Colonisation with ESKAPE organisms and Candida auris among primary caregivers and healthcare workers in a neonatal unit at a public sector tertiary South African hospital.

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Sampling as part of the study: *Microbiological surveillance of hospital environmental surfaces, medical equipment, healthcare workers, patients and patients' parents in a tertiary hospital in Soweto, South Africa.* (Ethics clearance certificate number: M19112)

Declaration

I, Nicki Rees, declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the branch of Clinical Microbiology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

……26th…day of ……October…….2022…….in……Johannesburg, South Africa

Abstract

Background: Nosocomial neonatal infection remains a significant cause of mortality and morbidity, particularly in the high care and intensive care settings. Among implicated pathogens ESKAPE- C organisms are considered particularly worrisome due to their virulence, ability to gain resistance and propensity to affect multiple sites. Transmission to neonates is postulated to occur through contact with colonised adults.

Objective: This study aims to describe the prevalence of colonisation of both primary caregivers and healthcare workers in contact with admitted neonates. As a secondary objective this study aims to identify the most common resistance patterns in ESKAPE-C organisms isolated from primary caregivers and healthcare workers in a neonatal unit. The overall aim of the study is to provide insight into how best to prevent hospital acquired infections in this group.

Methods: This cross-sectional prevalence study describes colonisation of healthcare workers (HCW) and primary caregivers in a neonatal unit in a tertiary South African hospital. Over one week in August 2021, twenty-five primary caregivers and twenty-nine healthcare workers submitted specimens which were processed for the identification of ESKAPE-C organisms. Susceptibility was performed on identified organisms.

Results: Of the healthcare worker participants 13,8% (4/29) were shown to be colonised with one or more ESKAPE-C pathogen, while 52% (13/25) of primary caregivers were shown to be colonised with one or more ESKAPE-C pathogens. Of the *S. aureus* organisms isolated 28,6% were MRSA, of the *A. baumannii* organisms isolated 66.7% were XDR and of the Enterobacteriaceae isolated 60% were ESBL producing. No CRE or VRE organisms were isolated in this study.

Conclusion: This study demonstrates that the prevalence of colonisation of healthcare workers and primary caregivers is significant and reinforces the need for stringent infection prevention and control strategies to prevent transmission to vulnerable neonates.

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Nomenclature and abbreviations

ESKAPE-C- *Enterobacter cloacae, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterococcus faecium and Candida auris*

Neonate- for the purposes of this study a neonate is defined as a newborn infant with no specific time frame applied (i.e., children admitted to the neonatal areas are defined as neonates regardless of their specific age)

NICU- Neonatal intensive care unit

TICU-Transitional intensive care unit

HCW- Healthcare worker-any person involved in providing healthcare directly or indirectly

XDR- Extensively drug resistant

MDR -Multidrug resistant

MDRO-Multidrug resistant organism

MRSA- Methicillin resistant *Staphylococcus aureus*

MSSA-Methicillin susceptible *Staphylococcus aureus*

MC&S- Microscopy culture and susceptibility testing

NHLS- National Health Laboratory Service

ESBL-Extended spectrum beta-lactamase

CRE-Carbapenem resistant Enterobacteriaceae

VRE-Vancomycin resistant Enterococci

PCR- Polymerase chain reaction

WHO-World Health Organization

HAI –Hospital acquired infection

UK-United Kingdom

CHBAH- Chris Hani Baragwanath Academic Hospital

VIDA- The Vaccines and Infectious Diseases Analytics

HPCSA-Heath Professions council of South Africa

BHI- Brain heart infusion broth

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Background

Hospital acquired infections (HAI) present a major diagnostic and therapeutic challenge in modern medicine. This is particularly true among the most vulnerable populations. Hospitalised neonates represent one of the most challenging of these populations because their immunocompromised state is often multifactorial. This population may be vulnerable due to their immature immune systems, co-existent prematurity, maternal comorbidities and long hospital stays[1,2]. Other contributing factors include increased need for invasive procedures and necessary frequent close contact with other persons[2]. Diagnosis of infection in neonates is challenging due to the difficulty of specimen collection (resulting in frequent contamination and insufficient quantity of specimen), unreliability of biomarkers in this age group and variability and non-specific nature of presenting features[3].

Not only is this population prone to nosocomial infection but management of these infections is difficult when it does occur. Many frequently used antibiotics are contraindicated or have not been adequately studied in neonates and others are known to cause serious side effects in this population group[3]. Negative outcomes due to nosocomial sepsis in neonates include increased mortality, neurodevelopmental delay, exposure to antibiotics with possible long-term sequelae and transmission of pathogens within the neonatal unit and beyond. Treatment of these infections presents a massive economic burden to both caregivers and hospitals[4].

‘The South African government seeks to reduce neonatal sepsis rates by 84% nationally by 2025’[5]; this reduction can only be achieved with ongoing surveillance, research, and innovation in infection prevention and control in this population.

ESKAPE organisms namely, *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species with the recent addition of *Candida auris* are the most common aetiological agents of hospital acquired infections worldwide [4]. These organisms are noted for their tendency to acquire antimicrobial resistance mechanisms, their virulence, and their ability to affect a wide variety of human sites. These organisms may survive in the environment or on the hands of caregivers or healthcare workers and may be spread via direct or fomite contact transmission in this manner [1].

As this type of infection presents a significant diagnostic and therapeutic challenge and the consequences of these infections are often severe, preventative strategies may be the preferred means of addressing this challenge. Some sources have cited hand washing as the ‘single most important intervention in interrupting the transmission of microorganisms’ [6] and therefore a vital component of neonatal hospital acquired infection prevention and control. It is important to note that hand hygiene is often emphasised with healthcare workers but may be somewhat neglected with regards to caregivers and visitors. Even where hand hygiene is emphasised, adherence to these strategies may be low[6].

In the setting of neonatal care areas, primary caregivers are exposed to the hospital environment as much as their babies because they are often required to stay at the hospital for the duration of their neonate’s admission. Primary caregivers’ prolonged presence in the hospital may be enough to establish colonisation of potential pathogens particularly ESKAPE-C organisms. This possible colonisation may lead to colonisation or infection of vulnerable neonates.

1.2 Literature review

The prevalence of colonisation with nosocomial pathogens in healthcare workers has been investigated with regards to methicillin-resistant *Staphylococcus aureus* (MRSA). Dulon et al. conducted a literature review of the MRSA carriage in healthcare workers in Europe and the United Kingdom (UK) in 2014 and found a pooled colonisation rate of 1,8%[7]. Hogan et al. conducted a cross-sectional study in 2016 in which they investigated nasal carriage of MRSA in Madagascan healthcare workers and students and found an 11% carriage rate of *S. aureus*[8]. In a UK study in 2018, 134 asymptomatic healthcare workers screening for MRSA carriage took place by obtaining swabs from their nares, throat, axillae and any wounds or skin rashes present, a carriage prevalence of 3.7% was found[9]. There is a paucity of data regarding the prevalence of carriage of methicillin-susceptible *S. aureus*(MSSA) in healthcare workers.

Rectovaginal *S. aureus* colonisation among mothers of neonates has been investigated. A large retrospective cohort study was performed in 2009 at the New York-Presbyterian Hospital/Columbia University Medical Center (NYP/CUMC). In this study, 13% and 0,7% of participants screened for rectovaginal colonisation were positive for MSSA and MRSA respectively[10].

Candida species hand carriage in hospital personnel was described by Yildirim et al. in 2007, this study found an overall carriage prevalence of 34.1%[11]. A colonisation prevalence of 6% was found in Kuwait in 2000 among 45 nurses who underwent oropharyngeal, and hand swabbing, however, no *C. auris* was isolated in this study[12]. A study conducted in New Delhi in 2019 found that 20 of 60 healthcare workers sampled (hands, aprons and stethoscope swabs) cultured a fungus, all three of the cultured Candida species in this study were cultured from the hands[13]. Transmission of Candida species from healthcare worker's hands to a neonate was demonstrated in a study conducted in Italy in 2001, in this study *C. parapsilosis* found on the

hands of two healthcare workers was found to be genetically indistinguishable from *C.parapsilosis* causing invasive infection in a neonate despite evidence that the neonate was not colonised with Candida species on admission[14].Overall there is a lack of data regarding epidemiology of Candida colonisation on the hands of healthcare workers and primary caregivers particularly with regards to the emerging pathogen *C. auris*

A systematic review conducted by Montoya et al. in 2016 aimed to determine the prevalence of multidrug-resistant organism (MDRO) colonisation on the hands of healthcare workers, 59 articles were reviewed. Two of the articles reviewed were African studies (one in Ethiopia and one in Egypt), both of which were cross sectional. The main finding of this systematic review was that the prevalence of common MDROs varies greatly by 'pathogen, care setting, culture acquisition method, study design and geography'[15]. This study emphasizes the need for African and more specifically South African studies aimed at determining the prevalence of colonisation with pathogenic organisms in healthcare workers.

Bulabula et al. described the studies done in Africa regarding colonisation of mothers of neonates with multidrug resistant Gram-negative bacilli in their 2020 systematic review and meta-analysis. In this study the overall prevalence of Extended Spectrum Beta-lactamase (ESBL) producing Enterobacteriaceae among pregnant and post-partum women was 17%[16]. This important review reinforces the concept that 'Maternal colonisation has been shown to be an important risk factor for neonatal colonisation and subsequent neonatal infection'[16] and contributes to the pool of knowledge regarding maternal colonisation in the African context. No studies exist either globally or in South Africa that determine the prevalence of colonisation of healthcare workers as well as primary caregivers of all seven ESKAPE-C pathogens.

