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Colonisation with extended spectrum beta-lactamase-producing and carbapenem-resistant Enterobacterales in children admitted to a paediatric referral hospital in South Africa

A DISSERTATION SUBMITTED

BY

STUDENT: Dr Babatunde Oluwatosin Ogunbosi

STUDENT NUMBER: OGNBAB002

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SUPERVISOR: Professor Brian Eley,

Head of the Paediatric Infectious Diseases Unit,

Red Cross War Memorial Children's Hospital,

Department of Paediatrics and Child Health, University of Cape Town

CO-SUPERVISOR: Dr. James Nuttall

Senior Lecturer, Paediatric Infectious Diseases Unit,

Red Cross War Memorial Children's Hospital,

Department of Paediatrics and Child Health, University of Cape Town

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Declaration

I, **Babatunde O. Ogunbosi** hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Abstract

Introduction:

There are few studies describing colonisation with extended spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) among children in sub-Saharan Africa. Colonisation often precedes infection and multi-drug-resistant Enterobacterales are important causes of invasive infection.

Methods:

In this prospective cross-sectional study, conducted between April and June 2017, 200 children in a tertiary academic hospital were screened by rectal swab for EBSL-PE and CRE. The resistance-conferring genes were identified using polymerase chain reaction technology. Risk factors for colonisation were also evaluated.

Results:

Overall, 48% (96/200) of the children were colonised with at least one ESBL-PE, 8 of these with 2 ESBL-PE, and one with a CRE (0.5%, 1/200). Common colonising ESBL-PE were *Klebsiella pneumoniae* (62.5%, 65/104) and *Escherichia coli* (34.6%, 36/104). The most frequent ESBL-conferring gene was $bla_{\text{CTX-M}}$ in 95% (76/80) of the isolates. No resistance-conferring gene was identified in the CRE isolate (*Enterobacter cloacae)*. Most of the *Klebsiella pneumoniae* isolate*s* were susceptible to piperacillin/tazobactam (86.2%) and amikacin (63.9%). Similarly, 94.4% and 97.2% of the *Escherichia coli* isolates were susceptible to piperacillin/tazobactam and amikacin, respectively. Hospitalisation for more than 7 days before study enrolment was associated with ESBL-PE colonisation.

Conclusion:

Approximately half of hospitalised children in this study were colonised with ESBL-PE. This highlights the need for improved infection prevention and control practices to limit the dissemination of these microorganisms.

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Finally, to the immortal, invincible and only wise God, be all glory, honour, adoration and gratitude for through Him and by Him only could this work have been possible. Thank you Lord Jesus.

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Abbreviations

AES	Advanced Expert System
ANOVA	Analysis of variance
aOR	Adjusted odds ratio
ART	Antiretroviral therapy
BSI	Bloodstream infection
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CRE	Carbapenem-resistant Enterobacterales
CTX	Cefotaxime
CTX-M	Cefotaxime - M type
ESBL	Extended spectrum beta-lactamase
ESBLs	Extended spectrum beta-lactamases
ESBL-PE	Extended spectrum beta-lactamase-producing Enterobacterales
GES	Guiana extended spectrum
GSH	Groote Schuur Hospital
HREC	Human Research Ethics Committee
ICU	Intensive care unit
IMP	Imipenemase

KPC	*Klebsiella pneumoniae* carbapenemase
MIC	Minimal inhibitory concentration
MDRO	Multidrug resistant organism
NDM	New Delhi metallo-beta-lactamase
NHLS	National Health Laboratory Service
NICU	Neonatal intensive care unit
OR	Odds ratio
OXA-48	Oxacillinase-48
PCR	Polymerase chain reaction
PEG	Percutaneous endoscopic gastrostomy
RCWMCH	Red Cross War Memorial Children's Hospital
SHV	Sulfhydryl variable
TEM	Temoneira
VIM	Verona Integron-Mediated

CHAPTER 1: Introduction

1.1 Context

Infection with multidrug-resistant organisms (MDRO) is becoming a threat to global health [1, 2]. An estimated 700,000 individuals died from MDRO annually in 2014 with an estimated increase in healthcare costs of 20-35 billion dollars in the USA alone [3, 4]. If the current trajectory of infection with MDRO continues, global estimates are that 10 million individuals will die annually by 2050, higher than the combined deaths from cancer and road traffic accidents, and healthcare costs will have escalated to 10 trillion dollars between now and then [3]. Added to this, MDROs pose great challenges to advances in medicine, especially with the use of technology in novel therapies and organ transplantation. Notable among the MDROs are extended spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) [5]. They are among the pathogens categorised as critical in the World Health Organisation list of priority antibiotic-resistant pathogens [5]. These organisms are notorious for causing healthcare-associated infection around the world, and there are growing concerns about their increasing contribution to community-acquired infections. Invasive infection with CRE is associated with high mortality, high cost of management, few therapeutic options and the absence of effective consensus treatment guidelines [6].

The ESBL-PE, particularly *Klebsiella pneumoniae (K. pneumoniae)* and *Escherichia coli (E. coli)*, possess genes which encode extended spectrum beta-lactamases (ESBLs) that hydrolyse the beta-lactam ring in most penicillins, cephalosporins including the oxyiminocephalosporins, and monobactams [7, 8]. These enzymes have no activity against the cephamycins such as cefoxitin and cefotetan, and carbapenems, and are inhibited by beta-lactam inhibitors such as clavulanic acid [8]. The genes that encode for these enzymes are carried on plasmids which often also carry genes conferring resistance to other antibiotics like the fluoroquinolones, aminoglycosides, tetracyclines, chloramphenicol, trimethoprim

and sulphonamides making ESBL-PE multidrug-resistant organisms. The ESBLs belong to the Ambler class A enzymes. More than 200 have been identified. Initially, Temoneira (TEM) and sulfhydryl *v*ariable (SHV) enzymes were discovered. Over time the cefotaxime (CTX)-M-type (CTX-M), enzymes have become the most important ESBLs globally [9-14]. The CTX-M enzymes are found in both *K. pneumoniae,* which is responsible for most healthcare-associated infections, and *E. coli* which is responsible for many community-acquired ESBL infections [11-15].

Carbapenem-resistant Enterobacterales employ several resistance-conferring mechanisms including mobile genetic elements such as plasmids that encode carbapenemases. The carbapenemases hydrolyse the carbapenem ring more efficiently than other beta-lactamases. The carbapenemase-encoding genes on plasmids can be transferred from one bacterium to another, facilitating local transmission and global spread.

There are few reports of ESBL-PE and CRE colonisation in children. Colonisation often precedes infection [16, 17]. Infection risk among children previously colonised with CRE have ranged from 3.4 to 28.2% [18-21]. Risk of colonised children progressing to infection is increased by existing metabolic disease, previous carbapenem use, neutropaenia and prior surgical procedures [20].

Many of the hospital-based paediatric studies on ESBL-PE colonisation are in neonates, often in neonatal intensive care units (NICUs). Prevalence ranged from 4.3% to 75% [22-24]. Studies of ESBL-PE colonisation among hospitalised paediatric patients outside the neonatal age range have recorded prevalence between 18.5% and 57.1% [25-27], whereas carriage in community settings is lower, ranging from 0.1% to 12.4% [28-32]. In some settings, community carriage has increased over time. For example, surveillance studies in Bolivia and Peru over a decade show a steady rise from 0.1% in 2002 to 12.4% in 2011 [28, 32, 33]. Community carriage is thought to act as a reservoir for the development of community-acquired ESBL infections, particularly *E. coli* infections.

Factors that predispose to ESBL-PE colonisation in the neonatal period include prior carbapenem, penicillin and aminoglycoside exposure, prolonged hospitalisation, early-onset pneumonia, prolonged antibiotic use, formula milk feeds and having an ESBL-PE colonised mother [22, 24, 34-38]. Risk factors for ESBL-PE colonisation in older children include mechanical ventilation, prolonged hospital stay, prior antibiotic use and prior hospitalisation [25, 26]. However, ESBL-producing *E coli* carriage documented among children in a very remote community in Senegal where prior antibiotic use was most unlikely suggests that other factors may play a role in promoting colonisation [29]. Conversely, breastfeeding confers protection against ESBL-PE colonisation in the new born period [37, 39]. In early studies, ESBL-PE colonisation was associated with the SHV enzyme. More recent studies suggest that the CTX-M enzyme now predominates. [9, 24, 35, 36, 39].

Studies in children have reported CRE colonisation rates of 0.5% to 29.5% [18-21, 40-48]. All the studies on CRE colonisation have documented *K. pneumoniae* colonisation [18-21, 43, 44] while two studies also documented *Serratia species, E. coli* and *Enterobacter species* colonisation [18, 21]. In Turkey, the predominant carbapenemases were New Delhi metallo-beta-lactamase (NDM), oxacillinase (OXA) and imipenemase (IMP) [19, 21]. *Klebsiella pneumoniae* carbapenemase (KPC) and NDM are frequently isolated in the United Kingdom [18], while KPC was the predominant carbapenem resistance gene in Italy [43] and Verona Integron-Mediated Metallo-beta-lactamase (VIM) in Greece [44]. Risk factors associated with CRE colonisation include carbapenem exposure, transfer between healthcare facilities, aminoglycoside exposure, surgical procedures, urinary catheterisation, nasogastric intubation and prolonged antibiotic administration [19-21, 43].

Despite the global spread of these organisms and the association of widespread and indiscriminate use of broad-spectrum antibiotics with their emergence, little is known about the epidemiology of ESBL-PE and CRE colonisation and infection on the African continent, especially in children. There have been reports of ESBL-PE infections in South Africa from

the early 1990s, but most reports are in adults [49]. One of the first reports in children described the treatment and outcome of a case series of invasive CRE infections [50]. To address the dearth of ESBL-PE and CRE colonisation research in children in Africa, the present study describes ESBL-PE and CRE colonisation including prevalence, factors associated with colonisation and the distribution of ESBL genes among ESBL-PE isolates.

1.2 Ethical considerations

The study was conducted in accordance with the Helsinki Declaration. The study protocol was approved by the Human Research Ethics Committee (HREC), Faculty of Health Sciences, University of Cape Town, reference number: HREC REF: 898/2016. The Research Committee at the Red Cross War Memorial Children's Hospital (RCWMCH) also approved the study.

Written informed consent was obtained in the preferred language (English, Afrikaans or isiXhosa) from the parents or legal guardian, and children aged 12 years and above before enrolment. Children aged between 7 years and above provided written informed assent before being enrolled into the study. The services of translators were employed where necessary during consenting, assenting and enrolment procedures.

Study subjects and their parents or legal guardian were informed of their ESBL-PE and/or CRE colonisation status. When colonisation was documented, the implications for clinical care and infection control practices were explained to the study subjects and parents or legal guardians. The result was also provided to the attending physicians and other healthcare providers involved in their care. Parents or legal guardians of study subjects who had already been discharged from hospital at the time of receipt of the results were informed telephonically.

1.2 Author guidelines for PLOS ONE Journal: See Appendix 7

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Chapter 2: Publication-ready manuscript

Colonisation with extended spectrum beta-lactamase-producing and carbapenem-resistant Enterobacterales in children admitted to a paediatric referral hospital in South Africa

Babatunde O. Ogunbosi,[1,2*,] Clinton Moodley[3,4], Preneshni Naicker[3,4], James Nuttall[1,2], Colleen Bamford[3,4], Brian Eley[1,2]

Authors' affiliation:

1. Paediatric Infectious Diseases Unit, Red Cross War Memorial Children's Hospital, Cape Town, South Africa

2. Department of Paediatrics and Child Health, University of Cape Town, Cape Town, South Africa

3. National Health Laboratory Service, Groote Schuur Hospital, Cape Town, South Africa

4. Division of Microbiology, University of Cape Town, Cape Town, South Africa

Authors' email addresses:

Babatunde O. Ogunbosi* – tundeogunbosi@yahoo.com

James Nuttall - james.nuttall@uct.ac.za

Preneshni Naicker - Preneshni.Naicker@lancet.co.za

Clinton Moodley - c.moodley@uct.ac.za

Colleen Bamford - colleen.bamford@pathcare.org

Brian Eley - brian.eley@uct.ac.za

*** Corresponding Author:** Babatunde O. Ogunbosi, Paediatric Infectious Diseases Unit, Red Cross War Memorial Children's Hospital Department of Paediatrics and Child Health, University of Cape Town, Cape Town, South Africa. E-mail: tundeogunbosi@yahoo.com (BOO)

Abstract

Introduction:

There are few studies describing colonisation with extended spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) among children in sub-Saharan Africa. Colonisation often precedes infection and multi-drug-resistant Enterobacterales are important causes of invasive infection.

Methods:

In this prospective cross-sectional study, conducted between April and June 2017, 200 children in a tertiary academic hospital were screened by rectal swab for EBSL-PE and CRE. The resistance-conferring genes were identified using polymerase chain reaction technology. Risk factors for colonisation were also evaluated.

Results:

Overall, 48% (96/200) of the children were colonised with at least one ESBL-PE, 8 of these with 2 ESBL-PE, and one with a CRE (0.5% (1/200)). Common colonising ESBL-PE were *Klebsiella pneumoniae* (62.5%, 65/104) and *Escherichia coli* (34.6%, 36/104). The most frequent ESBL-conferring gene was blaCTX-M in 95% (76/80) of the isolates. No resistance-conferring gene was identified in the CRE isolate (*Enterobacter cloacae*). Most of the *Klebsiella pneumoniae* isolates were susceptible to piperacillin/tazobactam (86.2%) and amikacin (63.9%). Similarly, 94.4% and 97.2% of the *Escherichia coli* isolates were susceptible to piperacillin/tazobactam and amikacin, respectively. Hospitalisation for more than 7 days before study enrolment was associated with ESBL-PE colonisation.

Conclusion:

Approximately half of the hospitalised children in this study were colonised with ESBL-PE. This highlights the need for improved infection prevention and control practices to limit the dissemination of these microorganisms.

Key Words: Colonisation, ESBL, CRE, children, Africa

INTRODUCTION

Infection with multidrug-resistant organisms is a threat to global health [1, 2]. Notable are the emergence of extended spectrum beta-lactamase-producing Enterobacterales (ESBL-PE) and carbapenem-resistant Enterobacterales (CRE) [3]. They are among pathogens categorised as critical on the World Health Organization list of priority antibiotic resistant pathogens [3]. These organisms are notorious for causing healthcare-associated infections, and there are growing concerns about their increasing contribution to community-acquired infections [4]. Invasive infection with CRE is associated with high mortality, high cost of management, few therapeutic options and the absence of effective consensus treatment guidelines [5].

Colonisation often precedes infection, however reports of ESBL-PE and CRE colonisation progressing to infection in children are rare [6, 7]. Most paediatric studies on ESBL-PE colonisation are in neonatal intensive care units (NICUs) with reported prevalence ranging from 4.3% to 75% [8-10]. Outside the neonatal age, ESBL-PE colonisation among hospitalised paediatric patients have recorded prevalence between 18.5% and 57.1% [11-13] while carriage in community settings is lower, ranging from 0.1% to 12.4% [14-18]. In some settings, community carriage has increased over time. For example, surveillance studies in Bolivia and Peru, over a decade, show a steady rise from 0.1% in 2002 to 12.4% in 2011 [14, 18, 19]. Community carriage may be a reservoir for the development of community-acquired ESBL-PE infections, particularly *Escherichia coli* infections [4].

Factors which predispose to ESBL-PE colonisation in the neonatal period include prior carbapenem, penicillin and aminoglycoside exposure, prolonged hospitalisation, early onset pneumonia, prolonged antibiotic use, formula milk feeds, and having an ESBL-PE colonised mother [8, 9, 20-25]. Risk factors for ESBL-PE colonisation in older children include mechanical ventilation, prolonged hospital stay, prior antibiotic use and prior hospitalisation [11, 12]. However, ESBL-producing *E. coli* carriage documented among children in a very

remote community in Senegal, where prior antibiotic use was most unlikely, suggested that other factors may play a role in promoting colonisation [15]. Conversely, breastfeeding confers protection against ESBL-PE colonisation in the new born period [23, 26]. In early studies, ESBL-PE colonisation was associated with the sulphydryl variable (SHV) enzyme. More recent studies suggest that the cefotaxime-hydrolyzing beta-lactamase, (CTX-M) enzyme now predominates [4, 9, 21, 22, 26, 27].

Studies in children have reported CRE colonisation rates of 0.5% to 29.5% [28-40]. All the studies on CRE colonisation have documented *Klebsiella pneumoniae* colonisation [31-33, 35, 37, 40] while two studies also documented colonisation with *Serratia spp., E. coli* and *Enterobacter spp.* [31, 40]. Though there are likely due to geographical variations, one or more carbapenemases have been reported on all continents [31-33, 35, 40]. Risk factors associated with CRE colonisation include carbapenem exposure, transfer-in from other health facilities, aminoglycoside exposure, surgical procedures, urinary catheterisation, nasogastric intubation and prolonged antibiotic administration [32, 35, 37, 40]. Infection risk among children previously colonised with CRE have ranged from 3.4 to 28.2% [31, 35, 37, 40]. Risk of children colonised with carbapenem-resistant *K. pneumoniae* progressing to infection is increased by existing metabolic disease, previous carbapenem use, neutropaenia and prior surgical procedures [37].

