Blood and virus detection on barber hair clippers

By

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ABSTRACT

Background. Bleeding from the popular clean-shave '*chiskop*' haircut was recently reported as prevalent in South Africa (SA), a country with 6.9 million HIV-infected people.

Objectives. To investigate the prevalence of barber hair clipper contamination with blood and HIV and hepatitis B viruses.

Methods. Fifty barbers from three townships in Cape Town, SA, were invited to participate. One clipper from each barber was collected immediately after it had been used for a clean-shave haircut. Each clipper was rinsed with phosphate-buffered saline and then submerged in viral medium. The polymerase chain reaction (PCR) was used to identify the blood-specific RNA marker haemoglobin beta (HBB), hepatitis B virus (HBV) and HIV.

Results. The clean-shave haircut was the most common haircut requested by clients (78%). Of the clippers collected, 42% were positive for HBB, confirming detection of blood, none were positive for HIV, and 4 (8%) were positive for HBV. Two clippers (clippers 16 and 20) were positive on qualitative HBV PCR. HBV DNA from clipper 16 clustered with genotype A sequences from SA, India, Brazil and Martinique, while clipper 20 clustered with SA genotype D sequences. The clipper 20 sequence was identical to a subtype D sequence (GenBank accession AY233291) from Gauteng, SA.

Conclusion. This study confirms that there is significant contamination of barber hair clippers with blood and blood-borne viruses. Hepatitis B was detected with enough DNA copies to pose a risk of transmitting infection. Although HIV was not detected in this small study, the risk of transmission should be quantified. Further studies to investigate barber clipper sterilization practices and whether the clean-shave hairstyle is an independent risk factor for HIV, HBV and hepatitis C virus infections are warranted. Public education on individual clipper ownership (as is the case with a toothbrush) should be advocated for clean-shave and blade-fade haircuts.

Dedication

I dedicate this work to my late father Bekamawabo Mapherson Spengane, my mother Monica Spengane, my godmother Nomthunzi Tungu and my siblings without their constant love and support none of my achievements would be possible.

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Contributions by authors

Zandile Spengane: Formulation of research question, hypothesis, literature review, aim, objetcives, methods, data collection in the field, data analysis, drafting of article and critical revision.

Professsor NP Khumalo: Assisted with conception and design of thesis, data analysis and interpretation, drafting of article, critical revision and final approval to be published.

Dr Mzudumile Ngwanya: Laboratory data analysis and interpretation, drafting of article, and critical revision.

Dr Steven Korsman: Laboratory data analysis and interpretation, drafting of article, critical revision

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Laboratory data analysis: K, Mkentane, L M, Davids, W, Zemany, M, Africa, M, Nicol
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Abbreviations:

AIDS	acquired immune deficiency syndrome
AKN	acne keloidalis nuchae
ART	antiretroviral therapy
CDC	center for disease control and prevention
EFV	efavirenz
FKN	folliculitis keloidalis nuchae
HBB	haemoglobin beta
HBV	hepatitis b virus
HIV	human immunodeficiency virus
HSV	herpes simplex virus
RNA	ribonucleic acid
RSB	road side barbers
SB	shop barbers
TDF	tenofovir
US	United States
UV	ultraviolet

CHAPTER 1: Literature review

1.1 Background to study

Human hair variation and common hair pathology

Human scalp hair varies significantly in hair curvature and that of African ancestry has the tightest curl.(1, 2) Older studies suggested that hair biochemistry did not vary with curvature.(3) However, this has recently been challenged with higher lipid content reported in curly, than straight hair.(4) Three dimensional reconstruction of scalp biopsies report that the hair follicle emerges from the scalp at right and acute angles in straight and curly hair respectively. Further, population data suggests that the afro-textured hair follicle is prone to specific forms of alopecia (hair loss) that predominantly affect women, traction alopecia,(5) central centrifugal cicatricial alopecia(6) and men, folliculitis keloidalis nuchae (FKN) (6). Traction alopecia is hair loss as a result of pulling during hair grooming that results in inflammation and injury to the hair follicles. Centrifugal cicatricial alopecia is hair loss most commonly seen in African women. The proposed pathogenesis is the use of caustic hair products and the use of hot-combs that induces hair follicle damage resulting in a specific pattern of hair loss (7). Folliculitis keloidalis nuchae (FKN) is common, but not exclusive, to African men. The difference in hair grooming practices between men and women likely explains this. FKN refers to inflammation of hair follicles that result in scarring alopecia (8). The lesions usually start as papules and pustules that heal with small or large keloids (8).

Hairstyles and hairstyle trends

Hair style trends for men have changed over the years from “big afro’s” of the 1960-70’s to the “blade-fade” haircut or “German-cut” that was popular in the 1920’s, 1990’s and still is today; to the current clean-shave (the *chiskop*) that started at the turn of the last century and is worn by 70% of black men in Cape Town Townships(6). The blade-fade or German-cut was originally worn by working class men in the 1920’s and was popular amongst the Hitler’s Wehrmacht soldiers in Germany, hence the name. The clean-shave haircut, is also worn by the majority of black men in South Africa and the African diaspora. Besides being a trend, this “chiskop” haircut is part of certain cultural rituals in different African tribes. The haircut is achieved by either a razor blade or by pressing the metal of the electric clipper directly onto the scalp without using the manufacturer supplied plastic combs. This gives a clean-shave hair cut similar to that achieved using a razor blade. Shaving pimples more commonly seen on the beard also occur on the scalp as transient papules and pustules. However, when these pimples evolve into permanent keloids on the back (nuchal) scalp they are characteristic of folliculitis keloidalis nuchae (FKN)/ Acne keloidalis nuchae (AKN) which has a prevalence of 10.5% in males (and 0.1% females) >18years (6). FKN prevalence was

highest in participants whose hair had been cut with razors (10.7%), followed by clippers (5.9%) and depilatory creams (0%) (8).

Haircut associated bleeding

On history taking, bleeding from the scalp as a result of the clean-shave haircut was recognized incidentally from patients participating in a population study (6). A follow up study investigating the determinants of FKN in males found that 32% of men (of unknown HIV status) reported that they had at least one occurrence of bleeding during a clean-shave haircut (9). The authors then investigated the possible health care risks of the clean-shave haircut, they found that 24.8% of HIV positive men in their sample also reported a history of bleeding during a clean-shave haircut(10). The clean-shave haircut was found to be an independent risk factor for scalp injury and haircut associated bleeding with or without the presence of FKN (10). Recently, invisible bleeding was detected from scalp swabs after professional clean-shave haircuts (11). A dermatologist examined the participant's scalp and no scalp visible injury was noted. Scalp swabs were taken and tested for blood-specific RNA markers (albumin and hemoglobin beta – HBB) (11). 37% of participant's scalp swabs were positive for HBB suggesting microscopic bleeding (11). The potential transmission of blood-borne viruses such as Hepatitis B (HBV) and the Human Immunodeficiency Virus (HIV) is most concerning. A study done in Ethiopia states that shared shaving equipment in barbershops is common practice (12), and accidental scratch by sharp equipment in barbershops may create an opportunity for HIV and other blood borne pathogens, to enter to the body easily (12).