1.3 Study Aim and Objectives

This study aims to describe the prevalence of colonisation of both primary caregivers and healthcare workers in contact with admitted neonates and in so doing provide insight into how best to prevent hospital acquired infections in this group.

Primary objectives:

1) To determine the prevalence of colonisation with ESKAPE-C organisms in primary caregivers in a neonatal unit in a tertiary South African hospital.
2) To determine the prevalence of ESKAPE-C organisms present on the hands of healthcare workers in a neonatal unit in a tertiary South African hospital.

Secondary objective:

3) To identify the most common resistance patterns in ESKAPE-C organisms isolated from primary caregivers and healthcare workers in a neonatal unit.

CHAPTER 2

METHODOLOGY

2.1 Research Questions

1.What is the prevalence of colonisation among healthcare workers with ESKAPE-C organisms in neonatal areas at a tertiary hospital in Johannesburg, South Africa?

2.What is the prevalence of colonisation among primary caregivers of admitted neonates with ESKAPE-C organisms at a tertiary hospital in Johannesburg, South Africa?

3.What are the common resistance patterns in ESKAPE-C organisms isolated from primary caregivers and healthcare workers at a tertiary hospital in Johannesburg, South Africa?

2.2 Research Design

This is a cross-sectional study that was conducted in the neonatal unit at Chris Hani Baragwanath Academic Hospital (CHBAH) including the neonatal intensive care unit (NICU) and the transitional intensive care unit (TICU).

2.3 Research Setting

CHBAH is the third largest hospital in the world and services roughly 60,000 patients per year in the Maternity unit alone. Approximately 17000 deliveries take place in the unit yearly of which 3500 are classified as low birth weight.[17] The Hospital is a teaching hospital for the University of Witwatersrand and therefore undergraduate and post-graduate students form part of the retinue of staff in the hospital. The neonatal intensive care unit at CHBAH comprises

two functional units occupying one area: the TICU (effectively a high-care unit) made up of 18 beds and the NICU made up of 12 beds. Hospital personnel rotate between the areas with doctors often seeing patients in both areas on a single day. Other healthcare workers including radiographers, physiotherapists and dieticians service this area as well as other areas in the hospital and personnel traffic in the unit is high out of necessity. The bed occupancy rates are generally high: 'On average, the bed occupancy rate is 95% for the beds used for mechanical ventilation and 180 - 200% for the rest of the NICU'[18]. This is due to the large population serviced by this hospital (Soweto and surrounds). Unavoidably high occupancy rates, infrastructure difficulties and the fact that the admitted patients are often critically ill mean that infection prevention and control are of paramount importance.

2.4 Inclusion and Exclusion Criteria

All healthcare workers and primary caregivers present at the time of sampling were asked to participate on a voluntary basis. Only those that did not wish to participate and primary caregivers younger than 18 years were excluded. Potential participants who were unable to provide consent were to be excluded but this population was not encountered. Primary caregiver participants were not excluded if they were unable to submit all three requested swabs (hand swabs, vaginal swabs and rectal swabs). Participants could choose to submit any combination of swabs based on their level of comfort. Healthcare workers were excluded only if they declined to participate. Participants were not asked to collect a vaginal specimen if they have vaginal bleeding, pain, or discomfort at the time of collection.

2.5 Materials and Methods

Samples were collected over a one-week period to allow adequate time for laboratory processing to take place. Collection took place in August 2021 and sample processing continued for three weeks after sampling. Each participant was interviewed by either a microbiology registrar or a trained Wits-VIDA nurse prior to specimen collection. All caregivers and healthcare workers present at the time of sampling were asked to participate. Primary caregivers were asked to submit hand, rectal and vaginal swabs while healthcare workers were asked to submit hand swabs only.

Research staff consisted of two Clinical microbiology registrars and two trained professional nurses as well as two microbiology technologists (who performed processing only), all of whom were registered with the Health Professionals' Council of South Africa (HPCSA). Potential primary caregiver participants were shown a video produced by the research team explaining the purpose of the research and the methods for swab collection (video available upon request from author). Participants were also provided with a written information sheet and verbal explanation by the research team. The information sheet was in English, where possible the research team explained the project in the language of the participants' choosing.

Participants were counselled, and written consent was obtained prior to sampling. Participants were free to withdraw participation at any point before, during or after sample collection and processing, and were provided with contact details in order to do this should they wish to. Participants were informed that participation was voluntary and anonymous. Questions and concerns of participants were addressed verbally by research staff. Participants were informed that participation had no impact on future treatment that they, or their family receive, and that no disease state would be diagnosed or treated by this research. Participants were informed that

organisms isolated may be stored for future studies but that their anonymity would be protected at all times.

2.5.1. Methods for specimen collection of hand swabs of primary caregivers and healthcare workers:

During collection, research staff donned appropriate Personal Protective Equipment: non-sterile gloves and plastic aprons, which were changed between participants. Research staff took all reasonable precautions to avoid contact with environmental hazards in the ward such as areas contaminated with biohazardous waste or sharp objects. Appropriate Personal Protective Equipment was worn by those processing samples including non-sterile gloves, goggles/visor, and mask as well a clean lab coat at all times while in the laboratory. All processing took place in a National Health Laboratory Service (NHLS) laboratory with appropriate safety measures in place, including a designated health and safety officer. Pre-labelled sterile swabs were inspected by the collecting microbiology registrar or professional nurse prior to sample collection for intact packaging, correct labelling and suitability to task.

Hand swabs were collected prior to vaginal and rectal swabs. Participants were not asked to wash hands prior to collection but were not excluded if hand washing had recently taken place. Primary caregivers were asked to hold out both hands in front of them palms facing upward while the sampler swabbed the palmer surface of both hands with the same swab including the web spaces and palmer surface of the thumb for approximately ten seconds. The participant was then asked to turn their hand over and the dorsal surface of the hand including the web spaces and dorsal surface of the thumb were swabbed for approximately ten seconds. Participants were not asked to remove any jewellery if present, but care was taken to swab the jewellery surface and skin under the jewellery if present. The swab was then placed into Amies transport medium (Delta lab, Spain) immediately after collection. Each participant's swabs

were placed in individual sealable plastic bags along with a working card before being placed in transport container. Swabs were transported in sturdy, intact cooling containers containing ice blocks on the same day as collection to the laboratory for processing. Care was taken to avoid contamination of the swab with environmental material during sample collection and transport.

2.5.2. Vaginal swab self-collection technique instructions given to each primary caregiver participant:

To take place in a private comfortable place. No need to shower/bathe prior to collection. Wash hands with soap and water prior to collection Open swab package but do not touch the cotton side of the swab or put it down. Undress from the waist down and get into a comfortable position (leg up on toilet or squatting). Insert the swab into the vagina about five centimetres from the opening (to the mark). Rotate the swab for ten seconds. Remove the swab Insert the swab into the gel and close the lid tightly

2.5.3. Rectal swab self-collection instructions given to each primary caregiver participant:

To take place in a private comfortable place No need to shower/bathe prior to collection. Wash hands with soap and water prior to collection. Undress from the waist down and get into a comfortable position (leg up on toilet or squatting). Open swab package but do not touch the cotton side of the swab or put it down. Insert the swab into the anus two to three centimetres from the opening (to the mark). Rotate swab for ten seconds. Remove the swab. Insert the swab into the gel and close the lid tightly

2.5.4. Culture identification and Antimicrobial susceptibility testing

Swabs were processed on the day that they were received. Each swab was placed in an individual Brain heart infusion (BHI) broth medium (Diagnostic media product, South Africa) and labelled with a specimen number as soon as it was received in the laboratory. Swabs were placed in BHI by breaking at the pre-scored area of the swab or by using clean flamed forceps and scissors if breaking failed. The BHI broth was incubated in ambient air at 35℃ (+/- 2℃) for 48 hours. The BHI was inspected for visible turbidity at twenty-four and forty-eight hours.

BHI that was turbid at either 24 or 48 hours was then plated out onto MacConkey agar (Diagnostic media product, South Africa), 5% Sheep Blood agar (Diagnostic media products, South Africa), Sabouraud-dextrose agar (Diagnostic media products, South Africa) and CHROMagar Staph aureus (Media-Mage, South Africa). Turbid broth was mixed using a vortex for thirty seconds prior to inoculation. Plating took place using ten microliter wire loops (Lasec, South Africa) with a flaming step between streaks to achieve single colonies. BHI broth that remained clear was recorded on the working card as 'no growth on BHI' and no further processing was required. Plates were examined at twenty-four and forty-eight hours for growth of colonies suggestive of ESKAPE-C organisms based on morphology and simple biochemical tests as per the standard operating procedure (see appendix E and F). If colonies on Sabouraud-dextrose agar (Diagnostic media products, South Africa) could not definitively be distinguished a Gram stain was performed and if a yeast like organism was observed further identification was carried out. If colonies were too mixed to perform identification on primary plates the organism of interest was plated out and incubated overnight for pure growth.

2.5.5. Morphological features of target organisms:

Enterococcus faecium: small off-white to grey round colonies with α-haemolysis or γ-haemolysis on 5% Sheep Blood agar (Diagnostic media product, South Africa).

Staphylococcus aureus: Pink to mauve colonies on CHROMagar Staph aureus

Klebsiella pneumoniae: Lactose fermenting on MacConkey agar), raised circular two to three millimetre colonies with entire margins, often mucoid. Indole negative.

Acinetobacter baumannii: Small one to two millimetre round convex colonies with an entire margin that are non-lactose fermenting but light pink to salmon in colour and opaque on MacConkey agar ().

Pseudomonas aeruginosa: non-lactose fermenting on MacConkey agar flat spreading two to three millimetre colonies with a wrinkled moist surface and a metallic sheen, oxidase positive.