Despite the global distribution of these organisms and the association of their emergence due to widespread and indiscriminate use of broad-spectrum antibiotics, little is known about the epidemiology of ESBL-PE and CRE colonisation and infection on the African continent, especially in children. There have been reports of ESBL-PE infections in South Africa from the early 1990s, and some more recent studies describing ESBL-PE infections, but few colonisation studies [41-44]. One of the first reports of CRE infection and colonisation in children in South Africa described the treatment and outcome of a case series of invasive CRE infections [45]. To address the dearth of ESBL-PE and CRE colonisation research in

children in Africa, we describe ESBL-PE and CRE colonisation at a children's hospital including prevalence, factors associated with ESBL-PE colonisation, and the distribution of ESBL genes among ESBL-PE isolates.

METHODS

Study design and setting

This prospective, cross-sectional study was conducted at Red Cross War Memorial Children's Hospital (RCWMCH) in Cape Town, South Africa, a 273-bedded tertiary hospital affiliated to the University of Cape Town academic complex and dedicated to the care of children aged 13 years and below. The hospital is a referral centre for sick children from the Western Cape Province, but also receives referrals from surrounding provinces.

Enrolment and sampling

Recruitment took place in four wards at RCWMCH, including two general medical wards and two surgical wards. All children admitted to the four wards were eligible for enrolment, except those who experienced colonisation or infection with ESBL-PE and/or CRE in the previous 1-year period.

A systematic sampling approach was employed for selecting study participants. From the daily ward list of children resident in the four wards, every third patient was approached for enrolment. If the inclusion criteria were not met, the next patient on the ward list was approached for enrolment. Enrolment took place from Monday to Friday. Between 3 and 8 patients were enrolled on any one day. Enrolment alternated between the medical and surgical patients to ensure that each patient type constituted a minimum of 45% of all enrolled study participants. Patient enrolment was completed between 3 April and 7 June 2017.

Data collection

Demographic and clinical information of the enrolled patients was entered on a study-specific structured data sheet. The information included age at enrolment, gender, source of admission – either from a health care facility or convalescence home, current diagnosis, surgical procedures, and intensive care unit (ICU) treatment in the current admission or the preceding 12 months. Information on antibiotic use was also collected such as the type and duration of antibiotic therapy in the 12 months preceding this admission or in the current admission. Information on out-of-hospital antibiotic exposure was mainly as reported by caregiver recall, which was limited. While information on in-hospital antibiotics used was collected from the patient's clinical records at RCWMCH, exposure(s) at other hospitals could not be ascertained and might have been missed.

Stool specimen collection

A soft tipped TransystemTM sterile transport swab (COPAN Italia S.a.A via Perotti 10, 25125 Brescia Italy) was used to collect rectal stool specimens in a quiet, comfortable room. Swabbing was performed by an experienced research nurse assisted by a chaperone and usually in the presence of the child's parent or legal guardian. All measures were taken to minimise patient discomfort. The specimens were transported to the National Health Laboratory Service (NHLS) medical microbiology laboratory, Groote Schuur Hospital (GSH), Cape Town, for processing within 6 hours of collection.

Microbiological Procedures

All microbiological procedures were performed at the medical microbiology laboratory, GSH, located 4.3 km from RCWMCH. Stool specimens were plated onto ChromID ESBL media (bioMérieux, Marcy l'Etoile, France) and incubated at 37°C for 24 hours. Suspected colonies were subcultured onto blood agar plates for pure growth. Purified isolates were identified using the Vitek 2® Gram negative card with susceptibility testing performed using

the AST GN-N255 card (bioMérieux, Marcy l'Etoile, France). Where necessary, this was supplemented with E-test (bioMérieux, Marcy l'Etoile, France) gradient diffusion to confirm minimal inhibitory concentrations (MICs) of ertapenem, imipenem and meropenem. MICs were interpreted according to the 2017 Clinical and Laboratory Standards Institute (CLSI) guidelines [46] while ESBLs and CRE were reported based on the Advanced Expert System (AES) interpretation of the Vitek 2® system. The susceptibility of Enterobacterales isolated was evaluated for the following antibiotics: ampicillin, co-amoxiclav, piperacillin-tazobactam, cefuroxime, cefoxitin, ceftriaxone, ceftazidime, cefepime, ertapenem, meropenem, imipenem, ciprofloxacin, gentamicin, amikacin, cotrimoxazole, tigecycline and colistin. All isolates were stored at -70°C on microbeads for further molecular testing for EBSL and carbapenemase-producing genes.

Molecular testing for common ESBL and carbapenemase genes

Stored isolates were grown overnight (18 - 24 hours) on McConkey agar plates, before sub-culturing onto 2% Blood agar. A loop-full of each culture was suspended in 750 µl BashingBead™ Buffer and pre-lysed on the Tissue Lyser in a ZR BashingBead™ Lysis Tube (Zymo Research Corporation), at 50Hz for 5 min. The Lysis tube was centrifuged at 10 000 rpm, and 200 µl of the supernatant extracted and purified using the QIAsymphony DSP Virus/Pathogen Kit (Qiagen), according to the manufacturer's recommendations.

Using gene-specific primers, selected commonly encountered ESBL and carbapenemase genes were detected and amplified by PCR reaction. The primers used are shown in Supplementary Table I and detailed methods are detailed in a recent publication [47]. The amplification products were analysed by agarose gel electrophoresis with 1.5%, and visualised with ethidium bromide and using ultraviolet light. Positive amplicons were confirmed with Sanger sequencing, and BLAST analysis.

Definitions

HIV-exposed but uninfected child: A child <18 months old in whom a positive HIV serological test was documented in either the mother or the child, but the HIV DNA PCR test was negative in the child who was not on antiretroviral therapy (ART).

HIV Infection: A positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test in any child < 18 months old, or 2 positive serological test results (HIV ELISA or HIV Rapid test) or a positive HIV DNA PCR result confirmed by either a HIV RNA PCR or repeat HIV DNA PCR test in a child > 18 months old were considered HIV-infected [48].

Extended Spectrum Beta-Lactamase Producing Enterobacterales (ESBL-PE):

Enterobacterales were categorised as ESBL-PE according to the Vitek 2® Advanced Expert System (AES) interpretation of the AST GN-N255 card. This categorisation is based solely on the pattern of susceptibility and resistance to different cephalosporins as the card lacks wells containing cephalosporins combined with a beta-lactamase inhibitor [49].

Carbapenem-resistant Enterobacterales (CRE): Enterobacterales which are resistant to any carbapenem antibiotic (minimum inhibitory concentration of ≥4 mcg/ml for doripenem, meropenem or imipenem, or ≥2 mcg/ml for ertapenem) according to 2017 CLSI breakpoints [46]. Resistance to carbapenems may result from several mechanisms including alteration of outer membrane permeability or the production of carbapenemases. Common carbapenemase genes include bla$_{NDM}$, bla$_{KPC}$, bla$_{GES}$, bla$_{VIM}$, bla$_{OXA\text{-}48\text{-}like}$ and bla$_{IMP}$ [46].

Co-morbidity: An underlying chronic medical condition for which the patient was receiving care at the time of enrolment.

Major surgery: An invasive operative procedure in which extensive resection is performed, e.g. a body cavity is entered, organs are removed, or normal anatomy is altered. In general,

if a mesenchymal barrier is opened (pleural cavity, peritoneum, meninges), the surgery is considered major [50].

Statistical analysis

Data were entered in SPSS Statistics Version 24.0 Software (IBM, Armonk, New York, USA) and analysed. Descriptive statistics, for continuous variables, was reported as medians with interquartile ranges or, where applicable as means and standard deviations. Categorical variables were reported as proportions and percentages. The 95% confidence interval (CI) for binomial proportions were estimated for mid-point prevalence estimates. Categorical variables were compared using either the uncorrected chi square test or Fisher's exact test while continuous variables were analysed using the Student's t test or analysis of variance (ANOVA). Non-normally distributed data were compared using Mann-Whitney U test. Two-tail p values <0.05 were considered statistically significant.

Univariate analyses were used to identify potential risk factors associated with rectal colonisation by ESBL-PE. All factors with a p value <0.2 on univariate analysis, and those biologically plausible or reported in literature, were then analysed in a binomial logistic regression model to identify factors independently associated with ESBL-PE colonisation. The binomial logistic regression model was built using a stepwise backward selection. The univariate results were reported using unadjusted odds ratios (ORs) and 95% confidence interval (95% CI), and the logistic regression results expressed as adjusted odds ratios (aORs) and 95% CI.

Ethical consideration

The study was conducted in accordance with the Helsinki Declaration. The study protocol was approved by the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, reference number: HREC REF: 898/2016. The Research Committee at the RCWMCH also approved the study.

Written informed consent was obtained in the preferred language (English, Afrikaans or isiXhosa) from the parents or legal guardian, and children aged 12 years and above before enrolment. Children aged between 7 years and above provided written informed assent before being enrolled into the study. The services of translators were employed where necessary during consenting, assenting and enrolment procedures.

Children and their legal guardian were informed of their ESBL-PE and/or CRE colonisation status. When colonisation was documented, the implications for care management, and infection control practices were explained. The result was also provided to the attending physicians and other health care providers involved in their care. Parents or legal guardian of children who had been discharged at the time of receipt of the results were informed telephonically.

RESULTS

Study participants

Of a total of 299 children who were selected for enrolment, 99 were excluded for various reasons. Thus, a total of 200 children were enrolled and completed the study. Supplementary Fig. 1 depicts participant enrolment, phenotypic and genotypic results.

Supplementary Fig 1. Flow chart depicting patient enrolment, phenotypic and genotypic testing results

Prevalence of ESBL-PE and CRE colonisation

Of 200 participants enrolled, 96 were colonized by at least one ESBL-PE, giving an ESBL-PE colonisation prevalence of 48% (95% CI 40.9-55.2%). Of these, 8 patients were colonised by two different ESBL-PE, prevalence of 4% (95% CI 1.9-7.5%). One patient was colonised by a carbapenem-resistant *Enterobacter cloacae (E. cloacae)* giving a CRE colonisation prevalence of 0.5% (95% CI 0.02–2.4%). Of the total 104 ESBL-PE isolates collected from

the 96 ESBL-PE colonized participants, *K. pneumoniae* accounted for 62.5% (65/104), *E. coli* 34.6% (36/104), *Klebsiella oxytoca (K. oxytoca)* 1.9% (2/104), and *E. cloacae* 1.0% (1/104).

Characteristics of participants

Of the 200 enrolled children, 60.5% were male, the median age was 12 months (range 4 days – 7 years and 4 months), and 59.6% (119/200) were less than 24 months of age. The HIV status was known in 153 patients; 3.5% (7/200) were HIV-infected, 19.5% (39/200) were HIV-exposed but uninfected and 53.5% (107/200) were HIV-unexposed. Of the 47 children with unknown HIV status, 89.4% (42/47) were enrolled in the surgical wards. The most frequent primary diagnoses were pneumonia, bone and soft tissue infection and acute diarrhoeal disease (Table 1). There was no significant difference in the proportions of colonised and non-colonised children with pre-existing co-morbid conditions (Table 2).

Table 1: Characteristics of the study subjects at the time of enrolment

	Total (N=200) n (%)	**Colonised (N=97) n (%)**	**Not colonised (N=103) n (%)**
Gender			
Male	121 (60.5)	58 (59.8)	63 (61.2)
Female	79 (39.5)	39 (40.2)	40 (38.8)
Age category			
≤28 days	9 (4.5)	6 (6.2)	3 (2.9)
>28 days – 12 months	89 (44.5)	50 (51.5)	39 (3.79)
>12 months - <60 months	47 (23.5)	20 (20.6)	27 (26.2)
≥60 months	55 (27.5)	21 (21.6)	34 (33.0)

Median age in months (IQR)	12 (2-68)	7 (2-45)	18 (4-82)
Median (IQR) days in hospital before enrolment	4 (2-9)	5 (2-12)	3 (2-7)
HIV status			
Infected	7 (3.5)	4 (4.1)	3 (2.9)
Exposed, uninfected	39 (19.5)	16 (16.5)	23 (22.3)
Unexposed, uninfected	107 (53.5)	65 (67.0)	42 (40.8)
Unknown	47 (23.5)	12 (2.4)	35 (34.0)
Primary clinical diagnosis			
Pneumonia	64 (32.0)	33 (34.0)	31 (30.1)
Bone and soft tissue infection	20 (10.0)	7 (7.2)	13 (12.6)
Acute diarrhoeal disease	11 (5.5)	8 (8.2)	3 (2.9)
Central nervous system malformation	10 (5.0)	6 (6.2)	4 (3.9)
Appendicitis	8 (4.0)	1 (1.0)	7 (6.8)
Meningitis	8 (4.0)	2 (2.1)	6 (5.8)
Urogenital malformations	7 (3.5)	2 (2.1)	5 (4.9)
Hydrocephalus	7 (3.5)	0 (0.0)	7 (6.8)
Hirschsprung disease	5 (2.5)	2 (2.1)	3 (2.9)
Trauma	5 (2.5)	2 (2.1)	3 (2.9)
Neonatal sepsis	4 (2.0)	4 (4.1)	0 (0.0)
Inguinal hernia	4 (2.0)	2 (2.1)	2 (1.9)
Tuberculosis	3 (1.5)	1 (1.0)	2 (1.9)
Solid tumour	3 (1.5)	2 (2.1)	1 (1.0)
Bloodstream infection	2 (1.0)	0 (0.0)	2 (1.9)

Table 2: Factors associated with extended spectrum beta-lactamase-producing Enterobacterales colonisation determined by univariate analyses and binomial logistic regression

	Colonised N=96 n (%)	Not colonised N=104 n (%)	OR (95% CI)	aOR (95% CI)
Male gender	38 (39.6)	41 (39.4)	1.01 (0.57-1.78)	
Age < 12 months	55 (57.3)	43 (41.3)	1.90 (1.09-3.34) *	1.36 (0.70-2.65)
Transferred to RCWMCH	10 (10.4)	9 (8.7)	1.23 (0.48-3.16)	
Previous RCWMCH admission	23 (24.0)	15 (14.4)	1.87 (0.91-3.84)	1.23 (0.52-2.91)
Current admission to medical ward (vs. surgical ward)	63 (65.6)	49 (47.1)	2.14 (1.21-3.79) *	0.78 (0.35-1.77)
Hospitalisation for >7 days before enrolment	40 (41.7)	20 (19.2)	3.0 (1.59-5.66) *	2.83 (1.40-5.72) *
Cardiac co-morbidity	8 (8.3)	4 (3.8)	2.27 (0.66-7.81)	
Gastrointestinal tract co-morbidity	20 (20.9)	22 (21.2)	0.98 (0.50-1.94)	
Neurological co-morbidity	17 (17.7)	16 (15.4)	1.18 (0.56-2.50)	
Concomitant chronic lung disease	7 (7.3)	3 (2.9)	2.65 (0.67-10.55)	2.67 (0.57-12.59)
Concomitant congenital anomaly	25 (26.0)	25 (24.0)	1.11 (0.59-2.11)	

Major surgery in current admission	12 (12.5)	23 (22.1)	0.50 (0.24-1.08)	0.40 (0.15-1.04)
Peripheral venous line	88 (91.7.7)	87 (83.7)	2.15 (0.88-5.24)	1.81 (0.65-5.02)
Nasotracheal intubation ± ventilation	8 (8.3)	4 (3.8)	2.27 (0.66-7.81)	
PEG feeding	6 (6.2)	1 (1.0)	6.87 (0.81-58.1)	
Nasogastric intubation	38 (39.6)	24 (23.1)	2.18 (1.18-4.03) *	1.60 (0.76-3.39)
Decreased level of consciousness	5 (5.2)	1 (1.0)	5.66 (0.65-49.3)	
Receiving gastric acid inhibitor therapy	12 (12.5)	6 (5.8)	2.33 (0.84-6.49)	
Receiving immunosuppressive therapy	11 (11.2)	11 (10.6)	1.09 (0.45-2.65)	
Admission to ICU in 12 months preceding current admission	6 (6.2)	2 (1.9)	3.40 (0.68-17.27)	
Admission to ICU during current admission	19 (19.8)	15 (14.4)	1.46 (0.70-3.08)	
Antibiotic administration in the 12-month period preceding admission	29 (30.2)	21 (20.2)	1.71 (0.90-3.27)	1.35 (0.63-2.90)

*p<0.05

OR Odds ratio, 95% CI 95% confidence interval, aOR adjusted Odds ratio, RCWMCH Red Cross War Memorial Children's Hospital, PEG percutaneous endoscopic gastrostomy, ICU intensive care unit.

Nine percent (18/200) of the enrolled children were transferred-in from another healthcare facility, primarily to the medical wards at RCWMCH, 72.2% (13/18). The median duration of hospital stay at the time of enrolment was 4 days (range 1-64 days) and 30% of the patients

had been hospitalised for more than 7 days prior to enrolment (Table 2). About a third of the patients, 32.5% (65/200), had been admitted into other wards in the course of the current admission, aside from the wards in which they were enrolled during the study, and the majority of these children were enrolled in the medical wards, 96.9% (63/65). Of the children admitted into other wards before enrolment, 52.3% (34/65) had been treated in the ICU. In all, 19% (38/200) had been treated in the ICU at RCWMCH either in the current admission or in the preceding 12 months (Table 2).