1.2 The HIV pandemic

There are 36.7 million people living with HIV globally(13). Nineteen million live in Eastern and Southern Africa, which consist of low and middle-income countries. Sub-Saharan Africa has the highest prevalence of HIV. There are many contributory factors for this such as social circumstances, poverty, migration, low level of education, family breakdown, substance abuse, early sexual debut and inter-generational sex. HIV is a large burden of disease in South Africa. The overall HIV prevalence rate in 2015 was approximately 11,2% of the total South African population(14). The total number of people living with HIV is estimated at 6,19 million in 2015 (14). New infections in 2015 were estimated at 1,22 million in the age group 15 – 49 years (14). Black people in South Africa represent 80% of the entire population, thus it is to be expected that the majority of people living with HIV are black South Africans. AIDS related deaths accounted for 30,5% of all deaths in 2015 (14).

1.3 Hepatitis B in Sub-Saharan Africa

Like HIV, Hepatitis B is also highly prevalent in Sub-Saharan Africa. Asia-Pacific and Sub-Saharan Africa are estimated to have 45% of the global population living with chronic hepatitis B infection (15). Chronic hepatitis B infection is estimated to affect 65 million people in Africa with 2.5 million of them in South Africa (15). Hepatitis B infection related deaths are on the rise, with a mortality between 500,000 - 1,2 million annually (15). A quarter of hepatitis B infected patients die from cirrhosis, liver failure or hepatocellular carcinoma (15). HIV and Hepatitis B co-infection in South Africa is not uncommon, with a variation between urban and rural areas. In South Africa the rate of positive HBsAg (hepatitis B surface antigen) is highest in the peri-urban areas at 22.9%, followed by the mining antiretroviral treatment (ART) group at 19.8% and 7.1% in the rural population attending ART clinics (16). Patients who test positive on their screening HIV rapid test are also tested for hepatitis B as part of their pre-antiretroviral treatment (ART) workup. The South African 2015 ART guidelines state that if an HIV positive patient also has hepatitis B co-infection they are eligible for ART irrespective of their CD4 count (17). The South African first line ART regimen is the fixed drug combination which is one tablet which contains three drugs: tenofovir, emitricitabine and efavirenz. In South Africa, patients with HIV and hepatitis B co-infection are kept on a tenofovir (TDF) containing regimen. Tenofovir (TDF) is one of the antiretrovirals recommended as first line therapy in the treatment of naïve chronic hepatitis B infection because of its high antiviral potency(18). Unfortunately a large proportion of the population who have asymptomatic hepatitis B infection are undiagnosed as they don't present to the health facilities or have presented for other reasons and had a negative HIV test, if tested at all.

1.3.1 Route of HIV and HBV transmission

The HIV and Hepatitis-B virus (HBV) spread from one individual to another via three known mechanisms. First and most common mechanism is through sexual intercourse were there is friction causing micro-abrasions in the genital mucosa and mixing of body fluids. The second mechanism is when fresh blood of an HIV positive and/or HBV positive individual comes into contact with blood of an uninfected person. This mode is common amongst drug users who share needles. The third mode of transmission is from mother to child in utero or through labor and breastfeeding. Campaigns developed at reducing the spread of HIV have been focused on promoting safe sex practices, being faithful to one partner and providing free condoms to schools, clinics and other public places. Government spends billions on antiretroviral treatment and testing campaigns. All mothers who are HIV positive get antiretrovirals irrespective of their CD4 counts and this has decreased mother to child transmission dramatically over the past couple of years.

Environmental Hepatitis B virus transmission has been well documented. It has been related to high concentration of HBV in the blood of an infected individual and its ability to remain infectious on inanimate surfaces (19). HIV can remain viable in fluid or dried medium for several days at room temperature (19). The HIV lipid envelope is thought to inhibit drying thus shields the viral proteins from dehydration (19). Van Bueren *et. al* stated that the ability of HIV to survive independent of a human host cell seems to be greater than cell associated virus (20). High HIV viral titers can stay virulent up to eight weeks outside the body (19). Thus the potential of viral spread from one client to another via contaminated hair-clippers or razor blades would depend on the viral concentration, titer or load of the infected individuals blood.

1.4 Knowledge of HIV transmission amongst barbers

Barber's knowledge of HIV and its mode of transmission was assessed in a study carried out in Ibadan, Nigeria. Barbers were assessed as having good awareness of HIV/AIDS (21). Eighty four percent of the barbers were aware that sharing non-sterile sharp instruments could potentially transmit HIV (21). Interestingly the authors reported that 10% of clippers were sterilized in the sessions, 72.5% were disinfected and 17.5% were decontaminated (21). A similar study done in Kanpur, India assessed the awareness of HIV and practices of shop and road side barbers (22). The total number of barbers enrolled in the study was 360 (22). This was made up of 270 shop barbers and 90 roadside barbers (22). Only 6.7% of all barbers knew all the modes of transmission of HIV (22). 46.7% of the roadside barbers had no knowledge of the nature HIV (22). No study yet, has looked at the practices and assessed knowledge of HIV and HBV amongst informal barbers in South African townships.

1.5 Grooming and cultural practices

South Africa has many tribes with different cultural practices. Our country has urban and rural areas. Certain cultural practices like circumcision and facial scarification are still practiced. Various grooming salons such as barbershops, beauty parlors (spa's) and nail grooming shops, utilize sharp instruments. A study done in Nigeria reported that HIV transmission through sharing of non-sterile sharp instruments such as those used for barbering and circumcision were not emphasized in the campaign against the transmission of HIV (21). The situation is similar in South Africa. South Africa has many informal barbershops based in townships, which operate in old shipping containers. Roadside barbershops are common in countries such as Ethiopia, Pakistan, and Bangladesh (12). Access to basic services such as clean running water, sanitation and proper sterilization of equipment in informal barbershops is questionable. Barbers use the same hair clipper on

many different clients on a daily basis. Some clients and barbers prefer using the old-fashioned stainless steel straight edge barber razor for cutting the beard and hair. Thus a history of bleeding from the scalp after a clean-shave haircut is of no surprise. To maintain the clean-shave haircut style, a client has to cut their hair at least twice a month (9). A small abrasion or cut on the scalp is enough to serve as an entry point for any microorganism to gain entry into the skin and cause infection.