Enterobacter species: Grey to off-white spreading round colonies with entire to undulate margins on 5% blood agar Rapid indole negative.

Candida auris: Large creamy whitish colonies on Sabouraud-dextrose agar often with a matt appearance (Diagnostic media product, South Africa)

Suspicious colonies were identified using VITEK®-2 (BioMérieux, France) as per manufacturer's instructions and antimicrobial susceptibilities were determined for ESKAPE-C isolates either by VITEK®-2 (BioMérieux, France) or using the Kirby-Bauer disk diffusion method. CLSI guidelines for antimicrobial susceptibility testing were used to classify organisms into either susceptible, resistant or intermediate categories. All ESKAPE-C organisms isolated were transported to Wits VIDA for storage. VITEK®-2 (BioMérieux, France) was used for identification as well as antimicrobial susceptibility testing per manufacturer's instructions and CLSI guidelines[19]. If antimicrobial susceptibility failed on VITEK®-2 (BioMérieux, France) manual susceptibilities were performed using the Kirby-Bauer method as per CLSI standards. Processing was recorded in a detailed manner on the

working card and signed and dated at each step by the person processing. Each finalised working card was reviewed, signed, and dated by a clinical microbiology registrar to signify that the processing and results were valid and conducted according to the standard operating procedure or if processing was not deemed valid repeat testing and troubleshooting was requested on a sample-by-sample basis.

2.6 Data Collection and Analysis

All specimen processing was recorded in detail on the prescribed working card by the microbiology technologist or registrar processing the specimen. Each swab was assigned a reference number prefaced by the area in which it was collected and participant population for example TICUH-01(TICU healthcare worker number 1). At no time were specimen or participant data loaded onto the NHLS laboratory information system or any other laboratory information system. All data management and analysis was performed using reference numbers to protect participants' anonymity. The research team was not aware of participants' identity while processing the specimens and analysing the data.

Descriptive statistics including proportions and percentages were used for analysis of the prevalence of colonisation among the selected population.

2.7 Ethical considerations

Ethics clearance was sought and approved by the Human Research Ethics Committee (HREC) of the University of Witwatersrand under certificate number M200973. Sampling was performed as part of the study *Microbiological surveillance of hospital environmental surfaces, medical equipment, healthcare workers, patients and patients' parents in a tertiary hospital in Soweto, South Africa.* (M191129)

CHAPTER 3

Results

Twenty-five primary caregivers and 29 healthcare workers consented to and underwent sampling. Of the 25 consenting primary caregivers seven individuals submitted rectal, vaginal and hand swabs one participant submitted hand and vaginal swabs while 17 submitted hand swabs only (figure 1).

Overall, 13 primary caregiver participants (13/25, 52%) and four healthcare worker participants (4/29,13.8%) were culture-positive for one or more of the ESKAPE-C organisms (figure 2). Of the 13 primary caregivers in which the target organisms were isolated 10 had ESKAPE-C organisms isolated from the hand swab (10/25 40%). Among primary caregivers *S. aureus* was the most commonly isolated organism(seven), followed by *E. faecium*(four). The most common Enterobacteriaceae isolated was *K. pneumoniae*(three). One *C. auris*, one *A. baumannii*, and one *E. cloacae* were isolated (figure 3). Among the healthcare workers one *C. auris*, two *A. baumannii* and one *E. cloacae* were isolated (figure 4). During the study period only *P. aeruginosa* of all the ESKAPE-C organisms was not isolated among the adults that are in frequent contact with admitted neonates. Figures 3 and 4 demonstrate the proportions of organisms isolated among participants who were culture-positive for ESKAPE-C organisms.

Of the seven *S. aureus* isolates two were Methicillin-resistant (28,6%) while five were Methicillin-susceptible. Of the five Enterobacteriaceae isolated two *K. pneumoniae* and one *E. cloacae* were ESBL producers (60%). Two of the three A. *baumannii* isolates were extensively drug-resistant(XDR) (66, 7%). No carbapenem-resistant Enterobacteriaceae (CRE) or vancomycin-resistant enterococci (VRE) were isolated in either the primary caregiver or healthcare worker group.

Both *A. baumannii* XDR organisms were isolated from the healthcare worker group while both MRSA organisms were isolated from the primary caregivers group. Of the ESBL producing isolates two were isolated from specimens taken from primary caregivers while one was isolated from a specimen taken from a healthcare worker.

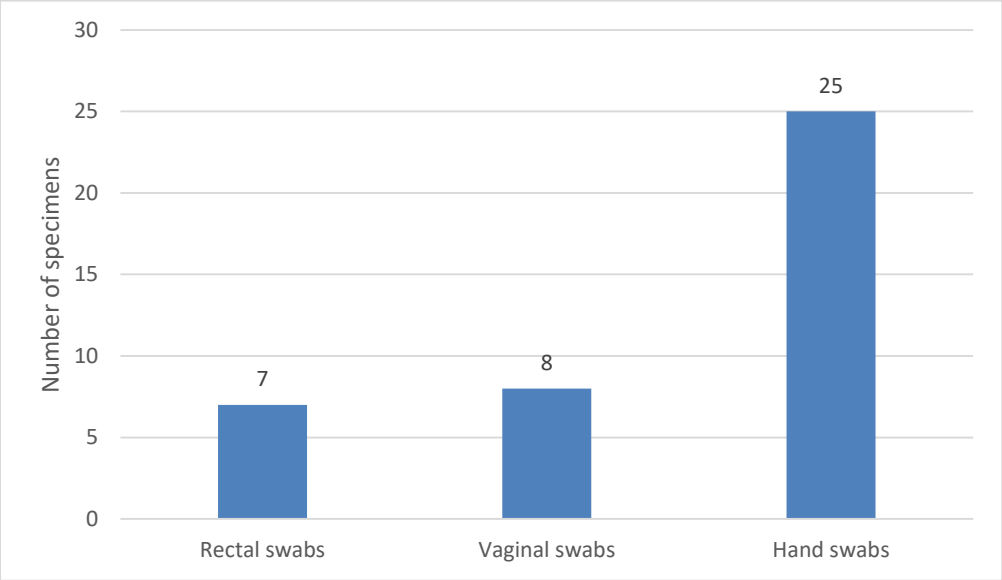

Figure 1: Proportion of specimens submitted by primary caregiver participants

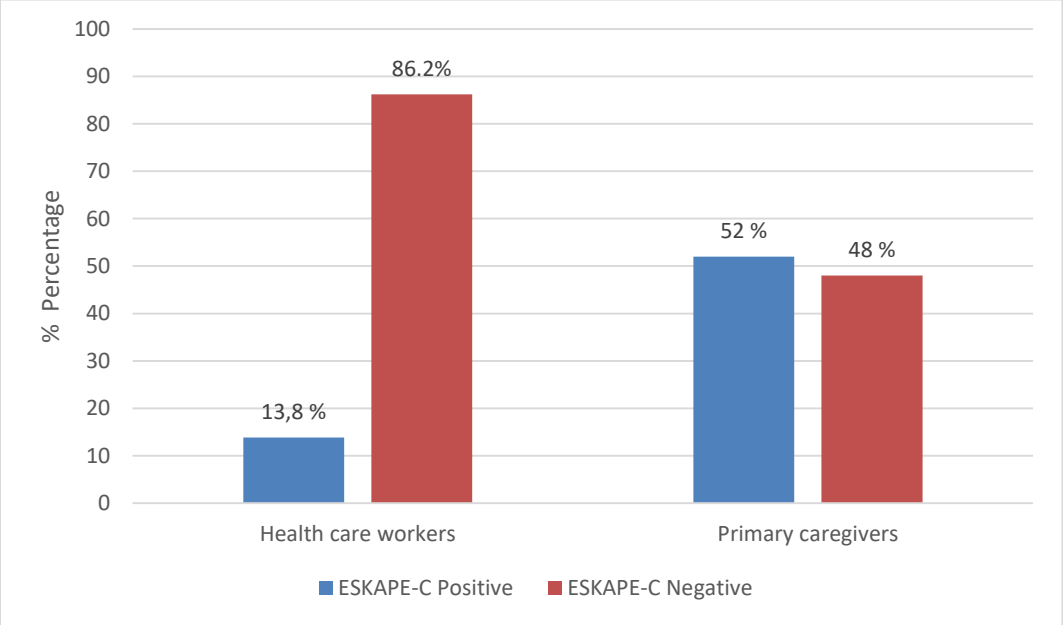

Figure 2: Percentage of ESKAPE-C culture-positive and negative isolates from healthcare workers and primary caregivers