Overall, 25% (50/200) of the participants reported antibiotic usage in the 12-month period before current hospitalisation, 29.9% (29/97) of colonised children and 20.4% (20/103) of non-colonised children. The most common antibiotics administered during this period were penicillins in 15.0% (30/200) of children, while 4.5% (9/200) received a carbapenem. During the current admission, 80.5% (165/200) had received one or more antibiotics, 84.5% (82/97) of colonised children and 76.7% (79/103) of non-colonised children. The antibiotics that these children received during the current period of hospitalisation included penicillins in 55.0% (110/200), aminoglycosides in 32% (64/200), cephalosporins in 19.5% (39/200), beta-lactam/beta-lactam inhibitor combination in 16% (32/200), carbapenems in 9% (18/200), cotrimoxazole in 7.5% (15/200), macrolides in 5.5% (11/200), metronidazole in 4.0% (8/200) and fluoroquinolones in 3.0% (6/200).

Factors associated with ESBL-PE colonisation

Using univariate analysis, age less than 12 months, admission to a medical ward at the time of study enrolment, hospitalisation for more than 7 days before study enrolment, and nasogastric intubation at the time of enrolment were significantly associated with ESBL-PE colonisation. However, on binomial logistic regression analysis, only hospitalisation for more than 7 days before study enrolment remained significantly associated with ESBL-PE colonisation ($p=0.013$) (Table 2).

Antibiotic susceptibility profile of the ESBL-PE and CRE isolates

Table 3 summarises the antibiotic susceptibility profiles of the 104 ESBL-PE isolates. All ESBL-PE isolates were resistant to the cephalosporins except for one *E. coli* isolate and one *E. cloacae* isolate which retained susceptibility to cefepime, using Vitek 2. All the ESBL-PE isolates were however susceptible to the carbapenems and colistin. Most of the *K. pneumoniae* (72.3%) were susceptible to piperacillin/tazobactam and to amikacin (86.2%). Similarly, 94.4% and 97.2% of the *E. coli* isolates were susceptible to piperacillin/tazobactam and amikacin, respectively (Table 3). Susceptibility to both piperacillin/tazobactam and amikacin was observed in 73.1% of all isolates, 64.6% and 91.7% of all ESBL-PE *K. pneumoniae* and *E. coli* isolates respectively. The CRE *E. cloacae* isolate exhibited intermediate susceptibility to ertapenem on E-test, but was susceptible to imipenem and meropenem.

Table 3: Antibiotic susceptibility profile of the extended spectrum beta-lactamase-producing Enterobacterales isolates showing proportion susceptible

Antibiotic	**Total N=104 (%)**	***Klebsiella pneumoniae* N=65 n (%)**	***Escherichia coli* N=36 n (%)**	***Klebsiella oxytoca* N=2 n (%)**	***Enterobacter cloacae* N=1 n (%)**
Cotrimoxazole	10 (9.6)	5 (7.7)	4 (11.1)	0 (0.0)	1 (100.0)
Ampicillin / amoxicillin	1 (1.0)	0 (0.0)	1 (2.8)	0 (0.0)	0 (0.0)
Amoxicillin plus clavulanic acid	19 (18.3)	1 (1.5)	16 (44.4)	2 (100.0)	0 (0.0)
Piperacillin plus tazobactam	83 (79.8)	47 (72.3)	34 (94.4)	2 (100.0)	0 (0.0)

Cefepime	2 (1.9)	0 (0.0)	1 (2.8)	0 (0.0)	1 (100.0)
Ciprofloxacin	687 (64.4)	41 (63.1)	23 (63.9)	2 (100.0)	1 (100.0)
Gentamicin	33 (31.7)	8 (12.3)	22 (61.1)	2 (100.0)	1 (100.0)
Amikacin	93 (89.4)	56 (86.2)	35 (97.2)	1 (50.0)	1 (100.0)
Tigecycline	104 (100.0)	65 (100.0)	36 (100.0)	2 (100.0)	0 (0.0)

Genetic testing of ESBL-PE and CRE Isolates

Of the 104 ESBL-PE and 1 CRE isolated from 97 colonised children, 94 isolates from 87 patients were available for genetic testing. Eighty ESBL-PE isolates (85.1%) from 73 patients had one or more resistance-conferring genes (Table 4). Of these, 25% (20/80) had only one resistant-conferring gene, 14 (17.5%) had 2 genes, and 46 (57.5%) had 3 genes identified. The most common resistance conferring gene was bla$_{CTX-M}$ found in 95% (76/80) of ESBL-PE i.e. in 98.2% (56/57) of *K. pneumoniae* isolates, 55.9% (19/34) of *E. coli* isolates, and the only *K. oxytoca* (1.3%) isolate tested. The bla$_{TEM}$ and bla$_{SHV}$ genes were found in 66.3% (53/80) and 13.8% (11/80) of ESBL-PE isolates respectively, often in combination with bla$_{CTX-M}$ gene. Three *E. coli* isolates had bla$_{TEM}$ as the only resistance gene, and one *K. pneumoniae* isolate had bla$_{SHV}$ as the only resistance gene. The 3 *E. coli* TEM-only positive amplicons were sequenced, and two were identified as TEM-1 genotypes i.e. narrow-spectrum beta-lactamase, and the remaining one as a TEM-135 genotype i.e. ESBL. The DNA from the SHV-only positive *K. pneumoniae* isolate was not sequenced as blaSHV is intrinsic to *K. pneumoniae* and would not be differentiated using conventional sequencing. For the two ESBL-PE isolates which retained susceptibility to cefepime, no gene was found in the *E. cloacae* isolate, while the *E. coli* isolate had both CTX-M and TEM genes.

Table 4: Frequency of genes conferring extended spectrum beta-lactam resistance to the colonising extended spectrum beta-lactamase-producing Enterobacterales isolates

ESBL Gene	**Extended spectrum beta-lactamase producing Enterobacterales**				**Total**
	N=104				
	Klebsiella pneumoniae	*Escherichia coli*	*Klebsiella oxytoca*	*Enterobacter cloacae*	
	n=65	*n=36*	*n=2*	*n=1*	
Not available	8	2	1	0	11
No gene found	0	12	0	1	13
No EBSL gene confirmed					
TEM 1 only	0	2	0	0	2
SHV 1 only	1	0	0	0	1
EBSL gene confirmed					
TEM-135 only	0	1	0	0	1
CTX-M only	0	16	0	0	16
CTX-M + TEM	1	2	1	0	4
CTX-M + SHV	10	0	0	0	10
TEM + SHV	0	0	0	0	0
CTXM + TEM + SHV	45	1	0	0	46

ESBL-PE, extended spectrum beta-lactamase-producing Enterobacteriaceae, ESBL, extended spectrum beta-lactamase, CTX-M, cefotaxime-hydrolyzing beta-lactamase, TEM, Temoneira resistance encoding genes, SHV, sulphydryl variable

The only CRE isolate, an *E. cloacae,* was negative for common CRE conferring genes, namely bla$_{NDM}$, bla$_{KPC}$, bla$_{OXA-48}$ and variants, bla$_{IMP}$, bla$_{VIM}$ and bla$_{GES}$.

DISCUSSION

In this prospective cross-sectional study, we showed that approximately 50% of children hospitalised in RCWMCH are colonised with ESBL-PE, predominately with *K. pneumoniae* and *E. coli*, findings which are consistent with previous paediatric colonisation studies [12, 13, 51]. The CRE colonisation prevalence was extremely low in our patients, but comparable to previous studies which also reported lower CRE colonisation [31, 35, 52]. Similarly, bloodstream infection (BSI) studies from our hospital have shown that while *K. pneumoniae* and *E. coli* are the predominant Gram-negative aetiological agents, CRE only sporadically caused invasive infection [45, 53]. Furthermore, of *K. pneumoniae* and *E. coli* isolates causing BSI at RCWMCH, 83% and 30% are ESBL-PE, respectively [42, 43].

This study was not designed to determine the timing of colonisation of ESBL-PE and CRE and hence we were unable to establish the extent of community versus hospital acquisition. Colonisation was documented in some patients who had only been hospitalised for one day, suggesting that some of the patients may have been colonised outside the hospital environment. However, the risk factor analysis showed that hospitalisation for more than 7 days before enrolment was an independent risk factor for ESBL-PE colonisation. This finding strongly suggests that the healthcare environment is an important site of colonisation and is consistent with previous BSI studies that showed that 95% and 55% of *K. pneumoniae* and *E. coli* BSIs respectively are hospital-acquired or healthcare-associated [42, 43].

The predominant ESBL gene identified was $bla_{CTX\text{-}M}$ (95%), often occurring in combination with other ESBL-conferring genes (78.9%). This was notable for the *K. pneumoniae* isolates, most of which carried more than one ESBL-conferring gene. In contrast, most *E. coli* isolates harboured only the $bla_{CTX\text{-}M}$ gene. This is in keeping with the global pattern where CTX-M has become the predominant ESBL-conferring gene in resistant *K. pneumoniae* and *E. coli* isolates [4, 21, 27]. It has been suggested that the CTX-M plasmid has adapted to *K. pneumoniae* resulting in better transmission efficiency [4, 27]. Three *E. coli* isolates each

harboured a TEM gene only. One isolate had a TEM-135 gene which encodes an enzyme that hydrolyses 3rd generation cephalosporins and thus confers ESBL-PE properties on the isolate [54]. This is unlike the TEM-1 gene found in the other 2 isolates, the first TEM gene which encodes an enzyme that hydrolyses earlier penicillins like ampicillin but not 3rd generation cephalosporins, and therefore does not confer ESBL-PE characteristics on the isolates [55]. Most *K. pneumoniae* isolates have chromosomal SHV genes which encodes enzymes that do not hydrolyse 3rd generation cephalosporins [55]. One of our *K. pneumoniae* isolates was shown to only carry an SHV gene. The ESBL-PE phenotype of the three isolates with TEM-1 only or SHV only genes might be due to hyperproduction of the encoded enzymes along with porin changes, or the presence of other ESBL's or enzymes not detected by this assay.

The antibiotic susceptibility profiles of the ESBL-PE isolates as summarised in Table 4 correlate with published data. High proportions of the *K. pneumoniae* and *E. coli* isolates were susceptible to piperacillin-tazobactam and to amikacin, similar to findings among ESBL-producing bloodstream isolates of *K. pneumoniae* and *E. coli* reported in previous studies from RCWMCH [42]. Despite these findings, a randomised control trial among adults that determined whether piperacillin-tazobactam was as effective as meropenem for treating BSI caused by *K. pneumoniae* or *E. coli* with non-susceptibility to third generation cephalosporins showed that the 30-day mortality was significantly higher in the piperacillin-tazobactam group, suggesting that piperacillin-tazobactam alone may no longer be the recommended definitive therapy in this context. The high proportion of *E. coli* (91.6%) susceptible to both piperacillin-tazobactam and amikacin suggest this may be a consideration in BSI caused by ESBL-PE E. coli. This is unlikely to be an option for ESBL-PE *K. pneumoniae* where only 64.6% were susceptible to both piperacillin-tazobactam and amikacin.

Study strengths and limitations

A strength of this study is that it is one of the first prospective studies to estimate the prevalence of ESBL-PE and CRE carriage in children hospitalised in sub-Saharan Africa. Limited funds for completing the microbiology investigation of the enrolled patients restricted our surveillance to one specimen per patient and prevented us from sampling the patients at multiple time points during hospitalisation, and prevented differentiation between community and hospital acquisition of ESBL-PE. Our sampling approach may have under-estimated the colonisation rate as multiple rectal swabs may have increased the yield of colonised children or serial sampling may have identified additional children who became colonised later on in the course of their hospital admission. The sample size of 200 children was not determined scientifically but influenced by the available funding. Thus, the study may have been underpowered to explore risk factors associated with colonisation definitively. Screening for genes conferring extended spectrum beta-lactam resistance was limited to the three commonly occurring resistance genes in our setting. Furthermore, we did not screen the isolates for the presence of Ambler group C beta-lactamases which produce similar antibiograms to the extended spectrum beta-lactamases. Lastly, relatedness of the ESBL-PE isolates was not evaluated. This may have assisted us to understand the extent of hospital transmission.

Notwithstanding these limitations the study showed that ESBL-PE colonisation prevalence is high in this setting, and prolonged hospitalisation is a risk factor for colonisation, suggesting the need for improved infection control practices.

CONCLUSION

The current study extends our understanding of ESBL-PE and CRE colonisation at our institution. Major findings were high ESBL-PE colonisation prevalence, very low CRE

colonisation prevalence, prolonged hospitalisation as an independent risk factor for ESBL-PE colonisation, and common resistance genes are responsible for conferring extended spectrum beta-lactam resistance. Further research is required to explore colonisation prevalence changes over time, quantify the extent of community versus hospital acquisition, determine relatedness of the isolates, and determine whether improved infection control practice can moderate colonisation rates.

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AUTHOR CONTRIBUTIONS

Conceptualization: Babatunde O. Ogunbosi, James Nuttall, Preneshni Naicker, Colleen Bamford, Brian Eley

Data curation: Babatunde O. Ogunbosi, Brian Eley

Formal Analysis: Babatunde O. Ogunbosi, Brian Eley

Funding acquisition: Babatunde O. Ogunbosi, Brian Eley

Investigation: Babatunde O. Ogunbosi, Preneshni Naicker, Clinton Moodley, Colleen Bamford

Methodology: Babatunde O. Ogunbosi, Clinton Moodley, Preneshni Naicker, Colleen Bamford, James Nuttall, Brian Eley

Project administration: Babatunde O. Ogunbosi, Clinton Moodley, Preneshni Naicker, Colleen Bamford, Brian Eley

Resources: Babatunde O. Ogunbosi, Brian Eley

Software: Babatunde O. Ogunbosi, Brian Eley

Supervision: Babatunde O. Ogunbosi, Brian Eley

Validation: Babatunde O. Ogunbosi, Preneshni Naicker, Clinton Moodley, Colleen Bamford, Brian Eley

Visualization: Babatunde O. Ogunbosi, Brian Eley

Writing – original draft: Babatunde O. Ogunbosi, Brian Eley

Writing – review & editing: Babatunde O. Ogunbosi, James Nuttall, Preneshni Naicker, Clinton Moodley, Colleen Bamford, Brian Eley

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Supplementary Table 1

References for ESBL and Carbapenemase PCR

Primer	Sequence 5' - 3'	Length	Reference
CARBAPENEMASES			
GesF	ATGCGCTTCATTCACGC	17	[1]
GesR	GCTCAGGATGAGTTGTG	17	[1]
ImpXF2	ATTGACACTCCATTTAC	17	[2]
ImpXR2	AACAACCAGTTTTGC	15	[2]
NdmF2	GGTTTGGCGATCTGGTTTTC	20	[3]
NdmR2	CGGAATGGCTCATCACGATC	20	[3]
Oxa48F	CGTGTATTAGCCTTATCG	18	[3]
Oxa48R	CGCTAACCACTTCTAGG	17	[3]
VimXF	GTGAGTATCCGACAGTC	17	[2]
VimXR	GAGCAAGTCTAGACCG	16	[2]
KpcF	TGTCACTGTATCGCCGTC	18	[4]
KpcR	CTCAGTGCTCTACAGAAAACC	21	[4]
ESBL's			
CTXM1F	CGCTTTGCGATGTGCAG	17	[1]
CTXM1R	ACCGCGATATCGTTGGT	17	[1]
SHVC	AGAAGGGTTATTCTTATTTGTCGC	24	[5]
SHVD	TCTTTCCGATGCCGCCGCCAGTCA	25	[5]
DEB	ATGAGTAAACTTGGTCTGAC	20	[6]
3061TEM	AGGAAGCAAAGCTGAAAGGAATCAAATTTGG	31	[1]

New Delhi Metallo-beta-lactamase (NDM), *Klebsiella pneumoniae* carbapenemase (KPC), Oxacillinase-48 (OXA-48) and variants, Imipenemase (IMP), Verona Integron-Mediated Metallo-beta-lactamase (VIM) and Guiana extended spectrum carbapenemase (GES). Temoneira (TEM), cefotaxime (CTX), cefotaxime (CTX)-M-type (CTX-M), sulf*h*ydryl *v*ariable (SHV)

References

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3. Manesen R, Bamford C, Smith M, Jacobson R, Moodley C, Williams S. Outbreak of carbapenem-resistant *Klebsiella* sp. in an academic hospital, Western Cape, May 2012. NationaI Institute for Communicable Diseases Surveillance Bulletin. 2012;10(4):82-6.