In South Africa, it is unknown whether the current methods used to clean hair-clippers and razor blades are virucidal and effective in disinfecting the instruments prior to utilizing them on another client. A study was conducted in Ethiopia to assess barber knowledge, attitude, and practices about sterilization of sharp equipment in their work place and to evaluate the microbiological efficacy of sterilization methods (12). They found heat as the preferred method for inactivating blood borne pathogens such as HIV (12). The use of open flames for sterilization has been well reported in African countries,(21) but there is no data on efficacy or adequate exposure time. The most common cleaning method used was the use of ethanol and sodium hypochlorate but the concentration, quantity and duration of cleaning of equipment was variable (12). Commercial methylated spirit contains ethyl alcohol (95%) and methyl alcohol (5%) is most commonly used to decontaminate barber equipment. Ethyl alcohol and methyl alcohol are not virucidal. Thus knowledge of the correct disinfectant and sterilization methods by barbers is important. The Ethiopian study found that, participants had poor knowledge on sterilization procedures (12). They attributed this to the fact that informal barbers operate in unlicensed barbers and carry out grooming practices without knowing important health safety principles (12). Many developing countries lack regulating bodies that monitor the correct health practices at barbershops. In developed countries activities of barbers are regulated through comprehensive training, licensing and monitoring programs (12).

1.6 Recommended equipment disinfection

The center for disease control and prevention (CDC), in the United States (US) developed the Guideline for Disinfection and Sterilization in Healthcare Facilities. Locally, the Cape Provincial Legislation, municipal health services by-laws of 2015 state that any person operating a salon must equip the premises with an adequate means to disinfect and sterilize instruments and equipment that may come into direct contact with any customer's hair or skin (23). Ultraviolet (UV) light is reported to effectively kill bacteria and viruses(24). Application of UV radiation in biologic safety cabinets causes the destruction of organisms on the surface of instruments. This microbicidal activity is highly dependent on the wavelength of UV radiation. Modern ultraviolet light sterilization units as well as specific

antiviral sprays are used internationally in hair salons. Quaternary ammonium disinfectant cleaners have virucidal activity effective enough to kill HSV (herpes simplex virus) in the presence of blood (25). HSV (herpes simplex virus) has similar properties to HIV and thus quaternary ammonium disinfectants could be used to decontaminate surfaces or tools with blood spills (25). New generation of accelerated hydrogen peroxide-based environmental surface disinfectants are bactericidal, virucidal, mycobactericidal and fungicidal (26). The formulation is safe to use and has a high compatibility profile for various materials in addition to being a fast acting, intermediate-level disinfectant (26). In the USA, disinfectants must be approved by the Environmental Protection Agency (EPA), which certifies the efficacy of products for infection control. The SA counterpart is the South African Bureau of Standards (SABS). Barbers are encouraged to use EPA- or SABS-approved disinfectants for their equipment. The problem with the above products is cost and availabilty. Many informal barbers operate in poverty stricken countries and communities. They themsleves are from a low socio-economic status and cannot afford these sterilization formulations and UV cabinets. Studies quoted state that informal barbers currently disinfect their instruments with ethanol and heat.

Summary

Sub-Saharan Africa has the highest prevalence of HIV. HIV/HBV co-infection is not uncommon. The clean-shave “chiskop” haircut is popular amongst African men. It’s also found to be an independent risk factor for scalp injury and haircut associated bleeding with or without the presence of FKN (10). The incidence and prevalence of barber clipper or razor contamination with blood and blood borne viruses in our setting is unknown. Currently there is no study in the literature that looks at informal barber practices in the South African townships. The methods of sterilization or disinfection of barber instruments in our townships is speculated but not verified. The potential transmission of blood-borne viruses such as Hepatitis B (HBV) and the Human Immunodeficiency Virus (HIV) using unsterilized barber clippers is a concern and possible risk. The aim of this study is to detect the prevalence of barber-clipper contamination with blood and the viruses (HIV and HBV). We want to investigate whether barbers, who predominantly cut clean-shave “chiskop” haircut versus longer haircuts, *i.e., at a distance from the scalp*, cause more or less haircut associated bleeding. We also want to explore the cleaning methods used by barbers. The objective is to swop clippers currently used by barbers for new ones and to test the clippers, after a haircut, for blood and viruses.

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Reference:

1. Khumalo NP, Doe PT, Dawber RP, Ferguson DJ. What is normal black African hair? A light and scanning electron-microscopic study. J Am Acad Dermatol. 2000;43(5 Pt 1):814-20.
2. Loussouarn G, Garcel AL, Lozano I, Collaudin C, Porter C, Panhard S, et al. Worldwide diversity of hair curliness: a new method of assessment. Int J Dermatol. 2007;46 Suppl 1:2-6.
3. Hrdy D, Baden HP. Biochemical variation of hair keratins in man and non-human primates. Am J Phys Anthropol. 1973;39(1):19-24.
4. Cruz CF, Fernandes MM, Gomes AC, Coderch L, Marti M, Mendez S, et al. Keratins and lipids in ethnic hair. International journal of cosmetic science. 2013;35(3):244-9.
5. Khumalo NP, Jessop S, Gumedze F, Ehrlich R. Hairdressing is associated with scalp disease in African schoolchildren. Br J Dermatol. 2007;157(1):106-10.
6. Khumalo NP, Jessop S, Gumedze F, Ehrlich R. Hairdressing and the prevalence of scalp disease in African adults. Br J Dermatol. 2007;157(5):981-8.
7. Bolognia J, Jorizzo, JL, Schaffer, VJ. Third Edition Dermatology. Third Edition ed: Elsevier Saunders; 2012.
8. Khumalo NP, Gumedze F, Lehloenya R. Folliculitis keloidalis nuchae is associated with the risk for bleeding from haircuts. Int J Dermatol.50(10):1212-6.
9. Khumalo NP. Folliculitis keloidalis nuchea, bleeding from haircuts, and potential HIV transmission. Int J Dermatol. 2012;51 Suppl 1:21-3, 4-6.
10. Khumalo NP, Gantsho N, Gumedze F, Mthebe T. Health risks of the clean-shave chiskop haircut. S Afr Med J.103(7):489-90.
11. Khumalo NP, Mkentane K, Muthukarapan C, Hardie D, Korsman S, Hu N, et al. Invisible bleeding from clean-shave haircuts: detection with blood specific RNA markers. Dermatology (Basel, Switzerland).227(3):197-201.
12. Biadgelegn F, Belyhun Y, Anagaw B, Woldeyohannes D, Moges F, Bekele A, et al. Potential risk of HIV transmission in barbering practice in Ethiopia: from public health and microbiological perspectives. BMC Public Health. 2012;12:707.
13. UNAIDS. Global AIDS Report Update 2016. Switzerland; 2016.
14. Lehohla P. Statistical release P0302
Mid-year population estimates, 2015. Pretoria, South Africa: Statistics South Africa, Africa SS; 2015.
15. Spearman CW, Sonderup MW. Preventing hepatitis B and hepatocellular carcinoma in South Africa: The case for a birth-dose vaccine. S Afr Med J. 2014;104(9):610-2.
16. King J, Hagemeister DT. Hepatitis B co-infection in HIV-infected patients receiving antiretroviral therapy at the TC Newman Anti Retroviral Treatment Clinic in Paarl, Western Cape2016.
17. Health NDo. National Consolidated Guidelines for the Prevention of mother-to-child transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults. Pretoria, South Africa: Department of Health; 2015.
18. Park JW, Kwak KM, Kim SE, Jang MK, Suk KT, Kim DJ, et al. Comparison of the long-term efficacy between entecavir and tenofovir in treatment- naive chronic hepatitis B patients. BMC gastroenterology. 2017;17(1):39.
19. Thompson SC, Boughton CR, Dore GJ. Blood-borne viruses and their survival in the environment: is public concern about community needlestick exposures justified? Aust N Z J Public Health. 2003;27(6):602-7.