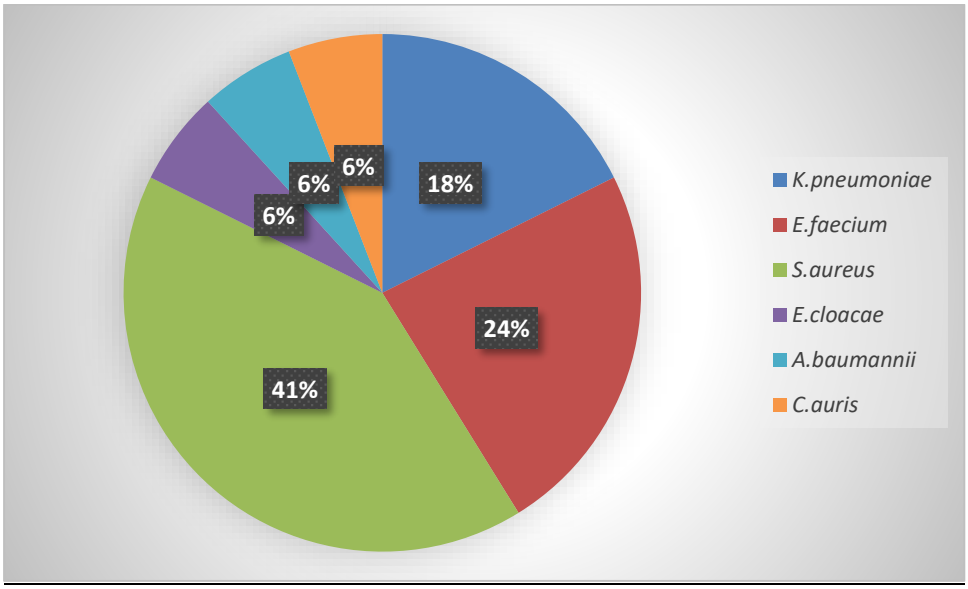

Figure 3: ESKAPE-C isolates among the 13 positive primary caregivers

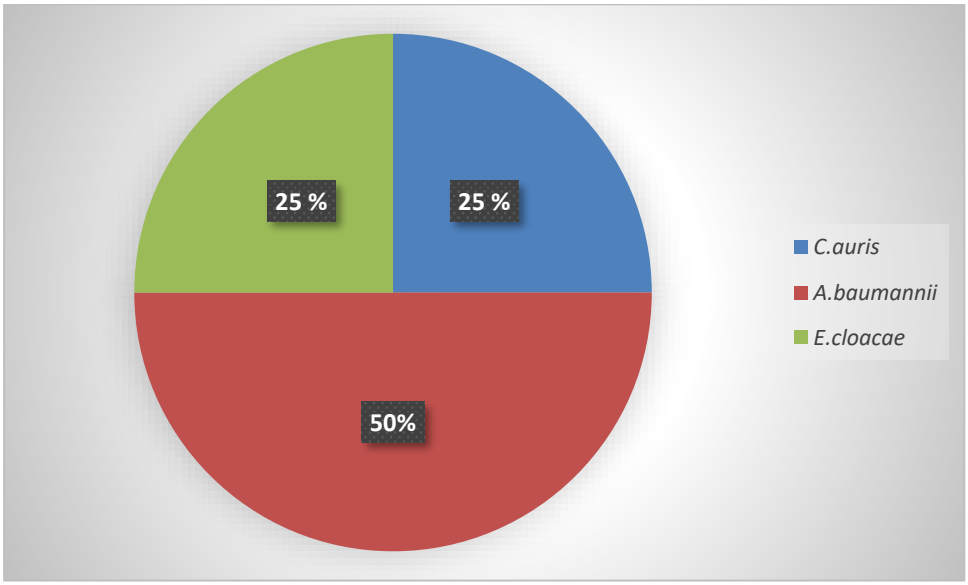

Figure 4: ESKAPE-C isolates among the 4 positive healthcare workers

CHAPTER 4

DISCUSSION

Infections are a leading cause of deaths of neonates and children under five years of age globally[20].Contaminated hands of mothers, other caregivers and HCW, as well as hospital equipment are recognised as major sources of infections in the neonates[21]. Empiric antibiotic selection may be informed by local epidemiological susceptibility data while infection control practices may be informed by transmission routes and dynamics. Data regarding colonisation of those in contact with neonates is therefore vital to optimise both treatment and prevention of infections with ESKAPE-C organisms. This study is, to the author's knowledge, unique in that it aims to describe the point prevalence of colonisation of all ESKAPE-C organisms in healthcare workers and primary caregivers in the neonatal intensive care areas in a large, tertiary South African hospital.

Lower prevalence of colonisation was found in healthcare workers (13.8%) as compared to primary caregivers (52%), however, the significance of this is unclear as HCW submitted only hand samples while some primary caregivers also submitted vaginal and rectal swabs, potentially increasing the yield in the primary caregiver group. This difference does not appear to be wholly explain the lower prevalence in healthcare workers as 40% of primary care givers still had ESKAPE-C organisms isolated from hand swabs only compared to 13.8% of healthcare workers. Another possible explanation for this finding is that healthcare workers spend less time in the hospital environment than the primary caregivers as most primary caregivers were lodging at the hospital for the duration of their child's admission while healthcare workers spend only their working hours in the hospital.

Sampling of hands is complex due to the nature of colonisation on hand surfaces and the distinction between transient and resident flora. Transient colonisers are organisms that temporarily reside on the upper surface of the skin and 'are responsible for cross-infections' [20]. Transient colonisers are organisms that are not normally found on the hands of healthy individuals outside of the hospital environment such as ESKAPE-C organisms. Resident flora are organisms that reside long-term on the hands and are usually of low virulence and commensal organisms. These organisms have low potential for transmission of infection[22]. In the present study some cases of transient colonisation may not have been detected due to the cross-sectional study design but may still be responsible for transmission within the neonatal unit.

C. auris was isolated on the hands of one healthcare worker. This organism is an emerging nosocomial pathogen first described in 2009[23] and is the most recent addition to and only fungus included in the ESKAPE-C group. This pathogen is of concern due to its intrinsic fluconazole resistance, its virulence as well as its potential to gain resistance to Amphotericin B. In South Africa, fluconazole and Amphotericin B are the two most available antifungals in the public sector. Schelenz et al.[23] described an outbreak of *C. auris* in a United Kingdom hospital in 2015-2016. They undertook extensive screening of healthcare workers by swabbing 'staff hands (agar impression plates), nose, axilla, groin and throat'[23] in order to elucidate the mode of transmission. Their study found only one out of 258 healthcare workers to be colonised with *C. auris* while 'environmental sampling of the clinical area surrounding colonized patients demonstrated contamination with *C. auris* of horizontal surfaces such as the floor around bed sites, trollies, radiators, windowsills, equipment monitors and key pads, and also one air sample'[23].This finding may indicate that environmental contamination and transient

colonisation of the hands of healthcare workers may be more significant in the transmission of *C. auris* than ongoing/resident colonisation of healthcare workers.

In the present study *S. aureus* was isolated in the primary caregiver group but not in the HCW group. The majority (71,4%) of these isolates were MSSA. The absence of positive HCW's samples is interesting as *S. aureus* colonisation has been well documented in healthcare workers globally[11,12,13]as well as approximately 20-30% of the general population[24]. This may be partially due to sampling of the hands rather than the nares which is the most common site of *S. aureus* colonisation[23], but small sample size may also have contributed to this surprising finding. Our 28% colonisation prevalence among primary care givers is comparable to that of the globally studied general population, most frequently cited as up to 30%[25]. There is a paucity of data in South African community populations demonstrating the colonisation prevalence of *S. aureus*. There is a need for research into general *S. aureus* colonisation prevalence for comparisons to be drawn with our findings and to establish whether hospital exposure influences colonisation of this organism.

Interestingly no *Pseudomonas aeruginosa* was isolated from either HCWs or primary caregivers from any sample type. This was the only ESKAPE-C organism not isolated. This is in keeping with the epidemiology of neonatal infections in South Africa as *P. aeruginosa* is rarely isolated compared with the other ESKAPE-C organisms. Pillay et al. found that *P. aeruginosa* made up only 7,8% of Gram-negative blood stream infections in neonates at Inkosi Albert Luthuli hospital in Kwazulu Natal, South Africa between 2014-2018[26]. It is also possible that environmental reservoirs play a greater role in *P. aeruginosa* transmission than the other ESKAPE-C organisms as it is noted to be ubiquitous in the enivironment[26]. It is unlikely that *P. aeruginosa* was missed in this study as it is unique and distinctive morphologically and biochemically.

A significant finding of the present study is that no CRE organisms were isolated from either group of participants in a unit in which CRE organisms are considered endemic. *K. pneumoniae* was found to be the predominant organism isolated from blood samples of neonates admitted to this unit in 2020 and about 30% of these were carbapenem-resistant (CRE) (unpublished data). In 2019, 63 positive CRE samples were isolated in the NICU and TICU i.e. the areas in which the present study was conducted in which no CRE organisms were isolated. In a 2016 study Bitterman et al. obtained rectal swabs from 177 voluntary healthcare worker participants in Israel and found no CRE colonisation among these participants in the setting of a hospital with an incidence of 2.6 cases per 1000 admissions[27]. The findings of the present study are in keeping with the Bitterman et al. findings although the sample types differ. This finding may be due to the transient rather than resident nature of the CRE colonisation on hands but may also be due to the lack of antibiotic pressure in this non-hospitalised healthy population resulting in an inability of these organisms to establish persistent colonisation in this group[27].

All four *E. faecium* organisms isolated were susceptible to vancomycin, a finding in keeping with the epidemiological profile of this organism reported in the 2018 National Department of Health Surveillance for Antimicrobial Resistance and Consumption of Antibiotics in South Africa. In this report only 5% of invasive *E. faecium* isolates were resistant to vancomycin[28]. This finding is also in keeping with the 2019 investigation performed by Madhi et al. in neonates at CHBAH. In this study minimally invasive tissue sampling was performed post-mortem within 36 hours of death. Microscopy, Culture & Susceptibility (MC&S) was performed on the resultant tissue specimens. All of the six Enterococci isolated in this study were susceptible to vancomycin[29].

The present study focused specifically on ESKAPE-C organisms due to their potential for drug resistance, their virulence, and their ability to affect multiple human sites. However, these

organisms do not by any means represent all organisms that cause infections in this group of patients. *E. coli*, group B streptococci, *Enterococcus faecalis*, Candida species other than *C. auris* and Coagulase-Negative Staphylococci (particularly where indwelling prosthetic devices are present) are also significant organisms implicated in neonatal infections in intensive care units; a comprehensive assessment of organism colonisation would need to include all these organisms.