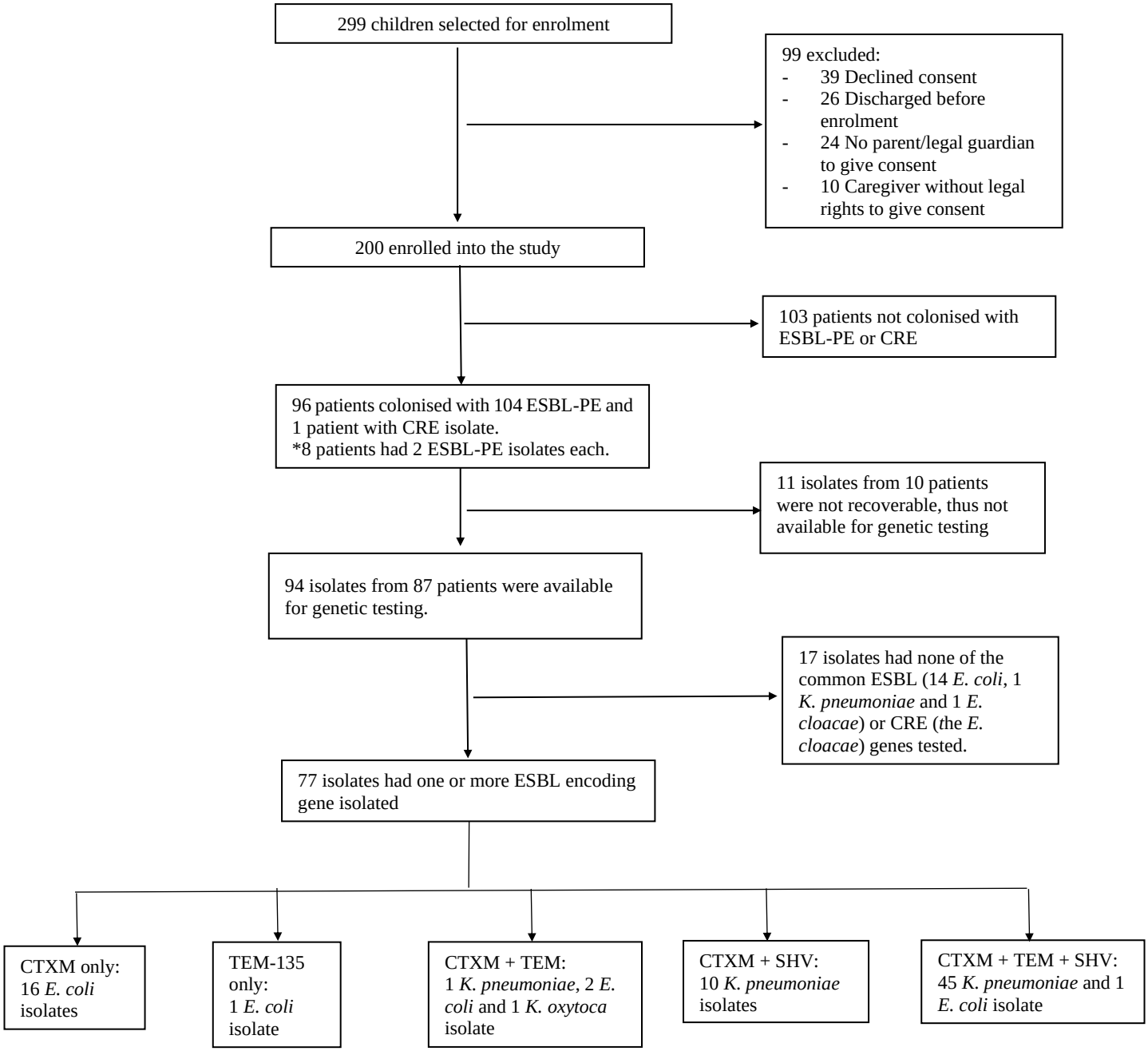
4. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, et al. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of Klebsiella pneumoniae. Antimicrob Agents Chemother. 2001;45(4):1151-61. doi: 10.1128/AAC.45.4.1151-1161.2001. PubMed PMID: 11257029; PubMed Central PMCID: PMCPMC90438.

5. Wang H, Kelkar S, Wu W, Chen M, Quinn JP. Clinical Isolates of Enterobacteriaceae Producing Extended-Spectrum β-Lactamases: Prevalence of CTX-M-3 at a Hospital in China. Antimicrobial Agents and Chemotherapy. 2003;47(2):790-3. doi: 10.1128/aac.47.2.790-793.2003.

6. Canica MM, Lu CY, Krishnamoorthy R, Paul GC. Molecular diversity and evolution of blaTEM genes encoding beta-lactamases resistant to clavulanic acid in clinical E. coli. J Mol Evol. 1997;44(1):57-65. PubMed PMID: 9010136.

Supplementary Figure 1

Flow diagram depicting patient enrolment and phenotypic and genotypic testing results



 # ESBL-PE, Extended spectrum beta-lactamase producing Enterobacterales, *CRE,* Carbapenem-resistant Enterobacterales, *CTX-M,* Cefotaxime-hydrolyzing beta-lactamase, *TEM,* Temoneira resistance encoding genes, *SHV,* Sulfhydryl variable, *K. pneumoniae - Klebsiella pneumoniae, E. coli - Escherichia coli, K oxytoca - Klebsiella oxytoca, E. cloacae – Enterobacter cloacae.*

APPENDICES

APPENDIX 1

ESBL-PE AND CRE RECTAL COLONISATION DATA COLLECTION FORM

ADMISSION DETAILS / DEMOGRAPHICS AND ANTHROPOMETRY

1. STUDY NO.: _____		**HOSPITAL STICKER**
2. Date enrolled: …… /……. /……. (DD/MM/YYYY)		
3. Date admitted: ...../……./…… (DD/MM/YYYY)		
4. Weight (Kg): ……	5. Height (cm): ……	
6. Gestational age at birth ……..	7. Birth weight (gm) ……	
8. Transferred in	Yes[1] No[2]	
9. Source of admission ……	10. Duration of stay at Source of admission .....days	
11. Previous ESBL-PE Infection(s): Yes[1] No[2]		
12. If Yes to No. 14		
13. Type of Previous ESBL-PE Infection(s):	14. Specify ID(s) of Previous ESBL-PE Infection(s)	
15.	16.	
17.	18.	
19. Previous CRE Infection(s): Yes [1] No [2]		
20. If Yes to No. 22		
21. Type of Previous CRE Infection(s):	22. Specify ID(s) of Previous CRE Infection(s)	
23.	24.	
25.	26.	
27. Ward admitted into …….	28. Residence on other wards in this hospital Yes [1] No [2]	29. If yes, which ward (s) ……

30. Date rectal swab collected…../…./…(DD/MM/YYYY)	31. Date of outcome ...../……./……(DD/MM/YYYY)
32. Outcome: Discharged home [1], Transferred out [2], Died [3]	
33. Any Red Cross War Memorial Children's Hospital admission in the last 1year? Yes [1] No [2]	
If Yes to No. 32, list admission details below	
Ward(s) admitted into	Dates of admission and discharge
34.	35. …… /……. /…….. to ...../……./…… (DD/MM/YYYY)
36.	37. …… /……. /…….. to ...../……./…… (DD/MM/YYYY)
38.	39. …… /……. /…….. to ...../……./…… (DD/MM/YYYY)

RISK FACTORS FOR COLONIZATION

Co-morbid Medical Condition								
40. Cardiac	Yes [1]	No [2]	41. Neurological	Yes [1]	No [2]	42. Congenital anomaly	Yes [1]	No [2]
43. Renal	Yes [1]	No [2]	44. Malignancy	Yes [1]	No [2]	45. Chronic Pulmonology	Yes [1]	No [2]
46. Genetic	Yes [1]	No [2]	47. Haematological	Yes [1]	No [2]	48. Solid Organ Transplant	Yes [1]	No [2]
49. Haematopoietic Stem Cell Transplant	Yes [1]	No [2]	50. Endocrine	Yes [1]	No [2]	51. Immunosuppressive therapy	Yes [1]	No [2]
52. Surgical	Yes [1]	No [2]	53. Primary Immune Deficiency	Yes [1]	No [2]	54. If yes, specify	<1 Month [1] ≥ 1 Month [2]	
55. Specify details of co-morbid medical condition(s)				56. Duration of co-morbid medical condition				
57. ……..				58. <6 weeks [1] ≥ 6 weeks [2]				
59. ……				60. <6 weeks [1] ≥ 6 weeks [2]				
61. ……..				62. <6 weeks [1] ≥ 6 weeks [2]				

63. HIV status	Infected [1] Exposed [2] Unexposed [3] Unknown [4]	64. Viral Load (Last 6mo) ……/ Log ……	65. CD4 (Last 6mo) ……../……..%

Co-morbid Surgical Condition

66. Surgery in the past	Yes [1]	No [2]	67. *Type: Minor [1] Major [2]	68. Specify name and date: _____
69. Surgery in this admission	Yes [1]	No [2]	70. *Type: Minor [1] Major [2]	71. Specify type and date: _____

Invasive Procedures during current admission

72. Peripheral venous line	Yes [1]	No [2]	73. Urethral catheter	Yes [1]	No [2]	74. PD catheter	Yes [1]	No [2]
75. Intubation / ventilation	Yes [1]	No [2]	76. Central venous line	Yes [1]	No [2]			
77. PEG tube feeding	Yes [1]	No [2]	78. Enteral feeding other than PEG	Yes [1]	No [2]	79. If yes, specify _____		
80. If yes, duration <1Month [1], ≥ 1 Month [2]			81. If yes, duration <1 Month [1], ≥ 1 Month [2]					

Other risk factors during current admission

82. Inhibitors of gastric acid production	Yes [1]	No [2]	83. If yes, specify type of inhibitor _____	84. Duration of use of inhibitor < 1 Month [1] ≥ 1 Month [2]
85. Neutropenia	Yes [1]	No [2]	86. <500cell/mm3 [1], 500-1000cell/mm3 [2], >1000cell/mm3 [3]	

87. LOC or impaired consciousness	Yes 1]	No [2]

ICU admission(s)

88. In previous admission in last 1 year	**Yes [1]**	**No [2]**
…… /……. /…….. to ...../……./…… (DD/MM/YYYY)		
…… /……. /…….. to ...../……./…… (DD/MM/YYYY)		
89. In current admission	**Yes [1]**	**No [2]**
…… /……. /…….. to ...../……./…… (DD/MM/YYYY)		
…… /……. /…….. to ...../……./…… (DD/MM/YYYY)		

ANTIBIOTIC HISTORY

94. Pre-admission antibiotic exposure in the last 1 Year: Yes[1] No[2]				
If yes:				
Class of antibiotic	Name of antibiotic	Date Started	Date Stopped	Duration of use
95. Carbapenem				
96. 2nd Gen. Cephalosporin				
97. 3rd Gen. Cephalosporin				
98. Penicillin				
99. Fluoroquinolone				
100. Macrolide				
101. Aminoglycoside				
102. Cotrimoxazole				
103. Piperacillin/Tazobactam				
104. Others……..				
105. Antibiotic use in this admission: Yes[1] No[2]				
If yes:				
Class of antibiotic	Name of antibiotic	Date Started	Date Stopped	Duration of use
106. Carbapenem				
107. 2nd Gen. Cephalosporin				
108. 3rd Gen. Cephalosporin				
109. Penicillin				
110. Fluoroquinolone				
111. Macrolide				
112. Aminoglycoside				
113. Cotrimoxazole				
114.Piperacillin/Tazobactam				
115. Others……..				

LABORATORY RESULTS

116. ID of Rectal swab isolate				ESBL		CRE	
				Yes [1]	No [2]	Yes [1]	No [2]
117. Molecular typing (MT) and MICs of Isolates							
ID of Isolate	MT	Antibiotic	MIC	Susceptibility S[1] I[2] R[3]			

* Minor surgery is any invasive operative procedure in which only skin or mucus membranes and connective tissue is resected e.g. vascular cut-down for catheter placement, implanting pumps in subcutaneous tissue. Major surgery is any invasive operative procedure in which a more extensive resection is performed, e.g. a body cavity is entered, organs are removed, or normal anatomy is altered. In general, if a mesenchymal barrier is opened (pleural cavity, peritoneum, meninges), the surgery is considered major. Ref: http://www.dar.emroy.edu/vetcare/surg_definitions.php

APPENDIX 2

INFORMED CONSENT FORM FOR PARENT(S)/GUARDIAN

IRB research approval number: IRB00001983

Title of the Research: Epidemiology of ESBL and CRE Colonization in Children admitted to Red Cross War Memorial Children's Hospital, Cape Town

Name and affiliation of Researcher: This study is being conducted by Dr Babatunde Ogunbosi, a Senior Registrar in Infectious Diseases Unit of the Department of Paediatrics and Child Health, Red Cross Children's Hospital under the supervision of Prof Brian Eley, Department of Paediatrics and Child Health, Red Cross Children's Hospital.

Sponsor of the Research: The investigator Dr Ogunbosi is being funded by the APFP which help to provide sub-specialty clinical and research training for paediatricians in Africa.

Purpose of Research: The purpose of this research is to describe the frequency and spectrum of multi-drug resistant enterobacteriaceae that colonize the gastrointestinal tracts of children hospitalised in Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town, and to evaluate risk factors associated with colonized children. Multi-drug resistant enterobacteriaceae are bacteria which usually reside in the gut and are resistant to some of the antibiotics that are used to treat serious infections. They can exist in the gut without causing infection, so the child is not sick from these organisms, this is called colonization. Some children can later develop infections from these organisms, which can be very difficult to treat. This research will determine the proportion of children carrying these organisms in their gastrointestinal tracts, but who are not sick from the organisms. The study will describe the characteristics of children who get colonized and also the risk factors associated with colonization. It will assist in easy identification, in future, of children that will be mostly colonized and therefore monitor them appropriately, as well as implement ways to prevent

spread to other children. It may also provide information on how to prevent other children from being colonized.

Procedure of the Research: Parents / guardians of children admitted to medical and surgical wards of the hospital will be approached for recruitment into the study. With your kind permission we will collect information with regards antibiotic use by your child/ward in the past year, and hospital admission or residence in a non-acute care health facility. Also information on admission into the intensive care unit and other areas in this hospital will be collected. In addition, information on the treatment given, especially antibiotics, in this admission, surgical and other procedures will also be collected.

Stool will be collected with rectal swabs/stool sample. A rectal swab has a sterile cotton wool mounted on a thin flexible plastic holder which does not cause pain nor harm. The sterile cotton wool on the rectal swab will be inserted into your child/ward's anus, the stool on it will be tested for the organisms mentioned earlier. If the stool on the rectal swab is not sufficient, then a bit of your child/ward's stool will be collected in a clean germ free container. No blood samples will be collected.

We hope to enroll about 200 children over the 8 - 12 month duration of the study.

Expected duration of research and of participant's involvement: Your child/ward will be involved when the rectal swab/stool sample is collected. The rectal swab/stool collection procedure will only take about 2-3minutes. However, we will continue to monitor his/her course in the hospital to update information being collected as part of the study.

Risks: The procedure is neither painful nor harmful in any way to your child/ward. Your child/ward might experience some discomfort as the rectal swab is being taken. We will ensure we do all we can to make the process most comfortable for your child/ward. We will also be available to address your questions and worries.

Costs: The cost of rectal swab/stool sample collection and testing will be borne by the research team, so overall there is no additional cost to you.

Benefits: Results of these tests will be promptly made available to you and the doctors for your child/ward's care. It will also assist the doctors to care better for other children in future. If your child/ward is colonized with a multi-drug resistant bacterium, we will teach you how to prevent spread to other members in your household.

Confidentiality: We will maintain utmost confidentiality at all times about information collected for this research. The information that we collect from you and entered into the computer for analysis will not be linked to your child/ward in anyway and your child/ward's name or any identifier, and will not be used in any publication or reports from this study. As part of our responsibility to conduct this research properly, officials from the University of Cape Town's Human Research Ethics Committee may have access to these records.

Voluntariness: Your participation in this study is entirely voluntary and you may also choose to withdraw at any stage of the study.

Alternatives to participation: If you choose not to participate, your child/ward will still receive all necessary treatment and it will not affect your child's right to receive treatment in the department or hospital in any way.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:

You are free to refuse to take part in this programme. You also have the right to withdraw at any given time if you choose.

Please note that some of the information that has been obtained about you and your child/ward before you chose to withdraw may have been used in reports and publications. These cannot be removed anymore. However, we promise to make sincere efforts to comply with your wishes as much as is possible.

What happens to research participants and communities when the research is over:

If you so wish, the researchers will inform you of the outcome of this research. However during the course of this research, you will be informed about any findings that may affect your child/ward's health.

Detailed contact information including contact address, telephone, fax, E-mail and any other contact information of the researcher, institutional IRB and head of institution:

This research has been approved by the Ethics Committee of the University of Cape Town and the HREC Convener can be reached at Prof Mark Blockman, E53, Ground Floor, Old Main Building, Groote Schuur Hospital, Observatory, Cape Town.

In addition, if you have any question about your participation in this research, you can contact the;

1. Investigator: Dr. Babatunde O. Ogunbosi
   Infectious Diseases Unit
   Department of Paediatrics and Child Health
   UCT
   Email: tundeogunbosi@yahoo.com
   Telephone: 074-3496244
2. Supervisor: Prof Brian Eley
   Infectious Diseases Unit
   Department of Paediatrics and Child Health
   UCT
   Email: brian.eley@uct.ac.za
   Telephone: 083-9477637

PLEASE KEEP A COPY OF THE SIGNED INFORMED CONSENT

CONSENT:

Statement of parent/guardian giving consent:

I have read the description of the research or have had it translated into the language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my child's participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop my child being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

NAME: _____

SIGNATURE: _____

DATE: _____

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including risks and benefits to make an informed decision.

