Bacteriome interactions in paediatric atopic dermatitis in a rural and urban South African cohort

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DECLARATION

I, **Gillian Ophelya Nondumiso Ndhlovu**, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise), and that neither the whole or any part thereof has been, is being, or is to be submitted for another degree in this or any other university. I authorise the University to reproduce for the purpose of research either the whole or any portion of the contents in any manner whatsoever.

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SUMMARY

Background: Alterations in the skin and nasal bacterial communities, associated with staphylococcal predominance (especially *Staphylococcus aureus*) and low bacterial diversity, are linked to the pathology and severity of atopic dermatitis (AD). This alteration of the skin and nasal bacterial communities may limit the effectiveness of antimicrobial treatment strategies. In addition to the predominance of *S. aureus*, recent studies have reported a proliferation and potential role of other staphylococcal species, particularly coagulase-negative *Staphylococcus* (CoNS), in AD pathology and severity. However, these data are not consistent. Reports additionally indicate that bacterial differences in the skin and nose may be influenced by geographical differences. Despite this, most studies on the alterations of the bacterial communities in AD have been in American, European, and Asian children, with no study involving sub-Saharan African children. Also, the risk of AD in early childhood differs based on rural or urban residency, with rural children showing a lower risk to disease than urban children. The difference is thought to be linked to variations in environmental exposures and microbiomes. However, there is a lack of studies that have comprehensively investigated differences in bacterial colonisation and the environmental risk associated between rural and urban children with AD. Therefore, this study explored the prevalence of skin and nasal *S. aureus* colonisation, its genetic diversity, and the clinical and socio-demographic factors associated with colonisation in early childhood AD compared to children without AD, in rural and urban South Africa. We also investigated the colonisation prevalence and biofilm propensity of CoNS with *S. aureus*, and their relationship with AD and disease severity. Furthermore, the study assessed the differences in the skin and nasal between bacteriome of children with and without AD.

Methods: We conducted a cross-sectional study in children aged 9 to 38 months with (cases) and without (controls) AD from Cape Town (urban) and Umtata (rural), South Africa, between February 2015 and May 2016. Swabs, from all participants, were collected from the anterior nares and nonlesional skin (healthy appearing skin, usually the middle of the back which is considered to have minimal “contamination” from lesional skin). Additionally, lesional skin specimens (from the most active area of eczematous skin with acute and/or chronic changes) were collected only from children with AD. These swabs were cultured for *S. aureus* and CoNS species using mannitol salt agar and Dnase agar. Staphylococcal biofilm formation was assessed using the crystal violet microtiter assay. Antibiotic susceptibility testing was

conducted for *S. aureus* only, using the disk diffusion method and interpreted using the EUCAST guidelines (2021). *Spa* typing and whole-genome sequencing were employed to characterise *S. aureus* clonal lineages and population structure, respectively. Skin and nasal bacteriome profiles were analysed using 16S rDNA V4-V5 amplicon sequencing. Categorical variables were compared using the Fisher's exact test. Risk factors for staphylococcal carriage were identified using questionnaires and analysed using multivariate logistic regression. Moreover, risk factors associated with alpha diversity were analysed using linear regression.

Results: Overall, 220 children (116 cases and 104 controls) were recruited (median age, 22.49 [IQR, 17–28]). *S. aureus* was detected in 124 specimens (104 cases and 20 controls). *S. aureus* colonisation was higher in cases than in controls, independent of geographic location (54% *vs.* 13%, $p < 0.001$ rural, and 70% vs. 35%, $p = 0.005$ urban). Severe AD was associated with higher *S. aureus* colonisation than moderate AD (86% vs. 52%, $p = 0.015$) among urban cases. Risk factors associated with *S. aureus* colonisation included having AD, in both rural (adjusted odds ratio [aOR] 7.54, [95% confidence interval 2.92;19.47]) and urban (aOR 4.2, 1.57;11.2) children. Among rural children, living in an electrified house that uses gas (aOR 4.08, [1.59;10.44]) or utilises kerosene and paraffin (aOR 2.88, [1.22;6.77]) for heating and cooking were associated with increased risk of *S. aureus* colonisation. However, exposure to farm animals (aOR 0.3, [0.11;0.83]) as well as living in a house that uses wood and coal (0.14, 0.04;0.49) or outdoor fire (aOR 0.31, [0.13;0.73]) were protective among rural children. *Spa* types t174 and t1476, and t272 and t1476 were dominant among urban and rural cases, respectively. Controls had no dominant *spa* types, regardless of geographic location. In urban cases, *spa* type t002 and t442 isolates were identified only in severe AD, t174 was more frequent in moderate AD and t1476 in severe AD.

Of the 124 *S. aureus* isolates, high-quality sequences from 96 isolates (79 cases and 17 controls) were analysed. The prevalence of phenotypic antibiotic resistance was low, and mostly in isolates from cases (gentamicin [10%], rifampicin [4%], chloramphenicol [4%], and multidrug resistance [9%]). Also, there was a high detection (99%) of penicillin (*blaZ)* and fosfomycin *fosB* or mutations in *murA* or *glpT*) in both cases and controls. Isolates from cases formed stronger biofilms than those from controls (76% vs. 35%, $p = 0.001$). Cases were associated with DNA damage repair genes (*lexA*, *ssbA*, *dinG* and *recF*), toxin genes (*hly* and *see*) and insertion sequences that carry antibiotic resistance genes (IS431R, ISLgar5 and

IS256). In contrast, genes associated with *S. aureus* adhesion (*ebh*, *fnbB* and *sasG*), predominated among controls.

The most common CoNS included *Staphylococcus epidermidis* (n=104), *Staphylococcus hominis* (n=53), *Staphylococcus haemolyticus* (n=38), and *Staphylococcus capitis* (n=28). CoNS and *S. aureus* commonly co-colonised nonlesional skin among cases (urban: 24% vs. 3%, $p = 0.037$ and rural 21% vs. 6*%,* $p < 0.001$), and the anterior nares of urban cases (24% vs. 0%, $p = 0.002$) compared with the control group. The nonlesional skin of the controls in the urban group was more frequently uncolonised with any staphylococci compared with the cases in the urban group (47% vs. 20%, $p = 0.015$). Colonisation with *S. capitis* on nonlesional skin and anterior nares was positively associated with more severe disease in rural (48.3±10.8 vs. 39.7±11.5, $p = 0.045$) and urban cases (74.9±10.3 vs. 38.4±13, $p = 0.004$), respectively. Antibiotic exposure (aOR 5.25 [1.68;16.38]) and having AD (aOR 4.67 [1.58;13.77]) were significant risk factors for CoNS colonisation among urban children.

Shannon diversity was higher on nonlesional skin specimens of controls compared to cases ($p = 0.025$), but only in the rural group when stratified by location ($p = 0.0003$). Shannon diversity on lesional skin was negatively associated with having a positive reaction to nut (adjusted beta -1.93 [-3.76;-0.09], $p = 0.039$), but in the anterior nares it was positively associated with having a mother with asthma (adjusted beta 0.49, [0.002;0.97], $p = 0.049$). On nonlesional skin, a positive reaction to wheat (1.04 [0.31;1.77], $p = 0.006$) and having a father with AD (adjusted beta 0.88[0.05;1.72], $p = 0.037$) were positively associated with Shannon diversity, however, having a father with asthma was negatively associated (adjusted beta -0.84, [-1.63;-0.06], $p = 0.035$). Bacterial community structures differed across specimen types in both cases (Anosin R=0.3736, $p < 0.0001$) and controls (Anosim R=0.7025, $p < 0.0001$). The relative abundance of *Streptococcus*, *Granulicatela*, *Veillonella* and *Prevotella* was high on the lesional skin of cases compared to the nonlesional skin of cases; *Anoxybacillus* and *Cutibacterium* were dominant on the nonlesional skin of cases compared to the nonlesional skin of controls. The relative abundance of *Staphylococcus*, *Veillonella* and *Sphingomonas* was higher in the anterior nares of cases compared to the anterior nares of controls. In contrast, controls had a higher relative abundance of *Haematobacter*, *Romboutsia* and *Ruminococcacaea* on nonlesional skin compared to the nonlesional skin of cases. *Ornithobacterium*, *Moraxella* and *Suttonella* were higher in the anterior nares of controls compared to the anterior nares of cases.

Conclusion: Children with AD were more likely to be colonised with *S. aureus*, including antibiotic resistant and strong biofilm forming strains, than healthy children. These strains possessed genomic features reported to drive AD pathology. We also show that AD disease, and not geography, is a strong influencer of the composition and diversity of the skin and nasal dysbiosis. Furthermore, unique bacterial taxa may be driving AD pathology, highlighting the need for their consideration in restoration of a protective skin and nasal bacteriome in AD, especially in sub-Saharan African children.

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ABBREVIATIONS

AD	Atopic dermatitis
agr	Accessory gene regulator
AIP	Autoinducing peptide
AMP(s)	Antimicrobial peptide(s)
ATCC	American Type Culture Collection
BA	Blood agar (sheep)
BURP	Based Upon Repeat Pattern
CI	Confidence interval
CC	Clonal complex
CoNS	Coagulase-negative *Staphylococcus*
DNase	Deoxyribonuclease
Esp	*Staphylococcus epidermidis* serine protease
FLG	Filaggrin
IQR	Interquartile range
MHA	Mueller-Hinton agar
Min	Minute(s)
MLST	Multilocus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Mannitol salt agar
MSSA	Methicillin-susceptible *Staphylococcus aureus*
MSCRAMMs	Microbial surface components recognising adhesive matrix molecules
Nuc	Thermonuclease gene
OR	Odds ratio
oSCORAD	Objective scoring of atopic dermatitis
PVL	Panton-Valentine Leukocidin
16S rRNA	16S ribosomal ribonucleic acid gene
SCORAD	Scoring of atopic dermatitis
SE	*Staphylococcus* enterotoxin
Sec	Second(s)
SNP	Single nucleotide polymorphisms
Spa	*Staphylococcus aureus* protein A
Spa-CC	*Spa*-clonal complex

ST	Sequence type
STGG	Skim milk-tryptone-glucose-glycerol
TEWL	Transepidermal water loss
Tsst	Toxic shock syndrome toxin
WGS	Whole genome sequencing
°C	Degrees Celsius
μL	Microlitres

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CHAPTER 1

The Microbiology of Atopic Dermatitis: A Review of the Literature

1.1. Atopic dermatitis and its epidemiology in early childhood

Atopic dermatitis (AD), a chronic inflammatory skin disease with marked clinical heterogeneity and natural history[1], affects about 20% of children and up to 10% of adults globally[2,3]. Although its prevalence varies widely across countries, the highest prevalence has been reported among high income countries, such as the United States of America and the United Kingdom[3]. However, an increase in prevalence in African and Asian countries, with historically low AD prevalence, has recently been reported[3]. Despite this, several challenges to assessing the epidemiology of AD still exist. Challenges include the lack of a globally accepted and implemented diagnostic test and a nomenclature for AD and differences in clinical features of AD across different races and ethnicities[2,4]. Most notably, AD prevalence decreases with age, with the onset of disease occurring in 75% of children within the first 6 months and 60% at 12 months of life[5].

1.2. Features of atopic dermatitis

AD has polymorphic manifestations depending on race and age[3,4]. Patients with AD usually present with persistent, relapsing, intensively itchy and dry eczematous skin lesions[6,7]. Moreover, patients typically have a compromised quality of life and struggle with physical discomfort and limited daily activity[8]. Patients with AD may experience emotional distress due to social stigma and are at a higher risk of mental disorders[9,10]. In early childhood, AD is the initially encountered allergic disease[11]. It is usually the precursor of the "atopic march"; the development of other allergic diseases, including asthma, allergic rhinitis, and food allergies in later childhood[11-13].

1.3. Mechanisms and pathophysiology of atopic dermatitis

AD is primarily a disease of altered skin barrier function and immunity, with a complex pathogenesis[14]. It results from the interaction between skin barrier deficiencies and the immune, microbiological, and environmental mechanisms that contribute to its clinical manifestation (Fig. 1.1)[15,16]. Structural and immunological deficiencies in AD affected skin play a significant role in the pathogenesis and exacerbation of AD. These promote skin microbial dysbiosis that is

associated with increased colonisation of pathogenic bacteria, particularly *Staphylococcus aureus*[14].

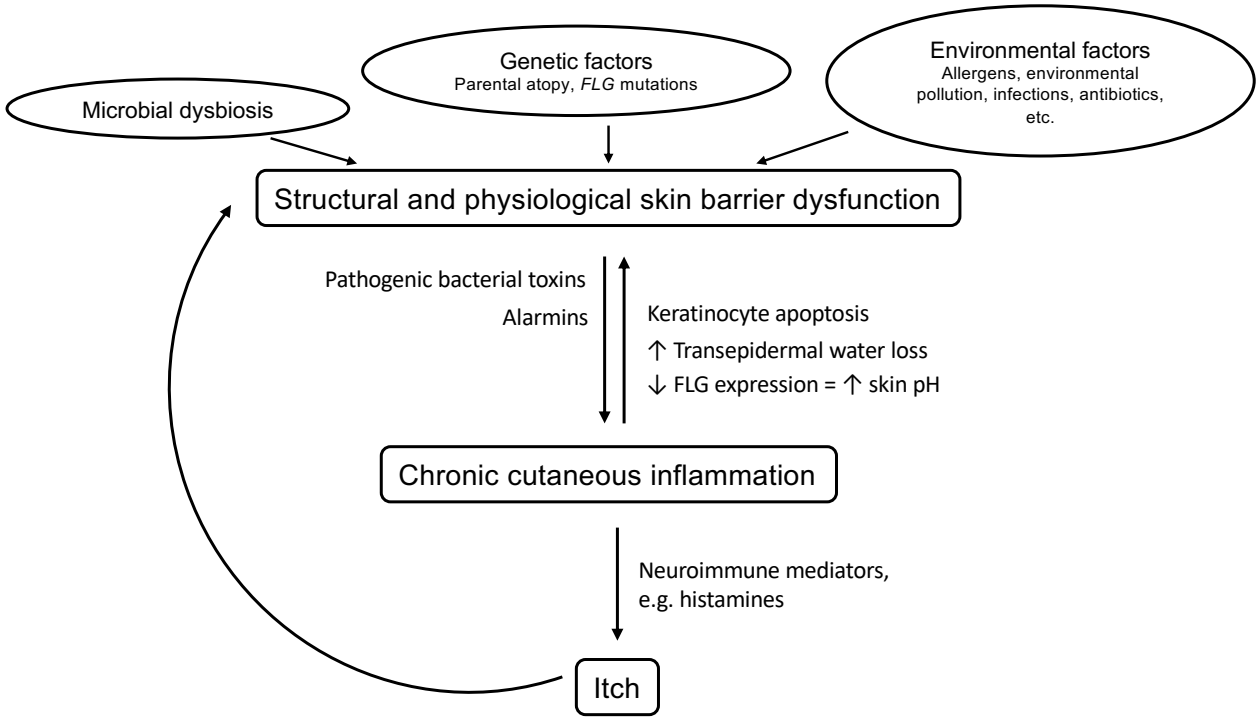


Figure 1.1. The mechanisms that contribute to the pathophysiology of atopic dermatitis. Structural and physiological skin barrier dysfunction is influenced by a parental history of atopy or filaggrin (FLG) mutations, microbial dysbiosis characterised by proliferation of pathogenic bacteria, and various environmental factors. The dysfunctional skin barrier then drives or promotes epidermal inflammation and subsequently itching both with independently further exacerbate skin barrier dysfunction. Details of the diagram modified from Sroka-Tomaszewska et al. and Zeigler et al. [15,16]

1.3.1. Skin barrier defects in the pathogenesis of atopic dermatitis

A dysfunctional skin barrier is one of the key features of AD. It is characterised by increased skin pH due to reduction of natural moisturising factor and ceramides, dryness due to increased transepidermal water loss (TEWL) and heightened epidermal proteolytic activity[17-24]. These result

in a dysfunctional epidermis that is sensitive to environmental insults and the overgrowth of pathogenic bacteria such as *S. aureus*[25-27]. Although keratinocytes on the outer layer of the skin produce antimicrobial peptides, AMPs (i.e., cathelicidins and β-defensins) that resist the colonisation of *S. aureus*, production of AMPs are reduced in AD, correlating with increased *S. aureus* colonisation [28,29].

1.3.2. Genetics of atopic dermatitis

Filaggrin, encoded by the *FLG* gene, is important in the formation and the maintenance of hydration of the stratum corneum, which is the outermost layer of the skin[30]. A major predisposing factor for AD is reduced or absent *FLG* expression [31], and individuals with deficient or reduced *FLG* expression, have an increased propensity to *S. aureus* colonisation[32-34]. However, associations between *FLG* mutations and AD are rarely observed in children of African ancestry. The African AD clinical phenotype is characterised by lichenification, chronicity and severe forms of the disease. Also, there is a strong association between *FLG* loss-of-function mutations and AD. These include R501X, 228del4, R2447X and S3247X mutations, which are mostly identified in European and some Asian populations[35]. Although these mutations lead to reduced *FLG* expression, no association with AD has been found in the few notable studies in South African, Ethiopian and in African American populations, despite the increase in AD prevalence contrasted by reduced filaggrin protein levels in skin[35-37]. Common *FLG* mutations do not significantly contribute to the genetic risk of AD, suggesting a non-*FLG* associated risk factor in these populations. A similar trend was observed among Japanese infants in a birth cohort study[38].

1.3.3. Dysregulation of epidermal immunity in atopic dermatitis

In addition to structural epidermal alterations, patients with AD have dominant local T helper type 2 (Th2)-mediated inflammation, characterised by an abundance of pro-inflammatory cytokines interleukin-1β (IL-1β), IL-4, IL-13, and interferon-γ (IFN-γ)[39]. These pro-inflammatory cytokines significantly decrease *FLG* expression in keratinocytes, thereby promoting barrier dysfunction[40]. The chronic activation of macrophages in response to the proliferation of pathogenic bacteria on skin lesions further contributes to chronic inflammation[41]. Also, disruption of the skin barrier and

pathogenic bacterial proliferation trigger the activation and stimulation of epidermal dendritic cells that in turn produce the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α.[42] This scenario further contributes to chronic epidermal inflammation. Chronic inflammation in AD to increases *S. aureus* adhesion and overgrowth in lesional skin[43,44]. In contrast, this phenomenon suppresses the growth of commensal bacteria[43,44]. Additionally, the proliferation of certain pathological bacteria has harmful consequences on the epidermal immunity. For example, *S. aureus* induces mast cell degranulation that induces pro-inflammatory responses and exacerbate epidermal damage[39].

1.3.4. Skin and nasal bacterial dysbiosis in atopic dermatitis

The skin bacteriota is important in maintaining the health of the skin barrier function by promoting homeostasis, regulating immune function, and facilitating resistance to pathogenic infections[45,46]. It is characterised by high microbial diversity and relative abundance of commensal bacteria that are thought to be protective against the development of AD[47,48]. The predominant genera of these commensal bacteria include *Propionibacterium, Corynebacterium, Streptococcus,* and *Staphylococcus* [49]. Other genera, such as *Gemella, Granulicatella, Rothia* and *Haemophilus*, have been reported to be present on healthy skin, at lower relative abundances[50]. Predominant genera are age dependent; with *Streptococcus* mostly associated with younger children, and *Propionibacterium* and *Corynebacterium* with adults[50]. However, these observations are not consistent[51]. In contrast, AD lesions, and to a lesser extent nonlesional AD skin, have a lower relative abundance of these skin commensal bacteria, especially *Propionibacterium* and *Corynebacterium*[47,52,53]. This correlates with increased relative abundance of the *Staphylococcus* genus[51], highlighting an antagonistic relationship between these skin commensal bacteria and *Staphylococcus*. Increased *Staphylococcus* abundance in AD is associated with low bacterial diversity[48,50,54-56]. Additionally, the low bacterial diversity in AD correlates with increased disease severity[51], highlighting the importance of skin bacterial shifts which are characterised by the increased abundance of *Staphylococcus* and low bacterial diversity in the pathogenesis of AD. Studies assessing skin bacterial changes in response to AD treatment have indeed demonstrated an improvement in clinical markers of disease associated with a decrease in the relative abundance of *Staphylococcus* and restoration of bacterial diversity[49,55,57]. Although alterations in the skin

bacterial community are the most studied in AD, recent studies show shifts in the skin fungal, mite and viral communities associated with disease parameters[58-61]. This suggests that other microbial taxa, in addition to the bacterial community, potentially influence the pathogenesis and exacerbation of AD.

Nasal colonisation with pathogenic bacteria is a risk factor for bacterial infections, such as skin and soft tissue infections[62,63]. Although the skin bacterial community is the most important in AD, shifts in the nasal bacterial community, characterised by the predominance of the *Staphylococcus* genus, have been observed in AD[64]. Similar to healthy skin, the nasal bacterial community differs according to age; with *Moraxella, Streptococcus, Dolosigranulum, Haemophilus* and *Neisseria* predominant in younger children[65], and *Propionibacterium* and *Corynebacterium* in adults[65,66]. However, other studies report the additional enrichment of *Staphylococcus* and *Moraxella*[67]. Totte et. al.[64] recently reported the enriched *Moraxella*, *Corynebacterium, Dolosigranulum* and *Staphylococcus* genera in the nasal microbiome of children with AD. Although an association with increased AD severity was noted, its contribution to AD was less than the skin bacteriota[64]. However, the exclusion of a control group in this cohort, limited the assessment of the "healthy nasal bacteriota". Except for *Staphylococcus*, these enriched genera are relatively abundant in healthy paediatric nasal bacteriota[65]. Therefore, this challenges their relevance in AD and disease severity and highlights the need for studies that will investigate i) differences in the nasal bacteriota of paediatric patients with AD and those without AD, and ii) relations with clinical markers of disease.

1.3.5. *S. aureus* colonisation in atopic dermatitis

The predominance of the genus *Staphylococcus* in AD skin is primarily due to the proliferation of *S. aureus*[49,68]. This suggests the importance of this bacterium in AD pathogenesis and flares. Indeed, clinical studies have demonstrated a strong association between skin *S. aureus* colonisation and AD flares and severity[69,70]. Patients with AD tend to have a higher prevalence and abundance of *S. aureus* on lesional and nonlesional skin, and the nose[14]. Moreover, birth cohort studies in the Netherlands, Switzerland, and Japan have shown that early life colonisation with *S. aureus* increases the risk of developing AD[38,71,72]. However, another birth cohort study in Denmark found

no relationship between early life *S. aureus* colonisation and the development of AD[73]. This suggests that while early life colonisation with *S. aureus* is frequently a predictor of the development of AD, this phenomenon is likely dependent on geography and race in which instance other bacteria may be implicated in the onset of AD [add refs: 38,71,72]. In addition to the increased skin colonisation with *S. aureus*, patients with AD are more likely to be colonised with this bacterium in the nose, particularly the anterior nares, compared to healthy individuals[63]. Frequent nasal colonisation in the first year of life is associated with both the onset of AD and increased disease severity[69,71]. Moreover, nasal colonisation is a risk factor for *S. aureus* infections outside the nose, including skin infections in AD[63,74,75].

1.3.5.1. *S. aureus* colonisation in health and atopic dermatitis

S. aureus colonisation of the skin of healthy, nonatopic individuals is rare, with 5–30% of the population being colonised[69]. The overabundance of *S. aureus* in AD lesions was first shown in 1974 by Leyden et. al.[76]. This group noted that more than 90% of adults with AD were colonised with *S. aureus* on AD lesions and these patients had comparatively higher loads of *S. aureus* compared to nonlesional skin. Over the years, more studies have supported this observation showing a higher relative abundance of *S. aureus*, which is associated with severe forms of AD [43,70], in patients with AD compared with their counterparts without AD[49,69,76-78]. Clinical studies have shown improvements in clinical disease scores in AD and the restoration of bacterial diversity observed in treatment regimens targeting the clearance of *S. aureus*[52,55,79]. This consolidates the importance of this bacterium in AD pathology. These support the notion that dysbiosis, linked with increased *S. aureus* abundance, has an active influence on AD disease activity. Another study showed that patients with AD, who were not colonised with *S. aureus*, also exhibited improved clinical disease scores with treatment, suggesting that clinical improvements cannot be attributed to a reduction in *S. aureus* colonisation alone[79]. This indicates that although dysbiosis associated with the overabundance of *S. aureus* plays an important role in AD pathogenesis, it is not the primary driver of the disease[51]. Moreover, another longitudinal study found that *S. aureus* colonisation was associated with AD cross-sectionally but not longitudinally[80]. In this study, the authors concluded that a causal relationship between *S. aureus* and AD was unlikely.

1.3.5.2. Consequences of *S. aureus* colonisation on the skin in the pathogenesis and exacerbation of atopic dermatitis

S. aureus colonisation in AD leads to epidermal damage and a hyperresponsive epidermal immunity that promotes disease flares[56]. The pathological effect of *S. aureus* in AD is underlined by its production of a variety of virulence factors that directly contribute to disease activity, by targeting epidermal structures and immune cells (Table 1.1). *S. aureus* strains on AD skin are typically associated with a higher prevalence of toxin production compared to *S. aureus* strains nonatopic skin[81-83]. *S. aureus* virulence factors include proteases, such as staphopain and staphylococcal V8 protease, that degrade host-derived AMPs and barrier proteins, resulting in the reduced ability of the host to clear *S. aureus* and promote barrier dysfunction, respectively[17,84,85]. The toxins produced degrade epidermal tight junctions (exfoliative toxins)[82,86] and inhibit terminal differentiation (EDIN), further contributing to the dismantling of the barrier integrity[87]. Moreover, *S. aureus* produces superantigenic toxins, such as staphylococcal enterotoxins and toxic shock syndrome toxin (TSST), which induce antigen-independent T cell activation and subsequent release of proinflammatory cytokines, causing the putative chronic inflammation in AD[88]. Other toxins produced, such as a-toxin, promote mast cell degranulation that leads to severe pruritus characteristic of AD lesions[87]. Not only do *S. aureus* virulence factors confer pathological colonisation, but they also allow *S. aureus* to evade host innate immune responses[88], thereby promoting persistent colonisation. In addition to immune evasion-associated virulence factors, *S. aureus* achieves persistent colonisation by producing biofilms[25]. These are clusters of microbes surrounded by a biomass, composed of polysaccharide intercellular adhesin (PIA), proteins, extracellular DNA (eDNA) or amyloid fibrils[25]. *S. aureus* virulence factors have been shown to accumulate in biofilms[89]. Biofilm growth provides resistance to host immune responses and antibiotic regimens targeted at eliminating *S. aureus*[43]. *S. aureus* biofilms are common in AD lesions and tend to correlate with increased disease severity[43,84,90]. Moreover, the pervasive inflammation in AD lesions promotes the overgrowth *S. aureus* biofilms[43], as such biofilm growth provides a persistent source of pathological *S. aureus* colonisation.

Table 1.1. *Staphylococcus aureus* virulence factors with clinically relevance in the pathophysiology and exacerbation of atopic dermatitis.

Function	Virulence factor	Mechanism of action and contribution to AD pathophysiology	Ref
Toxins	Superantigens	Target and bind to T-cells, resulting in their activation. This induces the production of proinflammatory cytokines.	44
	Leucocidins	Form pores on the cell membrane of leukocytes, resulting in cell lysis.	91
	Haemolysins	Form pores on the cell membrane of erythrocytes, resulting in cell lysis.	88
	Phenol soluble modulins	Promote host cell lysis and inflammation, and biofilm remodelling. This leads to the dispersal of *S. aureus* in biofilms, thereby promoting recurrent colonisation.	92,93
	Exfoliative toxins	Compromise skin barrier integrity by degrading the desmosomal cadherins that facilitate cell-to-cell adhesion in the epidermis.	86
Proteases	Staphopains	Degrade host-derived AMPs.	17
	V8 protease	Degrade epidermal barrier proteins.	17
Immune evasion	Staphylokinase	Increases the invasiveness of *S. aureus* through plasmin activation. This results in larger lesions with skin disruption and concomitant reduced clearance of pathobiont colonisation by the host.	91,94
	CHIP	Blocks the chemotaxis of neutrophils.	95
	SCIN	Interferes with the activation of the complement cascade.	95
	Staphylococcus protein A	Blocks opsonisation and subsequent phagocytosis which results in elimination, thereby promoting persistent colonisation. It also induces proinflammatory responses via the tumour necrosis factor receptor 1 that contribute to inflammation-induced skin barrier damage in AD lesions.	96,97
	Coagulase	Mediates the clotting of fibronectin, plasma, and blood.	88
Adhesion	MSCRAMMs	Binds to host-specific extracellular matrix proteins (including elastin, collagen, fibronectin and fibrinogen/epidermal cytokeratins), allowing for *S. aureus* adhesion and subsequent colonisation.	98,99

MSCRAMMs: microbial surface components recognising adhesive matrix molecules include elastin-binding, collagen-binding, fibronectin-binding proteins, and clumping factor proteins; **CHIP:** chemotaxis inhibitory protein; **SCIN:** staphylococcal complement inhibitor.

1.3.5.3. The molecular epidemiology of *S. aureus* in atopic dermatitis

The molecular characterisation of *S. aureus* is pivotal in understanding the strain biology, dissemination and the clinical outcomes associated with infections caused by this bacterium.[100] Molecular epidemiological studies have shown that patients with AD are colonised by *S. aureus* lineages that differ from those that are detected in individuals without AD.[101] *S. aureus* clonal complex, CC1 and CC5, are frequently detected among children with AD.[18,101-103] CC30 strains rarely colonise children with AD, but tend to be more common among healthy children.[18,101] This may suggest that AD selects for specific CCs that may contribute to AD disease activity or colonisation with certain CCs is a risk factor for the development of AD[56]. CC1 strains from patients with AD exhibited greater adherence to corneocytes[18], whereas CC30 strains from children without AD has reduced toxin production compared to AD-associated CCs.[104] Despite this, CCs associated with paediatric AD differ across geographical regions (Figure 1.2). CC30, which is predominant in children without AD in Ireland[18] and the United Kingdom,[101] is associated with AD in Brazilian children.[105-108] In other countries, such as Taiwan, where CC59 predominates,[109] and Spain, where CC30 predominates,[102,110] CCs are similar in children with and without AD. However, additional CCs (CC5, CC8 and CC45) are predominant among Spanish children with AD.[102,110] In Canada, children with AD are frequently colonised by CC1, CC5, CC8, CC15 and CC45,[103,111] while Japanese AD patients are limited to CC20.[112] However, it is unclear why some CCs, such as CC30, are common in children with AD in some settings but are not associated with AD in others. There are currently no molecular epidemiology studies involving sub-Saharan African children with AD prior to this study.

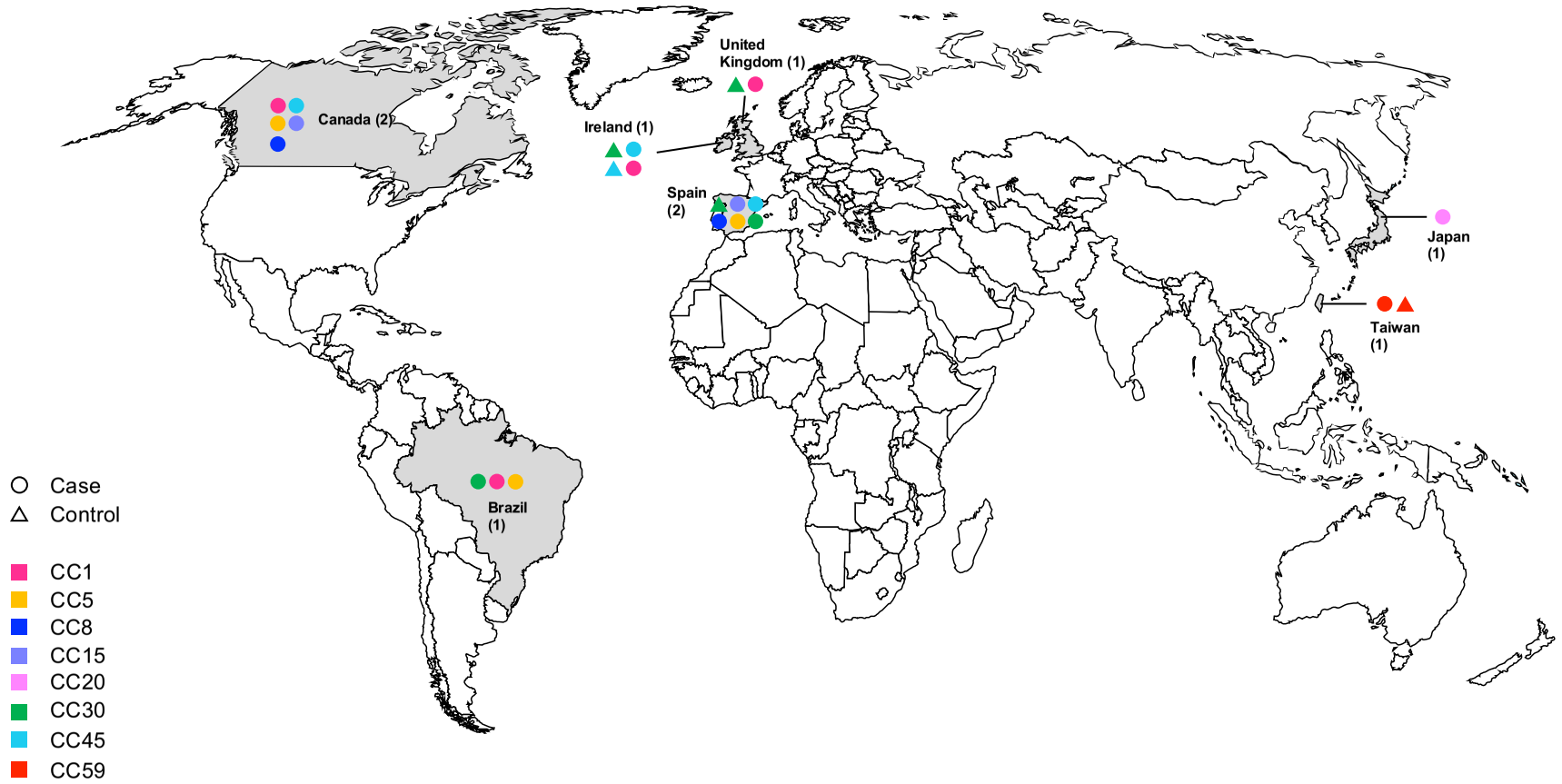


Figure 1.2. The global distribution of *S. aureus* clonal complexes in childhood AD. Clonal complex data available for countries shaded in grey. The number in brackets represents the number of independent studies conducted in each country. Data included in this figure was modified from the studies by Fleury et al.[18] in Ireland, Harkins et al.[101] in the United Kingdom, Cavalcante et al.[105-108] in Brazil, Lo et al.[109] in Taiwan, Rojo et al. and Benito et al.[102,110] in Spain, Yeung et al. and Balma-Mena et al.[103,111] in Canada, and Aziz et al.[112] in Japan.

1.3.5.4. The impact of antibiotic use in AD

Antibiotics, including fusidic acid, gentamicin, and clindamycin, are routinely used to treat *S. aureus* infections in AD[77,113]. Other antibiotics, such as mupirocin, are used to clear *S. aureus* nasal infections[114]. Although antibiotic use is usually associated with an improvement in clinical disease scores[115], antibiotic success is limited by rapid *S. aureus* recolonisation after discontinuation of treatment in AD patients[25,115], and is associated with significant perturbations of the skin bacterial community[116]. Additionally, *S. aureus* can develop resistance to almost all antibiotic classes[113]. There is an increasing prevalence of *S. aureus* resistant strains in children with AD[77,113,117], most importantly, methicillin-resistant *S. aureus* (MRSA) strains. These are associated with resistance to multiple antibiotics, which further limit the success of antibiotic regimes in AD[109,118]. Additionally, MRSA colonisation is associated with a greater reduction of skin commensal bacteria, lower microbial diversity and more severe disease compared to methicillin susceptible *S. aureus* (MSSA) colonisation in AD[54,119].

1.3.6. Bacterial interactions on the skin and anterior nares of individuals with and without atopic dermatitis: implications on *S. aureus* colonisation and virulence

Extensive interactions between bacteria occupying the same ecological niche exists. These competitive and synergistic interactions are important in shaping the composition and diversity of microbial communities[120]. In antagonistic interactions, bacteria either sequester or consume nutrient resources in a niche, preventing their competitors from accessing those nutrient resources; produce antimicrobials that inhibit the growth of their competitors; or produce factors that interfere with virulence signalling pathways[120-122]. However, bacteria may also synergise with each other for the degradation of nutritional sources for their utilisation[123], and to promote their persistent growth through the formation of and existence in mixed biofilm communities[124].

1.3.6.1. Implications of interactions between *S. aureus* and CoNS on *S. aureus* growth in health and AD

The colonisation and survival of *S. aureus* on the skin and nose can be hampered by resident commensal bacteria. Most of these commensal bacteria belong to the coagulase-negative *Staphylococcus* (CoNS) group, which includes *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Staphylococcus caprae* among others. However, other genera such as *Corynebacterium, Propionibacterium,* and *Streptococcus*, which are abundant on the skin and nose, have also antimicrobial effects against *S. aureus*[53,121,125]. CoNS directly limit *S. aureus* colonisation by producing AMPs, such as lugdinin (produced by *Staphylococcus lugdunensis*) and the serine protease, Esp (produced by *S. epidermidis*)[68,126-130]. They can also impair *S. aureus* survival by facilitating glycerol fermentation,[131] or by synergising with host immune and epidermal cells, augmenting their ability to eliminate *S. aureus*.[132,133] Moreover, CoNS production of proteases, such as Esp, limits the ability of *S. aureus* to form and destroy existing biofilms[127,134,135], which subsequently increases the susceptibility of *S. aureus* to be cleared by host immune responses[127]. This protective effect against *S. aureus* survival is generally limited to the skin and nose of individuals without AD[136]. In contrast, there is a reduction in the abundance of CoNS with antimicrobial effects against *S. aureus* in patients with AD[129]. This

relieves the growth suppressive effect on *S. aureus* by CoNS, resulting in the proliferation of this bacterium in patients with AD. Moreover, when present in AD, CoNS promote *S. aureus* growth and its pathological contribution to disease[136-138].

1.3.6.2. The effect of the interactions between *S. aureus* and CoNS on *S. aureus* pathogenic colonisation

Not only do CoNS impair *S. aureus* growth, but they can also suppress the production of virulence factors by *S. aureus* by inhibiting the activity of the accessory gene requlatory (agr) quorum sensing system[126,139]. Autoinducing peptides (AIPs) from other staphylococcal species or signalling inhibitory molecules produced by CoNS, inhibit the functioning of the *S. aureus* agr quorum sensing system via a process called quorum quenching[121,126,140,141]. Quorum quenching has been shown to be associated with reduced *S. aureus* virulence[134] haemolytic toxin production[142] and enhanced host immune responses against *S. aureus* in murine skin infection models[126]. These findings highlight the significance of commensal bacterial species in the prevention of *S. aureus* colonisation and pathogenic outcomes in *S. aureus* infections, which may have beneficial clinical effects in AD-associated *S. aureus* infections.

1.3.7. Contribution of other Staphylococcal species in atopic dermatitis flares

CoNS are opportunistic and a major cause of bacterial infections in hospital settings[143], however, they possess a lower pathogenic potential than to *S. aureus*[143,144]. This is primarily limited to biofilm formation, however, they may produce other virulence factors, such as phenol soluble modulins, metalloproteases and haemolysins[143,145]. Due to the large focus on *S. aureus*, the possible role of CoNS in the pathogenesis and pathophysiology of AD has been underappreciated, until recently. Traditionally, CoNS were considered infrequent in AD lesions compared to nonlesional skin[47,129], however, studies have reported their enrichment in AD; especially with *S. capitis, S. epidermidis* and *S. lugdunensis*[49,146-148]. CoNS are also associated with biofilm formation in AD lesions[98] and may induce epidermal damage and inflammation[149], which is associated with increased disease severity[148,150]. However, these data are not consistent with other studies that i) report no difference in CoNS colonisation between

children with and without AD[151], ii) report associations with milder forms of the disease[58,148,152] or, iii) report no difference between various forms of disease severity[64]. The differences underlying the enrichment of CoNS and their association with disease severity, possibly reflect species differences or host factors, such as race or geography.

1.3.8. Risk factors associated with atopic dermatitis and staphylococcal colonisation

Although AD is primarily influenced by intrinsic factors, such as epidermal barrier integrity and immunity; extrinsic factors, including environmental exposures, can contribute to disease activity and exacerbations. The potential role of environmental and socioeconomic factors in AD was initially inferred from the differences in the risk factors of AD, which vary across countries and regions[8]. Indeed, numerous environmental and socioeconomic factors, that increase the risk of AD, have been identified. These include overcrowding[153], carbohydrates and saturated fatty acid-rich diet[8] and high socioeconomic status or higher education level[153]. In contrast, a farm environment and associated animal exposure[154], exposure to UV[155] and an omega-3-polyunsaturated fatty acid-rich diet[156] are generally associated with a reduced risk of AD. These factors also influence the skin and nasal bacterial colonisation, which, in turn, affects disease activity and exacerbations in AD. For example, nitrosative stress from air pollution, a proxy for urban living, has bactericidal effects on skin staphylococcal commensal bacteria and reduces their biofilm forming ability[157]. Air pollution also reduces the abundance of nasal *Corynebacterium* in early childhood[158]. Moreover, individuals in rural and urban areas interact differently with their environments, influencing the composition of the skin and nasal bacterial communities[159]. Urban living is associated with reduced skin bacterial diversity and loss of protective skin commensals compared with rural living[159-161].

1.4. Scope and rationale of the thesis

Children under the age of five years have the highest burden of AD, however, limited studies focusing exclusively on defining changes in bacterial colonisation and their association with AD pathology have been published. Despite the significant role of the environment in influencing the skin and nasal bacterial colonisation[162], there is a scarcity of data comparing differences in bacterial colonisation between rural and urban children with AD. Furthermore,

studies involving children were mainly conducted in Caucasian[49,64,163] and Asian[164,165] children; with only one study including African children (specifically Egyptian). No studies were conducted in children from sub-Saharan African[51].

1.5. Overall aim

The overarching aim of the study was to investigate bacterial colonisation in early childhood AD, its association with disease severity and associated risk factors.

1.5.1. Specific aims

a. To determine the prevalence and genotypes of *S. aureus* in children, with and without AD, from rural and urban South African settings, and evaluate the risk factors for *S. aureus* colonisation in each geographic location.
b. To investigate the phenotypic and genomic features of *S. aureus* in AD compared to health in early childhood using a pan-genomic approach.
c. To assess patterns of staphylococcal colonisation and biofilm formation in children, with and without AD, from rural and urban South African settings.
d. To characterise the skin and nasal bacterial composition and diversity in rural and urban South African children with and without AD.

1.6. Statement of ethics

The parent study was approved by the Human Research and Ethics Committee of the Faculty of Health Science, University of Cape Town (HREC/REF: 451/2014) and the Western Cape Provincial Child Health Research Committee. Additional ethical approval specifically for the study was obtained from the Human Research and Ethics Committee of the Faculty of Health Science, University of Cape Town (HREC/REC: 668/2020). No additional data were collected other than those approved in the parent study. Written informed consent and assent were provided by guardians and participants, respectively. All data obtained and generated during the study were kept confidential. This research was conducted in accordance with the Declaration of Helsinki.

1.7. Thesis outline

CHAPTER 1 provides a review of the current literature on bacterial colonisation in AD in early childhood. We look at the prevalence of colonisation, the impact of colonisation, the relationship between *S. aureus* and coagulase-negative staphylococci and their consequence in disease propagation. We also summarise data on the role of the environment on bacterial colonisation of the skin and nose. Lastly, we highlight gaps in existing literature that we seek to fill with the present study.

CHAPTER 2 explores the molecular epidemiology of skin and nasal *S. aureus* colonisation in young children with and without AD. Here, we compare the prevalence of *S. aureus* isolates from the skin and nose of children with AD to those without clinical disease from rural and urban communities in South Africa as well as assess their relationship with disease severity in children with disease. We further explore the risk factors (i.e., clinical, socio-demographic, and environmental exposures) that are associated with *S. aureus* in each of the study sites using multivariate logistic regression analyses. Lastly, we describe the molecular epidemiology of *S. aureus*, i.e., the distribution of this bacterium's clonality determined by *spa* typing.

CHAPTER 3 describes and compares the genomic and phenotypic characteristics of *S. aureus* in AD to those from children without clinical disease. First, we report on the antibiotic susceptibility profiles and biofilm forming ability of *S. aureus* using the disc diffusion and crystal violet microtiter assay, respectively. We then assess the genomic differences between *S. aureus* strains from children with and without clinical disease using a pan-genomic approach.

CHAPTER 4 investigates the prevalence of CoNS on the skin and nose of children with AD compared to those without clinical. We then statistically explore the relationship between *S. aureus* and CoNS and their influence on disease severity. We conclude this chapter with the assessment of i) overall staphylococcal biofilm formation, ii) dual species biofilms of *S. aureus* and a co-colonising coagulase-negative and, iii) their correlation with disease severity.

CHAPTER 5 describes the changes in skin and nasal bacterial community structure and diversity in children with AD compared to healthy controls. We also explore the risk factors (i.e., clinical, socio-demographic, and environmental) that are associated with alpha diversity.

CHAPTER 6 summarises the key findings of the study and their implications, and further proposes the direction for future studies.

CHAPTER 2

Molecular epidemiology of *Staphylococcus aureus* in African children from rural and urban communities with atopic dermatitis

2.1. Abstract

Background: *Staphylococcus aureus* is associated with the exacerbation and severity of atopic dermatitis (AD). Studies have not investigated the colonisation dynamics of *S. aureus* lineages in African children with AD. We determined the prevalence and population structure of *S. aureus* in children, with and without AD, from rural and urban South African settings.

Methods: We conducted a study of AmaXhosa children aged 9-38 months with and without AD from rural Umtata and urban Cape Town, South Africa. *S. aureus* was screened from skin and nasal specimens using culture and clonal lineages were determined by *spa* typing. Logistic regression modelling was employed to assess risk factors associated with *S. aureus* colonisation.

Results: *S. aureus* colonisation was higher in cases compared to controls, independent of geographic location (54% vs. 13%, $p<0.001$ and 70% vs. 35%, $p=0.005$ in Umtata [rural] and Cape Town [urban], respectively). Severe AD was associated with higher colonisation compared with moderate AD (86% vs. 52%, p=0.015) among urban cases. Having AD was associated with colonisation in both rural (odds ratio [OR] 7.54, 95% CI 2.92;19.47) and urban (OR 4.2, 95% CI 1.57;11.2) children. In rural children, living in an electrified house that uses gas (OR 4.08, 95% CI 1.59;10.44) or utilises kerosene and paraffin (OR 2.88, 95% CI 1.22;6.77) for heating and cooking were associated with increased *S. aureus* colonisation. However, exposure to farm animals (OR 0.3, 95% CI 0.11;0.83) as well as living in a house that uses wood and coal (OR 0.14, 95% CI 0.04;0.49) or outdoor fire (OR 0.31, 95% CI 0.13;0.73) were protective. *Spa* types t174 and t1476, and t272 and t1476 were dominant among urban and rural cases, respectively, but no main *spa* type was observed among controls, independent of geographic location. In urban cases, *spa* type t002 and t442 isolates were only identified in severe AD, t174 was more frequent in moderate AD, and t1476 in severe AD.

Conclusion: The strain genotype of *S. aureus* differed by AD phenotypes and rural-urban settings. Continued surveillance of colonising *S. aureus* lineages is key in understanding the role of this bacterium in AD disease.

2.2. Introduction

Atopic dermatitis (AD) is a common childhood inflammatory skin disease that frequently presents in early childhood[101]. The prevalence of AD is high in developed countries, where it affects 10-20% of children[166]. However, recent epidemiological data indicate an increase in the prevalence of AD among children in developing countries, including South Africa[8,167,168]. The increasing prevalence of AD and allergy is also associated with urbanisation, with a lower prevalence and microbial-related protective environmental factors noted in rural areas[8,154]. Patients with AD usually suffer from persistent or relapsing itchy and dry eczematous skin lesions with inflammation and increased susceptibility to cutaneous *Staphylococcus aureus* (*S. aureus*) colonisation associated with perturbation of the skin microbial community[6,169]. In addition to skin colonisation, *S. aureus* has also been reported to colonise the nasal cavity as a primary reservoir for extra-nasal auto-transmission[69]. Skin and nasal *S. aureus* colonisation has been demonstrated in both AD patients and healthy individuals; however, a higher colonisation density and prevalence has been described in AD patients[69]. *S. aureus* colonisation has also been associated with AD pathogenesis[170], with colonisation preceding the clinical onset of AD in early childhood[72]. *S. aureus* produces a variety of virulence factors, including superantigens, proteases, as well as dermolytic and cytolytic toxins which contribute to the progression of AD[56]. Nonetheless, other staphylococcal species, including *S. epidermidis* and *S. haemolyticus*, have been implicated in the pathophysiology of AD by the degradation of epidermal structural proteins[48,149]. Molecular epidemiological studies have shown that while colonisation occurs in both AD patients and healthy individuals, the genetic background of colonising *S. aureus* strains differ across AD disease phenotypes and may influence disease pathogenesis and severity[101,103]. We hypothesised that geographic location affects *S. aureus* colonisation in AD and in health through distinct environmental exposures. Here, we report the prevalence and genotypes of *S. aureus* from skin and nasal samples of AmaXhosa AD and non-AD children in rural and urban South African settings. Additionally, we evaluated the risk factors for *S. aureus* colonisation in each geographic location.

2.3. Materials and methods

2.3.1. Study design, setting and population

We conducted a cross-sectional study of 220 children, with and without AD, aged 12–36 months (overall mean age 22.4 months; standard deviation 0.54 months) from February 2015 to May 2016 (Figure 2.1)[154,171]. Urban control subjects (n=50) were recruited as a sub-study from non-allergic, non-food-sensitised subjects participating in the South African Food Allergy (SAFFA) study at randomly selected creches in the Cape Town metropole. As creches are rarely found in the rural district, rural controls (n=54) were recruited from children of eligible age from the areas surrounding 10 district community health clinics in the rural Mqanduli district of Umtata. Patients with moderate-to-severe AD (n=56) were recruited from the Department of Paediatric Dermatology of the Red Cross War Memorial Children's Hospital in Cape Town and rural cases (n=60) from the Department of Dermatology, Nelson Mandela Academic Hospital, in Umtata. AD was clinically diagnosed by a dermatologist using the validated UK Working Party diagnostic criteria for AD[172]. Disease severity was determined using the objective SCORing of Atopic Dermatitis (oSCORAD) index as moderate (15–40) and severe (>40)[173]. A study nurse gathered environmental exposure data in addition to the clinical data.

2.3.2. Specimen collection and processing

Sterile Copan nylon-tipped flocked swabs (Cat. No. 516C; Copan Italia, Brescia, Italy) were used to collect samples from lesional (i.e., most active area of eczematous skin with acute and/or chronic changes) and non-lesional skin (i.e., area with the most normal-appearing skin – usually the back). The swab was pre-moistened with sterile distilled water and a 4 cm^2 area of the skin lesion was swabbed for at least 1 minute in a non-overlapping manner. In addition, nasal swabs were collected from all participants to determine the *S. aureus* carriage status according to previously described methodology[174]. The collected swabs were immediately placed into 1 ml skim milk-tryptone-glucose-glycerol (STGG), transported at 4°C to the laboratory within two hours of collection and frozen at -80 °C for subsequent batch processing. All lesional, non-lesional, and nasal swabs stored in STGG were allowed to thaw at room temperature, vortexed for 30 seconds and 100 µl were inoculated onto Mannitol Salt Agar (MSA) (National Health Laboratory Services [NHLS], Green Point Media Laboratory Cape Town, South Africa), and aerobically incubated at 37 °C for 48 hours. Isolates that were positive for both mannitol fermentation and Dnase production were presumptively identified as *S. aureus*[175].

2.3.3. Nucleic acid extraction

Recovered *S. aureus* isolates were aerobically subcultured onto 2% sheep blood agar at 37 °C overnight. Genomic DNA extraction was completed using a modified heat lysis method[176]. Briefly, colonies were re-suspended in AVE buffer (Qiagen, Hilden, Germany), instead of phosphate-buffered saline, and centrifuged at 13,000 × g for 2 minutes. Depending on the initial DNA concentration, the supernatant containing genomic DNA was diluted in AVE buffer to a final concentration range of 20-70 ng/µl.

2.3.4. Molecular identification and characterisation of *S. aureus* isolates

Isolates presumptively identified as *S. aureus* were screened for the thermonuclease (*nuc*) gene using species-specific primers as previously described[177]. *S. aureus* isolates were characterised by staphylococcal protein A (*spa*) typing, targeting the variable X-region of the gene using the conventional primers *spa*-1113F/*spa*-1514R[178,179]. Isolates that failed to yield a *spa* amplicon or had poor sequence quality were re-analysed using alternative *spa* primers T3F/1517R or 1095F/1517R[180,181]. Clustering was based on their genetic relatedness to *spa*-clonal complexes (*spa*-CCs), using the Based Upon Repeat Pattern (BURP) clustering algorithm of the Ridom Staph Type software (Ridom GmbH, Münster, Germany)[182]. PCR detection of the *nuc* gene was performed to rule out misidentification of isolates that failed to yield a *spa* amplicon[177].

2.3.5. Statistical analysis

All data analyses were performed using Stata version SE16.0 (1985-2019 StataCorp LP, Texas, USA). The significance threshold for all analyses was 0.05. Univariate and multivariate analyses to assess risk factors for *S. aureus* colonisation were performed using logistic regression and presented as odd ratios (OR) and adjusted Ors reported with a 95% confidence interval (CI). The level of statistical significance in the logistic regression analysis was determined by a Chi-square test. Variables that were significant determinants for colonisation were included in the multivariate logistic regression model. Comparison of categorical data was performed by the Chi-squared test unless otherwise stated. Comparison of means was performed using the t-test for two independent samples reported with a standard deviation (SD). Participants with missing data were excluded from the analyses relating to that variable.

2.4.Results

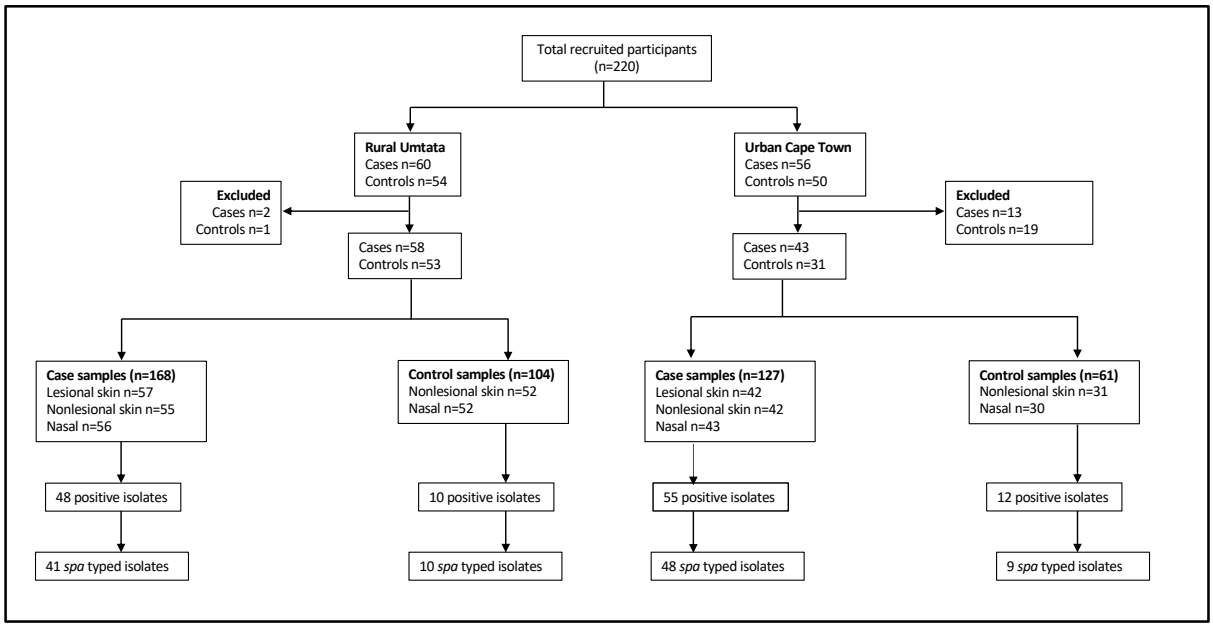


Figure 2.1. Flow chart of the processing of participants' samples. Eleven participants (eight cases and three controls) had one unavailable specimen for either the lesional skin, nonlesional skin or anterior nares.

2.4.1. Participant characteristics and *S. aureus* colonisation in cases and controls

Table 2.1. shows the characteristics of the participants in this study. Food allergy was common in cases than controls in both rural and urban groups. Additionally, urban cases were more likely to have a father with allergic rhinitis than urban controls. In the urban group, controls were more commonly immunised for all early childhood vaccinations than the cases, while there was no significant different between cases and controls in the rural group. Also, rural controls and their mothers were more likely have a farm animal at their home than rural cases. The use of gas and electricity for heating and cooking was prevalent in both rural and urban groups, however, the use of other household fuels was overall more common in the rural group than the urban group. Specifically, in the rural group, cases more frequently lived in houses that use electricity and gas or kerosene and paraffine, whereas controls more frequently lived in houses that use paraffine only, wood and fire, or an indoor fire.

A total of 185 (84 controls and 101 cases) children were assessed for *S. aureus* colonisation. Thirty-five participants were excluded (missing specimen) from the study analysis (Figure 2.1).

Of these, 79 (43%) were positive for *S. aureus* on at least one of the sample types. There was an overall higher prevalence of colonisation among urban participants compared to rural participants (55% [41/74] vs. 34% [38/111], $p = 0.006$). *S. aureus* was commonly detected from cases compared to controls in both rural (54% [31/58] vs. 13% [7/53], $p < 0.001$) and urban settings (70% [30/43] vs. 35% [11/31], $p = 0.005$). Also, cases were more frequently colonised on nonlesional skin compared to controls, and this was independent of geographic location (Table S2.1.). Among cases, colonisation was more common on lesional skin compared to nonlesional skin (rural: $p = 0.035$ and urban: $p = 0.021$) and the anterior nares (rural: $p = 0.008$). The prevalence of colonisation was common among urban cases with severe disease (86% [19/22] vs. 52% [11/21], $p = 0.015$), however, this was not associated with the sample type (Table S2.2.). Overall, these findings show that geographic location may influence the dynamics of *S. aureus* colonisation of the skin and nares in cases and controls, and this is dependent on the site of colonisation and disease severity.

Table 2.1. Participant characteristics of atopic dermatitis cases and healthy controls.

Explanatory variable	**Umtata**				**Cape Town**			
	Total, *n* (%)	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**	**Total, *n* (%)**	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**
Total	111 (100)	58 (52)	53 (48)	0.502[a]	74 (100)	43 (58)	31 (42)	**0.049**
Age (months)								
Mean [standard deviation]	21.27 [7.15]	21.03 [7.41]	21.53 [6.90]	0.718	24.19 [7.37]	23.98 [7.44]	24.48 [7.38]	0.773
Sex								
Female	42 (39)	24 (43)	18 (34)	0.431	36 (49)	19 (44)	17 (55)	0.480
Male	67 (61)	32 (57)	35 (66)		38 (51)	24 (56)	14 (45)	
AD severity								
Moderate	23 (40)	23 (40)	—		21 (49)	21 (49)	—	
Severe	35 (60)	35 (60)	—		22 (51)	22 (51)	—	
Atopic disease								
Food allergy	11 (10)	10 (17)	1 (2)	**0.009**	9 (12)	9 (21)	0 (0)	**0.008**
Asthma	0 (0)	0 (0)	0 (0)		1 (1)	1 (3)	0 (0)	1.000
Allergic rhinitis	7 (8)	1 (2)	6 (11)	0.242	1 (1)	1 (3)	0 (0)	1.000
Mode of birth								
Caesarean section	25 (23)	14 (24)	11 (21)	0.821	33 (46)	20 (49)	13 (42)	0.637
Vaginal	86 (77)	44 (76)	42 (79)		39 (54)	21 (51)	18 (58)	
Breastfeeding	35 (32)	10 (17)	25 (47)	0.001	9 (12)	7 (16)	2 (6)	0.288
Antibiotic exposure	92 (82)	49 (83)	43 (81)	0.810	54 (72)	30 (70)	24 (77)	0.598
Immunisation status								
Complete	107 (96)	56 (95)	52 (98)	0.620	64 (86)	33 (77)	31 (100)	**0.004**
Incomplete	4 (4)	3 (5)	1 (2)		10 (14)	10 (23)	0 (0)	
Large family [1]	62 (55)	30 (52)	32 (60)	0.445	24 (32)	14 (33)	10 (32)	1.000
Animal exposure	93 (84)	39 (67)	53 (100)	**0.001**	2 (3)	2 (6)	0 (0)	0.495
Parental education								
Primary	8 (7)	2 (3)	6 (11)	**0.001**	1 (1)	1 (2)	0 (0)	**0.025**
Secondary	70 (63)	31 (53)	39 (74)		33 (45)	14 (33)	19 (61)	
Tertiary	31 (28)	25 (43)	6 (11)		40 (54)	28 (65)	12 (39)	
Other	2 (2)	0 (0)	2 (4)		0 (0)	0 (0)	0 (0)	
Maternal factors								
Animal exposure	96 (86)	44 (76)	52 (98)	**0.001**	4 (60)	4 (11)	0 (0)	0.120

Pregnant smoking	1 (1)	0 (0)	1 (2)	0.482	3 (45)	0 (0)	3 (10)	0.094
Smoking	1 (1)	0 (0)	1 (2)	0.477	4 (6)	1 (3)	3 (10)	0.324
Asthma	2 (2)	2 (3)	0 (0)	0.496	6 (8)	4 (10)	2 (6)	1.000
Allergic rhinitis	4 (4)	4 (7)	0 (0)	0.120	5 (68)	4 (10)	1 (3)	0.387
Atopic dermatitis	2 (2)	2 (3)	0 (0)	0.496	3 (4)	2 (5)	1 (3)	1.000
Food allergy	3 (3)	2 (3)	1 (2)	1.000	1 (1)	1 (2)	0 (0)	1.000
Paternal factors								
Smoking	15 (14)	9 (16)	6 (12)	0.589	20 (31)	11 (31)	9 (31)	1.000
Asthma	3 (3)	3 (5)	0 (0)	0.245	0 (0)	0 (0)	0 (0)	
Allergic rhinitis	3 (3)	3 (5)	0 (0)	0.245	7 (10)	7 (17)	0 (0)	**0.018**
Atopic dermatitis	1 (1)	1 (2)	0 (0)	1.000	2 (3)	2 (5)	0 (0)	0.505
Food allergy	1 (1)	1 (2)	0 (0)	1.000	1 (1)	1 (2)	0 (0)	1.000
Household factors								
Electricity + gas	69 (62)	56 (97)	13 (25)	**0.001**	66 (99)	35 (97)	31 (100)	1.000
Kerosene + paraffin	64 (58)	44 (76)	20 (38)	**0.001**	43 (64)	21 (58)	22 (71)	0.317
Paraffin	38 (34)	6 (10)	32 (60)	**0.001**	0 (0)	0 (0)	0 (0)	
Indoor fire	4 (4)	2 (3)	2 (4)	1.000	0 (0)	0 (0)	0 (0)	
Outdoor fire	49 (44)	12 (21)	37 (70)	**0.001**	0 (0)	0 (0)	0 (0)	
Wood + fire	31 (28)	4 (7)	27 (51)	**0.001**	0 (0)	0 (0)	0 (0)	

Bold text indicates statistical significance. AD, atopic dermatitis; CI, confidence interval; IQR, interquartile range; [1] Large family is arbitrarily defined as 6 or more members living within one household.