This study may provide background for further studies particularly research using molecular techniques to demonstrate relatedness of organisms cultured from clinical samples (both screening and diagnostic) and those colonising the adults in close contact with admitted neonates. These studies may in turn provide the basis for interventional studies aimed at reducing colonisation in healthcare workers and primary caregivers.

The present study highlights the need for comprehensive infection prevention and control strategies. The presence of colonisation of healthcare workers and primary caregivers of ESKAPE-C organisms may result in colonisation and infection in neonates. Infection can be interrupted by appropriate handwashing (as per the WHO '5 moments of hand hygiene')[30], correct use of Personal Protective Equipment and the use of colonisation screening and contact precautions where applicable. The implementation of these steps requires evidence-based protocols, dedicated infection prevention and control teams as well as ongoing training and participation by all adults in the neonatal care environments.

Limitations

This study had several limitations. The small number of participants in this sampling phase limits the generalisability of the findings and is a major limitation of the study. In this study numbers were limited due to reluctance to participate in both participant groups as well as

funding limitations. Healthcare workers may have been reluctant to participate due to fears of punitive action despite assurances that this study is anonymous. Perceived time limitations due to short staffing may also have contributed. We found that doctors particularly felt they did not have time to participate. In most cases where primary caregivers volunteered to participate, they felt unable to provide all three requested swab types. This may have led to an underestimation of colonisation in this group. Self-swabbing may become the norm in South Africa and the rest of the world for a variety of microbiological specimens[31], participation in such studies may improve with increased familiarity with this type of sampling. Among healthcare workers we only requested hand swabs. This may have led to an underestimation of colonisation due to undetected reservoirs at other sites for example, the gastrointestinal tract or the nares. Although this limitation may be mitigated by findings of other researchers suggesting that 'Cross-transmission in this setting is most probably related to transfer of bacteria through HCWs' hands or contact with surfaces, and not to personal gastrointestinal carriage of the bacteria.'[25] Increased participant numbers as well as more complete specimen collection would have provided more accurate data but may also have increased unacceptability to participants.

Another limitation of this study is that it was cross-sectional, we are thus unable to give insight into colonisation incidence over time. Also, the prevalences we found may not be typical of the setting, for instance if they vary seasonally or are affected by length of stay/employment in the neonatal unit. Hand colonisation of ESKAPE-C organisms may be transient in nature in some individuals[22] therefore on-going sampling may have provided more insight into true occurrence of colonisation with these organisms. We are also unable to determine if colonisation in either group is transient or sustained as only one sampling episode took place. It may be useful to establish extent of sustained colonisation among healthcare workers and primary caregivers to determine if decolonisation strategies would be applicable to this group.

Culture based methods for the identification and categorisation of the studied organisms are labour intensive, time sensitive and require highly skilled labour. Since morphology was used in the identification method it is possible that some ESKAPE-C isolates were missed due to the subjective nature of this technique. Standard operating procedures and additional biochemical tests were included to mitigate this possibility, but this remains a potential limitation. In future, similar studies may be conducted using multiplex Polymerase chain reaction based methods and this may increase the efficiency of the research and well as the sensitivity of ESKAPE-C detection.

Participants were not asked about hospital admission during pregnancy or preceding pregnancy. Lack of data regarding primary caregivers' previous healthcare contacts and other risk factors for colonisation means that we are unable to assert that all cases of colonisation were established due to exposure to the hospital environment during the current admission. A more thorough medical history of the participants would have allowed us to categorise participants into those likely colonised due to prior healthcare sector contact and those likely colonised due to the current healthcare sector contact. This categorisation would allow us to identify modifiable and non-modifiable risk factors for colonisation.

E. coli was not included in this study as it does not comprise one of the ESKAPE-C pathogens and is not considered as critical as these organisms in terms of hospital acquisition and spread of drug resistance mechanisms. The exclusion of this organism as well as the other members of the family Enterobacteriaceae mean that the proportion of ESBLs identified in this study applies to *K. pneumoniae* and Enterobacter species only and is not generalizable to the rest of the Enterobacteriaceae family. This study is therefore not a comprehensive representation of colonisation of all potentially pathogenic organisms but specifically the ESKAPE-C group.

Conclusion

To the authors knowledge this is the first study in South Africa describing the prevalence of colonisation with ESKAPE-C organisms among healthcare workers and caregivers. This study aimed to describe the cross-sectional prevalence of ESKAPE-C organisms in healthcare workers and primary caregivers in a tertiary hospital in South Africa and found a prevalence of 13,8% in healthcare workers and 52% in primary caregivers. Colonisation prevalence may be underestimated in this investigation as only hands were swabbed in the healthcare worker population excluding other reservoirs or sites of colonisation. Of the Enterobacteriaceae isolated 60% percent were ESBL-producing, 28,6% of the *S. aureus* isolated were methicillin resistant and 66.7% of all *A. baumannii* isolates were extensively-drug resistant.

These findings indicate that colonisation and subsequent contact with adults may be an important means of transmission of ESKAPE-C organisms to admitted neonates. Currently no standardised recommendations exist regarding decolonisation techniques in healthcare workers or primary caregivers to prevent colonisation/infection in neonatal patients, but this may be an interesting and useful area for further research. Handwashing is frequently cited as an effective method to interrupt transmission of these organisms and this study reinforces this vital concept.

Future research into the epidemiology of colonisation in these populations may include molecular detection techniques as well as a longer sampling periods in order to yield a more comprehensive appreciation of the colonisation dynamics leading to neonatal infection. With longer sampling periods and molecular techniques levels of relatedness between clinical and research isolates may be established proving the link between adult colonisation and neonatal infection, potentially leading to new methods of transmission interruption.

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Raw data is available upon request from the author, this includes working cards as well as collated data.

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Appendix A: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr N Rees

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200973

NAME: (Principal Investigator)	Dr N Rees
DEPARTMENT:	School of Pathology Department of Clinical Microbiology and Infectious Diseases Medical School University
PROJECT TITLE:	Colonisation with ESKAPE organisms and Candida auris amongst primary caregivers and healthcare workers in a neonatal unit at a public sector tertiary South African hospital
DATE CONSIDERED:	2020/10/02
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr L Mothibi
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2020/11/13

This clearance certificate is valid for 5 years from the date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **September** and will therefore reports and re-certification will be due early in the month of **September** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

16/11/20
Date

Appendix B: Participant information film script and illustrations

Script: Participant information video

Scene 1: This scene will be a recording of a microbiology registrar talking. It is to establish the disclaimers of the video and let the audience know what to expect. The chosen Doctor is to help build trust with the audience by using a relatable character.

Voice over(VO): Please make sure you speak slowly and clearly. Pause between each point.

Line 1: We will be a doing a study where we will be looking for the germs that can cause infections in babies in the hospital environment. This includes germs on healthcare workers, moms of babies admitted and on babies' skin.

Line 2: The study will help us know the type of germs and what medication we can use to get rid of the germs. It will help us improve cleaning in the areas of concern in the environment and encourage better hand washing practices.

Line 3: We will not know your identity.

Line 4: We are not testing for any diseases. We are only looking for infections that affect new-borns.

Line 5: This will not affect the care your baby or you receive.

Line 6: We are also collecting specimens from healthcare workers, babies and the environment.

Line 7: We will be taking an anal and vaginal swab

Line 8: This will not be painful.

Line 9: Please fill in a consent form

Line 10: Feel free to ask us any questions you have.

Line 11: Thank you.

Introduction to swabs: This introduction is so that the audience knows where to take the swabs and the procedure before.

Title board: Where to do your swabs.

VO: where to do your swabs.

Scene: Showing the audience where to take the swab.

VO: Do the swab in the bathroom or a private place.

Animation: Show the user with the swabs in a bathroom, or a room where they draw the curtains/close the door showing privacy.

Scene 2: Vaginal Swabs – we need to show an animation of each step as clearly visually as possible. All steps should be literal; I think universal symbols for correct/incorrect can be used such as a tick or x when showing steps. I would recommend when showing a human using a person of color to keep this relatable to our target audience. Full bodies/faces need not be used, this could be done with just hands and the genitals to keep it neutral. It will be helpful to animate the actual swabs and tools etc. if possible, to eliminate confusion.

Title Board: How to do the vaginal swab

VO: How to do the vaginal swab

Step 1: Wash your hands

VO: Wash your hands with soap and water.

Animation: Show the user washing hands with soap.

Step 3: Opening of the swab

VO: Open the swab packet. Do not touch the cotton side of the swab or put it down.

Animation: Show the user owning the package. I think we need to show what the cotton side looks like and have a big red X over the user touching it as well as a big red X showing the user putting this down.

Step 4: Undress to expose your vagina and get into a comfortable position (leg up on toilet or squatting)

VO: Remove clothing to uncover your vagina. Get into a comfortable position.

Animation: Show the user removing pants/underwear. A visual or the user squatting/putting leg on toilet. Maybe use ticks to indicate this is correct.

Step 5: Insert the swab into the vagina about 5cm from the opening

VO: Put the swab into the vagina about 5cm from opening.

Animation: Action to mimic the VO, please make sure the 5cm is clear. There is no mark on the swab.

Step 6: Rotate the swab for 10 seconds then remove.

VO: Turn the swab for 10 seconds then remove.

Animation: Can we have a 10 second countdown to indicate 10 seconds showing the swab rotating.

Step 7: Insert the swab into the gel and close the lid tightly

VO: Put the swab into the gel and close the lid tightly.

Animation: Action to mimic the VO

Step 8: Hand washing.

VO: Wash your hands with soap and water

Animation: Action to mimic the VO

Scene 3: Rectal Swabs, again make sure the animation leaves no room for imagination or confusion.

Title Board: How to do the rectal swab

VO: How to do the rectal swab.

Step 1: Wash your hands

VO: Wash your hands with soap and water.

Animation: Show the user washing hands with soap.