NAME: _____

SIGNATURE: _____

DATE: _____

APPENDIX 3

INFORMED CONSENT FORM FOR ADOLESCENTS

IRB research approval number: IRB00001983

Title of the Research: Epidemiology of ESBL and CRE Colonization in Children admitted to Red Cross War Memorial Children's Hospital, Cape Town

Name and affiliation of Researcher: This study is being conducted by Dr Babatunde Ogunbosi, a Senior Registrar in Infectious Diseases Unit of the Department of Paediatrics and Child Health, Red Cross Children's Hospital under the supervision of Prof Brian Eley, Department of Paediatrics and Child Health, Red Cross Children's Hospital.

Sponsor of the Research: The investigator Dr Ogunbosi is being funded by the APFP which help to provide sub-specialty clinical and research training for paediatricians in Africa.

Purpose of Research: The purpose of this research is to describe the frequency and spectrum of multi-drug resistant enterobacteriaceae that colonize the gastrointestinal tracts of children hospitalised in Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town, and to evaluate risk factors associated with colonized children. Multi-drug resistant enterobacteriaceae are bacteria which usually reside in the gut and are resistant to some of the antibiotics that are used to treat serious infections. They can exist in the intestine without causing infection, so the child is not sick from these organisms, this is called colonization. Some children can later develop infections from these organisms, which can be very difficult to treat. This research will determine the proportion of children carrying these organisms in their intestine, but who are not sick from the organisms. The study will describe the characteristics of children who get colonized and also the risk factors associated with colonization. It will assist in easy identification, in future, of children that will be mostly colonized and therefore monitor them appropriately, as well as implement ways to prevent

spread to other children. It may also provide information on how to prevent other children from being colonized.

Procedure of the Research: Parents / guardians of children admitted to medical and surgical wards of the hospital and the adolescent will be approached for recruitment into the study. With your kind permission we will collect information with regards antibiotic you have received in the past year, and hospital admission or residence in a non-acute care health facility. Also, information on admission into the intensive care unit and other areas in this hospital will be collected. In addition, information on the treatment given, especially antibiotics, in this admission, surgical and other procedures will also be collected.

Stool will be collected with rectal swabs/stool sample. A rectal swab has a sterile cotton wool mounted on a thin flexible plastic holder which does not cause pain nor harm. The sterile cotton wool on the rectal swab will be inserted into your anus, the stool on it will be tested for the organisms mentioned earlier. If the stool on the rectal swab is not sufficient, then a bit of your stool will be collected in a clean germ-free container. No blood samples will be collected. We hope to enroll about 200 children over the 8 – 12 month duration of the study.

Expected duration of research and of participant's involvement: You will be involved when the rectal swab/stool sample is collected. The rectal swab/stool collection procedure will only take about 2-3minutes. However, we will continue to monitor you in the course of hospital stay to update information being collected as part of the study.

Risks: The procedure is neither painful nor harmful in any way to you. You might experience some discomfort as the rectal swab is being taken. We will ensure we do all we can to make the process most comfortable for you. We will also be available to address your questions and worries.

Costs: The cost of rectal swab/stool sample collection and testing will be borne by the research team, so overall there is no additional cost to you or your parent/guardian.

Benefits: Results of these tests will be promptly made available to you and the doctors for your care. It will also assist the doctors to care better for other children in future. If you are colonized with a multi-drug resistant bacterium, we will teach you and your parent/guardian how to prevent spread to other members in your household.

Confidentiality: We will maintain utmost confidentiality at all times about information collected for this research. The information that we collect from you and entered into the computer for analysis will not be linked to you in anyway and your name or any identifier, and will not be used in any publication or reports from this study. As part of our responsibility to conduct this research properly, officials from the University of Cape Town's Human Research Ethics Committee may have access to these records.

Voluntariness: Your participation in this study is entirely voluntary and you may also choose to withdraw at any stage of the study.

Alternatives to participation: If you choose not to participate, you will still receive all necessary treatment and it will not affect your right to receive treatment in the department or hospital in any way.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:

You are free to refuse to take part in this programme. You also have the right to withdraw at any given time if you choose.

Please note that some of the information that has been obtained about you before you chose to withdraw may have been used in reports and publications. These cannot be removed anymore. However, we promise to make sincere efforts to comply with your wishes as much as is possible.

What happens to research participants and communities when the research is over:

If you so wish, the researchers will inform you of the outcome of this research. However, during the course of this research, you will be informed about any findings that may affect your health.

Detailed contact information including contact address, telephone, fax, E-mail and any other contact information of the researcher, institutional IRB and head of institution:

This research has been approved by the Ethics Committee of the University of Cape Town and the HREC Convener can be reached at Prof Mark Blockman, E53, Ground Floor, Old Main Building, Groote Schuur Hospital, Observatory, Cape Town.

In addition, if you have any question about your participation in this research, you can contact the;

1. Investigator: Dr. Babatunde O. Ogunbosi
   Infectious Diseases Unit
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   Email: tundeogunbosi@yahoo.com
   Telephone: 074-3496244

2. Supervisor: Prof Brian Eley
   Infectious Diseases Unit
   Department of Paediatrics and Child Health
   UCT
   Email: brian.eley@uct.ac.za
   Telephone: 083-9477637

PLEASE KEEP A COPY OF THE SIGNED INFORMED CONSENT

CONSENT:

Statement of adolescent giving consent:

I have read the description of the research or have had it translated into the language I understand. I have also talked it over with the doctor to my satisfaction. I understand that my participation is voluntary. I know enough about the purpose, methods, risks and benefits of the research study to judge that I want to take part in it. I understand that I may freely stop being part of this study at any time. I have received a copy of this consent form and additional information sheet to keep for myself.

NAME: _____

SIGNATURE: _____

DATE: _____

Statement of person obtaining informed consent:

I have fully explained this research to _____ and have given sufficient information, including risks and benefits to make an informed decision.

NAME: _____

SIGNATURE: _____

DATE: _____

APPENDIX 4

INFORMED ASSENT FORM FOR CHILDREN AGED 7 YEARS AND ABOVE

IRB research approval number: IRB00001983

Title of the Research: Epidemiology of ESBL and CRE Colonization in Children admitted to Red Cross War Memorial Children's Hospital, Cape Town

Name and affiliation of Researcher: My name is Dr Babatunde Ogunbosi, a Senior Registrar in Infectious Diseases Unit of the Department of Paediatrics and Child Health, Red Cross Children's Hospital working under the supervision of Prof Brian Eley, Department of Paediatrics and Child Health, Red Cross Children's Hospital. Our job is to prevent infections in children and also to take care of children who get infections so they can get well again.

Sponsor of the Research: The investigator, Dr Ogunbosi, is being funded by the APFP which help to provide sub-specialty clinical and research training for paediatricians in Africa.

Introduction and purpose of study: We will be asking you to take part in this study. We have also spoken to your parent/guardian about the study and they are also aware we will be asking you take part in this study. Their consent is also needed for you to take part in the study, but should you decline to be part of the study, we will respect your decision and will not include you in the study. You may choose to discuss this over with your parents or friends, and if you need further information about the study, feel free to seek clarification from us or any doctor at any point in the study.

The purpose of this research is to describe the frequency and spectrum of multi-drug resistant bacteria called enterobacteriaceae that colonise the gastrointestinal tracts of children hospitalised in Red Cross War Memorial Children's Hospital (RCWMCH), Cape Town, and to evaluate risk factors associated with colonized children. Multi-drug resistant enterobacteriaceae are bugs which usually reside in the intestines and are resistant to some

of the antibiotics that are used to treat serious infections. They can exist in the gut without causing infection, so the child is not sick from these organisms, this is called colonization. When children develop infections from these organisms, they can be very difficult to treat. This research will determine the proportion of children carrying these organisms in their intestines, but who are not sick from the organisms. The study will describe the characteristics of children who carry these organisms and also the risk factors associated with that. It will assist in easy identification, in future, of children that will be mostly affected in such a way and therefore help in monitor them appropriately, as well as implement ways to prevent spread of the organisms to other children. It may also provide information on how to prevent other children from acquiring these organisms.

Why ask you to participate in the study?

We are carrying out the study in children, aged between 1 month and 18 years. Children admitted into this and other wards in Red Cross Children's Hospital who do not already have infections by the organisms mentioned earlier, will be invited to take part.

Voluntariness: Your participation in this study is entirely voluntary and you may also choose to opt out at any stage of the study.

Alternatives to participation: If you choose not to participate, you will still receive all necessary treatment and it will not affect your right to receive treatment in this hospital in any way.

Procedure of the Research: Should you decide to be part of the study, we will collect information with regards any antibiotic you have received in the past year and hospital admission or stay in a non-acute care health facility. Also, information about admission into the ICU and other areas in this hospital will be collected. In addition, information on the treatment given, especially antibiotics, in this admission, surgical and other procedures will be collected.

Stool will be collected with rectal swabs/stool sample. A rectal swab has a sterile cotton wool mounted on a thin flexible plastic holder which does not cause pain nor harm. The sterile cotton wool on the rectal swab will be inserted into your anus. The stool on it will be tested for the organisms mentioned earlier. If the stool on the rectal swab is not sufficient, then a bit of your stool will be collected in a clean germ-free container. No blood samples will be collected.

Expected duration of research and of participant's involvement: We will be collecting information about you for this research throughout the course of your admission in this hospital. However, the rectal swab/stool sample collection procedure takes only about 2-3minutes. We hope to enroll about 200 children over the 8-12 month duration of the study.

Risks: The rectal swab/stool sample collection procedure is neither painful nor harmful in any way. You might experience some discomfort. We will ensure we do all we can to make the process most comfortable for you. We will take all standard precautions to ensure that we do not pass infection to you and we will be available to address your questions and worries.

Benefits: Results of these tests will be promptly made available to your parent/guardian and your doctors for your care. It will also assist doctors to care better for other children in future. If you are colonized with a multi-drug resistant bacterium, we will teach you how to prevent spreading it to other members in your household.

Confidentiality: We will maintain utmost confidentiality at all times about information collected for this research. The information that we collect from you and enter into the computer for analysis will not be linked to you and your name or any identifier, and will not be used in any publication or reports from this study.

Consequences of participants' decision to withdraw from research and procedure for orderly termination of participation:

You are free to refuse to take part in this programme. You also have the right to withdraw at any given time if you so choose.

Please note that some of the information that has been obtained about you before you chose to withdraw may have been used in reports and publications. These cannot be removed anymore. However, we promise to make sincere efforts to comply with your wishes as much as is possible.

What happens to research participants and communities when the research is over?

If you so wish, the researchers will inform you and your parent/guardian of the outcome of this research. However, during the course of this research, you and your parent/guardian will be informed about any findings that may affect your health.

Contact information:

Should you need to contact anyone with respect to this study, we can be reached at Dr. Babatunde O. Ogunbosi, African Paediatric Fellowship Programme, Department Of Paediatrics And Child Health, Red Cross Children's Hospital, Klipfontein Road, 5th Floor, ICH Building, Rondebosch, 7700. Telephone: 0743496244 Email: tundeogunbosi@yahoo.com **Or** Prof Brian Eley, Department Of Paediatrics And Child Health, Red Cross Children's Hospital, Klipfontein Road, 5th Floor, ICH Building, Rondebosch, 7700, Telephone: 083-9477637; Email: brian.eley@uct.ac.za

This research has been approved by the Ethics Committee of the University of Cape Town and the HREC Convener can be reached at Prof Mark Blockman, E53, Ground Floor, Old Main Building, Groote Schuur Hospital, Observatory, Cape Town.

PLEASE KEEP A COPY OF THE SIGNED INFORMED ASSENT

ASSENT:

Statement of child giving consent:

I have had this study explained to me in detail in a manner I understand. I understand that my participation is voluntary. The risks and benefits of the research study to judge that I want to

take part in it have been explained to me. I understand that I may freely stop being part of this study at any time. I have received a copy of this assent form and additional information sheet to keep for myself.

I agree to take part in the study.

NAME: _____

SIGNATURE: _____

DATE: _____

WITNESS

NAME: _____

SIGNATURE: _____

DATE: _____

Statement of person obtaining informed assent:

I have fully explained this research to _____.

I have given sufficient information, including risks and benefits to make an informed decision. I confirm that the participant has freely given assent to take part in the study.

NAME: _____

SIGNATURE: _____

DATE: _____

APPENDIX 5

ETHICS APPROVAL, HUMAN RESEARCH ETHICS COMMITTEE, FACULTY OF HEALTH SCIENCES, UNIVERSITY OF CAPE TOWN

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

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

26 January 2017

HREC REF: 898/2016

Prof B Eley
Paediatric & Child Health
Red Cross War Memorial Children's Hospital
Rondebosch

Dear Prof Eley

PROJECT TITLE: EPIDEMIOLOGY OF EXTENDED SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE AND CARBAPENEM RESISTANT ENTEROBACTERIACEAE COLONIZATION IN CHILDREN ADMITTED TO RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL CAPE TOWN (MPhil candidate B Ogunbosi)

Thank you for your response letter dated 22 January 2017, addressing the issues raised by the Human Research Ethics Committee (HREC).

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30 January 2018.

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)

We acknowledge that the student, B Ogunbosi will also be involved in this study.

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 before the research may occur.

Yours sincerely

Signature removed to avoid exposure online

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.

HREC 898/2016

APPENDIX 6

STUDY APPROVAL, RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN

Western Cape Government
Health

Dr Jane Kawadza
Manager: Medical Services
Email: Jane.Kawadza@Westerncape.gov.za
Tel: +27 21 658 5788 fax: +27 21 658 5166
RXH: RCC61

Prof B Eley
Red Cross War Memorial Children's Hospital

Dear Prof B Eley

APPROVAL OF RESEARCH

PROJECT TITLE: EPIDEMIOLOGY OF EXTENDED SPECTRUM BETA-LACTAMASE- PRODUCING ENTEROBACTERIACEAE AND CARBAPENEM RESISTANT ENTEROBACTERIACEAE COLONIZATION IN CHILDREN ADMITTED TO RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN

It is a pleasure to inform you that approval is hereby granted to conduct the above-mentioned study at Red Cross War Memorial Children's Hospital.

Yours sincerely,

Signature removed to avoid exposure online

Dr J Kawadza
Manager: Medical Services
Date: 23.02.17

APPENDIX 7

2020 ETHICS RENEWAL/ANNUAL PROGRESS REPORT

UNIVERSITY OF CAPE TOWN HEALTH SCIENCES FACULTY — FACULTY OF HEALTH SCIENCES
UNIVERSITY OF CAPE TOWN Human Research Ethics Committee

FHS016: Annual Progress Report / Renewal

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-Oct-2020
☐ Not approved	See attached comments		
Signature Chairperson of the HREC	signature removed	Date Signed	11/6/19

Comments to PI from the HREC
Thank you for the deviation document signature removed

Principal investigator to complete the following:

1. Protocol Information

Date (when submitting this form)	10 June 2019		
HREC REF Number	698/2016	Current Ethics Approval was granted until	28 February 2019
Protocol title	EPIDEMIOLOGY OF EXTENDED SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE AND CARBAPENEM RESISTANT ENTEROBACTERIACEAE COLONIZATION IN CHILDREN ADMITTED TO RED CROSS WAR MEMORIAL CHILDREN'S HOSPITAL, CAPE TOWN		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ✓ No		
If yes, could you please provide the HREC Ref's for all sub-studies? Note: A separate FHS016 must be submitted for each sub-study			
Principal Investigator	Brian Eley		
Department / Office Internal Mail Address	Room 520, 5th floor ICH building, Red Cross Children's Hospital, Klipfontein Road, Rondebosch, 7701		

APPENDIX 8

INSTRUCTIONS TO AUTHORS – PLOS ONE

Submission Guidelines

Related information for authors

- Submission system
- Journal scope and publication criteria
- Getting started guide
- Guidelines for revisions
- Publication fees

Style and Format

File format Manuscript files can be in the following formats: DOC, DOCX, or RTF. Microsoft Word documents should not be locked or protected.

LaTeX manuscripts must be submitted as PDFs. Read the LaTeX guidelines.

Length Manuscripts can be any length. There are no restrictions on word count, number of figures, or amount of supporting information.

We encourage you to present and discuss your findings concisely.

Font Use a standard font size and any standard font, except for the font named "Symbol". To add symbols to the manuscript, use the Insert → Symbol function in your word processor or paste in the appropriate Unicode character.

Headings	Limit manuscript sections and sub-sections to 3 heading levels. Make sure heading levels are clearly indicated in the manuscript text.
Layout and spacing	Manuscript text should be double-spaced. Do not format text in multiple columns.
Page and line numbers	Include page numbers and line numbers in the manuscript file. Use continuous line numbers (do not restart the numbering on each page).
Footnotes	Footnotes are not permitted. If your manuscript contains footnotes, move the information into the main text or the reference list, depending on the content.
Language	Manuscripts must be submitted in English. You may submit translations of the manuscript or abstract as supporting information. Read the supporting information guidelines.
Abbreviations	Define abbreviations upon first appearance in the text. Do not use non-standard abbreviations unless they appear at least three times in the text. Keep abbreviations to a minimum.
Reference style	PLOS uses "Vancouver" style, as outlined in the ICMJE sample references. See reference formatting examples and additional instructions below.