2.4.2. Risk factors associated with *S. aureus* colonisation across the locations

Tables 2.2a and 2.2b show the logistic regression models to assess the effect of various risk factors on colonisation with *S. aureus* in children from rural and urban children. The univariate models revealed that having AD was associated with colonisation in both rural (odds ratio [OR] 7.54, 95% CI [confidence interval] 2.92;19.47) and urban (OR 4.2, 95% CI 1.57;11.2) children. Additionally, living in an electrified house that also utilises gas (OR 4.08, 95% CI 1.59;10.44) or uses kerosene and paraffin (OR 2.88, 95% CI 1.22;6.77) for heating and cooking increased the odds of colonisation with *S. aureus* among the rural children. Exposure to farm animals (OR 0.3, 95% CI 0.11;0.83) and use of wood and coal (OR 0.14, 95% CI 0.04;0.49) or an outdoor fire (OR 0.31, 95% CI 0.13;0.73) within a household were associated with reduced odds of colonisation. In the rural group, only having AD (adjusted odds ratio [aOR] 8.02, 95% CI 1.28;50.37) and the use of wood and coal (aOR 0.14, 95% CI 0.02;0.99) were associated with *S. aureus* colonisation in the multivariate model.

Table 2.2a. Unconditional logistic regression analysis of child, parental, domestic, and environmental characteristics associated with *S. aureus* colonisation in Umtata participants.

Explanatory variable	Colonised [a], *n* (%)	Not colonised, *n* (%)	OR [95% CI]	p-value	aOR [95% CI]	p-value
AD: case	31 (28)	27 (24)	7.54 [2.92–19.47]	**0.000**	8.02 [1.28–50.37]	**0.026**
Sex: male	21 (19)	46 (42)	0.74 [0.33–1.67]	0.469	0.83 [0.32–2.16]	0.696
Child characteristics						
Breastfeeding	10 (9)	25 (23)	0.69 [0.29–1.63]	0.395	1.46 [0.48–4.47]	0.503
Allergic rhinitis	1 (1)	6 (7)	0.43 [0.05–3.79]	0.449	Excluded	
Asthma [§]	0 (0)	0 (0)	Omitted [d]		Excluded	
Food allergy	5 (5)	6 (5)	1.69 [0.48–5.95]	0.413	Excluded	
Mode of delivery: vaginal	29 (26)	57 (51)	0.9 [0.36–2.29]	0.833	Excluded	
Incomplete immunisation status	2 (2)	2 (2)	1.97 [0.27–14.58]	0.506	Excluded	
Antibiotic exposure	33 (30)	58 (52)	1.71 [0.57–5.12]	0.34	1.54 [0.39–6]	0.536
Large family size [b]	15 (14)	35 (32)	0.71 [0.32–1.57]	0.395	0.94 [0.36–2.44]	0.903
Animal exposure [c]	27 (24)	65 (59)	0.3 [0.11–0.83]	**0.021**	0.53 [0.11–2.54]	0.429
Fossil fuel exposure						
Electricity + gas	31 (28)	38 (34)	4.08 [1.59–10.44]	**0.003**	0.35 [0.05–2.47]	0.295
Kerosene + paraffin	28 (25)	36 (32)	2.88 [1.22–6.77]	**0.015**	0.69 [0.19–2.49]	0.571
Indoor fire	1 (1)	3 (3)	0.63 [0.06–6.27]	0.694	Excluded	
Outdoor fire	10 (9)	39 (35)	0.31 [0.13–0.73]	**0.008**	0.54 [0.17–1.67]	0.283
Wood + coal	3 (3)	28 (25)	0.14 [0.04–0.49]	**0.002**	0.14 [0.02–0.99]	**0.048**
Maternal factors						
Allergic rhinitis	0 (0)	4 (4)	Omitted [d]		Excluded	
Asthma	1 (1)	1 (1)	1.95 [0.12–32]	0.641	Excluded	
Atopic dermatitis	1 (1)	1 (1)	1.95 [0.12–32]	0.641	Excluded	
Food allergy	1 (1)	2 (2)	0.96 [0.08–10.93]	0.973	Excluded	
Smoking	0 (0)	1 (1)	Omitted [d]		Excluded	
Pregnant smoker	0 (0)	1 (1)	Omitted [d]		Excluded	
Animal exposure [c]	31 (28)	65 (59)	0.55 [0.18–1.64]	0.28	1.93 [0.37–10.16]	0.438
Paternal factors						
Allergic rhinitis [§]	0 (0)	3 (3)	Omitted [d]		Excluded	
Asthma [§]	1 (1)	2 (2)	0.96 [0.08–10.93]	0.973	Excluded	

Atopic dermatitis [§]	0 (0)	1 (1)	Omitted [d]		Excluded	
Food allergy [§]	0 (0)	1 (1)	Omitted [d]		Excluded	
Smoking	5 (5)	10 (9)	0.94 [0.3–2.98]	0.267	Excluded	

AD, atopic dermatitis; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; [§] No within group variance; [a] Colonisation with *Staphylococcus aureus*; [b] Large family size is arbitrarily defined as 6 or more members within a household; [c] Animal exposure refers to farm animals; [d] Independent variables omitted due to dependency in the regression model. Bold text indicates statistical significance.

Table 2.2b. Unconditional logistic regression analysis of child, parental, domestic, and environmental characteristics associated with *S. aureus* colonisation in Cape Town participants.

Explanatory variable	**Colonised [a], *n* (%)**	**Not colonised, *n* (%)**	**OR [95% CI]**	**p-value**
AD: case	30 (41)	13 (18)	4.2 [1.57–11.2]	0.004
Sex: male	19 (26)	19 (25)	0.74 [0.33–1.67]	0.469
Child characteristics				
Breastfeeding	6 (8)	3 (4)	1.71 [0.39–7.45]	0.472
Atopic dermatitis				
Allergic rhinitis	1 (1)	0 (0)	Omitted [d]	
Asthma [§]	1 (1)	0 (0)	Omitted [d]	
Food allergy	6 (8)	3 (4)	1.71 [0.39–7.45]	0.472
Mode of delivery: vaginal	22 (31)	17 (24)	0.95 [0.37–2.43]	0.921
Incomplete immunisation status	8 (11)	2 (3)	3.76 [0.74–19.09]	0.11
Antibiotic exposure	31 (42)	23 (31)	1.35 [0.48–3.77]	0.57
Large family size [b]	9 (12)	8 (11)	0.88 [0.3–2.61]	0.816
Animal exposure [c]	1 (1)	1 (1)	0.86 [0.05–14.3]	0.915
Fossil fuel exposure				
Electricity + gas	36 (54)	30 (45)	Omitted [d]	
Kerosene + paraffin	20 (30)	23 (34)	0.43 [0.15–1.23]	0.116
Indoor fire	0 (0)	0 (0)	Omitted [d]	
Outdoor fire	0 (0)	0 (0)	Omitted [d]	
Wood + coal	0 (0)	0 (0)	Omitted [d]	
Maternal factors				
Allergic rhinitis	0 (0)	5 (7)	Omitted [d]	
Asthma	3 (4)	3 (4)	0.76 [0.14–4.06]	0.751
Atopic dermatitis	1 (1)	2 (3)	0.38 [0.03–4.33]	0.432
Food allergy	1/73	0 (0)	Omitted [d]	
Smoking	3 (4)	1 (1)	2.65 [0.26–26.82]	0.41
Pregnant smoker	2 (3)	1 (1)	1.76 [0.15–20.45]	0.65
Animal exposure [c]	3 (5)	1 (2)	2.64 [0.26–26.76]	0.412

Paternal factors				
Allergic rhinitis [§]	5 (7)	2 (3)	2.08 [0.38–11.52]	0.4
Asthma [§]	0 (0)	0 (0)	Omitted [d]	
Atopic dermatitis [§]	2 (3)	0 (0)	Omitted [d]	
Food allergy [§]	1 (1)	0 (0)	Omitted [d]	
Smoking	13 (20)	7 (11)	1.86 [0.62–5.54]	0.267

AD, atopic dermatitis; OR, odds ratio; CI, confidence interval; [§] No within group variance; [a] Colonisation with *Staphylococcus aureus*. [b] Large family size is arbitrarily defined by more than 6 members within a household; [c] Animal exposure refers to exposure to farm animals; [d] Independent variables omitted due to dependency in the regression model.

2.4.3. Clonal lineages of recovered *S. aureus* isolates

Of the 125 skin and nasal *S. aureus* isolates that were recovered, only 108 isolates were *spa* typed (Figure 2.1). Seventeen isolates were excluded due to their failure to amplify the *spa* gene using the described primers or poor sequence quality for *spa* type assignment, despite repeated sequencing. BURP analysis grouped 41 *spa* types into seven *spa*-clonal complexes (*spa*-CCs) and 18 *spa* types were singletons (Figure 2.2.). Among children with *spa* typed isolates, 25% (19/76) were colonised with one *spa* type, while 7% (5/76) were colonised with different *spa* types on at least two of the sample types that were positive for *S. aureus*. One rural case toddler was colonised with *spa* type t062 on lesional skin and anterior nares, and with *spa* type t1399 on nonlesional skin, both belonging to the same *spa*-CC (*spa*-CC NF3). The most frequent *spa* types were *spa*-CC002/t002 (*spa*-CC/*spa* type; 8%), *spa* cluster 4/t272 (9%), *spa* cluster 6/t174 (14%) and *spa* cluster 5/t1476 (18%). We also identified four new (t15783, t18354, t18750 and t19774) and one unassigned *spa* types (i.e., txAC), respectively.

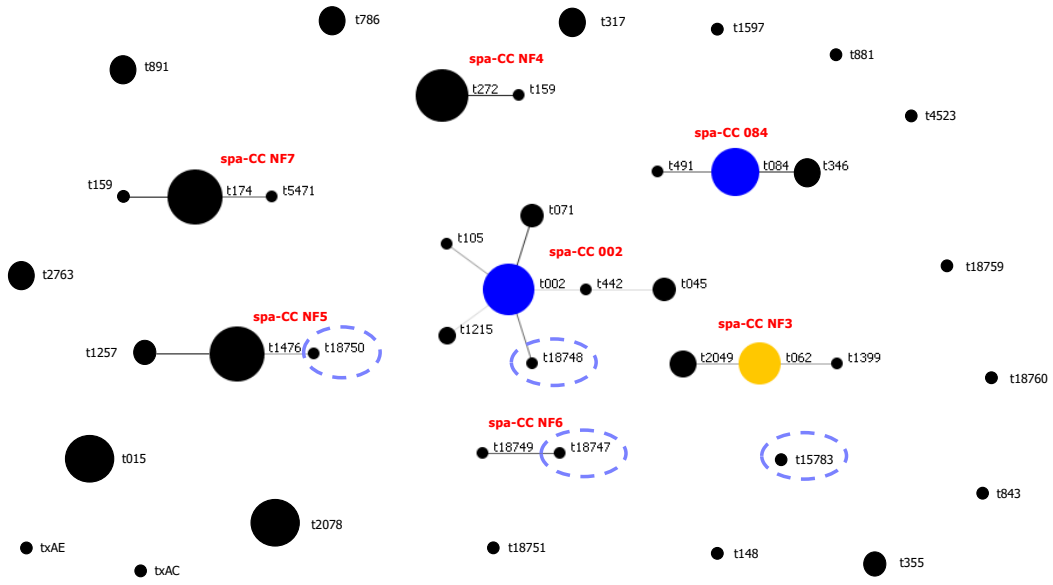


Figure 2.2. Based Upon Repeat Pattern (BURP) clustering analysis on identified *spa* types. The cluster analysis was conducted using a cost of four and a minimum number of repeats of five. *Spa* types with five or fewer repeats were excluded. The blue circles represent the founder of the cluster (i.e., the *spa* type with the highest finder score), the yellow circle is the group sub-founder. The circle size is proportional to the number of isolates with the same spa type.

Dotted purple ovals indicate novel *spa* types identified in this cohort. The distance between the *spa* types does not reflect degree of genetic difference.

2.4.4. Distribution of *S. aureus* spa clonal lineages across locations by AD disease and severity

We observed a distinction in the *spa* clonal lineages colonising rural and urban children. Here, *spa* cluster 4 was frequently identified among rural children (18% [9/51] vs. 4% [2/57], p = 0.015) and *spa* cluster 6 in urban children (23% [13/57] vs. 6% [3/51], p = 0.013) compared to their respective counterparts based for all sample types (Table 2.3). The diversity of *spa* types, indicated by the number of detected representative *spa* types, among cases was higher compared with controls in both locations (Figure 2.3). Additionally, we observed a significant difference in the distribution of *spa* clonal lineages between urban cases and controls *(p* = 0.009), with *spa* cluster 5/t1476 and *spa* cluster 6/t174 identified as predominant among cases. There was no overall difference between rural cases and controls (p = 0.224), albeit *spa* cluster 4/t272 and *spa* cluster 5/t1476 were the dominant *spa* clonal lineages among cases, with no single most dominant *spa* clonal lineage among controls (Figure 2.3). Also, there was a significant difference in the distribution of *spa* clonal lineages among urban cases based on AD severity (p = 0.001). In these cases, *spa*-CC002 (t002 and t442) isolates were identified in severe AD, *spa* cluster 6/t174 was more frequent in moderate AD, and *spa* cluster 5/t1476 in severe AD. Although no significant difference was observed between AD severity and the identified *spa* types in rural cases (p = 0.126), *spa* cluster 3 (t062 and t1399) isolates were detected only in moderate cases while *spa* cluster 5 (t1476 and t1257) isolates predominated in severe cases (Figure 2.4).

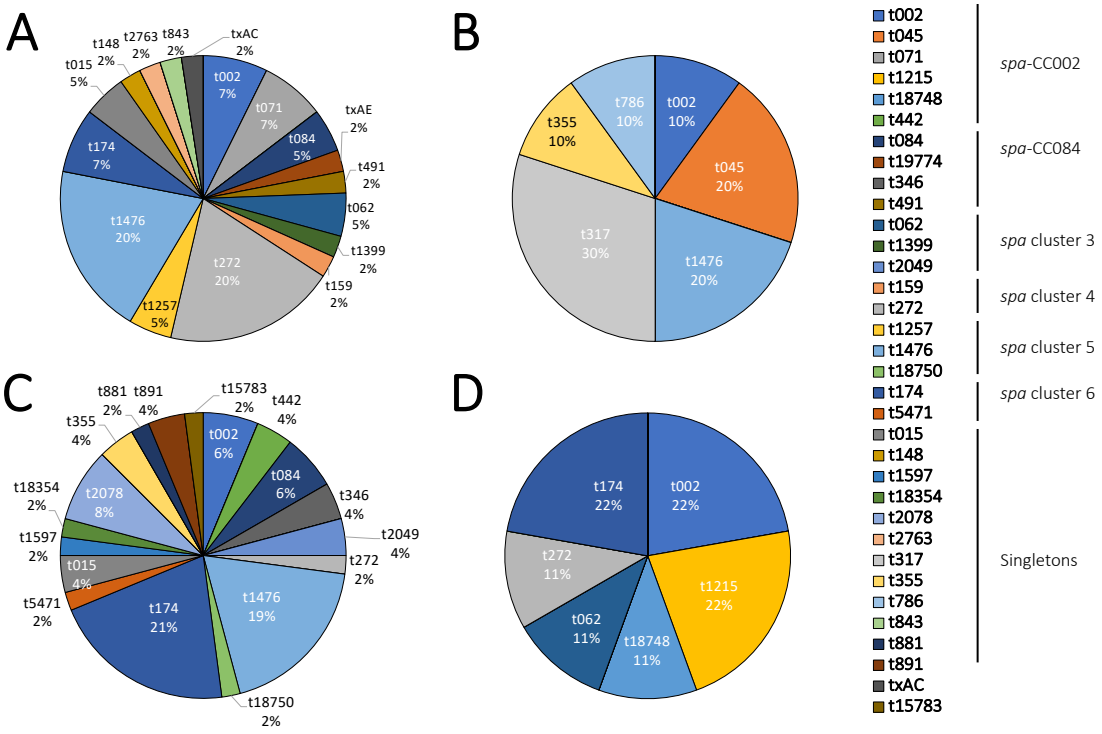


Figure 2.3. The distribution of *spa* types by the disease phenotype, stratified by location. (A) rural cases, (B) rural controls, (C) urban cases, and (D) urban controls. Percentages were calculated by the number of isolates for a *spa* type divided by the total number of *spa* types in each group.

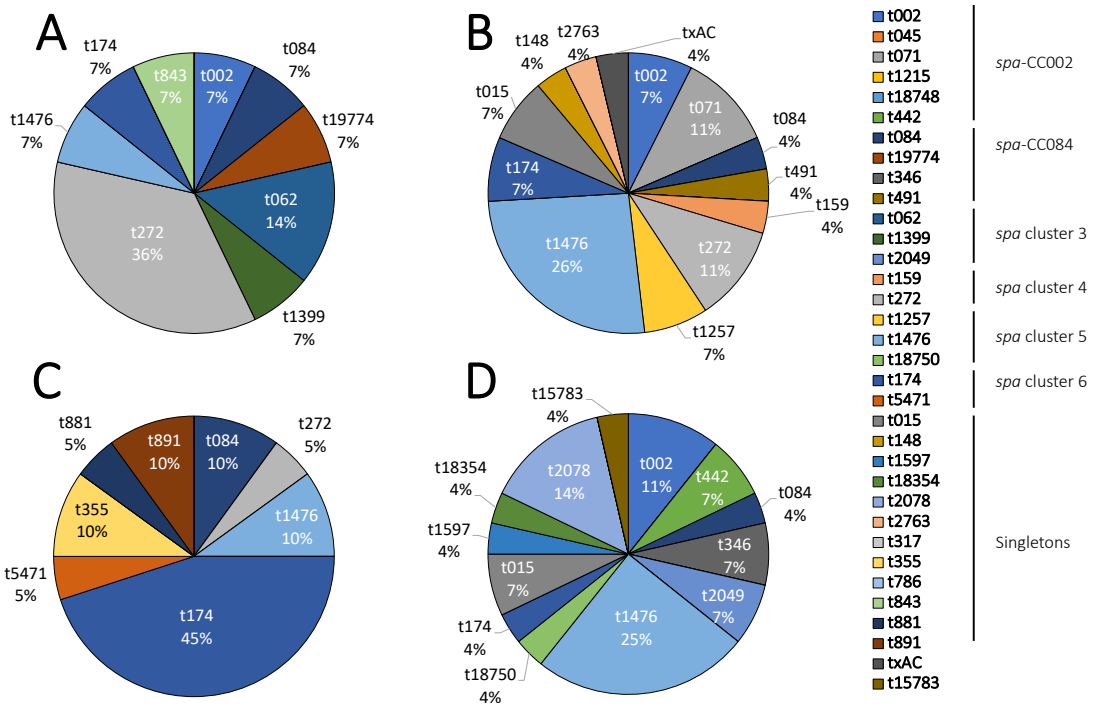


Figure 2.4. The distribution of *spa* types by disease severity. (A) rural moderate, (B) rural severe, (C) urban moderate, and (D) urban severe. Percentages were calculated by the number of isolates for a *spa* type divided by the total number of *spa* types in each group.

Table 2.3. The distribution of clonal lineages of *S. aureus* isolates among Umtata and Cape Town participants.

***Spa*-CC**	**Umtata**			**Cape Town**		
	No. of isolates (%)	**No. of *spa* types (%)**	***spa* types (no. of isolates)**	**No. of isolates (%)**	**No. of *spa* types (%)**	***spa* types (no. of isolates)**
spa-CC002	9	3 (14)	t002 (4); t045 (2); t071 (3)	10	4 (19)	t002 (5); t1215 (2); t18748 (1); t442 (2)
spa-CC084	3	2 (10)	t084 (2); t491 (1); t19774 (1)	5	2 (10)	t084 (3); t346 (2)
spa cluster 3	3	2 (10)	t062 (2); t1399 (1)	3	2 (10)	t062 (1); t2049 (2)
spa cluster 4	9	2 (10)	t159 (1); t272 (8)	2	1 (5)	t272 (2)
spa cluster 5	12	2 (10)	t1476 (10); t1257 (2)	10	2 (10)	t1476 (9); t18750 (1)
spa cluster 6	3	1 (5)	t174 (3)	13	2 (10)	t174 (12); t5471 (1)
Singletons	10	7 (33)	t015 (2); t148 (1); t2763 (1); t317 (3); t355 (1); t786 (1); t843 (1)	13	7 (33)	t015 (2); t18354 (1); t1597 (1); t2078 (4); t335 (2); t881 (1); t891 (2)
Unaligned/*spa* types with unknown repeat succession	2	2 (10)	txAC (1)	1	1 (5)	t15783 (1)
Total	51	21		57	21	

Bold text indicates *spa* types that were identified in only one location.

2.5.Discussion

In this chapter, we sought to determine the prevalence and molecular epidemiology of *S. aureus* colonising the skin and anterior nares of rural and urban South Africa AmaXhosa children with (cases) and without (controls) AD. We report that i) while both rural and urban children with AD are more frequently colonised with *S. aureus*, particularly on affected or lesional skin, than their equivalents without AD, ii) rural living more significantly influenced *S. aureus* colonisation. Also, the *spa* clonal lineages identified presently varied based on geographic location and were associated with AD disease and severity.

Patients with AD generally have a defective epidermal barrier, hyperactive epidermal immunity, and microbial dysbiosis associated with the proliferation pathogenic bacteria such as *S. aureus*[170]. The predominance of *S. aureus* is consistently linked with acute disease flares and more severe AD[43,70]. This is due to the immunological and physiological changes in AD lesions which promote the proliferation of *S. aureus*.[14] We found a higher prevalence of *S. aureus* colonisation among cases than controls in both locations (55% vs. 13% in the rural group and 70% vs. 35% in the urban group). This observation is consistent with a similar study in Italian children that reported a prevalence of 57% vs. 20% in cases compared with controls[63]. This likely indicates that the prevalence of *S. aureus* in AD in early childhood is independent of population and geographic location highlighting the importance of this bacterium in the pathology of AD[63,69].

The lesional skin of patients with AD is more susceptible to *S. aureus* colonisation compared with unaffected nonlesional skin, with a reported colonisation prevalence of 23–70% and 6–39% in cases and controls, respectively[69,77,151]. Similarly, among cases, we noted that the lesional skin was more frequently colonised with *S. aureus* compared with the nonlesional skin in both urban and rural groups. Moreover, studies have reported a similar colonisation prevalence on the lesional skin and the anterior nares of patients with AD, with nasal colonisation presumed to be the primary source of the increased skin colonisation in AD[33,69]. In contrast, lesional skin was more frequently colonised compared with the anterior nares among rural cases. However, this was not observed among urban children. This likely indicates transient nasal colonisation hence the relatively low frequency of colonisation, or a potential non-nasal source of *S. aureus* that is promoting *S. aureus* colonisation among rural cases[63,183].

Moreover, this suggests that the rural and urban environments distinctly influence nasal colonisation in this study.

As mentioned, epidermal barrier dysfunction, especially in patients with severe AD, is linked with increased *S. aureus* colonisation on lesional skin[49,184]. Consistent with previous studies[49,69,151], children with severe disease were more commonly colonised with *S. aureus* for all sample types. However, this observation was observed only in urban cases and not found in rural cases. It is known that skin microbial colonisation differs across geographies and may explain the varied susceptibility to skin diseases in different regions and populations[162]. In this regard, each of the rural and urban communities in this study represent a geographic population that is uniquely affected by *S. aureus* colonisation in AD pathology. Additionally, the rural and urban populations, independent of disease, are generally distinct populations with varied sensitisation patterns to environmental exposures[171] and inflammatory immune responses[185]. These may, in turn, affect microbial colonisation and its contribution to AD pathology.

Epidemiological studies have shown that exposures in rural and urban environments influence bacterial colonisation on the skin and nasal cavity differently[74,186]. The relationship between *S. aureus* colonisation and AD is well established in the literature. In these studies, colonisation with *S. aureus* precedes the clinical onset of AD in early childhood and is linked to increased disease severity[72]. In line with previous studies[70], we observed that having AD increased the odds of colonisation with *S. aureus*. These findings likely support the involvement of *S. aureus* in AD pathology. Also, exposure to air pollutants can exert damage to the epidermal barrier[187], which makes the skin more susceptible to colonisation with bacterial pathogens such as *S. aureus*[20]. In this study, we noted that living in a house that uses kerosene and paraffin was associated with increased odds of colonisation among rural children. The burning of kerosene and paraffin release fine air particulates[188] that consequently induce or exacerbate epidermal damage resulting in increased likelihood of colonisation with *S. aureus*. Interestingly, living in a house that burns wood and coal or uses outdoor fire, which also release fine air pollutants that may induce cutaneous irritation, for cooking and heating reduced the odds of colonisation with *S. aureus* also among rural children. It is unclear why the burning of kerosene and paraffin, and wood and coal affect *S. aureus* colonisation differently. However, the effect of environmental air pollutants in children is a function of exposure time[189]. Therefore, although the children are living in homes that use wood and coal or an outdoor fire, they might have limited exposure to the produced particulates, which restrict the possible effect on skin

irritation and increased susceptibility to *S. aureus*. This is likely because the burning of wood and coal or an outdoor fire might be considered "dirty fuels" that release huge amounts of smoke; therefore, guardians may consequently limit the exposure time of the children. Electricity and biogas are relatively "clean fuels" with minimal air pollution emission at the household level[190]. Interestingly, living in an electrified house that also utilises gas increased the odds of colonisation with *S. aureus* in the rural group. Also, animals are typically reservoirs for human *S. aureus* colonisation[191], however, we found that rural children living in a house with farm animals had lower odds of *S. aureus* colonisation. This observation possibly indicates the absence of or minimal direct interaction between children and animals. Therefore, there are few to no animal-to-human transmission events of *S. aureus*. Collectively, these findings indicate the importance of the immediate environment in shaping bacterial colonisation and the potential implication thereof in the AD pathology.

The distribution of bacterial strains varies across different geographies[192]. We presently observed diversity in the colonising *spa* clonal lineages prevalent in the rural and urban groups. Here, the *spa* types belonging to the *spa* cluster 4 (previously associated with multi-locus sequence type [MLST] clonal complex [CC]121[193]) was common in the rural group whereas the urban group was predominantly colonised with *spa* cluster 6 (CC1[194]) isolates. This finding further supports studies that posit that geographic location likely influences the colonisation dynamics of childhood skin and nares[101-103,195]. Moreover, both cases and controls in the urban group exhibited distinct *S. aureus spa* clonal lineages. In contrast, there was no difference in the *S. aureus* lineages colonising rural cases and controls. These findings highlight that the rural and urban locations in this study each provide a specific niche for the selection of certain *S. aureus* clonal lineages to prevail[196]. The prevailing clonal lineages then influence the population structure and associated colonisation dynamics in these communities[197]. This warrants the need for future studies to investigate features or factors that are specific to each of these communities that influence the *S. aureus* population structure and its association with AD disease in this cohort.

It is unclear whether a relationship between AD disease severity and the clonal lineages of the colonising *S. aureus* isolates exists, with some studies demonstrating a relationship[102,103] whereas others do not[33,198]. Despite this, we observed distinct *S. aureus* clonal lineages in cases with moderate or severe disease in the urban group. Specifically, the *spa* clonal lineages *spa* cluster 5 (CC5[199]) and *spa* cluster 6 (CC1[194]) were the most common in severe and moderate

disease, respectively. Also, *spa*-CC002 (CC5[103]) isolates were detected only among urban cases with severe AD. These findings are consistent with an investigation in Spanish children that reported a high prevalence of CC5 isolates in those with severe AD[102] and that of CC1 isolates in Danish children with moderate disease[33]. However, they contradict a study that demonstrated the predominance of CC5 in moderate disease among Canadian children with AD[103]. In rural cases, although there was no significant diversity of *spa* clonal lineages based on disease severity, *spa* cluster 3 (CC5[193,200]) isolates were identified only in moderate disease whereas *spa* cluster 6 (CC1[194]) isolates were prevalent in severe disease. While the identification of *spa* cluster 3 (CC5) isolates in children with moderate AD is similar to a previous report[103], The predominance of *spa* cluster 6 (CC1) isolates in severe AD contradicts studies which reported CC1 isolates more commonly among children with moderate AD[33]. It remains unclear why certain *S. aureus* clonal lineages prevail in moderate disease and others in severe disease, and why these differ between the rural and urban groups in this cohort. However, these findings suggest that location may be important in the role of bacterial clonality in AD disease. As such, future investigations ought to determine the factors that underline the differences of *S. aureus* clonal lineages based on disease severity and these affect AD disease pathology.

This chapter has some limitations. Firstly, BURP analysis excludes *spa* types with less than five repeats[182]. Secondly, we predicted the corresponding MLST CCs of the *S. aureus spa* types identified in this study by inferring data from previous studies (Table S2.3). Furthermore, we were unable to *spa* type 14% (17/125) because of reasons mentioned above. This limitation emphasises the need for whole-genome sequencing (WGS) to provide comprehensive genomic characterisation of *S. aureus* isolates in cases and controls[101]. Also, while the present indicates the prevalence of *S. aureus* among cases, especially those with severe disease, these findings do not necessarily indicate the involvement of this bacterium in AD pathology. That is, *S. aureus* may either directly contribute to AD pathology or other bacteria are key in driving disease and *S. aureus* proliferates only because of the pathological changes associated with AD. Therefore, studies need to evaluate if and how *S. aureus* contributes to AD pathology in this cohort.

2.6.Conclusion

In conclusion, this chapter shows that children with AD are more frequently colonised with *S. aureus* compared to those without AD. The strain biology (clonal lineages) of colonising *S. aureus* is a unique signature of AD and disease severity; however, this depends on rural or urban residency. Our findings indicate that geographic location is important in driving the colonisation epidemiology and population structure of *S. aureus* in early childhood AD in South Africa. Future studies need to examine the mechanisms within the rural and urban environments that contribute to *S. aureus* colonisation and its relationship with AD and disease severity. Understanding the mechanisms of *S. aureus* colonisation will provide insights into if and how this bacterium contributes to AD pathology among South African children. This information will consequently guide population-targeted treatment strategies directed at alleviating the potential

2.7.Supplementary data

Table S2.1. Participant colonisation among all, rural and urban cases and controls.

	Total			**Umtata**			**Cape Town**		
	Case, *n* (%)	**Control, *n* (%)**	**p-value**	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**
Lesional skin	42 (42)			22 (39)			20 (48)		
Non-lesional skin	32 (33)	10 (12)	0.001	16 (29)	5 (10)	0.011	16 (38)	5 (16)	0.04
Anterior nares	28 (28)	12 (15)	0.028	9 (16)	5 (10)	0.318	19 (44)	7 (23)	0.067

Bold text indicates statistical significance.

Table S2.2. Colonisation in cases stratified by disease severity among all, rural and urban cases.

	Total			**Umtata**			**Cape Town**		
	Moderate, *n* (%)	**Severe, *n* (%)**	**p-value**	**Moderate, *n* (%)**	**Severe, *n* (%)**	**p-value**	**Moderate, *n* (%)**	**Severe, *n* (%)**	**p-value**
Lesional skin	13 (32)	29 (51)	0.059	5 (24)	17 (49)	0.066	8 (40)	12 (55)	0.346
Non-lesional skin	13 (30)	19 (36)	0.562	6 (27)	10 (31)	0.753	7 (33)	9 (43)	0.525
Anterior nares	11 (26)	17 (31)	0.562	3 (14)	6 (18)	0.655	8 (38)	11(50)	0.432

Table S2.3. Extrapolated MLST sequence types and clonal complexes for *spa* types identified in the present study.

***Spa* CC**	***Spa* type**	**Ridom repeats**	**Extrapolated ST**	**Extrapolated CC**	**Ref.**
spa-CC002	t002	26-23-17-34-17-20-17-12-17-16	ST5	CC5	103,201
	t045	26-17-20-17-12-17-16	ST5, ST225	CC5	103
	t071	26-23-23-17-34-17-20-17-12-17-16	ST5	CC5	102
	t442	35-17-34-17-20-17-12-17-16	ST487	CC5	103
	t1215	26-23-17-34-20-17-12-17-17-16	ST5	CC5	202,203
	t18748 [a]	26-23-23-17-34-17-20-17-34-17-20-17-12-17-16			
spa-CC084	t084	07-23-12-34-34-12-12-23-02-12-23	ST15, ST18	CC15	103
	t346	07-23-12-34-12-12-23-02-12-23		CC15	103
	t491	26-23-12-34-34-12-12-23-02-12-23	ST1036	CC15	103,194
	t19774	07-23-20-12-34-12-23-02-12-23			
spa cluster 3	t062	26-23-17-12-17-16	ST15, ST965, ST5	CC5	193,200
	t1399	26-23-17-12-17			
	t2049	26-23-17-17-16			
spa cluster 4	t159	14-44-13-12-17-17-23-18-17	ST121	CC121	193
	t272	14-44-13-12-17-17-17-23-18-17	ST121	CC121	194
spa cluster 5	t1476	11-10-17-34-24-34-22-25	ST8	CC5/CC8	199,204
	t18750	11-10-17-34-24-22-25			
	t1257	11-19-34-05-17-34-24-34-22-25	ST612	CC8	205
spa cluster 6	t174	14-21-16-34-33-13	ST1	CC1	194
	t5471	35-21-16-34-33-13			
Singletons	t015	08-16-02-16-34-13-17-34-16-34	ST45	CC45	103,201, 206
	t317	08-17-23-18-23-18-17	ST121		
	t355	07-56-12-17-16-16-33-31-57-12	ST152	CC152	207,208
	t786	07-12-21-17-13-34-34-33-34	ST88	CC88	206
	t891	26-23-13-23-31-05-17-25-17-25-28	ST22	CC22	209,210
	t881	07-06-17-34-34-22-34	ST1370	CC20	211
	t843	04-82-17-25-17-25-25-16-17		CC130	212
	t148	07-23-12-21-12-17-20-17-12-12-17	ST1434	CC72	201
	t1597	15-12-17-20-17-12-12-17			
	t2078	04-13-21-12-17-20-17-12-17-17			
	t2763	26-13-17-34-16-13			
	t15783	07-23-21-24-33-17-17			
spa types with unknown repeat succession	txAC	r26-r08-r02-r05			

MLST, multilocus sequence typing; ST, sequence type; CC, clonal cluster;
[§]Underlined text indicates sequence types that were obtained from the *spa* server (http://spaserver.ridom.de/).

CHAPTER 3

Investigating genome diversity of *Staphylococcus aureus* associated with paediatric atopic dermatitis in South Africa

3.1. Abstract

Background: *Staphylococcus aureus* frequently colonises the skin and nose of patients with atopic dermatitis (AD), a disease associated with skin barrier dysfunction and chronic cutaneous inflammation. Published genomic studies on AD-associated *S. aureus* in paediatric populations in sub-Saharan Africa are limited. This study investigated the diversity of *S. aureus* in AD than healthy participants in early childhood using a pan-genomic approach.

Methods: We conducted a cross-sectional study of children (aged 9–38 months) with and without AD from rural and urban South African settings. *S. aureus* was isolated from skin and nasal samples and confirmed by PCR detection of the *S. aureus* species-specific thermonuclease gene. We performed whole-genome sequencing (WGS) and subsequent *in silico* analyses to identify clonal lineages, virulence, and antibiotic resistance genetic determinants. The antibiotic susceptibility and biofilm capability of the *S. aureus* isolates were also assessed.

Results: Of the 124 *S. aureus* isolates, high-quality sequences from 96 isolates (79 cases and 17 controls) were analysed. Isolates from cases showed phenotypic resistance to gentamicin (10%), rifampicin (4%), chloramphenicol (4%), and exhibited multidrug resistance (9%). Also, isolates from cases formed stronger biofilms than those from controls (76% vs. 35%, p = 0.001). Cases were associated with DNA damage repair genes (*lexA*, *ssbA, dinG* and *recF*), toxin genes (*hly* and *see*) and insertion sequences that carry antibiotic resistance genes (IS431R, ISLgar5 and IS256). In contrast, genes associated with *S. aureus* adhesion (*ebh*, *fnbB* and *sasG*) predominated among controls. There was no significant difference in the distribution of immune evasion cluster types between cases and controls. However, IEC type G was identified only among cases.

Conclusion: This study revealed that AD-associated *S. aureus* has phenotypic and genetic features important for successful pathogenic colonisation and survival. Further studies are needed to assess the pathological implications of colonisation of various *S. aureus* lineages *in vivo* to elucidate their pathological contribution to AD pathogenesis and pathophysiology.

3.2. Introduction

Atopic dermatitis (AD) is a chronic or recurrent inflammatory skin disease, affecting about 20% of children and up to 3% of adults globally[213]. The prevalence of childhood AD is increasing, especially in countries with a previously low prevalence[213]. This increase includes countries in sub-Saharan Africa that currently have a prevalence of 14%[213]. Patients with AD are frequently colonised with *Staphylococcus aureus* on the skin and nose[14]. Moreover, *S. aureus* colonisation in AD is often associated with flares and increased disease severity[70]. Because of frequent *S. aureus* infections, antibiotics are routinely used in AD. However, *S. aureus* can develop resistance to virtually all classes of antibiotics[113]. The effectiveness of antibiotic use in AD is limited by rapid *S. aureus* recolonisation after discontinuation of treatment[25,115]. Furthermore, the indiscriminate and excessive use of antibiotics leads to significant alterations in the skin microbial community[116]. These factors collectively lead to limited clinical improvement and cause a relapse of AD clinical manifestations. Colonisation with methicillin-resistant *S. aureus* (MRSA) strains that are typically associated with multidrug resistance, limits the success of antibiotic regimes in AD[109,118]. Moreover, these strains often lead to complications in patients with AD, including being associated with more severe forms of AD than in patients colonised by methicillin-susceptible *S. aureus* (MSSA) strains[50,119].

S. aureus colonisation is common in AD lesions and is strongly correlated with increased disease severity. Biofilms protect *S. aureus* from antibiotics, phagocytosis, and host antimicrobial peptides targeted at its elimination, thereby permitting its growth and persistence on skin lesions[43]. *S. aureus* biofilms are commonly detected in skin lesions of children with AD[43,84,98]. This phenomenon is accentuated by the putatively disrupted skin barrier and chronic inflammation[43,84]. Also, colonisation with biofilm-forming *S. aureus* is associated with more severe AD in both paediatric and adult patients[90,214]. However, a high frequency of non-producers has been noted among Brazilian children with AD[105]. This discrepancy suggests differences in the biofilm-forming ability of *S. aureus* that might be strain or lineage-specific. However, no data exist to support this hypothesis.

S. aureus directly contributes to AD pathology through the myriad of virulence factors it produces. These include adhesins, toxins, antigens, and proteases that enable this bacterium to adhere to host tissues and compromise skin homeostasis, leading to the disrupted skin barrier function and inflammation[88]. For example, superantigen toxins, such as staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin (TSST), directly bind to and activate T cells inducing in the secretion of proinflammatory cytokines[88]. Additionally, *S. aureus* proteases (staphopains and V8) and toxins

(exfoliative toxin and epidermal cell differentiation inhibitor) degrade epidermal structural proteins and inhibit the differentiation of epidermal cells, thereby leading to barrier dysfunction and epidermal hyperplasia[17,84,85,87]. Studies using a gene-targeted approach to assess the presence of specific *S. aureus* virulence factors, with known clinical relevance in AD pathology have demonstrated a higher frequency of colonisation with strains carrying virulence factor genes compared to healthy individuals[14,215]. Moreover, the carriage of virulence factor genes is associated with increased AD disease severity[14,215]. However, these data are not consistent[38,216,217].

Some genes that are associated with antibiotic resistance, biofilm formation, and virulence in *S. aureus* are carried on mobile genetic elements (MGEs). These MGEs include plasmids, bacteriophages (prophages) and staphylococcal pathogenicity islands (SaPIs), which are central to the adaptation of *S. aureus* in different environments[218]. For example, SE-encoding genes are borne on plasmids, prophages and SaPIs[219], while genes of the immune evasion cluster are carried on prophages[220]. The loss or gain of MGEs influences *S. aureus* survival and adaptation in AD[101]. Lineage-specificity has been observed among MGEs[91,221]. In other words, certain virulence factors and antibiotic resistance genes associated with MGEs are limited to specific lineages. In AD, emerging data suggest that specific *S. aureus* lineages are adapted for survival and the induction of disease activity[101]. For instance, among European children, clonal complex (CC) 1 is associated with AD, while CC30 is common among children without AD[101,110]. These differences in *S. aureus* lineages appear to be accompanied by differences in the carriage of virulence factors and adaptability to AD[222]. However, the data remains limited.

Most published studies comparing *S. aureus* genomics between patients with AD and healthy individuals focused on a limited repertoire of virulence and antibiotic resistance genes[14]. Few studies have assessed the complete genomes of *S. aureus* in AD, and how these are associated with disease in early childhood[101,113]. It is important to identify genomic markers of *S. aureus* that are linked to AD pathology so to distinguish *S. aureus* strains from children with AD from those carried by children without AD. Moreover, data from sub-Saharan Africa are not available.

We, therefore, investigated the factors important for *S. aureus* adaptation on the skin and anterior nares of children with AD compared with healthy paediatric participants, by exploring the genomic variability using a pan-genomic approach.

3.3. Materials and Methods

3.3.1. Collection of *S. aureus* isolates

This study included 124 *S. aureus* isolates recovered from the skin (lesional and nonlesional) and anterior nares of children with and without AD from Umtata and Cape Town, South Africa, as previously described (HREC/REF: 451/2014)[223]. Briefly, the isolates were inoculated on mannitol salt agar (National Health Laboratory Services [NHLS], Green Point Media Laboratory, Cape Town, South Africa) and incubated at 37 °C for 48 hours. Isolates were identified as presumptive *S. aureus* based on colony morphology, mannitol fermentation and DNase production[175]. Isolates were confirmed as *S. aureus* by PCR detection of the species-specific thermonuclease (*nuc*) gene[177,224].

3.3.2. Assessment of biofilm formation of *S. aureus* isolates

Biofilm formation was assessed *in vitro* using the crystal violet biofilm microtiter assay (MTA), and interpreted as previously described[225]. Briefly, a single colony from an 18-24-hour old culture was inoculated into 3 mL of 3% tryptic soy broth supplemented with 0.1% glucose (gTSB; Merck, South Africa), followed by incubation with shaking for 24 hours. The broth cultures were diluted (1:100) in a flat-bottomed 96-well microtiter plate (Lasec, South Africa) and statically incubated in ambient air at 37 °C for 24 hours. *S. aureus* ATCC 29213 and uninoculated gTSB were included as positive and negative controls, respectively. Planktonic bacterial suspensions were discarded, plates were washed three times with sterile reverse osmosis (RO)-filtered water and dried at 60 °C for 1 hour. The formed biofilms were stained with 200 µL of 0.1% crystal violet and incubated for 10 minutes at room temperature, before discarding the excess crystal violet. After that, 200 µL of 30% acetic acid was added to each well and incubated at room temperature for 30 minutes. The absorbance was measured at 492 nm using the Mindray MR 96A ELISA Microplate Reader (Vacutec, South Africa). Biofilm biomass was analysed using OD readings as follows: (OD>OD negative control [OD_c], non-producer; OD_c<OD<2·OD_c, weak producer; 2·OD_c<OD<4·OD_c, moderate producer; and OD>4·OD_c, strong producer).

3.3.3. Antibiotic susceptibility testing of *S. aureus* isolates

Antibiotic susceptibility testing (AST) was conducted using the Kirby-Bauer disk diffusion method with the following antibiotics: cefoxitin (30 µg), mupirocin (5 µg and 200 µg), chloramphenicol (30

µg), rifampicin (5 µg), clindamycin (2 µg), erythromycin (15 µg), trimethoprim-sulfamethoxazole (23.75µg and 1.25µg, respectively), tetracycline (30 µg) and gentamicin (10 µg). Cefoxitin resistance was tested as a surrogate to detect methicillin resistance[226]. *S. aureus* ATCC 25923 was used as the quality control. The D-test was used to distinguish constitutive and inducible resistance to the macrolide-lincosamide-streptogramin B (MLS_B) group of antibiotics[227]. AST was conducted and interpreted according to the 2021 EUCAST guidelines and breakpoints. Isolates identified as resistant to at least three classes of antibiotics were classified as multidrug-resistant (MDR)[228].

3.3.4. DNA extraction, library preparation, and whole-genome sequencing

Total DNA was obtained from isolates streaked on 2% blood agar (National Health Laboratory Services, Green Point Media Laboratory Cape Town, South Africa) after overnight incubation at 37 °C. A loopful of the isolate was emulsified in 200 mL RO water, transferred to a ZR Bashing Bead lysis tube (0.1 & 0.5 mm; Zymo Research, USA) with 750 mL of ATL buffer (Qiagen, Germany) and processed at 50 Hz for 5 minutes. Samples were purified using the Maxwell Buccal Purification kit (Promega, USA), according to the manufacturer's instructions. DNA was quantified using the Biotium Accuclear Ultra-high sensitivity dsDNA Quantitative kit using the Mosquito LV liquid platform, Bravo WS, and BMG FLUOstar Omega plate reader. Samples were cherrypicked to 200 ng/120 mL using the Tecan liquid handling platform and sheared to 450 bp using a Covaris LE220 instrument. Post sheared samples (up to 4 × 96 well plates) were purified using SPRISelect beads and rearrayed into a 1 × 384 plate on the Hamilton STAR. Library construction (ER, A-tailing, and ligation) was performed using the 'NEB Ultra II custom kit' on an Agilent Bravo WS automation system. PCR set-up was performed using KapaHiFi Hot start mix and 384 well UDI tag barcodes on the Agilent Bravo WS automation system. PCR conditions were as follows: initial incubation at 95 °C for 5 mins followed by five cycles of 98 °C for 30 seconds, 65 °C for 30 seconds and 72 °C for 1 minute, and a final incubation step at 72 °C for 10 minutes. Post PCR plates were pooled using equal volumes and purified using Agencourt AMPure XP SPRI beads on the Hamilton STAR. Libraries were quantified using an Agilent Bioanalyser, normalised to 2.8 nM, and prepared for sequencing on a NovaSeq.

3.3.5. Genome assembly, MLST analysis, gene prediction and gene clustering

Pre-processed reads were *de novo* assembled using the short read assembler Velvet[229]. The species identity of each genome was confirmed using KmerFinder version 3.2.[230,231] accessed through the Center for Genomic Epidemiology (CGE; https://www.genomicepidemiology.org/). Only high-

quality genomes with <150 contigs and N50 >50,000[232] were included in the subsequent analyses. *In silico* multi-locus sequence typing (MLST) and *spa* typing were conducted to identify the sequence types (STs) and *spa* types of the isolates using the MLST tool version 2.0 and spaTyper version 1.0, respectively; accessed through CGE. MLST clonal complexes (CCs) were extrapolated from the predicted ST identities using PubMLST (accessed on https://pubmlst.org/). The genes encoding *S. aureus* virulence factors, including toxins and host immune evasion factors, were detected by VirulenceFinder version 2.0[233] accessed through CGE and the Virulence Factors of Pathogenic Bacteria (VFPB) database[234]. Genetic determinants for antibiotic resistance were identified using the Resistance Gene Identifier version 5.2.0; accessed via the Comprehensive Antibiotic Resistance Database (CARD)[235] and ResFinder[236] accessed through CGE. The criteria for detecting AMR genes were set to perfect (100% identity) and strict (>95% identity) hits and the sequences were compared with the curated reference sequences in the CARD. For ResFinder, the selection criterion was set at a 90% sequence similarity threshold with a minimum length of 60%. The staphylococcal cassette chromosome *mec* (SCC*mec*) types of *mecA*-positive MRSA isolates were determined by the SCC*mec*Finder version 1.2.[237]. Identification of putative plasmids was performed by screening the genome assemblies for plasmid replicon (*rep*) genes using PlasmidFinder[238] accessed through CGE. The size (in base pairs [bp]) and associated genes of plasmids identified by PlasmidFinder were obtained from the NCBI database[239]. The PHAge Search Tool Enhanced Release (PHASTER) algorithm[240] was used to identify prophage sequences from the *S. aureus* genomes. Only "intact" or "complete" (with a PHASTER score >90) prophages were considered for further analyses. The associated genes of the predicted prophage sequences were obtained from the NCBI database[239]. The most similar prophage was further queried against bacteriophage-related taxa (38018 [bacteriophage sp.], 10699 [Siphoviridae], 10662 [Myoviridae], 10744 [Podoviridae], 10841 [Microviridae], 2100421 [uncultured Caudovirales phage], 28883 [Caudovirales], 12333 [unclassified bacteriophages], 79205 [unclassified dsDNA phages] and 102294 [unclassified Caudovirales]) in the NCBI database[239], to predict the prophage family and genus based on their maximum homology. Insertion sequence (IS) elements were identified from Prokka version 1.14.6[241] annotated genomes using the ISfinder database[242]. Analyses were limited to replicon, prophage, and IS elements identified in 10 or more genomes. For these, we extracted data on virulence factor genes, transposons and genes conferring resistance to antibiotics and metals, and reported the prevalence.

3.3.6. Phylogenetic tree construction and annotation

Single nucleotide polymorphism WGS phylogenetic analysis was conducted using the Reference sequence Alignment based Phylogeny builder (REALPHY version 1.13)[243] with default settings. *S. aureus* NCTC 8325 was used as a reference. The generated phylogenetic trees were visualised and annotated using the iTOL (Interactive Tree of Life) tool version 6[244].

3.3.7. Pan-genome and pangenome-wide association study of *S. aureus*

Genes were then predicted from these assemblies using Prokka. Using the predicted genes, we constructed a gene-based pan-genome for these isolates using Panaroo version 1.2.8 with default settings[245]. We performed pan-GWAS (genome-wide association study) based on disease status (case/control) in Pyseer[246], using a gene presence/absence file generated in Panaroo as an input to define genes that are enriched in *S. aureus* genomes from cases and controls.

3.3.8. Statistical analyses

Unless stated otherwise, all statistical analyses were performed in STATA SE 15.1 (StataCorp LP, Texas, USA). The two-sample *z*-test was applied to compare the proportions. Cohen's kappa statistic was performed to assess the agreement between phenotypic and genotypic susceptibility and resistance to antibiotics. The κ coefficient was interpreted as no agreement (κ<0-0.2), minimal agreement (κ = 0.21-0.39), weak agreement (κ = 0.4-0.59), moderate agreement (κ = 0.6-0.79), strong agreement (κ = 0.8-0.9), and almost perfect agreement (κ >0.9)[247].

3.4. Results

3.4.1. Participant characteristics and *S. aureus* population structure

Table 3.1 describes the characteristics of the study participants. Of the 124 *S. aureus* isolates from cases and controls, high-quality genomes from 96 isolates (79 cases and 17 controls) were obtained. The average size of the assembled genomes was 2.81 Mb (2.71 – 3.6 Mb), with an average of 120 (10 – 1196) contigs per genome. Each genome had an average of 2685 (2538 – 4585) predicted protein sequences and an average GC content of 32.7% (32.6% – 32.9%). The *S. aureus* isolates were grouped into 20 STs that clustered into nine CCs and three singletons (Figure 3.1). Of these, CC5 (ST5, ST650, and ST4005) and CC8 (ST8, ST72, and ST612) isolates were predominant, representing 24% (23/96) and 25% (24/96) of the isolates, respectively (Table S3.1). CC8 and CC15 isolates were more common among cases (28% [22/79] vs. 12% [2/17], p = 0.1648 and 13% [10/79] vs. 6% [1/17], p=0.426, respectively) and CC121 among controls (24% [4/17] vs. 9% [7/79], p = 0.085), but without statistical significance. CC5 isolates were prevalent among cases (22% [17/79]) and controls (29% [5/17]). CC45, CC22 and CC30, isolates were detected only in cases.

Table 3.1. Participant characteristics

	Cases	**Controls**
No. of participants	107	87
Male, % (n/N) [a]	54 (58/104)	59 (51/87)
Rural, % (n/N)	57 (61/107)	61 (53/87)
Age in months, median (range)	22.4 (9–38)	22.1 (12–36)
Objective SCORAD	41.5 (21.4–82.2)	NA
No. of *S. aureus* isolates	104	20

[a] Sex data is not available for three participants

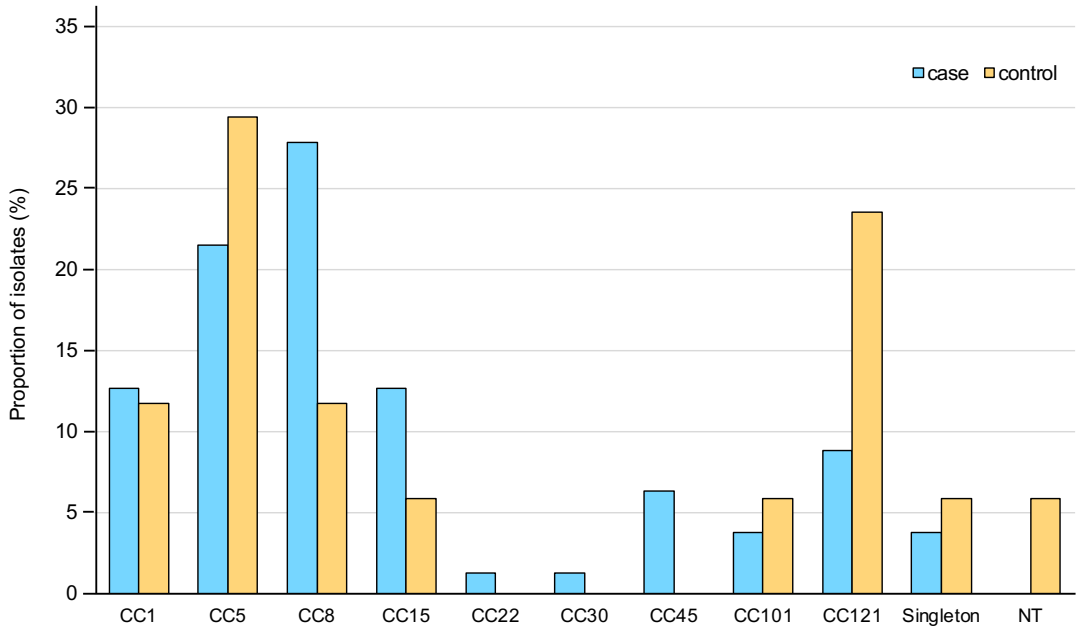


Figure 3.1. Genetic diversity of *S. aureus* associated with AD**.** CC1 (ST1), CC5 (ST5, ST4005, ST650), CC8 (ST8, ST72 and ST612), CC15 (ST15, ST199 and ST2126), CC22 (ST22), CC30 (ST31), CC45 (ST45 and SST508), CC101 (ST101) and CC121 (ST121). Singletons include ST12 (n=2), ST20 (n=1) and ST88 (n=1).

3.4.2. Distribution of mobile genetic elements (MGEs) among cases and controls

Plasmid analysis revealed 27 unique plasmids based on *rep* genes, with an average of 2.7 and 2.8 plasmid replicons *per* isolate in cases and controls, respectively. Plasmid replicons were not identified in four isolates (all from cases). Lineage-association was limited to three plasmids, namely pBORa53 (CC8), SAP074A (CC5), and SAP057A (CC121) (Table S3.2). We limited the examination of plasmid-associated genes to replication sequences which were identified in 10 or more isolates (Table 3.2). Plasmids pN315, pSJH101 and pMSSA476, carrying penicillin (*blaZ*) and cadmium (*cadD*) resistance genes, were the most frequently identified in both cases (47% [37/79], 19% [17/79], and 41% [32/79], respectively) and controls (41% [7/17], 35% [6/17], and 24% [4/17], respectively). Plasmid pE-1(EDINA), which carries the enterotoxin E (*see*), *edinA*, as well as cadmium and arsenic resistance genes, was more common among controls than cases (29% [5/17] vs. 6% [5/79], p=0.005).

Table 3.2. The distribution of plasmids, present in at least ten genomes, in cases and controls.

Plasmid	Size	Associated genes	Cases (n [%])	Controls (n [%])	*p*-value
pN315	24653 bp	*cadD, blaZ, blaR1, blaI, arsR, arsC, Tn552*	37 (47)	7 (41)	0.671
MSSA476	20652 bp	*cadD, blaI, blaR1, blaZ*	32 (41)	4 (24)	0.1897
pSJH101	30429 bp	*cadD, blaZ, blaR1*	17 (19)	6 (35)	0.2274
pS0385p1	5246 bp	*tetB*	16 (20)	3 (18)	0.8067
pSaa6159	20730 bp	*blaI, blaZ*	16 (20)	2 (12)	0.416
pBORa53	17334 bp	*blaR1, blaZ, cadD, cadX*	11 (14)	2 (12)	0.8134
pDLK1	2402 bp	*ermC*	10 (13)	1 (6)	0.4262
pSAS	20652 bp	*cadD, cadC, blaR1, blaZ*	10 (13)	1 (6)	0.3497
pETB	38211 bp	*etB, cadD*	7 (9)	3 (18)	0.282
pE-1(EDINA)	34986 bp	*entE, edinA, cadX, cadD, arsR, Tn 431*	5 (6)	5 (29)	0.0047
pSK156	45052 bp	*cadD, blaI, blaR1, blaZ, antP, qacA/B*	5 (6)	2 (12)	0.4342

We predicted 392 prophage genomes from 96 *S. aureus* isolates, of which, 150 (38%) were intact/complete. The predicted 150 intact prophages were identified in 95% (91/96) of the isolates: 96% (76/79) of cases and 88% (15/17) of controls. All predicted intact prophages belonged to the family *Siphoviridae* and various genera including Biseptimavirus, Dubowvirus, Peeveelvirus, Phietavirus, and Triavirus (Table S3.3). Phage integrase genes were found in 68% (102/150) of the intact prophages. The predicted intact prophages were grouped into 31 distinct prophages. Cases and controls had an average of 1.6 and 1.2 intact prophages per isolate, respectively. We did not observe any major lineage association with the identified intact prophages (Table S3.3). However, some prophages, such as *Staphylococcus* phages phiETA (CC121), SA97, and phiN315 (CC5), and phage 71 (CC45) only had one lineage (Table S3.3). There was no difference in the number of prophages per isolate between cases and controls (Figure S3.1C). We limited our analysis to prophages that were identified in at least ten genomes (Tables S3.3 and 3.3). Of these, *Staphylococcus* prophage phi2958PVL was the most common (34% [33/96]) and was more frequently identified among cases than controls (41% [32/79] vs. 6% [1/17], $p = 0.0064$) (Table 3.3). The prevalence of staphylococcal prophages phiJB and P282 (23% [18/79] cases vs. 18% [3/17] controls, $p = 0.642$ and 20% [16/79] cases vs. 12% [2/17] controls, $p = 0.416$, respectively) were similar among cases and controls (Table 3.3).

Table 3.3. The distribution of prophages, present in at least ten genomes, in cases and controls.

Most similar prophage hit	Associated genes[†]	Case [n (%)]	Control [n (%)]	*p*-value
Staphylococcus prophage phi2958PVL	*lukF-PV*, *lukS-PV*, *vapE*, *clpP*	32 (41%)	1 (6%)	0.0064
Staphylococcus prophage phiJB	*yopX* of the type III secretion system	18 (23%)	3 (18%)	0.6420
Staphylococcus prophage P282	immune evasion cluster genes *chp*, *sak*, *scn* and *clpP*	16 (20%)	2 (12%)	0.416

[†] The information of the associated genes was obtained from the NCBI database.