Step 3: Opening of the swab

VO: Open the swab packet. Do not touch the cotton side of the swab or put it down.

Animation: Show the user owning the package. I think we need to show what the cotton side looks like and have a big red X over the user touching it as well as a big red X showing the user putting this down.

Step 5: Insert the swab into the anus about 5cm from the opening

VO: Put the swab into the anus about 3cm from opening.

Animation: Action to mimic the VO, please make sure the 3cm is clear. There is no mark on the swab.

Step 6: Rotate the swab for 10 seconds then remove.

VO: Turn the swab for 10 seconds.

Animation: Can we have a 10 second countdown to indicate 10 seconds showing the swab rotating.

Step 7: Insert the swab into the gel and close the lid tightly

VO: Put the swab into the gel and close the lid tightly.

Animation: Action to mimic the VO

Step 8: Hand washing.

VO: Wash your hands with soap and water

Animation: Action to mimic the VO

Conclusion: What to do with the swabs.

Scene: Give both specimens to the researcher to package and send to the lab along with your hand swabs

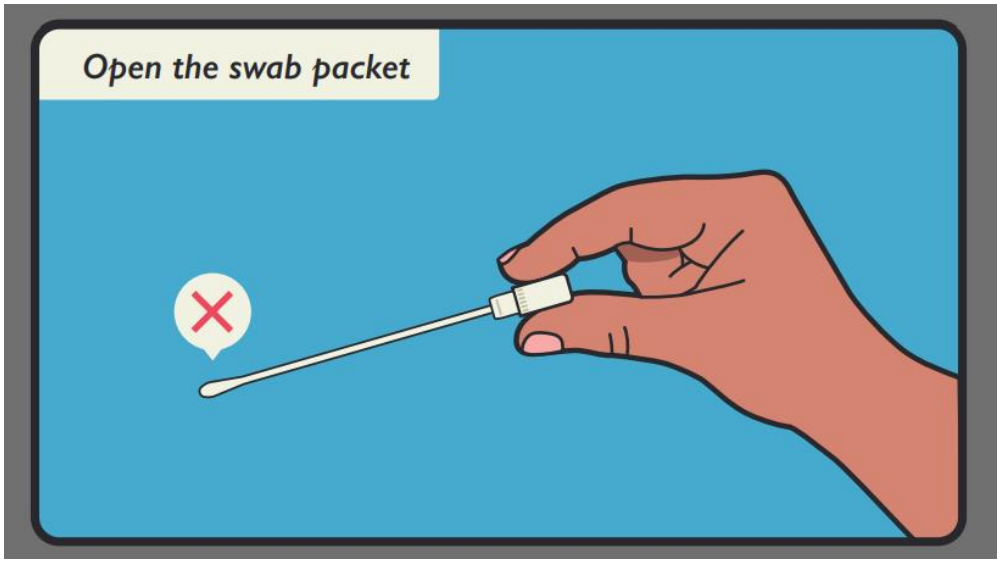
VO: When you are done. Give both containers to the researcher.

Animation: Animation to mimic VO.

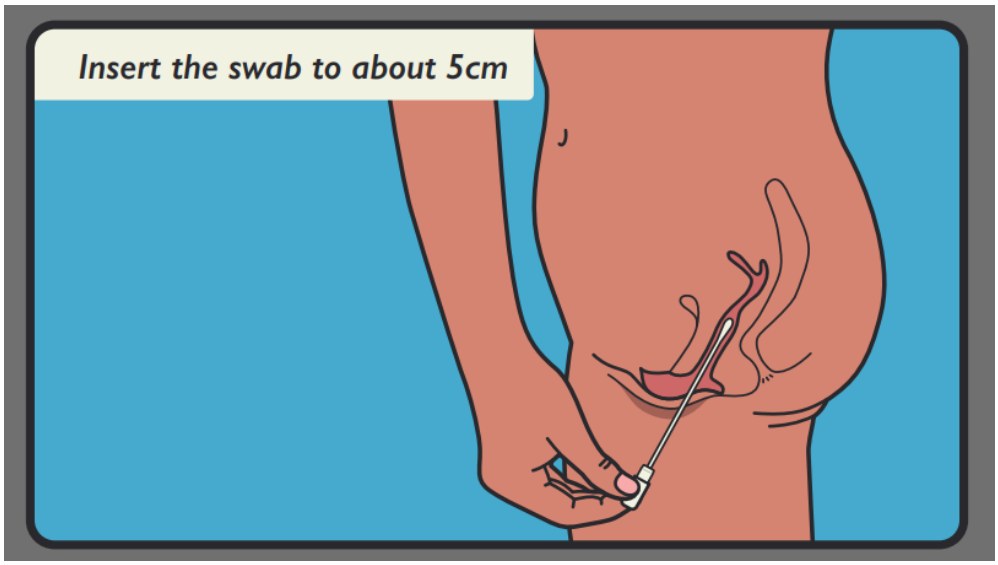
Final: This is to end off the video and reinforce the messaging that they may ask any questions.

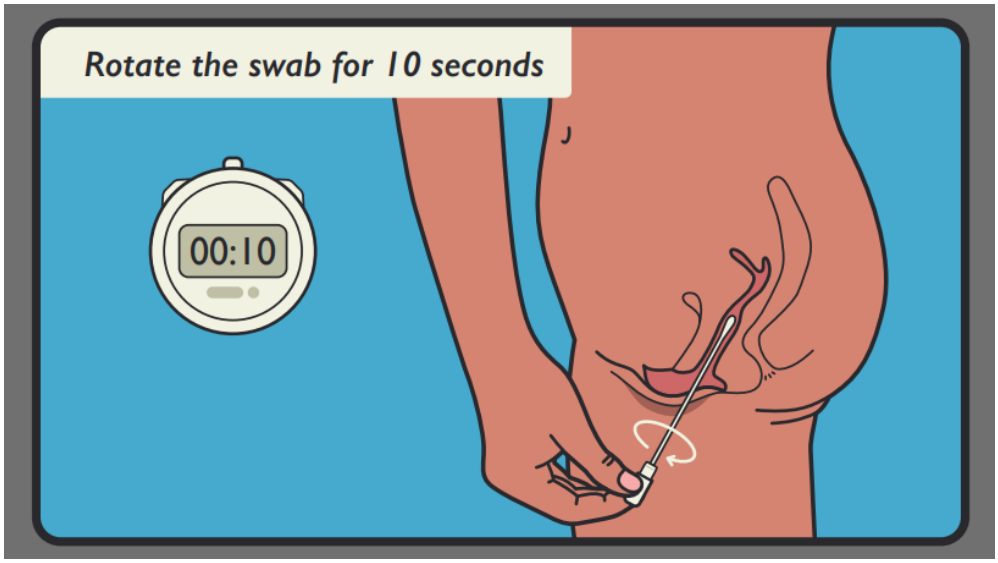
End Board: Thank you. Please feel free to ask any questions.

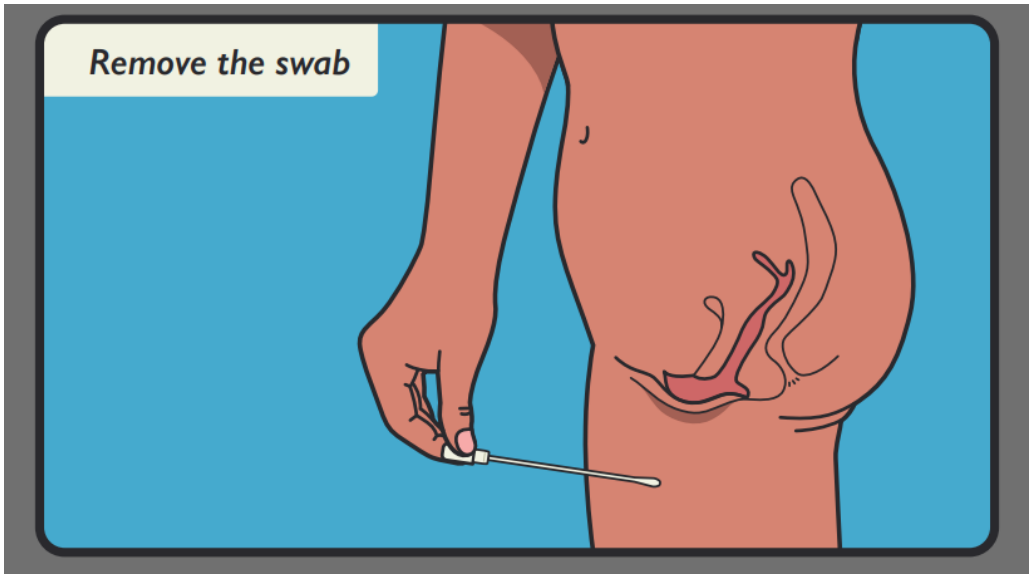
VO: Thank you.Please feel free to ask any questions.