Equations

We recommend using MathType for display and inline equations, as it will provide the most reliable outcome. If this is not possible, Equation Editor or Microsoft's Insert→Equation function is acceptable.

Avoid using MathType, Equation Editor, or the Insert→Equation function to insert single variables (e.g., "$a^2 + b^2 = c^2$"), Greek or other symbols (e.g., β, Δ, or ′ [prime]), or mathematical operators (e.g., x, ≥, or ±) in running text. Wherever possible, insert single symbols as normal text with the correct Unicode (hex) values.

Do not use MathType, Equation Editor, or the Insert→Equation function for only a portion of an equation. Rather, ensure that the entire equation is included. Equations should not contain a mix of different equation tools. Avoid "hybrid" inline or display equations, in which part is text and part is MathType, or part is MathType and part is Equation Editor.

Nomenclature

Use correct and established nomenclature wherever possible.

Units of measurement	Use SI units. If you do not use these exclusively, provide the SI value in parentheses after each value. Read more about SI units.
Drugs	Provide the Recommended International Non-Proprietary Name (rINN).
Species names	Write in italics (e.g., *Homo sapiens*). Write out in full the genus and species, both in the title of the manuscript and at the first mention of an organism in a paper. After

	first mention, the first letter of the genus name followed by the full species name may be used (e.g., *H. sapiens*).
Genes, mutations, genotypes, and alleles	Write in italics. Use the recommended name by consulting the appropriate genetic nomenclature database (e.g., HGNC for human genes; we strongly recommend using this tool to check against previously approved names). It is sometimes advisable to indicate the synonyms for the gene the first time it appears in the text. Gene prefixes such as those used for oncogenes or cellular localization should be shown in roman typeface (e.g., v-fes, c-MYC).
Allergens	The systematic allergen nomenclature of the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Sub-committee should be used for manuscripts that include the description or use of allergenic proteins. For manuscripts describing new allergens, the systematic name of the allergen should be approved by the WHO/IUIS Allergen Nomenclature Sub-Committee prior to manuscript publication. Examples of the systematic allergen nomenclature can be found at the WHO/IUIS Allergen Nomenclature site.

Copyediting manuscripts

Prior to submission, authors who believe their manuscripts would benefit from professional

editing are encouraged to use language-editing and copyediting services. Obtaining this service is the responsibility of the author, and should be done before initial submission. These services can be found on the web using search terms like "scientific editing service" or "manuscript editing service."

Submissions are not copyedited before publication.

Submissions that do not meet the *PLOS ONE* publication criterion for language standards may be rejected.

Manuscript Organization

Manuscripts should be organized as follows. Instructions for each element appear below the list.

Beginning section	*The following elements are required, in order:* • Title page: List title, authors, and affiliations as first page of manuscript • Abstract • Introduction
Middle section	*The following elements can be renamed as needed and presented in any order:* • Materials and Methods • Results • Discussion

- Conclusions (optional)

Ending section

The following elements are required, in order:

- Acknowledgments
- References
- Supporting information captions (if applicable)

Other elements

- Figure captions are inserted immediately after the first paragraph in which the figure is cited. Figure files are uploaded separately.
- Tables are inserted immediately after the first paragraph in which they are cited.
- Supporting information files are uploaded separately.

 Please refer to our downloadable sample files to ensure that your submission meets our formatting requirements:

- Download sample title, author list, and affiliations page (PDF)
- Download sample manuscript body (PDF)

Viewing Figures and Supporting Information in the compiled submission PDF

The compiled submission PDF includes low-resolution preview images of the figures after the reference list. The function of these previews is to allow you to download the entire submission as quickly as possible. Click the link at the top of each preview page to download a high-resolution version of each figure. Links to download Supporting Information files are also available after the reference list.

Parts of a Submission

Title

Include a full title and a short title for the manuscript.

Title	Length	Guidelines	Examples
Full title	250 characters	Specific, descriptive, concise, and comprehensible to readers outside the field	Impact of cigarette smoke exposure on innate immunity: A *Caenorhabditis elegans* model
			Solar drinking water disinfection (SODIS) to reduce childhood diarrhoea in rural Bolivia: A cluster-randomized, controlled trial
Short title	100 characters	State the topic of the study	Cigarette smoke exposure and innate immunity
			SODIS and childhood diarrhoea

Titles should be written in sentence case (only the first word of the text, proper nouns, and genus names are capitalized). Avoid specialist abbreviations if possible. For clinical trials, systematic reviews, or meta-analyses, the subtitle should include the study design.

Author list

Authorship requirements

All authors must meet the criteria for authorship as outlined in the authorship policy. Those

who contributed to the work but do not meet the criteria for authorship can be mentioned in the Acknowledgments. Read more about Acknowledgments.

The corresponding author must provide an ORCID iD at the time of submission by entering it in the user profile in the submission system. Read more about ORCID.

Author names and affiliations

Enter author names on the title page of the manuscript and in the online submission system.

On the title page, write author names in the following order:

- First name (or initials, if used)
- Middle name (or initials, if used)
- Last name (surname, family name)

Each author on the list must have an affiliation. The affiliation includes department, university, or organizational affiliation and its location, including city, state/province (if applicable), and country. Authors have the option to include a current address in addition to the address of their affiliation at the time of the study. The current address should be listed in the byline and clearly labeled “current address.” At a minimum, the address must include the author’s current institution, city, and country.

If an author has multiple affiliations, enter all affiliations on the title page only. In the submission system, enter only the preferred or primary affiliation. Author affiliations will be listed in the typeset PDF article in the same order that authors are listed in the submission.

Author names will be published exactly as they appear in the manuscript file. Please double-check the information carefully to make sure it is correct.

Corresponding author

The submitting author is automatically designated as the corresponding author in the submission system. The corresponding author is the primary contact for the journal office and the only author able to view or change the manuscript while it is under editorial consideration.

The corresponding author role may be transferred to another coauthor. However, note that transferring the corresponding author role also transfers access to the manuscript. (To designate a new corresponding author while the manuscript is still under consideration, watch the video tutorial below.)

Only one corresponding author can be designated in the submission system, but this does not restrict the number of corresponding authors that may be listed on the article in the event of publication. Whoever is designated as a corresponding author on the title page of the manuscript file will be listed as such upon publication. Include an email address for each corresponding author listed on the title page of the manuscript.

 **How to select a new corresponding author in Editorial Manager**

Consortia and group authorship

If a manuscript is submitted on behalf of a consortium or group, include its name in the manuscript byline. Do not add it to the author list in the submission system. You may include the full list of members in the Acknowledgments or in a supporting information file.

PubMed only indexes individual consortium or group author members listed in the article byline. If included, these individuals must qualify for authorship according to our criteria.

Read the group authorship policy.

Author contributions

Provide at minimum one contribution for each author in the submission system. Use the CRediT taxonomy to describe each contribution. Read the policy and the full list of roles.

Contributions will be published with the final article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and we expect that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

PLOS ONE will contact all authors by email at submission to ensure that they are aware of the submission.

Cover letter

Upload a cover letter as a separate file in the online system. The length limit is 1 page.

The cover letter should include the following information:

- Summarize the study's contribution to the scientific literature
- Relate the study to previously published work
- Specify the type of article (for example, research article, systematic review, meta-analysis, clinical trial)
- Describe any prior interactions with PLOS regarding the submitted manuscript
- Suggest appropriate Academic Editors to handle your manuscript (see the full list of Academic Editors)

- List any opposed reviewers

IMPORTANT: Do not include requests to reduce or waive publication fees in the cover letter. This information will be entered separately in the online submission system.

Read about publication fee assistance.

Title page

The title, authors, and affiliations should all be included on a title page as the first page of the manuscript file.

 Download our sample title, author list, and affiliations page (PDF)

Abstract

The Abstract comes after the title page in the manuscript file. The abstract text is also entered in a separate field in the submission system.

The Abstract should:

- Describe the main objective(s) of the study
- Explain how the study was done, including any model organisms used, without methodological detail
- Summarize the most important results and their significance
- Not exceed 300 words

Abstracts should not include:

- Citations
- Abbreviations, if possible

Introduction

The introduction should:

- Provide background that puts the manuscript into context and allows readers outside the field to understand the purpose and significance of the study
- Define the problem addressed and why it is important
- Include a brief review of the key literature
- Note any relevant controversies or disagreements in the field
- Conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved

Materials and Methods

The Materials and Methods section should provide enough detail to allow suitably skilled investigators to fully replicate your study. Specific information and/or protocols for new methods should be included in detail. If materials, methods, and protocols are well established, authors may cite articles where those protocols are described in detail, but the submission should include sufficient information to be understood independent of these references.

Protocol documents for clinical trials, observational studies, and other **non-laboratory** investigations may be uploaded as supporting information. We recommend depositing **laboratory protocols** at protocols.io. Read detailed instructions for depositing and sharing your laboratory protocols.

Human or animal subjects and/or tissue or field sampling

Methods sections describing research using human or animal subjects and/or tissue or field sampling must include required ethics statements. For details, consult the reporting guidelines for specific study types.

Data

PLOS journals require authors to make all data underlying the findings described in their manuscript fully available without restriction, with rare exception.

Large data sets, including raw data, may be deposited in an appropriate public repository. See our list of recommended repositories.

For smaller data sets and certain data types, authors may provide their data within supporting information files accompanying the manuscript. Authors should take care to maximize the accessibility and reusability of the data by selecting a file format from which data can be efficiently extracted (for example, spreadsheets or flat files should be provided rather than PDFs when providing tabulated data).

For more information on how best to provide data, read our policy on data availability. PLOS does not accept references to "data not shown."

Cell lines

Methods sections describing research using cell lines must state the origin of the cell lines used. See the reporting guidelines for cell line research.

Laboratory protocols

To enhance the reproducibility of your results, we recommend and encourage you to deposit laboratory protocols in protocols.io, where protocols can be assigned their own persistent digital object identifiers (DOIs).

To include a link to a protocol in your article:

1. Describe your step-by-step protocol on protocols.io
2. Select **Get DOI** to issue your protocol a persistent digital object identifier (DOI)
3. Include the DOI link in the Methods section of your manuscript using the following format provided by protocols.io:
   http://dx.doi.org/10.17504/protocols.io.[PROTOCOL DOI]

At this stage, your protocol is only visible to those with the link. This allows editors and reviewers to consult your protocol when evaluating the manuscript. You can make your protocols public at any time by selecting **Publish** on the protocols.io site. Any referenced protocol(s) will automatically be made public when your article is published.

New taxon names

Methods sections of manuscripts adding new zoological, botanical, or fungal taxon names to the literature must follow the guidelines for new taxon names.

Results, Discussion, Conclusions

These sections may all be separate, or may be combined to create a mixed Results/Discussion section (commonly labeled “Results and Discussion”) or a mixed Discussion/Conclusions section (commonly labeled “Discussion”). These sections may be further divided into subsections, each with a concise subheading, as appropriate. These sections have no word limit, but the language should be clear and concise.

Together, these sections should describe the results of the experiments, the interpretation of these results, and the conclusions that can be drawn.

Authors should explain how the results relate to the hypothesis presented as the basis of the study and provide a succinct explanation of the implications of the findings, particularly in relation to previous related studies and potential future directions for research.

PLOS ONE editorial decisions do not rely on perceived significance or impact, so authors should avoid overstating their conclusions. See the *PLOS ONE* Criteria for Publication for more information.

Acknowledgments

Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution.

Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named.

PLOS journals publicly acknowledge the indispensable efforts of our editors and reviewers on an annual basis. To ensure equitable recognition and avoid any appearance of partiality, do not include editors or peer reviewers—named or unnamed—in the Acknowledgments.

Do not include funding sources in the Acknowledgments or anywhere else in the manuscript file. Funding information should only be entered in the financial disclosure section of the submission system.

References

Any and all available works can be cited in the reference list. Acceptable sources include:

- Published or accepted manuscripts
- Manuscripts on preprint servers, providing the manuscript has a citable DOI or arXiv URL.

Do not cite the following sources in the reference list:

- Unavailable and unpublished work, including manuscripts that have been submitted but not yet accepted (e.g., “unpublished work,” “data not shown”). Instead, include those data as supplementary material or deposit the data in a publicly available database.
- Personal communications (these should be supported by a letter from the relevant authors but not included in the reference list)

References are listed at the end of the manuscript and numbered in the order that they appear in the text. In the text, cite the reference number in square brackets (e.g., “We used the techniques developed by our colleagues [19] to analyze the data”). PLOS uses the numbered citation (citation-sequence) method and first six authors, et al.

Do not include citations in abstracts.

Make sure the parts of the manuscript are in the correct order *before* ordering the citations.

Formatting references

Because all references will be linked electronically as much as possible to the papers they cite, proper formatting of the references is crucial.

PLOS uses the reference style outlined by the International Committee of Medical Journal Editors (ICMJE), also referred to as the “Vancouver” style. Example formats are listed below. Additional examples are in the ICMJE sample references.

A reference management tool, EndNote, offers a current style file that can assist you with the formatting of your references. If you have problems with any reference management program, please contact the source company's technical support.

Journal name abbreviations should be those found in the National Center for Biotechnology Information (NCBI) databases.

Source	Format
Published articles	Hou WR, Hou YL, Wu GF, Song Y, Su XL, Sun B, et al. cDNA, genomic sequence cloning and overexpression of ribosomal protein gene L9 (rpL9) of the giant panda (Ailuropoda melanoleuca). Genet Mol Res. 2011;10: 1576-1588. Devaraju P, Gulati R, Antony PT, Mithun CB, Negi VS. Susceptibility to SLE in South Indian Tamils may be influenced by genetic selection pressure on TLR2 and TLR9 genes. Mol Immunol. 2014 Nov 22. pii: S0161-5890(14)00313-7. doi: 10.1016/j.molimm.2014.11.005. Note: A DOI number for the full-text article is acceptable as an alternative to or in addition to traditional volume and page numbers. When providing a DOI, adhere to the format in the example above with both the label and full DOI included at the end of the reference (doi: 10.1016/j.molimm.2014.11.005). Do not provide a shortened DOI or the URL.
Accepted, unpublished articles	Same as published articles, but substitute “Forthcoming” for page numbers or DOI.

Source	Format
Online articles	Huynen MMTE, Martens P, Hilderlink HBM. The health impacts of globalisation: a conceptual framework. Global Health. 2005;1: 14. Available from: http://www.globalizationandhealth.com/content/1/1/14
Books	Bates B. Bargaining for life: A social history of tuberculosis. 1st ed. Philadelphia: University of Pennsylvania Press; 1992.
Book chapters	Hansen B. New York City epidemics and history for the public. In: Harden VA, Risse GB, editors. AIDS and the historian. Bethesda: National Institutes of Health; 1991. pp. 21-28.
Deposited articles (preprints, e-prints, or arXiv)	Krick T, Shub DA, Verstraete N, Ferreiro DU, Alonso LG, Shub M, et al. Amino acid metabolism conflicts with protein diversity. arXiv:1403.3301v1 [Preprint]. 2014 [cited 2014 March 17]. Available from: https://128.84.21.199/abs/1403.3301v1 Kording KP, Mensh B. Ten simple rules for structuring papers. BioRxiv [Preprint]. 2016 bioRxiv 088278 [posted 2016 Nov 28; revised 2016 Dec 14; revised 2016 Dec 15; cited 2017 Feb 9]: [12 p.]. Available from: https://www.biorxiv.org/content/10.1101/088278v5 doi: 10.1101/088278
Published media (print or online newspapers and magazine articles)	Fountain H. For Already Vulnerable Penguins, Study Finds Climate Change Is Another Danger. The New York Times. 2014 Jan 29 [Cited 2014 March 17]. Available from: http://www.nytimes.com/2014/01/30/science/earth/climate-change-taking-toll-on-penguins-study-finds.html
New media (blogs, web	Allen L. Announcing PLOS Blogs. 2010 Sep 1 [cited 17 March 2014]. In: PLOS Blogs [Internet]. San Francisco: PLOS 2006 - . [about 2

Source	Format
sites, or other written works)	screens]. Available from: http://blogs.plos.org/plos/2010/09/announcing-plos-blogs/.
Masters' theses or doctoral dissertations	Wells A. Exploring the development of the independent, electronic, scholarly journal. M.Sc. Thesis, The University of Sheffield. 1999. Available from: http://cumincad.scix.net/cgi-bin/works/Show?2e09
Databases and repositories (Figshare, arXiv)	Roberts SB. QPX Genome Browser Feature Tracks; 2013 [cited 2013 Oct 5]. Database: figshare [Internet]. Available from: http://figshare.com/articles/QPX_Genome_Browser_Feature_Tracks/701214
Multimedia (videos, movies, or TV shows)	Hitchcock A, producer and director. Rear Window [Film]; 1954. Los Angeles: MGM.

Supporting information

Authors can submit essential supporting files and multimedia files along with their manuscripts. All supporting information will be subject to peer review. All file types can be submitted, but files must be smaller than 20 MB in size.