20. van Bueren J, Simpson RA, Jacobs P, Cookson BD. Survival of human immunodeficiency virus in suspension and dried onto surfaces. J Clin Microbiol. 1994;32(2):571-4.
21. Arulogun OS, Adesoro MO. Potential risk of HIV transmission in barbering practice among professional barbers in Ibadan, Nigeria. Afr Health Sci. 2009;9(1):19-25.
22. Nath B, Kumari R, Midha T, Vaswani ND, Lekhwani S. Profession of barbering: Unexplored issues in HIV/AIDS. Clinical Epidemiology and Global Health. 2013;1(2):67-73.
23. Government WC. Provincial Gazette 7389. In: Gazette WCP, editor. Cape Town: Province of Western Cape; May 2015. p. 108.
24. Russell AD HW, Ayliffe GAJ. Principles and practices of disinfection, preservation and sterilization: Oxford: Blackwell Science; 1999 1999.
25. Weber DJ, Barbee SL, Sobsey MD, Rutala WA. The effect of blood on the antiviral activity of sodium hypochlorite, a phenolic, and a quaternary ammonium compound. Infect Control Hosp Epidemiol. 1999;20(12):821-7.
26. Omidbakhsh N, Sattar SA. Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. Am J Infect Control. 2006;34(5):251-7.

Blood and virus detection on barber clippers

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Background. Bleeding from the popular clean-shave '*chiskop*' haircut was recently reported as prevalent in South Africa (SA), a country with 6.9 million HIV-infected people.
Objectives. To investigate the prevalence of barber hair clipper contamination with blood and HIV and hepatitis B viruses.
Methods. Fifty barbers from three townships in Cape Town, SA, were invited to participate. One clipper from each barber was collected immediately after it had been used for a clean-shave haircut. Each clipper was rinsed with phosphate-buffered saline and then submerged in viral medium. The polymerase chain reaction (PCR) was used to identify the blood-specific RNA marker haemoglobin beta (HBB), hepatitis B virus (HBV) and HIV.
Results. The clean-shave haircut was the most common haircut requested by clients (78%). Of the clippers collected, 42% were positive for HBB, confirming detection of blood, none were positive for HIV, and 4 (8%) were positive for HBV. Two clippers (clippers 16 and 20) were positive on qualitative HBV PCR. HBV DNA from clipper 16 clustered with genotype A sequences from SA, India, Brazil and Martinique, while clipper 20 clustered with SA genotype D sequences. The clipper 20 sequence was identical to a subtype D sequence (GenBank accession AY233291) from Gauteng, SA.
Conclusion. This study confirms that there is significant contamination of barber hair clippers with blood and blood-borne viruses. Hepatitis B was detected with enough DNA copies to pose a risk of transmitting infection. Although HIV was not detected in this small study, the risk of transmission should be quantified. Further studies to investigate barber clipper sterilisation practices and whether the clean-shave hairstyle is an independent risk factor for HIV, HBV and hepatitis C virus infections are warranted. Public education on individual clipper ownership (as is the case with a toothbrush) should be advocated for clean-shave and blade-fade haircuts.

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Human scalp hair varies significantly in curvature, and individuals of African ancestry have hair with the tightest curl.[1,2] Population data suggest that hair follicles with this texture are prone to specific forms of alopecia (hair loss) that predominantly affect women (traction alopecia[3] and central centrifugal cicatricial alopecia[4]) and men (folliculitis keloidalis nuchea[4]).

Hairstyle trends for men have changed over the years, from the 'big afros' of the 1960s and 1970s to the 'blade-fade' haircut or German cut (originally worn by working class men in the 1920s and popular among Hitler's Wehrmacht soldiers in Germany, hence the name) that became popular in the 1990s and the current clean-shave or '*chiskop*' that became fashionable at the turn of the last century and is worn by 70% of black men in Cape Town townships.[4] The *chiskop* is worn by the majority of black men in South Africa (SA) and the African diaspora. Besides being a trend, this haircut is part of certain cultural rituals in various African and Indian (tonsure) tribes. The haircut is achieved either by using a razor blade or by pressing the metal shears of an electric clipper directly onto the scalp without using the manufacturer-supplied plastic stages (combs). This gives a clean-shave haircut similar to that achieved using a razor blade.

Shaving pimples, more commonly seen in the beard area, also occur on the scalp as transient papules and pustules. However, when these pimples evolve into permanent keloids on the back (nucheal) scalp they are characteristic of folliculitis keloidalis nuchea (FKN), also commonly and incorrectly called acne keloidalis nuchea, which has a prevalence of 10.5% in males and 0.1% females aged >18 years.[4] The prevalence of FKN was found to be highest in individuals whose hair had been cut with razors (10.7%), followed by clippers (5.9%), while no case was seen in those who used depilatory creams.[5]

A history of haircut-associated bleeding as a result of clean-shave haircuts was an unexpected finding in 32% of male participants from a population study[4] in which HIV status was unknown. This was later confirmed in a later study where a history of bleeding was reported in 24.8% of HIV-positive men.[5,6] Invisible bleeding was recently detected from scalp swabs after professional clean-shave haircuts (with no visible injury on the scalp when examined by a dermatologist) in 37% of participants using genetic testing for blood-specific RNA markers (albumin and haemoglobin beta (HBB)).[7] The potential transmission of blood-borne viruses such as hepatitis B (HBV) and HIV is most concerning. A study in Ethiopia reported that sharing shaving equipment in barber shops is common practice,[8] and an accidental scratch by sharp equipment in barber shops may create an opportunity for HIV and other blood-borne pathogens to enter the body.[8] Currently there is no study in the literature that has looked at informal barber practices in SA townships. The prevalence of contamination of haircut clippers or razors with blood and blood-borne viruses in our setting is unknown. Methods of sterilisation

or disinfection of barber instruments in our townships have been speculated on but not verified.

Objectives

The primary objective was to test clippers that had just been used for a haircut for blood and viruses. Our intention was to detect the prevalence of clipper contamination with blood, HIV and HBV, to ascertain whether barbers who mainly cut clean-shave *chiskop* haircuts caused more or less haircut-associated bleeding than barbers who did longer haircuts, i.e. at a distance from the scalp, and to investigate the cleaning methods used by barbers.