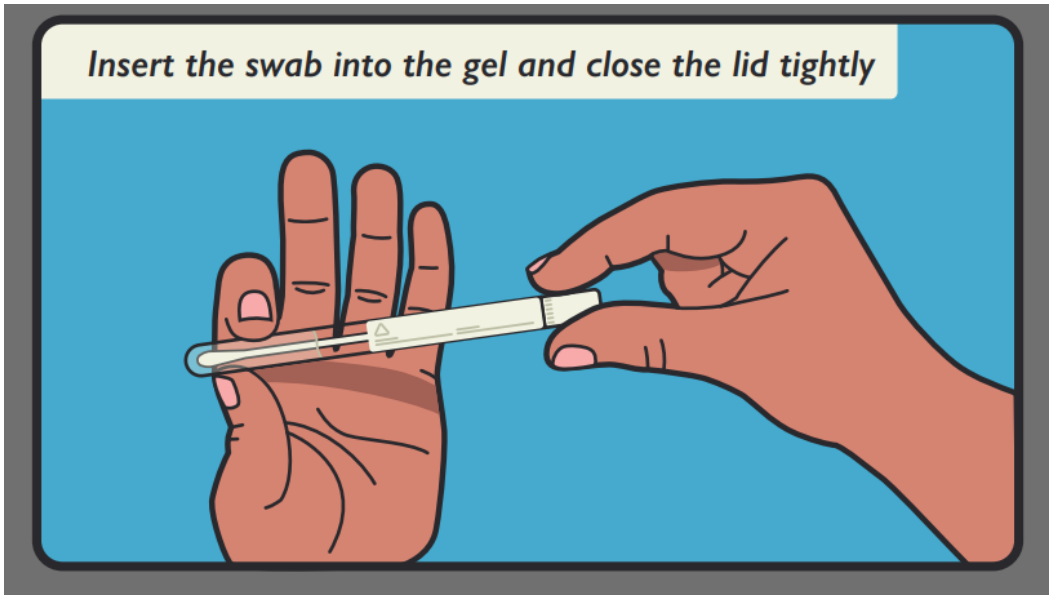


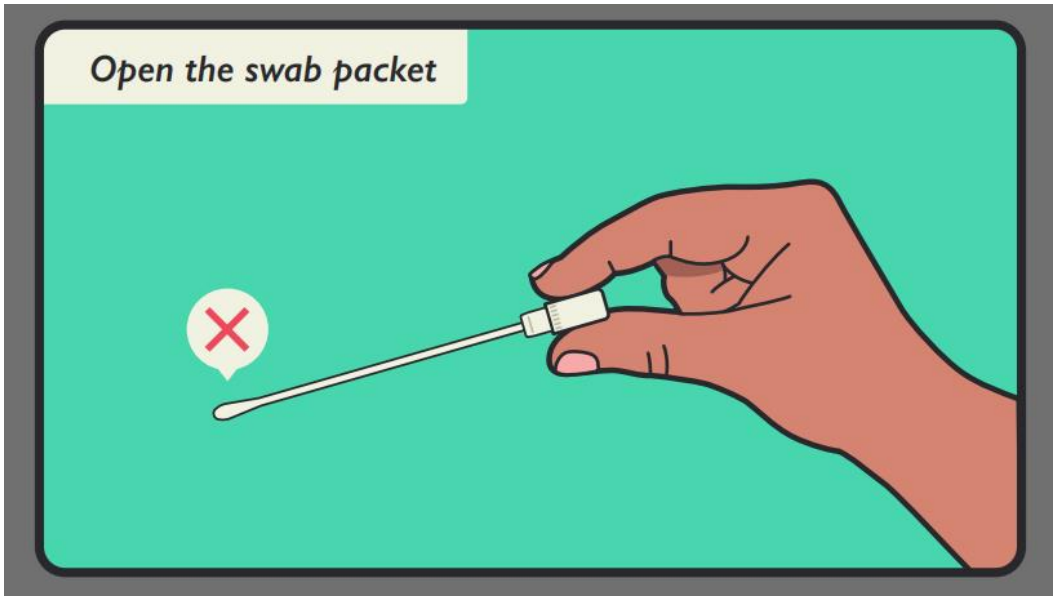

Undress and get into a comfortable position

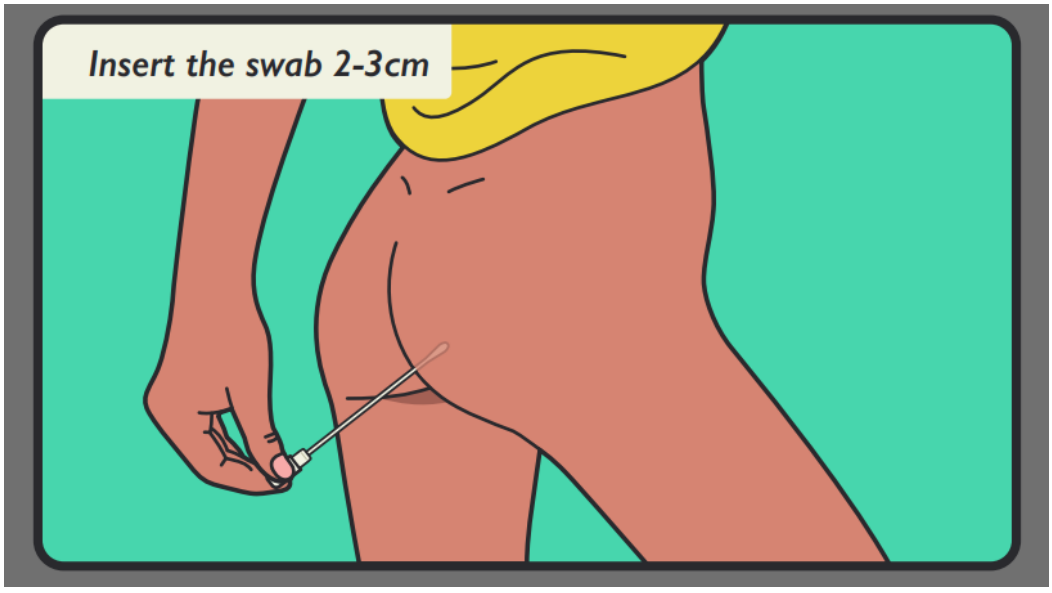

Insert the swab to about 5cm

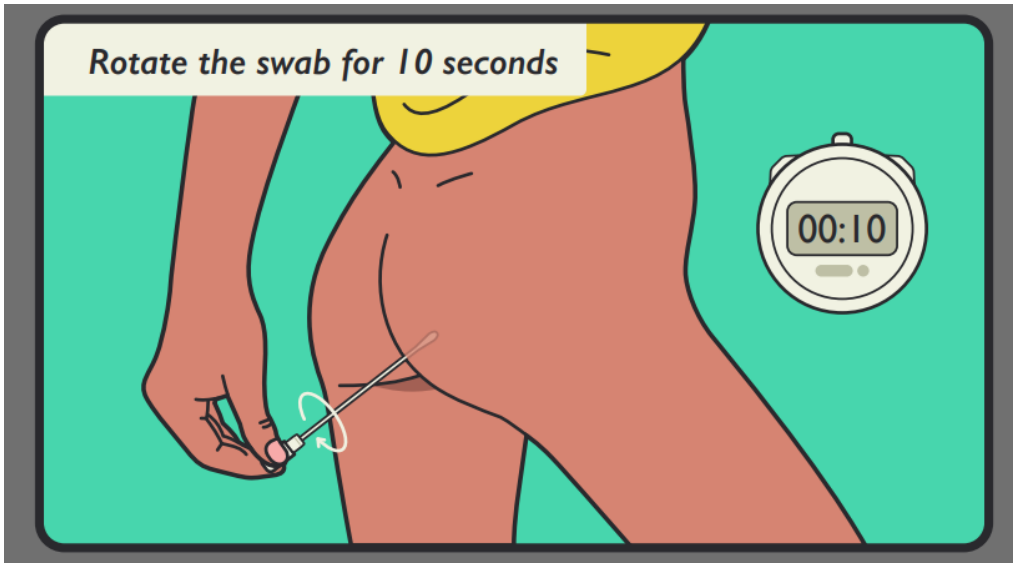

Rotate the swab for 10 seconds
00:10

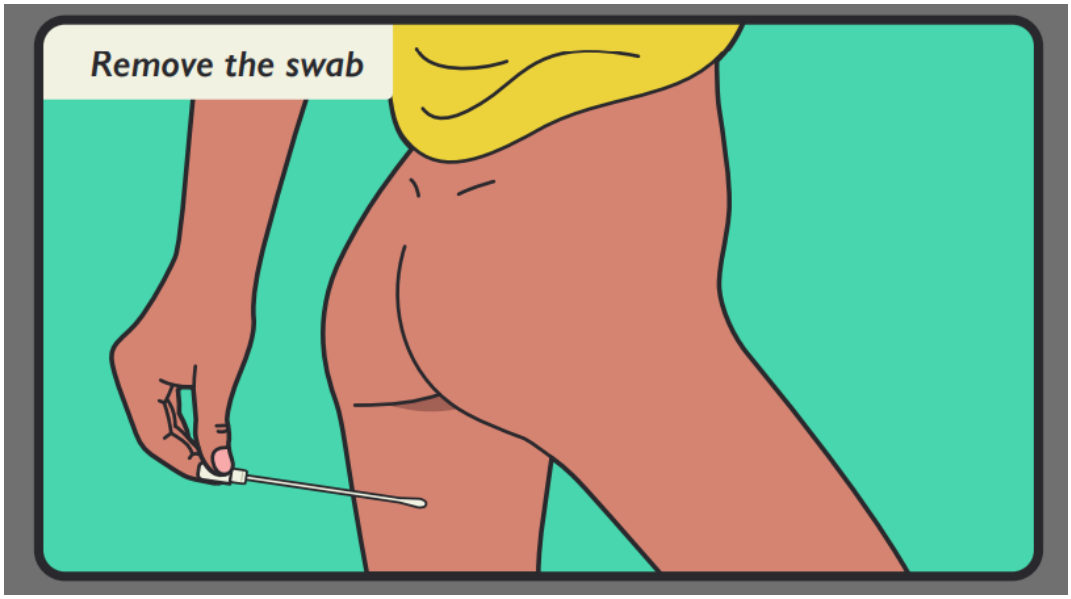

Remove the swab

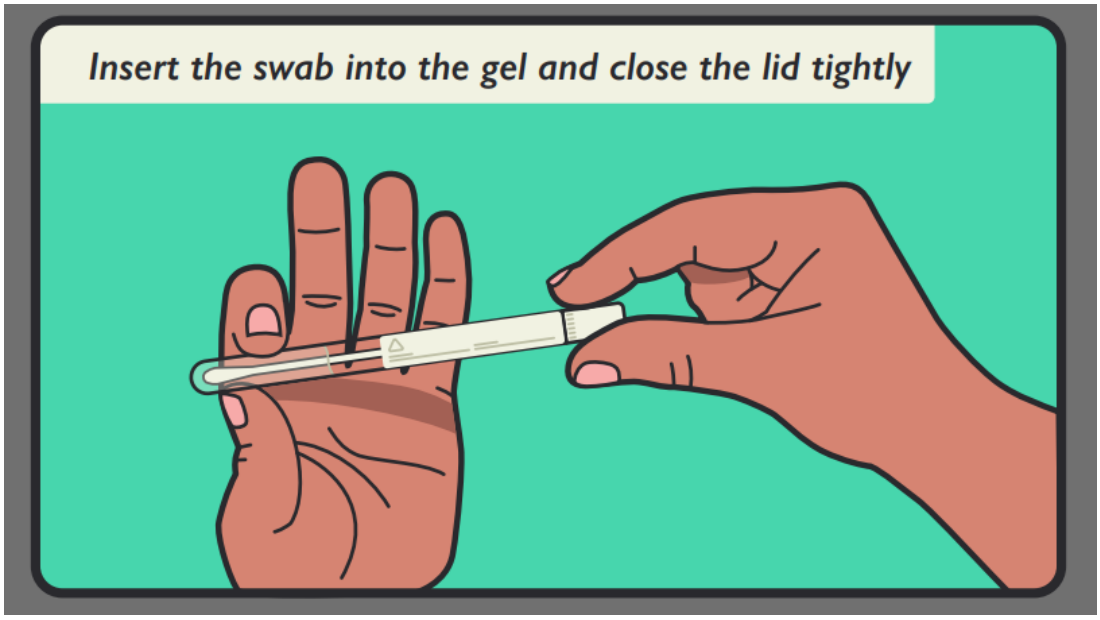

Insert the swab into the gel and close the lid tightly


Open the swab packet


Insert the swab 2-3cm


Rotate the swab for 10 seconds
00:10


Remove the swab


Insert the swab into the gel and close the lid tightly

Appendix C: Consent forms

Consent to participate in the research project:

Colonisation with ESKAPE organisms and Candida auris among primary caregivers and healthcare workers in a neonatal unit at a public sector tertiary South African hospital.

I ……. agree to participate in this research project. The research has been explained to me and I understand what my participation will involve.

I agree that my participation will remain anonymous YES NO (please circle)

I agree that the information I provide may be used YES NO

anonymously by other researchers following this study

…… (signature)

…… (name of participant)

…… (date)

Consent to participate in the research project:

Colonisation with ESKAPE organisms and Candida auris among primary caregivers and healthcare workers in a neonatal unit at a public sector tertiary South African hospital.

I …… agree to participate in this research project. The research has been explained to me and I understand what my participation will involve.

I agree that my participation will remain anonymous YES NO (please circle)

I agree that the information I provide may be used YES NO

anonymously by other researchers following this study

…… (signature)

…… (name of participant)

…… (date)

Appendix D: Participant information Sheet

STUDY INFORMATION DOCUMENT: PARENTS TO ADMITTED NEONATES

STUDY TITLE: MICROBIOLOGICAL SURVEILLANCE OF HOSPITAL ENVIRONMENTAL SURFACES, MEDICAL EQUIPMENT, HEALTHCARE WORKERS, PATIENTS AND PATIENTS' PARENTS IN A TERTIARY HOSPITAL IN SOWETO, SOUTH AFRICA.

To CHBAH neonatal unit parents/caregivers,

We will be conducting a research study and in this study, we plan to determine the microbial contamination of the hospital environment, equipment, healthcare workers, caregivers and neonates admitted to the neonatal unit at CHBAH.Research is a process used in seeking new knowledge. In this study we want to learn the colonization rates of environment, equipment, patients, parents and staff which will give an indication of the effectiveness of regular cleaning and disinfection of instruments, and hands of healthcare workers. Data collected from the study will help in decisions to take appropriate and targeted measures to prevent hospital-acquired-infections in this unit.

We are inviting you as a caregiver to a neonate admitted in the unit to take part in this research study.

What is involved in the study:

1. The study investigators will collect samples from the environment, staff, patients and caregivers and these will be analysed in the laboratory to determine the types of organisms on all the surfaces.
2. The study will be conducted from February 2020.
3. The caregivers' anatomical sites that will be swabbed will be:
    - Hands
    - Groin
    - Rectum
4. Hand sampling will be performed by study investigator at pre-arranged time and will take less than 30 minutes.
5. Hand swab samples will be transported immediately to the participating laboratory in an intact, sturdy cooler box and processed on arrival.
6. Groin and rectal swabbing will be self-administered by the caregivers.
7. Each neonatal unit bathroom will be provided with two opaque boxes, one containing packs for self-administered swabbing and one for completed swabs.
8. Participating caregivers will use one moistened swab per area i.e. groin (left and right), underarm (left and right), nares (left and right) and rectal and placed immediately into the transport medium.
9. Swabs will then be placed in the sealable plastic bags with the completed consent form, and placed in the opaque box for completed swabs.

10. Self-administered swabs will be collected daily at 08:00 from all sites and transported to participating laboratories in an intact, sturdy cooler box and processed on the day of arrival.

Risks of being involved in the study:

There are no risks associated with being involved in this study.

Benefits of being in the study:

1. There is no direct benefit to the participant however the study findings will benefit neonates who will be admitted to this unit in future.
2. There will be no payment or costs associated with participation.