Authors may use almost any description as the item name for a supporting information file as long as it contains an "S" and number. For example, "S1 Appendix" and "S2 Appendix," "S1 Table" and "S2 Table," and so forth.

Supporting information files are published exactly as provided, and are not copyedited.

Supporting information captions

List supporting information captions at the end of the manuscript file. Do not submit captions in a separate file.

The file number and name are required in a caption, and we highly recommend including a one-line title as well. You may also include a legend in your caption, but it is not required.

Example caption

S1 Text. Title is strongly recommended. Legend is optional.

In-text citations

We recommend that you cite supporting information in the manuscript text, but this is not a requirement. If you cite supporting information in the text, citations do not need to be in numerical order.

Read the supporting information guidelines for more details about submitting supporting information and multimedia files.

Figures and tables

Figures

Do not include figures in the main manuscript file. Each figure must be prepared and submitted as an individual file.

Cite figures in ascending numeric order at first appearance in the manuscript file.

Read the guidelines for figures and requirements for reporting blot and gel results.

Figure captions

Figure captions must be inserted in the text of the manuscript, immediately following the paragraph in which the figure is first cited (read order). Do not include captions as part of the figure files themselves or submit them in a separate document.

At a minimum, include the following in your figure captions:

- A figure label with Arabic numerals, and "Figure" abbreviated to "Fig" (e.g. Fig 1, Fig 2, Fig 3, etc). Match the label of your figure with the name of the file uploaded at submission (e.g. a figure citation of "Fig 1" must refer to a figure file named "Fig1.tif").
- A concise, descriptive title

The caption may also include a legend as needed.

Read more about figure captions.

Tables

Cite tables in ascending numeric order upon first appearance in the manuscript file.

Place each table in your manuscript file directly after the paragraph in which it is first cited (read order). Do not submit your tables in separate files.

Tables require a label (e.g., "Table 1") and brief descriptive title to be placed above the table. Place legends, footnotes, and other text below the table.

Read the guidelines for tables.

Statistical reporting

Manuscripts submitted to *PLOS ONE* are expected to report statistical methods in sufficient detail for others to replicate the analysis performed. Ensure that results are rigorously

reported in accordance with community standards and that the statistical methods employed are appropriate for the study design.

Consult the following resources for additional guidance:

- SAMPL guidelines, for general guidance on statistical reporting
- *PLOS ONE* guidelines, for clinical trials requirements
- *PLOS ONE* guidelines, for systematic review and meta-analysis requirements
- EQUATOR, for specific reporting guidelines for a range of other study types

Reporting of statistical methods

In the methods, include a section on statistical analysis that reports a detailed description of the statistical methods. In this section:

- List the name and version of any software package used, alongside any relevant references
- Describe the technical details or procedures required to reproduce the analysis
- Provide the repository identifier for any code used in the analysis (See our code-sharing policy.)

Statistical reporting guidelines:

- Identify research design and independent variables as being between- or within-subjects
- For pre-processed data:
    - Describe any analysis carried out to confirm the data meets the assumptions of the analysis performed (e.g. linearity, co-linearity, normality of the distribution).

    - If data were transformed include this information, with a reason for doing so and a description of the transformation performed
- Provide details of how outliers were treated and your analysis, both with the full dataset and with the outliers removed
- If relevant, describe how missing/excluded data were handled
- Define the threshold for significance (alpha)
- If appropriate, provide sample sizes, along with a description of how they were determined. If a sample size calculation was performed, specify the inputs for power, effect size and alpha. Where relevant, report the number of independent replications for each experiment.
- For analyses of variance (ANOVAs), detail any post hoc tests that were performed
- Include details of any corrections applied to account for multiple comparisons. If corrections were not applied, include a justification for not doing so
- Describe all options for statistical procedures. For example, if t-tests were performed, state whether these were one- or two-tailed. Include details of the type of t-test conducted (e.g. one sample, within-/between-subjects).
- For step-wise multiple regression analyses:
    - Report the alpha level used
    - Discuss whether the variables were assessed for collinearity and interaction
    - Describe the variable selection process by which the final model was developed (e.g., forward-stepwise; best subset). See SAMPL guidelines.
- For Bayesian analysis explain the choice of prior trial probabilities and how they were selected. Markov chain Monte Carlo settings should be reported.

Reporting of statistical results

Results must be rigorously and appropriately reported, in keeping with community standards.

- **Units of measurement.** Clearly define measurement units in all tables and figures.
- **Properties of distribution.** It should be clear from the text which measures of variance (standard deviation, standard error of the mean, confidence intervals) and central tendency (mean, median) are being presented.
- **Regression analyses.** Include the full results of any regression analysis performed as a supplementary file. Include all estimated regression coefficients, their standard error, p-values, and confidence intervals, as well as the measures of goodness of fit.
- **Reporting parameters.** Test statistics (F/t/r) and associated degrees of freedom should be provided. Effect sizes and confidence intervals should be reported where appropriate. If percentages are provided, the numerator and denominator should also be given.
- **P-values.** Report exact p-values for all values greater than or equal to 0.001. P-values less than 0.001 may be expressed as $p < 0.001$, or as exponentials in studies of genetic associations.
- **Displaying data in plots.** Format plots so that they accurately depict the sample distribution. 3D effects in plots can bias and hinder interpretation of values, so avoid them in cases where regular plots are sufficient to display the data.
- **Open data.** As explained in PLOS's Data Policy, be sure to make individual data points, underlying graphs and summary statistics available at the time of publication. Data can be deposited in a repository or included within the Supporting Information files.

Data reporting

All data and related metadata underlying the findings reported in a submitted manuscript should be deposited in an appropriate public repository, unless already provided as part of the submitted article.

See instructions on providing underlying data to support blot and gel results

Read our policy on data availability.

Repositories may be either subject-specific (where these exist) and accept specific types of structured data, or generalist repositories that accept multiple data types. We recommend that authors select repositories appropriate to their field. Repositories may be subject-specific (e.g., GenBank for sequences and PDB for structures), general, or institutional, as long as DOIs or accession numbers are provided and the data are at least as open as CC BY. Authors are encouraged to select repositories that meet accepted criteria as trustworthy digital repositories, such as criteria of the Centre for Research Libraries or Data Seal of Approval. Large, international databases are more likely to persist than small, local ones.

See our list of recommended repositories.

To support data sharing and author compliance of the PLOS data policy, we have integrated our submission process with a select set of data repositories. The list is neither representative nor exhaustive of the suitable repositories available to authors. Current repository integration partners include Dryad and FlowRepository. Please contact data@plos.org to make recommendations for further partnerships.

Instructions for PLOS submissions with data deposited in an integration partner repository:

- Deposit data in the integrated repository of choice.
- Once deposition is final and complete, the repository will provide you with a dataset DOI (provisional) and private URL for reviewers to gain access to the data.
- Enter the given data DOI into the full Data Availability Statement, which is requested in the Additional Information section of the PLOS submission form. Then provide the URL passcode in the Attach Files section.

If you have any questions, please email us.

Accession numbers

All appropriate data sets, images, and information should be deposited in an appropriate public repository. See our list of recommended repositories.

Accession numbers (and version numbers, if appropriate) should be provided in the Data Availability Statement. Accession numbers or a citation to the DOI should also be provided when the data set is mentioned within the manuscript.

In some cases authors may not be able to obtain accession numbers of DOIs until the manuscript is accepted; in these cases, the authors must provide these numbers at acceptance. In all other cases, these numbers must be provided at full submission.

Identifiers

As much as possible, please provide accession numbers or identifiers for all entities such as genes, proteins, mutants, diseases, etc., for which there is an entry in a public database, for example:

- Ensembl
- Entrez Gene
- FlyBase
- InterPro
- Mouse Genome Database (MGD)
- Online Mendelian Inheritance in Man (OMIM)
- PubChem

Identifiers should be provided in parentheses after the entity on first use.

Striking image

You can choose to upload a "Striking Image" that we may use to represent your article online in places like the journal homepage or in search results.

The striking image must be derived from a figure or supporting information file from the submission, i.e., a cropped portion of an image or the entire image. Striking images should ideally be high resolution, eye-catching, single panel images, and should ideally avoid containing added details such as text, scale bars, and arrows.

If no striking image is uploaded, we will designate a figure from the submission as the striking image.

Striking images should not contain potentially identifying images of people. Read our policy on identifying information.

The PLOS licenses and copyright policy also applies to striking images.

Additional Information Requested at Submission

Financial Disclosure Statement

This information should describe sources of funding that have supported the work. It is important to gather these details prior to submission because your financial disclosure statement cannot be changed after initial submission without journal approval. If your manuscript is published, your statement will appear in the Funding section of the article.

Enter this statement in the Financial Disclosure section of the submission form. Do not include it in your manuscript file.

The statement should include:

- Specific grant numbers
- Initials of authors who received each award
- Full names of commercial companies that funded the study or authors
- Initials of authors who received salary or other funding from commercial companies
- URLs to sponsors' websites

Also state whether any sponsors or funders (other than the named authors) played any role in:

- Study design
- Data collection and analysis
- Decision to publish
- Preparation of the manuscript

If they had no role in the research, include this sentence: "The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript."

If the study was unfunded, include this sentence as the Financial Disclosure statement: "The author(s) received no specific funding for this work."

Read our policy on disclosure of funding sources.

Competing interests

This information should not be in your manuscript file; you will provide it via our submission system.

All potential competing interests must be declared in full. If the submission is related to any patents, patent applications, or products in development or for market, these details, including patent numbers and titles, must be disclosed in full.

Read our policy on competing interests.

Manuscripts disputing published work

For manuscripts disputing previously published work, it is *PLOS ONE* policy to invite a signed review by the disputed author during the peer review process. This procedure is aimed at ensuring a thorough, transparent, and productive review process.

If the disputed author chooses to submit a review, it must be returned in a timely fashion and contain a full declaration of all competing interests. The Academic Editor will consider any such reviews in light of the competing interest.

Authors submitting manuscripts disputing previous work should explain the relationship between the manuscripts in their cover letter, and will be required to confirm that they accept the conditions of this review policy before the manuscript is considered further.

Related manuscripts

Upon submission, authors must confirm that the manuscript, or any related manuscript, is not currently under consideration or accepted elsewhere. If related work has been submitted to *PLOS ONE* or elsewhere, authors must include a copy with the submitted article. Reviewers will be asked to comment on the overlap between related submissions.

We strongly discourage the unnecessary division of related work into separate manuscripts, and we will not consider manuscripts that are divided into “parts.” Each submission to *PLOS ONE* must be written as an independent unit and should not rely on any work that has not already been accepted for publication. If related manuscripts are submitted to *PLOS ONE*, the authors may be advised to combine them into a single manuscript at the editor's discretion.

Read our policies on related manuscripts.

Preprints

PLOS encourages authors to post preprints as a way to accelerate the dissemination of research and supports authors who wish to share their work early and receive feedback before formal peer review. Deposition of manuscripts with preprint servers does not impact consideration of the manuscript at any PLOS journal.

Authors posting on bioRxiv or medRxiv may submit directly to relevant PLOS journals through the direct transfer to journal service.

Authors submitting manuscripts in the life sciences to *PLOS ONE* may opt-in to post their work on bioRxiv during the *PLOS ONE* initial submission process.

Read more about preprints.

Learn how to post a preprint to bioRxiv during *PLOS ONE* initial submission.

Guidelines for Specific Study Types

Registered Reports

Submission and format requirements for Registered Report Protocols and Registered Reports are similar to those for a regular submission and may be specific to your study type. For instance, if your Registered Report Protocol submission is about a Clinical Trial or a Systematic Review, follow the appropriate guidelines.

For Registered Report Protocols:

- Provide enough methodological detail to make the study reproducible and replicable
- Confirm that data will be made available upon study completion in keeping with the PLOS Data policy

- Include ethical approval or waivers, if applicable
- Preliminary or pilot data may be included, but only if necessary to support the feasibility of the study or as a proof of principle
- For meta-analyses or a Clinical Trials, consider using the protocol-specific reporting guidelines PRISMA-P or SPIRIT respectively

For more guidance on format and presentation of a protocol, consult the sample template hosted by the Open Science Framework. Discipline-specific and study-specific templates are also available.

If data need to be collected, modified or processed specifically for your study, or if participants need to be recruited specifically for your study, then it should occur only after your Registered Report Protocol is accepted for publication.

For Registered Report Research Articles:

- Report the results of all planned analyses and, if relevant, detail and justify all deviations from the protocol.
- The manuscript may also contain exploratory, unplanned analyses.

Read more about Registered Report framework.

Human subjects research

All research involving human participants must have been approved by the authors' Institutional Review Board (IRB) or by equivalent ethics committee(s), and must have been conducted according to the principles expressed in the Declaration of Helsinki. Authors should be able to submit, upon request, a statement from the IRB or ethics committee indicating approval of the research. We reserve the right to reject work that we

believe has not been conducted to a high ethical standard, even when formal approval has been obtained.

Subjects must have been properly instructed and have indicated that they consent to participate by signing the appropriate informed consent paperwork. Authors may be asked to submit a blank, sample copy of a subject consent form. If consent was verbal instead of written, or if consent could not be obtained, the authors must explain the reason in the manuscript, and the use of verbal consent or the lack of consent must have been approved by the IRB or ethics committee.

All efforts should be made to protect patient privacy and anonymity. Identifying information, including photos, should not be included in the manuscript unless the information is crucial and the individual has provided written consent by completing the Consent Form for Publication in a PLOS Journal (PDF). Download additional translations of the form from the Downloads and Translations page. More information about patient privacy, anonymity, and informed consent can be found in the International Committee of Medical Journal Editors (ICMJE) Privacy and Confidentiality guidelines.

Manuscripts should conform to the following reporting guidelines:

- Studies of diagnostic accuracy: STARD
- Observational studies: STROBE
- Microarray experiments: MIAME
- Other types of health-related research: Consult the EQUATOR web site for appropriate reporting guidelines

Methods sections of papers on research using human subjects or samples must include ethics statements that specify:

- **The name of the approving institutional review board or equivalent committee(s)**. If approval was not obtained, the authors must provide a detailed statement explaining why it was not needed
- **Whether informed consent was written or oral**. If informed consent was oral, it must be stated in the manuscript:
    - Why written consent could not be obtained
    - That the Institutional Review Board (IRB) approved use of oral consent
    - How oral consent was documented

For studies involving humans categorized by race/ethnicity, age, disease/disabilities, religion, sex/gender, sexual orientation, or other socially constructed groupings, authors should:

- Explicitly describe their methods of categorizing human populations
- Define categories in as much detail as the study protocol allows
- Justify their choices of definitions and categories, including for example whether any rules of human categorization were required by their funding agency
- Explain whether (and if so, how) they controlled for confounding variables such as socioeconomic status, nutrition, environmental exposures, or similar factors in their analysis

In addition, outmoded terms and potentially stigmatizing labels should be changed to more current, acceptable terminology. Examples: “Caucasian” should be changed to “white” or “of [Western] European descent” (as appropriate); “cancer victims” should be changed to “patients with cancer.”

For papers that include identifying, or potentially identifying, information, authors must download the Consent Form for Publication in a PLOS Journal, which the individual, parent, or guardian must sign once they have read the paper and been informed about the

terms of PLOS open-access license. The signed consent form should not be submitted with the manuscript, but authors should securely file it in the individual's case notes and the methods section of the manuscript should explicitly state that consent authorization for publication is on file, using wording like:

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

For more information about *PLOS ONE* policies regarding human subjects research, see the Publication Criteria and Editorial Policies.

Clinical trials

Clinical trials are subject to all policies regarding human research. *PLOS ONE* follows the World Health Organization's (WHO) definition of a clinical trial:

A clinical trial is any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes [...] Interventions include but are not restricted to drugs, cells and other biological products, surgical procedures, radiologic procedures, devices, behavioural treatments, process-of-care changes, preventive care, etc.

All clinical trials must be registered in one of the publicly-accessible registries approved by the WHO or ICMJE (International Committee of Medical Journal Editors). Authors must provide the trial registration number. Prior disclosure of results on a clinical trial registry site will not affect consideration for publication. We reserve the right to inform authors' institutions or ethics committees, and to reject the manuscript, if we become aware of unregistered trials.

PLOS ONE supports prospective trial registration (i.e. before participant recruitment has begun) as recommended by the ICMJE's clinical trial registration policy. **Where trials were not publicly registered before participant recruitment began**, authors must:

- Register all related clinical trials and confirm they have done so in the Methods section
- Explain in the Methods the reason for failing to register before participant recruitment

Clinical trials must be reported according to the relevant reporting guidelines, i.e. CONSORT for randomized controlled trials, TREND for non-randomized trials, and other specialized guidelines as appropriate. The intervention should be described according to the requirements of the TIDieR checklist and guide. Submissions must also include the study protocol as supporting information, which will be published with the manuscript if accepted.

Authors of manuscripts describing the results of clinical trials must adhere to the CONSORT reporting guidelines appropriate to their trial design, available on the CONSORT Statement web site. Before the paper can enter peer review, authors must:

- Provide the registry name and number in the methods section of the manuscript
- Provide a copy of the trial protocol as approved by the ethics committee and a completed CONSORT checklist as supporting information (which will be published alongside the paper, if accepted). This should be named S1 CONSORT Checklist.
- Include the CONSORT flow diagram as the manuscript's “Fig 1”

Any deviation from the trial protocol must be explained in the paper. Authors must explicitly discuss informed consent in their paper, and we reserve the right to ask for a copy of the patient consent form.