Methods

This was a prospective cross-sectional study. Aerial maps were used to sample 50 barber shops from three townships, the populations of which reflect the racial (apartheid) segregation of the previous SA government. Langa and Gugulethu townships have a population of predominantly black African ancestry and Bonteheuwel a population of predominantly mixed-race ancestry. The maps of Gugulethu and Langa, the two larger townships, were divided into four, then five barbers were selected from each section. The smaller Bonteheuwel township was divided into two, and five barbers were selected from each section. Basic demographic data were collected. Consenting barbers then performed one haircut, immediately after which they gave their clipper to the investigator; in exchange they received a new clipper. Methods used by the barbers to clean the clippers after use on each client were documented.

Each clipper was placed into a ziplock bag at room temperature, after which the bag was sealed and taken to a laboratory for virological sampling of the clipper. At the laboratory the clipper was submerged in a petri dish with 1.5 mL of viral medium, plugged into an electrical port and switched on to allow maximum wash through the teeth. The viral medium was then aspirated using a pipette and the residue was placed into a tube and stored at –80ºC until viral analysis.

Total nucleic acid was extracted from 800 µL of wash using the EasyMag system (bioMérieux, Netherlands). For HIV testing, qualitative nested reverse-transcription polymerase chain reaction (RT-PCR) was performed to detect HIV RNA present, as follows.

A 160 bp region of *gag* (nucleotide position 1494 - 1653 on the reference HIV HXB2 genome) was amplified by one-step RT-PCR using the SuperScript One-Step RT-PCR System with Platinum *Taq* (Invitrogen, Thermo Fisher Scientific, USA) in 50 µL final volume reaction with primers GAG A (forward, 5'-AGAGAACCAAGGGGAAGTGA-3') and GAG B (reverse, 5'-TCTCTAAAGGGTTCCTTTGG-3') each at 0.6 µM, and 10 µL nucleic acid eluate. Reverse transcription was carried out at 50°C for 30 minutes and PCR at 94°C for 2 minutes, followed by 40 cycles at 94°C for 25 seconds, 40°C for 30 seconds and 68°C for 45 seconds, with a final extension step of 68°C for 7 minutes. Nested PCR was performed using SuperTherm Taq DNA polymerase (Separation Scientific, SA) in a 50 µL final reaction volume with primers GAG C (forward, 5'-CATAGCAGGAACTACTAGTA-3') and GAG D (reverse, 5'-TCCTTGTCTTATGTCCAGAA-3'), each at 1 µM, with 2 µL prenested PCR product. PCR cycling conditions were the same as described above. PCR products were analysed under ultraviolet (UV) light after 2% agarose gel electrophoresis.[6,9]

For HBV testing, 600 µL of wash was tested with the quantitative Roche COBAS AmplPrep/COBAS TaqMan HBV Test, version 2 (Roche Diagnostics, Germany). Samples with detectable HBV DNA were then tested on a qualitative nested PCR[11] to obtain products for sequencing, as follows. A 189 bp region of the HBV *pre-S1* gene was amplified by nested PCR using SuperTherm *Taq* DNA polymerase (Separation Scientific) in a 50 µL final reaction volume. First-round primers were as follows: HBV1 (forward) TGGGAACAAGAKCTAC; HBV2 (reverse) GAACTGGAGCCACCAG; the final primer concentration was 0.4 µM. Nested PCR primers were as follows: HBV3 (forward) AATCCMGATTGGGACYTCAA; HBV4 (reverse) TCCTRACTGSCGATTGGT; final primer concentration 1 µM. Cycling conditions were identical for both rounds, and were as follows: 94°C for 2 minutes, followed by 35 cycles of 94°C for 20 seconds, 50°C for 30 seconds and 72°C for 45 seconds, with a final extension step of 72°C for 7 minutes. PCR products were analysed under UV light after 2% agarose gel electrophoresis. Both qualitative HIV and HBV PCRs were performed on the Applied Biosystems GeneAmp PCR System 9700 (Applied Biosystems, USA).

HBV PCR products obtained were sent to Inqaba Biotechnical Industries (SA) for bidirectional Sanger sequencing. Chromatograms were edited in FinchTV (Geospiza, USA). Sequences were aligned using ClustalX[12] and manipulated in BioEdit.[13] The maximum-likelihood tree was drawn from an alignment of 188 nucleotide positions in MEGA6[14] using the Tamura-Nei evolutionary model.[15] Bootstrap support was calculated with 1 000 replicates.

For comparison, HBV sequences representing most genotypes were selected from GenBank, including sequences from SA. The following sequences, with GenBank accession numbers listed by genotype, were chosen to represent the different genotypes: genotype A: AY576430, AY576434, GQ355536, GQ355565, GQ355572, GQ355575, HE974375, HE974376, HQ646554, HQ646556, JF784220, KC752150, KF476003, KF476015, KF476018 and X51970; genotype B: AB033554, AF100309 and D00329_JP; genotype C: AB644284, AB644286 and GQ184326; genotype D: AF280817, AY233291, AY233295, AY576433, EU594430, FJ904447, GQ184322, GQ205389, HE974377, JX898722, KC012652, KF170740 and KF476030; genotype E: AP007262, DQ060824 and HE974380; genotype F: AB116654 and AF223965; genotype G: AP007264 and GU565217; genotype H: AB298362 and AY090454; genotype I: FJ023674 and FJ882615. Orangutan HBV, AF193864, was used to root the tree.

For the blood-specific markers, hair clippers were rinsed with phosphate-buffered saline (PBS) and RNA was extracted from the PBS using the QIAgen RNeasy kit (Qiagen, SA). Briefly, total RNA was extracted according to the manufacturer's instructions. The eluted RNA was used for cDNA synthesis with a reverse-transcription enzyme (Fermentas, SA) and random hexamer primers. Conventional PCR was performed using primer sets for the blood-specific marker, with HBB RNA primer sequences as follows: (forward) CAC CTG GAC AAC CTC AAG; (reverse) AAT TCAC CCC ACC AGT GCA. PCR products were detected with a 3130xl Genetic Analyzer (Applied Biosystems). Briefly, a 1 µL aliquot of neat PCR product was added to 8 µL Hi-Di Formamide and 0.2 µL of GeneScan 500 Rox size standard (Applied Biosystems). The samples were heated to 95°C before being loaded onto the analyser. The conditions used for capillary electrophoresis were as follows: samples were injected through a 36 cm capillary filled with pop7 polymer, (ThermoFisher Scientific, USA) at a temperature of 60°C. Samples were injected for 3 seconds using an injection voltage of 1.2 kV and electrophoresed for ~20 minutes. The raw data were analysed using GeneMapper Software v4.0 (ThermoFisher Scientific).

Ethics clearance

Ethics approval for this study was obtained from the Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town (ref. no. 208/2013).

Results

All invited barbers agreed to participate, and a total of 50 clippers were collected. Twenty-one (42%) of all clippers were positive for the blood RNA marker HBB (5/20, 7/10 and 9/20 from townships 1, 2 and 3, respectively). Clippers from township 2 had a significantly higher prevalence of blood contamination at 72%, compared with 33% of those from townships 1 and 3 (p=0.023). Table 1 shows the characteristics of the study participants according to hairstyle.