S. aureus isolates from cases and controls had an average of 10 and 9 IS elements, respectively. Table 3.4 shows the distribution of IS elements in cases and controls. ISSau3 was detected in all case and control isolates. ISSau5 was only identified in cases (14% [11/79]) and ISSep3 was more prevalent in cases than controls (54% [43/79] vs. 29% [5/17], $p = 0.061$), but without statistical significance. Also, ISCsp1 and ISSau6 were more common among controls than cases (71% [12/17] vs. 37% [29/79], $p = 0.0104$ and 53% [9/17] vs. 23% [18/79], $p = 0.0121$, respectively). Transposon Tn552 was common in both cases and controls (62% [49/79] vs. 76% [13/17], $p = 0.2586$).

Table 3.4. The distribution of IS elements, present in at least ten genomes, in cases and controls.

IS element	IS family	Species	Case (n [%])	Control (n [%])	*p*-value
ISSau3	IS1182	*Staphylococcus aureus*	79 (100)	17 (100)	–
IS1181	ISL3	*Staphylococcus aureus*	48 (61)	11 (65)	0.762
ISSep3	IS200/IS605	*Staphylococcus epidermidis*	43 (54)	5 (29)	0.061
IS1252	IS30	Enterococcus sp.	39 (49)	5 (29)	0.134
ISBli29	ISNCY	*Brevibacterium linens*	31 (39)	10 (59)	0.139
ISCsp1	IS3	Clostridium sp.	29 (37)	12 (71)	0.0104
ISSsu9	IS1595	*Streptococcus suis*	33 (42)	7 (41)	0.964
ISSau6	IS6	*Staphylococcus aureus*	18 (23)	9 (53)	0.0121
IS655	IS3	*Bacillus halodurans*	16 (20)	2 (12)	0.416
IS431R	IS6	*Staphylococcus aureus*	16 (20)	2 (12)	0.416
ISSau2	IS3	*Staphylococcus aureus*	10 (13)	4 (24)	0.249
ISSau8	ISL3	*Staphylococcus aureus*	8 (10)	4 (24)	0.1296
ISSau5	IS30	*Staphylococcus aureus*	11 (14)	0 (0)	–
ISSep1	IS1182	*Staphylococcus epidermidis*	10 (13)	1 (6)	0.426

3.4.3. Prevalence of phenotypic and genotypic antibiotic non-susceptibility

Most of the *S. aureus* isolates (60% [58/96]) were susceptible to all tested antibiotics, independent of disease status: 61% (48/79) cases vs. 59% (10/17) controls, p=0.882 (Figure 3.2). Phenotypic resistance to chloramphenicol (4% [3/77]), gentamicin (9% [7/77]) and rifampicin (4% [3/78]) was low and only observed among cases. Phenotypic resistance to tetracycline and erythromycin were similar between cases and controls (22% [17/77] cases vs. 18% [3/17] controls, p = 0.686 and 16% [12/77] vs. 12% [2/17], p = 0.689, respectively). All isolates with clindamycin resistance (12% [11/95]) exhibited erythromycin-induced resistance, independent of disease status. Moreover, the prevalence of MRSA was low in both cases (8% [6/78]) and controls (6% [1/17]). Phenotypic MDR isolates were only detected in cases (9% [7/79]) (Figure 3.2).

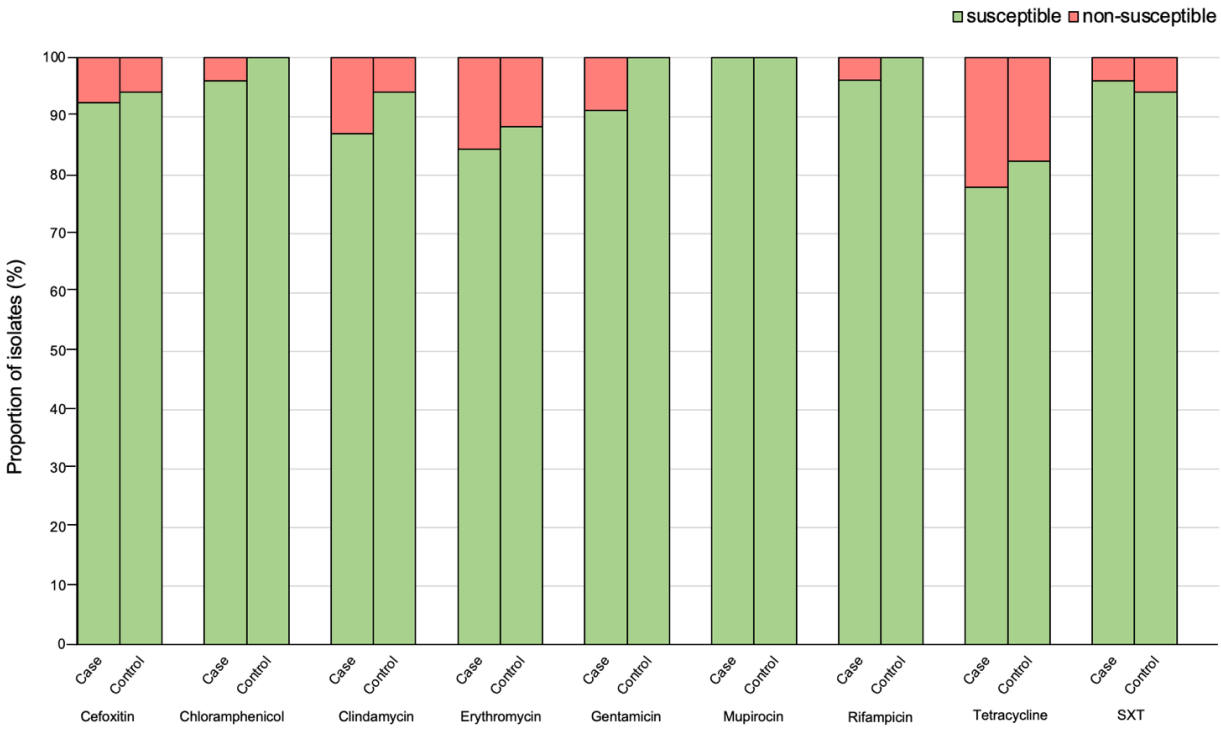


Figure 3.2. Phenotypic antibiotic resistance in cases and controls. SXT, trimethoprim-sulfamethoxazole.

In-silico determination of antibiotic resistance revealed ARGs for methicillin (*mecA*), tetracycline (*tet45, tetM*, and *tetK*), gentamicin (*acc*(*6'*)-*le-aph*(*2''*)-*la*), rifampicin (double mutation in RpoB; $H_{481}N$ and $I_{527}M$), chloramphenicol (*cat*(*pC221*)), and MLS_B (*ermC* and *msrA*) (Figure 3.3). Three case isolates exhibited rifampicin resistance; two of these isolates exhibited resistance via base substitution ($H_{481}N$ and $I_{527}M$) in the RpoB gene. There was moderate to almost perfect concordance between phenotypic and genotypic resistance to all antibiotics tested, except for trimethoprim-sulfamethoxazole (Table S3.5 and Figure S3.3). We also observed ARGs for penicillin (*blaZ*), fosfomycin (*fosB* or mutations in *murA* or *glpT*), and ciprofloxacin (mutations in ParC [S80F] and or GyrA [S84L]) which were not tested phenotypically. The *blaZ* was identified in 99% (95/96) of isolates: 100% (79/79) in cases and 94% (16/17) in controls. Fosfomycin ARGs were the second most common among all isolates (82%, 79/96) and did not differ between cases and controls ([66/79] vs. [13/17], p=0.493). Ciprofloxacin mutations were detected only among cases (9% [7/79]). The *blaZ* gene was detected in all CCs, while *cat* (*pC221*) and *msrA* were detected only among CC5 isolates. Furthermore, RpoB mutations ($H_{481}N$ and $I_{527}M$), the *tet45* gene and mutations in parC [S80F] and gyrA [S84L] were detected only among CC8 isolates. The *ermC* gene was identified in CC8 and

CC121 isolates. MRSA was identified in cases comprising the multidrug-resistant CC5-ST5-SCC*mec*IV and CC8-ST612-SCC*mec*IV (Figures 3.6 and S3.3).

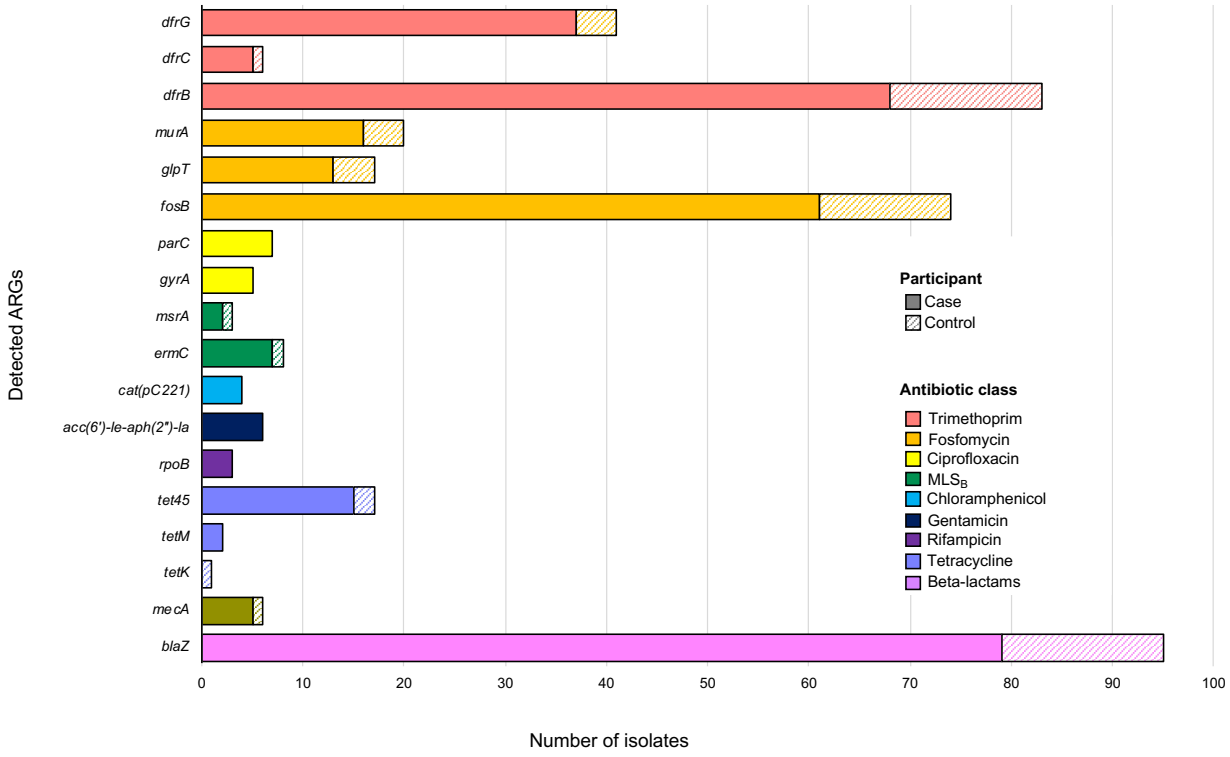


Figure 3.3. The distribution of antimicrobial resistance genes (ARGs) in *S. aureus* from cases and controls. MLS_B, macrolide-lincosamide-streptogramin B.

3.4.4. Phenotypic biofilm formation, and adhesion- and biofilm-associated genes

Strong biofilm-producing isolates were the most common (72% [69/96]), followed by moderate (17% [16/96]) and weak (11% [11/96]) producers when disease status was not considered. Comparison between cases and controls revealed that strong producers were common in cases (76% [60/79] vs. 35% [6/17], $p = 0.001$), while moderate (14% [11/79] cases vs. 29% [5/17] controls, $p = 0.120$) and weak producers (10% [8/79] cases vs. 18% [3/17] controls, $p = 0.377$) were similar in the two groups. Moreover, biofilm formation between cases and controls did not differ based on *S. aureus* lineage (Figure 3.4) and was not associated with phenotypic antibiotic resistance (Table S3.6).

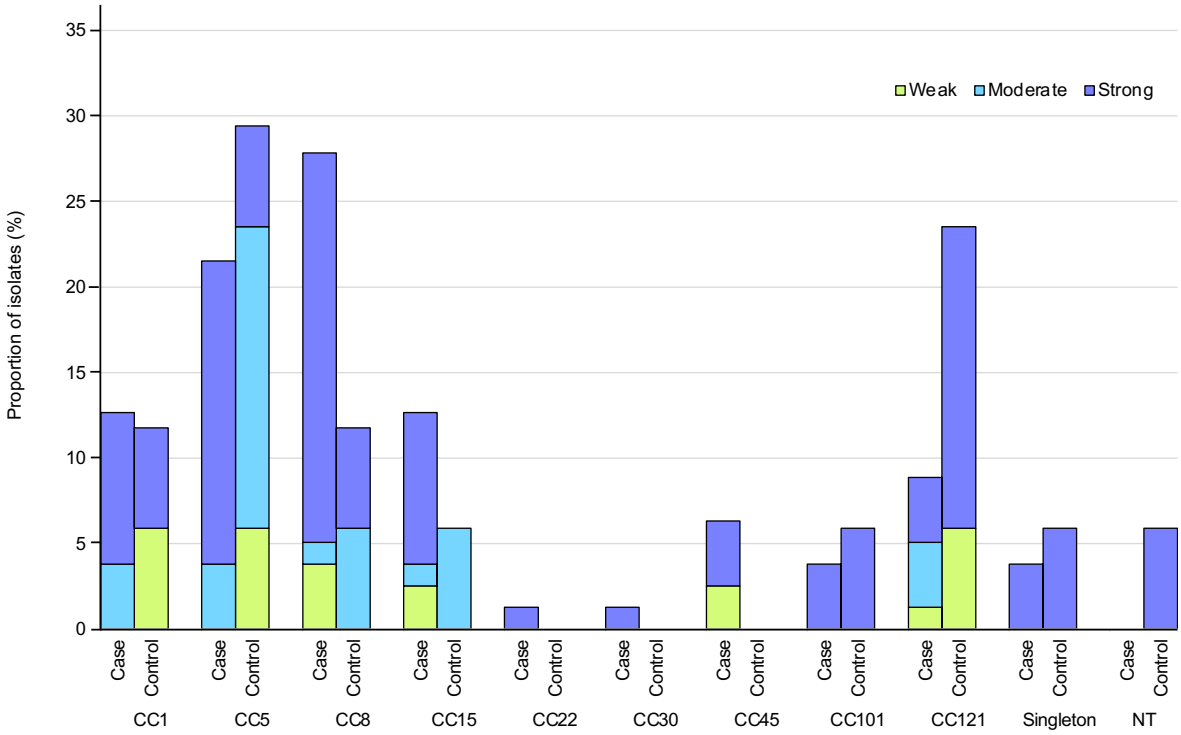


Figure 3.4. Distribution of biofilm phenotypes in cases and controls based on *S. aureus* lineages. Singletons include ST12 (n=2), ST20 (n=1) and ST88 (n=1).

Polysaccharide intercellular adhesin (PIA) genes, *icaA* and *icaC,* were detected in all isolates, while 90% (86/96) of isolates were *icaB*-positive (89% [70/79] cases vs. 94% [16/17] controls, p=0.685) and 10% were *icaD*-positive (11% [9/79] cases vs. 6% [1/17] controls, p=0.685). Other frequently identified (>70%) genes included *fnbpA/B, clfA/B, ebh,* and *sasG*, (Figure 3.5). In contrast, *eap* was only identified in 16% (15/96) of isolates (18% [14/79] cases vs. 6% [1/17] controls, p=0.295), while *bap* was detected in none.

3.4.5. Prevalence of virulence genes

Generally, there was a low prevalence of staphylococcal superantigen genes, except for the enterotoxin gene cluster (EGC; which comprises *seg*, *sei*, *sem*, *sen*, *seo*, and *seu*) (48% [46/96]) (Figure 3.5). About 20% (20/96) of the isolates analysed were positive the cytolysin genes *lukF-PV* and *lukS-PV*. All the isolates in this study were positive for *hla* and the *hlg* operon. More than 90% (90/96) of all isolates were *lukED*-positive. The toxin genes *edinC* (5% [4/79]), *etB* (4% [3/79]) and *sed* (9% [7/79]) were detected only in cases. Furthermore, *edinA* and *seb* were more frequently identified among controls

than cases (24% [4/17] vs. 6% [5/79], p=0.049 and 29% [5/17] vs. 1% [1/79], p=0.001, respectively) (Figure 3.5).

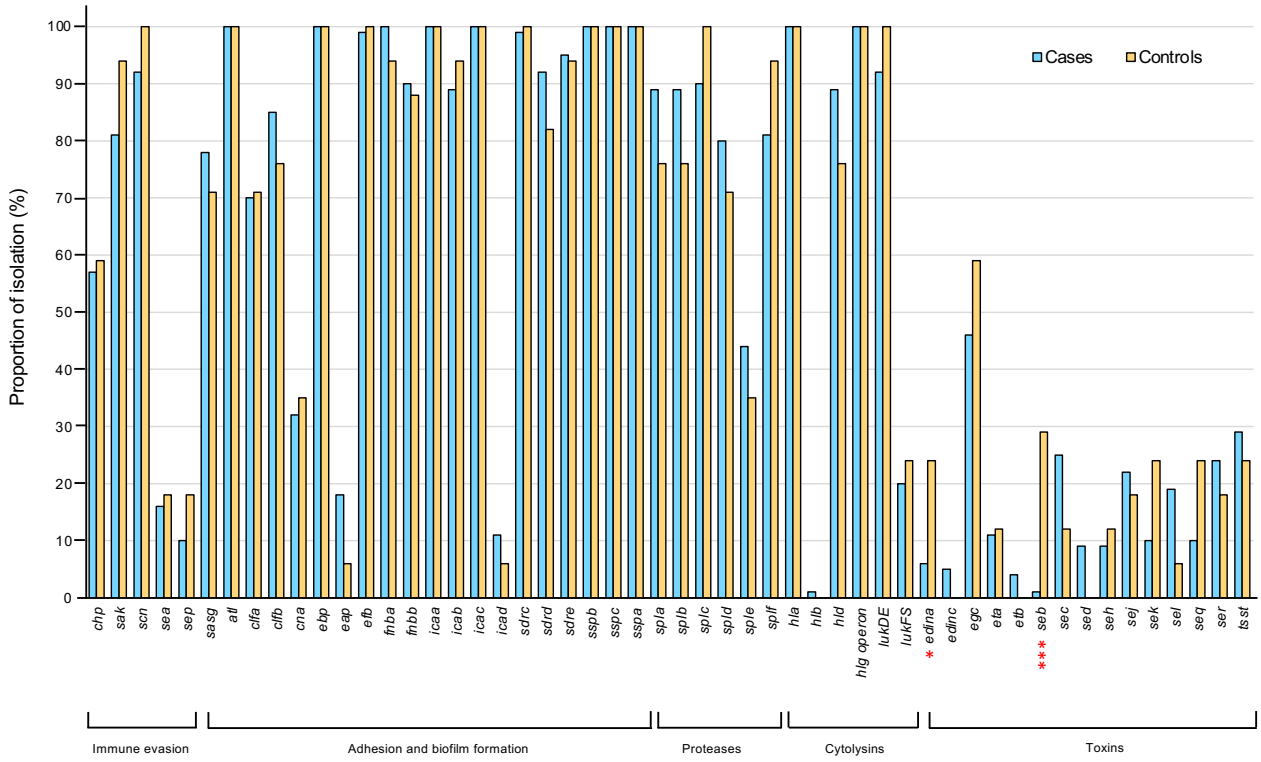


Figure 3.5. The distribution of virulence-associated genes in cases and controls, grouped by functional class. * p<0.05; ***p<0.001.

The phylogenetic tree in Figure 3.6. shows clustering of the respective *S. aureus* isolates according to CCs with distinct antibiotic and virulence gene profiles. Concerning virulence gene profiles, we observed that CC1 isolates were *seh*-positive, while 58% (7/12) and 75% (9/12) of CC1 *S. aureus* isolates were positive for the *lukFS-PV* and *sea/sek/seq* genes, respectively. The *egc* cluster was present in all CC5-ST5, CC8-ST72, CC121 and CC45-ST508 isolates. Most CC8-ST8 isolates carried *tsst* (78% [14/18]) and *sej* (72% [13/18]). The exfoliative toxin genes (*eta* and *etb*) were detected only in CC15-ST15 (n=4; *eta* only) and CC121 (n=8; *eta* and or *etb*). The epidermal cell differentiation inhibitor (EDIN) genes were identified only in CC5-ST5 (n=9, *edinA*) and CC121 (n=4; *edinC*). *S. aureus* assigned with CC15-ST15 exhibited the least virulence genes, possessing only the haemolysin genes (*hla, hlb*, and the *hlg* operon). In contrast, CC5-ST5 and CC1 isolates possessed an average of 7.8 (range=5–11) and 9 (range=5–11) virulence genes, respectively.

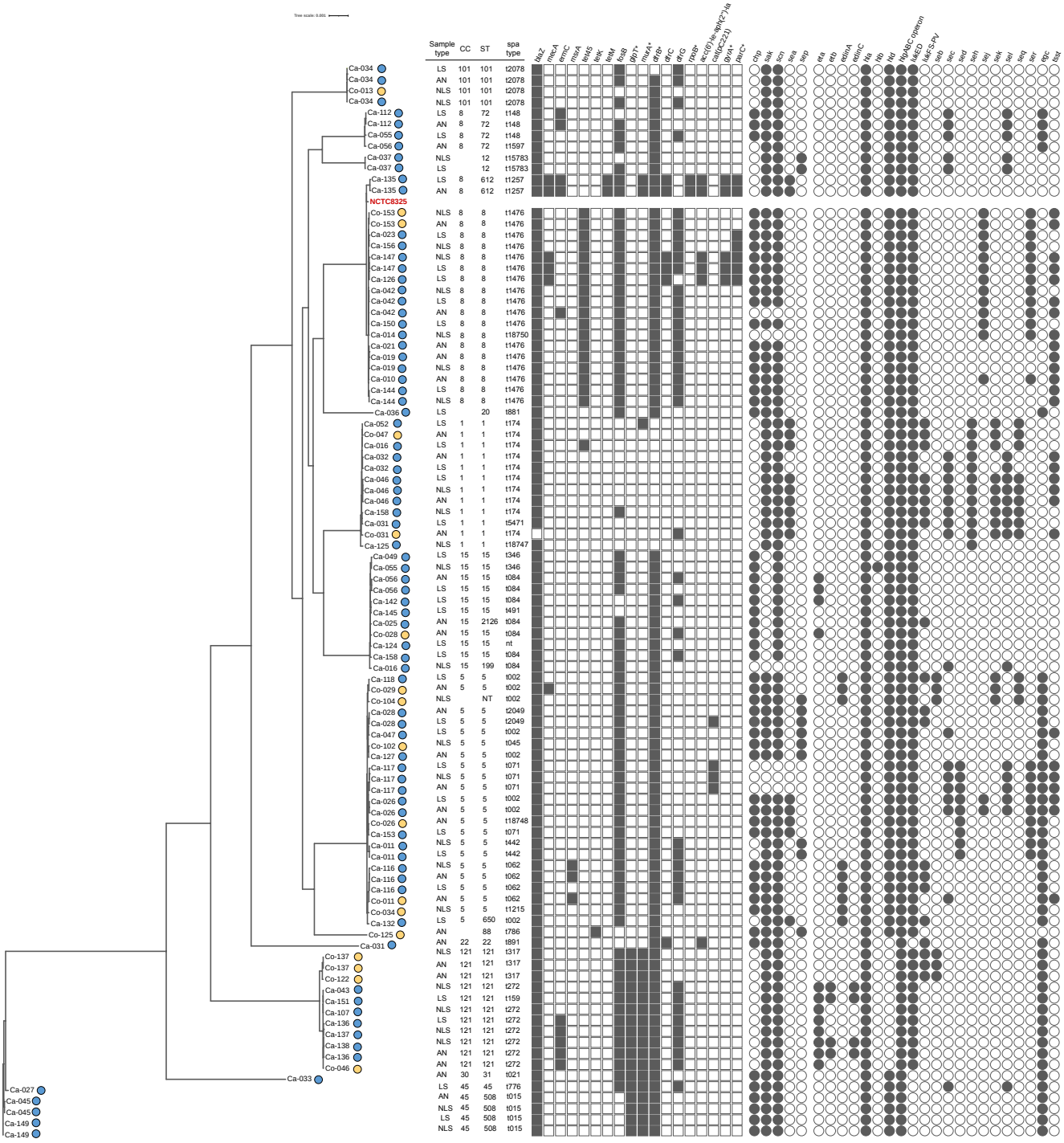


Figure 3.6. A maximum-likelihood tree *S. aureus* isolates based on SNPs in the core genome of *S. aureus*. The blue and yellow circles represent cases and controls, respectively. Grey and white indicate the presence and absence of the respective genes, respectively. AN, anterior nares; LS, lesional skin; NLS, nonlesional skin. The tree is annotated with CC (clonal complex), ST (sequence type), spa type, antibiotic resistance determinants, immune evasion cluster, and virulence factor data. Antibiotic resistance genes: *blaZ*, penicillin; *mecA*, methicillin; *ermC* and *msrA*, MLS_B; *tet45*, *tetK* and *tetM*,

tetracycline; *fosB*, *glpT* and *murA*, fosfomycin; *dfrB*, *dfrC* and *dfrG*, trimethoprim; *rpoB*, rifamycin; *acc(6')-le-aph(2'')-la*, gentamicin; *cat(pC221)*, chloramphenicol; *gyrA* and *parC*, ciprofloxacin. Antibiotic resistance in antibiotic genes with an asterix (*) indicates point mutations to the gene. Immune evasion cluster genes: *chp*, chemotaxis inhibitory protein precursor; *sak*, staphylokinase; *scn*, staphylococcal component inhibitor; *sea/p*, staphylococcus enterotoxins A/P. Virulence factor genes: *eta*/*b*, exfoliative toxins A/B; *edinA/C*, epidermal cell differentiation inhibitors A/C; *hla/b/d/g*, haemolysins α/β/δ/γ; *lukED*, leukotoxin ED, *lukFS-PV*, Panton-Valentine leukocidin; *seb-r*, staphylococcal enterotoxins B-R; egc, enterotoxin gene cluster; and *tsst*, toxic shock syndrome toxin.

3.4.6. Distribution of the immune evasion cluster genes

The prevalence of the immune evasion cluster (IEC) genes was as follows: most isolates carried *scn* (94%, 90/96), followed by *sak* (83%, 80/96), *chp* (57%, 55/96), *sea* (17%, 16/96) and *sep* (11% [11/96]); with frequencies similar between cases and controls (Figure 3.5). Six isolates, all from cases, did not carry any of the IEC genes. Of the various IEC groups, type B (*sak-chp-scn*) was predominant (37% [33/90]), followed by type E (*sak-scn*; 22% [20/90]), type D (*sea-sak-scn*; 13% [12/90]), type C (*chp-scn*; 11% [10/90]), type F (*sep-sak-chp-scn*; 8% [7/90]), and type A (*sea-sak-chp-scn*) and type G (*sep-sak-scn*); each with four isolates (4%). There were no significant differences in the distribution of IEC types between cases and controls, but type G isolates were identified only among cases (Figure 3.7). We observed some lineage-specific distribution of IEC types in cases and controls (Figures 3.6 and S3.4). CC101 and CC121 isolates were exclusively type E, CC8-ST8 (except for two CC8-ST8 isolates which carried none of the IEC genes) and CC45-ST508 were type B, while CC15-ST15 were type C. Also, CC1 isolates were mostly type D (75% [9/12]) but also type E (25% [3/12]). In contrast, CC5 exhibited high IEC type diversity representing types A, B, F, and G.

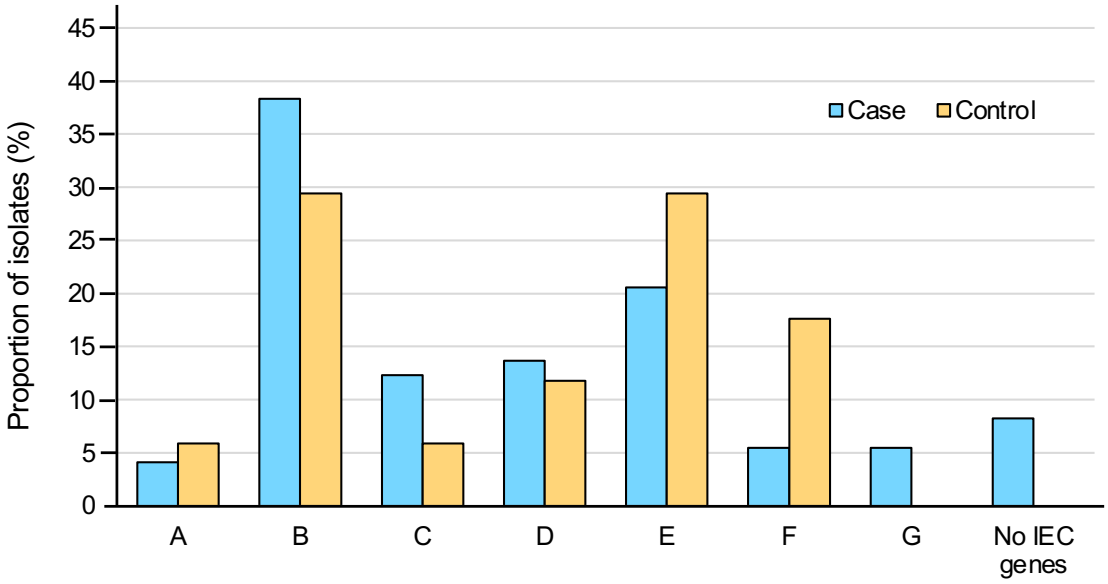


Figure 3.7. The distribution of immune evasion cluster (IEC) types in cases and controls. IEC types A (*sea-sak-chp-scn*), B (*sak-chp-scn*), C (*chp-scn*), D (*sea-sak-scn*), E (*sak-scn*), F (*sep-sak-chp-scn*), and G (*sep-sak-scn*). All comparisons between cases and controls were without statistical significance.

3.4.7. Pan-genome-wide association study (Pan-GWAS)

We performed a pan-GWAS analysis using Pyseer to identify genes that are differentially associated with the case and control groups. We found 277 genes that were differentially enriched in cases and controls. Of these, 56 were annotated as known proteins (Table 3.5) and the remaining 221 as hypothetical proteins. The genes *ebh*, *fnbB* and *group_1994* [*sasG*] were enriched in the control group. Two variants of the tyrosine recombinase XerC predominated in controls, while variant (*xerC_5*) predominated in cases. Interestingly, *lexA*, *ssbA, dinG* and *recF_2*, were enriched in the case group. Two variants of IS1181 (group_802 and group_965) were each predominant in the case and control groups, respectively. Other IS elements such as ISSau6 and IS431R [*group_998*] were more common in controls, while IS431R [*group_1619* and *group_1618*], ISSsu9 [*group_1518*], ISSep1 [*group_501*] and IS256 [*group_489*] were associated with cases. Moreover, the transposon Tn552 was associated with cases. Among ARGs, *tetK* was dominant among controls while *ermC*, *tetM* and *aacA-aphD* were associated with cases. Virulence factor genes, *hylB* and *see*, were common among cases, while *sea* and *see* predominated the control group. Moreover, the Type VII secretion system protein EsaG, encoded by *essG*, was associated with cases.

Table 3.5. Pan-genome wide association of case and control genomes showing 56 annotated genes.

Variant	Annotation	Allele frequency	Beta [standard error]	*p*-value†
ebh	Extracellular matrix-binding protein homologue	97.9%	-5.49 [2.39]	0.00295
ltaE	Low specificity L-threonine aldolase	97.9%	-5.49 [2.39]	0.00295
fnbB	Fibronectin-binding protein B	90.6%	-2.49 [2.30]	0.0443
pre_2	Plasmid recombination enzyme type 2	46.9%	-3.91 [1.40]	0.00911
pre_1	Plasmid recombination enzyme type 1	46.9%	-3.91 [1.40]	0.00911
ssbA_3~~~ssbA_2	Single-stranded DNA-binding protein A	42.7%	2.11 [2.04]	0.0449
group_802	ISL3 family transposase IS1181	40.6%	4.78 [1.65]	0.00548
group_1994 [sasG]	Surface protein G; Putative surface protein	39.6%	-2.41 [1.30]	0.0377
blaI	Penicillase repressor	35.4%	-4.25 [1.06]	0.0065
xerC_5~~~xerC_2	Tyrosine recombinase XerC	35.4%	-4.25 [1.06]	0.0065
xerC_3~~~xerC_4	Tyrosine recombinase XerC	35.4%	-4.25 [1.06]	0.0065
blaR12	Regulatory protein BlaR1	35.4%	-4.25 [1.06]	0.0065
group_1176 [blaZ]	Beta-lactamase	35.4%	-4.25 [1.06]	0.0065
acuI	Putative acrylyl-CoA reductase AcuI	34.4%	-3.16 [0.897]	0.0111
ssbA_1~~~ssbA_3~~~ssbA_5 ~~~ssbA_4~~~ssbA_2	Single-stranded DNA-binding protein A	34.4%	2.74 [1.86]	0.0393
see_1~~~see~~~sea_2	Enterotoxin type E	28.1%	4.83 [1.80]	0.0039
group_998	IS6 family transposase ISSau6; IS6 family transposase IS431R	26%	-5.03 [0.693]	0.00267
ssbA_4~~~ssbA_5~~~ssbA_1 ~~~ssbA_2	Single stranded DNA-binding protein A	24%	-3.52 [0.698]	0.0124
group_1220 [entG]	Enterotoxin type G	22.9%	-2.60 [0.715]	0.0491
sea_6~~~see	Enterotoxin type A	22.9%	-2.60 [0.715]	0.0491
essG_9~~~essG_8~~~essG_4 ~~~essG_7	Type VII secretion system protein EsaG	20.8%	3.99 [1.23]	0.00577
repN_1~~~repN_2	Replication initiation protein	19.8%	-5.09 [0.688]	0.00278
tetK	Tetracycline resistance protein TetK	19.8%	-5.09 [0.688]	0.00278
group_965	ISL3 family transposase IS1181	18.8%	-5.07 [0.687]	0.00314
pdhD_2~~~garB	Dihydrolipoyl dehydrogenase; Glutathione amide reductase	18.8%	-5.07 [0.687]	0.00314
merR1	Mercuric resistance operon regulatory protein	18.8%	-5.07 [0.687]	0.00314
merB	Alkylmercury lyase	18.8%	-5.07 [0.687]	0.00314
essG_10~~~essG_2~~~essG_9 ~~~essG_3~~~essG_8~~~essG_7	Type VII secretion system protein EsaG	18.8%	3.90 [1.16]	0.00731
merA	Mercuric reductase	18.8%	-5.07 [0.687]	0.00314
lexA_3	LexA repressor	17.7%	2.62 [1.68]	0.0412
hylB~~~hylB_1	Hyaluronate lyase	16.7%	25.6 [0.999]	0.0153
ermC	Erythromycin resistance protein C	11.5%	3.65 [1.30]	0.0189

lytO	Probable autolysin	9.38%	3.74 [1.12]	0.00835
ssbA_2	Single stranded DNA-binding protein A	8.33%	6.35 [1.16]	0.000413
lexA_1~~~lexA_3	LexA repressor	7.29%	29.7 [0.63]	0.0365
group_1665	DegV domain-containing protein	6.25%	3.28 [1.18]	0.0399
group_1619	IS6 family transposase IS431R	6.25%	3.28 [1.18]	0.0399
group_1518	IS1595 family transposase ISSsu9	6.25%	3.28 [1.18]	0.0399
aacA-aphD	Bifunctional aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase	6.25%	3.28 [1.18]	0.0399
thyA_2	Thymidylate synthase	6.25%	3.28 [1.18]	0.0399
group_1618	IS6 family transposase IS431R	5.12%	3.44 [1.10]	0.0386
group_1144 [dinG_1]	3'-5' exonuclease DinG	3.12%	5.59 [0.68]	0.00352
mecR1	Methicillin resistance mecR1 protein	3.12%	3.54 [0.84]	0.011
bcglA	Restriction enzyme BgcI subunit alpha	3.12%	5.59 [0.68]	0.00352
group_501	IS1182 family transposase ISSep1	3.12%	3.54 [0.84]	0.011
group_489	IS256 family transposase IS256	3.12%	5.05 [0.707]	0.00371
bin3	Putative transposon Tn552 DNA-invertase bin3	2.08%	5.49 [0.527]	0.00295
group_1103 [bcglB]	Restriction enzyme Bgcl subunit beta	2.08%	5.49 [0.527]	0.00295
group_988	IS256 family transposase ISLgar5	2.08%	5.49 [0.527]	0.00295
xerC_5	Tyrosine recombinase XerC	2.08%	5.49 [0.527]	0.00295
dauA_2	C4-dicarboxylic acid transporter DauA	2.08%	5.49 [0.527]	0.00295
dauA_1	C4-dicarboxylic acid transporter DauA	2.08%	5.49 [0.527]	0.00295
pknD	Serine/threonine-protein kinase PknD	2.08%	5.49 [0.527]	0.00295
rep	Replication initiation protein	2.08%	5.49 [0.527]	0.00295
tetM	Tetracycline resistance protein TetM	2.08%	5.49 [0.527]	0.00295
recF_2	DNA repair protein recF	2.08%	5.49 [0.527]	0.00295

Beta represents the slope of regression, with a negative value showing genes enriched in controls and a positive value showing genes enriched in cases.

† The *p*-value of association, adjusted for population structure.

3.5. Discussion

This report describes the phenotypic and genomic features of *S. aureus* in South African children with and without AD. We demonstrated that *S. aureus* associated with cases and controls do not differ in their antibiotic resistance and carriage of virulence factors. However, *S. aureus* from cases tended to produce stronger biofilms and carried genetic features that are linked to increased DNA damage repair, antibiotic resistance, and virulence with potential clinical relevance to AD pathology. In contrast, control strains were enriched with genes related to *S. aureus* adhesion.

We observed a low prevalence of antibiotic resistance in this cohort that did not significantly differ between cases and controls. These observations are similar to previous reports in Irish[113], Polish[77], and Italian[43] paediatric patients with AD. MRSA strains were also low in both cases (8%) and controls (6%), as previously demonstrated[113]. Moreover, MDR *S. aureus* isolates were detected only among cases, suggesting frequent use of antibiotics or exposure to resistant strains, possibly from a hospital environment. However, prevalence is low in this geographic setting and may not reflect the distribution of MDR strains among children with AD. More studies need to be conducted to explore the antibiotic resistance profiles between *S. aureus* isolates from children with and without AD. Such studies will help better our understanding on whether antimicrobial resistance phenotypes have any association with the AD causing propensity of *S. aureus*.

We observed a higher prevalence of strong biofilm producers in cases than in controls, which is similar to previous studies[43,84,98]. Biofilm formation is associated with increased disease severity and may contribute towards AD inflammation due to occlusion of sweat glands[43,63,98]. Biofilms, particularly strong biofilms, protect *S. aureus* from i) environmental factors (including antibiotics), ii) the bactericidal effects of host antimicrobial peptides (AMPs) and, iii) host immune responses[25]. Similarly, strong biofilm producing strains were significantly more common among cases than controls. This indicates that *S. aureus* strains from cases are better able to form biofilms and are better adapted to persistent colonisation. Additionally, strong biofilm-producing strains exhibit higher resistance to antibiotics and host-derived AMPs than weak biofilm producers, leading to significant *S. aureus* growth and persistence[43,84]. In contrast, we did not observe any association between biofilm phenotype and resistance/susceptibility to any of the phenotypically tested antibiotics. Overall, these findings suggest that strong biofilm formation is the primary driver of persistent colonisation in children with AD, with antibiotic resistance likely not influencing this phenomenon. Also, persistent colonisation with *S. aureus* in AD may be detrimental to the child as this provides a continuous source of virulence

factors that will further perpetuate the clinical manifestations of AD and contribution to disease flares. This highlights the need for treatment strategies in AD to have anti-biofilm capabilities for significant effectiveness in reducing *S. aureus* colonisation and mitigating its harmful effects.

S. aureus produces a variety of toxins, cytotoxins, proteases, antigens and adhesins that are key to its successful colonisation, pathogenicity, and survival[88]. In AD, these factors usually promote harmful colonisation of *S. aureus* which contributes to or exacerbates disrupted skin barrier functionality and aberrant cutaneous inflammation[88]. Epidemiological studies have shown that AD-associated *S. aureus* exhibits more virulence genes than controls[69,81,106,110], which is also linked to increased disease severity[110]. Among the cases in this study, we observed the predominance of *hylB* and *see*, which encode hyaluronate lyase and enterotoxin type E, respectively. Enterotoxin type E belongs to the staphylococcal enterotoxin group, which is a group of toxins that induces T cell-dependent inflammatory responses in AD[88]. The role of hyaluronate lyase, in AD, has not been defined. However, its importance in the pathology of subcutaneous infections, promotion of inflammatory responses, degradation of epidermal proteins, and driving biofilm formation[248-250], which are features associated with AD pathology, have been shown. These results show that hyaluronate lyase likely plays a significant, but unstudied, role in driving AD pathology in this cohort.

Immune evasion by *S. aureus* is mediated by the immune evasion cluster (IEC), which modulates innate immune responses in humans[251]. IEC includes SCIN, and a varying combination of SAK, CHIPS and staphylococcal enterotoxins, SEA or SEP, across different IEC types[220]. We detected IEC genes in the *S. aureus* control group and most (92%) of the cases. None of the IEC genes significantly differed based on disease status. Further, cases and controls did not differ substantially in the distribution of IEC types. Similar to previous reports[220,251,252], IEC type B, independent of disease status, whereas the type G isolates were identified only among cases[102]. Although the specific functions of the IEC genes and their contribution to AD are addressed in the literature[110,253], the clinical relevance of different IEC types in AD is lacking and warrants further study.

Insertion sequences (IS) are small MGEs and are generally between 700–2,500 base pairs in size, affecting the genomic plasticity, diversity, and pathogenic potential of *S. aureus*[254]. They contribute to bacterial genomic alteration through numerous mechanisms, including gene inactivation, genomic rearrangement, and reduction, and can also regulate the expression of neighbouring genes[255]. ISFinder revealed that ISCsp1 and ISSau6 were more common among controls than in cases. This was supported by our pan-genomic analysis which revealed an association of ISSau6, but not ISCsp1, with controls.

Cases revealed a predominance of IS431R, ISSsu9, ISSep1, ISLgar5 and IS256. The specific functions of ISSau6, ISSsu9 and ISSep1 are poorly studied[256-258]. However, they may be involved in regulating cadmium resistance[259], dissemination of ARGs[260] or termination of the transcription of highly transcribable genes[261], through mechanisms not yet described in literature. Most of the IS elements found in cases were linked to the carriage and transmission of ARGs, reflecting the enrichment of multiple ARGs presently observed in cases. For example, IS431R contains *ermC*[262], and the IS256 family (IS256 and ISLgar5 detected presently) is associated with multidrug resistance[263]. Moreover, the IS256 family has been shown to influence biofilm formation[264], which might reflect the high prevalence of strong biofilm producers observed among the cases in this study.

There is increasing evidence that specific *S. aureus* lineages are adapted for induction of disease activity and survival in AD patients[18,103,113,152]. In this study, we noted no significant difference in the clonality of *S. aureus* isolates from cases and controls. However, CC8 isolates predominated among cases, while CC121 isolates were the most common among controls. In contrast, CC5 isolates were similarly common in both cases and controls. The specific features or mechanisms that favour the predominance of certain lineages in AD remain unknown[56]. However, it has been suggested that in AD, differences in the virulence potential across lineages are associated with the proliferation of some lineages and other lineages are associated with asymptomatic colonisation in controls [101].

Factors associated with AD, such as chronic inflammation and the biofilm environment of *S. aureus*, have been demonstrated to induce DNA damage in *S. aureus*[265,266]. These mediators of DNA damage have a bactericidal effect on this bacterium[265]. Despite this, *S. aureus* usually survives and persistently colonises patients with AD[56]. This indicates the presence of efficient DNA damage repair mechanisms in *S. aureus* from patients with AD. We presently found an association between the case group and the genes that are important in the repair of DNA damage via the SOS response, namely *lexA*, *ssbA, dinG* and *recF*[267-269]. This data suggests that the AD environment selects for strains that have efficient repair mechanisms which circumvent the detrimental effects of this DNA damage, allowing for *S. aureus* survival.

Limitations to this study include a small sample size. Additionally, the cross-sectional design limits the assessment of whether these strains persist or are replaced by new ones. This is important because it has been shown that changes in *S. aureus* carriage strains are associated with increased disease severity[270]. Our investigation focused on the recovery of a single colony which does not provide a comprehensive understanding of the clonality of *S. aureus* colonization in AD[152]. Lastly, we noted a

higher prevalence of incomplete and questionable prophages compared to intact prophages. This is likely because intact prophages are usually under strong selection or genetic degradation for rapid deletion from bacterial genomes[271]. Moreover, since genomes are divided into contigs, some prophage sequences may have been split into different contigs and therefore misidentified as incomplete or questionable prophages by PHASTER[272]. The PHASTER tool also relies on previously annotated prophage sequences therefore cannot identify novel prophage sequences.

3.6. Conclusion

In this chapter, we provide an understanding of the pan-genomic differences of *S. aureus* in early childhood AD, in a previously unstudied population in South Africa. Although we did not observe a clear phylogenetic and clonal differentiation of *S. aureus* based on AD disease, specific phenotypic and genetic signatures distinguished AD isolates from controls. Specifically, AD-associated strains exhibited phenotypic features related to biofilm formation, and genomic features associated with DNA damage repair and toxins which are important for the persistent colonisation and propagation of AD disease. The findings from our study emphasise the need for further *in-vivo* studies to assess the pathological implications of colonisation with these strains. This data will explain the functional differences in *S. aureus* strains that are adapted for colonization in patients with AD compared to healthy children.

3.7. Supplementary Materials

Table S3.1. Distribution of sequence types in cases and controls

Sequence type	Cases, n (%)	Controls, n (%)	*p*-value
ST1	10 (12.6)	2 (11.8)	0.920
ST101	3 (3.8)	1 (5.9)	0.696
ST12	2 (2.5)	0 (0)	0.507
ST121	7 (8.9)	4 (23.5)	0.085
ST15	8 (10.1)	1 (5.9)	0.586
ST199	1 (1.2)	0 (0)	0.641
ST20	1 (1.2)	0 (0)	0.641
ST2126	1 (1.2)	0 (0)	0.641
ST22	1 (1.2)	0 (0)	0.641
ST31	1 (1.2)	0 (0)	0.641
ST4005	3 (3.8)	0 (0)	0.414
ST45	1 (1.2)	0 (0)	0.641
ST5	13 (16.4)	5 (29.4)	0.214
ST508	4 (5.1)	0 (0)	0.343
ST612	2 (2.5)	0 (0)	0.507
ST650	1 (1.2)	0 (0)	0.641
ST72	4 (5.1)	0 (0)	0.343
ST8	16 (20.2)	2 (11.8)	0.416
ST88	0 (0)	1 (5.9)	0.030
Unknown ST	0 (0)	1 (5.9)	0.030

Table S3.2. Predicted plasmids.

Plasmid	Species of origin	Size	No. of genomes	Accession no.	Associated CCs
pIM13	*Bacillus subtilis*	2246 bp	1	M13761	CC8 (1)
pUSA01	*Staphylococcus aureus*	3125 bp	2	NC007790	CC45 (2)
pETB	*Staphylococcus aureus*	38211 bp	10	NC003265	CC45 (3), CC121 (7)
pSJH101	*Staphylococcus aureus*	30429 bp	23	CP000737	CC5 (8), CC8 (13), ST20 (1), NT (1)
pSAS	*Staphylococcus aureus*	20652 bp	11	BX571858	CC1 (6), CC8 (4), CC30 (1)
ORF1(EDINA)	*Staphylococcus aureus*	34986 bp	10	AP003089	CC5 (8), ST88 (1), NT (1)
pWBG759	*Staphylococcus aureus*	28384 bp	23	GQ900401	CC5 (8), CC8 (13), ST20 (1), NT (1)
pNE131	*Staphylococcus epidermidis*	2355 bp	1	NC001390	CC8 (1)
MSSA476	*Staphylococcus aureus*	20652 bp	36	BX571857	CC1 (1), CC8 (28)
pBORa53	*Staphylococcus aureus*	18334 bp	13	AY917098	CC8 (13)
pSK156	*Staphylococcus aureus*	45052 bp	7	GQ900448	CC5 (7)
pSaa6159	*Staphylococcus aureus*	20730 bp	18	CP002115	CC1 (1), CC8 (1), CC15 (11), CC45 (1), CC101 (4)
pTW20	*Staphylococcus aureus*	29585 bp	8	FN433597	CC5 (6), CC8 (2)
pWBG745	*Staphylococcus aureus*	38204 bp	2	GQ900389	CC1 (1), CC15 (1)
pWBG746	*Staphylococcus aureus*	33702 bp	1	GQ900390	NT (1)
SAP057A	*Staphylococcus aureus*	39308 bp	4	NC013334	CC121 (4)
SAP074A	*Staphylococcus aureus*	27268 bp	8	GQ900426	CC5 (8)
SAP102A	*Staphylococcus aureus*	36082 bp	1	GQ900496	CC22 (1)
SAP105A	*Staphylococcus epidermidis*	26236 bp	1	GQ900452	CC5 (1)
pLW043	*Staphylococcus aureus*	57889 bp	3	AE017171	CC8 (3)
repA(pWBG759)	*Staphylococcus aureus*	28384 bp	1	GQ900401	ST20 (1)
repC(Cassette)	*Staphylococcus aureus*	68256 bp	1	AB037671	ST88 (1)
repC(pS0385p1)	*Staphylococcus aureus*	5246 bp	19	AM990993	CC8 (18), ST88 (1)
repD(pK214)	*Lactococcus lactis*	29871 bp	1	NC009751	CC5 (1)
repD(pTZ4)	*Staphylococcus aureus*	4555 bp	3	NC010111	CC5 (3)
repL(pDLK1)	*Staphylococcus aureus*	2402 bp	11	GU562624	CC8 (6), CC121 (5)
repSAP001(pN315)	*Staphylococcus aureus*	24653 bp	44	AP003139	CC1 (7), CC5 (2), CC8 (16), CC15 (11), CC22 (1), CC30 (1), CC45 (1) CC101 (4), ST88 (1)

Abbreviations: CC, clonal complex; ST, sequence type; NT, non-typeable (according to the MLST tool 2.0 in https://www.genomicepidemiology.org/).
Only predicted plasmids in bold (identified in ten or more genomes) were included in the main results and discussion.

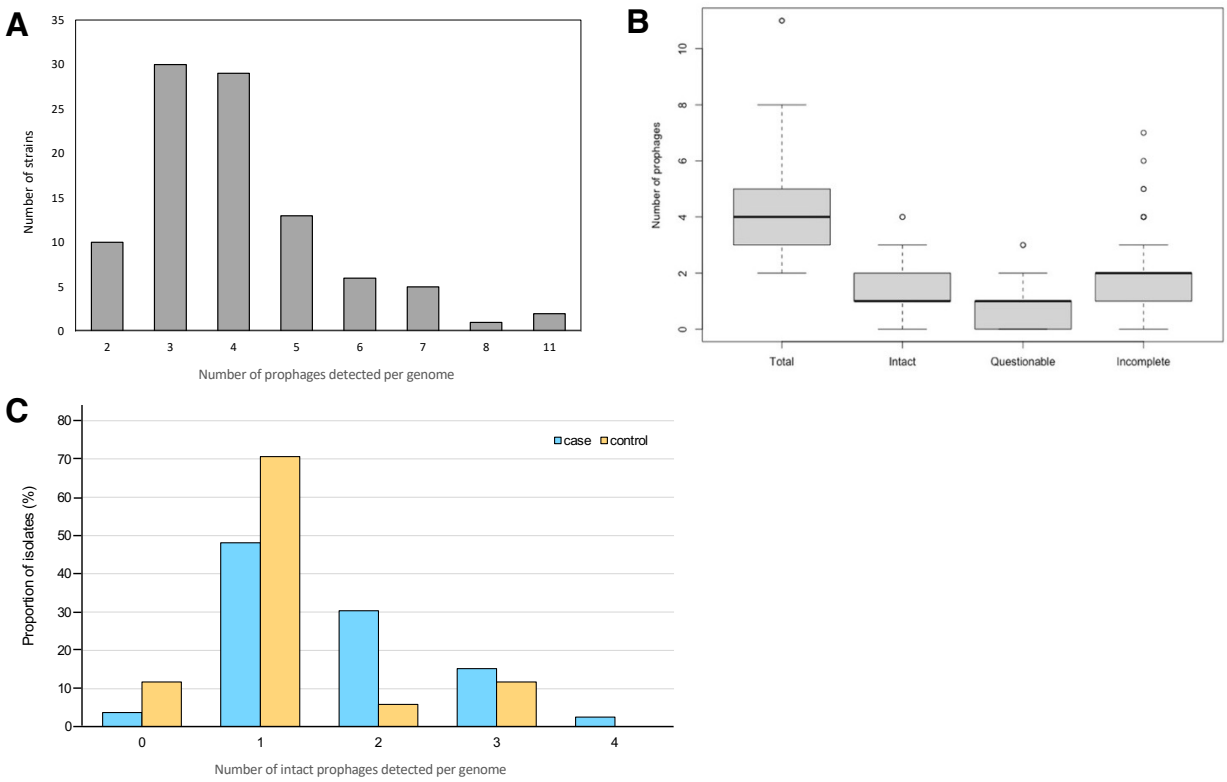


Figure S3.1. The distribution of prophages in *S. aureus* isolates. (A) Bar plot showing the distribution of total prophages (including intact, questionable, and incomplete) in *S. aureus* isolates. (B) Box plot showing the total, intact, questionable, and incomplete prophages of *S. aureus*. (C) Number of intact prophages per isolate.

Table S3.3. Predicted intact prophages.

Most similar phage hit	No. of genomes	No. with integrase	Integrase group†	Predicted genus	Predicted family	Associated CCs
Staphylococcus phage P282	18	10	Sa3int	Biseptimavirus	Siphoviridae	CC8 (16), CC45 (2)
Staphylococcus phage SA1014ruMSSAST7	2	0		Biseptimavirus		ST12 (2)
Staphylococcus phage SA345ruMSSAST8	1	1		Biseptimavirus		CC1 (1)
Staphylococcus phage phiN315	4	3	Sa3int	Biseptimavirus		CC5 (4)
Staphylococcus phage phiSa2wa ST22	1	1	Sa2int	Biseptimavirus		CC22 (1)
Staphylococcus phage 11	2	2	Sa5int	Dubowvirus		CC5 (1), CC15 (1)
Staphylococcus phage 53	9	2	Sa7int	Dubowvirus		CC5 (3), CC8 (6)
Staphylococcus phage SA97	5	3	Sa1int	Dubowvirus		CC5 (5)
Staphylococcus phage phiETA2	1	1	Sa2int	Dubowvirus		CC1 (1)
Staphylococcus phage phiNM2	1	1	Sa7int	Dubowvirus		CC8 (1)
Staphylococcus phage AJ 2017	8	6		Peeveelvirus		CC1 (6), CC121 (2)
Staphylococcus phage SA780ruMSSAST101	4	0		Peeveelvirus		CC101 (4)
Staphylococcus phage SA7	3	2		Peeveelvirus		CC1 (2), CC8 (1)
Staphylococcus phage tp310-1	1	1	Sa2int	Peeveelvirus		CC5 (1)
Staphylococcus phage tp310-3	1	1	Sa3int	Peeveelvirus		CC22 (1)
Staphylococcus phage 71	4	2		Phietavirus		CC45 (4)
Staphylococcus phage 88	1	1	Sa5int	Phietavirus		CC1 (1)
Staphylococcus phage 96	5	5	Sa9int	Phietavirus		CC1 (4), CC15 (1)
Staphylococcus phage B236	1	0	Sa1int	Phietavirus		CC8 (1)
Staphylococcus phage phiETA3	3	3	Sa3int	Phietavirus		CC15 (3)
Staphylococcus phage phiETA	8	7	Sa1int	Phietavirus		CC121 (8)
Staphylococcus phage phiJB	21	14	Sa6int	Phietavirus		CC1 (3), CC5 (7), CC8 (4), CC15 (2), CC45 (1), ST12 (2), ST88 (1), NT (1)
Staphylococcus phage phiMR11	1	1	Sa12int	Phietavirus		CC15 (1)
Staphylococcus phage 47	1	1	Sa2int	Triavirus		CC1 (1)
Staphylococcus phage Ipla35	1	1		Triavirus		CC121 (1)
Staphylococcus phage SA13	1	1		Triavirus		CC5 (1)
Staphylococcus phage StauST398-2	2	2		Triavirus		CC15 (1), CC121 (1)
Staphylococcus phage YMC/09/04/R1988	1	0	Sa2int	Triavirus		CC5 (1)
Staphylococcus phage phi2958PVL	33	30	Sa2int	Triavirus		CC1 (9), CC5 (6), CC8 (6), CC15 (6), CC30 (1), CC45 (5)

Staphylococcus phage PT1028	5	0	Not assigned	Unclassified		CC8 (2), CC121 (3)
Staphylococcus phage SPbeta like	1	1	Not assigned	Unclassified		CC8 (1)

Abbreviations: CC, clonal complex; ST, sequence type; NT, non-typeable (according to the MLST tool 2.0 in https://www.genomicepidemiology.org/).

Only predicted prophages in bold (identified in ten or more genomes) were included in were included in the main results and discussion.

[†]Data on integrase groups obtained from [273], [274], and [275].

Table S3.4. Predicted insertion sequence elements.

IS element	IS family	Species of origin	No. of genomes	Length (bp)	Inverted repeat length (bp)†	ORF§	Accession no.	Associated CCs
IS1181	ISL3	*Staphylococcus aureus*	59	1513	17/23	439 (120-1439)	L14544	CC5 (22), CC8 (24), CC22 (1), CC121 (10), ST12 (1), NT (1)
IS1252	IS30	*Enterococcus* sp.	44	1065	21/28	226 (75-755)	L38972	CC8 (20), CC15 (11), CC22 (2), CC30 (1), CC45 (5), CC101 (4), ST88 (1)
IS232	IS21	*Bacillus thuringiensis*	1	2184	48/67	431 (93-1388) 250 (1378-2130)	M38370	CC22 (1)
IS256	IS256	*Staphylococcus aureus*	3	1324	17/26	390 (102-1274)	M18086	CC8 (2), CC22 (1)
IS257-1	IS6	*Staphylococcus aureus*	3	791	21/26	224 (57-731)	X53952	CC8 (3)
IS257-2	IS6	*Staphylococcus aureus*	2	790	20/26	221 (57-722)	X53951	CC8 (2)
IS257-3	IS6	*Staphylococcus aureus*	3	789	23/27	224 (57-731)	X53951	CC8 (3)
IS257R1	IS6	*Staphylococcus aureus*	6	790	18/20	224 (57-731)	X13290	CC8 (5), CC22 (1)
IS257R2	IS6	*Staphylococcus aureus*	5	789	23/27	224 (57-731)	X13290	CC8 (5)
IS431R	IS6	*Staphylococcus aureus*	18	790	17/20	224 (57-731)	M18437	CC8 (18)
IS431mec	IS6	*Staphylococcus aureus*	7	790	18/20	224 (57-731)	X53818	CC5 (1), CC8 (6)
IS655	IS3	*Bacillus halodurans*	18	1221	9/15	94 (80-364) 288 (325-1191) 370 (80-1191)	NC_002570	CC1 (12), CC30 (1), CC45 (5)
IS712	IS21	*Lactococcus lactis*	1	2163	41/51	407 (81-1304) 252 (1316-2074)	NC_009004	CC22 (1)
ISBli29	ISNCY	*Brevibacterium linens*	41	4278	32/38	197 (704-111) 119 (1060-701) 208 (1259-1885)	CP014869	CC1 (11), CC5 (3), CC8 (2), CC15 (11), CC30 (1), CC45 (4),

						480 (1905-3347) 264 (3445-4239)		CC101 (4), CC121 (3), ST88 (1), NT (1)
ISClsp1	IS3	*Clostridium* sp.	41	1222	25/41	96 (78-368) 291 (329-1204) 375 (78-1204)	NZ_AAQV01000022	CC5 (22), CC22 (1), CC45 (1), CC101 (4), CC121 (11), ST88 (1), NT (1)
ISEfa8	IS3	*Enterococcus faecium*	6	1366	30/41	96 (75-365) 305 (320-1237) 387 (75-1237	NZ_AAAK03000001	CC22 (1), CC45 (5)
ISLgar5	IS256	*Lactococus garvieae*	2	1336	21/26	396 (95-1285)	AKFO01000017.1	CC8 (2)
ISSau1	IS30	*Staphylococcus aureus*	1	1070	20/26	315 (114-1061)	NC_002952	CC30 (1)
ISSau2	IS3	*Staphylococcus aureus*	14	1600	29/40	251 (34-789) 297 (705-1601) 521 (34-1601)	NC_002952	CC22 (1), CC30 (1), CC121 (11), ST12 (1)
ISSau3	IS1182	*Staphylococcus aureus*	96	1946	13/16	548 (280-1926)	NC_002952	CC1 (12), CC5 (22), CC8 (24), CC15 (11), CC22 (1), CC30 (1), CC45 (5), CC101 (4), CC121 (11), ST12 (2), ST20 (1), ST88 (1), NT (1)
ISSau5	IS30	*Staphylococcus aureus*	11	1136	7/12	163 (104-595) 175 (597-1124) 340 (104-1124)	NC_002952	CC8 (8), CC15 (1), CC121 (1), ST20 (1)
ISSau6	IS6	*Staphylococcus aureus*	27	793	21/22	224 (59-733)	NC_002952	CC5 (13), CC8 (1), CC22 (1), CC45 (3), CC121 (6), ST20 (1), ST88 (1), NT (1)
ISSau8	ISL3	*Staphylococcus aureus*	12	1498	19/24	438 (141-1457)	NC_007622	CC1 (1), CC121 (11)
ISSep1	IS1182	*Staphylococcus epidermidis*	11	1936	19/28	559 (271-1950)	NC_004461	CC5 (1), CC8 (9), CC22 (1)
ISSep2	IS110	*Staphylococcus epidermidis*	1	1564		337 (442-1455)	NC_004461	ST20 (1)
ISSep3	IS200/IS605	*Staphylococcus epidermidis*	48	740		160 (133-615)	NC_004461	CC1 (12), CC8 (24), CC15 (11), CC22 (1)

ISSsu9	IS1595	*Streptococcus suis*	40	1796	22/26	341 (89-1114) 157 (1217-1690)	NC_012926	CC1 (1), CC5 (6), CC8 (17), CC15 (3), CC22 (1), CC101 (4), CC121 (8)

Abbreviations: bp, base pair; IS, insertion element; ORF, open reading frame; CC, clonal complex; ST, sequence type; NT, non-typeable (according to the MLST tool 2.0 in https://www.genomicepidemiology.org/).