The methods section must include the name of the registry, the registry number, and the URL of your trial in the registry database for each location in which the trial is registered.

Animal research

All research involving vertebrates or cephalopods must have approval from the authors' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee(s), and must have been conducted according to applicable national and international guidelines. Approval must be received prior to beginning research.

Manuscripts reporting animal research must state in the Methods section:

- The full name of the relevant ethics committee that approved the work, and the associated permit number(s).
- Where ethical approval is not required, the manuscript should include a clear statement of this and the reason why. Provide any relevant regulations under which the study is exempt from the requirement for approval.
- Relevant details of steps taken to ameliorate animal suffering.

Example ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Minnesota (Protocol Number: 27-2956). All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

Authors should always state the organism(s) studied in the Abstract. Where the study may be confused as pertaining to clinical research, authors should also state the animal model in the title.

To maximize reproducibility and potential for re-use of data, we encourage authors to follow the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for all submissions describing laboratory-based animal research and to upload a completed ARRIVE Guidelines Checklist to be published as supporting information.

Non-human primates

Manuscripts describing research involving non-human primates must report details of husbandry and animal welfare in accordance with the recommendations of the Weatherall report, *The use of non-human primates in research*, including:

- Information about housing, feeding, and environmental enrichment.
- Steps taken to minimize suffering, including use of anesthesia and method of sacrifice, if appropriate.

Random source animals

Manuscripts describing studies that use random source (e.g. Class B dealer-sourced in the USA), shelter, or stray animals will be subject to additional scrutiny and may be rejected if sufficient ethical and scientific justification for the study design is lacking.

Unacceptable euthanasia methods and anesthetic agents

Manuscripts reporting use of a euthanasia method(s) classified as unacceptable by the American Veterinary Medical Association or use of an anesthesia method(s) that is widely prohibited (e.g., chloral hydrate, ether, chloroform) must include at the time of initial submission, scientific justification for use in the specific study design, as well as

confirmation of approval for specific use from their animal research ethics committee. These manuscripts may be subject to additional ethics considerations prior to publication.

Humane endpoints

Manuscripts reporting studies in which death of a regulated animal (vertebrate, cephalopod) is a likely outcome or a planned experimental endpoint, must comprehensively report details of study design, rationale for the approach, and methodology, including consideration of humane endpoints. This applies to research that involves, for instance, assessment of survival, toxicity, longevity, terminal disease, or high rates of incidental mortality.

Definition of a humane endpoint

A humane endpoint is a predefined experimental endpoint at which animals are euthanized when they display early markers associated with death or poor prognosis of quality of life, or specific signs of severe suffering or distress. Humane endpoints are used as an alternative to allowing such conditions to continue or progress to death following the experimental intervention ("death as an endpoint"), or only euthanizing animals at the end of an experiment. Before a study begins, researchers define the practical observations or measurements that will be used during the study to recognize a humane endpoint, based on anticipated clinical, physiological, and behavioral signs. Please see the NC3Rs guidelines for more information. Additional discussion of humane endpoints can be found in this article: Nuno H. Franco, Margarida Correia-Neves, I. Anna S. Olsson (2012) How "Humane" Is Your Endpoint? — Refining the Science-Driven Approach for Termination of Animal Studies of Chronic Infection. PLoS Pathog 8(1): e1002399 doi.org/10.1371/journal.ppat.1002399.

Full details of humane endpoints use must be reported for a study to be reproducible and for the results to be accurately interpreted.

For studies in which death of an animal is an outcome or a planned experimental endpoint, authors should include the following information in the Methods section of the manuscript:

- The specific criteria (i.e. humane endpoints) used to determine when animals should be euthanized.
- The duration of the experiment.
- The numbers of animals used, euthanized, and found dead (if any); the cause of death for all animals.
- How frequently animal health and behavior were monitored.
- All animal welfare considerations taken, including efforts to minimize suffering and distress, use of analgesics or anaesthetics, or special housing conditions.

If humane endpoints were not used, the manuscript should report:

- A scientific justification for the study design, including the reasons why humane endpoints could not be used, and discussion of alternatives that were considered.
- Whether the institutional animal ethics committee specifically reviewed and approved the anticipated mortality in the study design.

Observational and field studies

Methods sections for submissions reporting on any type of field study must include ethics statements that specify:

- Permits and approvals obtained for the work, including the full name of the authority that approved the study; if none were required, authors should explain why

- Whether the land accessed is privately owned or protected
- Whether any protected species were sampled
- Full details of animal husbandry, experimentation, and care/welfare, where relevant

Paleontology and archaeology research

Manuscripts reporting paleontology and archaeology research must include descriptions of methods and specimens in sufficient detail to allow the work to be reproduced. Data sets supporting statistical and phylogenetic analyses should be provided, preferably in a format that allows easy re-use. Read the policy.

Specimen numbers and complete repository information, including museum name and geographic location, are required for publication. Locality information should be provided in the manuscript as legally allowable, or a statement should be included giving details of the availability of such information to qualified researchers.

If permits were required for any aspect of the work, details should be given of all permits that were obtained, including the full name of the issuing authority. This should be accompanied by the following statement:

All necessary permits were obtained for the described study, which complied with all relevant regulations.

If no permits were required, please include the following statement:

No permits were required for the described study, which complied with all relevant regulations.

Manuscripts describing paleontology and archaeology research are subject to the following policies:

- **Sharing of data and materials.** Any specimen that is erected as a new species, described, or figured must be deposited in an accessible, permanent repository (i.e., public museum or similar institution). If study conclusions depend on specimens that do not fit these criteria, the article will be rejected under *PLOS ONE*'s data availability criterion.
- **Ethics.** *PLOS ONE* will not publish research on specimens that were obtained without necessary permission or were illegally exported.

Systematic reviews and meta-analyses

A systematic review paper, as defined by The Cochrane Collaboration, is a review of a clearly formulated question that uses explicit, systematic methods to identify, select, and critically appraise relevant research, and to collect and analyze data from the studies that are included in the review. These reviews differ substantially from narrative-based reviews or synthesis articles. Statistical methods (meta-analysis) may or may not be used to analyze and summarize the results of the included studies.

Reports of systematic reviews and meta-analyses must include a completed PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to accompany the main text. Blank templates are available here:

- Checklist: PDF or Word document
- Flow diagram: PDF or Word document

Authors must also state in their "Methods" section whether a protocol exists for their systematic review, and if so, provide a copy of the protocol as supporting information and provide the registry number in the abstract.

If your article is a systematic review or a meta-analysis you should:

- State this in your cover letter

- Select “Research Article” as your article type when submitting
- Include the PRISMA flow diagram as Fig 1 (required where applicable)
- Include the PRISMA checklist as supporting information

Meta-analysis of genetic association studies

Manuscripts reporting a meta-analysis of genetic association studies must report results of value to the field and should be reported according to the guidelines presented in *Systematic Reviews of Genetic Association Studies* by Sagoo *et al.*

On submission, authors will be asked to justify the rationale for the meta-analysis and how it contributes to the base of scientific knowledge in the light of previously published results. Authors will also be asked to complete a checklist (DOCX) outlining information about the justification for the study and the methodology employed. Meta-analyses that replicate published studies will be rejected if the authors do not provide adequate justification.

Personal data from third-party sources

For all studies using personal data from internet-based and other third-party sources (e.g., social media, blogs, other internet sources, mobile phone companies), data must be collected and used according to company/website Terms and Conditions, with appropriate permissions. All data sources must be acknowledged clearly in the Materials and Methods section.

Read our policy on data availability.

In the Ethics Statement, authors should declare any potential risks to individuals or individual privacy, or affirm that in their assessment, the study posed no such risks. In addition, the following Ethics and Data Protection requirements must be met.

For interventional studies, which impact participants' experiences or data, the study design must have been prospectively approved by an Ethics Committee, and informed consent is required. The Ethics Committee may waive the requirement for approval and/or consent.

For observational studies in which personal experiences and accounts are not manipulated, consultation with an Ethics or Data Protection Committee is recommended. Additional requirements apply in the following circumstances:

- If information used could threaten personal privacy or damage the reputation of individuals whose data are used, an Ethics Committee should be consulted and informed consent obtained or specifically addressed.
- If authors accessed any personal identifying information, an Ethics or Data Protection Committee should oversee data anonymization. If data were anonymized and/or aggregated before access and analysis, informed consent is generally not required.

Note that Terms of Use contracts do not qualify as informed consent, even if they address the use of personal data for research.

See our reporting guidelines for human subjects research.

Cell lines

Authors reporting research using cell lines should state when and where they obtained the cells, giving the date and the name of the researcher, cell line repository, or commercial source (company) who provided the cells, as appropriate.

Authors must also include the following information for each cell line:

For *de novo* (new) cell lines, including those given to the researchers as a gift, authors must follow our policies for human subjects research or animal research, as appropriate. The ethics statement must include:

- Details of institutional review board or ethics committee approval; AND
- For human cells, confirmation of written informed consent from the donor, guardian, or next of kin

For established cell lines, the Methods section should include:

- A reference to the published article that first described the cell line; AND/OR
- The cell line repository or company the cell line was obtained from, the catalogue number, and whether the cell line was obtained directly from the repository/company or from another laboratory

Authors should check established cell lines using the ICLAC Database of Cross-contaminated or Misidentified Cell Lines to confirm they are not misidentified or contaminated. Cell line authentication is recommended – e.g., by karyotyping, isozyme analysis, or short tandem repeats (STR) analysis – and may be required during peer review or after publication.

Blots and gels

Please review *PLOS ONE's* requirements for reporting blot and gel results and providing the underlying raw images.

Antibodies

Manuscripts reporting experiments using antibodies should include the following information:

- The name of each antibody, a description of whether it is monoclonal or polyclonal, and the host species.
- The commercial supplier or source laboratory.
- The catalogue or clone number and, if known, the batch number.
- The antigen(s) used to raise the antibody.
- For established antibodies, a stable public identifier from the Antibody Registry.

The manuscript should also report the following experimental details:

- The final antibody concentration or dilution.
- A reference to the validation study if the antibody was previously validated. If not, provide details of how the authors validated the antibody for the applications and species used.

We encourage authors to consider adding information on new validations to a publicly available database such as Antibodypedia or CiteAb.

Small and macromolecule crystal data

Manuscripts reporting new and unpublished three-dimensional structures must include sufficient supporting data and detailed descriptions of the methodologies used to allow the reproduction and validation of the structures. All novel structures must have been deposited in a community endorsed database prior to submission (please see our list of recommended repositories).

Small molecule single crystal data

Authors reporting X-Ray crystallographic structures of small organic, metal-organic, and inorganic molecules must deposit their data with the Cambridge Crystallographic Data Centre (CCDC), the Inorganic Crystal Structure Database (ICSD), or similar community

databases providing a recognized validation functionality. Authors are also required to include the relevant structure reference numbers within the main text (e.g. the CCDC ID number), as well as the crystallographic information files (.cif format) as Supplementary Information, along with the checkCIF validation reports that can be obtained via the International Union of Crystallography (IUCr).

Macromolecular structures

Authors reporting novel macromolecular structures must have deposited their data prior to initial submission with the Worldwide Protein Data Bank (wwPDB), the Biological Magnetic Resonance Data Bank (BMRB), the Electron Microscopy Data Bank (EMDB), or other community databases providing a recognized validation functionality. Authors must include the structure reference numbers within the main text and submit as Supplementary Information the official validation reports from these databases.

Methods, software, databases, and tools

PLOS ONE will consider submissions that present new methods, software, databases, or tools as the primary focus of the manuscript if they meet the following criteria:

Utility

The tool must be of use to the community and must present a proven advantage over existing alternatives, where applicable. Recapitulation of existing methods, software, or databases is not useful and will not be considered for publication. Combining data and/or functionalities from other sources may be acceptable, but simpler instances (i.e. presenting a subset of an already existing database) may not be considered. For software, databases, and online tools, the long-term utility should also be discussed, as relevant. This discussion may include maintenance, the potential for future growth, and the stability of the hosting, as applicable.

Validation

Submissions presenting methods, software, databases, or tools must demonstrate that the new tool achieves its intended purpose. If similar options already exist, the submitted manuscript must demonstrate that the new tool is an improvement over existing options in some way. This requirement may be met by including a proof-of-principle experiment or analysis; if this is not possible, a discussion of the possible applications and some preliminary analysis may be sufficient.

Availability

If the manuscript's primary purpose is the description of new software or a new software package, this software must be open source, deposited in an appropriate archive, and conform to the Open Source Definition. If the manuscript mainly describes a database, this database must be open-access and hosted somewhere publicly accessible, and any software used to generate a database should also be open source. If relevant, databases should be open for appropriate deposition of additional data. Dependency on commercial software such as Mathematica and MATLAB does not preclude a paper from consideration, although complete open source solutions are preferred. In these cases, authors should provide a direct link to the deposited software or the database hosting site from within the paper. If the primary focus of a manuscript is the presentation of a new tool, such as a newly developed or modified questionnaire or scale, it should be openly available under a license no more restrictive than CC BY.

Software submissions

Manuscripts whose primary purpose is the description of new software must provide full details of the algorithms designed. Describe any dependencies on commercial products or operating system. Include details of the supplied test data and explain how to install and run

the software. A brief description of enhancements made in the major releases of the software may also be given. Authors should provide a direct link to the deposited software from within the paper.

Database submissions

For descriptions of databases, provide details about how the data were curated, as well as plans for long-term database maintenance, growth, and stability. Authors should provide a direct link to the database hosting site from within the paper.

Read the PLOS policy on sharing materials and software.

New taxon names

Zoological names

When publishing papers that describe a new zoological taxon name, PLOS aims to comply with the requirements of the International Commission on Zoological Nomenclature (ICZN). Effective 1 January 2012, the ICZN considers an online-only publication to be legitimate if it meets the criteria of archiving and is registered in ZooBank, the ICZN's official registry.

For proper registration of a new zoological taxon, we require two specific statements to be included in your manuscript.

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Anochetus boltoni *Fisher* ***sp. nov.*** *urn:lsid:zoobank.org:act:B6C072CF-1CA6-40C7-8396-534E91EF7FBB*

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Please also insert the following text into the **Methods** section, in a sub-section to be called "Nomenclatural Acts":

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix "http://zoobank.org/". The LSID for this publication is: urn:lsid:zoobank.org:pub: XXXXXXX. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: PubMed Central, LOCKSS [author to insert any additional repositories].

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Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

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Solanum aspersum S.Knapp, sp. nov. [urn:lsid:ipni.org:names:77103633-1] Type: Colombia. Putumayo: vertiente oriental de la Cordillera, entre Sachamates y San Francisco de Sibundoy, 1600-1750 m, 30 Dec 1940, J. Cuatrecasas 11471 (holotype, COL; isotypes, F [F-1335119], US [US-1799731]).

Journal staff will contact IPNI to obtain the GUID (LSID) after your manuscript is accepted for publication, and this information will then be added to the manuscript during the production phase

In the **Methods** section, include a sub-section called “Nomenclature” using the following wording:

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In addition, new names contained in this work have been submitted to IPNI, from where they will be made available to the Global Names Index. The IPNI LSIDs can be resolved and the associated information viewed through any standard web browser by appending the LSID contained in this publication to the prefix http://ipni.org/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

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Effective January 2012, the description or diagnosis of a new taxon can be in either Latin or English. This does not affect the requirements for scientific names, which are still to be Latin.

Also effective January 2012, the electronic PDF represents a published work according to the ICN for algae, fungi, and plants. Therefore the new names contained in the electronic publication of PLOS article are effectively published under that Code from the electronic edition alone, so there is no longer any need to provide printed copies.

Additional information describing recent changes to the Code can be found here.

For proper registration of the new taxon, we require two specific statements to be included in your manuscript.

In the **Results** section, the globally unique identifier (GUID), currently in the form of a Life Science Identifier (LSID), should be listed under the new species name, for example:

Hymenogaster huthii. Stielow et al. 2010, sp. nov.
[urn:lsid:indexfungorum.org:names:518624]

You will need to contact either Mycobank or Index Fungorum to obtain the GUID (LSID). Please do this as early as possible to avoid delay of publication upon acceptance of your manuscript. It is your responsibility to provide us with this information so we can include it in the final published paper. Effective January 2013, all papers describing new fungal species must reference the identifier issued by a recognized repository in the protologue in order to be considered effectively published.

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substitute appropriately if you have submitted to Index Fungorum using the prefix http://www.indexfungorum.org/Names/NamesRecord.asp?RecordID=.

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In addition, new names contained in this work have been submitted to MycoBank from where they will be made available to the Global Names Index. The unique MycoBank number can be resolved and the associated information viewed through any standard web browser by appending the MycoBank number contained in this publication to the prefix http://www.mycobank.org/MB/. The online version of this work is archived and available from the following digital repositories: [INSERT NAMES OF DIGITAL REPOSITORIES WHERE ACCEPTED MANUSCRIPT WILL BE SUBMITTED (PubMed Central, LOCKSS etc)].

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Qualitative research

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