No clippers were positive for HIV. Four clippers (8%) were positive for HBV. Two clippers (clippers 16 and 20) were positive on qualitative HBV PCR. HBV DNA from clipper 16 clustered with genotype A sequences from SA, India, Brazil, and Martinique, while clipper 20 clustered with SA genotype D sequences. The clipper 20 sequence was identical to a subtype D sequence (GenBank accession AY233291) from Gauteng, SA[15] (Fig. 1).

In the communities with a predominantly black African population, townships 1 and 3, the *chiskop* haircut was the style most commonly requested by clients. In township 2, the population of which is predominantly of mixed-race ancestry, no client requested a *chiskop*; instead 6 had a brush cut, 3 a blade-fade and 1 a German cut (the scalp margins are shaved with a blade in the blade-fade and with a clipper in the German cut; however, the result is the same). All the barbers cleaned the clippers after each client, but the cleaning agents varied. Most barbers (82%) used disinfection with methylated spirits after using a brush to remove hair, and 8% used an open flame. All the barbers who used an open flame to clean their clippers (8% of the total sample) were from township 2 and usually cut the blade-fade style (p=0.013).

Discussion

Four clippers were contaminated with HBV. The HBV sequences identified are in keeping with other sequences from SA, where the main hepatitis B genotypes are A and D.[15,16] The use of only 188 nucleotide positions for phylogenetic analysis limits the conclusions that can be reached, but is adequate to show that our sequences cluster with sequences obtained from our region by other researchers. We found concentrations of HBV DNA of between <20 and 45 IU/mL in clipper wash. Welzel *et al.*[17] calculated a ratio of 1 IU:10 DNA copies in their laboratory. The 2009 chronic hepatitis B practice guidelines[18] indicate a ratio of 1 IU:5 DNA copies. This amounts to approximately 300 DNA copies washed from clipper 16. Levels of HBV DNA in blood vary considerably, depending on the stage of infection, whether infection is acute or chronic, e-antigen positivity or negativity, and other factors. Viral loads are reported to reach as high as 10 $\log^{10}$ IU/mL serum/plasma,[19-22] and in our diagnostic laboratory we have seen occasional samples with an estimated viral load of up to 15 $\log^{10}$ IU/mL plasma. Viral loads (log copies/mL) of 4.3, 5.9, 6.2 and 5.2 have been reported in urine, saliva, tears and sweat, respectively.[23] Hepatitis B transmission has been linked to body fluids other than blood,[23,24] as well as to the sharing of toothbrushes and razors.[25] The risk of transmission between healthcare workers and patients has been well documented.[24,26] There is therefore a risk of clipper contamination from minor bleeding on the scalp. The infectious dose of hepatitis B has been estimated at 10 - 100 viruses. A study in chimpanzees determined the ID50 of HBV (the minimum infectious dose of HBV, or the dose at which 50% of chimpanzees would be infected[27]) in five chimpanzees to be ~10 DNA copies for both genotypes A and C.[27] In a subsequent study, the same team found that a dose of 2.6 - 4.6 DNA copies from pre-acute-phase chimpanzee serum caused infection in 3/3 chimeric mice with transplanted human hepatocytes, while a dose of 200 - 350 DNA copies from late acute-phase serum caused infection in only 1/3 mice,[28] suggesting that virus during early acute infection is more able

Table 1. Characteristics of study participants according to hairstyle

Characteristic	Total, *N* (%)	Clean-shave haircut barbers, *n* (%)	Other haircut barbers, *n* (%)	*p*-value*
Site				<0.0001
Township 1 (Langa)	20 (40.0)	19 (48.7)	1 (9.1)	
Township 2 (Bonteheuwel)	10 (20.0)	0	10† (90.9)	
Township 3 (Gugulethu)	20 (40.0)	20 (51.3)	0	
Total	50	39	11	
Age group of clients serviced (years)				0.317
5 - 17	6 (12.0)	6 (15.4)	0	
18 - 60	44 (88.0)	33 (84.6)	11 (100)	
Total	50	39	11	
Cleaning methods				0.013
Brush and spirit	41 (82.0)	35 (89.8)	6 (54.5)	
Spirit only	5 (10.0)	3 (7.7)	2 (18.2)	
Open flame	4 (8.0)	1 (2.5)	3 (27.3)	
Total	50	39	11	
Hepatitis B virus				0.643
Positive	4 (8.0)	3 (7.7)	1 (9.1)	
Negative	46 (92.0)	36 (92.3)	10 (90.9)	
Total	50	39	11	
Blood marker (HBB)				0.023
Positive	21 (42.0)	13 (33.3)	8 (72.7)	
Negative	29 (58.0)	26 (66.7)	3 (27.3)	
Total	50	39	11	

HBB = haemoglobin beta.
*p-value based on χ^2 test of association for difference between clean-shave and other hairstyles.
†6 brush-cut, 3 blade-fade, 1 German cut.