Only insertion sequences in bold (identified in ten or more genomes) were included in the main results and discussion.

† left of slash is number of matching nucleotides, right of slash is total length of inverted repeat.

§ first number is amino acid length, range in parentheses is transcription start and end positions.

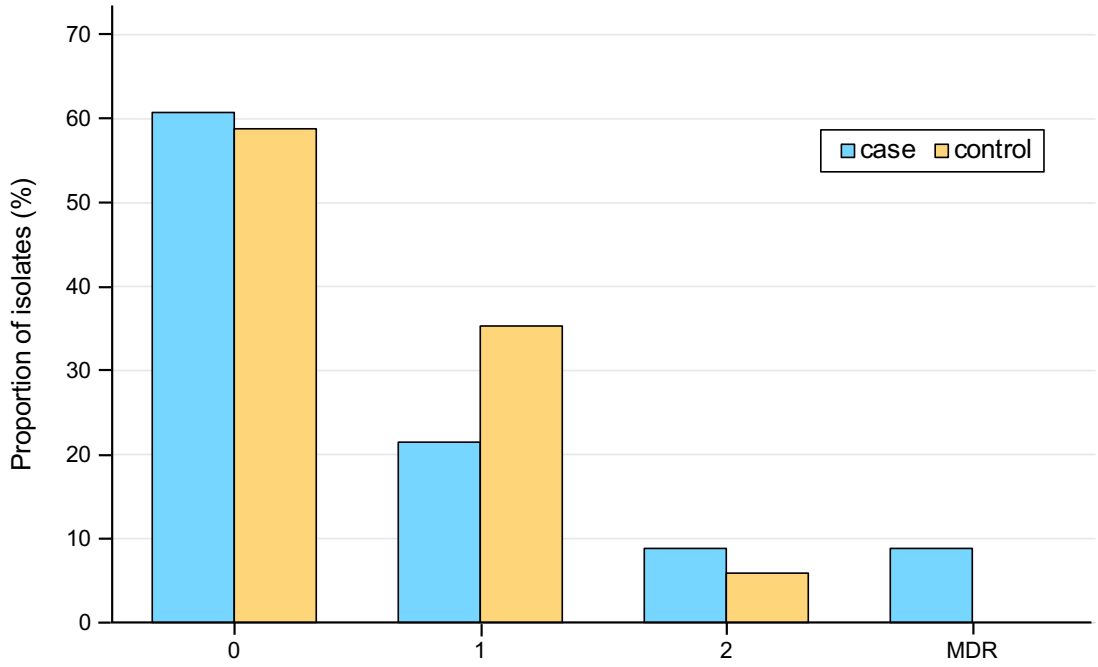


Figure S3.2. Barplot representing the number of antibiotic classes to which *S. aureus* strains were phenotypically resistant. MDR, multidrug resistant. All comparisons between cases and controls were without statistical significance.

Table S3.5. Agreement between phenotypes and genotypes of antibiotic resistance.

Antibiotic	Phenotypic resistance (n [%])	Genotypic resistance (n [%])	Agreement	Kappa	Comment on agreement	p-value
Tetracycline	20 (21%)	20 (21%)	91.67%	0.7474	Moderate	<0.0001
Erythromycin	14 (15%)	11 (12%)	94.79%	0.7706	Moderate	<0.0001
Clindamycin	11 (12%)	8 (8%)	96.88%	0.8252	Strong	<0.0001
Cefoxitin	7 (7%)	6 (6%)	98.96%	0.9175	Almost perfect	<0.0001
Gentamicin	7 (7%)	6 (6%)	98.96%	0.9175	Almost perfect	<0.0001
Chloramphenicol	4 (4%)	4 (4%)	100%	1	Almost perfect	<0.0001
Trimethoprim-sulfamethoxazole	4 (4%)	45 (47%)	56.1%	0	None	0.5
Rifampicin	3 (3%)	2 (2%)	98.96%	0.7949	Moderate	<0.0001
Mupirocin	0	0	—	—		—

Table S3.6. Relationship between biofilm formation and phenotypic antibiotic resistance.

Antibiotic phenotype		Biofilm phenotype			*p*-value
		Weak (n [%])	Moderate (n [%])	Strong (n [%])	
Cefoxitin	Susceptible	9 (9.5%)	15 (15.8%)	64 (67.4%)	0.268
	Resistant	2 (2.1%)	1 (1.1%)	4 (4.2%)	
Chloramphenicol	Susceptible	11 (11.6%)	15 (15.8%)	65 (68.4%)	0.744
	Intermediate	0	0	1 (1.1%)	
	Resistant	0	1 (1.1%)	2 (2.1%)	
Clindamycin	Susceptible	11 (11.7%)	14 (14.8%)	58 (61.7%)	0.522
	Resistant	0	2 (2.1%)	9 (9.6%)	
Erythromycin	Susceptible	11 (11.7%)	13 (13.8%)	56 (59.6%)	0.422
	Resistant	0	3 (3.2%)	11 (11.7%)	
Gentamicin	Susceptible	9 (9.5%)	16 (16.8%)	63 (66.3%)	0.314
	Intermediate	1 (1.1%)	0	1 (1.1%)	
	Resistant	1 (1.1%)	0	4 (4.2%)	
Mupirocin	Susceptible	11 (11.6%)	16 (16.8%)	68 (71.6%)	–
	Resistant	0	0	0	
Rifampicin	Susceptible	11 (11.6%)	16 (16.8%)	65 (68.4%)	1.000
	Resistant	0	0	3 (3.2%)	
Tetracycline	Susceptible	8 (8.5%)	14 (14.9%)	52(55.3%)	0.568
	Resistant	3 (3.2%)	2 (2.1%)	15 (16%)	
SXT	Susceptible	10 (10.6%)	16 (17%)	64 (68.1%)	0.497
	Resistant	1 (1.1%)	0	3 (3.2%)	

Abbreviations: SXT, trimethoprim-sulfamethaxazole.

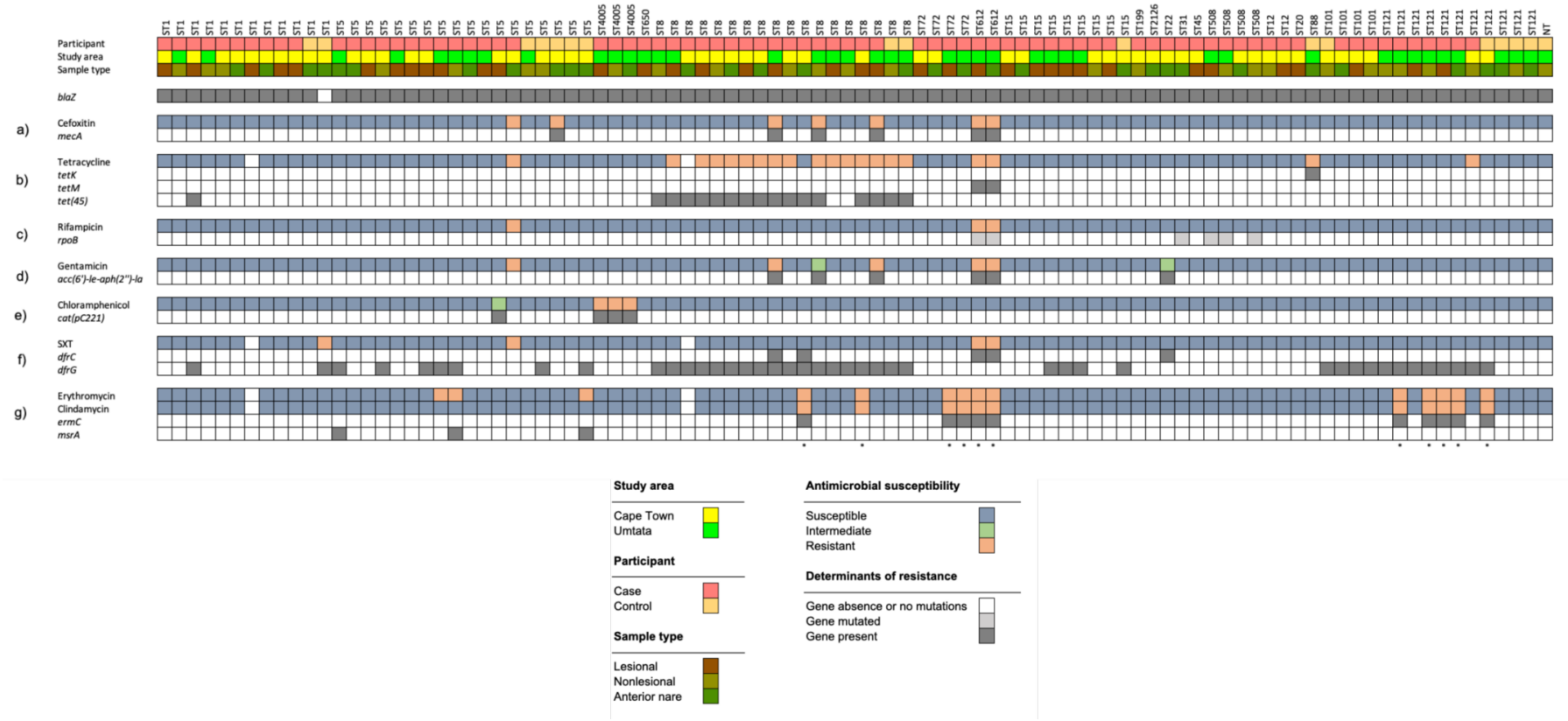


Figure S3.3. Resistome and phenotypic antibiotic susceptibility of *S. aureus* isolates based on *in silico* predicted sequence types. Asterix (*) under g) indicates isolates that exhibited phenotypic erythromycin-inducible clindamycin resistance based on the D-zone test. AST was not performed for the isolates with white boxes for tetracycline, rifampicin, gentamicin, chloramphenicol, erythromycin, and clindamycin due to lack of viability.

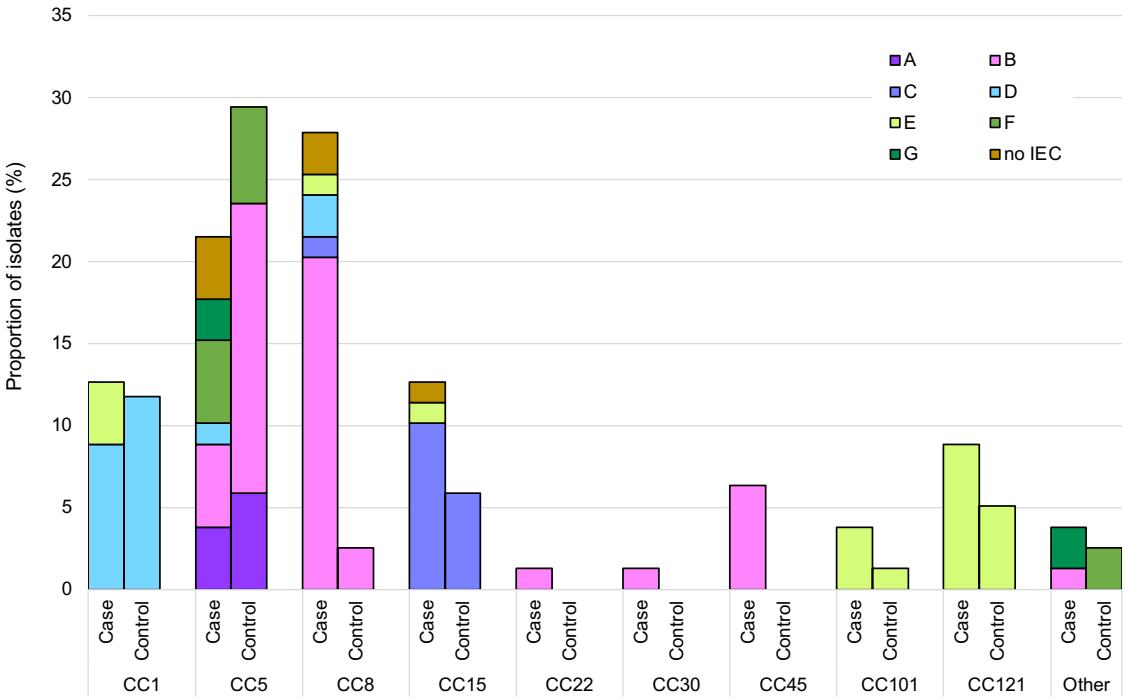


Figure S3.4. The distribution of immune evasion cluster types by clonal complex between cases and controls.

CHAPTER 4

Skin and nasal colonisation of coagulase-negative staphylococci are associated with atopic dermatitis among South African children

4.1. Abstract

Background: Skin colonisation with coagulase-negative staphylococci (CoNS) is generally beneficial, but recent investigations suggest its association with flares and atopic dermatitis (AD) severity. However, this relationship remains unclear. Therefore, we sought to assess patterns of staphylococcal colonisation and biofilm formation in children, with and without AD, from rural and urban South African settings.

Methods: We conducted a cross-sectional study of AD-affected and non-atopic AmaXhosa children aged 9-38 months from rural Umtata and urban Cape Town, South Africa. CoNS isolates were recovered from lesional and nonlesional skin samples, and the anterior nares of participants. Identification of staphylococci was achieved by MALDI-TOF mass spectrometry. The microtiter plate assay assessed *in-vitro* biofilm formation.

Results: CoNS and *S. aureus* commonly co-colonised nonlesional skin among cases (urban: 24% vs. 3%, $p = 0.037$ and rural 21% vs. 6%, $p < 0.001$), and anterior nares in urban cases (24% vs. 0%, $p = 0.002$). *S. capitis* colonisation on nonlesional skin and anterior nares was positively associated with more severe disease in rural (48.3±10.8 vs. 39.7±11.5, $p = 0.045$) and urban (74.9±10.3 vs. 38.4±13, $p = 0.004$) cases, respectively. Biofilm formation was similar between cases and controls, independent of rural-urban living.

Conclusion: CoNS colonisation is associated with AD and disease severity and may be implicated in AD exacerbations. Studies are needed to understand their underlying pathological contribution in AD pathogenesis.

4.2. Introduction

The human skin and nose are populated by a diverse community of bacteria, viruses, fungi and mites[39]. The genus *Staphylococcus* represents the largest bacterial group on healthy skin and nose (in particular the anterior nares)[49,276]. The coagulase-negative staphylococci (CoNS), comprising *Staphylococcus epidermidis*, *Staphylococcus hominis* and *Staphylococcus haemolyticus*, represents the largest bacterial group on the skin and anterior nares[132,277]. This group performs numerous important functions. In the skin, CoNS interact with host epidermal and immune cells to maintain skin homeostasis and protect against opportunistic infections[278]. They competitively prevent *Staphylococcus aureus* colonisation on healthy skin and anterior nares by producing various bacteriocins and antimicrobial peptides (AMPs)[278]. Moreover, CoNS may antagonise *S. aureus* colonisation through glycerol fermentation[131,279] and synergising with host AMPs[278]. CoNS can also inhibit the expression of *S. aureus* virulence factors, thereby limiting its pathological colonisation[278].

Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by red, intensely itchy, and dry, inflamed skin lesions, often with an altered skin microbial community compared to healthy individuals[280]. *S. aureus* is the primary bacterial pathogen associated with AD disease flares[56]. Further, studies have shown that the frequency and relative abundance of CoNS having anti-*S. aureus* activity is reduced in AD due to the over-proliferation of *S. aureus*[129]. Sometimes, the proliferation of CoNS during AD flares coincides with *S. aureus* overgrowth[49]. In such instances, CoNS are proposed to compensate for the increase in *S. aureus* colonisation or synergise with *S. aureus*, leading to augmented *S. aureus* growth and pathological potential[49,137,138]. However, there are conflicting findings on the shifts in CoNS colonisation and prevalence during AD flares compared to healthy skin, and on their correlation with AD severity in both children and adults[57,58,150,281]. Although CoNS have a much lower pathological potential than *S. aureus*, antigens of *S. haemolyticus* and *S. epidermidis* can induce dysfunctional immune responses that contribute to chronic skin damage in AD[149,282]. Also, some *S. epidermidis* strains contribute to the pathogenesis of AD through the production of the cysteine protease EcpA, which promotes epidermal damage and inflammation[149]. Further, a recent study provided evidence of an increased abundance of *S. epidermidis* and *S. hominis* in Netherton Syndrome and ichthyosis vulgaris[58],

highlighting the potential pathological role of CoNS in skin diseases. In this regard, research on AD is rapidly shifting towards understanding how CoNS may contribute to its pathogenesis.

The disrupted skin barrier in AD provides a unique environment that enhances staphylococcal binding through the exposure of the epidermal extracellular matrix components[18,25]. This scenario consequently triggers the formation of biofilms, a key determinant for the chronicity of *S. aureus* and CoNS colonisation on AD skin[25]. Also, the putative pro-inflammatory environment in AD promotes staphylococcal biofilm growth[43]. *S. aureus* and *S. epidermidis* biofilms are generally present on AD skin and are associated with disease severity[43,98,283]. *In-vitro* studies have shown that *S. epidermidis* may antagonise[127] or cooperate[90,284] with *S. aureus* in biofilm formation. These studies on the interactions of CoNS in mixed biofilms with *S. aureus* have primarily included healthy individuals[127,137,165] and are limited in patients with AD[90]. Moreover, no study has compared these interactions between AD patients and healthy individuals to assess whether they differ based on AD disease status.

This chapter explores the skin and nasal staphylococcal colonisation patterns in AD cases and healthy controls across urban and rural environments. It also evaluates biofilm formation of staphylococcal species, including *S. aureus* and CoNS interactions in mixed-species biofilms.

4.3. Materials and Methods

4.3.1. Study population

Between February 2015 and May 2016, 220 AmaXhosa (same ethnolinguistic background) children with (cases) and without (controls) AD aged 9-38 months (mean, 22.5 months; standard deviation, 7.3 months) were recruited from Umtata, South Africa and Cape Town, South Africa. Children, with a diagnosis of AD based on the United Kingdom Working Party diagnosis of atopic eczema[172], were recruited through the Red Cross Children's War Memorial Hospital (Cape Town, South Africa) and Nelson Mandela Academic Hospital (Umtata, South Africa). AD severity was measured at the time of specimen collection, using the objective SCORing of Atopic Dermatitis (oSCORAD) index[173]. Children of a similar age but without a clinical diagnosis of AD, were

recruited from community early development centres in Cape Town and Umtata. Swabs were collected from the anterior nares and nonlesional skin (area with most normal-appearing skin, usually the back) of both cases and controls, as previously described[223]. Lesional skin (most active area of eczematous skin with acute and or chronic changes) swabs, from cases only, were also collected. Collected swabs were placed in STGG and stored at -80 °C for subsequent batch processing.

4.3.2. Bacterial isolation and species identification

To recover the CoNS isolates, 20 µL of each specimen was inoculated on mannitol salt agar (MSA) (National Health Laboratory Services [NHLS], South Africa), streaked for single colony growth and incubated at 37 °C in ambient air for 48 hours. All morphologically distinct colonies were selected for further analysis. Species identification was performed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. All identified CoNS isolates were stored at -20 °C in skim milk-tryptone-glucose-glycerine (STGG) medium (NHLS, South Africa) for further batch processing. *S. aureus* isolates included in the analyses within this study were recovered previously[223].

4.3.3. Assessment of *in vitro* mono-species and dual-species biofilm formation

Biofilm formation was assessed *in-vitro* using the crystal violet microtiter assay[225]. Staphylococcal isolates in STGG were streaked on 2% blood agar and incubated overnight at 37 °C in ambient air. A single colony was inoculated into 3 mL 3% tryptic soy broth supplemented with 0.1% glucose (gTSB; Merck, South Africa) and incubated with shaking for 24 hours. Thereafter, the broth culture was diluted at 1:100 (in total volume of 200 µL) for mono-species biofilms and 1:1 (100 µL:100 µL) each diluted at 1:100 for dual staphylococcal biofilms, in a flat-bottomed 96-well microtiter plate (Lasec, South Africa) as previously described[90]. Plates were then statically incubated in ambient air at 37 °C for 24 hours. *S. aureus* ATCC 29213 and uninoculated gTSB were included as positive and negative controls, respectively. Planktonic bacterial suspensions were discarded, wells were rinsed three times in sterile reverse osmosis (RO)-filtered water and dried at 60 °C for 1 hour. The formed biofilms were stained with 200 µL 0.1% crystal violet and

incubated at room temperature for 10 minutes. Excess crystal violet was discarded, the stained biofilms were solubilised with 200 µL 30% acetic acid and thereafter incubated at room temperature for 30 minutes. The absorbance was measured at 492 nm using the Mindray MR 96A ELISA Microplate Reader (Vacutec, South Africa). Biofilm biomass was analysed using OD readings as previously described[225]: (OD>OD negative control [OD_c], non-producer; OD_c<OD-<2·OD_c, weak producer; 2·OD_c<OD<4·OD_c, moderate producer; and OD>4·OD_c, strong producer).

4.3.4. Statistical analysis

We used R studio version 4.0.4 to conduct statistical analyses. Species with less than ten isolates were arbitrarily considered rare in this cohort and classified as "rare CoNS." The Fisher's exact test was performed for comparisons between categorical variables. Differences in continuous dependent variables, by a categorical variable, were assessed using either the Wilcoxon rank-sum test or the Kruskal-Wallis test. We used the Bonferroni method to adjust for multiple comparisons. All analyses were two-tailed. The log2 fold change method was used to compare the fold change of the biofilm biomass from dual CoNS-*S. aureus* biofilms compared to mono-species *S. aureus* biofilms. The effect on biofilm biomass was arbitrarily classified based on a log2 fold change as follows: "no effect" (> -0.1 to <0.1); "weak, positive" (0.1 to <0.4); "moderate, positive" (0.4 to <0.9); "strong, positive" (>0.9); "weak, negative" (-0.1 to < -0.4); "moderate, negative" (-0.4 to < -0.9). Correlation between continuous variables was assessed using Pearson's correlation. A *p*-value of <0.05 was considered statistically significant.

4.4. Results

4.4.1. Staphylococcal colonisation patterns and associated risk factors

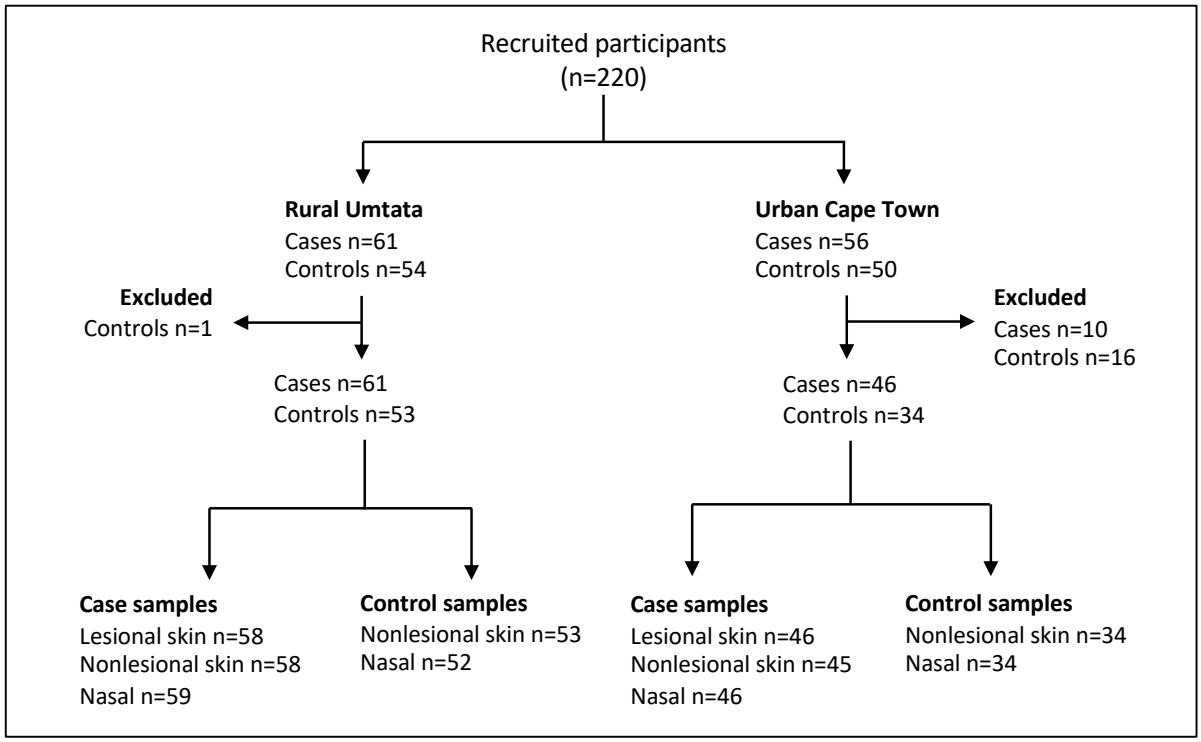


Figure 4.1. Flow chart of study participant specimens included in the analyses. Twenty-seven participants were excluded from analysis due to the unavailability of samples.

Table 4.1. Participant characteristics.

	AD cases	non-AD controls
No. of participants	107	87
Male, %	54 (58/104) [a]	59 (51/87)
Rural, %	57 (61/107)	61 (53/87)
Age, mean (range)	22.39 (9–38)	22.61 (12–36)
Objective SCORAD, mean (range)	41.51 (21.4–82.2)	—
No. of staphylococcal isolates %	78 (296/381)	22 (85/381)

[a] Data of participant sex is not available for all analysed participants

A total of 194 (87 controls and 107 cases) children were assessed for *S. aureus* colonisation. Twenty-seven participants were excluded (missing specimen) from the study analysis (Figure 4.1). We recovered 381 staphylococcal isolates from lesional skin (n=112), nonlesional skin (cases: n=117; controls: n=60) and anterior nares (cases: n=67; controls: n=25), from cases and controls (Figure 4.1). These isolates represented 16 different staphylococcal species including *S. aureus* (n=129), *S. epidermidis* (n=104), *S. hominis* (n=53), *S. haemolyticus* (n=38), *S. capitis* (n=28), *S. saprophyticus* (n=10), *S. warneri* (n=5), *S. cohnii* (n=4), *S. equorum* (n=2), *S. pasteuri* (n=2), *S. caprae* (n=1), *S. lentus* (n=1), *S. lugdunensis* (n=1), *S. nepalensis* (n=1), *S. sciuri* (n=1), and *S. succinus* (n=1). Antibiotic exposure (adjusted odds ratios, aOR [95% confidence interval, 5.25 [1.68;16.38]) and having AD (4.67 [1.58;13.77]) are risk factors for CoNS colonisation among urban children (Table S4.1).

The distribution of staphylococcal colonisation in cases and controls by geographic location is shown in Figure 4.2. Colonisation with *S. aureus* or CoNS only, or both was common on lesional skin in both rural and urban cohorts. In the urban cohort, controls were more frequently not colonised with any *Staphylococcus* species than cases (47% [16/34] vs. 20% [9/45], $p = 0.015$). Co-colonisation with CoNS species and *S. aureus* on nonlesional skin was more prevalent in cases than controls, from both rural (21% [21/58] vs. 6% [3/53], $p < 0.001$) and urban (24% [9/45] vs. 3% [1/34], $p = 0.037$) settings. Similarly, the colonisation rates for either *S. aureus* or CoNS only, were higher on the nonlesional skin of cases than controls, independent of geographic location (Figure 4.2). There was no co-colonisation with CoNS species and *S. aureus* in the anterior nares of urban controls (Figure 4.2).

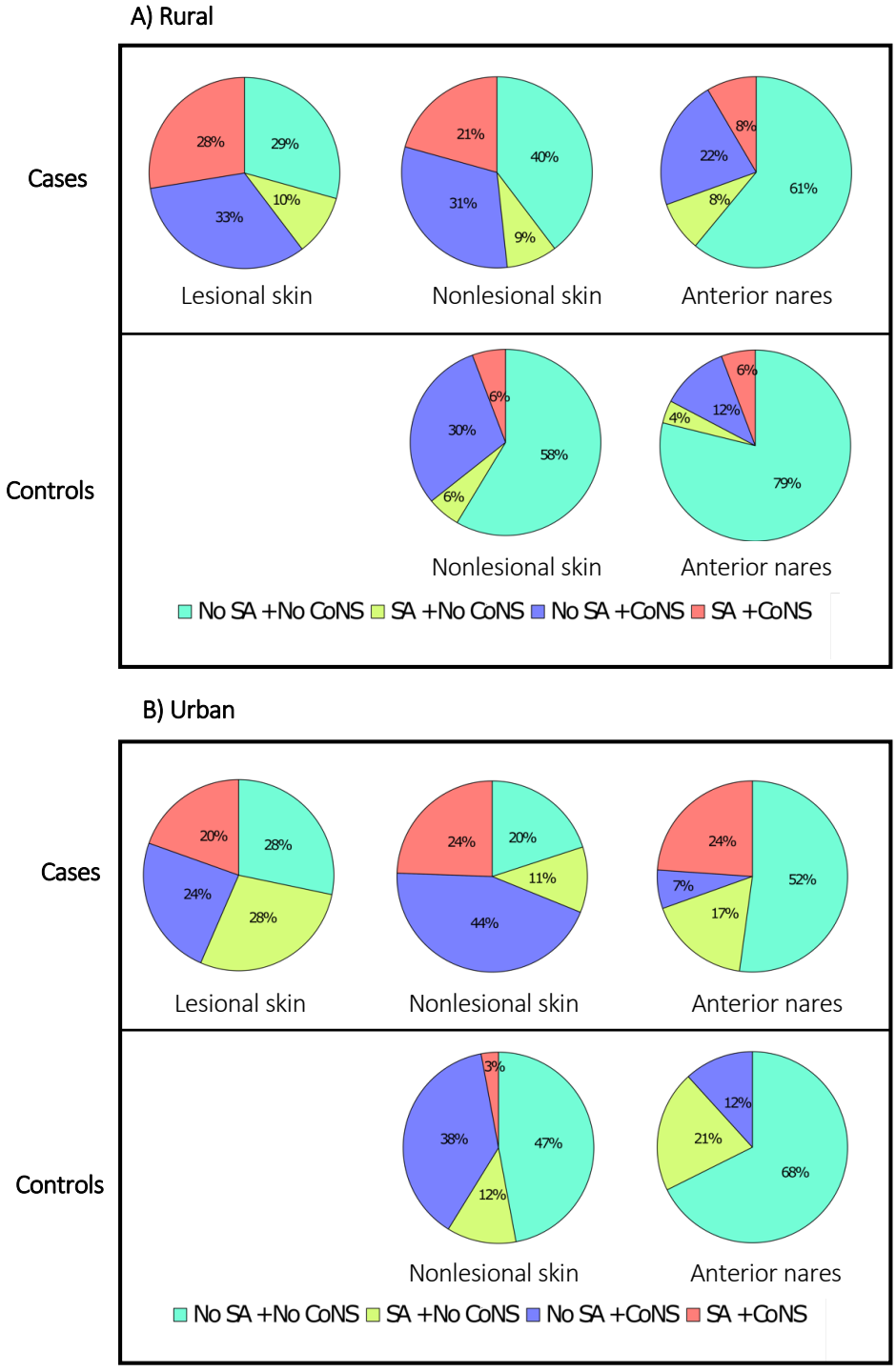


Figure 4.2. The distribution of *S. aureus* and CoNS colonisation on lesional and nonlesional skin, and anterior nares of (A) rural and (B) urban cases and controls. CoNS colonisation is defined as

colonisation with at least one CoNS species. SA, *S. aureus*; CoNS, coagulase-negative staphylococci.

S. haemolyticus was isolated from the nonlesional skin of rural cases only, and not from the control group (10% [6/58) vs. 0% [0/58], $p = 0.029$). Moreover, the prevalence of *S. haemolyticus* was similar among lesional cases in the rural (10%) and urban (11%) cohorts (Tables 4.2 and 4.3). *S. epidermidis* was also significantly more frequent in the anterior nares of rural cases compared to controls (27% [16/59] vs. 10% [5/52], $p = 0.029$) (Table 4.2). The rare staphylococcal group was found in the nonlesional skin of rural controls but not cases (11% [6/53] vs. 0% [0/58], $p = 0.009$) (Table 4.2). Overall, colonisation with most recovered CoNS species on AD lesional skin did not significantly differ from nonlesional skin and anterior nares in both geographic locations. However, a few exceptions were observed. *S. capitis* was more frequently reported in nonlesional skin (20% [9/45] vs. 3% [1/34], $p = 0.038$) of cases compared to controls in the urban cohort (Table 4.3). When CoNS species were compared between sample types, a higher colonisation prevalence of *S. hominis* was observed in nonlesional skin compared to lesional skin ($p = 0.016$) and anterior nares ($p = 0.0004$) of urban cases. This trend was also observed with *S. capitis* nonlesional skin colonisation compared to anterior nares ($p = 0.027$) but not with AD lesional skin ($p = 0.069$) among urban cases.

Table 4.2. CoNS species identified on the skin and anterior nares of cases and controls from Umthatha (rural).

Species	**Lesional skin**	**Nonlesional skin**			**Anterior nares**		
	Case, *n* (%) N=58	**Case, *n* (%) N=58**	**Control, *n* (%) N=53**	**p-value**	**Case, *n* (%) N=59**	**Control, *n* (%) N=52**	***p*-value**
S. epidermidis	24 (41.4)	17 (29.3)	8 (15.1)	0.116	16 (27.1)	5 (9.6)	**0.029**
S. capitis	3 (5.2)	7 (12.1)	1 (1.9)	0.066	2 (3.4)	0 (0)	0.498
S. haemolyticus	6 (10.3)	6 (10.3)	0 (0)	**0.029**	2 (3.5)	0 (0)	0.498
S. hominis	6 (10.3)	8 (13.8)	10 (18.9)	0.448	1 (1.7)	2 (3.8)	0.597
S. saprophyticus	2 (3.4)	0 (0)	1 (1.9)	0.465	0 (0)	1 (1.9)	0.465
Rare CoNS[a]	4 (6.9)	0 (0)	6 (11.3)	**0.009**	2 (3.4)	1 (1.9)	1.000

[a]Rare CoNS include *S. caprae*, *S. cohnii*, *S. equorum, S. lentus*, *S. lugdunensis, S. nepalensis, S. pasteuri, S. sciuri, S. succinus* and *S. warneri*. Underlined bold text indicates statistical significance.

Table 4.3. CoNS species identified on the skin and anterior nares of cases and controls from Cape Town (urban).

Species	**Lesional skin**	**Nonlesional skin**			**Anterior nares**		
	Case, *n* (%) N=46	**Case, *n* (%) N=45**	**Control, *n* (%) N=34**	**p-value**	**Case, *n* (%) N=46**	**Control, *n* (%) N=34**	***p*-value**
S. epidermidis	10 (21.7)	10 (22.2)	3 (8.8)	0.140	8 (17.4)	3 (8.8)	0.338
S. capitis	3 (6.5)	9 (20)	1 (2.9)	**0.038**	2 (4.3)	0 (0)	0.505
S. haemolyticus	5 (10.9)	9 (20)	8 (23.5)	0.784	2 (4.3)	0 (0)	0.505
S. hominis	4 (8.7)	13 (28.9)	7 (20.6)	0.602	1 (2.2)	1 (2.9)	1.000
S. saprophyticus	0 (0)	4 (8.9)	1 (2.9)	0.388	1 (2.2)	0 (0)	1.000
Rare CoNS[a]	1 (2.2)	1 (2.2)	3 (8.8)	0.307	1 (2.2)	0 (0)	1.000

[a]Rare CoNS include *S. caprae*, *S. cohnii*, *S. equorum, S. lentus*, *S. lugdunensis, S. nepalensis, S. pasteuri, S. sciuri, S. succinus* and *S. warneri*. Underlined bold text indicates statistical significance.

4.4.2. Colonisation with CoNS is associated with AD severity

AD severity, as determined by oSCORAD scores, was highest amongst rural cases co-colonised by *S. aureus* and CoNS on lesional skin (median [interquartile range, IQR], 54.5 [49.5;71.5]). This was compared to those without staphylococcal colonisation (40.5 [18;54.5], $p = 0.0083$), and those colonised by CoNS only (35 [23;65], $p = 0.047$) (Figure 4.3, *upper panel*). A similar pattern of higher severity scores was observed among rural cases co-colonised with *S. aureus* and CoNS on nonlesional skin compared to those without colonisation. However, it did not reach statistical significance (Figure 4.3, *middle panel*). In urban cases, lower severity scores were associated with the absence of staphylococcal colonisation on nonlesional skin (28 [9;41]) compared to cases colonised with *S. aureus* only (56 [51;75], $p = 0.014$), and to those co-colonised with *S. aureus* and CoNS (39.5 [25;63], $p = 0.035$) (Figure 4.3, *middle panel*). Higher AD severity scores were also associated with co-colonisation of *S. aureus* and CoNS in the anterior nares of cases from urban (58 [4;79] vs. 31 [13.5;57.5], $p = 0.018$) and rural (76.5 [68;81.5] vs. 44 [23;62], $p = 0.0059$) settings when compared to cases without staphylococcal colonisation (Figure 4.3, *lower panel*). Moreover, urban cases showed lower AD severity scores when colonised with CoNS only (31 [13.5;57.5]) compared to no colonisation at all (9 [6;22], $p = 0.031$) in their anterior nares (Figure 4.3, *lower panel*). When considering individual CoNS species, a significant positive association between higher oSCORAD scores and *S. capitis* colonisation was observed for the nonlesional skin of rural cases (48.3±10.8 vs. 39.7±11.5, $p = 0.045$, Table 4.4). This was also noted with the anterior nares of urban cases compared with those not colonised (74.9±10.3 vs. 38.4±13, $p = 0.004$, Table 4.5).

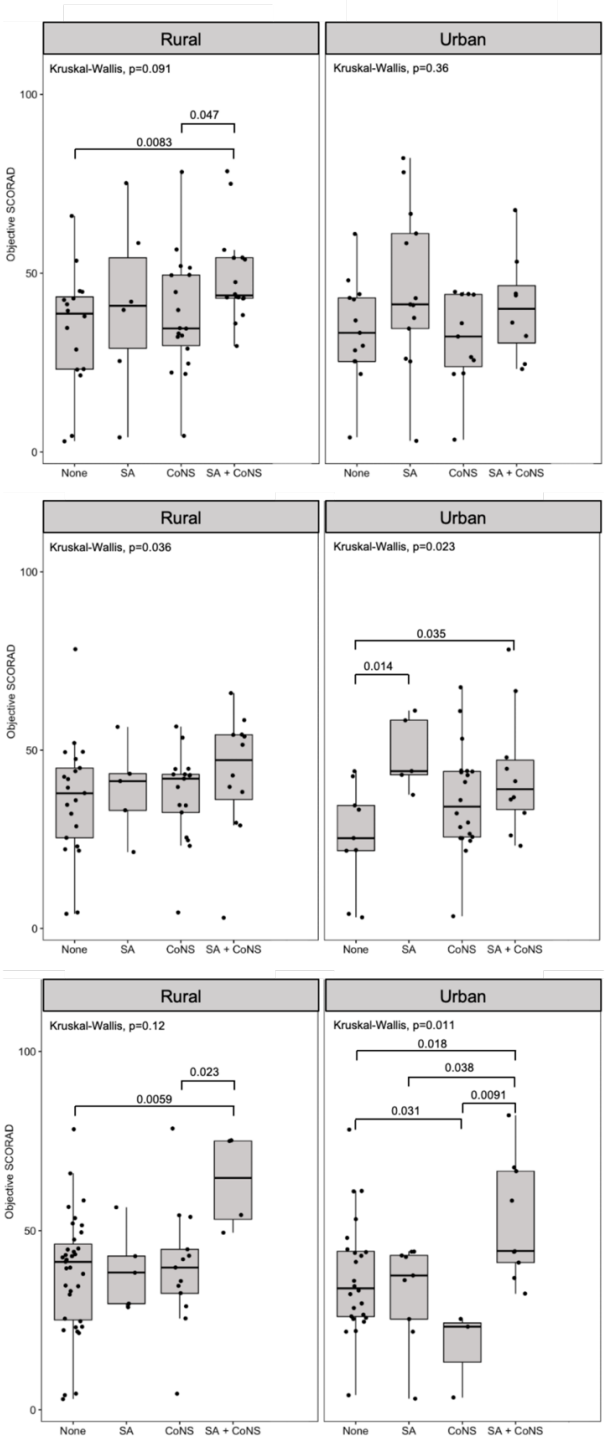


Figure 4.3. Relationship between objective SCORAD and staphylococcal colonisation on lesional skin (upper panels), nonlesional skin (middle panels), and anterior nares (lower panels). None, no staphylococcal colonisation; SA, *Staphylococcus aureus*; CoNS, coagulase-negative *Staphylococcus*; SA + CoNS, simultaneous colonisation with *S. aureus* and CoNS.

Table 4.4. Mean values of objective SCORAD based on the presence or absence of CoNS in rural cases.

Species	Lesional skin			Nonlesional skin			Anterior nares		
	Sample + $\mu \pm$ SD	Sample – $\mu \pm$ SD	p-value	Sample + $\mu \pm$ SD	Sample – $\mu \pm$ SD	p-value	Sample + $\mu \pm$ SD	Sample – $\mu \pm$ SD	p-value
S. epidermidis	45.2 ± 13.7	41.3 ± 14.1	0.209	39.9 ± 11.6	41.2 ± 11.9	0.931	47.1 ± 16.5	40.9 ± 12.7	0.283
S. capitis	48.8 ± 22.8	42.6 ± 13.5	0.967	48.3 ± 10.8	39.7 ± 11.5	**0.045**	64.4 ± 15	41.8 ± 13.8	0.061
S. haemolyticus	52.4 ± 16	41.8 ± 13.4	0.060	44 ± 10.6	40.4 ± 11.6	0.497	52 ± 32.7	42.3 ± 13.4	0.781
S. hominis	37.9 ± 9.2	43.6 ± 14.4	0.367	37.5 ± 6.4	41.3 ± 12.3	0.409	NA	42.5 ± 14.1	–
S. saprophyticus	40.8 ± 3.5	42.7 ± 14.1	0.918	NA	40.8 ± 13.7	–	NA	42.6 ± 13.9	–
Rare CoNS[a]	41.4 ± 16	43.1 ± 14	0.967	NA	40.8	–	NA	42.4 ± 14	–

NA represents observations that are too few for mean and standard deviation calculation. Sample +, CoNS present; Sample -, CoNS absent; μ, mean; SD, standard deviation. Underlined bold text indicates statistical significance. *P*-values were determined using the Wilcoxon rank-sum test.
[a]Rare CoNS include *S. caprae*, *S. cohnii*, *S. equorum, S. lentus*, *S. lugdunensis, S. nepalensis, S. pasteuri, S. sciuri, S. succinus* and *S. warneri*.

Table 4.5. Mean values of objective SCORAD based on the presence or absence of CoNS in urban cases.

Species	Lesional skin			Nonlesional skin			Anterior nares		
	Sample + $\mu \pm$ SD	Sample – $\mu \pm$ SD	p-value	Sample + $\mu \pm$ SD	Sample – $\mu \pm$ SD	p-value	Sample + $\mu \pm$ SD	Sample – $\mu \pm$ SD	p-value
S. epidermidis	37.5 ± 9	40.8 ± 16.2	0.951	37.7 ± 17.7	39.4 ± 12.6	0.318	40.1 ± 12.2	40 ± 15.7	0.678
S. capitis	46.1 ± 20.6	38.6 ± 13.1	0.366	40.7 ± 20.2	39.9 ± 13.6	0.818	74.9 ± 10.3	38.4 ± 13	**0.004**
S. haemolyticus	32.6 ± 9.2	39.7 ± 13.8	0.327	41.2 ± 17.9	39.8 ± 14.3	0.94	49.5 ± 24.2	39.6 ± 14.6	0.515
S. hominis	30.5 ± 10.6	39.9 ± 13.6	0.195	37.1 ± 11.6	41.3 ± 16	0.573	NA	40 ± 15	–
S. saprophyticus	NA	40 ± 14.9	–	34.8 ± 13.1	40.6 ± 15.1	0.554	NA	40.1 ± 15	–
Rare CoNS[a]	NA	39.3 ± 13.7	–	NA	40 ± 15	–	NA	40.3 ± 15	–

NA represents observations that are too few for mean and standard deviation calculation. Sample +, CoNS present; Sample -, CoNS absent; μ, mean; SD, standard deviation. Underlined bold text indicates statistical significance. *P-values* were determined using the Wilcoxon rank-sum test.
[a]Rare CoNS include *S. caprae*, *S. cohnii*, *S. equorum, S. lentus*, *S. lugdunensis, S. nepalensis, S. pasteuri, S. sciuri, S. succinus* and *S. warneri*.

4.4.3. Biofilm formation of staphylococcal species in monocultures and mixed cultures

We observed an overall modest-to-high prevalence of strong biofilm-producing-staphylococci (43%–72%), regardless of disease status, sample type or geographic location (Figure 4.4). When specific species were considered, *S. aureus* and *S. hominis* isolates mostly formed strong biofilms, whereas *S. epidermidis*, *S. capitis* and *S. saprophyticus* exhibited a high distribution of both strong and moderate biofilm producers (Figures S4.1–S4.3, S4.5, and S4.6). This phenomenon was independent of disease status and geographic location. In contrast, *S. haemolyticus* and the rare *Staphylococcus* group showed no clear distribution of biofilm phenotype (Figures S4.4 and S4.7). Staphylococcal isolates that are do not form biofilms were identified only in the rural group. We further assessed the change in the biofilm biomass in mixed species biofilm cultures from *S. aureus* only biofilm cultures to determine the effect of co-colonising CoNS of *S. aureus* biofilm biomass *in vitro* (Figure 4.5). Due to the small number of pairs, we did not stratify the analysis by geographic location. In both cases and controls, a positive fold change, i.e., increased in biofilm biomass in mixed biofilm cultures although at varying degrees, was common. However, there was no significant change in the biofilm biomass of mixed biofilms from mono-species *S. aureus* biofilms between cases and controls. Also, there was no significant association between the fold change in biofilm biomass in mixed biofilms and disease severity (Figure 4.6).

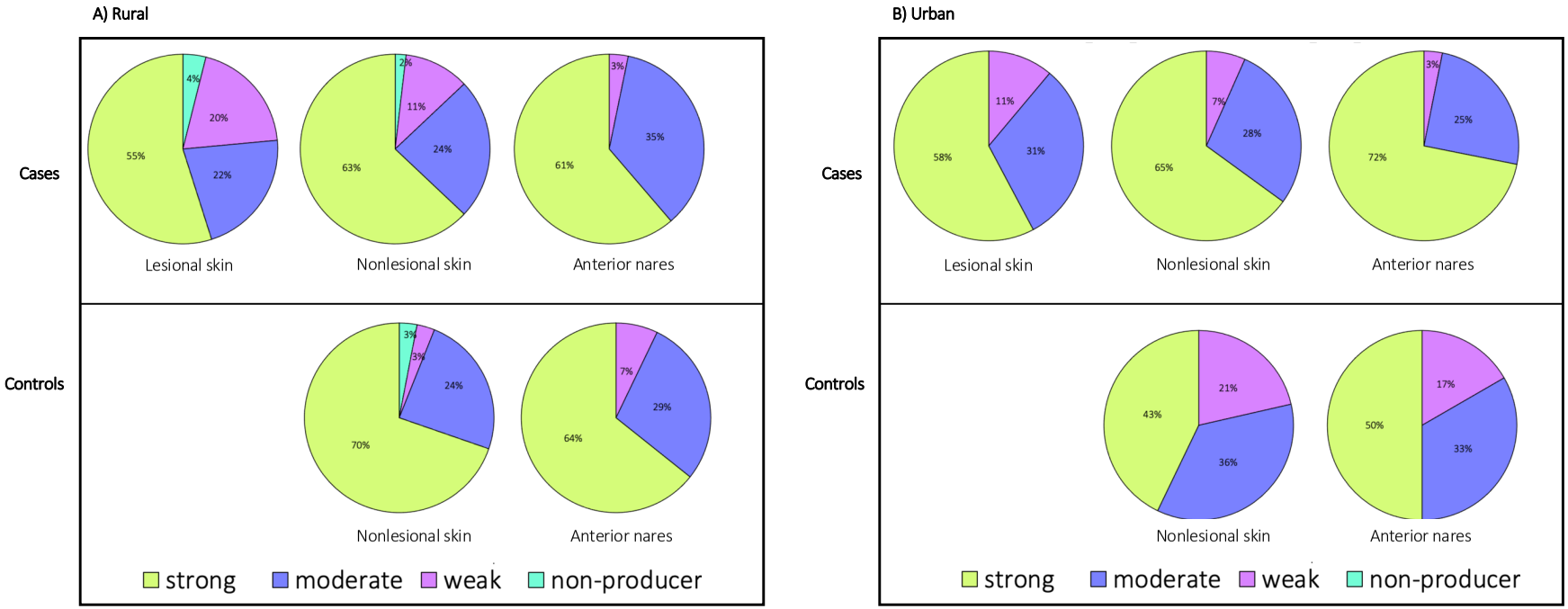


Figure 4.4. Prevalence of staphylococcal biofilm propensity in mono-species biofilms in (A) rural and (B) urban cases and controls.

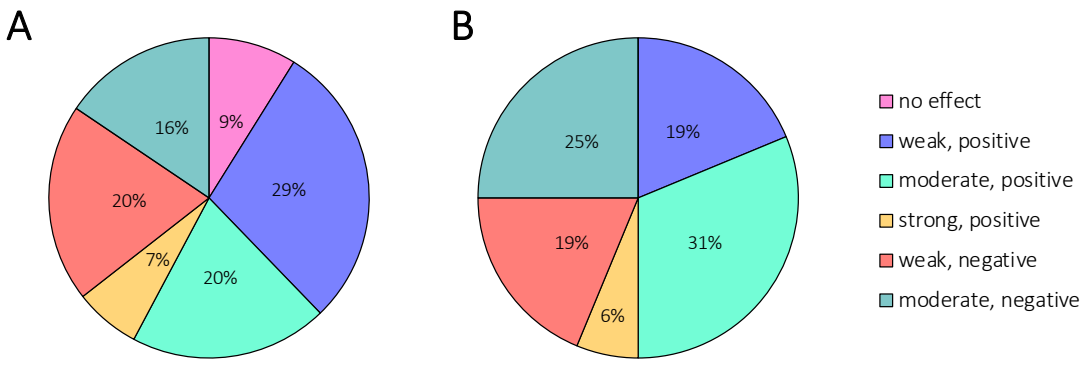


Figure 4.5. The effect of co-colonising CoNS on *S. aureus* biofilm biomass in co-culture biofilms in (A) cases and (B) controls. The effect was calculated based on a $\log_2$ fold change from *S. aureus* mono-species biofilm biomass. Effect on biofilm biomass was arbitrarily classified based on $\log_2$ fold change as follows: "no effect" (> -0.1 to <0.1); "weak, positive" (0.1 to <0.4); "moderate, positive" (0.4 to <0.9); "strong, positive" (>0.9); "weak, negative" (-0.1 to < -0.4); "moderate, negative" (-0.4 to < -0.9). A positive change resulted in increased biofilm biomass and a negative change resulted in a decreased biofilm biomass in the mixed species biofilm culture compared to the mono-biofilm culture of the analogous *S. aureus*.

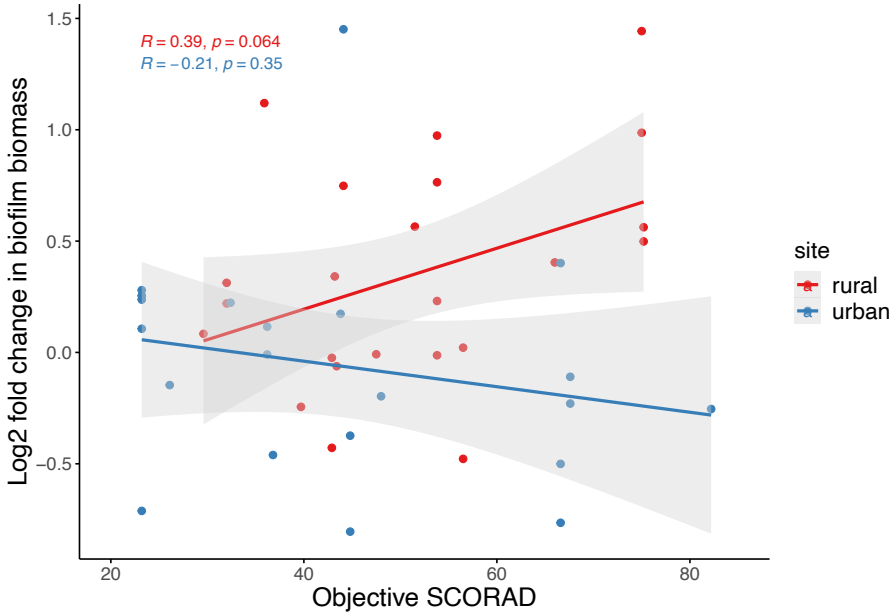


Figure 4.6. Pearson's correlation between objective SCORAD and the log fold change of biofilm biomass from mono-species *S. aureus* biofilms to co-biofilm cultures of CoNS and *S. aureus* in rural and urban cases.

4.5. Discussion

In this chapter, we report on the colonisation and biofilm formation of *Staphylococcus* species in rural and urban South African children with and without AD. We show that children with AD are more frequently colonised with CoNS than those without AD, especially on the nonlesional skin. We also show a relationship between increased disease severity and being co-colonised with *S. aureus* and CoNS, although this mostly dependent on geographic location. Moreover, staphylococcal biofilm formation was similar between cases and controls indicating that biofilm formation possibly does not have a significant contribution in the harmful colonisation of staphylococci in AD pathology.

We found a higher prevalence of *S. capitis* and *S. haemolyticus* on the nonlesional skin of urban and rural cases, respectively, compared with the nonlesional skin of controls. The prevalence of *S. capitis* on the nonlesional skin of patients with AD compared with those without AD has been demonstrated[150]. *S. hominis* was recently shown to reduce *S. aureus* colonisation and improve disease symptoms in patients with AD in a recent randomised phase I clinical trial, highlighting the importance of this bacterium in skin health[68]. In contrast, the lesional skin of patients with AD, especially those with severe disease, tend to have a reduced prevalence and abundance of *S. hominis* compared with the nonlesional skin of both AD patients and those without AD[72,148,150]. However, these data are inconsistent with an increase in prevalence noted elsewhere[146] and other studies reporting no change in *S. hominis* prevalence in patients with AD[151]. Presently, we found that fewer urban were colonised with *S. hominis* on the lesional skin compared with their nonlesional skin as reported previously[148]. This indicates that the changes in the skin in patients with AD discourages the growth of *S. hominis*, hence limiting its protective effects. Moreover, the difference in the prevalence of *S. hominis* between the rural and urban groups suggest a potential influence of the environment in colonisation dynamics of this bacterium. Urban and rural environments distinctly influence the composition and diversity of the skin microbial community, especially in early childhood[285], with their unique environmental exposures and how the people interact with their immediate enviornments[286]. Rural residents tend to have more diverse skin microbial communities than those in urban communities[161]. From this scenario we posit that rural living supports the acquisition of a more diverse skin bacteriome[154], encouraging the survival of "protective CoNS", despite AD disease. In contrast, these "protective CoNS" are limited in individuals living in urban areas, and their absence is particularly experienced in AD cases where *S. aureus* growth is uninhibited[287].

Chronic epidermal inflammation in patients with AD has been shown to influence staphylococcal colonisation, with the dominance of the T helper type 2 and T helper type 17 driven inflammation augmenting the growth of *S. aureus*, especially in severe disease[152]. CoNS mostly promote anti-inflammatory responses on the skin[288] and epidermal inflammation in AD inhibits their growth[43]. Also, the prevailing immunological profiles in AD vary across rural and urban groups; with those living in rural communities frequently associated with microinflammation compared with those in urban communities[150,289,290]. This possibly explains the differences in CoNS colonisation between rural and urban groups in this study. Collectively, these findings indicate that while some CoNS species are beneficial on healthy skin, they can adapt and proliferate in AD lesions, consequently contributing to disease severity. In contrast, other CoNS, such as *S. hominis*, are inhibited. The mechanisms by which this adaption, or lack thereof, occurs remains unknown and warrants further study.

Adult patients with AD co-colonised with *S. aureus* and CoNS on the skin and anterior nares have lower IgE levels than those colonised with only *S. aureus*[78]. Lower IgE levels tend to correlate with less severe disease[291,292]. Therefore, this suggests that patients with AD who are co-colonised with *S. aureus* and CoNS are more likely to have less severe disease. However, this might depend on the co-colonising CoNS species and/or strains as a positive correlation between increased disease severity and co-colonisation with CoNS and *S. aureus* has also been reported[148]. Presently, children with AD that were co-colonised with *S. aureus* and CoNS on the lesional skin, the nonlesional skin, or the anterior nares had higher disease severity scores. However, this observation depended on geographic location. It has been posited that the concurrent increase in *S. aureus* and CoNS colonisation reflects a compensatory effect of CoNS in response to *S. aureus* proliferation in AD, particularly in severe AD[49]. In contrast, CoNS may cooperate with *S. aureus*, promoting its deleterious effects in AD[136,138]. Nonetheless, our analysis is limited to correlating the co-detection of *S. aureus* and CoNS with disease severity. Therefore, future studies are needed to assess the clinical relevance of this co-colonisation, and if and how it contributes to AD pathology and disease severity.

Data on the relationship between the colonisation of specific CoNS species on AD skin and disease severity remains inconsistent[147,148,152]. We found that rural and urban cases who were colonised with *S. capitis* on the nonlesional skin and the anterior nares, respectively, had higher disease severity scores than those who were not colonised. This observation is similar a

previous report by Edslev et. al.[148] who demonstrated a positive relationship between increased disease severity and colonisation with *S. capitis*. However, an absence of correlation has also been demonstrated[152]. Our findings indicate that *S. capitis* may contribute to AD pathology in this cohort, through mechanisms not yet described in the literature. We also found no association between *S. hominis* and severity scores as previously demonstrated[152].However, another study reported that patients with AD colonised with *S. hominis* tended to have less severe disease[148]. It is unclear why there are conflicting associations between the colonisation of CoNS and disease severity. However, these possibly indicate differences at the host level[293] that vary across ethnicities, age groups, and geographic location; therefore distinctly influencing CoNS colonisation in skin diseases[162], and their relationship with AD disease severity. Collectively, these findings indicate that the relationship between CoNS and AD severity depends on the colonising CoNS species and possibly strains.

Epidermal barrier damage in AD promotes staphylococcal adhesion to the skin by exposing the underlying extracellular matrix component to which these bacteria bind[18,294]. Upon binding to the epidermis, staphylococci then form biofilm communities that allow them to persistently colonise the skin[295]. *S. aureus* and *S. epidermidis* strains that produce strong biofilm are commonly found in patients with AD, especially in those with more severe disease[90,286]. Presently, there was a modest-to-high prevalence of strong biofilm-producing staphylococcal isolates in both cases and controls, for all sample types and in both the rural and urban groups. CoNS can either antagonise or synergise with *S. aureus* in mixed biofilm cultures[137,284]. However, the relationship between these interactions and AD pathology is poorly studies and mostly limited to patients with mild disease[90]. Compared to biofilms of *S. aureus* monocultures, we found no overall difference in the fold change in mixed biofilms of co-colonising *S. aureus* and CoNS did not differ with AD disease and disease severity. Collectively, these findings indicate that the ability for staphylococci to form biofilms and the outcome of the interaction between co-colonising CoNS and *S. aureus* in mixed biofilm cultures are likely innate characteristics of CoNS and *S. aureus* and therefore do not influence AD pathology in this cohort. Despite these findings, it is important to note that epidermal changes in AD affect the ability of staphylococci to form biofilms *in vivo*. For example, an increased pH (a common feature of the skin of patients with AD)[26] and the antimicrobial peptide cathelicidin LL-37 found on the skin[84] reduce staphylococcal biofilm formation *in vitro*[296]. Moreover, *S. aureus* and *S. epidermidis* grow differently at high pH in dual-species biofilms *in vitro*[297], thus affecting their cooperation in biofilm growth and consequently, biofilm biomass. In contrast,

staphylococcal biofilm production is encouraged by other host factors, such as chronic inflammation[43]. The dissimilar effect of various features of AD disease on staphylococcal biofilm formation highlight the need for *in vivo* studies using murine[298] or *ex vivo* human skin[299] models, of healthy and AD skin, to understand how these features of disease influence staphylococcal biofilm formation and if and how these contribute to AD pathology.