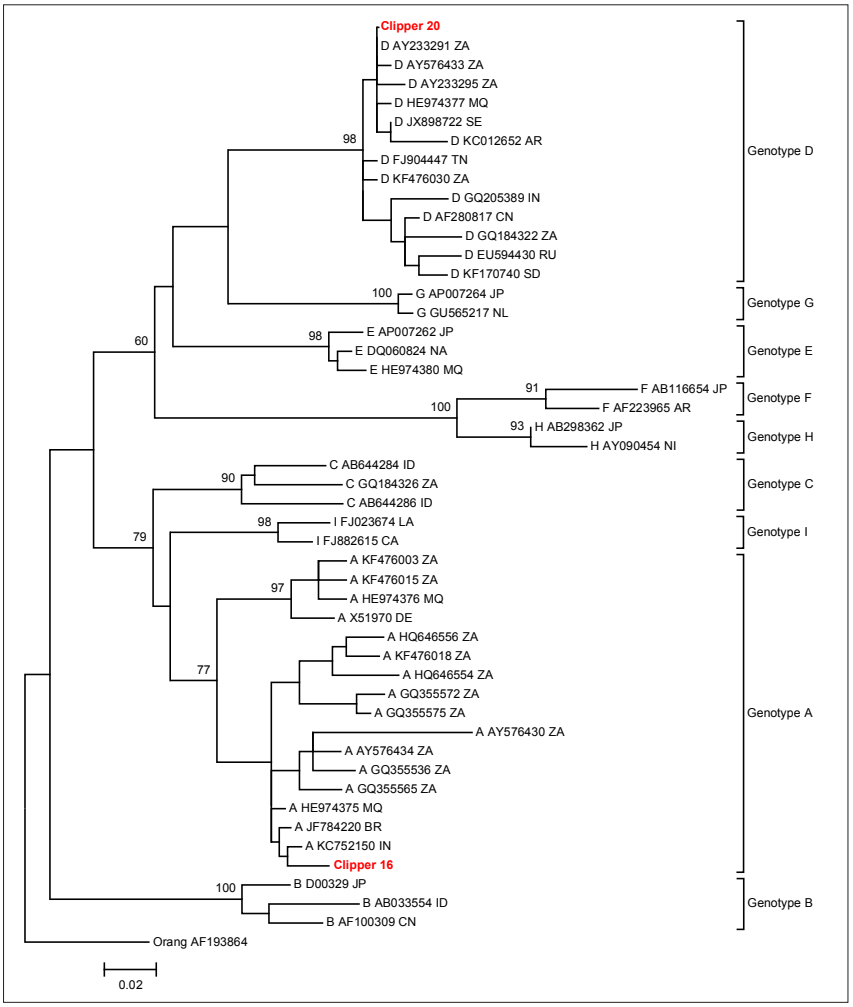

Fig. 1. A maximum-likelihood tree constructed in MEGA6 using an alignment of 188 nucleotide sequences. Selected bootstrap support above 60% is shown. Study samples are shown in red. The tree is rooted on an orangutan HBV sequence. Sequences for comparison have names starting with genotype, followed by Genbank accession number, ending with country ISO 3166-1 abbreviation (AR = Argentina; BR = Brazil; CA = Canada; CN = China; DE = Germany; ID = Indonesia; IN = India; LA = Laos; MQ = Martinique; NA = Namibia; NI = Nicaragua; RU = Russia; SD = Sudan; SE = Sweden; TN = Tunisia; ZA = South Africa; scale bar = number of substitutions per site.)

to initiate a new infection when transmitted, possibly because in later infection virus in serum may be bound to antibodies.

Commercial methylated spirit contains ethyl alcohol (95%) and methyl alcohol (5%), and our findings indicate that it is commonly used to decontaminate barber equipment. Ethyl alcohol and methyl alcohol are not virucidal. The use of open flames for sterilisation has been reported from African countries,[29] but there are no data on efficacy or adequate exposure time. UV light is reported to kill bacteria and viruses effectively.[30] Application of UV radiation in biological safety cabinets destroys organisms on the surface of instruments. This microbicidal activity is highly dependent on the wavelength of UV radiation. Modern UV light sterilisation units as well as specific antiviral sprays are used internationally in hair salons. Quaternary ammonium disinfectant cleaners have virucidal activity effective enough to kill herpes simplex virus (HSV) in the presence of blood.[31] HSV has similar properties to HIV, and quaternary ammonium disinfectants could therefore be used to decontaminate surfaces or tools with blood spills.[31] The new generation of accelerated hydrogen peroxide-based environmental surface disinfectants are bactericidal, virucidal, mycobactericidal and fungicidal.[32] The formulation is safe to use and has a high compatibility profile for various materials in addition to being a fast-acting intermediate-level disinfectant.[32] In the USA, disinfectant must be approved by the Environmental Protection Agency (EPA), which certifies the efficacy of products for infection control. The SA counterpart is the South African Bureau of Standards (SABS). Barbers are encouraged to use EPA- or SABS-approved disinfectants for their equipment.

In our study, the low levels of HBV DNA copies on the clippers represent a possible public healthcare risk. We are not aware of cases of HBV or HIV infection resulting from clipper use in our setting. We are unable to identify when each clipper became contaminated with virus, and we do not know how virus levels on clippers decline as the clippers move through hair when they are used on subsequent clients. It is unlikely that the HBV levels detected on the clippers would pose a risk to a client, but closer to the time of the source contamination there may have been levels that did pose such a risk. We are also unable to determine whether the viral DNA levels detected represent infectious virus, or damaged virus no longer able to cause infection. HIV viral loads are typically lower than HBV viral loads, and with similar clipper use this may explain why we did not detect HIV RNA on any clippers. However, closer in time to the source contamination, clippers may have had levels of infectious HIV that could have posed a risk to clients.

The results show that clippers used to mostly cut the longer blade-fade hairstyle were associated with more bleeding markers, although one would expect that the clippers used to cut the clean-shave *chiskop* would have had more blood markers as a result of the close contact of the blade with the scalp. Our results showed that 72% of the clippers used to cut the longer blade-shade were positive for HBB, compared with 33% of the clippers used for the *chiskop* cut. One plausible explanation could be the small sample size in township 2. There was a statistically significant difference in cleaning methods used by the barbers cutting the *chiskop* and blade-fade hairstyles. Of the barbers using clippers to cut the blade-fade, only 54% used a brush and spirit, while 27% used other methods such as open flames to clean clippers.

While it has been reported that an open flame is used to clean clippers in other African countries, there are no data on the efficacy of this method for disinfection or sterilisation.[29] Many developing countries lack regulating bodies that monitor health practices at barber shops. In developed countries, activities of barbers are regulated through comprehensive training, licensing and monitoring programmes.[8] Lack of knowledge about correct methods of sterilisation and disinfection may be directly

associated with the lack of formal trade training of the barbers. Barbers' level of education may be a contributory factor.

Study limitations

The hepatitis B and HIV status of clients was unknown. There are some confounding factors that could have contributed to our results, such as the skill of the barbers in the different townships and the methods they used to cut the hairstyles. The small sample size of township 2 could also have skewed our results. The amount of time spent cleaning the clippers with the different methods was not measured. This is also a possible confounding factor and could have contributed to the results. A study with a bigger sample size could obtain results of better quality.

Conclusions

This study confirmed that there is significant contamination of barber hair clippers with blood and blood-borne viruses. Hepatitis B was detected with enough DNA copies to pose a risk of infection. Although HIV was not detected, the clinical significance of contamination of clippers with blood, especially with regard to the transmission of blood-borne infections, warrants further study. Further scientific scrutiny is required to quantify infection risk and investigate whether the clean-shave hairstyle is an independent risk factor for HIV, HBV and hepatitis C virus (HCV) infections as well as HIV/HBV and HIV/HCV co-infections. Finally, barber hygiene practices may require upscaling and access to sterilisation facilities. In the meantime, public education recommending individual clipper ownership for close-shave *chiskop* and blade-fade haircuts should be advocated.

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1. Khumalo NP, Doe PT, Dawber RP, Ferguson DJ. What is normal black African hair? A light and scanning electron-microscopic study. J Am Acad Dermatol 2000;43(5 Pt 1):814-820. https://doi.org/10.1067/mjd.2000.107958
2. Loussouarn G, Garcel AL, Lozano I, et al. Worldwide diversity of hair curliness: A new method of assessment. Int J Dermatol 2007;46(Suppl 1):2-6. https://doi.org/10.1111/j.1365-4632.2007.03453.x
3. Khumalo NP, Jessop S, Gumedze F, Ehrlich R. Hairdressing is associated with scalp disease in African schoolchildren. Br J Dermatol 2007;157(1):106-110. https://doi.org/10.1111/j.1365-2133.2007.07987.x
4. Khumalo NP, Jessop S, Gumedze F, Ehrlich R. Hairdressing and the prevalence of scalp disease in African adults. Br J Dermatol 2007;157(5):981-988. https://doi.org/10.1111/j.1365-2133.2007.08146.x
5. Khumalo NP, Gumedze F, Lehloenya R. Folliculitis keloidalis nuchae is associated with the risk for bleeding from haircuts. Int J Dermatol 2011;50(10):1212-1216. https://doi.org/10.1111/j.1365-4632.2010.04655.x
6. Khumalo NP, Gantsho N, Gumedze F, Mthebe T. Health risks of the clean-shave chiskop haircut. S Afr Med J 2013;103(7):489-490. https://doi.org/10.7196/SAMJ.6675
7. Khumalo NP, Muthukarapan C, Hardy D, et al. Invisible bleeding from clean-shave haircuts, detection with blood specific RNA markers. Dermatology 2013;227(3):197-201. https://doi.org/10.1159/000353529
8. Biadgelegn F, Belyhun Y, Anagaw B, et al. Potential risk of HIV transmission in barbering practice in Ethiopia: From public health and microbiological perspectives. BMC Public Health 2012;12:707. https://doi.org/10.1186/1471-2458-12-707
9. Engelbrecht S, van Rensburg EJ. Detection of southern African human immunodeficiency virus type 1 subtypes by polymerase chain reaction: Evaluation of different primer pairs and conditions. J Virol Methods 1995;55(3):391-400. https://doi.org/10.1016/0166-0934(95)00088-7
10. Hardie DR, Kannemeyer J, Stannard LM. DNA single strand conformation polymorphism identifies five defined strains of hepatitis B virus (HBV) during an outbreak of HBV infection in an oncology unit. J Med Virol 1996;49(1):49-54. https://doi.org/10.1002/(SICI)1096-9071(199605)49:1<49::AID-JMV8>3.0.CO;2-K
11. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22(22):4673-4680. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC308517/ (accessed 12 March 2018).
12. Hall TA. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 1999:41:95-98. http://brownlab.mbio.ncsu.edu/JWB/papers/1999Hall1.pdf (accessed 2 March 2018).
13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013;30(12):2725-2729. https://doi.org/10.1093/molbev/mst197
14. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 1993;10(3):512-526. https://doi.org/10.1093/oxfordjournals.molbev.a040023
15. Kimbi GC, Kramvis A, Kew MC. Distinctive sequence characteristics of subgenotype A1 isolates of hepatitis B virus from South Africa. J Gen Virol 2004;85(Pt 5):1211-1220. https://doi.org/10.1099/vir.0.19749-0
16. Andersson MI, Maponga TG, Ijaz S, et al. The epidemiology of hepatitis B virus infection in HIV-infected and HIV-uninfected pregnant women in the Western Cape, South Africa. Vaccine 2013;31(47):5579-5584. https://doi.org/10.1016/j.vaccine.2013.08.028
17. Welzel TM, Miley WJ, Parks TL, Goedert JJ, Whitby D, Ortiz-Conde BA. Real-time PCR assay for detection and quantification of hepatitis B virus genotypes A to G. J Clin Microbiol 2006;44(9):3325-3333. https://doi.org/10.1128/JCM.00024-06
18. Lok AS, McMahon BJ. Chronic hepatitis B: Update 2009. Hepatology 2009;50(3):661-662. https://doi.org/10.1002/hep.23190
19. Ciupe SM, Ribeiro RM, Nelson PW, Perelson AS. Modeling the mechanisms of acute hepatitis B virus infection. J Theor Biol 2007;247(1):23-35. https://doi.org/10.1016/j.jtbi.2007.02.017
20. Ganem D, Prince AM. Hepatitis B virus infection – natural history and clinical consequences. N Engl J Med 2004;350(11):1118-1129. https://doi.org/10.1056/NEJMra031087
21. Ribeiro RM, Lo A, Perelson AS. Dynamics of hepatitis B virus infection. Microbes Infect 2002;4(8):829-835. https://doi.org/S1286457902016039
22. Whalley SA, Murray JM, Brown D, et al. Kinetics of acute hepatitis B virus infection in humans. J Exp Med 2001;193(7):847-854. https://doi.org/10.1084/jem.193.7.847
23. Komatsu H, Inui A, Sogo T, Tateno A, Shimokawa R, Fujisawa T. Tears from children with chronic hepatitis B virus (HBV) infection are infectious vehicles of HBV transmission: Experimental transmission of HBV by tears, using mice with chimeric human livers. J Infect Dis 2012;206(4):478-485. https://doi.org/10.1093/infdis/jis293
24. Hu DJ, Kane MA, Heymann DL. Transmission of HIV, hepatitis B virus, and other bloodborne pathogens in health care settings: A review of risk factors and guidelines for prevention. Bull World Health Organ 1991;69(5):623-630. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2393247/ (accessed 12 March 2018).
25. Goh KT, Ding JL, Monteiro EH, Oon CJ. Hepatitis B infection in households of acute cases. J Epidemiol Community Health 1985;39(2):123-128. https://doi.org/10.1136/jech.39.2.123
26. Corden S, Ballard AL, Ijaz S, et al. HBV DNA levels and transmission of hepatitis B by health care workers. J Clin Virol 2003;27(1):52-58. https://doi.org/S1386653202001270
27. Komiya Y, Katayama K, Yugi H, et al. Minimum infectious dose of hepatitis B virus in chimpanzees and difference in the dynamics of viremia between genotype A and genotype C. Transfusion 2008;48(2):286-294. https://doi.org/10.1111/j.1537-2995.2007.01522.x
28. Tabuchi A, Tanaka J, Katayama K, et al. Titration of hepatitis B virus infectivity in the sera of pre-acute and late acute phases of HBV infection: Transmission experiments to chimeric mice with human liver repopulated hepatocytes. J Med Virol 2008;80(12):2064-2068. https://doi.org/10.1002/jmv.21320
29. Arulogun OS, Adesoro MO. Potential risk of HIV transmission in barbering practice among professional barbers in Ibadan, Nigeria. Afr Health Sci 2009;9(1):19-25. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2932524/ (accessed 12 March 2018).
30. Russell AD, Hugo WB, Ayliffe GAJ. Principles and Practices of Disinfection, Preservation and Sterilization. Oxford: Blackwell Science, 1999.
31. Weber DJ, Barbee SL, Sobsey MD, Rutala WA. The effect of blood on the antiviral activity of sodium hypochlorite, a phenolic, and a quaternary ammonium compound. Infect Control Hosp Epidemiol 1999;20(12):821-827. https://doi.org/10.1086/501591
32. Omidbakhsh N, Sattar SA. Broad-spectrum microbicidal activity, toxicologic assessment, and materials compatibility of a new generation of accelerated hydrogen peroxide-based environmental surface disinfectant. Am J Infect Control 2006;34(5):251-257. https://doi.org/10.1016/j.ajic.2005.06.002

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