The strengths of this study comprise the inclusion a unique rural and urban cohort provided insights into the differences between staphylococcal colonisation in children, with and without AD, across communities with distinct environmental exposures. Our analyses included all recoverable CoNS species on the skin and anterior nares. These analyses provided a comprehensive characterisation of the colonisation and biofilm propensity of staphylococci, and how they associate with disease severity in early childhood AD. Nonetheless, we have some limitations. These include using a culture-dependent technique to describe staphylococcal colonisation patterns, which may underestimate the prevalence of colonisation because it relies bacterial viability and colonies to form on solid media for detection. Also, we focused our analyses on the presence/absence of the detected staphylococci which limited evaluation of differences in bacterial abundance (based on colony-forming units). The cross-sectional design do not permit the assessment of the causality between staphylococcal colonisation and biofilm phenotypes with AD severity. Additionally, all cases had used emollients before sampling, which may affect the patterns of staphylococcal colonisation presently observed [300]. This study also has small sample size which was further reduced by the multiple levels of stratification, i.e., by disease status, geographic location, and sample type. This means that some of our significant observations may be chance findings. Therefore, we advocate for future studies which will investigate CoNS colonisation in AD in a larger cohort. Despite these limitations, this chapter provides unique knowledge on skin and nasal staphylococcal colonisation and biofilm formation, and their association with disease severity in early childhood AD, and how these factors vary across rural and urban communities

4.6. Conclusion

In this chapter we show that CoNS, particularly *S. capitis*, are associated with AD severity and may be important in AD pathology through mechanisms not yet described in the literature. Also, the importance of CoNS in AD pathology depends on their interactions with *S. aureus.* Our study highlights that while *S. aureus* remains the most studied *Staphylococcus* species in

the pathogenesis of AD, CoNS may contribute to AD exacerbations. We also show the need for further *in vivo* studies on specific CoNS species or strains, and how they contribute to AD exacerbation. Understanding the dynamics of the pathological role of CoNS will allow their targeting by therapeutic strategies, aimed at countering complications from CoNS-associated infections in AD.

4.7. Supplementary Materials

Table S4.1. Unconditional logistic regression analysis of child, parental, domestic, and environmental characteristics associated with CoNS colonisation in Umtata and Cape Town participants.

Explanatory variable	**Umtata (rural)**				**Cape Town (urban)**			
	OR [95% CI]	***p*-value**	**aOR [95% CI] [a]**	***p*-value**	**OR [95% CI]**	***p*-value**	**aOR [95% CI] [a]**	***p*-value**
AD: case	4.14 [1.83-9.35]	0.001	2.55 [0.92-7.06]	0.071	3.6 [1.36-9.51]	0.010	4.67 [1.58-13.77]	0.005
Sex: male	0.70 [0.31-1.57]	0.385	Removed		0.71 [0.28-1.81]	0.479	Removed	
Child characteristics								
Allergic rhinitis	1.89 [0.34-10.33]	0.463	Removed		Omitted			
Asthma	Omitted				Omitted			
Food allergy	1.00 [0.27-3.63]	0.995	Removed		5.32 [0.64-44.40]	0.123	Removed	
Breastfeeding	0.67 [0.29-1.51]	0.332	Removed		1.64 [0.40-6.62]	0.490	Removed	
Mode of delivery: vaginal	1.71 [0.70-4.18]	0.236	Removed		0.55 [0.21-1.46]	0.231	Removed	
Incomplete immunisation status [d]	1.74 [1.17-17.28]	0.637	Removed		6.05 [0.73-50.00]	0.095	Removed	
Antibiotic exposure	1.21 [0.45-3.26]	0.703	Removed		3.99 [1.44-11.09]	0.008	5.25 [1.68-16.38]	0.004
Large family	0.79 [0.37-1.71]	0.557	Removed		0.92 [0.30-2.82]	0.879	Removed	
Farm animal exposure	0.17 [0.04-0.76]	0.021	0.35 [0.07-1.80]	0.207	Omitted			
Fossil fuel exposure								
Electricity + gas	1.84 [0.84-4.05]	0.130	Removed		Omitted			
Kerosene + paraffin	3.01 [1.36-6.66]	0.007	2.59 [0.84-8.01]	0.099	0.92 [0.34-2.45]	0.870	Removed	
Indoor fire	1.74 [0.17-17.28]	0.637			Omitted			
Outdoor fire	0.40 [0.18-0.87]	0.022	0.76 [0.29-1.95]	0.565	Omitted			
Wood + coal	0.41 [0.18-0.96]	0.040	1.79 [0.49-6.58]	0.380	Omitted			
Maternal factors								

Allergic rhinitis	1.74 [0.17-17.28]	0.637	Removed		Omitted			
Asthma	Omitted				2.77 [0.31-24.96]	0.365	Removed	
Atopic dermatitis	Omitted				Omitted			
Food allergy	Omitted				Omitted			
Smoking	Omitted				1.77 [0.17-17.94]	0.628	Removed	
Pregnant smoker	Omitted				1.12 [0.10-12.96]	0.930	Removed	
Animal exposure	0.39 [0.10-1.47]	0.163	Removed		0.51 [0.07-3.88]	0.517		
Paternal factors								
Allergic rhinitis	1.14 [0.10-13.00]	0.914	Removed		4.04 [0.47-34.73]	0.203	Removed	
Asthma	1.14 [0.10-13.00]	0.914	Removed		Omitted			
Atopic dermatitis	Omitted				Omitted			
Food allergy	Omitted				Omitted			
Smoking	0.59 [0.20-1.77]	0.345	Removed		1.43 [0.47-4.36]	0.529	Removed	

[a] Variables without statistical significance in the univariate model were excluded from the multivariate model.

[b] A large family was arbitrarily defined by more than six members within a household.

[c] Variables omitted due to dependency in the regression model.

[d] Immunisation to routine childhood vaccines.

Abbreviations: AD, atopic dermatitis; OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval.

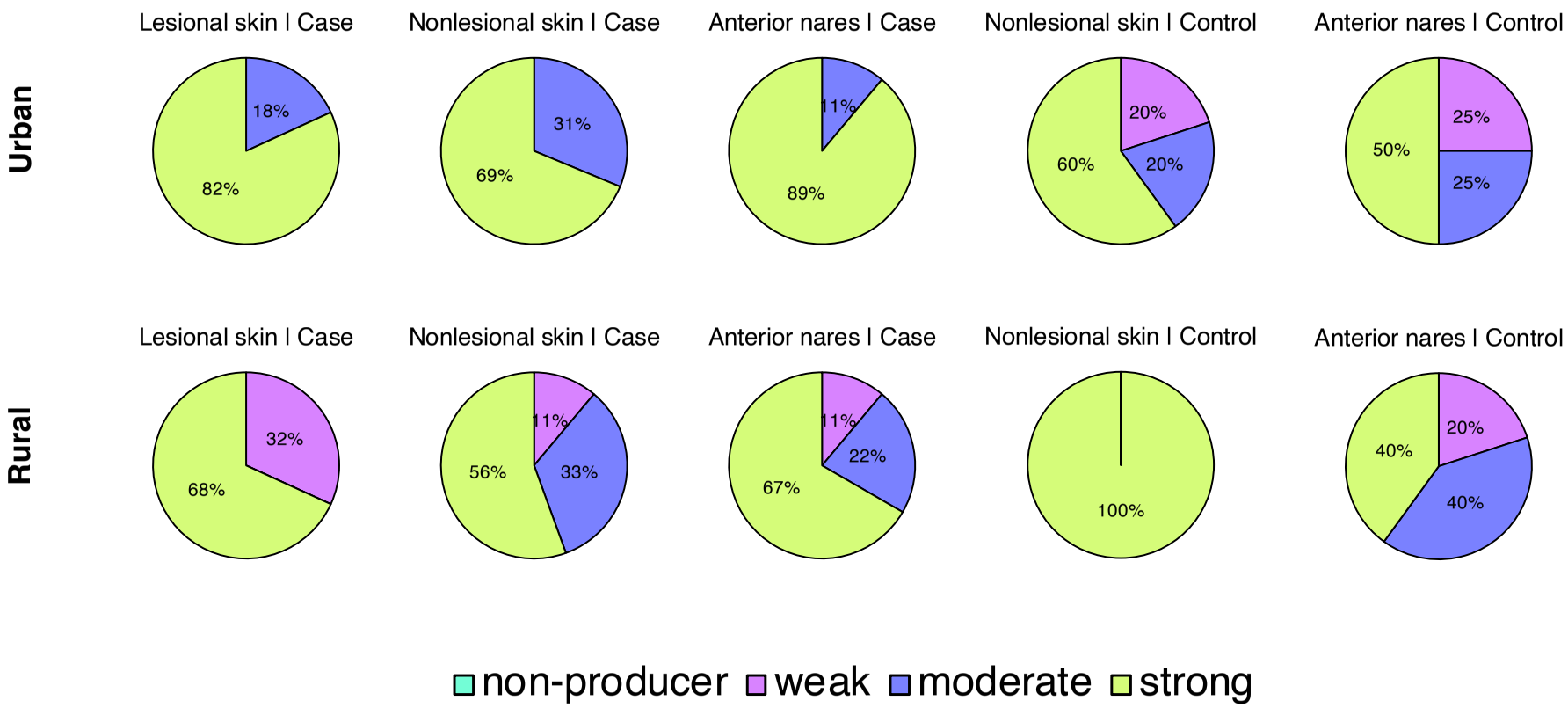


Figure S4.1. Biofilm strength of *S. aureus* isolates from urban (*upper*) and rural (*lower*) children with and without AD. The number of isolates recovered from the sample types from urban cases are n=22 for the lesional skin, n=16 for the nonlesional skin, and n=18 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=5 for the nonlesional skin, and n=8 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=22 for the lesional skin, n=18 for the nonlesional skin, and n=9 for

the anterior nares. The number of isolates recovered from the sample types from rural cases are n=6 for the nonlesional skin, and n=5 for the anterior nares.

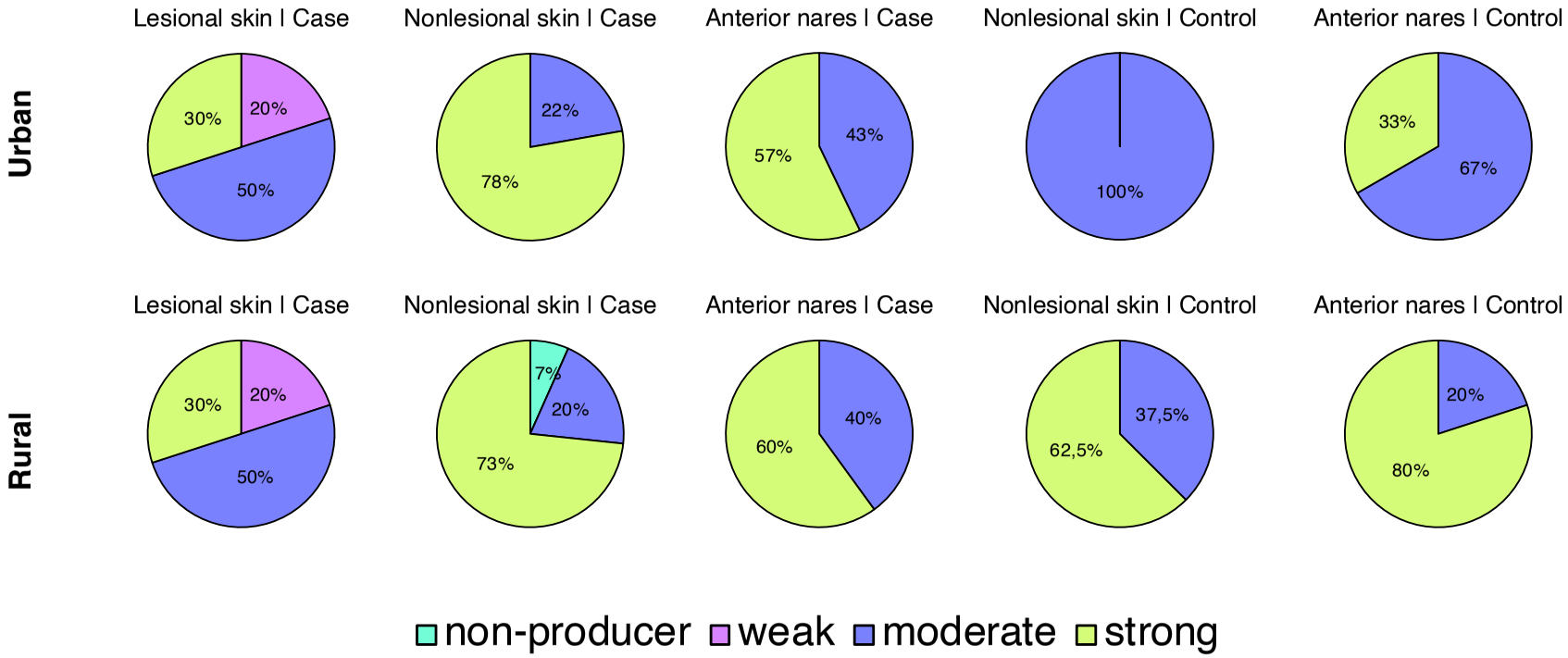


Figure S4.2. Biofilm strength of *S. epidermidis* isolates from urban (*upper*) and rural (*lower*) children with and without AD. The number of isolates recovered from the sample types from urban cases are n=10 for the lesional skin, n=9 for the nonlesional skin, and n=7 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=3 for the nonlesional skin, and n=3 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=10 for the lesional skin, n=15 for the nonlesional skin, and n=15 for

the anterior nares. The number of isolates recovered from the sample types from rural cases are n=8 for the nonlesional skin, and n=5 for the anterior nares.

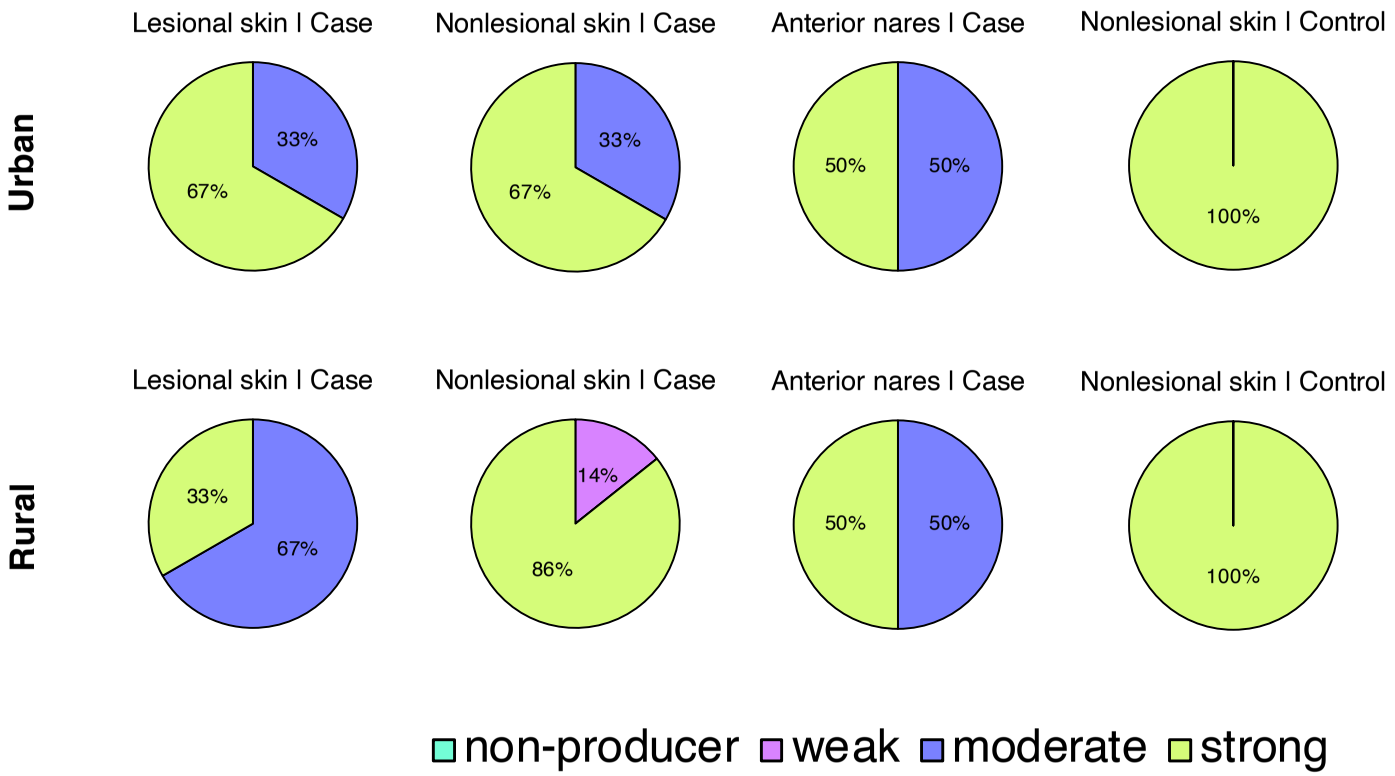


Figure S4.3. Biofilm strength of *S. capitis* isolates from urban (*upper*) and rural (*lower*) children without AD. The number of isolates recovered from the sample types from urban cases are n=3 for the lesional skin, n=9 for the nonlesional skin, and n=2 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=1 for the nonlesional skin, and n=0 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=3 for the lesional skin, n=7 for the nonlesional skin, and n=2 for the anterior nares. The number of isolates recovered from the sample types from rural cases are n=1 for the nonlesional skin, and n=0 for the anterior nares.

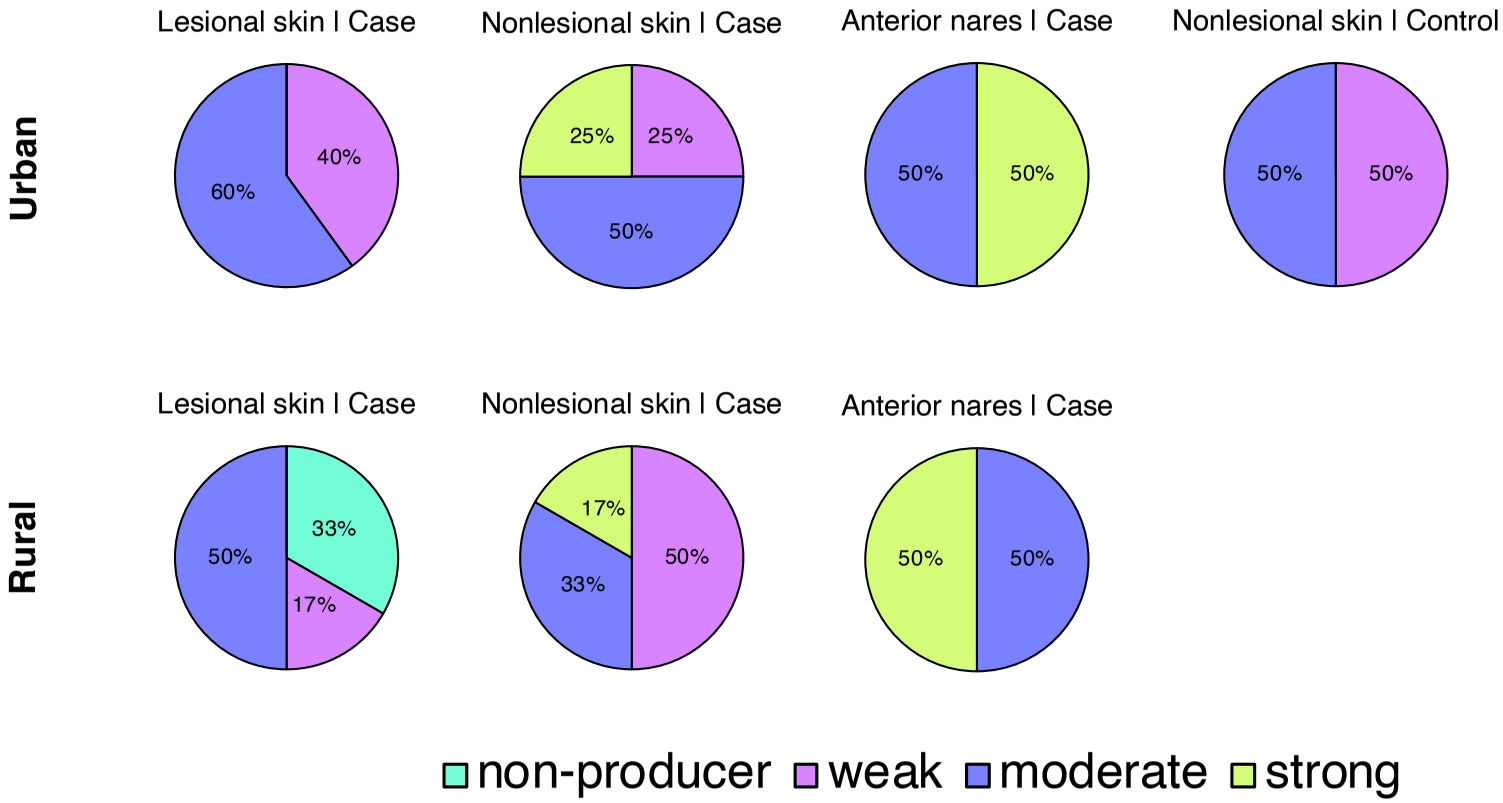


Figure S4.4. Biofilm strength of *S. haemolyticus* isolates in urban (*upper*) and rural (*lower*) children with and without AD. The number of isolates recovered from the sample types from urban cases are n=5 for the lesional skin, n=8 for the nonlesional skin, and n=2 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=8 for the nonlesional skin, and n=0 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=6 for the lesional skin, n=6 for the nonlesional skin, and n=0 for the

anterior nares. The number of isolates recovered from the sample types from rural cases are n=0 for the nonlesional skin, and n=0 for the anterior nares.

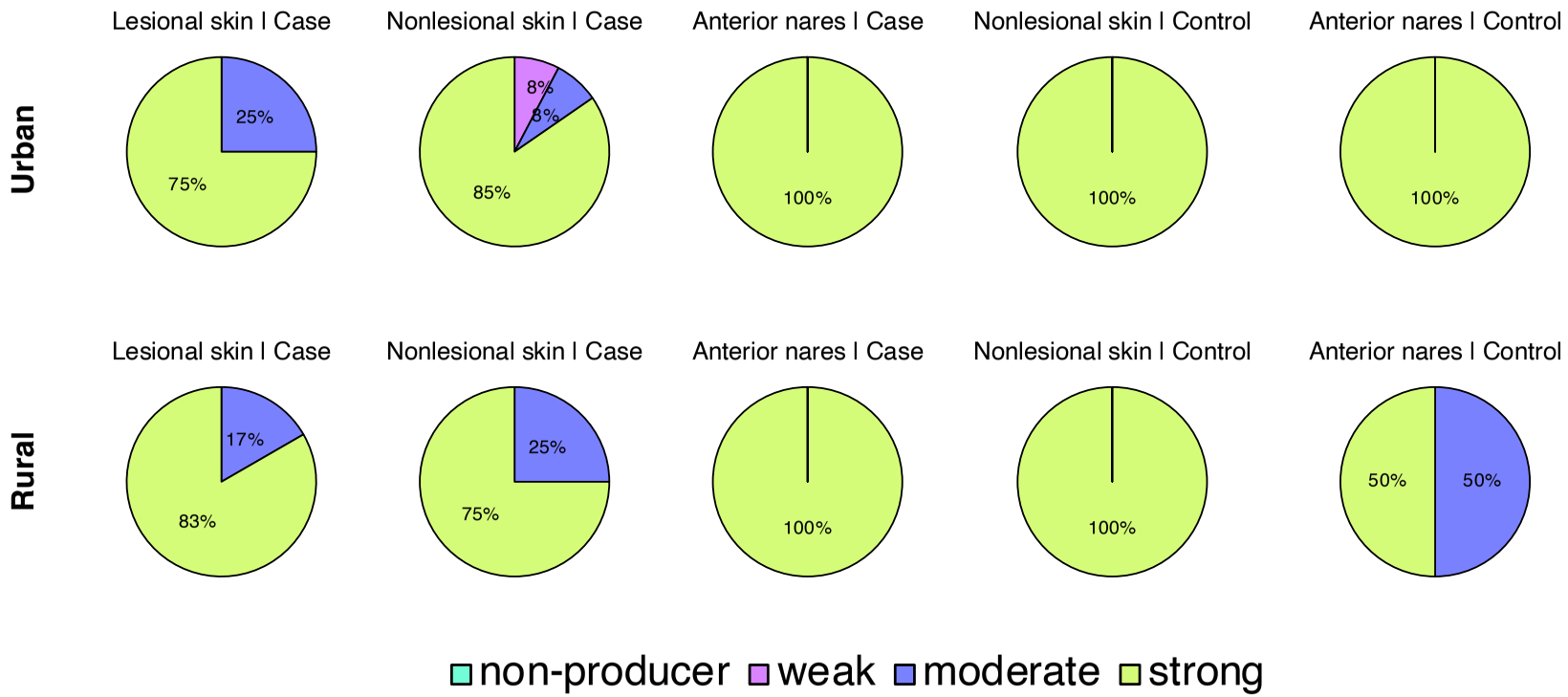


Figure S4.5. Biofilm strength of *S. hominis* isolates from urban (*upper*) and rural (*lower*) children with and without AD. The number of isolates recovered from the sample types from urban cases are n=4 for the lesional skin, n=13 for the nonlesional skin, and n=1 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=7 for the nonlesional skin, and n=1 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=6 for the lesional skin, n=8 for nonlesional skin, and n=1 for the anterior nares. The number of isolates recovered from the sample types from rural cases are n=10 for the nonlesional skin, and n=2 for the anterior nares.

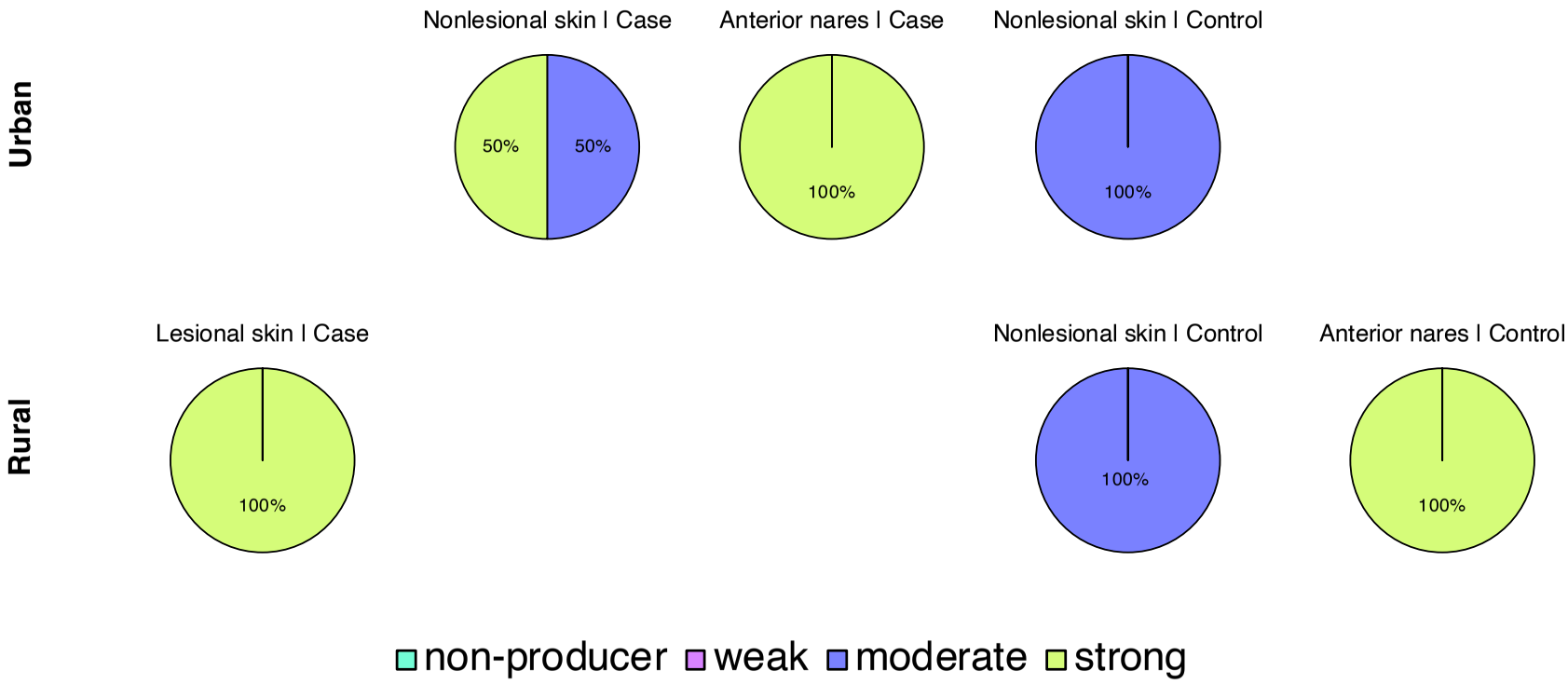


Figure S4.6. Biofilm strength of *S. saprophyticus* isolates from urban (*upper*) and rural (*lower*) children with and without AD. The number of isolates recovered from the sample types from urban cases are n=0 for the lesional skin, n=4 for the nonlesional skin, and n=1 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=1 for the nonlesional skin, and n=0 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=1 for the lesional skin, n=0 for the nonlesional skin, and n=0 for

the anterior nares. The number of isolates recovered from the sample types from rural cases are n=1 for the nonlesional skin, and n=1 for the anterior nares.

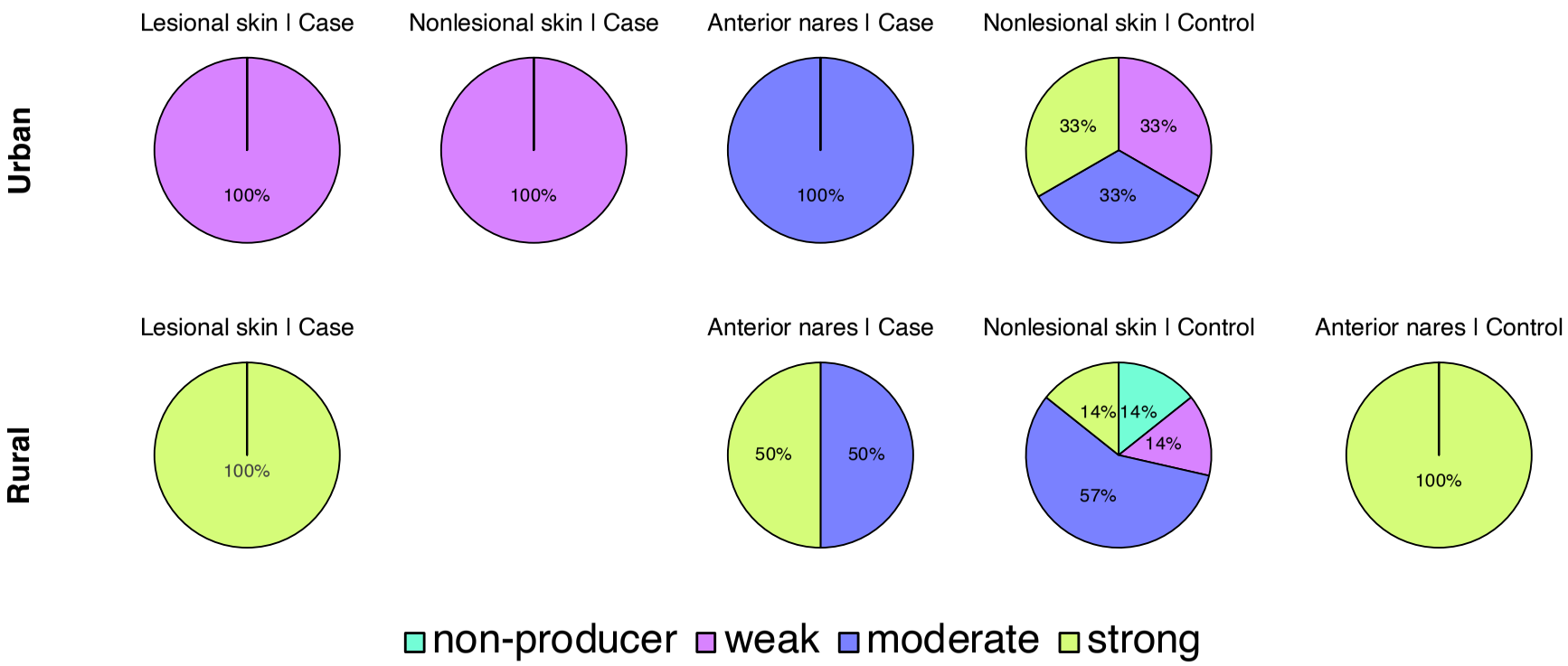


Figure S4.7. Biofilm strength of rare staphylococcal species isolates from urban (*upper*) and rural (*lower*) children with and without AD. Rare staphylococcal species are species that has less than ten recovered isolates. These included *Staphylococcus warneri*, *Staphylococcus cohnii, Staphylococcus equorum*, *Staphylococcus pasteuri*, *Staphylococcus caprae, Staphylococcus lentus*, *Staphylococcus lugdunensis*, *Staphylococcus nepalensis*, *Staphylococcus sciuri*, and *Staphylococcus succinus*. The number of isolates recovered from the sample types from urban cases are

n=1 for the lesional skin, n=1 for the nonlesional skin, and n=1 for the anterior nares. The number of isolates recovered from the sample types from urban controls are n=3 for the nonlesional skin, and n=0 for the anterior nares. The number of isolates recovered from the sample types from rural cases were n=3 for the lesional skin, n=0 for the nonlesional skin, and n=2 for the anterior nares. The number of isolates recovered from the sample types from rural cases are n=7 for the nonlesional skin, and n=1 for the anterior nares.

CHAPTER 5

Skin and nasal bacteriomes in early childhood atopic dermatitis in rural and urban South African communities

5.1. Abstract

Background: Alterations in the skin and nasal bacterial communities are associated with the pathogenesis and flares of atopic dermatitis (AD). However, few studies have investigated these putative alterations in children from diverse environments, and it remains unstudied in children from sub-Saharan Africa.

Methods: We conducted a cross-sectional study of 220 AmaXhosa children, aged 9–38 months, with and without AD from rural and urban settings in South Africa. Specimens from cases (lesional skin, nonlesional skin and anterior nares) and controls (nonlesional skin and anterior nares) were collected, and their bacteriome profiles were assessed using 16S rRNA sequencing. We also explored the association of environmental and clinical factors with bacterial diversity using linear regression modelling.

Results: Shannon diversity was higher on nonlesional skin specimens of controls compared with lesional (p = 0.019) and nonlesional (p = 0.025) skin samples of cases; but only in the rural group when stratified for location (p = 0.0003). The relative abundance of *Streptococcus*, *Granulicatela*, *Veillonella* and *Prevotella* was high in lesional skin among cases compared to nonlesional skin of cases and controls. *Anoxybacillus* and *Cutibacterium* was high on nonlesional skin of cases compared with nonlesional skin of controls. The relative abundances of *Staphylococcus*, *Veillonella* and *Sphingomonas* was high in the anterior nares of cases than the anterior nares of controls. In contrast, controls had a higher relative abundance of *Haematobacter*, *Romboutsia* and *Ruminococcacaea* on nonlesional skin compared with the nonlesional skin of cases, and *Ornithobacterium*, *Moraxella* and *Suttonella* were more enriched in the anterior nares of controls than the anterior nares of cases.

Conclusion: Our results indicate that AD disease, and not geography, has a stronger influence on the composition and diversity of the skin and nasal bacteriota. Also, unique taxa, other than the *Staphylococcus* genus, are likely driving AD pathology in South African children, relative to children in other countries or regions. These data highlight the need for new treatment strategies, targeting the restoration of the skin and nasal bacteriota in AD that are specific to sub-Saharan African children.

5.2. Introduction

Allergic diseases such as atopic dermatitis (AD), allergic rhinitis and food allergy, affect about 20% people globally[301]. Evidence suggests that rural living is protective against the development of allergic diseases[154]. Specifically, microbial exposure in a natural environment is thought to mediate development of allergic disease(s) in early childhood[285,302,303]. Rural environments have higher microbial diversity (i.e., with greater microbial richness and evenness) than urban environments and are predominated by bacteria that are associated with protection against the development of allergic diseases[304,305]. The skin and nasal microbiota (shaped by the environmental microbiota) of rural children tends to have greater diversity and have microbial community composition and structure that significantly differs from urban residents[51,161,302]. The lower microbial diversity in urban spaces and children is attributed to environmental exposures, such as air pollution, which can have harmful effects on the commensal members of the skin and nasal microbiota thereby affecting microbial diversity[157,285]. Therefore, depending on rural or urban living, the association between microbial exposure and allergic diseases in the natural environment suggests that distinct microbial factors drive allergy in early childhood. However, there are limited data on the differences in skin and nasal microbial diversity and structure in early childhood allergic diseases, specifically AD, across rural and urban environments.

AD is a chronic inflammatory skin disease that affects about 20% children and up to 3% adults globally[213]. Among children, the prevalence of AD is highest in children under 3 years[306]. Although AD is usually associated with developed, high-income countries[3], recent data show increasing prevalence of AD among children in countries with a previously low prevalence, such as African countries[213]. Moreover, the risk of AD is higher among urban children than rural children[307]. AD is characterised by a defective skin barrier, chronic cutaneous inflammation and microbial dysbiosis on the skin and in the nose[64]. Dysbiosis of the skin microbiota in AD, especially in older children and adults, is characterised by a predominance of the *Staphylococcus* species and reduced microbial diversity[48]. It is also associated with a reduction in the relative abundance of bacterial genera such as *Streptococcus*, *Prevotella* and *Corynebacterium*, on both the affected and unaffected skin of patients with AD, although the latter is at a lower degree[164]. These changes are correlated with increased disease severity[51]. In addition to microbial changes on AD skin, evidence suggests that changes in the nasal microbiota may also be involved in AD pathology[64]. The importance of the nasal bacterial

community in AD is that it can serve as a reservoir of pathobionts that have clinical relevance in AD and other skin and soft tissue infections[62,63]. Therefore, changes in the nasal bacterial community likely influence skin bacterial shifts with clinical relevance to AD pathology[15]. The predominance of the bacterial genera *Staphylococcus*, *Moraxella, Corynebacterium* and *Dolosigranulum* have been observed in children with AD[64,163] and were linked to increased AD severity. However, their contribution to AD was less than the skin microbiota[64]. Since neither study included a control group[64,163] and the identified taxa (except for *Staphylococcus*) are also enriched in the nose of healthy children[65], it is unclear if the predominance of these bacteria contributes to AD pathology or if they merely reflect the natural nasal colonisation dynamics in these cohorts.

Despite having the highest disease prevalence, few studies have focused exclusively on the skin and nasal microbiota of children with AD under the age of 5 years. The skin microbiota of younger children has been shown to differ from that in adolescents and adults[50]. Evidence shows that ethnicity affects the sebum and moisture content of the skin. Here, the skin of people of African ancestry tends to be more sebaceous and susceptible to transepidermal water loss (i.e., is more inclined to be dry) than other races[308,309]. This is important as the sebum and moisture content (dry, sebaceous or moist) of the skin affects the composition and structure of the skin microbial community[310]. This scenario therefore suggests differences in the skin microbiota across ethnicities based on variations in moisture and sebum content[160]. Moreover, the skin and nasal microbiota vary across different environments (rural vs. urban) and is thought to be linked to susceptibility to microbial diseases associated with the skin or nose[162]. However, studies investigating shifts in the skin and nasal microbiomes, associated with AD pathology, have primarily involved Western[49,64,163] and Asian[164,165] children, with only one study in Africa including Egyptian children[51]. This is relevant given that comprehensive data on the skin and nasal microbiota has been shown to aid in the development of restorative therapies for AD[311-313]. This includes therapies such as dupilumab[313,314], corticosteroids[312,315], emollients[150], and *Lactobacillus* probiotics[311]. While these therapies have shown significant improvements in the skin and nasal microbiomes to health, it is important to understand that a healthy microbiome is defined by microbiomes that have been characterised in Western and Asian populations. However, it is known that the composition and diversity of healthy skin and nasal microbiomes vary across races or ethnicities, and geographic location[162,310]. Therefore, it is plausible that the effectiveness of these therapies may be limited in non-Western and non-

Asian populations as they are formulated to restore skin and nasal microbiomes to levels that are not relevant to these populations.

In this chapter, we aimed to characterise the skin and nasal microbial composition and diversity of rural and urban South African children, with and without AD, in a previously unstudied population. We also explored the association between environmental and clinical factors with microbial diversity.

5.3. Methodology

5.3.1. Study design and participants

We conducted a cross-sectional study of urban and rural AmaXhosa children (n=220) aged 9 – 38 months, with and without AD, from Cape Town, South Africa and Umtata, South Africa. AD diagnosis was based on the U.K. working party diagnosis of atopic eczema[172]. AD patients with moderate-to-severe disease were recruited between February 2015 and May 2016 through the Red Cross Children's War Memorial Hospital (Cape Town, South Africa) and Nelson Mandela Academic Hospital (Umtata, South Africa). Children of a similar age group, without a clinical diagnosis of AD, were recruited from community early development centres in Cape Town and Umtata. Clinical, environmental, and socio-demographic data were collected through questionnaires. Details of the study participants and recruitment are described elsewhere[223].

5.3.2. Statement of ethical approval

The parent study was approved by the Human Research and Ethics Committee of the Faculty of Health Sciences, University of Cape Town (HREC/REF: 451/2014) and the Western Cape Provincial Child Health Research Committee. Additional ethical approval, specifically for this study, was obtained from the Human Research and Ethics Committee of the Faculty of Health Science, University of Cape Town (HREC/REC: 668/2020). No additional data, other than that approved in the parent study, were collected. Written informed consent and assent were provided by guardians and participants, respectively. All data obtained and generated during the study were kept confidential. This research was conducted in accordance with the Declaration of Helsinki.

5.3.3. Sample collection

Sterile Copan nylon-tipped flocked swabs (Cat. no. 516C; Copan Italia, Brescia, Italy) were used to collect samples from lesional (the most active area of eczematous skin with acute and/or chronic changes) and non- lesional (area with the most normal-appearing skin, usually the back) skin. The swab was pre-moistened with sterile distilled water and a 4 cm^2 area of the skin lesion was swabbed for at least 1 minute in a non-overlapping manner. Additionally, nasal swabs were collected from all participants, as previously described[174]. The collected swabs were immediately placed into 1 ml skim milk-tryptone-glucose-glycerol (STGG), transported at 4 °C to the laboratory within two hours of collection and frozen at −80 °C for subsequent batch processing.

5.3.4. DNA isolation and 16S rRNA sequencing

Skin and nasal specimens, stored at -80 °C in skim milk-tryptone-glycerol-glucose (STGG), were thawed and 400 µl was aliquoted into lysozyme (25 mg/mL, Sigma Alderich fine Chemicals Biosciences, FisherScientific) and incubated for 30 minutes at 37 °C. Post incubation, the aliquots were transferred into ZR BashingBead™ Lysis tubes containing 0.5 mm beads and lysed using a Tissuelyser (Qiagen) for 5 minutes at 50 Hz. The lysates were centrifuged at 1,000 rpm (10,640 × g) for 2 minutes. Total DNA was isolated using the DSP Virus/Pathogen Mini Kit® (Qiagen, Germany) and eluted in 70 µL elution buffer. Bacterial mock community cells (Zymo Research Corp., CA, United States) were extracted and included as the extraction control on each plate. Neat STGG, Milli-Q ultrapure water (Millipore Sigma, Burlington, MA, United States), lysozyme, and neat STGG with swab were also included as non-template extraction controls. For 16S ribosomal RNA (rRNA) gene analysis, we amplified the V4-V5 regions of the 16S rRNA gene, using the Earth Microbiome Project primers, as previously described[316]. Samples were barcoded and pooled in batches of 300, prior to sequencing on a MiSeq v3 300 cycle run, which produces 13 Gbp per run.

5.3.5. *Details of bioinformatic steps and quality control*

Demultiplexed reads were quality filtered, followed by denoising and chimera removal using the DADA2 module in QIIME 2 (version 2021.4.0), preserving an average of 75% of the reads for each sample post quality control. Default parameters were used except for maximum

expected error: 4.0 and length truncation: 250 bp for forward truncation (--p-trunc-len-f) and 200 bp for reverse truncation (--p-trunc-len-r). Amplicon sequence variants or ASVs (n=16694) were generated and subsequently clustered into operational taxonomic units or OTUs (n=3184) at 95% identity using the "cluster-features-de-novo" method in q2-vsearch (version 2021.4). Singleton OTU sequences were filtered out. Taxonomic annotation for all resultant sequences were assigned against SILVA (version 138) using the "classify-sklearn" classification method with a Naive Bayes classifier in QIIME 2.

5.3.6. Statistical analysis

All statistical analyses were performed using the R statistical software (version 4.0.4). Amplicon Sequence Variants (ASV) were merged at the genus level and 3% prevalence filtering was implemented for each sample type (anterior nares only and skin [both lesional skin and nonlesional skin]) before analysis. Genus-relative abundances were visualised using stacked bar charts. The Shannon diversity index was employed to determine the alpha diversity (richness and evenness) between cases and controls for nonlesional skin and anterior nares samples, between lesional and nonlesional skin samples in cases, and between rural and urban children for each sample type[317]. A linear regression model was used to determine the association between the Shannon diversity index for each sample type (lesional skin, nonlesional skin and anterior nares) and the participant's clinical, environmental, and socio-demographic factors. Disease status (case or control; except for lesional skin, which was only available for cases), and area (Cape Town or Umtata) were adjusted for in all models. In the multivariate model, exposure to animals, parental education and smoking were selected for adjustment. Additional variables, with a p-value <0.1 in the univariate analysis, were included in the multivariate model. Non-metric Multi-dimensional Scaling (NMDS) was employed to visualise beta diversity. Analysis of Similarities (Anosim) was used to compare the beta diversity between disease status or sample types. We employed ANCOM2 on unrarefied data with False Discovery Rate (FDR) Benjamini-Hochberg correction cut-off at 0.05 to determine differentially abundant taxa.

5.4. Results

5.4.1. Participant and sample characteristics

Table 5.1 outlines the clinical, environmental, and socio-demographic information of the study participants in Cape Town (urban group) and Umtata (rural group). Of the 220 recruited children, 185 (111 rural and 74 urban) were included in the analysis. The remaining 35 were excluded due to in availability of samples as described in Chapter 2. The median age was 21.27 (standard deviation, 7.15) and 24.19 (7.37) months in the rural and urban groups, respectively. The distribution of sex was similar in both geographical groups. In both rural and urban groups, food allergy was commonly reported among cases compared to controls (rural: 17% vs. 2%, p = 0.009, and urban: 21% vs. 0%, p = 0.008). Features associated with rural living, such as exposure to domestic/farm animals and the use of non-electric sources for cooking/heating were mostly associated with the rural group, with a significantly variable frequency between cases and controls.

Table 5.1. Characteristics of the study participants.

Explanatory variable	**Umtata**				**Cape Town**			
	Total, *n* (%)	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**	**Total, *n* (%)**	**Case, *n* (%)**	**Control, *n* (%)**	**p-value**
Total	111 (100)	58 (52)	53 (48)	0.502[a]	74 (100)	43 (58)	31 (42)	0.049[a]
Age (months)								
Mean [standard deviation]	21.27 [7.15]	21.03 [7.41]	21.53 [6.90]	0.718	24.19 [7.37]	23.98 [7.44]	24.48 [7.38]	0.773
Sex								
Female	42 (39)	24 (43)	18 (34)	0.431	36 (49)	19 (44)	17 (55)	0.480
Male	67 (61)	32 (57)	35 (66)		38 (51)	24 (56)	14 (45)	
AD severity								
Moderate	23 (40)	23 (40)	—		21 (49)	21 (49)	—	
Severe	35 (60)	35 (60)	—		22 (51)	22 (51)	—	
Atopic disease								
Food allergy	11 (10)	10 (17)	1 (2)	**0.009**	9 (12)	9 (21)	0 (0)	**0.008**
Asthma	0 (0)	0 (0)	0 (0)		1 (1)	1 (3)	0 (0)	1.000
Allergic rhinitis	7 (8)	1 (2)	6 (11)	0.242	1 (1)	1 (3)	0 (0)	1.000
Mode of birth								
Caesarean section	25 (23)	14 (24)	11 (21)	0.821	33 (46)	20 (49)	13 (42)	0.637
Vaginal	86 (77)	44 (76)	42 (79)		39 (54)	21 (51)	18 (58)	
Breastfeeding	35 (32)	10 (17)	25 (47)	**0.001**	9 (12)	7 (16)	2 (6)	0.288
Antibiotic exposure	92 (82)	49 (83)	43 (81)	0.810	54 (72)	30 (70)	24 (77)	0.598
Immunisation status								
Complete	107 (96)	56 (95)	52 (98)	0.620	64 (86)	33 (77)	31 (100)	0.004
Incomplete	4 (4)	3 (5)	1 (2)		10 (14)	10 (23)	0 (0)	
Large family [1]	62 (55)	30 (52)	32 (60)	0.445	24 (32)	14 (33)	10 (32)	1.000
Animal exposure	93 (84)	39 (67)	53 (100)	**0.001**	2 (3)	2 (6)	0 (0)	0.495
Parental education								
Primary	8 (7)	2 (3)	6 (11)	**0.001**	1 (1)	1 (2)	0 (0)	**0.025**
Secondary	70 (63)	31 (53)	39 (74)		33 (45)	14 (33)	19 (61)	
Tertiary	31 (28)	25 (43)	6 (11)		40 (54)	28 (65)	12 (39)	
Other	2 (2)	0 (0)	2 (4)		0 (0)	0 (0)	0 (0)	
Maternal factors								
Animal exposure	96 (86)	44 (76)	52 (98)	**0.001**	4 (60)	4 (11)	0 (0)	0.120

Pregnant smoking	1 (1)	0 (0)	1 (2)	0.482	3 (45)	0 (0)	3 (10)	0.094
Smoking	1 (1)	0 (0)	1 (2)	0.477	4 (6)	1 (3)	3 (10)	0.324
Asthma	2 (2)	2 (3)	0 (0)	0.496	6 (8)	4 (10)	2 (6)	1.000
Allergic rhinitis	4 (4)	4 (7)	0 (0)	0.120	5 (68)	4 (10)	1 (3)	0.387
Atopic dermatitis	2 (2)	2 (3)	0 (0)	0.496	3 (4)	2 (5)	1 (3)	1.000
Food allergy	3 (3)	2 (3)	1 (2)	1.000	1 (1)	1 (2)	0 (0)	1.000
Paternal factors								
Smoking	15 (14)	9 (16)	6 (12)	0.589	20 (31)	11 (31)	9 (31)	1.000
Asthma	3 (3)	3 (5)	0 (0)	0.245	0 (0)	0 (0)	0 (0)	
Allergic rhinitis	3 (3)	3 (5)	0 (0)	0.245	7 (10)	7 (17)	0 (0)	0.018
Atopic dermatitis	1 (1)	1 (2)	0 (0)	1.000	2 (3)	2 (5)	0 (0)	0.505
Food allergy	1 (1)	1 (2)	0 (0)	1.000	1 (1)	1 (2)	0 (0)	1.000
Household factors								
Electricity + gas	69 (62)	56 (97)	13 (25)	**0.001**	66 (99)	35 (97)	31 (100)	1.000
Kerosene + paraffin	64 (58)	44 (76)	20 (38)	**0.001**	43 (64)	21 (58)	22 (71)	0.317
Paraffin	38 (34)	6 (10)	32 (60)	**0.001**	0 (0)	0 (0)	0 (0)	
Indoor fire	4 (4)	2 (3)	2 (4)	1.000	0 (0)	0 (0)	0 (0)	
Outdoor fire	49 (44)	12 (21)	37 (70)	**0.001**	0 (0)	0 (0)	0 (0)	
Wood + fire	31 (28)	4 (7)	27 (51)	**0.001**	0 (0)	0 (0)	0 (0)	

Bold text indicates statistical significance. AD, atopic dermatitis; CI, confidence interval; IQR, interquartile range; [1] A large family is arbitrarily defined as 6 or more members living within one household.

5.4.2. Bacterial composition in the skin and anterior nares in cases and controls

At the genus level, the dominant bacteria found in the anterior nares of both cases and controls included *Moraxella*, *Streptococcus*, *Haemophilus* and *Corynebacterium* (Figure 5.1A). However, we observed specific predominance of *Ornithobacterium* and *Dolosigranulum* among controls and cases, respectively. This was also observed when stratified by location (Figure S5.1). The relative abundance of *Staphylococcus*, *Corynebacterium*, *Streptococcus*, *Prevotella*, *Acinetobacter* and *Chryseobacterium* was high for both lesional skin and nonlesional skin samples in both cases and controls (Figure 5.1B), and within each location (Figure S5.2).

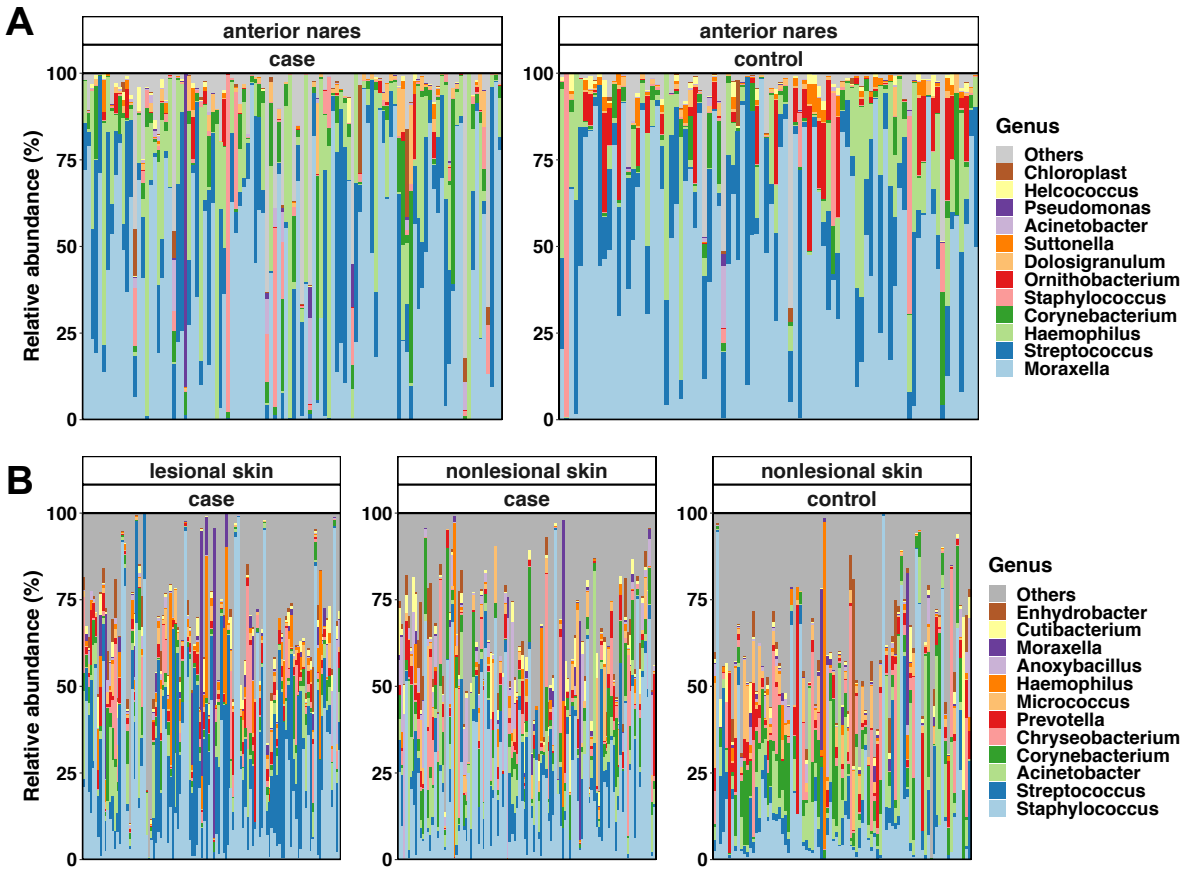


Figure 5.1. Relative abundance of the top 12 bacterial genera of the anterior nares (A) and skin (B) samples from cases and controls.

5.4.3. Skin and nasal bacterial diversity

A lower alpha diversity was observed on the nonlesional skin of cases compared to the nonlesional skin of controls (Shannon diversity index $p = 0.025$; Figure 5.2A, *left panel*). However, when stratified by location, this observation was detected only in the rural group (Shannon diversity index $p < 0.001$, Figure 5.2A, *right panel*) and not in the urban group (Shannon diversity index $p = 0.29$; Figure 5.2A, *middle panel*). The alpha diversity of the anterior nares of cases and controls was similar (Shannon diversity index $p = 0.86$; Figure 5.2B, *left panel*), even when stratified by location (urban group, Shannon diversity index $p = 0.84$ and rural group, Shannon diversity index $p = 0.96$; Figure 5.2B, *middle and right panels*). The alpha diversity between lesional and nonlesional skin in the overall cohort (Shannon diversity index $p = 0.7$), and within geographic groups (urban group, Shannon diversity index $p = 0.72$ and rural group, Shannon diversity index $p = 0.43$) (Figure S5.3A) were similar among cases. In contrast, nonlesional skin samples of controls had a higher alpha diversity than lesional skin of cases (Shannon diversity index $p = 0.019$) (Figure S5.3B). However, when stratified by location, this observation was limited to the rural group (Shannon diversity index $p = 0.0002$) (Figure S5.3B). Geographic group comparisons revealed that nonlesional skin samples of rural controls had a higher alpha diversity than urban controls (Shannon diversity index $p < 0.0001$; Figure S5.4). However, this was not observed for other sample type comparisons. Also, there was no difference in the alpha diversity for each sample type between cases with severe and moderate disease (Figure 5.3), even when stratified by geographic location (Figure S5.5).

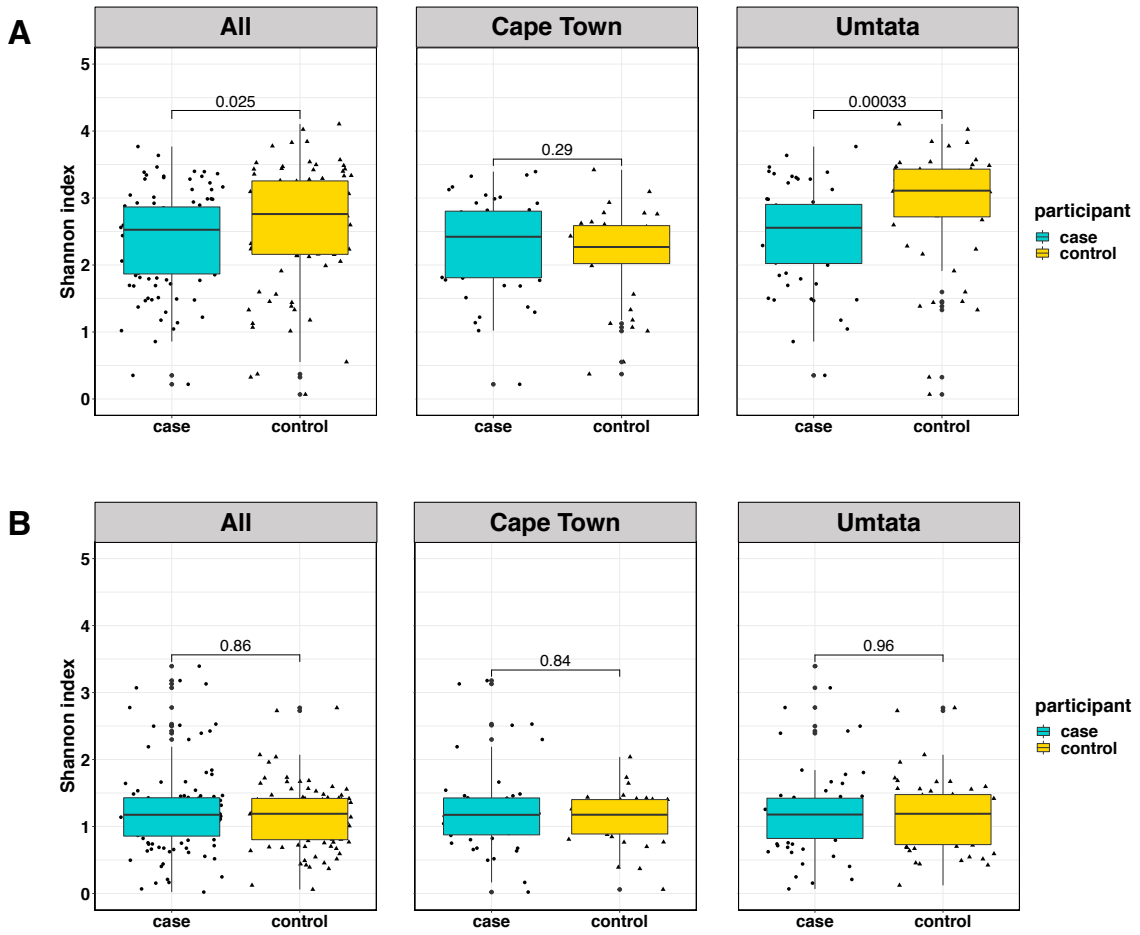


Figure 5.2. Boxplots comparing the Shannon diversity index of (A) nonlesional skin and (B) anterior nares samples between cases and controls. All represents comparisons independent of location.

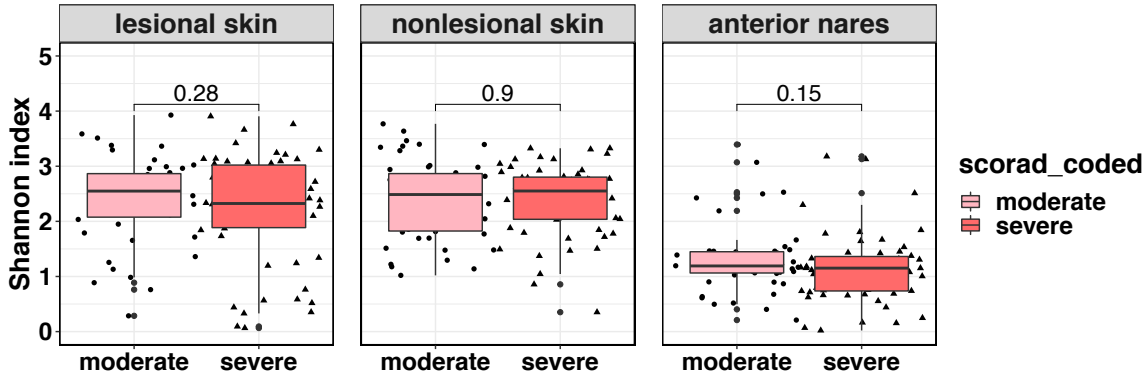


Figure 5.3. Boxplots comparing the Shannon diversity index between cases with moderate and severe AD for each sample type.

5.4.4. Relationship between clinical, demographic, and environmental factors with bacterial diversity

We employed linear regression modelling to assess the relationship between alpha diversity and the participants' clinical, socio-demographic, and environmental factors for each sample type, adjusting for disease status and location (Tables 5.2 – 5.4). The trivariate model revealed that children born to parents without any allergy, have a higher Shannon diversity index on lesional skin (beta coefficient, 0.49 [95% CI, 0.08 – 0.9]) and anterior nares (0.32 [0.06 – 0.59]), compared with the nonlesional skin of children with asthmatic fathers (-0.75 [-1.51 – 0]). The Shannon diversity index was lower on the lesional skin of children with allergic rhinitis (-1.52 [-2.65;-0.43]) or those who had a skin prick test (SPT) allergic reaction to nuts (-1.96 [-3.65; -0.27]), as well as in the anterior nares of those with a SPT allergic reaction to fish (-0.68 [-1.34;-0.03]). In contrast, a positive reaction to wheat (0.9 [0.15;1.66]) or consumption of *amasi* (0.31 [0;0.61]), were associated a higher bacterial diversity index on nonlesional skin. Living in an electrified house, that also uses gas for heating and/or cooking, was associated with a lower bacterial diversity on nonlesional skin (-0.35 [-0.68;-0.01]). Our multivariate model showed that a SPT reaction to nuts (-1.93 [-3.76;-0.09]) and being born to an asthmatic father (-0.84 [-1.63;-0.06]), were associated with a lower bacterial diversity on lesional skin and nonlesional skin, respectively. Interestingly, a higher bacterial diversity was observed in the anterior nares of children born to mothers with asthma (0.49 [0.002;0.97]), and on nonlesional skin of those born of fathers with AD (0.88 [0.05;1.72]) or with a positive reaction to wheat (1.04 [0.31;1.77]), was observed.

Table 5.2. The relationship between the Shannon diversity index of nonlesional skin samples and clinical and environmental factors.

Explanatory variable		**No. of observations (%)**	**Coefficient [95% CI]**	**p-value**	**Adjusted coefficient [95% CI]**	**p-value**
Sex	Female	86 (45.3)	Reference			
	Male	104 (54.7)	0.16[-0.06 – 0.38]	0.143	0.19[-0.03 – 0.41]	0.085
Family size	Small	105 (54.1)	Reference			
	Large	89 (45.9)	0.08[-0.15 – 0.3]	0.498		
Ever breastfed	No	150 (78.1)	Reference			
	Yes	42 (21.6)	-0.08[-0.35 – 0.19]	0.575		
Allergic rhinitis †	No	152 (95.6)	Reference			
	Yes	7 (4.4)	0.53[-0.06 – 1.12]	0.076		
Maternal smoking	No	181 (97.3)	Reference			
	Yes	5 (2.7)	-0.24[-0.93 – 0.45]	0.501	0.34[-0.4 – 1.08]	0.364
Paternal smoking	No	147 (81.2)	Reference			
	Yes	34 (16.6)	-0.08[-0.37 – 0.21]	0.57	-0.17[-0.44 –0.11]	0.231
Parental education	Other	2 (1)	Reference			
	Primary	9 (4.7)	0.92[-0.22 – 2.06]	0.114	0.95[-0.15 – 2.06]	0.091
	Secondary	104 (54.2)	0.23[-0.82 – 1.28]	0.666	0.45[-0.57 – 1.47]	0.387
	Tertiary	77 (40.1)	-0.02[-1.08 – 1.05]	0.974	0.15[-0.89 – 1.2]	0.772
Mode of delivery	C-section	59 (31.1)	Reference			
	Vaginal	131 (68.9)	-0.1[-0.34 – 0.14]	0.397		
Cat ownership	No	144 (77.8)	Reference			
	Yes	41 (22.2)	-0.05[-0.32 – 0.23]	0.737		
Dog ownership	No	93 (50.2)	Reference			
	Yes	92 (49.7)	-0.04[-0.28 – 0.19]	0.712		

Farm animal contact	No	92 (49.7)	Reference			
	Yes	93 (50.2)	0.32[-0.04 – 0.68]	0.085	0.11 [-0.32 – 0.53]	0.622
Antibiotic exposure	No	40 (20.8)	Reference			
	Yes	152 (79.2)	-0.12[-0.39 – 0.15]	0.389		
Probiotic exposure	No	185 (96.9)	Reference			
	Yes	6 (3.1)	-0.28[-0.9 – 0.35]	0.382		
Amasi consumption	No	31 (16.1)	Reference			
	Yes	161 (83.9)	**0.31[0 – 0.61]**	**0.047**		
Electricity + gas	No	41 (22.2)	Reference			
	Yes	144 (77.8)	**-0.35[-0.68 – -0.01]**	**0.041**	-0.14[-0.6 – 0.31]	0.522
Paraffin	No	151 (81.6)	Reference			
	Yes	34 (18.4)	0.22[-0.12 – 0.55]	0.2	0.08[-0.33 – 0.5]	0.686
Indoor fire	No	181 (97.8)	Reference			
	Yes	4 (2.2)	0.04[-0.73 – 0.8]	0.923		
Outdoor fire	No	135 (73)	Reference			
	Yes	50 (27)	0.15[-0.16 – 0.46]	0.337	-0.06[-0.42 – 0.3]	0.736
Electricity	No	148 (80)	Reference			
	Yes	37 (20)	-0.04[-0.34 – 0.26]	0.8	-0.01[-0.31 – 0.28]	0.921
Gas	No	180 (97.3)	Reference			
	Yes	5 (2.7)	-0.74[-1.42 – -0.06]	0.032	-0.41[-1.11 – 0.28]	0.241
Kerosene + paraffin	No	75 (40.5)	Reference			
	Yes	110 (59.5)	-0.01[-0.24 – 0.22]	0.917		
Wood + coal	No	156 (84.3)	Reference			
	Yes	29 (15.7)	0.29[-0.05 – 0.64]	0.098	0.18 [-0.22 – 0.59]	0.364
Peanut reaction	No	186 (98.4)	Reference			

	Yes	3 (1.6)	-0.07[-0.95 – 0.81]	0.874		
Nut reaction	No	187 (99.5)	Reference			
	Yes	1 (0.5)	-0.34[-1.84 – 1.17]	0.657		
Cow milk reaction	No	184 (97.4)	Reference			
	Yes	5 (2.6)	0.08[-0.6 – 0.77]	0.811		
Egg reaction	No	181 (95.8)	Reference			
	Yes	8 (4.2)	0.06[-0.5 – 0.61]	0.838		
Wheat reaction	No	188 (97.9)	Reference			
	Yes	4 (2.1)	**0.9[0.15 – 1.66]**	**0.019**	**1.04[0.31 – 1.77]**	**0.006**
Fish reaction	No	190 (99)	Reference			
	Yes	2 (1)	0.8[-0.27 – 1.86]	0.142		
Reported food allergy	No	173 (90.1)	Reference			
	Yes	19 (9.9)	0.24[-0.14 – 0.61]	0.214		
Maternal no allergy	No	28 (14.7)	Reference			
	Yes	163 (85.3)	-0.19[-0.5 – 0.13]	0.247		
Maternal asthma	No	183 (95.8)	Reference			
	Yes	8 (4.2)	0.07[-0.48 – 0.62]	0.813		
Maternal allergic rhinitis	No	182 (95.3)	Reference			
	Yes	9 (4.7)	0.26[-0.26 – 0.78]	0.319		
Maternal atopic dermatitis	No	186 (97.2)	Reference			
	Yes	5 (2.8)	0.18[-0.51 – 0.86]	0.614		
Maternal food allergy	No	188 (98.4)	Reference			
	Yes	3 (1.6)	0.22[-0.65 – 1.1]	0.615		
Paternal no allergy	No	188 (98.4)	Reference			
	Yes	3 (1.6)	0.06[-0.28 – 0.4]	0.732		

Paternal asthma	No	187 (97.9)	Reference			
	Yes	4 (2.1)	**-0.75[-1.51 – 0]**	**0.05**	**-0.84[-1.63 – -0.06]**	**0.035**
Paternal allergic rhinitis	No	180 (94.2)	Reference			
	Yes	11 (5.8)	0.05[-0.43 – 0.53]	0.836		
Paternal atopic dermatitis	No	188 (98.4)	Reference			
	Yes	3 (1.6)	**0.83[-0.04 – 1.7]**	**0.062**	**0.88[0.05 – 1.72]**	**0.037**
Paternal food allergy	No	189 (99)	Reference			
	Yes	2 (1)	0.22[-0.85 – 1.29]	0.688		
Maternal animal exposure	No	86 (44.3)	Reference			
	Yes	98 (55.7)	0.2[-0.16 – 0.55]	0.282	-0.08[-0.5 – 0.33]	0.701
Immunisation status	No	177 (92.1)	Reference			
	Yes	15 (7.8)	0.31[-0.11 – 0.72]	0.144		

† Allergic rhinitis was excluded from the trivariate model because of missing variables.

Table 5.3. The relationship between the Shannon diversity index of anterior nares samples and clinical and environmental factors.

Explanatory variables		No. of observations (%)	Coefficient [95% CI]	p-value	Adjusted coefficient [95% CI]	p-value
Sex	Female	82 (43.4)	Reference			
	Male	107 (56.6)	-0.07[-0.23 – 0.1]	0.424	-0.003 [-0.19 – 0.18]	0.973
Family size	Small	102 (52)	Reference			
	Large	94 (48)	0.04[-0.13 – 0.21]	0.638		
Ever breastfed	No	145 (75.9)	Reference			
	Yes	46 (24.1)	0.05[-0.15 – 0.25]	0.609		
Allergic rhinitis	No	148 (89.7)	Reference			
	Yes	9 (10.3)	-0.23[-0.58 – 0.13]	0.21		
Maternal smoking	No	181 (97.8)	Reference			
	Yes	5 (2.2)	-0.37[-0.89 – 0.15]	0.157	-0.22 [-0.82 – 0.2]	0.461
Paternal smoking	No	146 (80.7)	Reference			
	Yes	35 (19.3)	0.02[-0.19 – 0.24]	0.821	-0.02 [-0.24 – 0.2]	0.846
Parental education	Other	2 (1)	Reference			
	Primary	10 (5.2)	0.43[-0.45 – 1.31]	0.337	0.37 [-0.51 – 1.24]	0.411
	Secondary	105 (55)	0.33[-0.49 – 1.14]	0.43	0.31 [-0.49 – 1.12]	0.446
	Tertiary	74 (38.7)	0.22[-0.61 – 1.05]	0.607	0.17 [-0.65 – 0.99]	0.684
Mode of delivery	C-section	58 (30.7)	Reference			
	Vaginal	131 (69.3)	0.16[-0.03 – 0.34]	0.094	0.14 [-0.05 – 0.33]	0.157
Cat ownership	No	143 (77.3)	Reference			
	Yes	42 (22.7)	0.02[-0.19 – 0.22]	0.881		
Dog ownership	No	93 (50.3)	Reference			
	Yes	92 (49.7)	-0.02[-0.2 – 0.16]	0.81		
Farm animal contact	No	88 (47.6)	Reference			

	Yes	97 (52.4)	0.08[-0.21 – 0.37]	0.58	-0.007 [-0.31 – 0.3]	0.961
Antibiotic exposure	No	40 (20.9)	Reference			
	Yes	151 (79.1)	0.02[-0.18 – 0.23]	0.819		
Probiotic exposure	No	184 (96.8)	Reference			
	Yes	6 (3.2)	-0.18[-0.65 – 0.3]	0.467		
Amasi consumption	No	33 (17.3)	Reference			
	Yes	158 (82.7)	-0.1[-0.32 – 0.13]	0.401		
Electricity + gas	No	44 (23.9)	Reference			
	Yes	141 (76.1)	-0.14[-0.39 – 0.12]	0.293	-0.05 [-0.42 – 0.31]	0.776
Paraffin	No	148 (80)	Reference			
	Yes	37 (20)	0.08[-0.16 – 0.33]	0.501	0.03 [-0.28 – 0.33]	0.863
Indoor fire	No	181 (97.8)	Reference			
	Yes	4 (2.2)	-0.3[-0.87 – 0.28]	0.312		
Outdoor fire	No	136 (73.5)	Reference			
	Yes	49 (26.5)	0.13[-0.1 – 0.36]	0.264	0.16 [-0.11 – 0.43]	0.251
Electricity	No	149 (80.5)	Reference			
	Yes	36 (19.5)	-0.14[-0.36 – 0.09]	0.241	-0.02 [-0.27 – 0.22]	0.841
Gas	No	180 (97.3)	Reference			
	Yes	5 (2.7)	-0.18[-0.7 – 0.34]	0.493	-0.16 [-0.74 – 0.42]	0.581
Kerosene + paraffin	No	76 (41.1)	Reference			
	Yes	109 (48.9)	0.08[-0.09 – 0.25]	0.353		
Wood + coal	No	153 (82.7)	Reference			
	Yes	32 (17.3)	-0.13[-0.39 – 0.13]	0.325	-0.26 [-0.56 – 0.05]	0.098
Peanut reaction	No	185 (98.4)	Reference			
	Yes	3 (1.6)	-0.15[-0.82 – 0.51]	0.653		

Nut reaction	No	187 (99.5)	Reference			
	Yes	1 (0.5)	0.24[-0.9 – 1.38]	0.679		
Cow milk reaction	No	182 (96.8)	Reference			
	Yes	6 (3.2)	-0.39[-0.87 – 0.09]	0.109		
Egg reaction	No	180 (95.7)	Reference			
	Yes	8 (4.3)	0.02[-0.4 – 0.44]	0.916		
Wheat reaction	No	187 (97.9)	Reference			
	Yes	4 (2.1)	-0.18[-0.76 – 0.4]	0.537		
Fish reaction	No	188 (98.4)	Reference			
	Yes	3 (1.6)	**-0.68[-1.34 – -0.03]**	**0.041**	0.56 [-1.24 – 0.11]	0.102
Food allergy	No	171 (89.5)	Reference			
	Yes	20 (10.5)	-0.13[-0.41 – 0.15]	0.363		
Maternal no allergy	No	28 (14.7)	Reference			
	Yes	162 (85.3)	0.07[-0.17 – 0.31]	0.585		
Maternal asthma	No	183 (96.3)	Reference			
	Yes	7 (3.7)	**0.42[-0.03 – 0.86]**	**0.065**	**0.49 [0.002 – 0.97]**	**0.049**
Maternal allergic rhinitis	No	181 (95.3)	Reference			
	Yes	9 (4.7)	-0.31[-0.7 – 0.08]	0.118		
Maternal atopic dermatitis	No	185 (97.4)	Reference			
	Yes	5 (2.6)	-0.34[-0.85 – 0.18]	0.198		
Maternal food allergy	No	186 (97.9)	Reference			
	Yes	4 (2.1)	0.21[-0.37 – 0.78]	0.474		
Paternal no allergy	No	21 (11.1)	Reference			
	Yes	169 (88.9)	**0.32[0.06 – 0.59]**	**0.018**	-0.05 [-0.53 – 0.43]	0.831
Paternal asthma	No	186 (97.9)	Reference			

	Yes	4 (2.1)	0.14[-0.44 – 0.71]	0.638		
Paternal allergic rhinitis	No	180 (94.7)	Reference			
	Yes	10 (5.3)	-0.37[-0.74 – 0]	0.053	-0.33 [-0.96 – 0.31]	0.311
Paternal atopic dermatitis	No	187 (98.4)	Reference			
	Yes	3 (1.6)	-0.59[-1.25 – 0.07]	0.082	-0.626 [-1.43 – 0.18]	0.125
Paternal food allergy	No	188 (98.9)	Reference			
	Yes	2 (1.1)	-0.07[-0.88 – 0.74]	0.863		
Maternal animal exposure	No	83 (45.1)	Reference			
	Yes	101 (54.9)	-0.15[-0.43 – 0.13]	0.283		
Immunisation status	Complete	176 (92.1)	Reference			
	Incomplete	15 (7.9)	0.04[-0.28 – 0.35]	0.814		

Table 5.4. The relationship between the Shannon diversity index of lesional skin samples and clinical and environmental factors.

Explanatory variables		**No. of observations (%)**	**Coefficient [95% CI]**	**p-value**	**Adjusted coefficient [95% CI]**	**p-value**
Sex	Female	48 (46.2)	Reference			
	Male	56 (52.8)	-0.28[-0.62 – 0.06]	0.108	-0.16 [-0.44 – 0.36]	0.842
Family size	Small	61 (58.1)	Reference			
	Large	46 (43.8)	-0.01[-0.36 – 0.35]	0.974		
HIV exposure	Exposed	23 (23.2)	Reference			
	Unexposed	76 (76.8)	-0.13[-0.55 – 0.3]	0.56		
Ever breastfed	No	89 (84)	Reference			
	Yes	17 (16)	-0.35[-0.82 – 0.12]	0.139		
Allergic rhinitis	No	68 (97.1)	Reference			
	Yes	2 (2.9)	**-1.54[-2.65 – -0.43]**	**0.007**		
Maternal smoking	No	99 (99)	Reference			
	Yes	1 (1)	0.38[-1.46 – 2.22]	0.68	-0.09 [-2.67 – 2.49]	0.945
Paternal smoking	No	78 (79.6)	Reference			
	Yes	20 (10.4)	-0.01[-0.47 – 0.46]	0.979	0.01 [-0.47 – 0.49]	0.962
Parental education	Primary	3 (2.8)	Reference			
	Secondary	46 (43.4)	-0.66[-1.72 – 0.39]	0.216	-0.6 [-1.73 – 0.54]	0.298
	Tertiary	57 (53.8)	-0.79[-1.84 – 0.26]	0.139	-0.55 [-1.7 – 0.61]	0.347
Mode of delivery	C-section	35 (33.7)	Reference			
	Vaginal	69 (76.3)	0.12[-0.26 – 0.5]	0.534		
Cat ownership	No	84 (84.8)	Reference			
	Yes	15 (15.2)	0.42[-0.09 – 0.93]	0.106		
Dog ownership	No	54 (54.5)	Reference			
	Yes	45 (45.5)	0.19[-0.19 – 0.57]	0.327		

Farm animal contact	No	55 (55.6)	Reference			
	Yes	44 (44.4)	0.26[-0.22 – 0.73]	0.283	0.06 [-0.45 – 0.57]	0.813
Antibiotic exposure	No	23 (21.7)	Reference			
	Yes	83 (78.3)	0.09[-0.34 – 0.51]	0.685		
Probiotic exposure	No	101 (96.2)	Reference			
	Yes	4 (3.8)	-0.21[-1.11 – 0.7]	0.654		
Amasi consumption	No	7 (6.6)	Reference			
	Yes	99 (93.4)	-0.08[-0.78 – 0.62]	0.822		
Electricity + gas	No	4 (4)	Reference			
	Yes	95 (96)	-0.02[-0.95 – 0.91]	0.966	-0.06 [-1.32 – 1.19]	0.922
Paraffin	No	91 (91.9)	Reference			
	Yes	8 (8.1)	-0.22[-0.91 – 0.47]	0.529	-0.22 [-1 – 0.56]	0.576
Indoor fire	No	97 (97)	Reference			
	Yes	2 (3)	1.05[-0.24 – 2.34]	0.11		
Outdoor fire	No	86 (86.9)	Reference			
	Yes	13 (13.1)	0.4[-0.17 – 0.96]	0.169	0.2 [-0.42 – 0.81]	0.522
Electricity	No	75 (75.8)	Reference			
	Yes	24 (24.2)	0.21[-0.26 – 0.68]	0.373	-0.03 [-0.55 – 0.49]	0.902
Gas	No	97 (97)	Reference			
	Yes	2 (3)	0.6[-0.69 – 1.9]	0.358	0.69 [-1.14 – 2.51]	0.456
Kerosene + paraffin	No	32 (32.3)	Reference			
	Yes	67 (67.7)	-0.29[-0.69 – 0.11]	0.156		
Wood + coal	No	95 (96)	Reference			
	Yes	4 (4)	0.65[-0.28 – 1.59]	0.168	-0.06 [-1.27 – 1.56]	0.928
Peanut reaction	No	100 (97.1)	Reference			

	Yes	3 (2.9)	-0.55[-1.55 – 0.46]	0.285		
Nut reaction	No	102 (99)	Reference			
	Yes	1 (1)	**-1.96[-3.65 – -0.27]**	**0.024**	**-1.93 [-3.76 – -0.09]**	**0.039**
Cow milk reaction	No	96 (93.2)	Reference			
	Yes	7 (6.8)	-0.49[-1.17 – 0.18]	0.15		
Egg reaction	No	95 (92.2)	Reference			
	Yes	8 (7.8)	-0.3[-0.93 – 0.34]	0.355		
Wheat reaction	No	102 (96.2)	Reference			
	Yes	4 (3.8)	0.73[-0.16 – 1.63]	0.108		
Fish reaction	No	102 (96.2)	Reference			
	Yes	4 (3.8)	-0.51[-1.41 – 0.4]	0.269		
Food allergy	No	86 (81.1)	Reference			
	Yes	20 (18.9)	-0.2[-0.64 – 0.24]	0.361		
Maternal no allergy	No	24 (22.9)	Reference			
	Yes	81 (77.1)	**0.49[0.08 – 0.9]**	**0.018**	0.43 [-0.05 – 0.92]	0.083
Maternal asthma	No	99 (94.3)	Reference			
	Yes	6 (5.7)	-0.36[-1.11 – 0.4]	0.348		
Maternal allergic rhinitis	No	97 (92.4)	Reference			
	Yes	8 (7.6)	-0.09[-0.75 – 0.56]	0.779		
Maternal atopic dermatitis	No	100 (95.2)	Reference			
	Yes	5 (4.9)	-0.31[-1.13 – 0.51]	0.456		
Maternal food allergy	No	102 (97.1)	Reference			
	Yes	3 (2.9)	-0.81[-1.84 – 0.23]	0.125		
Paternal no allergy	No	17 (16.2)	Reference			
	Yes	88 (83.8)	0.08[-0.4 – 0.55]	0.747		

Paternal asthma	No	102 (97.1)	Reference			
	Yes	3 (2.9)	-0.19[-1.25 – 0.87]	0.726		
Paternal allergic rhinitis	No	95 (90.5)	Reference			
	Yes	10 (9.5)	0.01[-0.6 – 0.61]	0.985		
Paternal atopic dermatitis	No	102 (97.1)	Reference			
	Yes	3 (2.9)	-0.36[-1.41 – 0.69]	0.494		
Paternal food allergy	No	103 (98.1)	Reference			
	Yes	2 (1.9)	0.39[-0.88 – 1.67]	0.543		
Maternal animal exposure	No	50 (50.5)	Reference			
	Yes	49 (49.5)	0.2[-0.27 – 0.67]	0.402		
Immunisation status	Complete	92 (86.8)	Reference			
	Incomplete	14 (13.2)	0.04[-0.49 – 0.56]	0.887		

5.4.5. Beta-diversity

Clustering of microbiomes, from both skin and nasal bacterial communities, was based on the sample type, in the whole cohort (Figure 5.4), and within each area (Figure S5.6), in both cases and controls. Nonlesional skin samples were significantly dissimilar between cases and controls (Figures 5.5 and S5.7). In contrast, samples from the anterior nares of cases and controls did not cluster differently in the overall cohort (Figure 5.5), but when stratified by geographical location, were dissimilar between rural cases and controls, with no dissimilarities in the urban group (Figure S5.7). Moreover, clustering was dissimilar between nonlesional skin and anterior nares from rural and urban controls (Figure S5.8); with no significant dissimilarity between sample types from rural and urban cases (Figure S5.8).

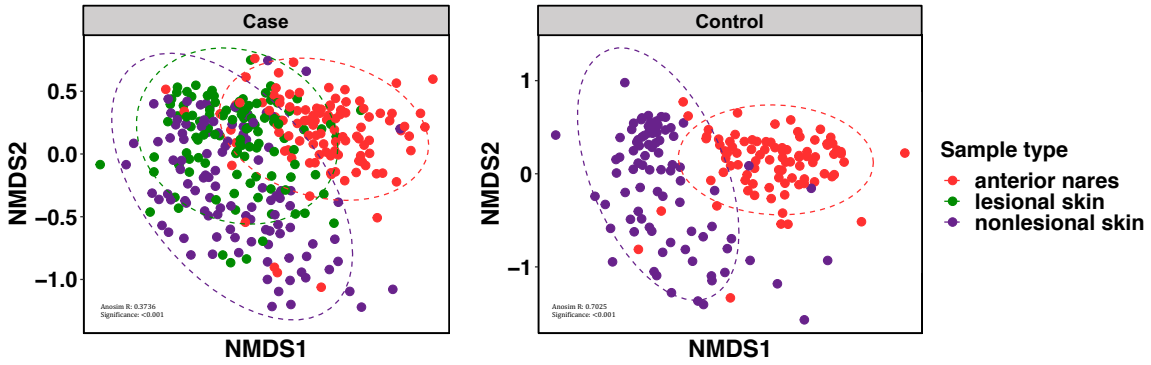


Figure 5.4. Non-metric multidimensional scaling distance matrixes based on Bray-Curtis dissimilarities between skin and anterior nares samples from cases and controls irrespective of geographical location.

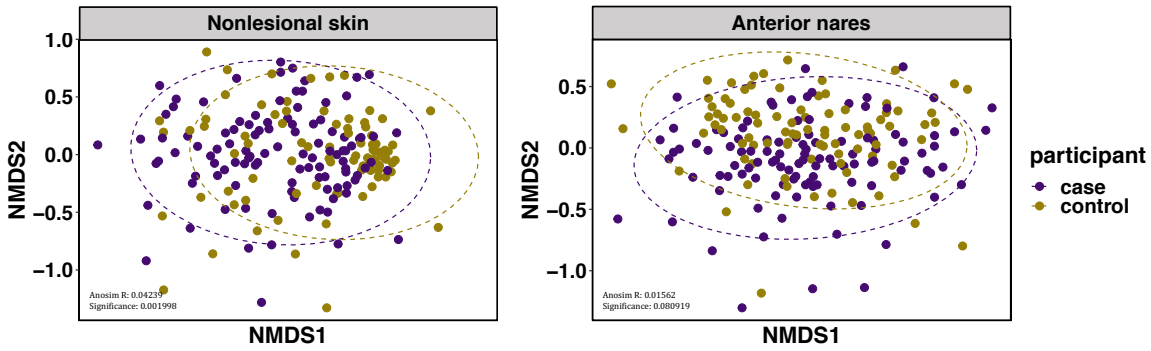


Figure 5.5. Non-metric multidimensional scaling distance matrixes based on Bray-Curtis dissimilarities of nonlesional skin and anterior nares samples between cases and controls irrespective of geographical location.

5.4.6. Differentially abundant taxa on nonlesional skin

We identified 14 genera that were differentially abundant in the nonlesional skin samples of cases and controls (Figure 5.6B). The relative abundance of UCG-005 (*Ruminococcaceae*), *Rothia*, *Romboutsia*, *Lachnoanaerobaculum*, *Haematobacter* and *Christensenellaceae* was significantly lower in the nonlesional skin samples of cases compared with controls. In contrast, UCG-010 (*Lachnospiraceae*), *Rikenellaceae*, *Geobacillus*, *Finegoldia*, *Cutibacterium*, *Brochothrix*, *Anoxybacillus* and *Alistipes* were significantly enriched in the nonlesional skin samples of cases than controls. When stratified by location, the increased relative abundance of two genera, *Anoxybacillus* and *Geobacillus*, in the urban group and four genera, *Alistepes, Brochothrix*, *Rothia* and *Flavobacterium*, in the rural group was detected in the nonlesional skin samples of cases and controls (Figures S5.9 and S5.10). In contrast, no taxa in the urban group were more abundant in the nonlesional skin of controls, compared to cases (Figure S5.10), but 22 genera such as *Corynebacterium* and *Brachybacterium* were enriched in nonlesional skin of controls than cases in the rural group (Figure S5.9).

5.4.7. Differentially abundant taxa on lesional skin and nonlesional skin

Numerous genera were differentially abundant between lesional skin and nonlesional skin samples among cases (Figure 5.6). Here, there was a reduction in the relative abundance of *Undibacterium*, *Serratia*, *Lautropia*, *Geobacillus*, *Fusobacterium*, *Capnocytophaga*, *Anoxybacillus* and *Abiotropia* on lesional skin. In contrast, *Veillonella*, *Streptococcus*, *Prevotella*, *Porphyromonas*, *Neisseria*, *Moraxella*, *Leptotrichia*, *Haemophilus*, *Granulicatella*, *Gemella*, *Alloprevotella* and *Actinomyces* were enriched on lesional skin than nonlesional skin. When stratified by location, there was overrepresentation of *Capnocytophaga* in urban cases, whereas rural cases showed *Rothia*, *Aggregatibacter*, *Lachnoanaerobaculum, Capnocytophaga* and *Abiotrophia* on the lesional skin compared to nonlesional skin. Interestingly, compared to the nonlesional skin of cases, *Fusobacterium* was enriched on the lesional skin in the rural group (Figure S5.9) but reduced in the urban group (Figure S5.10). Nonlesional skin samples of controls showed more abundant taxa among rural controls than urban controls (Figure S5.11). Lesional skin samples of controls had a high relative abundance of bacterial genera such as *Streptococcus*, *Veillonella, Haemophilus*, *Neisseria*, *Gemella* and *Staphylococcus* compared with nonlesional skin of controls (Figure 5.6C). Moreover, both

rural and urban groups exhibited a Except for *Staphylococcus*, similar findings were observed within the rural and urban groups (Figures S5.9 and S5.10).

5.4.8. Differentially abundant taxa on the anterior nares

Among anterior nares samples, 10 genera were differentially abundant in cases compared with controls (Figure 5.6D). *Veillonella*, UCG-005 (*Ruminococcaceae*), *Staphylococcus*, *Sphingomonas* and *Anoxybacillus* were significantly abundant in the anterior nares of cases compared with controls, while the relative abundance of *Suttonella*, *Ornithobacterium*, *Moraxella*, *Helcococcus*, *Cutibacterium* was reduced. When stratified by location, *Suttonella* and *Ornithobacterium* remained reduced in the anterior nares of cases than the controls for both locations (Figures S5.9 and S5.10). Additionally, we identified *Gracilibacteria*, which was reduced in the urban group, and *Moraxella*, *Helcococcus* and *Cutibacterium* in the rural group. *Anoxybacillus* was increased only in the urban group, whereas *Veillonella*, UCG-005 (*Ruminococcaceae*), *Sphingomonas* only in the rural group.

5.4.9 Differentially abundant taxa based on AD severity for each sample type

No bacterial genera were differentially abundant on the nonlesional skin or the anterior nares of cases with moderate or severe disease. However, lesional skin samples of cases with severe disease were enriched with *Stenotrophomonas*, compared with those with moderate disease (CLR mean difference = 0.005; W statistic = 144), when geographic location was not considered. There were no differentially abundant taxa for all sample types when stratified by rural or urban living.

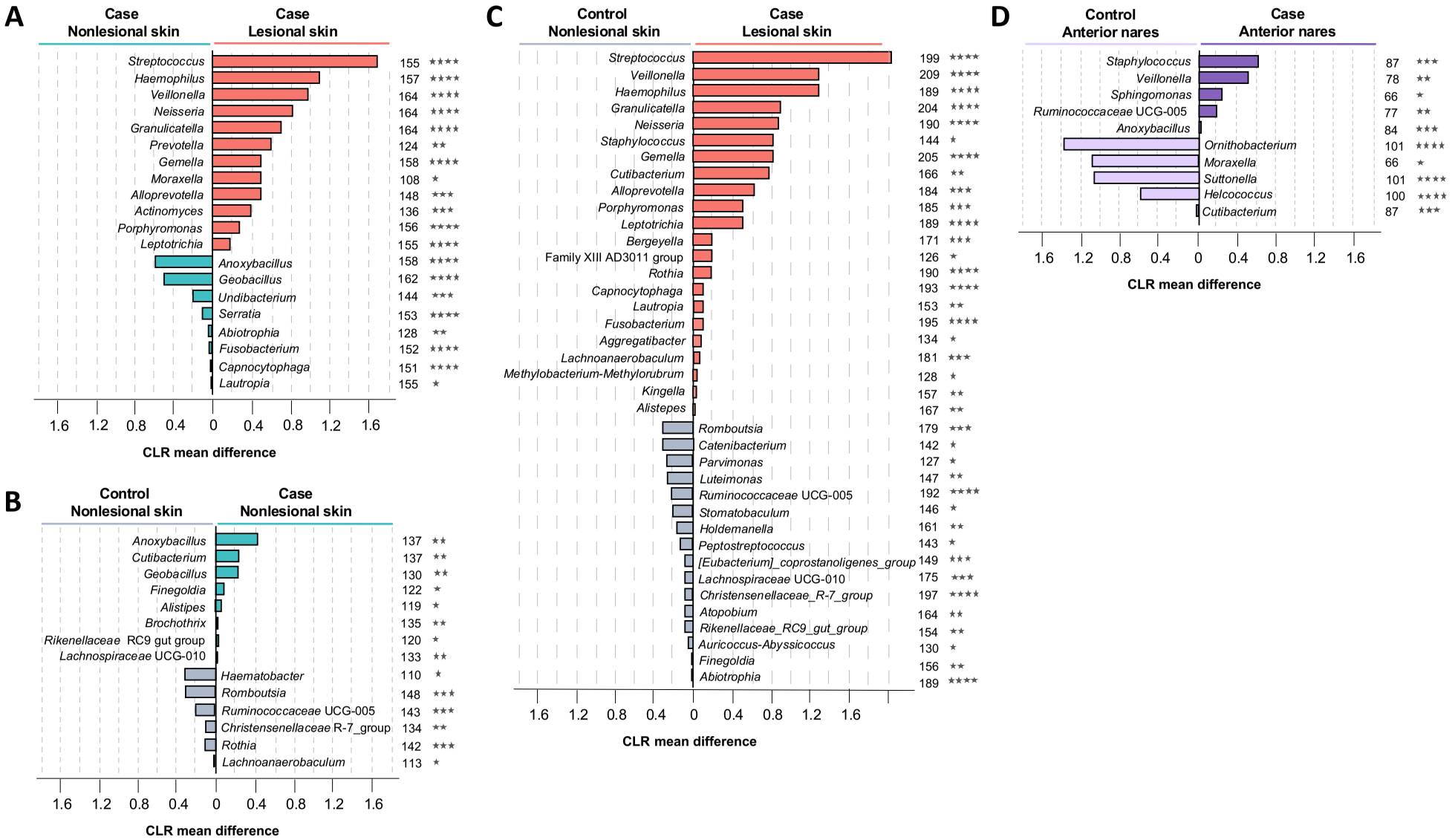

A
Case
Nonlesional skin
Case
Lesional skin
Streptococcus 155 ****
Haemophilus 157 ****
Veillonella 164 ****
Neisseria 164 ****
Granulicatella 164 ****
Prevotella 124 **
Gemella 158 ****
Moraxella 108 *
Alloprevotella 148 ***
Actinomyces 136 ***
Porphyromonas 156 ****
Leptotrichia 155 ****
Anoxybacillus 158 ****
Geobacillus 162 ****
Undibacterium 144 ***
Serratia 153 ****
Abiotrophia 128 **
Fusobacterium 152 ****
Capnocytophaga 151 ****
Lautropia 155 *
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference
B
Control
Nonlesional skin
Case
Nonlesional skin
Anoxybacillus 137 **
Cutibacterium 137 **
Geobacillus 130 **
Finegoldia 122 *
Alistipes 119 *
Brochothrix 135 **
Rikenellaceae RC9 gut group 120 *
Lachnospiraceae UCG-010 133 **
Haematobacter 110 *
Romboutsia 148 ***
Ruminococcaceae UCG-005 143 ***
Christensenellaceae R-7_group 134 **
Rothia 142 ***
Lachnoanaerobaculum 113 *
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference
C
Control
Nonlesional skin
Case
Lesional skin
Streptococcus 199 ****
Veillonella 209 ****
Haemophilus 189 ****
Granulicatella 204 ****
Neisseria 190 ****
Staphylococcus 144 *
Gemella 205 ****
Cutibacterium 166 **
Alloprevotella 184 ***
Porphyromonas 185 ***
Leptotrichia 189 ****
Bergeyella 171 ***
Family XIII AD3011 group 126 *
Rothia 190 ****
Capnocytophaga 193 ****
Lautropia 153 **
Fusobacterium 195 ****
Aggregatibacter 134 *
Lachnoanaerobaculum 181 ***
Methylobacterium-Methylorubrum 128 *
Kingella 157 **
Alistepes 167 **
Romboutsia 179 ***
Catenibacterium 142 *
Parvimonas 127 *
Luteimonas 147 **
Ruminococcaceae UCG-005 192 ****
Stomatobaculum 146 *
Holdemanella 161 **
Peptostreptococcus 143 *
[Eubacterium]_coprostanoligenes_group 149 ***
Lachnospiraceae UCG-010 175 ***
Christensenellaceae_R-7_group 197 ****
Atopobium 164 **
Rikenellaceae_RC9_gut_group 154 **
Auricoccus-Abyssicoccus 130 *
Finegoldia 156 **
Abiotrophia 189 ****
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference
D
Control
Anterior nares
Case
Anterior nares
Staphylococcus 87 ***
Veillonella 78 **
Sphingomonas 66 *
Ruminococcaceae UCG-005 77 **
Anoxybacillus 84 ***
Ornithobacterium 101 ****
Moraxella 66 *
Suttonella 101 ****
Helcococcus 100 ****
Cutibacterium 87 ***
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference

Figure 5.6. ANCOM2 of differentially abundant genera for skin and anterior nares samples between cases and controls. Differentially abundant genera (A) between lesional and nonlesional skin of cases, (B) between the nonlesional skin of cases and controls, (C) between lesional skin of cases and nonlesional skin of controls, and (D) between the anterior nares of cases and controls. An ANCOM detection level of ≥0.6 was considered significant. The raw W statistic (indicates the number of times the null hypothesis (that the average abundance of a given genus in a group is equal to that in the other group) was rejected) for each comparison is shown right of each bar. The stars right of the W statistic indicates the ANCOM detection levels: one star represents ≥0.6, two stars represents ≥0.7, three stars represent ≥0.8, and four stars represent ≥0.9. The length of each bar represents the centred log-ratio (CLR) mean difference in relative abundance.

5.5. Discussion

This chapter investigated the differences in the diversity and structure of the skin and nasal bacteriomes of rural and urban South African children, with and without AD. We report that the bacterial (alpha) diversity and community structure of the skin, but not of the anterior nares, is significantly distinct between South African children with and without AD, with greater diversity in healthy children. However, these observations were limited to rural children when geographic location was considered. Moreover, lesional skin of both rural and urban cases had a predominance of *Streptococcus*, *Veillonella* and *Granulicatela* compared with nonlesional skin of both cases and controls. Also, *Veillonella*, *Sphingomonas* and *Ruminococcaceae* were enriched in the anterior nares of cases compared to controls, although this was mostly limited to the rural group.

Skin bacterial dysbiosis is a key feature of AD regardless of age, with a low alpha diversity on the skin lesions of patients with AD compared to the unaffected skin (nonlesional skin) of both patients and controls[50,64,318,319]. In this study, the alpha diversity of the lesional skin of cases was not different from the nonlesional skin of cases, but it was significantly lower than the nonlesional skin of controls. Although, these findings were limited to the rural group when geographic location was considered. The difference in alpha diversity is driven by the bloom of a single bacterial taxa, particularly *Staphylococcus*, because of AD-associated changes in the epidermal function and immunity[51,312]. Therefore, these data suggest potential proliferation of *Staphylococcus* on lesional skin, that is higher than the nonlesional skin of controls, but similar to the nonlesional skin of cases. As with skin dysbiosis, patients with AD tend to have a lower bacterial diversity than controls due to the proliferation of *Staphylococcus*, and subsequent reduction in bacterial diversity[318]. In contrast, we found no difference in the nasal alpha diversity in children with AD compared to controls, independent of location. This indicates a possible absence of taxa driving dysbiosis. Moreover, the low skin and nasal bacterial diversity in patients with AD is associated with increased disease severity, also owing to staphylococcal predominance[64,318]. However, bacterial diversity between cases with severe and moderate disease was similar for each sample type, even when stratified by rural and urban living. Taken together, these findings indicate that skin bacterial dysbiosis, especially among rural children, is key in AD pathology in this cohort. However, nasal bacterial dysbiosis and disease severity are not implicated.

In line with previous studies[302], we found a negative association of child and/or parental allergic disease with bacterial diversity, regardless of sample type. Interestingly, a higher bacterial diversity was found in the anterior nares of children with asthmatic mothers, and on nonlesional skin of those born of fathers with AD or have a positive reaction to wheat, Although the relationship between wheat allergy and increased bacterial diversity is unclear in the literature, wheat allergy tends to be associated with the enrichment of bacteria such as *Ruminococcaceae*[320], which are linked to high bacterial diversity[321]. Also, early life consumption of *amasi*, a South African fermented milk drink that tends to be protective against the development of allergy[154], was associated with higher bacterial diversity on nonlesional skin. Moreover, living in an electrified house that also uses gas for heating and/or cooking (usually a marker for urbanisation), was associated with a lower bacterial diversity on nonlesional skin[302,322]. This finding supports studies that demonstrated a lower skin bacterial diversity with increasing urbanisation[322] may lead to an increase in the incidence of immune-mediated skin diseases[323].

We identified a high relative abundance of certain genera, including *Streptococcus*, *Veillonella*, *Gemella*, *Prevotella*, *Porphyromonas*, *Leptotrichia*, *Haemophilus*, *Granulicatella*, *Alloprevotella* on lesional skin compared to nonlesional skin of cases and controls. Zheng et. al.[164] and Song et. al.[165] reported a lower relative abundance of *Veillonella*, *Granulicatella*, *Prevotella*, *Porphyromonas* and *Leptotrichia* in Chinese infants with AD compared to healthy infants. However, these genera have been shown to be enriched in some children with AD[50,51] and can be pathogenic in other diseases such as endocarditis and skin and soft tissue infections[324,325]. In this regard, their predominance on lesional skin in this study suggests that they might contribute to AD pathology.

Streptococcus, *Haemophilus* and *Gemella* are sometimes enriched in AD, with some members of the genera *Streptococcus* and *Gemella* associated with the development of childhood allergic disease[51,326-328]. The relative abundance of *Streptococcus* tends to be reduced on lesional skin compared nonlesional skin of children with and without AD[50,164,329]. However, other studies report an increased relative abundance of *Streptococcus* on AD lesions compared to nonlesional skin of both cases and controls[51,326]. A longitudinal birth cohort study in the United Kingdom revealed that children affected by AD at 12 months of age had a high relative abundance of the family *Streptococcacaea*[47]. Moreover, a high relative abundance of *Streptococcus* was previously reported in Egyptian children[51] and Singaporean adults[326]. Group

A *Streptococcus* (GAS; especially in co-infection with *Staphylococcus aureus*), *Streptococcus bovis*, and *Streptococcus viridans* are more common in children with AD than in healthy children and can exacerbate chronic skin inflammation[151,330-332]. Also, children with GAS typically have a greater facial involvement of AD lesions and a higher risk of invasive infections and hospitalisations[328]. These data suggest that some members of the genus *Streptococcus* play a role in AD pathology, in mechanisms yet to be described, and may be linked to inducing pro-inflammatory responses and local tissue destruction[333]. However, the predominant *Streptococcus* species on lesional skin in our cohort cannot be determined because the sequencing of the V4-V5 region of 16S rRNA employed in this study, is limited to the genus level phylogenetic resolution. In this regard, we recommend the employment of high-resolution sequencing or a species-specific qPCR to identify the species that are driving the enrichment of *Streptococcus* in this cohort.

The contribution of *Veillonella*, *Prevotella*, *Porphyromonas*, *Leptotrichia*, *Granulicatella*, *Alloprevotella* and *Actinomyces* in AD flares is limited in the literature, although their clinical relevance in other inflammatory diseases have been demonstrated. For example, increased *Prevotella* abundance is implicated in diseases with an inflammation-dependent aetiology[334]. Specifically, *Prevotella* stimulates epithelial cells to produce proinflammatory cytokines interleukin (IL)-6 and IL-8, which drive T helper 17 (Th-17) immune responses[334]. Th-17 immune responses are associated with chronic inflammation in AD lesions[335]. Similarly, *Granulicatella*[336,337], *Leptotrichia*[338] and *Porphyromonas*[339] also induce IL-6 and IL-8 secretion, as well as other proinflammatory cytokines such as IL-1β, MCP-1 and TNF-α. The predominance of these genera and their proposed induction of the pro-inflammatory IL-6/IL-8 signalling in AD is supported by findings in a previous study conducted on this cohort, which reported high circulating IL-6 and IL-8 cytokines levels in cases, particularly rural cases[289]. Moreover, IL-6 and IL-8 are generally upregulated on the skin of patients with AD (both lesional and nonlesional) compared to the skin of healthy controls[340,341], and indicate severe inflammation on AD lesions[342]. Therefore, the enrichment of these genera may contribute to AD pathology by driving the putative cutaneous inflammation.

Nonlesional skin, while not under the same immunological and physiological changes as AD lesions, exhibits changes in the bacterial community, distinguishing it from the healthy skin of controls[7]. Specifically, the nonlesional skin of cases has a lower alpha diversity, which is accompanied by the enrichment of *Staphylococcus* (especially *Staphylococcus aureus*),

compared to controls[69,318]. However, changes in the alpha diversity of nonlesional skin in patients with AD are not as significant as those on lesional skin. Similarly, the bacterial diversity on the nonlesional skin of cases was lower than the controls in this study. However, this observation was without the putative concomitant proliferation of the genus *Staphylococcus*. Presently, *Cutibacterium* and *Finegoldia,* among other taxa, were enriched on nonlesional AD skin compared to healthy skin. While the predominance of *Finegoldia* is expected[343], the relative abundance of *Cutibacterium* (a lipophilic genus) tends to decrease on AD nonlesional skin[344].

Recent studies demonstrated that the nasal microbiota in paediatric patients with AD was dominated by *Staphylococcus, Moraxella, Corynebacterium* and *Dolosigranulum*[64,163]. However, these studies did not include a control group to define the "healthy nasal microbiota". Moreover, except for *Staphylococcus*, these genera exist in a high relative abundance in a healthy paediatric nasal bacteriota[65]. It is therefore unclear whether the observed enrichment of these genera has implications in AD pathology or reflects the natural colonisation dynamics of these bacterial genera in the nose in these cohorts. Similarly, in this study, the anterior nares of cases were predominated by *Staphylococcus* and other genera, such as the pro-inflammatory genus *Veillonella*, which is enriched in children with asthma and allergic rhinitis[345,346]. This was different from the anterior nares of controls, which were expectedly predominated by normal nasal residents, including *Moraxella*, *Ornithobacterium*, and other genera (*Suttonella* and *Helcococcus*) which have been demonstrated to have a positive correlation with *Ornithobacterium*[347]. These findings highlight that bacterial shifts in the nasal cavity of children with AD lead to a bacterial community dominated by inflammation-driving bacteria, which may propagate AD pathology.

Only lesional skin samples showed a differential abundance based on disease severity, with the lesional skin of severe cases having a higher relative abundance of *Stenotrophomonas* than those with moderate disease. However, this was not maintained when stratified by geographic location. Dominance of the genus *Stenotrophomonas* has been reported in adult patients with AD[348], and in other inflammatory skin diseases such as radiotherapy-induced dermatitis associated with chronic ulcers[349]. Some members of this genus, such as *Stenotrophomas maltophilia*, are opportunistic pathogens that cause cellulitis, and wound and soft tissue infections[350]. This bacterium also induces neutrophil-dependent inflammation that leads to epithelial damage and is associated with increased levels of the cytokines IL-1β, IL-6, TARC

and MIP-1α[351]. Of note, these cytokines are involved in AD pathology and were previously noted to be increased among the cases in this cohort[289]. Interestingly, *Stenotrophomonas* has also been reported to be enriched in paediatric patients with AD who responded positively to an emollient treatment[55]. Taken together, this finding suggests that *Stenotrophomas* may be contributing to severe disease by promoting inflammation and epithelial damage. However, because of the widespread use of emollients among the cases in this study, the predominance of this genus may also be a consequence of the treatment rather than an active propagator of severe AD. This highlights the need for further studies into the contribution, if any, of *Stenotrophomas* in the pathology of AD in this population.

Environmental exposures may influence the skin and nasal microbial diversity and composition, which contributes to the development of allergic diseases, especially in early childhood[285,302,303]. Rural and urban environments have distinct exposures with which their residents interact, which consequently uniquely influence the diversity and composition of the skin and nasal microbiota[159,161,302]. This leads to disparities in the skin and nasal microbiota of the residents of each geographical locality[285]. Indeed, the skin microbiota of rural residents exhibit greater diversity and has a different bacterial community structure to urban residents[51,161,302]. Similarly, we observed that nonlesional skin of rural controls showed a higher diversity, which was also dissimilar, from the urban controls. This supports the importance of the environment-specific exposures in shaping the skin microbiota[352]. Although similar alpha diversity was observed, rural and urban living contributed to distinct bacterial communities in the anterior nares of controls. This suggests that healthy controls, regardless of rural-urban living, have diverse nasal communities, however, the specific taxa enriched in the anterior nares differ; most likely reflecting the differences in microbial exposures that shape the nasal microbiota. In contrast to controls, we found that the bacterial structure of the skin and anterior nares of cases did not differ between rural and urban environments. This suggests that AD pathology is stronger than the local environment, and its associated bacterial exposures, in determining bacterial diversity and structure.

The indoor microbiome in rural and urban environments reflect their respective external environments, with higher microbial diversity among rural homes than urban homes[304,305]. As previously mentioned, rural-urban living strongly influences the skin and nasal microbial composition. Here, rural residents harbour bacterial communities reflective of the natural

environment, compared to urban residents who influence the microbial communities in urban and industrial spaces[159,161,353]. *Ruminococcaceae* and *Lachnospiraceae*, which are associated with farm environments and lower rates of allergic disease[354,355], were enriched in house dust in a rural environment (specifically rural controls compared to the urban group)[305]. Presently, these taxa were predominant on nonlesional skin samples of rural controls, compared to rural cases and urban controls. Moreover, we observed the predominance of *Sphingomonas*, which is mostly found in soil, plants and water[356], and which is common in rural environments compared to urban environments[322]. Also, *Chloroplast*, a marker of exposure to phytoplankton[357], which also predominates in indoor rural environments[322], was enriched on skin samples of the rural group (regardless of disease status) compared to the urban group. In contrast, human-associated genera, including *Enhydrobacter* and *Hymenobacter*, which are common in urban environments, were increased in healthy urban children compared to healthy rural children[322]. Similarly, the indoor microbiota of children with AD is predominated by human-associated genera such as *Actinomyces*, *Finegoldia*, *Micrococcus*, *Prevotella* and *Cutibacterium*[358]. Of note, these genera, except for *Micrococcus*, presently predominated in lesional and nonlesional skin samples of cases. The predominance of human-associated genera in indoor environments supports the notion that human activity influences the composition and diversity of the indoor environment[353]. However, this may also indicate a potential role of these genera in shaping childhood immunity, in a manner that increases the propensity of developing AD[358]. Collectively, these findings indicate that differences in bacterial exposure from the immediate/indoor environment drive the establishment of skin and nasal bacteriomes in early childhood[352], and may be linked to AD and urban living.

The strengths of this study include the inclusion of children, with and without AD, to define shifts in the bacteriota with AD. Also, the differences in bacterial diversity and composition in distinct environments, and their association with bacterial diversity was assessed. Limitations to our study include the cross-sectional design, which limits the assessment of the changes of the skin and nasal microbiomes in early childhood, the establishment of a causal relationship between these bacterial communities and, exacerbations experienced by children with AD. Our sample size, although moderate, is reduced by the multiple levels of stratification by disease status (case and control), sample type (lesional skin, nonlesional skin and anterior nares), and geographic location (rural and urban). Moreover, the amplification of the V4-V5 region of the 16S rRNA gene limits the taxonomic resolution to the genus-level that precludes the identification of the specific bacterial species associated with disease phenotypes. Recent

studies have demonstrated the involvement of fungi (mycome) and viruses (virome) in AD pathology[59,326], however, this analysis was limited to the bacterial component of the microbiome. Also, the use of emollients, which alters the skin bacterial community[55,300], was not restricted among cases before sampling.

5.6. Conclusion

In conclusion, this study shows differences in skin and nasal bacterial composition and diversity in healthy children depend on rural-urban living. However, AD disease appears to be a stronger influence than geography. Our findings also show shifts in the skin and nasal bacterial communities in children with AD, that likely contribute to the epidermal inflammation and, consequently, epidermal damage, that are specific to this cohort. This suggests that bacterial taxa driving AD pathology among South African children are different from those in other countries and ethnicities. Since current strategies are founded on data from children in Western societies, our data highlight the importance of new treatment strategies for the restoration of the skin and nasal microbiota in AD, that are specific to sub-Saharan African children for managing AD.

5.7. Supplementary Materials

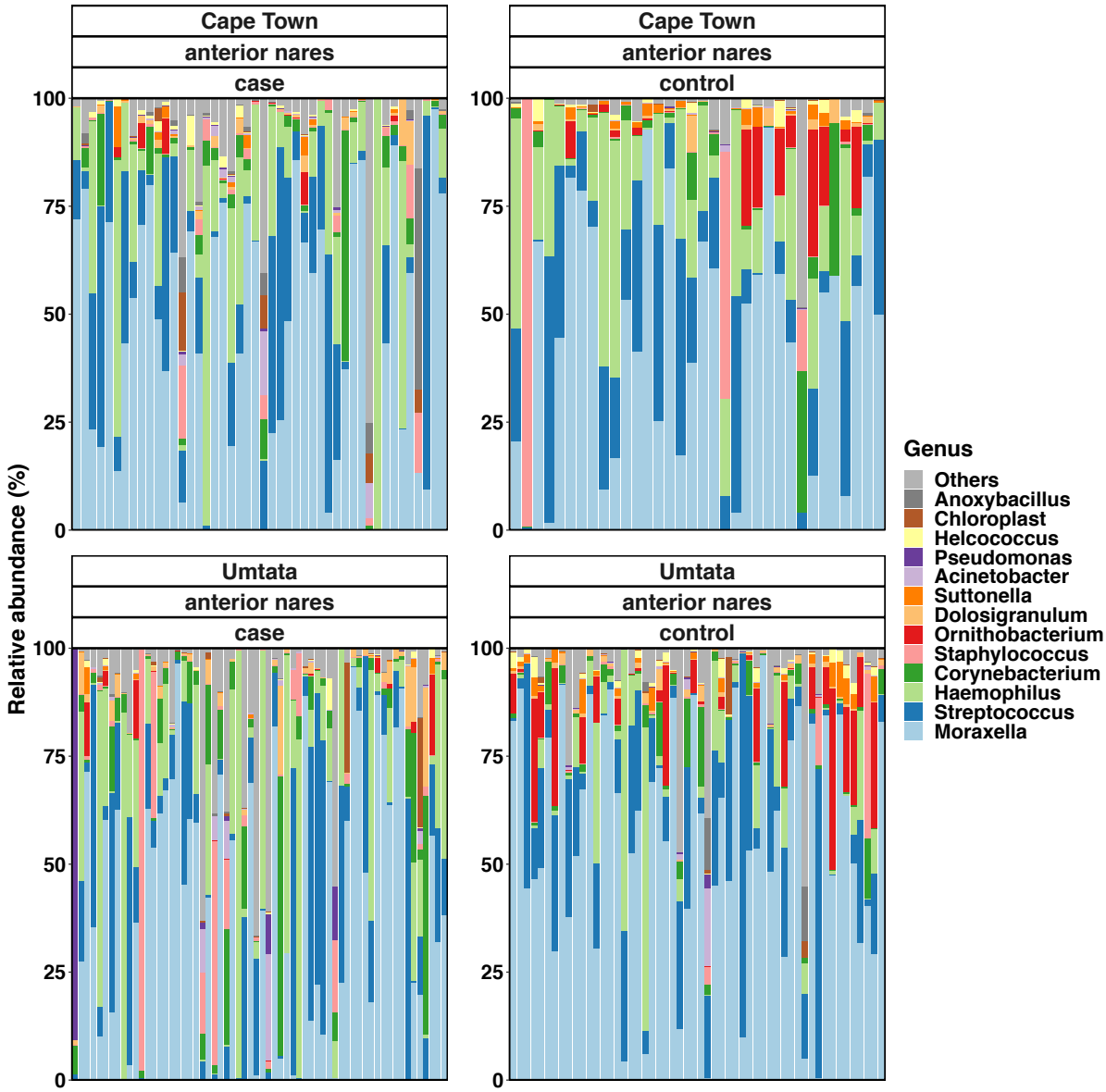


Figure S5.1. Relative abundance of the top 12 bacterial genera in the anterior nares of cases and controls.

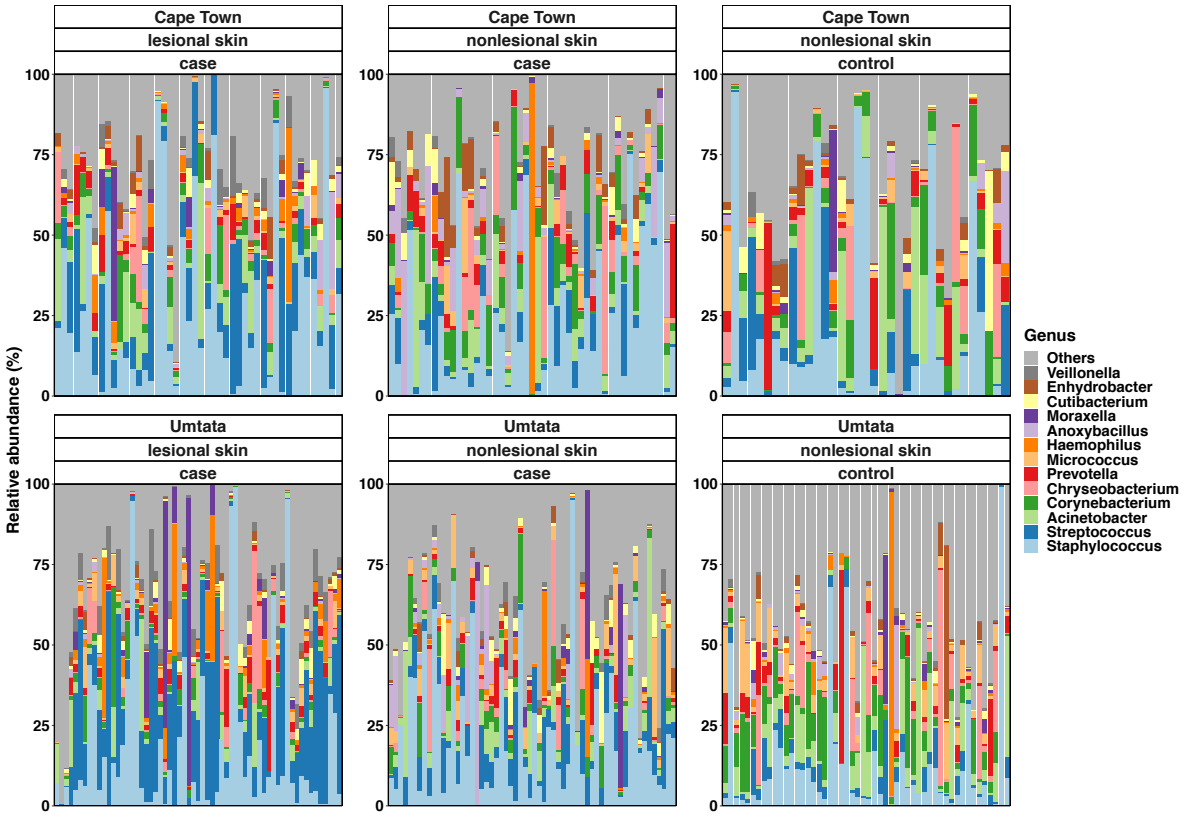


Figure S5.2. Relative abundance of the top 12 bacterial genera from skin samples of cases and controls.

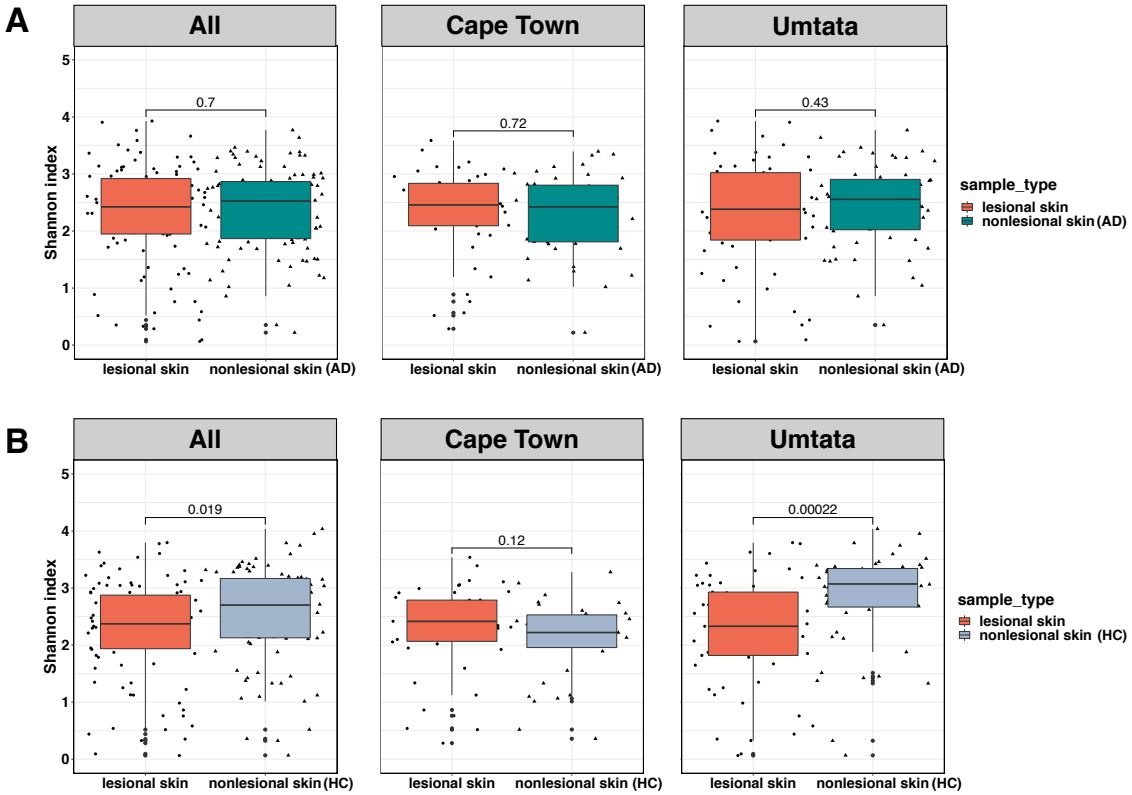


Figure S5.3. Boxplots comparing Shannon diversity index of lesional skin to nonlesional skin samples of both cases (A) and controls (B). AD, atopic dermatitis; HC, healthy control.

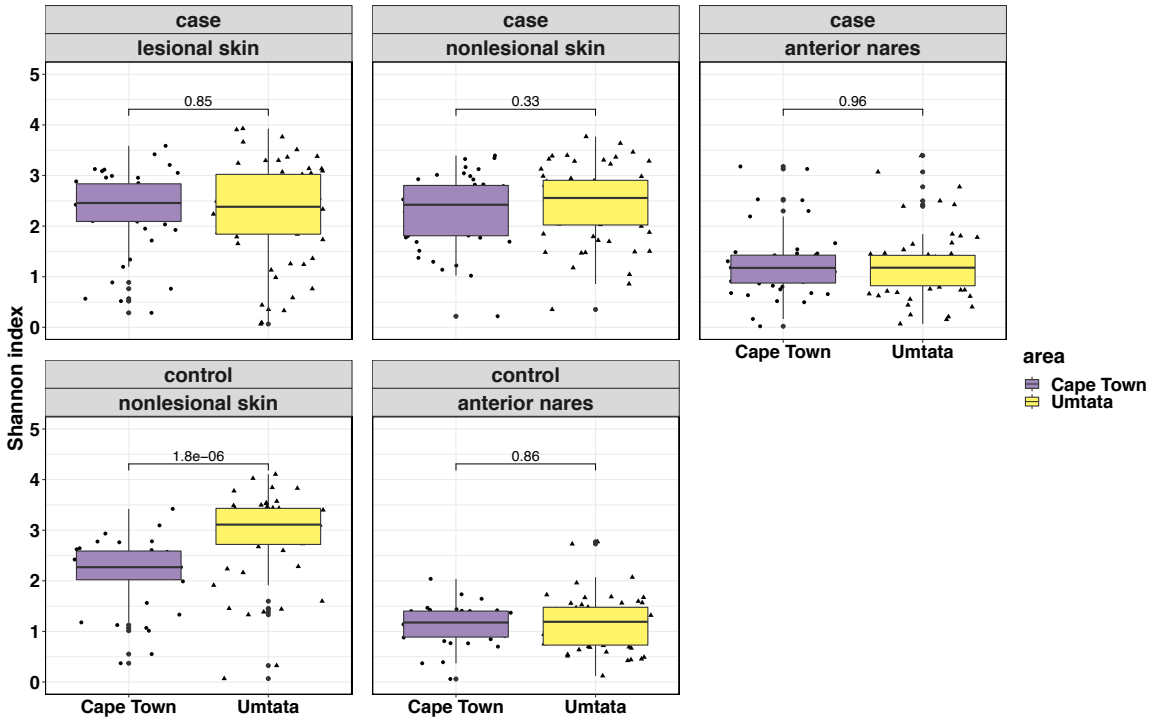


Figure S5.4. Boxplots showing the Shannon diversity index of each sample type between the rural and urban groups.

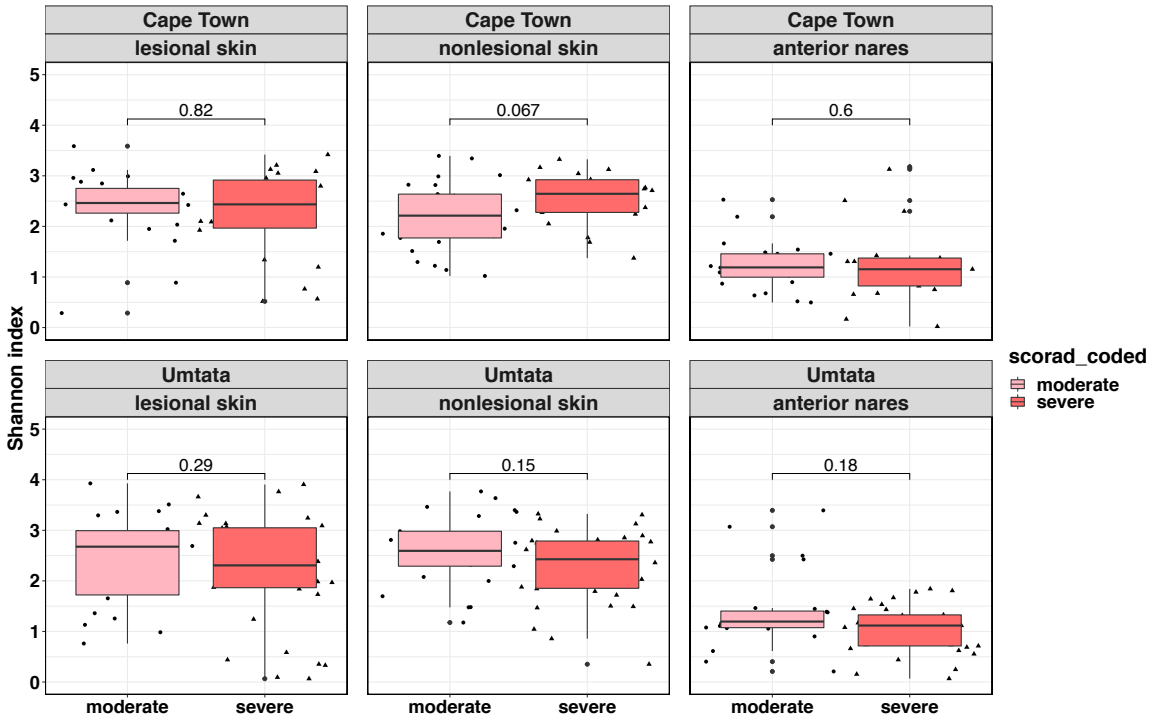


Figure S5.5. Boxplots showing the Shannon diversity index for each sample type among cases from each geographic group.

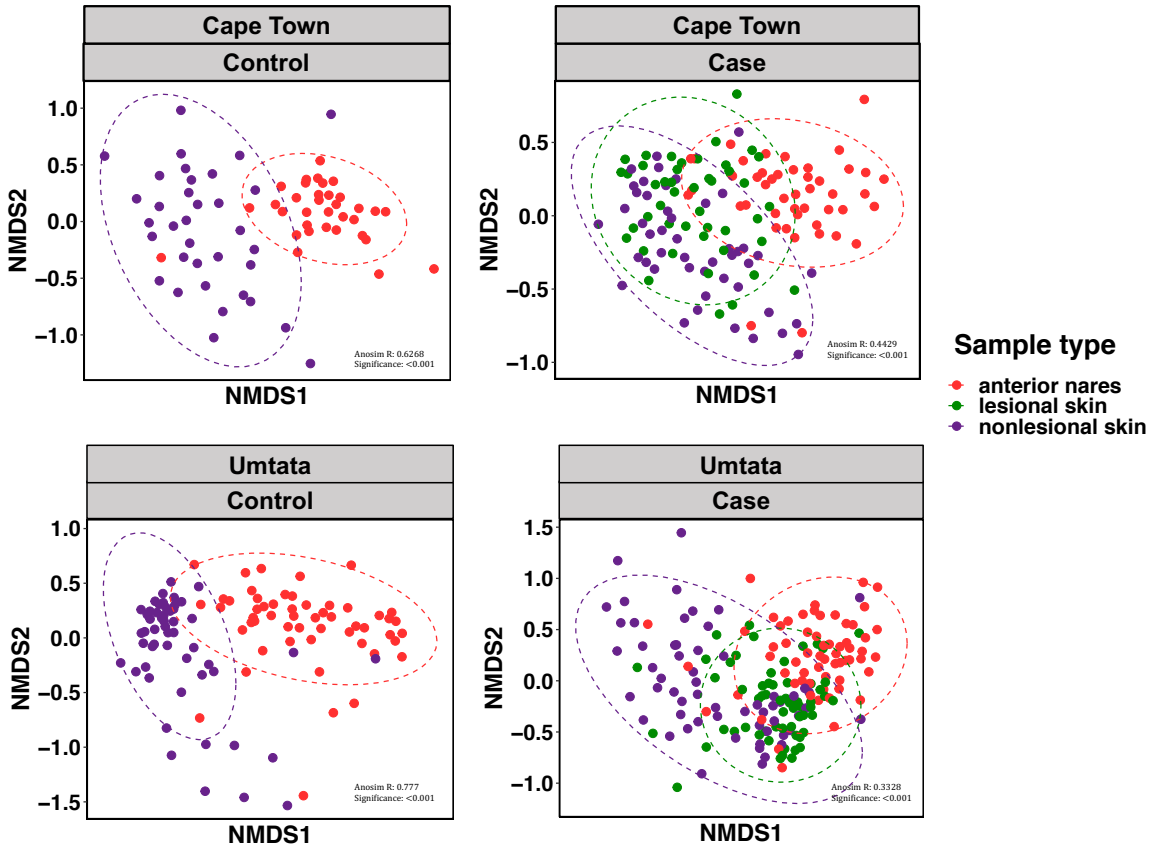


Figure S5.6. Nonmetric multidimensional scaling of Bray–Curtis dissimilarities plot showing the microbiota for nose, lesional and nonlesional skin.

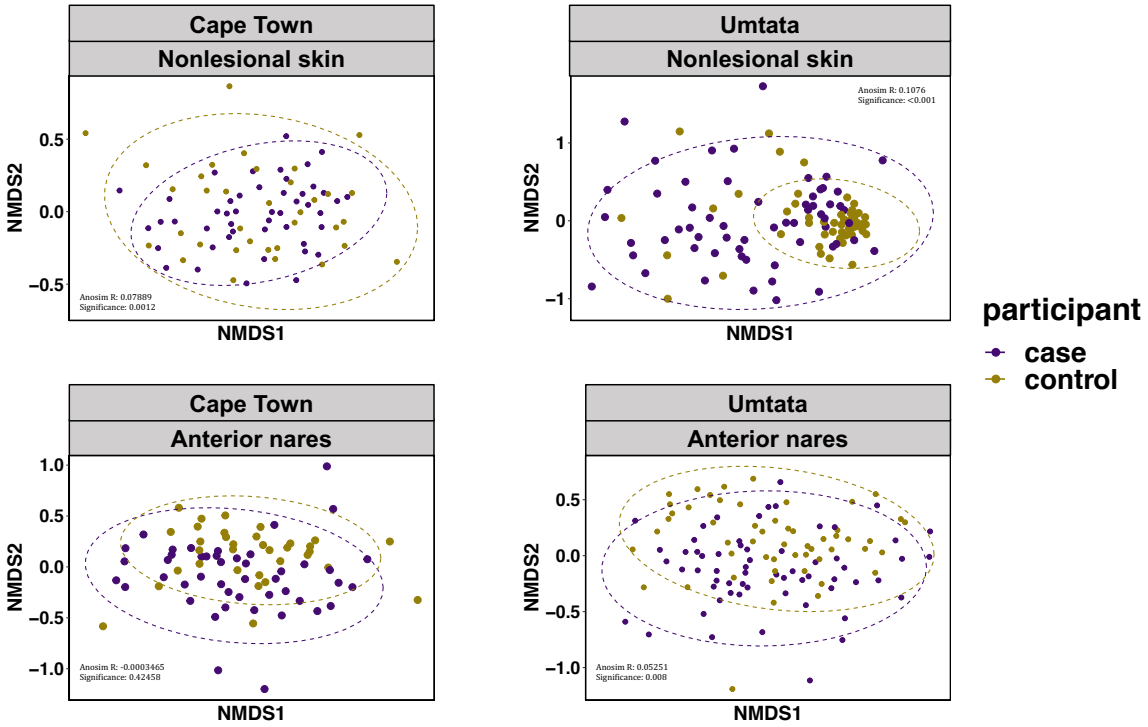


Figure S5.7. Non-metric multidimensional scaling distance matrixes based on Bray-Curtis dissimilarities of nonlesional skin and anterior nares samples between cases and controls in each geographic group.

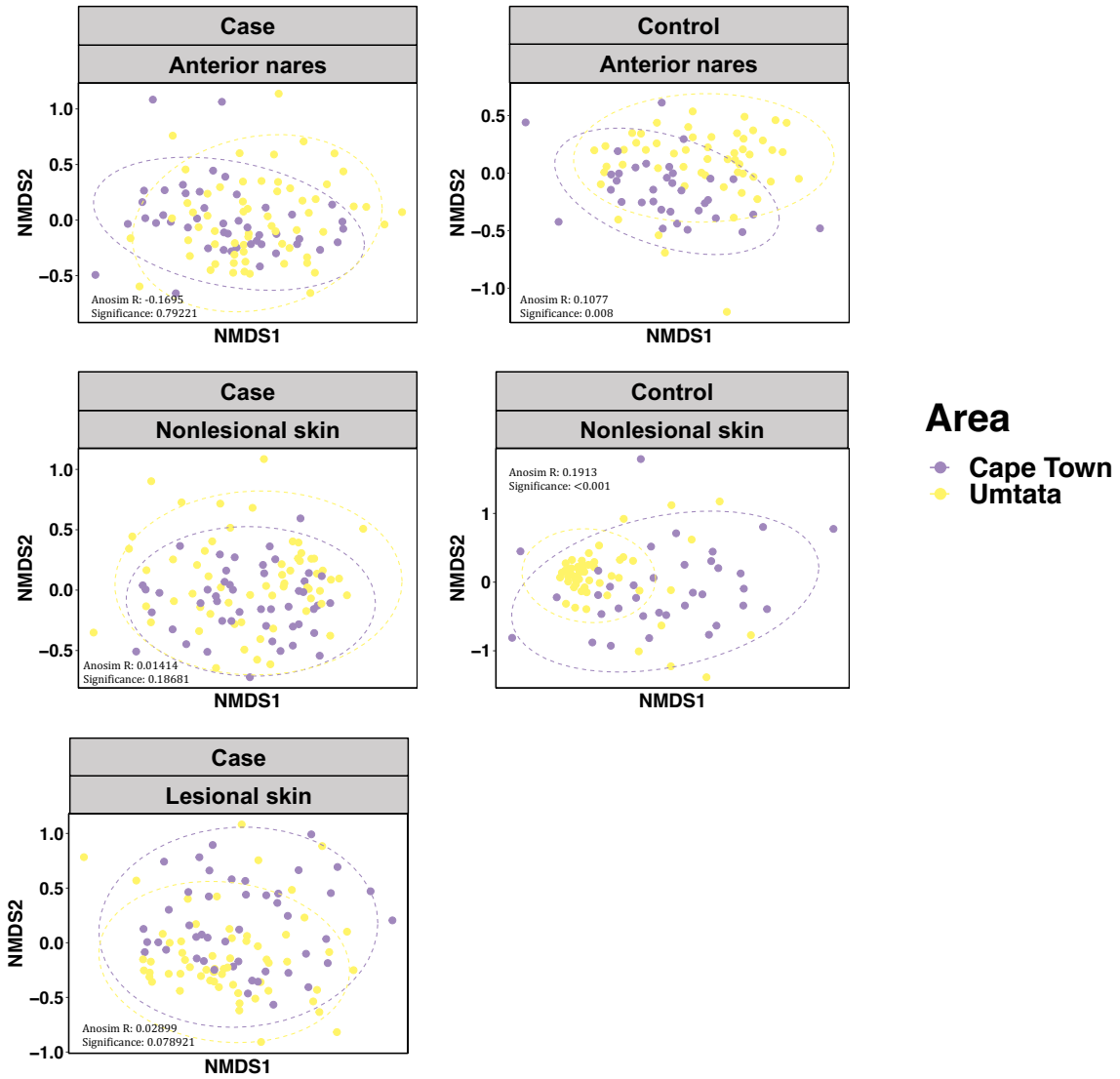


Figure S5.8. Non-metric multidimensional scaling distance matrixes based on Bray-Curtis dissimilarities of each sample type between the rural and urban groups.

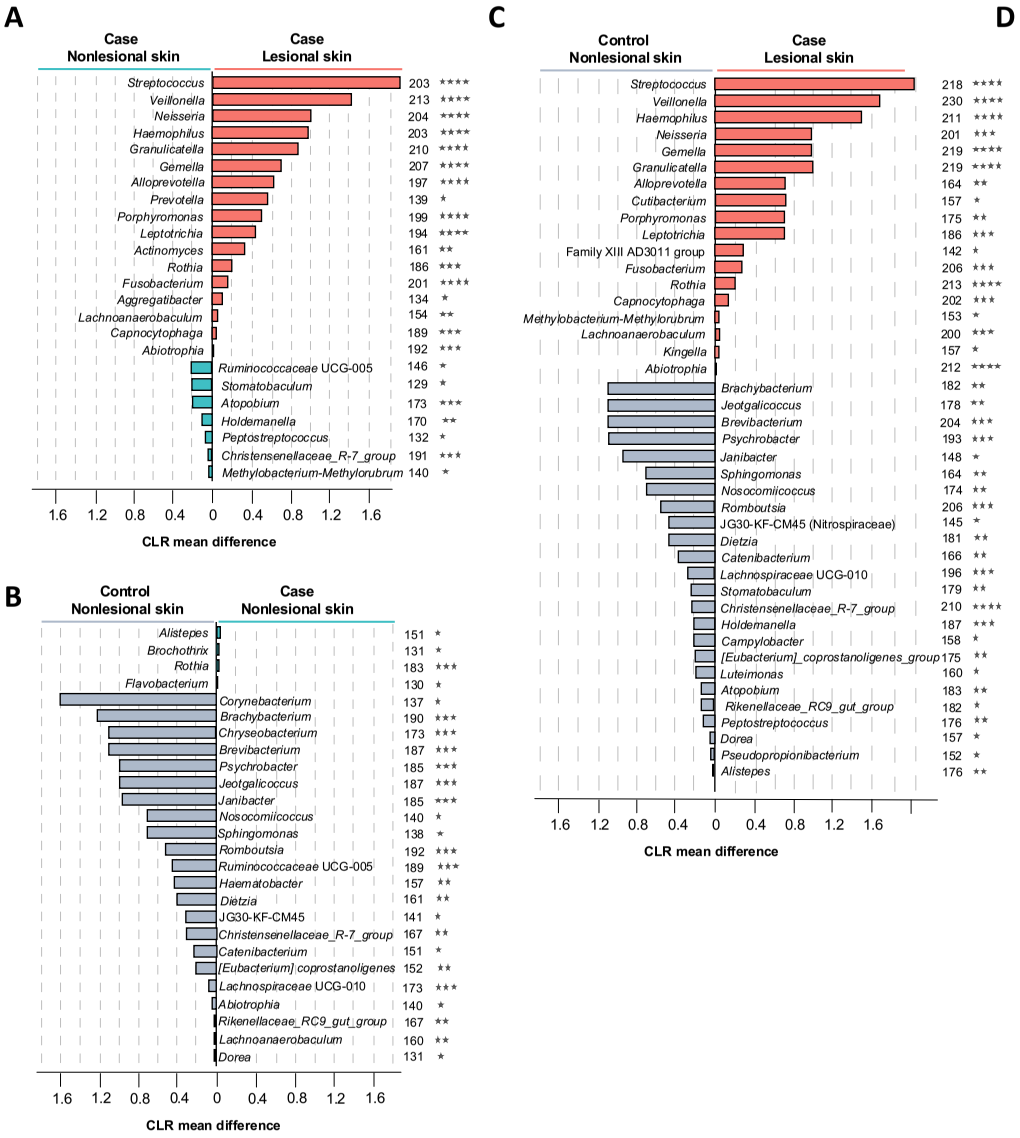

A
Case
Nonlesional skin
Case
Lesional skin
Streptococcus 203 ****
Veillonella 213 ****
Neisseria 204 ****
Haemophilus 203 ****
Granulicatella 210 ****
Gemella 207 ****
Alloprevotella 197 ****
Prevotella 139 *
Porphyromonas 199 ****
Leptotrichia 194 ****
Actinomyces 161 **
Rothia 186 ***
Fusobacterium 201 ****
Aggregatibacter 134 *
Lachnoanaerobaculum 154 **
Capnocytophaga 189 ***
Abiotrophia 192 ***
Ruminococcaceae UCG-005 146 *
Stomatobaculum 129 *
Atopobium 173 ***
Holdemanella 170 **
Peptostreptococcus 132 *
Christensenellaceae_R-7_group 191 ***
Methylobacterium-Methylorubrum 140 *
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference
B
Control
Nonlesional skin
Case
Nonlesional skin
Alistepes 151 *
Brochothrix 131 *
Rothia 183 ***
Flavobacterium 130 *
Corynebacterium 137 *
Brachybacterium 190 ***
Chryseobacterium 173 ***
Brevibacterium 187 ***
Psychrobacter 185 ***
Jeotgalicoccus 187 ***
Janibacter 185 ***
Nosocomiicoccus 140 *
Sphingomonas 138 *
Romboutsia 192 ***
Ruminococcaceae UCG-005 189 ***
Haematobacter 157 **
Dietzia 161 **
JG30-KF-CM45 141 *
Christensenellaceae_R-7_group 167 **
Catenibacterium 151 *
[Eubacterium] coprostanoligenes 152 **
Lachnospiraceae UCG-010 173 ***
Abiotrophia 140 *
Rikenellaceae_RC9_gut_group 167 **
Lachnoanaerobaculum 160 **
Dorea 131 *
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference
C
Control
Nonlesional skin
Case
Lesional skin
Streptococcus 218 ****
Veillonella 230 ****
Haemophilus 211 ****
Neisseria 201 ***
Gemella 219 ****
Granulicatella 219 ****
Alloprevotella 164 **
Cutibacterium 157 *
Porphyromonas 175 **
Leptotrichia 186 ***
Family XIII AD3011 group 142 *
Fusobacterium 206 ***
Rothia 213 ****
Capnocytophaga 202 ***
Methylobacterium-Methylorubrum 153 *
Lachnoanaerobaculum 200 ***
Kingella 157 *
Abiotrophia 212 ****
Brachybacterium 182 **
Jeotgalicoccus 178 **
Brevibacterium 204 ***
Psychrobacter 193 ***
Janibacter 148 *
Sphingomonas 164 **
Nosocomiicoccus 174 **
Romboutsia 206 ***
JG30-KF-CM45 (Nitrospiraceae) 145 *
Dietzia 181 **
Catenibacterium 166 **
Lachnospiraceae UCG-010 196 ***
Stomatobaculum 179 **
Christensenellaceae_R-7_group 210 ****
Holdemanella 187 ***
Campylobacter 158 *
[Eubacterium]_coprostanoligenes_group 175 **
Luteimonas 160 *
Atopobium 183 **
Rikenellaceae_RC9_gut_group 182 *
Peptostreptococcus 176 **
Dorea 157 *
Pseudopropionibacterium 152 *
Alistepes 176 **
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference
D
Control
Anterior nares
Case
Anterior nares
Veillonella 92 ***
Ruminococcaceae UCG-005 93 ***
Sphingomonas 82 **
Moraxella 94 ***
Ornithobacterium 110 ****
Suttonella 110 ****
Helcococcus 110 ****
Cutibacterium 80 **
1.6 1.2 0.8 0.4 0 0.4 0.8 1.2 1.6
CLR mean difference

Figure S5.9. ANCOM of differentially abundant genera for skin and anterior nares samples between cases and controls in Umtata. Differentially abundant genera (A) between lesional and nonlesional skins of cases, (B) between the nonlesional skin of cases and controls, (C) between lesional skin of cases and nonlesional skin of controls, and (D) between the anterior nares of cases and controls. An ANCOM detection level of ≥0.6 was considered significant. The raw W statistic (indicates the number of times the null hypothesis (that the average abundance of a given genus in a group is equal to that in the other group) was rejected)for each comparison is shown right of each bar. The stars right of the W statistic indicates the ANCOM detection levels: one star represents ≥0.6, two stars represents ≥0.7, three stars represent ≥0.8, and four stars represent ≥0.9. The length of each bar represents the centred log-ratio (CLR) mean difference in relative abundance.

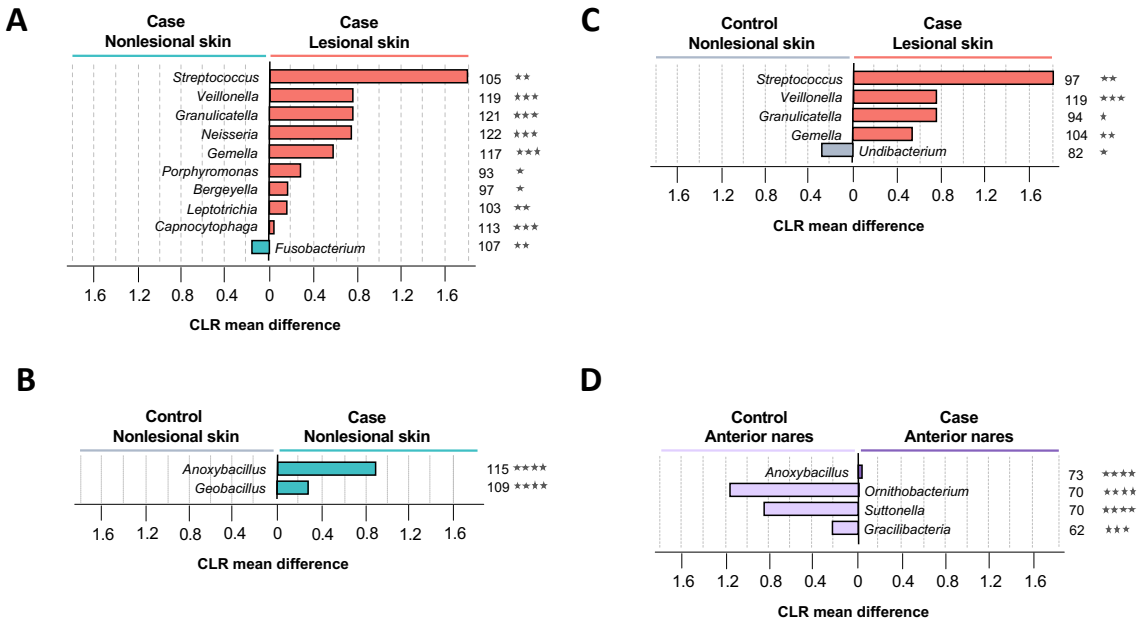
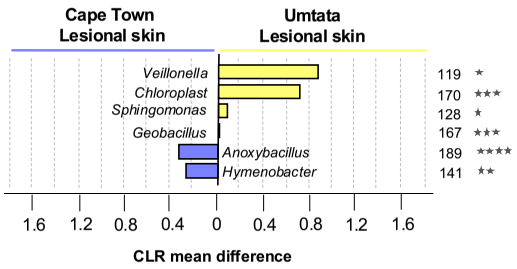
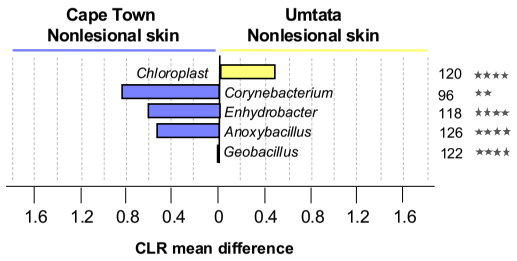
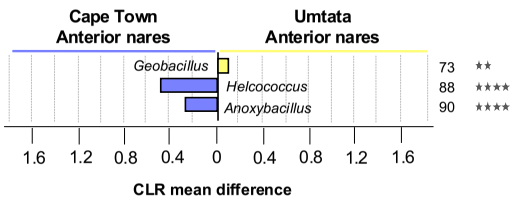


Figure S5.10. ANCOM of differentially abundant genera for skin and anterior nares samples between cases and controls in Cape Town**.** Differentially abundant genera (A) between lesional and nonlesional skins of cases, (B) between the nonlesional skin of cases and controls, (C) between lesional skin of cases and nonlesional skin of controls, and (D) between the anterior nares of cases and controls. An ANCOM detection level of ≥0.6 was considered significant. The raw W statistic (indicates the number of times the null hypothesis (that the average abundance of a given genus in a group is equal to that in the other group) was rejected) for each comparison is shown right of each bar. The stars right of the W statistic indicates the ANCOM detection levels: one star represents ≥0.6, two stars represents ≥0.7, three stars represent ≥0.8, and four stars represent ≥0.9. The length of each bar represents the centred log-ratio (CLR) mean difference in relative abundance.

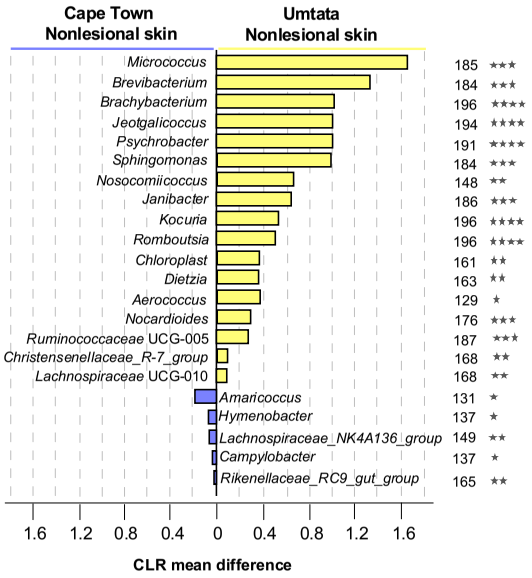
Cases







Controls



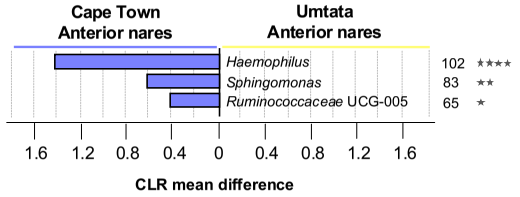


Figure S5.11. ANCOM of differentially abundant genera between Cape Town and Umtata participants. **Cases panel -** Differentially abundant genera between lesional skin samples of Cape Town and Umtata cases (*left panel*), between the nonlesional skin samples of Cape Town and Umtata cases (*middle panel*), and between the anterior nares of Cape Town and Umtata cases (*right panel*). **Controls panel** - Differentially abundant genera between the nonlesional skin samples of Cape Town and Umtata controls (*left panel*) and between the anterior nares of Cape Town and Umtata controls (*right panel*). An ANCOM detection level of ≥0.6 was considered significant. The raw W statistic (indicates the number of times the null hypothesis (that the average abundance of a given genus in a group is equal to that in the other group) was rejected) for each comparison is shown right of each bar. The stars right of the W statistic indicates the ANCOM detection levels: one star represents ≥0.6, two stars represents ≥0.7, three stars represent ≥0.8, and four stars represent ≥0.9. The length of each bar represents the centred log-ratio (CLR) mean difference in relative abundance.

CHAPTER 6

General conclusion and future consideration

The work presented in this thesis provides insights into the skin and nasal bacterial colonisation in South African children with AD, a previously unstudied population, and how these are affected by rural and urban living. First, it focused on characterising staphylococcal colonisation and determining the clinical, environmental, and socio-demographic risk factors associated with staphylococcal colonisation. The dynamics of *S. aureus* to identify genomic differences between children with and without AD was also investigated. Lastly, the changes in skin and nasal bacterial community structure and diversity were assessed.

Genomic epidemiology of *S. aureus* in atopic dermatitis (Chapters 2 and 3)

Colonisation with *S. aureus* is common in children with AD, regardless of rural or urban living, specifically children with severe disease. Moreover, factors associated with rural residency, such as farm animals and use of fossil fuels (e.g., coal and wood) for cooking and heating, were found to have a greater effect on skin and nasal *S. aureus* colonisation. However, due to the limited sample size of this cohort and the biased selection criteria, there is a need for future studies with a larger sample size, and unbiased selection strategy to allow for a detailed risk factor analysis.

The dominant *S. aureus* genotypes (clonal lineages) between cases and controls using *spa* typing was investigated. This study showed that specific *spa* types were associated with cases, independent of rural and urban living, with no prevalent *spa* type among controls. Based on these findings, analysis to explore the pan-genomic differences of *S. aureus* between cases and controls was investigated. The analyses were limited to case-vs.-control due to the low number of available isolates. The *S. aureus* isolates of cases were associated with antibiotic resistance, strong biofilm forming ability, DNA damage repair and virulence genes that promote chronic inflammation and epidermal damage. In contrast, isolates from controls were mostly associated with adhesion. These observations reflect possible adaptational differences in the *S. aureus* strains that are associated with health and AD. Here, strains in children with AD showing a virulence potential to drive AD pathology and evade antibiotic and immune responses targeted at their elimination, while those in controls are more adapted for a non-pathogenic existence. Nonetheless, there is a need for future functional studies to provide understanding on the pathological implications, or lack thereof, of colonisation with these strains.

Coagulase-negative staphylococci in atopic dermatitis (Chapter 4)

Based on the shifting interest in literature suggesting the involvement of CoNS in the pathology of AD, this study assessed the prevalence of CoNS in AD and how they relate to disease severity and the presence of *S. aureus*. Cases were frequently co-colonised with CoNS and *S. aureus* than controls. Moreover, this trend was associated with increased disease severity compared to those colonised with either *S. aureus* or CoNS only, or without any staphylococcal colonisation. These observations were independent of rural and urban living. Different CoNS species were associated with cases depending on rural and urban living, with *S. epidermidis* and *S. haemolyticus* common among rural cases, and *S. capitis* among urban cases. However, *S. capitis* was associated with more severe disease in both rural and urban cases. These observations indicate a potential role of *S. capitis* in driving AD pathology and exacerbating disease.

Skin and nasal microbiomes in atopic dermatitis (Chapter 5)

Bacterial dysbiosis on the skin and anterior nares is common in AD and leads to increased disease severity. It is associated with the predominance of the *Staphylococcus* genus, loss of bacterial diversity, and decrease in the relative abundance of commensal genera such as *Streptococcus and Corynebacterium*. The shifts in the bacterial community between cases and controls from the rural and urban environments were investigated. The bacterial diversity and community structure of the skin, and not the anterior nares, were significantly different in AD, with lower diversity in AD. However, these observations were limited to the rural group. Moreover, lesional skin of both rural and urban cases had a predominance of *Streptococcus*, *Veillonella* and *Granulicatela* compared to nonlesional skin of both cases and controls. Also, *Veillonella*, *Sphingomonas* and *Ruminococcaceae* were enriched on the anterior nares of cases compared to controls, although this was mostly limited to the rural group. These genera, especially *Streptococcus* and *Veillonella*, are known to induce chronic inflammation and epithelial tissue damage in other diseases. Therefore, these genera might also drive AD pathology in a likewise manner. Moreover, *Streptococcus*, *Veillonella* and *Granulicatela*, might be important in AD pathology and exacerbations on skin lesions. However, the data implicating these genera in driving AD pathogenesis is limited in the literature and highlights the need for future studies to understand their contribution in AD pathogenesis.

Overall, the work presented in this thesis supports the predominance of *S. aureus* in AD and highlights potential pathological adaptation of these strains in children with AD. Further, it provided evidence that CoNS may be important in AD pathology. However, this trend was observed when they are co-colonised with *S. aureus*. Moreover, the specific implicated CoNS species differ across rural and urban environments possibly in mechanisms yet to be described in the literature. Lastly, bacterial taxa such as *Streptococcus* and *Veillonella* may be contributing more significantly to AD pathology on AD lesions compared to the traditionally described *Staphylococcus* genus. It should be highlighted that the findings from this study are largely observational. As such, there is a need for longitudinal studies which will reveal whether there is a causal relationship between bacterial signatures in childhood AD observed in this study and the development along with exacerbation of AD. Despite this, these findings point to the need for new treatment strategies targeting the restoration of the skin and nasal bacteriome in AD that are specific sub-Saharan African children in the management of AD. Such treatment options that are specific to this population will ensure that AD is appropriately managed in this population. This will likely lead to treatment outcomes and improving the quality of life of children living with AD in South Africa. Therefore, there is an urgent need for the public health in South Africa, and Africa by extension, to adapt policies that will focus on driving these medical initiatives, especially considering the increasing prevalence of childhood AD among African populations.

Final perspectives

In addition to the work presented in this thesis, we propose the following investigations in future studies:

i. Perform *in vivo* investigation murine to understand the pathological consequences of *S. aureus*
ii. Perform *in vivo* investigation using AD murine models to elucidate the role of *S. capitis* in AD pathology.
iii. Characterise the *Streptococcus* species that predominate in cases and perform WGS to understand the genomic features that distinguish the specific strains in cases from controls.
iv. As a continuation from iii), conduct *in vivo* investigations to understand the mechanisms by which the *Streptococcus* species drive AD pathology.

v. Characterise the viral and fungal communities and their association with markers of disease to evaluate which viral and fungal profiles are associated with disease and disease severity.

vi. Characterise the metabolome to evaluate the metabolomic signatures that are associated AD among South African children.

REFERENCES

1. Simpson EL, Villarreal M, Jepson B, et al. Patients with Atopic Dermatitis Colonized with Staphylococcus aureus Have a Distinct Phenotype and Endotype. *J Invest Dermatol.* 2018.
2. Silverberg JI. Public Health Burden and Epidemiology of Atopic Dermatitis. *Dermatol Clin.* 2017;35(3):283-289.
3. Laughter MR, Maymone MBC, Mashayekhi S, et al. The global burden of atopic dermatitis: lessons from the Global Burden of Disease Study 1990-2017. *Br J Dermatol.* 2021;184(2):304-309.
4. Torrelo A. Atopic dermatitis in different skin types. What is to know? *J Eur Acad Dermatol Venereol.* 2014;28 Suppl 3:2-4.
5. Kay J, Gawkrodger DJ, Mortimer MJ, Jaron AG. The prevalence of childhood atopic eczema in a general population. *J Am Acad Dermatol.* 1994;30(1):35-39.
6. Eyerich K, Eyerich S, Biedermann T. The Multi-Modal Immune Pathogenesis of Atopic Eczema. *Trends Immunol.* 2015;36(12):788-801.
7. Polanska A, Danczak-Pazdrowska A, Silny W, Jenerowicz D, Olek-Hrab K, Osmola-Mankowska A. Nonlesional skin in atopic dermatitis is seemingly healthy skin - observations using noninvasive methods. *Wideochir Inne Tech Maloinwazyjne.* 2013;8(3):192-199.
8. Nutten S. Atopic dermatitis: global epidemiology and risk factors. *Ann Nutr Metab.* 2015;66 Suppl 1:8-16.
9. Gochnauer H, Valdes-Rodriguez R, Cardwell L, Anolik RB. The Psychosocial Impact of Atopic Dermatitis. *Adv Exp Med Biol.* 2017;1027:57-69.
10. Xie QW, Dai X, Tang X, Chan CHY, Chan CLW. Risk of Mental Disorders in Children and Adolescents With Atopic Dermatitis: A Systematic Review and Meta-Analysis. *Front Psychol.* 2019;10:1773.
11. Carlsten C, Dimich-Ward H, Ferguson A, et al. Atopic dermatitis in a high-risk cohort: natural history, associated allergic outcomes, and risk factors. *Ann Allergy Asthma Immunol.* 2013;110(1):24-28.
12. Silverberg JI, Simpson EL. Association between severe eczema in children and multiple comorbid conditions and increased healthcare utilization. *Pediatr Allergy Immunol.* 2013;24(5):476-486.
13. Yaneva M, Darlenski R. The link between atopic dermatitis and asthma- immunological imbalance and beyond. *Asthma Res Pract.* 2021;7(1):16.
14. Ogonowska P, Gilaberte Y, Baranska-Rybak W, Nakonieczna J. Colonization With Staphylococcus aureus in Atopic Dermatitis Patients: Attempts to Reveal the Unknown. *Front Microbiol.* 2020;11:567090.
15. Sroka-Tomaszewska J, Trzeciak M. Molecular Mechanisms of Atopic Dermatitis Pathogenesis. *Int J Mol Sci.* 2021;22(8).
16. Ziegler SF. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Curr Opin Immunol.* 2010;22(6):795-799.
17. Williams MR, Nakatsuji T, Sanford JA, Vrbanac AF, Gallo RL. Staphylococcus aureus Induces Increased Serine Protease Activity in Keratinocytes. *J Invest Dermatol.* 2017;137(2):377-384.
18. Fleury OM, McAleer MA, Feuillie C, et al. Clumping Factor B Promotes Adherence of Staphylococcus aureus to Corneocytes in Atopic Dermatitis. *Infect Immun.* 2017;85(6).
19. Berents TL, Carlsen KC, Mowinckel P, et al. Skin Barrier Function and Staphylococcus aureus Colonization in Vestibulum Nasi and Fauces in Healthy Infants and Infants with Eczema: A Population-Based Cohort Study. *PLoS One.* 2015;10(6):e0130145.
20. Addor FA, Takaoka R, Rivitti EA, Aoki V. Atopic dermatitis: correlation between non-damaged skin barrier function and disease activity. *Int J Dermatol.* 2012;51(6):672-676.
21. Kottner J, Lichterfeld A, Blume-Peytavi U. Transepidermal water loss in young and aged healthy humans: a systematic review and meta-analysis. *Arch Dermatol Res.* 2013;305(4):315-323.
22. Gioia F, Celleno L. The dynamics of transepidermal water loss (TEWL) from hydrated skin. *Skin Res Technol.* 2002;8(3):178-186.
23. Tabata N, Tagami H, Kligman AM. A twenty-four-hour occlusive exposure to 1% sodium lauryl sulfate induces a unique histopathologic inflammatory response in the xerotic skin of atopic dermatitis patients. *Acta Derm Venereol.* 1998;78(4):244-247.
24. Jinnestal CL, Belfrage E, Back O, Schmidtchen A, Sonesson A. Skin barrier impairment correlates with cutaneous Staphylococcus aureus colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. *Int J Dermatol.* 2014;53(1):27-33.
25. Di Domenico EG, Cavallo I, Capitanio B, et al. Staphylococcus aureus and the Cutaneous Microbiota Biofilms in the Pathogenesis of Atopic Dermatitis. *Microorganisms.* 2019;7(9).

26. Jang H, Matsuda A, Jung K, et al. Skin pH Is the Master Switch of Kallikrein 5-Mediated Skin Barrier Destruction in a Murine Atopic Dermatitis Model. *J Invest Dermatol.* 2016;136(1):127-135.
27. Cork MJ, Danby SG, Vasilopoulos Y, et al. Epidermal barrier dysfunction in atopic dermatitis. *J Invest Dermatol.* 2009;129(8):1892-1908.
28. Howell MD, Boguniewicz M, Pastore S, et al. Mechanism of HBD-3 deficiency in atopic dermatitis. *Clin Immunol.* 2006;121(3):332-338.
29. Sieprawska-Lupa M, Mydel P, Krawczyk K, et al. Degradation of human antimicrobial peptide LL-37 by Staphylococcus aureus-derived proteinases. *Antimicrob Agents Chemother.* 2004;48(12):4673-4679.
30. Kezic S, Kemperman PM, Koster ES, et al. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *J Invest Dermatol.* 2008;128(8):2117-2119.
31. Palmer CN, Irvine AD, Terron-Kwiatkowski A, et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet.* 2006;38(4):441-446.
32. Miajlovic H, Fallon PG, Irvine AD, Foster TJ. Effect of filaggrin breakdown products on growth of and protein expression by Staphylococcus aureus. *J Allergy Clin Immunol.* 2010;126(6):1184-1190 e1183.
33. Clausen ML, Edslev SM, Andersen PS, Clemmensen K, Krogfelt KA, Agner T. Staphylococcus aureus colonization in atopic eczema and its association with filaggrin gene mutations. *Br J Dermatol.* 2017;177(5):1394-1400.
34. van Drongelen V, Haisma EM, Out-Luiting JJ, Nibbering PH, El Ghalbzouri A. Reduced filaggrin expression is accompanied by increased Staphylococcus aureus colonization of epidermal skin models. *Clin Exp Allergy.* 2014;44(12):1515-1524.
35. Thawer-Esmail F, Jakasa I, Todd G, et al. South African amaXhosa patients with atopic dermatitis have decreased levels of filaggrin breakdown products but no loss-of-function mutations in filaggrin. *J Allergy Clin Immunol.* 2014;133(1):280-282 e281-282.
36. Winge MC, Bilcha KD, Lieden A, et al. Novel filaggrin mutation but no other loss-of-function variants found in Ethiopian patients with atopic dermatitis. *Br J Dermatol.* 2011;165(5):1074-1080.
37. Margolis DJ, Mitra N, Gochnauer H, et al. Uncommon Filaggrin Variants Are Associated with Persistent Atopic Dermatitis in African Americans. *J Invest Dermatol.* 2018;138(7):1501-1506.
38. Nakamura Y, Takahashi H, Takaya A, et al. Staphylococcus Agr virulence is critical for epidermal colonization and associates with atopic dermatitis development. *Sci Transl Med.* 2020;12(551).
39. Lunjani N, Ahearn-Ford S, Dube FS, Hlela C, O'Mahony L. Mechanisms of microbe-immune system dialogue within the skin. *Genes Immun.* 2021.
40. Howell MD, Kim BE, Gao P, et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol.* 2009;124(3 Suppl 2):R7-R12.
41. Kasraie S, Werfel T. Role of macrophages in the pathogenesis of atopic dermatitis. *Mediators Inflamm.* 2013;2013:942375.
42. Novak N. An update on the role of human dendritic cells in patients with atopic dermatitis. *J Allergy Clin Immunol.* 2012;129(4):879-886.
43. Di Domenico EG, Cavallo I, Bordignon V, et al. Inflammatory cytokines and biofilm production sustain Staphylococcus aureus outgrowth and persistence: a pivotal interplay in the pathogenesis of Atopic Dermatitis. *Sci Rep.* 2018;8(1):9573.
44. Cardona ID, Cho SH, Leung DY. Role of bacterial superantigens in atopic dermatitis : implications for future therapeutic strategies. *Am J Clin Dermatol.* 2006;7(5):273-279.
45. Yamazaki Y, Nakamura Y, Nunez G. Role of the microbiota in skin immunity and atopic dermatitis. *Allergol Int.* 2017;66(4):539-544.
46. Sanford JA, Gallo RL. Functions of the skin microbiota in health and disease. *Semin Immunol.* 2013;25(5):370-377.
47. Kennedy EA, Connolly J, Hourihane JO, et al. Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. *J Allergy Clin Immunol.* 2017;139(1):166-172.
48. Bjerre RD, Bandier J, Skov L, Engstrand L, Johansen JD. The role of the skin microbiome in atopic dermatitis: a systematic review. *Br J Dermatol.* 2017;177(5):1272-1278.
49. Kong HH, Oh J, Deming C, et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* 2012;22(5):850-859.
50. Shi B, Bangayan NJ, Curd E, et al. The skin microbiome is different in pediatric versus adult atopic dermatitis. *J Allergy Clin Immunol.* 2016;138(4):1233-1236.
51. Ramadan M, Solyman S, Yones M, Abdallah Y, Halaby H, Hanora A. Skin Microbiome Differences in Atopic Dermatitis and Healthy Controls in Egyptian Children and Adults, and Association with Serum Immunoglobulin E. *OMICS.* 2019;23(5):247-260.

52. Kwon S, Choi JY, Shin JW, et al. Changes in Lesional and Non-lesional Skin Microbiome During Treatment of Atopic Dermatitis. *Acta Derm Venereol.* 2018.
53. Francuzik W, Franke K, Schumann RR, Heine G, Worm M. Propionibacterium acnes Abundance Correlates Inversely with Staphylococcus aureus: Data from Atopic Dermatitis Skin Microbiome. *Acta Derm Venereol.* 2018;98(5):490-495.
54. Shi B, Leung DYM, Taylor PA, Li H. Methicillin-Resistant Staphylococcus aureus Colonization Is Associated with Decreased Skin Commensal Bacteria in Atopic Dermatitis. *J Invest Dermatol.* 2018;138(7):1668-1671.
55. Seite S, Flores GE, Henley JB, et al. Microbiome of affected and unaffected skin of patients with atopic dermatitis before and after emollient treatment. *J Drugs Dermatol.* 2014;13(11):1365-1372.
56. Geoghegan JA, Irvine AD, Foster TJ. Staphylococcus aureus and Atopic Dermatitis: A Complex and Evolving Relationship. *Trends Microbiol.* 2018;26(6):484-497.
57. Xu Z, Liu X, Niu Y, et al. Skin benefits of moisturising body wash formulas for children with atopic dermatitis: A randomised controlled clinical study in China. *Australas J Dermatol.* 2020;61(1):e54-e59.
58. Moosbrugger-Martinz V, Hackl H, Gruber R, et al. Initial Evidence of Distinguishable Bacterial and Fungal Dysbiosis in the Skin of Patients with Atopic Dermatitis or Netherton Syndrome. *J Invest Dermatol.* 2021;141(1):114-123.
59. Bjerre RD, Holm JB, Palleja A, Solberg J, Skov L, Johansen JD. Skin dysbiosis in the microbiome in atopic dermatitis is site-specific and involves bacteria, fungus and virus. *BMC Microbiol.* 2021;21(1):256.
60. Han SH, Cheon HI, Hur MS, et al. Analysis of the skin mycobiome in adult patients with atopic dermatitis. *Exp Dermatol.* 2018;27(4):366-373.
61. Edslev SM, Andersen PS, Agner T, et al. Identification of cutaneous fungi and mites in adult atopic dermatitis: analysis by targeted 18S rRNA amplicon sequencing. *BMC Microbiol.* 2021;21(1):72.
62. Johnson RC, Ellis MW, Lanier JB, Schlett CD, Cui T, Merrell DS. Correlation between nasal microbiome composition and remote purulent skin and soft tissue infections. *Infect Immun.* 2015;83(2):802-811.
63. Pascolini C, Sinagra J, Pecetta S, et al. Molecular and immunological characterization of Staphylococcus aureus in pediatric atopic dermatitis: implications for prophylaxis and clinical management. *Clin Dev Immunol.* 2011;2011:718708.
64. Totte JEE, Pardo LM, Fieten KB, et al. Nasal and skin microbiomes are associated with disease severity in paediatric atopic dermatitis. *Br J Dermatol.* 2019;181(4):796-804.
65. Oh J, Conlan S, Polley EC, Segre JA, Kong HH. Shifts in human skin and nares microbiota of healthy children and adults. *Genome Med.* 2012;4(10):77.
66. De Boeck I, Wittouck S, Wuyts S, et al. Comparing the Healthy Nose and Nasopharynx Microbiota Reveals Continuity As Well As Niche-Specificity. *Front Microbiol.* 2017;8:2372.
67. Zhou Y, Mihindukulasuriya KA, Gao H, et al. Exploration of bacterial community classes in major human habitats. *Genome Biol.* 2014;15(5):R66.
68. Nakatsuji T, Hata TR, Tong Y, et al. Development of a human skin commensal microbe for bacteriotherapy of atopic dermatitis and use in a phase 1 randomized clinical trial. *Nat Med.* 2021.
69. Totte JE, van der Feltz WT, Hennekam M, van Belkum A, van Zuuren EJ, Pasmans SG. Prevalence and odds of Staphylococcus aureus carriage in atopic dermatitis: a systematic review and meta-analysis. *Br J Dermatol.* 2016;175(4):687-695.
70. Tauber M, Balica S, Hsu CY, et al. Staphylococcus aureus density on lesional and nonlesional skin is strongly associated with disease severity in atopic dermatitis. *J Allergy Clin Immunol.* 2016;137(4):1272-1274 e1273.
71. Lebon A, Labout JA, Verbrugh HA, et al. Role of Staphylococcus aureus nasal colonization in atopic dermatitis in infants: the Generation R Study. *Arch Pediatr Adolesc Med.* 2009;163(8):745-749.
72. Meylan P, Lang C, Mermoud S, et al. Skin Colonization by Staphylococcus aureus Precedes the Clinical Diagnosis of Atopic Dermatitis in Infancy. *J Invest Dermatol.* 2017;137(12):2497-2504.
73. Skov L, Halkjaer LB, Agner T, Frimodt-Moller N, Jarlov JO, Bisgaard H. Neonatal colonization with Staphylococcus aureus is not associated with development of atopic dermatitis. *Br J Dermatol.* 2009;160(6):1286-1291.
74. Sollid JU, Furberg AS, Hanssen AM, Johannessen M. Staphylococcus aureus: determinants of human carriage. *Infect Genet Evol.* 2014;21:531-541.
75. Sakr A, Bregeon F, Mege JL, Rolain JM, Blin O. Staphylococcus aureus Nasal Colonization: An Update on Mechanisms, Epidemiology, Risk Factors, and Subsequent Infections. *Front Microbiol.* 2018;9:2419.
76. Leyden JJ, Marples RR, Kligman AM. Staphylococcus aureus in the lesions of atopic dermatitis. *Br J Dermatol.* 1974;90(5):525-530.

77. Wrobel J, Tomczak H, Jenerowicz D, Czarnecka-Operacz M. Skin and nasal vestibule colonisation by Staphylococcus aureus and its susceptibility to drugs in atopic dermatitis patients. *Ann Agric Environ Med.* 2018;25(2):334-337.
78. Blicharz L, Usarek P, Mlynarczyk G, Skowronski K, Rudnicka L, Samochocki Z. Nasal Colonization by Staphylococci and Severity of Atopic Dermatitis. *Dermatitis.* 2020;31(3):215-222.
79. Bourrain M, Ribet V, Calvez A, Lebaron P, Schmitt AM. Balance between beneficial microflora and Staphylococcus aureus colonisation: in vivo evaluation in patients with atopic dermatitis during hydrotherapy. *Eur J Dermatol.* 2013;23(6):786-794.
80. Hu C, Duijts L, van Meel ER, et al. Association between nasal and nasopharyngeal bacterial colonisation in early life and eczema phenotypes. *Clin Exp Allergy.* 2021.
81. Na SY, Roh JY, Kim JM, Tamang MD, Lee JR. Analysis of Colonization and Genotyping of the Exotoxins of Staphylococcus aureus in Patients with Atopic Dermatitis. *Ann Dermatol.* 2012;24(4):413-419.
82. Tomi NS, Kranke B, Aberer E. Staphylococcal toxins in patients with psoriasis, atopic dermatitis, and erythroderma, and in healthy control subjects. *J Am Acad Dermatol.* 2005;53(1):67-72.
83. Bunikowski R, Mielke ME, Skarabis H, et al. Evidence for a disease-promoting effect of Staphylococcus aureus-derived exotoxins in atopic dermatitis. *J Allergy Clin Immunol.* 2000;105(4):814-819.
84. Sonesson A, Przybyszewska K, Eriksson S, et al. Identification of bacterial biofilm and the Staphylococcus aureus derived protease, staphopain, on the skin surface of patients with atopic dermatitis. *Sci Rep.* 2017;7(1):8689.
85. Hirasawa Y, Takai T, Nakamura T, et al. Staphylococcus aureus extracellular protease causes epidermal barrier dysfunction. *J Invest Dermatol.* 2010;130(2):614-617.
86. Bukowski M, Wladyka B, Dubin G. Exfoliative toxins of Staphylococcus aureus. *Toxins (Basel).* 2010;2(5):1148-1165.
87. Rerknimitr P, Otsuka A, Nakashima C, Kabashima K. The etiopathogenesis of atopic dermatitis: barrier disruption, immunological derangement, and pruritus. *Inflamm Regen.* 2017;37:14.
88. Seiti Yamada Yoshikawa F, Feitosa de Lima J, Notomi Sato M, Alefe Leuzzi Ramos Y, Aoki V, Leao Orfali R. Exploring the Role of Staphylococcus Aureus Toxins in Atopic Dermatitis. *Toxins (Basel).* 2019;11(6).
89. Graf AC, Leonard A, Schauble M, et al. Virulence Factors Produced by Staphylococcus aureus Biofilms Have a Moonlighting Function Contributing to Biofilm Integrity. *Mol Cell Proteomics.* 2019;18(6):1036-1053.
90. Gonzalez T, Stevens ML, Baatyrbek Kyzy A, et al. Biofilm propensity of Staphylococcus aureus skin isolates is associated with increased atopic dermatitis severity and barrier dysfunction in the MPAACH pediatric cohort. *Allergy.* 2021;76(1):302-313.
91. McCarthy AJ, Witney AA, Lindsay JA. Staphylococcus aureus temperate bacteriophage: carriage and horizontal gene transfer is lineage associated. *Front Cell Infect Microbiol.* 2012;2:6.
92. Cheung GY, Joo HS, Chatterjee SS, Otto M. Phenol-soluble modulins--critical determinants of staphylococcal virulence. *FEMS Microbiol Rev.* 2014;38(4):698-719.
93. Williams MR, Costa SK, Zaramela LS, et al. Quorum sensing between bacterial species on the skin protects against epidermal injury in atopic dermatitis. *Sci Transl Med.* 2019;11(490).
94. Peetermans M, Vanassche T, Liesenborghs L, et al. Plasminogen activation by staphylokinase enhances local spreading of S. aureus in skin infections. *BMC Microbiol.* 2014;14:310.
95. Thammavongsa V, Kim HK, Missiakas D, Schneewind O. Staphylococcal manipulation of host immune responses. *Nat Rev Microbiol.* 2015;13(9):529-543.
96. Kobayashi SD, DeLeo FR. Staphylococcus aureus protein A promotes immune suppression. *mBio.* 2013;4(5):e00764-00713.
97. Yao Y, Kozman A, Al-Hassani M, et al. Identification of staphylococcal protein A in infected atopic dermatitis lesions. *J Invest Dermatol.* 2010;130(10):2502-2504.
98. Allen HB, Vaze ND, Choi C, et al. The presence and impact of biofilm-producing staphylococci in atopic dermatitis. *JAMA Dermatol.* 2014;150(3):260-265.
99. O'Brien LM, Walsh EJ, Massey RC, Peacock SJ, Foster TJ. Staphylococcus aureus clumping factor B (ClfB) promotes adherence to human type I cytokeratin 10: implications for nasal colonization. *Cell Microbiol.* 2002;4(11):759-770.
100. Messina JA, Thaden JT, Sharma-Kuinkel BK, Fowler VG, Jr. Impact of Bacterial and Human Genetic Variation on Staphylococcus aureus Infections. *PLoS Pathog.* 2016;12(1):e1005330.
101. Harkins CP, Pettigrew KA, Oravcova K, et al. The Microevolution and Epidemiology of Staphylococcus aureus Colonization during Atopic Eczema Disease Flare. *J Invest Dermatol.* 2018;138(2):336-343.

102. Benito D, Aspiroz C, Gilaberte Y, et al. Genetic lineages and antimicrobial resistance genotypes in Staphylococcus aureus from children with atopic dermatitis: detection of clonal complexes CC1, CC97 and CC398. *J Chemother.* 2016;28(5):359-366.
103. Yeung M, Balma-Mena A, Shear N, et al. Identification of major clonal complexes and toxin producing strains among Staphylococcus aureus associated with atopic dermatitis. *Microbes Infect.* 2011;13(2):189-197.
104. Su M, Lyles JT, Petit Iii RA, et al. Genomic analysis of variability in Delta-toxin levels between Staphylococcus aureus strains. *PeerJ.* 2020;8:e8717.
105. Cavalcante FS, Saintive S, Carvalho Ferreira D, et al. Methicillin-resistant Staphylococcus aureus from infected skin lesions present several virulence genes and are associated with the CC30 in Brazilian children with atopic dermatitis. *Virulence.* 2021;12(1):260-269.
106. Cavalcante FS, Abad ED, Lyra YC, et al. High prevalence of methicillin resistance and PVL genes among Staphylococcus aureus isolates from the nares and skin lesions of pediatric patients with atopic dermatitis. *Braz J Med Biol Res.* 2015;48(7):588-594.
107. Cavalcante FS, Alvarenga C, Saintive S, Dios Abad E, Carvalho Ferreira D, Netto Dos Santos KR. Staphylococcus aureus nasal isolates may have the same genetic profile in atopic dermatitis paediatric patients and their close contacts. *J Med Microbiol.* 2020;69(6):850-853.
108. Abad ED, Ferreira DC, Cavalcante FS, et al. High incidence of acquiring methicillin-resistant Staphylococcus aureus in Brazilian children with Atopic Dermatitis and associated risk factors. *J Microbiol Immunol Infect.* 2020;53(5):724-730.
109. Lo WT, Wang SR, Tseng MH, Huang CF, Chen SJ, Wang CC. Comparative molecular analysis of meticillin-resistant Staphylococcus aureus isolates from children with atopic dermatitis and healthy subjects in Taiwan. *Br J Dermatol.* 2010;162(5):1110-1116.
110. Rojo A, Aguinaga A, Monecke S, Yuste JR, Gastaminza G, Espana A. Staphylococcus aureus genomic pattern and atopic dermatitis: may factors other than superantigens be involved? *Eur J Clin Microbiol Infect Dis.* 2014;33(4):651-658.
111. Balma-Mena A, Lara-Corrales I, Zeller J, et al. Colonization with community-acquired methicillin-resistant Staphylococcus aureus in children with atopic dermatitis: a cross-sectional study. *Int J Dermatol.* 2011;50(6):682-688.
112. Aziz F, Hisatsune J, Yu L, et al. Staphylococcus aureus Isolated from Skin from Atopic-Dermatitis Patients Produces Staphylococcal Enterotoxin Y, Which Predominantly Induces T-Cell Receptor Valpha-Specific Expansion of T Cells. *Infect Immun.* 2020;88(2).
113. Harkins CP, McAleer MA, Bennett D, et al. The widespread use of topical antimicrobials enriches for resistance in Staphylococcus aureus isolated from patients with atopic dermatitis. *Br J Dermatol.* 2018;179(4):951-958.
114. Dadashi M, Hajikhani B, Darban-Sarokhalil D, van Belkum A, Goudarzi M. Mupirocin resistance in Staphylococcus aureus: A systematic review and meta-analysis. *J Glob Antimicrob Resist.* 2020;20:238-247.
115. Blazewicz I, Jaskiewicz M, Bauer M, et al. Decolonization of Staphylococcus aureus in patients with atopic dermatitis: a reason for increasing resistance to antibiotics? *Postepy Dermatol Alergol.* 2017;34(6):553-560.
116. Blaser MJ. The theory of disappearing microbiota and the epidemics of chronic diseases. *Nat Rev Immunol.* 2017;17(8):461-463.
117. Briscoe CC, Reich P, Fritz S, Coughlin CC. Staphylococcus aureus antibiotic susceptibility patterns in pediatric atopic dermatitis. *Pediatr Dermatol.* 2019;36(4):482-485.
118. Jung MY, Chung JY, Lee HY, Park J, Lee DY, Yang JM. Antibiotic Susceptibility of Staphylococcus aureus in Atopic Dermatitis: Current Prevalence of Methicillin-Resistant Staphylococcus aureus in Korea and Treatment Strategies. *Ann Dermatol.* 2015;27(4):398-403.
119. Jagadeesan S, Kurien G, Divakaran MV, Sadanandan SM, Sobhanakumari K, Sarin A. Methicillin-resistant Staphylococcus aureus colonization and disease severity in atopic dermatitis: a cross-sectional study from South India. *Indian J Dermatol Venereol Leprol.* 2014;80(3):229-234.
120. Stubbendieck RM, May DS, Chevrette MG, et al. Competition among Nasal Bacteria Suggests a Role for Siderophore-Mediated Interactions in Shaping the Human Nasal Microbiota. *Appl Environ Microbiol.* 2019;85(10).
121. Hardy BL, Dickey SW, Plaut RD, et al. Corynebacterium pseudodiphtheriticum Exploits Staphylococcus aureus Virulence Components in a Novel Polymicrobial Defense Strategy. *mBio.* 2019;10(1).
122. Stubbendieck RM, Vargas-Bautista C, Straight PD. Bacterial Communities: Interactions to Scale. *Front Microbiol.* 2016;7:1234.
123. Deng YJ, Wang SY. Synergistic growth in bacteria depends on substrate complexity. *J Microbiol.* 2016;54(1):23-30.

124. Zupancic J, Raghupathi PK, Houf K, Burmolle M, Sorensen SJ, Gunde-Cimerman N. Synergistic Interactions in Microbial Biofilms Facilitate the Establishment of Opportunistic Pathogenic Fungi in Household Dishwashers. *Front Microbiol.* 2018;9:21.
125. Regev-Yochay G, Trzcinski K, Thompson CM, Malley R, Lipsitch M. Interference between Streptococcus pneumoniae and Staphylococcus aureus: In vitro hydrogen peroxide-mediated killing by Streptococcus pneumoniae. *J Bacteriol.* 2006;188(13):4996-5001.
126. Paharik AE, Parlet CP, Chung N, et al. Coagulase-Negative Staphylococcal Strain Prevents Staphylococcus aureus Colonization and Skin Infection by Blocking Quorum Sensing. *Cell Host Microbe.* 2017;22(6):746-756 e745.
127. Iwase T, Uehara Y, Shinji H, et al. Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. *Nature.* 2010;465(7296):346-349.
128. Hardy BL, Bansal G, Hewlett KH, et al. Antimicrobial Activity of Clinically Isolated Bacterial Species Against Staphylococcus aureus. *Front Microbiol.* 2019;10:2977.
129. Nakatsuji T, Chen TH, Narala S, et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. *Sci Transl Med.* 2017;9(378).
130. Zipperer A, Konnerth MC, Laux C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. *Nature.* 2016;535(7613):511-516.
131. Traisaeng S, Herr DR, Kao HJ, Chuang TH, Huang CM. A Derivative of Butyric Acid, the Fermentation Metabolite of Staphylococcus epidermidis, Inhibits the Growth of a Staphylococcus aureus Strain Isolated from Atopic Dermatitis Patients. *Toxins (Basel).* 2019;11(6).
132. Brown MM, Horswill AR. Staphylococcus epidermidis-Skin friend or foe? *PLoS Pathog.* 2020;16(11):e1009026.
133. Bitschar K, Sauer B, Focken J, et al. Lugdunin amplifies innate immune responses in the skin in synergy with host- and microbiota-derived factors. *Nat Commun.* 2019;10(1):2730.
134. Chen C, Krishnan V, Macon K, Manne K, Narayana SV, Schneewind O. Secreted proteases control autolysin-mediated biofilm growth of Staphylococcus aureus. *J Biol Chem.* 2013;288(41):29440-29452.
135. Vandecandelaere I, Depuydt P, Nelis HJ, Coenye T. Protease production by Staphylococcus epidermidis and its effect on Staphylococcus aureus biofilms. *Pathog Dis.* 2014;70(3):321-331.
136. Burian M, Bitschar K, Dylus B, Peschel A, Schittek B. The Protective Effect of Microbiota on S. aureus Skin Colonization Depends on the Integrity of the Epithelial Barrier. *J Invest Dermatol.* 2017;137(4):976-979.
137. Fredheim EGA, Flaegstad T, Askarian F, Klingenberg C. Colonisation and interaction between S. epidermidis and S. aureus in the nose and throat of healthy adolescents. *Eur J Clin Microbiol Infect Dis.* 2015;34(1):123-129.
138. Boldock E, Surewaard BGJ, Shamarina D, et al. Human skin commensals augment Staphylococcus aureus pathogenesis. *Nat Microbiol.* 2018;3(8):881-890.
139. Chin D, Goncheva MI, Flannagan RS, et al. Coagulase-negative staphylococci release a purine analog that inhibits Staphylococcus aureus virulence. *Nat Commun.* 2021;12(1):1887.
140. Peng P, Baldry M, Gless BH, et al. Effect of Co-inhabiting Coagulase Negative Staphylococci on S. aureus agr Quorum Sensing, Host Factor Binding, and Biofilm Formation. *Front Microbiol.* 2019;10:2212.
141. Glatthardt T, Campos JCM, Chamon RC, et al. Small Molecules Produced by Commensal Staphylococcus epidermidis Disrupt Formation of Biofilms by Staphylococcus aureus. *Appl Environ Microbiol.* 2020;86(5).
142. Traber KE, Lee E, Benson S, et al. agr function in clinical Staphylococcus aureus isolates. *Microbiology.* 2008;154(Pt 8):2265-2274.
143. Argemi X, Hansmann Y, Prola K, Prevost G. Coagulase-Negative Staphylococci Pathogenomics. *Int J Mol Sci.* 2019;20(5).
144. Heilmann C, Ziebuhr W, Becker K. Are coagulase-negative staphylococci virulent? *Clin Microbiol Infect.* 2019;25(9):1071-1080.
145. Franca A, Gaio V, Lopes N, Melo LDR. Virulence Factors in Coagulase-Negative Staphylococci. *Pathogens.* 2021;10(2).
146. Soares J, Lopes C, Tavaria F, Delgado L, Pintado M. A diversity profile from the staphylococcal community on atopic dermatitis skin: a molecular approach. *J Appl Microbiol.* 2013;115(6):1411-1419.
147. Hon KL, Tsang YC, Pong NH, Leung TF, Ip M. Exploring Staphylococcus epidermidis in atopic eczema: friend or foe? *Clin Exp Dermatol.* 2016;41(6):659-663.
148. Edslev SM, Olesen CM, Norreslet LB, et al. Staphylococcal Communities on Skin Are Associated with Atopic Dermatitis and Disease Severity. *Microorganisms.* 2021;9(2).
149. Cau L, Williams MR, Butcher AM, et al. Staphylococcus epidermidis protease EcpA can be a deleterious component of the skin microbiome in atopic dermatitis. *J Allergy Clin Immunol.* 2020.

150. Tay ASL, Li C, Nandi T, et al. Atopic dermatitis microbiomes stratify into ecologic dermotypes enabling microbial virulence and disease severity. *J Allergy Clin Immunol.* 2021;147(4):1329-1340.
151. Bilal JA, Ahmad MI, Robaee AA, Alzolibani AA, Shobaili HA, Al-Khowailed MS. Pattern of bacterial colonization of atopic dermatitis in saudi children. *J Clin Diagn Res.* 2013;7(9):1968-1970.
152. Byrd AL, Deming C, Cassidy SKB, et al. Staphylococcus aureus and Staphylococcus epidermidis strain diversity underlying pediatric atopic dermatitis. *Sci Transl Med.* 2017;9(397).
153. Kowalska-Oledzka E, Czarnecka M, Baran A. Epidemiology of atopic dermatitis in Europe. *J Drug Assess.* 2019;8(1):126-128.
154. Levin ME, Botha M, Basera W, et al. Environmental factors associated with allergy in urban and rural children from the South African Food Allergy (SAFFA) cohort. *J Allergy Clin Immunol.* 2020;145(1):415-426.
155. Bonamonte D, Filoni A, Vestita M, Romita P, Foti C, Angelini G. The Role of the Environmental Risk Factors in the Pathogenesis and Clinical Outcome of Atopic Dermatitis. *Biomed Res Int.* 2019;2019:2450605.
156. Kantor R, Silverberg JI. Environmental risk factors and their role in the management of atopic dermatitis. *Expert Rev Clin Immunol.* 2017;13(1):15-26.
157. Janvier X, Alexandre S, Boukerb AM, et al. Deleterious Effects of an Air Pollutant (NO2) on a Selection of Commensal Skin Bacterial Strains, Potential Contributor to Dysbiosis? *Front Microbiol.* 2020;11:591839.
158. Gisler A, Korten I, de Hoogh K, et al. Associations of air pollution and greenness with the nasal microbiota of healthy infants: A longitudinal study. *Environ Res.* 2021;202:111633.
159. Selway CA, Mills JG, Weinstein P, et al. Transfer of environmental microbes to the skin and respiratory tract of humans after urban green space exposure. *Environ Int.* 2020;145:106084.
160. Skowron K, Bauza-Kaszewska J, Kraszewska Z, et al. Human Skin Microbiome: Impact of Intrinsic and Extrinsic Factors on Skin Microbiota. *Microorganisms.* 2021;9(3).
161. Ying S, Zeng DN, Chi L, et al. The Influence of Age and Gender on Skin-Associated Microbial Communities in Urban and Rural Human Populations. *PLoS One.* 2015;10(10):e0141842.
162. Gupta VK, Paul S, Dutta C. Geography, Ethnicity or Subsistence-Specific Variations in Human Microbiome Composition and Diversity. *Front Microbiol.* 2017;8:1162.
163. van Mierlo MMF, Pardo LM, Fieten KB, et al. The Skin and Nose Microbiome and Its Association with Filaggrin Gene Mutations in Pediatric Atopic Dermatitis. *Dermatology.* 2022:1-11.
164. Zheng Y, Wang Q, Ma L, et al. Alterations in the skin microbiome are associated with disease severity and treatment in the perioral zone of the skin of infants with atopic dermatitis. *Eur J Clin Microbiol Infect Dis.* 2019;38(9):1677-1685.
165. Song L, Wang Q, Zheng Y, et al. Cheek Microbial Communities Vary in Young Children with Atopic Dermatitis in China. *Dermatology.* 2020;236(2):160-169.
166. Gur Cetinkaya P, Sahiner UM. Childhood atopic dermatitis: current developments, treatment approaches, and future expectations. *Turk J Med Sci.* 2019;49(4):963-984.
167. Williams H, Stewart A, von Mutius E, et al. Is eczema really on the increase worldwide? *J Allergy Clin Immunol.* 2008;121(4):947-954 e915.
168. Civelek E, Sahiner UM, Yuksel H, et al. Prevalence, burden, and risk factors of atopic eczema in schoolchildren aged 10-11 years: a national multicenter study. *J Investig Allergol Clin Immunol.* 2011;21(4):270-277.
169. Mernelius S, Carlsson E, Henricson J, et al. Staphylococcus aureus colonization related to severity of hand eczema. *Eur J Clin Microbiol Infect Dis.* 2016;35(8):1355-1361.
170. Magnifico I, Petronio Petronio G, Venditti N, et al. Atopic Dermatitis as a Multifactorial Skin Disorder. Can the Analysis of Pathophysiological Targets Represent the Winning Therapeutic Strategy? *Pharmaceuticals (Basel).* 2020;13(11).
171. Botha M, Basera W, Facey-Thomas HE, et al. Rural and urban food allergy prevalence from the South African Food Allergy (SAFFA) study. *J Allergy Clin Immunol.* 2019;143(2):662-668 e662.
172. Williams HC, Burney PG, Pembroke AC, Hay RJ. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol.* 1994;131(3):406-416.
173. Oranje AP. Practical issues on interpretation of scoring atopic dermatitis: SCORAD Index, objective SCORAD, patient-oriented SCORAD and Three-Item Severity score. *Curr Probl Dermatol.* 2011;41:149-155.
174. Dube FS, Kaba M, Whittaker E, Zar HJ, Nicol MP. Detection of Streptococcus pneumoniae from Different Types of Nasopharyngeal Swabs in Children. *PLoS One.* 2013;8(6):e68097.
175. Kateete DP, Kimani CN, Katabazi FA, et al. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Ann Clin Microbiol Antimicrob.* 2010;9:23.

176. Leung MH, Oriyo NM, Gillespie SH, Charalambous BM. The adaptive potential during nasopharyngeal colonisation of Streptococcus pneumoniae. *Infect Genet Evol.* 2011;11(8):1989-1995.
177. Brakstad OG, Aasbakk K, Maeland JA. Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. *J Clin Microbiol.* 1992;30(7):1654-1660.
178. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol.* 2004;42(2):792-799.
179. Stegger M, Andersen PS, Kearns A, et al. Rapid detection, differentiation and typing of methicillin-resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecA(LGA251). *Clin Microbiol Infect.* 2012;18(4):395-400.
180. Votintseva AA, Fung R, Miller RR, et al. Prevalence of Staphylococcus aureus protein A (spa) mutants in the community and hospitals in Oxfordshire. *BMC Microbiol.* 2014;14:63.
181. Shopsin B, Gomez M, Montgomery SO, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains. *J Clin Microbiol.* 1999;37(11):3556-3563.
182. Mellmann A, Weniger T, Berssenbrugge C, et al. Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms. *BMC Microbiol.* 2007;7:98.
183. Fard-Mousavi N, Mosayebi G, Amouzandeh-Nobaveh A, Japouni-Nejad A, Ghaznavi-Rad E. The Dynamic of Staphylococcus aureus Nasal Carriage in Central Iran. *Jundishapur J Microbiol.* 2015;8(7):e20760.
184. Park HY, Kim CR, Huh IS, et al. Staphylococcus aureus Colonization in Acute and Chronic Skin Lesions of Patients with Atopic Dermatitis. *Ann Dermatol.* 2013;25(4):410-416.
185. Cooper PJ, Amorim LD, Figueiredo CA, et al. Effects of environment on human cytokine responses during childhood in the tropics: role of urban versus rural residence. *World Allergy Organ J.* 2015;8(1):22.
186. Andersen PS, Larsen LA, Fowler VG, Jr., Stegger M, Skov RL, Christensen K. Risk factors for Staphylococcus aureus nasal colonization in Danish middle-aged and elderly twins. *Eur J Clin Microbiol Infect Dis.* 2013;32(10):1321-1326.
187. Valacchi G, Sticozzi C, Pecorelli A, Cervellati F, Cervellati C, Maioli E. Cutaneous responses to environmental stressors. *Ann N Y Acad Sci.* 2012;1271:75-81.
188. Lam NL, Smith KR, Gauthier A, Bates MN. Kerosene: a review of household uses and their hazards in low- and middle-income countries. *J Toxicol Environ Health B Crit Rev.* 2012;15(6):396-432.
189. Wangchuk T, Mazaheri M, Clifford S, et al. Children's personal exposure to air pollution in rural villages in Bhutan. *Environ Res.* 2015;140:691-698.
190. Puzzolo E, Zerriffi H, Carter E, et al. Supply Considerations for Scaling Up Clean Cooking Fuels for Household Energy in Low- and Middle-Income Countries. *Geohealth.* 2019;3(12):370-390.
191. Verkade E, Kluytmans J. Livestock-associated Staphylococcus aureus CC398: animal reservoirs and human infections. *Infect Genet Evol.* 2014;21:523-530.
192. Campbell SJ, Deshmukh HS, Nelson CL, et al. Genotypic characteristics of Staphylococcus aureus isolates from a multinational trial of complicated skin and skin structure infections. *J Clin Microbiol.* 2008;46(2):678-684.
193. Yu F, Liu Y, Lv J, et al. Antimicrobial susceptibility, virulence determinant carriage and molecular characteristics of Staphylococcus aureus isolates associated with skin and soft tissue infections. *Braz J Infect Dis.* 2015;19(6):614-622.
194. Rijnders MI, Deurenberg RH, Boumans ML, et al. Population structure of Staphylococcus aureus strains isolated from intensive care unit patients in the netherlands over an 11-year period (1996 to 2006). *J Clin Microbiol.* 2009;47(12):4090-4095.
195. Egyir B, Guardabassi L, Esson J, et al. Insights into nasal carriage of Staphylococcus aureus in an urban and a rural community in Ghana. *PLoS One.* 2014;9(4):e96119.
196. Ruffing U, Alabi A, Kazimoto T, et al. Community-Associated Staphylococcus aureus from Sub-Saharan Africa and Germany: A Cross-Sectional Geographic Correlation Study. *Sci Rep.* 2017;7(1):154.
197. Clausen ML, Edslev SM, Norreslet LB, Sorensen JA, Andersen PS, Agner T. Temporal variation of Staphylococcus aureus clonal complexes in atopic dermatitis: a follow-up study. *Br J Dermatol.* 2019;180(1):181-186.
198. Kim DW, Park JY, Park KD, et al. Are there predominant strains and toxins of Staphylococcus aureus in atopic dermatitis patients? Genotypic characterization and toxin determination of S. aureus isolated in adolescent and adult patients with atopic dermatitis. *J Dermatol.* 2009;36(2):75-81.
199. Schaumburg F, Ngoa UA, Kosters K, et al. Virulence factors and genotypes of Staphylococcus aureus from infection and carriage in Gabon. *Clin Microbiol Infect.* 2011;17(10):1507-1513.

200. Li T, Lu H, Wang X, et al. Molecular Characteristics of Staphylococcus aureus Causing Bovine Mastitis between 2014 and 2015. *Front Cell Infect Microbiol.* 2017;7:127.
201. Benito D, Lozano C, Jimenez E, et al. Characterization of Staphylococcus aureus strains isolated from faeces of healthy neonates and potential mother-to-infant microbial transmission through breastfeeding. *FEMS Microbiol Ecol.* 2015;91(3).
202. Rasigade JP, Laurent F, Lina G, et al. Global distribution and evolution of Panton-Valentine leukocidin-positive methicillin-susceptible Staphylococcus aureus, 1981-2007. *J Infect Dis.* 2010;201(10):1589-1597.
203. Schaumburg F, Alabi AS, Mombo-Ngoma G, et al. Transmission of Staphylococcus aureus between mothers and infants in an African setting. *Clin Microbiol Infect.* 2014;20(6):O390-396.
204. Omuse G, Van Zyl KN, Hoek K, et al. Molecular characterization of Staphylococcus aureus isolates from various healthcare institutions in Nairobi, Kenya: a cross sectional study. *Ann Clin Microbiol Antimicrob.* 2016;15(1):51.
205. Perovic O, Iyaloo S, Kularatne R, et al. Prevalence and Trends of Staphylococcus aureus Bacteraemia in Hospitalized Patients in South Africa, 2010 to 2012: Laboratory-Based Surveillance Mapping of Antimicrobial Resistance and Molecular Epidemiology. *PLoS One.* 2015;10(12):e0145429.
206. Seidl K, Leimer N, Palheiros Marques M, et al. Clonality and antimicrobial susceptibility of methicillin-resistant Staphylococcus aureus at the University Hospital Zurich, Switzerland between 2012 and 2014. *Ann Clin Microbiol Antimicrob.* 2015;14:14.
207. Boswihi SS, Udo EE, Monecke S, et al. Emerging variants of methicillin-resistant Staphylococcus aureus genotypes in Kuwait hospitals. *PLoS One.* 2018;13(4):e0195933.
208. Kpeli G, Darko Otchere I, Lamelas A, et al. Possible healthcare-associated transmission as a cause of secondary infection and population structure of Staphylococcus aureus isolates from two wound treatment centres in Ghana. *New Microbes New Infect.* 2016;13:92-101.
209. Antiabong JF, Kock MM, Maphanga TG, Salawu AM, Mbelle NM, Ehlers MM. Trends in the Genetic Background of Methicillin-Resistant Staphylococcus Aureus Clinical Isolates in a South African Hospital: An Institutional-Based Observational Study. *Open Microbiol J.* 2017;11:339-351.
210. Garbacz K, Piechowicz L, Podkowik M, Mroczkowska A, Empel J, Bania J. Emergence and spread of worldwide Staphylococcus aureus clones among cystic fibrosis patients. *Infect Drug Resist.* 2018;11:247-255.
211. Hata E, Katsuda K, Kobayashi H, Uchida I, Tanaka K, Eguchi M. Genetic variation among Staphylococcus aureus strains from bovine milk and their relevance to methicillin-resistant isolates from humans. *J Clin Microbiol.* 2010;48(6):2130-2139.
212. Jamrozy DM, Fielder MD, Butaye P, Coldham NG. Comparative genotypic and phenotypic characterisation of methicillin-resistant Staphylococcus aureus ST398 isolated from animals and humans. *PLoS One.* 2012;7(7):e40458.
213. Gilaberte Y, Perez-Gilaberte JB, Poblador-Plou B, Bliek-Bueno K, Gimeno-Miguel A, Prados-Torres A. Prevalence and Comorbidity of Atopic Dermatitis in Children: A Large-Scale Population Study Based on Real-World Data. *J Clin Med.* 2020;9(6).
214. Blicharz L, Michalak M, Szymanek-Majchrzak K, et al. The Propensity to Form Biofilm in vitro by Staphylococcus aureus Strains Isolated from the Anterior Nares of Patients with Atopic Dermatitis: Clinical Associations. *Dermatology.* 2021;237(4):528-534.
215. Nada HA, Gomaa NI, Elakhras A, Wasfy R, Baker RA. Skin colonization by superantigen-producing Staphylococcus aureus in Egyptian patients with atopic dermatitis and its relation to disease severity and serum interleukin-4 level. *Int J Infect Dis.* 2012;16(1):e29-33.
216. Kozman A, Yao Y, Bina P, et al. Encoding a superantigen by Staphylococcus aureus does not affect clinical characteristics of infected atopic dermatitis lesions. *Br J Dermatol.* 2010;163(6):1308-1311.
217. Mempel M, Lina G, Hojka M, et al. High prevalence of superantigens associated with the egc locus in Staphylococcus aureus isolates from patients with atopic eczema. *Eur J Clin Microbiol Infect Dis.* 2003;22(5):306-309.
218. Malachowa N, DeLeo FR. Mobile genetic elements of Staphylococcus aureus. *Cell Mol Life Sci.* 2010;67(18):3057-3071.
219. Rajkovic A, Jovanovic J, Monteiro S, et al. Detection of toxins involved in foodborne diseases caused by Gram-positive bacteria. *Compr Rev Food Sci Food Saf.* 2020;19(4):1605-1657.
220. van Wamel WJ, Rooijakkers SH, Ruyken M, van Kessel KP, van Strijp JA. The innate immune modulators staphylococcal complement inhibitor and chemotaxis inhibitory protein of Staphylococcus aureus are located on beta-hemolysin-converting bacteriophages. *J Bacteriol.* 2006;188(4):1310-1315.
221. McCarthy AJ, Lindsay JA. The distribution of plasmids that carry virulence and resistance genes in Staphylococcus aureus is lineage associated. *BMC Microbiol.* 2012;12:104.

222. Hwang J, Thompson A, Jaros J, Blackcloud P, Hsiao J, Shi VY. Updated understanding of Staphylococcus aureus in atopic dermatitis: From virulence factors to commensals and clonal complexes. *Exp Dermatol.* 2021;30(10):1532-1545.
223. Ndhlovu GON, Abotsi RE, Shittu AO, et al. Molecular epidemiology of Staphylococcus aureus in African children from rural and urban communities with atopic dermatitis. *BMC Infect Dis.* 2021;21(1):348.
224. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. Rapid PCR detection of Staphylococcus aureus clonal complex 398 by targeting the restriction-modification system carrying sau1-hsdS1. *J Clin Microbiol.* 2011;49(2):732-734.
225. Stepanovic S, Vukovic D, Hola V, et al. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *APMIS.* 2007;115(8):891-899.
226. Fernandes CJ, Fernandes LA, Collignon P, Australian Group on Antimicrobial R. Cefoxitin resistance as a surrogate marker for the detection of methicillin-resistant Staphylococcus aureus. *J Antimicrob Chemother.* 2005;55(4):506-510.
227. Yao W, Xu G, Li D, et al. Staphylococcus aureus with an erm-mediated constitutive macrolide-lincosamide-streptogramin B resistance phenotype has reduced susceptibility to the new ketolide, solithromycin. *BMC Infect Dis.* 2019;19(1):175.
228. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect.* 2012;18(3):268-281.
229. Zerbino DR. Using the Velvet de novo assembler for short-read sequencing technologies. *Curr Protoc Bioinformatics.* 2010;Chapter 11:Unit 11 15.
230. Larsen MV, Cosentino S, Lukjancenko O, et al. Benchmarking of methods for genomic taxonomy. *J Clin Microbiol.* 2014;52(5):1529-1539.
231. Hasman H, Saputra D, Sicheritz-Ponten T, et al. Rapid whole-genome sequencing for detection and characterization of microorganisms directly from clinical samples. *J Clin Microbiol.* 2014;52(1):139-146.
232. Manara S, Pasolli E, Dolce D, et al. Whole-genome epidemiology, characterisation, and phylogenetic reconstruction of Staphylococcus aureus strains in a paediatric hospital. *Genome Med.* 2018;10(1):82.
233. Kleinheinz KA, Joensen KG, Larsen MV. Applying the ResFinder and VirulenceFinder web-services for easy identification of acquired antibiotic resistance and E. coli virulence genes in bacteriophage and prophage nucleotide sequences. *Bacteriophage.* 2014;4(1):e27943.
234. Liu B, Zheng D, Jin Q, Chen L, Yang J. VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic Acids Res.* 2019;47(D1):D687-D692.
235. Alcock BP, Raphenya AR, Lau TTY, et al. CARD 2020: antibiotic resistome surveillance with the comprehensive antibiotic resistance database. *Nucleic Acids Res.* 2020;48(D1):D517-D525.
236. Florensa AF, Kaas RS, Clausen P, Aytan-Aktug D, Aarestrup FM. ResFinder - an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microb Genom.* 2022;8(1).
237. Kaya H, Hasman H, Larsen J, et al. SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data. *mSphere.* 2018;3(1).
238. Carattoli A, Hasman H. PlasmidFinder and In Silico pMLST: Identification and Typing of Plasmid Replicons in Whole-Genome Sequencing (WGS). *Methods Mol Biol.* 2020;2075:285-294.
239. Boratyn GM, Camacho C, Cooper PS, et al. BLAST: a more efficient report with usability improvements. *Nucleic Acids Res.* 2013;41(Web Server issue):W29-33.
240. Arndt D, Grant JR, Marcu A, et al. PHASTER: a better, faster version of the PHAST phage search tool. *Nucleic Acids Res.* 2016;44(W1):W16-21.
241. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics.* 2014;30(14):2068-2069.
242. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res.* 2006;34(Database issue):D32-36.
243. Bertels F, Silander OK, Pachkov M, Rainey PB, van Nimwegen E. Automated reconstruction of whole-genome phylogenies from short-sequence reads. *Mol Biol Evol.* 2014;31(5):1077-1088.
244. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 2021;49(W1):W293-W296.
245. Tonkin-Hill G, MacAlasdair N, Ruis C, et al. Producing polished prokaryotic pangenomes with the Panaroo pipeline. *Genome Biol.* 2020;21(1):180.
246. Lees JA, Galardini M, Bentley SD, Weiser JN, Corander J. pyseer: a comprehensive tool for microbial pangenome-wide association studies. *Bioinformatics.* 2018;34(24):4310-4312.

247. McHugh ML. Interrater reliability: the kappa statistic. *Biochem Med (Zagreb).* 2012;22(3):276-282.
248. Makris G, Wright JD, Ingham E, Holland KT. The hyaluronate lyase of Staphylococcus aureus - a virulence factor? *Microbiology (Reading).* 2004;150(Pt 6):2005-2013.
249. Lee JH, Kim YG, Park S, Hu L, Lee J. Phytopigment Alizarin Inhibits Multispecies Biofilm Development by Cutibacterium acnes, Staphylococcus aureus, and Candida albicans. *Pharmaceutics.* 2022;14(5).
250. Dreno B, Pecastaings S, Corvec S, Veraldi S, Khammari A, Roques C. Cutibacterium acnes (Propionibacterium acnes) and acne vulgaris: a brief look at the latest updates. *J Eur Acad Dermatol Venereol.* 2018;32 Suppl 2:5-14.
251. Verkaik NJ, Benard M, Boelens HA, et al. Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization. *Clin Microbiol Infect.* 2011;17(3):343-348.
252. Silva V, Almeida F, Carvalho JA, et al. Emergence of community-acquired methicillin-resistant Staphylococcus aureus EMRSA-15 clone as the predominant cause of diabetic foot ulcer infections in Portugal. *Eur J Clin Microbiol Infect Dis.* 2020;39(1):179-186.
253. Jian Y, Zhao L, Zhao N, et al. Increasing prevalence of hypervirulent ST5 methicillin susceptible Staphylococcus aureus subtype poses a serious clinical threat. *Emerg Microbes Infect.* 2021;10(1):109-122.
254. Kuroda M, Ohta T, Uchiyama I, et al. Whole genome sequencing of meticillin-resistant Staphylococcus aureus. *Lancet.* 2001;357(9264):1225-1240.
255. Siguier P, Gourbeyre E, Chandler M. Bacterial insertion sequences: their genomic impact and diversity. *FEMS Microbiol Rev.* 2014;38(5):865-891.
256. Talat A, Khalid S, Majeed HAR, Khan AU. Whole-genome sequence analysis of multidrug-resistant Staphylococcus epidermidis ST35 strain isolated from human ear infection of an Iraqi patient. *J Glob Antimicrob Resist.* 2020;21:318-320.
257. Wan TW, Higuchi W, Khokhlova OE, et al. Genomic comparison between Staphylococcus aureus GN strains clinically isolated from a familial infection case: IS1272 transposition through a novel inverted repeat-replacing mechanism. *PLoS One.* 2017;12(11):e0187288.
258. Wang L, Si W, Xue H, Zhao X. Characterization of a functional insertion sequence ISSau2 from Staphylococcus aureus. *Mob DNA.* 2018;9:3.
259. Bojang A, Baines SL, Donovan L, et al. Genomic investigation of Staphylococcus aureus recovered from Gambian women and newborns following an oral dose of intra-partum azithromycin. *J Antimicrob Chemother.* 2019;74(11):3170-3178.
260. Harmer CJ, Hall RM. An analysis of the IS6/IS26 family of insertion sequences: is it a single family? *Microb Genom.* 2019;5(9).
261. McGavin MJ, Arsic B, Nickerson NN. Evolutionary blueprint for host- and niche-adaptation in Staphylococcus aureus clonal complex CC30. *Front Cell Infect Microbiol.* 2012;2:48.
262. Zhu Y, Zhang W, Liu S, Schwarz S. Identification of an IS431-derived translocatable unit containing the erm(C) gene in Staphylococcus aureus. *J Antimicrob Chemother.* 2021;76(4):1102-1104.
263. Frisch MB, Castillo-Ramirez S, Petit RA, 3rd, et al. Invasive Methicillin-Resistant Staphylococcus aureus USA500 Strains from the U.S. Emerging Infections Program Constitute Three Geographically Distinct Lineages. *mSphere.* 2018;3(3).
264. Hennig S, Ziebuhr W. A transposase-independent mechanism gives rise to precise excision of IS256 from insertion sites in Staphylococcus epidermidis. *J Bacteriol.* 2008;190(4):1488-1490.
265. Ha KP, Clarke RS, Kim GL, et al. Staphylococcal DNA Repair Is Required for Infection. *mBio.* 2020;11(6).
266. Podlesek Z, Zgur Bertok D. The DNA Damage Inducible SOS Response Is a Key Player in the Generation of Bacterial Persister Cells and Population Wide Tolerance. *Front Microbiol.* 2020;11:1785.
267. McRobbie AM, Meyer B, Rouillon C, Petrovic-Stojanovska B, Liu H, White MF. Staphylococcus aureus DinG, a helicase that has evolved into a nuclease. *Biochem J.* 2012;442(1):77-84.
268. Huang YH, Huang CY. SAAV2152 is a single-stranded DNA binding protein: the third SSB in Staphylococcus aureus. *Oncotarget.* 2018;9(29):20239-20254.
269. Liu L, Ingmer H, Vestergaard M. Genome-Wide Identification of Resveratrol Intrinsic Resistance Determinants in Staphylococcus aureus. *Antibiotics (Basel).* 2021;10(1).
270. Lomholt H, Andersen KE, Kilian M. Staphylococcus aureus clonal dynamics and virulence factors in children with atopic dermatitis. *J Invest Dermatol.* 2005;125(5):977-982.
271. Lawrence JG, Hendrix RW, Casjens S. Where are the pseudogenes in bacterial genomes? *Trends Microbiol.* 2001;9(11):535-540.
272. Marques AT, Tanoeiro L, Duarte A, Goncalves L, Vitor JMB, Vale FF. Genomic Analysis of Prophages from Klebsiella pneumoniae Clinical Isolates. *Microorganisms.* 2021;9(11).

273. Nepal R, Houtak G, Shaghayegh G, et al. Prophages encoding human immune evasion cluster genes are enriched in Staphylococcus aureus isolated from chronic rhinosinusitis patients with nasal polyps. *Microb Genom.* 2021;7(12).
274. Goerke C, Pantucek R, Holtfreter S, et al. Diversity of prophages in dominant Staphylococcus aureus clonal lineages. *J Bacteriol.* 2009;191(11):3462-3468.
275. Kraushaar B, Hammerl JA, Kienol M, et al. Acquisition of virulence factors in livestock-associated MRSA: Lysogenic conversion of CC398 strains by virulence gene-containing phages. *Sci Rep.* 2017;7(1):2004.
276. Kumpitsch C, Koskinen K, Schopf V, Moissl-Eichinger C. The microbiome of the upper respiratory tract in health and disease. *BMC Biol.* 2019;17(1):87.
277. Luna PN, Hasegawa K, Ajami NJ, et al. The association between anterior nares and nasopharyngeal microbiota in infants hospitalized for bronchiolitis. *Microbiome.* 2018;6(1):2.
278. Parlet CP, Brown MM, Horswill AR. Commensal Staphylococci Influence Staphylococcus aureus Skin Colonization and Disease. *Trends Microbiol.* 2019;27(6):497-507.
279. Fluhr JW, Darlenski R, Surber C. Glycerol and the skin: holistic approach to its origin and functions. *Br J Dermatol.* 2008;159(1):23-34.
280. Langan SM, Irvine AD, Weidinger S. Atopic dermatitis. *Lancet.* 2020;396(10247):345-360.
281. Nath S, Kumari N, Bandyopadhyay D, et al. Dysbiotic Lesional Microbiome With Filaggrin Missense Variants Associate With Atopic Dermatitis in India. *Front Cell Infect Microbiol.* 2020;10:570423.
282. Hoeger PH, Niggemann B, Schroeder C. Enhanced basal and stimulated PMN chemiluminescence activity in children with atopic dermatitis: stimulatory role of colonizing staphylococci? *Acta Paediatr.* 1992;81(6-7):542-546.
283. Allen HB, Mueller JL. A novel finding in atopic dermatitis: film-producing Staphylococcus epidermidis as an etiology. *Int J Dermatol.* 2011;50(8):992-993.
284. Formosa-Dague C, Speziale P, Foster TJ, Geoghegan JA, Dufrene YF. Zinc-dependent mechanical properties of Staphylococcus aureus biofilm-forming surface protein SasG. *Proc Natl Acad Sci U S A.* 2016;113(2):410-415.
285. Lehtimaki J, Karkman A, Laatikainen T, et al. Patterns in the skin microbiota differ in children and teenagers between rural and urban environments. *Sci Rep.* 2017;7:45651.
286. Blicharz L, Michalak M, Szymanek-Majchrzak K, et al. The Propensity to Form Biofilm in vitro by Staphylococcus aureus Strains Isolated from the Anterior Nares of Patients with Atopic Dermatitis: Clinical Associations. *Dermatology.* 2020:1-7.
287. Callewaert C, Ravard Helffer K, Lebaron P. Skin Microbiome and its Interplay with the Environment. *Am J Clin Dermatol.* 2020;21(Suppl 1):4-11.
288. Baviera G, Leoni MC, Capra L, et al. Microbiota in healthy skin and in atopic eczema. *Biomed Res Int.* 2014;2014:436921.
289. Lunjani N, Tan G, Dreher A, et al. Environment-dependent alterations of immune mediators in urban and rural south African children with atopic dermatitis. *Allergy.* 2021.
290. Mbow M, de Jong SE, Meurs L, et al. Changes in immunological profile as a function of urbanization and lifestyle. *Immunology.* 2014;143(4):569-577.
291. Laske N, Niggemann B. Does the severity of atopic dermatitis correlate with serum IgE levels? *Pediatr Allergy Immunol.* 2004;15(1):86-88.
292. Kumar MK, Singh PK, Patel PK. Clinico-immunological profile and their correlation with severity of atopic dermatitis in Eastern Indian children. *J Nat Sci Biol Med.* 2014;5(1):95-100.
293. Merriman JA, Mueller EA, Cahill MP, et al. Temporal and Racial Differences Associated with Atopic Dermatitis Staphylococcusaureus and Encoded Virulence Factors. *mSphere.* 2016;1(6).
294. Towell AM, Feuillie C, Vitry P, et al. Staphylococcus aureus binds to the N-terminal region of corneodesmosin to adhere to the stratum corneum in atopic dermatitis. *Proc Natl Acad Sci U S A.* 2021;118(1).
295. Pickering AC, Vitry P, Prystopiuk V, et al. Host-specialized fibrinogen-binding by a bacterial surface protein promotes biofilm formation and innate immune evasion. *PLoS Pathog.* 2019;15(6):e1007816.
296. Nostro A, Cellini L, Di Giulio M, et al. Effect of alkaline pH on staphylococcal biofilm formation. *APMIS.* 2012;120(9):733-742.
297. Stewart EJ, Payne DE, Ma TM, et al. Effect of Antimicrobial and Physical Treatments on Growth of Multispecies Staphylococcal Biofilms. *Appl Environ Microbiol.* 2017;83(12).
298. Abdul Hamid AI, Nakusi L, Givskov M, Chang YT, Marques C, Gueirard P. A mouse ear skin model to study the dynamics of innate immune responses against Staphylococcus aureus biofilms. *BMC Microbiol.* 2020;20(1):22.

299. Ashrafi M, Novak-Frazer L, Bates M, et al. Validation of biofilm formation on human skin wound models and demonstration of clinically translatable bacteria-specific volatile signatures. *Sci Rep.* 2018;8(1):9431.
300. Glatz M, Jo JH, Kennedy EA, et al. Emollient use alters skin barrier and microbes in infants at risk for developing atopic dermatitis. *PLoS One.* 2018;13(2):e0192443.
301. Dierick BJH, van der Molen T, Flokstra-de Blok BMJ, et al. Burden and socioeconomics of asthma, allergic rhinitis, atopic dermatitis and food allergy. *Expert Rev Pharmacoecon Outcomes Res.* 2020;20(5):437-453.
302. Hanski I, von Hertzen L, Fyhrquist N, et al. Environmental biodiversity, human microbiota, and allergy are interrelated. *Proc Natl Acad Sci U S A.* 2012;109(21):8334-8339.
303. Mariani J, Favero C, Spinazze A, et al. Short-term particulate matter exposure influences nasal microbiota in a population of healthy subjects. *Environ Res.* 2018;162:119-126.
304. Alenius H, Pakarinen J, Saris O, et al. Contrasting immunological effects of two disparate dusts - preliminary observations. *Int Arch Allergy Immunol.* 2009;149(1):81-90.
305. Mahdavinia M, Greenfield LR, Moore D, et al. House dust microbiota and atopic dermatitis; effect of urbanization. *Pediatr Allergy Immunol.* 2021;32(5):1006-1012.
306. Pedersen CJ, Uddin MJ, Saha SK, Darmstadt GL. Prevalence and psychosocial impact of atopic dermatitis in Bangladeshi children and families. *PLoS One.* 2021;16(4):e0249824.
307. Schram ME, Tedja AM, Spijker R, Bos JD, Williams HC, Spuls PI. Is there a rural/urban gradient in the prevalence of eczema? A systematic review. *Br J Dermatol.* 2010;162(5):964-973.
308. Girardeau S, Mine S, Pageon H, Asselineau D. The Caucasian and African skin types differ morphologically and functionally in their dermal component. *Exp Dermatol.* 2009;18(8):704-711.
309. Pappas A, Fantasia J, Chen T. Age and ethnic variations in sebaceous lipids. *Dermatoendocrinol.* 2013;5(2):319-324.
310. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol.* 2011;9(4):244-253.
311. Alam MJ, Xie L, Yap YA, Marques FZ, Robert R. Manipulating Microbiota to Treat Atopic Dermatitis: Functions and Therapies. *Pathogens.* 2022;11(6).
312. Khadka VD, Key FM, Romo-Gonzalez C, et al. The Skin Microbiome of Patients With Atopic Dermatitis Normalizes Gradually During Treatment. *Front Cell Infect Microbiol.* 2021;11:720674.
313. Olesen CM, Ingham AC, Thomsen SF, et al. Changes in Skin and Nasal Microbiome and Staphylococcal Species Following Treatment of Atopic Dermatitis with Dupilumab. *Microorganisms.* 2021;9(7).
314. Lee SJ, Kim SE, Shin KO, Park K, Lee SE. Dupilumab Therapy Improves Stratum Corneum Hydration and Skin Dysbiosis in Patients With Atopic Dermatitis. *Allergy Asthma Immunol Res.* 2021;13(5):762-775.
315. Gonzalez ME, Schaffer JV, Orlow SJ, et al. Cutaneous microbiome effects of fluticasone propionate cream and adjunctive bleach baths in childhood atopic dermatitis. *J Am Acad Dermatol.* 2016;75(3):481-493 e488.
316. Ul-Hasan S, Bowers RM, Figueroa-Montiel A, et al. Community ecology across bacteria, archaea and microbial eukaryotes in the sediment and seawater of coastal Puerto Nuevo, Baja California. *PLoS One.* 2019;14(2):e0212355.
317. Shannon CE. The mathematical theory of communication. 1963. *MD Comput.* 1997;14(4):306-317.
318. Clausen ML, Agner T, Lilje B, Edslev SM, Johannesen TB, Andersen PS. Association of Disease Severity With Skin Microbiome and Filaggrin Gene Mutations in Adult Atopic Dermatitis. *JAMA Dermatol.* 2018;154(3):293-300.
319. Olesen CM, Clausen ML, Agner T, et al. Altered Maturation of the Skin Bacterial Communities in Infants with Atopic Dermatitis. *Acta Derm Venereol.* 2022.
320. Caio G, Lungaro L, Segata N, et al. Effect of Gluten-Free Diet on Gut Microbiota Composition in Patients with Celiac Disease and Non-Celiac Gluten/Wheat Sensitivity. *Nutrients.* 2020;12(6).
321. Gu X, Sim JXY, Lee WL, et al. Gut Ruminococcaceae levels at baseline correlate with risk of antibiotic-associated diarrhea. *iScience.* 2022;25(1):103644.
322. Shan Y, Guo J, Fan W, et al. Modern urbanization has reshaped the bacterial microbiome profiles of house dust in domestic environments. *World Allergy Organ J.* 2020;13(8):100452.
323. Lehtimaki J, Thorsen J, Rasmussen MA, et al. Urbanized microbiota in infants, immune constitution, and later risk of atopic diseases. *J Allergy Clin Immunol.* 2021;148(1):234-243.
324. Mehmood M, Jaffar NA, Nazim M, Khasawneh FA. Bacteremic skin and soft tissue infection caused by Prevotella loescheii. *BMC Infect Dis.* 2014;14:162.
325. Saladi L, Zeana C, Singh M. Native Valve Endocarditis due to Veillonella Species: A Case Report and Review of the Literature. *Case Rep Infect Dis.* 2017;2017:4896186.
326. Chng KR, Tay AS, Li C, et al. Whole metagenome profiling reveals skin microbiome-dependent susceptibility to atopic dermatitis flare. *Nat Microbiol.* 2016;1(9):16106.

327. Dzidic M, Abrahamsson TR, Artacho A, Collado MC, Mira A, Jenmalm MC. Oral microbiota maturation during the first 7 years of life in relation to allergy development. *Allergy.* 2018;73(10):2000-2011.
328. Sugarman JL, Hersh AL, Okamura T, Howard R, Frieden IJ. A retrospective review of streptococcal infections in pediatric atopic dermatitis. *Pediatr Dermatol.* 2011;28(3):230-234.
329. Liu Y, Wang S, Dai W, et al. Distinct Skin Microbiota Imbalance and Responses to Clinical Treatment in Children With Atopic Dermatitis. *Front Cell Infect Microbiol.* 2020;10:336.
330. Adachi J, Endo K, Fukuzumi T, Tanigawa N, Aoki T. Increasing incidence of streptococcal impetigo in atopic dermatitis. *J Dermatol Sci.* 1998;17(1):45-53.
331. Akiyama H, Yamasaki O, Kanzaki H, Tada J, Arata J. Streptococci isolated from various skin lesions: the interaction with Staphylococcus aureus strains. *J Dermatol Sci.* 1999;19(1):17-22.
332. Hayakawa K, Hirahara K, Fukuda T, Okazaki M, Shiohara T. Risk factors for severe impetiginized atopic dermatitis in Japan and assessment of its microbiological features. *Clin Exp Dermatol.* 2009;34(5):e63-65.
333. Tsatsaronis JA, Ly D, Pupovac A, et al. Group A Streptococcus Modulates Host Inflammation by Manipulating Polymorphonuclear Leukocyte Cell Death Responses. *J Innate Immun.* 2015;7(6):612-622.
334. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. *Immunology.* 2017;151(4):363-374.
335. Klonowska J, Glen J, Nowicki RJ, Trzeciak M. New Cytokines in the Pathogenesis of Atopic Dermatitis-New Therapeutic Targets. *Int J Mol Sci.* 2018;19(10).
336. Karched M, Bhardwaj RG, Tiss A, Asikainen S. Proteomic Analysis and Virulence Assessment of Granulicatella adiacens Secretome. *Front Cell Infect Microbiol.* 2019;9:104.
337. Alkandari SA, Bhardwaj RG, Ellepola A, Karched M. Proteomics of extracellular vesicles produced by Granulicatella adiacens, which causes infective endocarditis. *PLoS One.* 2020;15(11):e0227657.
338. Graves DT, Correa JD, Silva TA. The Oral Microbiota Is Modified by Systemic Diseases. *J Dent Res.* 2019;98(2):148-156.
339. Bugueno IM, Batool F, Keller L, Kuchler-Bopp S, Benkirane-Jessel N, Huck O. Porphyromonas gingivalis bypasses epithelial barrier and modulates fibroblastic inflammatory response in an in vitro 3D spheroid model. *Sci Rep.* 2018;8(1):14914.
340. Murata S, Kaneko S, Morita E. Interleukin-8 Levels in the Stratum Corneum as a Biomarker for Monitoring Therapeutic Effect in Atopic Dermatitis Patients. *Int Arch Allergy Immunol.* 2021;182(7):592-606.
341. Guttman-Yassky E, Diaz A, Pavel AB, et al. Use of Tape Strips to Detect Immune and Barrier Abnormalities in the Skin of Children With Early-Onset Atopic Dermatitis. *JAMA Dermatol.* 2019;155(12):1358-1370.
342. Amarbayasgalan T, Takahashi H, Dekio I, Morita E. Interleukin-8 content in the stratum corneum as an indicator of the severity of inflammation in the lesions of atopic dermatitis. *Int Arch Allergy Immunol.* 2013;160(1):63-74.
343. Neumann A, Bjorck L, Frick IM. Finegoldia magna, an Anaerobic Gram-Positive Bacterium of the Normal Human Microbiota, Induces Inflammation by Activating Neutrophils. *Front Microbiol.* 2020;11:65.
344. Hrestak D, Matijasic M, Cipcic Paljetak H, Ledic Drvar D, Ljubojevic Hadzavdic S, Peric M. Skin Microbiota in Atopic Dermatitis. *Int J Mol Sci.* 2022;23(7).
345. Thorsen J, Rasmussen MA, Waage J, et al. Infant airway microbiota and topical immune perturbations in the origins of childhood asthma. *Nat Commun.* 2019;10(1):5001.
346. Chen M, He S, Miles P, et al. Nasal Bacterial Microbiome Differs Between Healthy Controls and Those With Asthma and Allergic Rhinitis. *Front Cell Infect Microbiol.* 2022;12:841995.
347. Coleman A, Zaugg J, Wood A, et al. Upper Respiratory Tract Microbiome of Australian Aboriginal and Torres Strait Islander Children in Ear and Nose Health and Disease. *Microbiol Spectr.* 2021;9(2):e0036721.
348. Dekio I, Sakamoto M, Hayashi H, Amagai M, Suematsu M, Benno Y. Characterization of skin microbiota in patients with atopic dermatitis and in normal subjects using 16S rRNA gene-based comprehensive analysis. *J Med Microbiol.* 2007;56(Pt 12):1675-1683.
349. Ramadan M, Hetta HF, Saleh MM, Ali ME, Ahmed AA, Salah M. Alterations in skin microbiome mediated by radiotherapy and their potential roles in the prognosis of radiotherapy-induced dermatitis: a pilot study. *Sci Rep.* 2021;11(1):5179.
350. Falagas ME, Kastoris AC, Vouloumanou EK, Dimopoulos G. Community-acquired Stenotrophomonas maltophilia infections: a systematic review. *Eur J Clin Microbiol Infect Dis.* 2009;28(7):719-730.

351. Di Bonaventura G, Pompilio A, Zappacosta R, et al. Role of excessive inflammatory response to Stenotrophomonas maltophilia lung infection in DBA/2 mice and implications for cystic fibrosis. *Infect Immun.* 2010;78(6):2466-2476.
352. Manus MB, Kuthyar S, Perroni-Maranon AG, Nunez-de la Mora A, Amato KR. Infant Skin Bacterial Communities Vary by Skin Site and Infant Age across Populations in Mexico and the United States. *mSystems.* 2020;5(6).
353. McCall LI, Callewaert C, Zhu Q, et al. Home chemical and microbial transitions across urbanization. *Nat Microbiol.* 2020;5(1):108-115.
354. Kirjavainen PV, Karvonen AM, Adams RI, et al. Farm-like indoor microbiota in non-farm homes protects children from asthma development. *Nat Med.* 2019;25(7):1089-1095.
355. Birzele LT, Depner M, Ege MJ, et al. Environmental and mucosal microbiota and their role in childhood asthma. *Allergy.* 2017;72(1):109-119.
356. White DC, Sutton SD, Ringelberg DB. The genus Sphingomonas: physiology and ecology. *Curr Opin Biotechnol.* 1996;7(3):301-306.
357. Lin Y, Gifford S, Ducklow H, Schofield O, Cassar N. Towards Quantitative Microbiome Community Profiling Using Internal Standards. *Appl Environ Microbiol.* 2019;85(5).
358. Ta LDH, Tay CJX, Lay C, et al. Household environmental microbiota influences early-life eczema development. *Environ Microbiol.* 2021;23(12):7710-7722.

APPENDIX A: Declaration of ethical clearance

Recent ethical clearance (as of July 2022).

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

FHS017: Annual Progress Report / Renewal

Record Reviews/Audits/Collection of Biological Specimens/Repositories/Databases/Registries

HREC office use only (FWA00001637; IRB00001938)			
This serves as notification of annual approval, including any documentation described below.			
☑ Approved	Annual progress report	Approved until/next renewal date	30-6-23
☐ Not approved	See attached comments		
Signature Chairperson of the HREC/ Designee		Date Signed	7/7/2022

Note: Please note that incomplete submissions will not be reviewed.
Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.

Please clarify your plan for research-related activities during COVID-19 lockdown

HUMAN RESEARCH ETHICS COMMITTEE
06 JUL 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	5th July 2022		
HREC REF Number	668 / 2020	Current Ethics Approval was granted until	30th June 2022
Protocol title	Microbiome interactions in atopic dermatitis of rural and urban South African populations (Sub-study linked to 451/2014)		
Principal Investigator	Dr. Felix Dube		
Department / Office Internal Mail Address	Department of Molecular and Cell Biology University of Cape Town 22 University Avenue North | Molecular Biology Building Second floor, Room 227 | Upper Campus, Rondebosch, 7700 Email: sizwe.dube@uct.ac.za |Tel: +27 021 650 3861		
1.1 Does this protocol receive US Federal funding?		☐ Yes	☑ No

2. Protocol status (tick ✓)

☑	Research-related activities are ongoing
☐	Data collection is complete, data analysis only
Please indicate (in the block below) the titles and HREC reference numbers of any projects currently making use of the Database/registry/repository.	

3. Protocol summary

Total number of records or specimens collected, reviewed or stored since the original approval		14000
Total number of records or specimens collected, reviewed or stored since last progress report		N/A
Have any research-related outputs (e.g. publications, abstracts, conference presentations) resulted from this research? If yes, please list and attach with this report.	☑ Yes	☐ No

4. Signature

16 February 2022 Page 1 of 2 FHS017
(Note: Please complete the Closure form (FHS019) if the study is completed within the approval period)

Signature of PI		Date	5th July 2022

Original application of ethical clearance for sub-study from parent study (HREC: 451/2014)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room G50- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-enquiries@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

29 October 2020

HREC REF: 668/2020

Dr F Dube
Department of Molecular and Cell Biology & IDM
Room 227, Cell Biology Building
Email: sizwe.dube@uct.ac.za

Dear Dr Dube

PROJECT TITLE: MICROBIOME INTERACTIONS IN ATOPIC DERMATITIS OF RURAL AND URBAN SOUTH AFRICAN POPULATIONS. SUB-STUDY LINKED TO 451/2014.

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study, subject to providing a new FHS 013 application form pg.1 with the correct expiry date for 451/2014.

The HREC query whether students will have time to review 2500-3000 folders, please clarify.

This approval is subject to strict adherence to the HREC recommendations regarding research involving human participants during COVID -19, dated 17 March 2020 & 06 July 2020.

Approval is granted for one year until the 30 October 2021.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

HREC/REF:668/2020sa

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
NHREC-registration number: REC-210208-007

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI), and Declaration of Helsinki (2013) guidelines. The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

HREC/REF:668/2020sa

APPENDIX B: Publications, conference proceedings and statement of work done by the candidate

Publications

Part of the data shown in Chapters 2 (**Figures 2.1, 2.3 and 2.4, and Tables 2.1, 2.2a, 2.2b, 2.3, S2.1, S2.2, and S2.3**) and 4 (**Figures 4.1–4.6, and Tables 2.1–2.5, and S2.1**) are contained in the following publications:

Chapter 2:

Gillian O. N. Ndhlovu, Regina E. Abotsi, Adebayo O. Shittu, Shima M. Abdulgader, Dorota Jamrozy, Christopher L. Dupont, Avumile Mankahla, Mark P. Nicol, Carol Hlela, Michael E. Levin, Nonhlanhla Lunjani, and Felix S. Dube. **Molecular epidemiology of *Staphylococcus aureus* in African children from rural and urban communities with atopic dermatitis**. *BMC Infectious Diseases (2021) 21:348*. DOI: 10.1186/s12879-021-06044-4.

Statement by candidate: All data presented in chapter 2 included in the publication were produced from experiments conducted by candidate. The interpretation of the results, statistical analyses, and presentation of all figures were completed by the candidate. The candidate also prepared the first draft of the manuscript publication and addressed all valuable feedback from co-authors and reviewers from the journal.

Chapter 4:

Gillian O. N. Ndhlovu, Felix S. Dube, Rasalika T. Moonsamy, Avumile Mankahla, Carol Hlela, Michael E. Levin, Nonhlanhla Lunjani, Adebayo O. Shittu, Shima M. Abdulgader. **Skin and nasal colonization of coagulase-negative staphylococci are associated with atopic dermatitis among South African toddlers**. *PLoS ONE 17(3): e0265326*. DOI: 10.1371/journal. pone.0265326.

Statement by candidate: All data presented in chapter 4 included in the publication were produced from experiments conducted by candidate. The interpretation of the results, statistical analyses, and presentation of all figures were completed by the candidate. The candidate also

prepared the first draft of the manuscript publication and addressed all valuable feedback from co-authors and reviewers from the journal.

Abstract Publication

Chapter 2:

Gillian O. N. Ndhlovu, Regina E. Abotsi, and Felix S. Dube. **The molecular epidemiology of Staphylococcus aureus lineages in paediatric atopic dermatitis in rural and urban South Africa: A case–control study**. *International Journal of Infectious Diseases Volume 101, Supplement 1, 413, December 01, 2020.* DOI: 10.1016/j.ijid.2020.09.1083. **The International Society for Infectious Diseases 2020, Kuala Lumpur,** Malaysia – February 2020 [Poster presentation – conference cancelled due to the 2019/2020 global COVID pandemic).

Statement by candidate: All data presented in chapter 2 included in the publication were produced from experiments conducted by candidate. The interpretation of the results, statistical analyses, and presentation of all figures were completed by the candidate. The candidate also prepared the abstract for the. Conference was cancelled due to the 2019 COVID pandemic, however, permission was granted to publish the submitted abstract.

Conference Proceedings

Part of the data shown in Chapter 2 (**Figures 2.3 and 2.4, and Tables 2.2a and 2.2b**), Chapter 3 (Figures 3.1 and S3.2), and Chapter 4 (**Figures 4.1–4.6, and Tables 2.1–2.5, and S2.1**) are contained in the following conference proceedings:

Chapter 2:

Gillian O.N. Ndhlovu, Regina E. Abotsi, and Felix S. Dube. **Molecular epidemiology of *Staphylococcus aureus* in African children from rural and urban communities with atopic dermatitis**. **8th FIDSSA Congress 2019, Johannesburg, South Africa** – November 2019 [Oral presentation].

Statement by candidate: All data presented in chapter 2 included in the conference presentation were produced from experiments conducted by candidate. The interpretation of the results, statistical analyses, and presentation of all figures were completed by the candidate. The candidate also prepared the presentation and presented the work.

Chapter 3:

Gillian O.N. Ndhlovu, Froodia Ngondoh, Adebayo Shittu, Felix Dube. **Investigating genome diversity of Staphylococcus aureus associated with paediatric atopic dermatitis in South Africa**. **American Society of Microbiology 2022, Washington D.C., United States of America – June 2022** [Poster presentation].

Statement by candidate: All data presented in chapter 3 included in the conference presentation were produced from experiments conducted by candidate. The interpretation of the results, statistical analyses, and presentation of all figures were completed by the candidate. The candidate also prepared the poster presentation and presented the work.

Chapter 4:

Gillian O.N. Ndhlovu, Felix S. Dube, Rasalika T. Moonsamy, Adebayo O. Shittu, Shima M. Abdulgader. **Staphylococcal interactions in biofilms in paediatric atopic dermatitis in a South African cohort. South African Society for Microbiology, Virtual – June 2021** [Poster presentation].

Statement by candidate: All data presented in chapter 4 included in the conference presentation were produced from experiments conducted by candidate. The interpretation of the results, statistical analyses, and presentation of all figures were completed by the candidate. The candidate also prepared the poster presentation and presented the work.