Participation is voluntary:

Refusal to participate will involve no penalty or loss of benefits to the participant. The participant may discontinue participation at any time without penalty, or loss of benefits to which the participant is otherwise entitled. There is no requirement to provide a reason for withdrawing and any data collected on such a person will in default be destroyed, unless the participant specifically consents to its retention.

Confidentiality:

Personal information will not be required and any information that can lead to participant's personal identification will be treated in the strictest confidence and will only be available to the research investigators and supervisors. The only exceptions - and all of them are rare - would normally be:

1. personal information may be disclosed if required by law
2. the Human Research Ethics Committees of the University may exceptionally require personal data to respond to a formal complaint, or for a compliance audit
3. the South African Health Products Regulatory Authority (SAHPRA), which is the successor body to the South African Medicines Control Council (SAMCC), might conceivably require access to personal data, if conducting an investigation into a drug trial

This study has been submitted for approval by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.
If you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Professor Clement Penny, who may be contacted on telephone number 011 717 2301, or by e-mail on Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za

Thank you for reading this Study Information Sheet.

Contact details:

Dr Fikile Mabena, Principal Investigator, telephone no. 0825002178, or by e-mail at Fikile.Mabena@wits.ac.za,

Prof Sithembiso Velaphi, Supervisor, on telephone no. 011 9339430, or by e-mail at Sithembiso.Velaphi@wits.ac.za

Professor CB Penny, Chairperson of the Human Research Ethics Committee (Medical) at the University of Witwatersrand, on telephone no. 011 717 2301, or by e-mail at Clement.Penny@wits.ac.za.
Ms. Z Ndlovu or Mr Rhulani Mkansi, Committee Secretariat, telephone nos.: 011 717 2700 or 1234, or by e-mail at: Zanele.Ndlovu@wits.ac.za or Rhulani.Mkansi@wits.ac.za

WITS UNIVERSITY

STUDY INFORMATION DOCUMENT: HEALTHCARE WORKERS

STUDY TITLE: MICROBIOLOGICAL SURVEILLANCE OF HOSPITAL ENVIRONMENTAL SURFACES, MEDICAL EQUIPMENT, HEALTHCARE WORKERS, PATIENTS AND PATIENTS' PARENTS IN A TERTIARY HOSPITAL IN SOWETO, SOUTH AFRICA.

To CHBAH neonatal unit staff members,

We will be conducting a research study and in this study, we plan to determine the microbial contamination of the hospital environment, equipment, healthcare workers, caregivers and neonates admitted to the neonatal unit at CHBAH.Research is a process used in seeking new knowledge. In this study we want to learn the colonization rates of environment, equipment, patients, parents and staff which will give an indication of the effectiveness of regular cleaning and disinfection of instruments, and hands of healthcare workers. Data collected from the study will help in decisions to take appropriate and targeted measures to prevent hospital-acquired-infections in this unit.

We are inviting you as a healthcare worker in the unit to take part in this research study.

What is involved in the study:

11. The study investigators will collect samples from the environment, staff, patients and caregivers and these will be analysed in the laboratory to determine the types of organisms on all the surfaces.
12. The study will be conducted from February 2020.

13. The staff members' anatomical sites that will be swabbed will be:
    - Hands
14. Hand sampling will be performed by study investigator at pre-arranged time and will take less than 30 minutes.
15. Hand swab samples will be transported immediately to the participating laboratory in an intact, sturdy cooler box and processed on arrival.

Risks of being involved in the study:

There are no risks associated with being involved in this study.

Benefits of being in the study:

3. There is no direct benefit to the participant however the study findings will benefit neonates who will be admitted to this unit in future.
4. There will be no payment or costs associated with participation.

Participation is voluntary:

Refusal to participate will involve no penalty or loss of benefits to the participant. The participant may discontinue participation at any time without penalty, or loss of benefits to which the participant is otherwise entitled. There is no requirement to provide a reason for withdrawing and any data collected on such a person will in default be destroyed, unless the participant specifically consents to its retention.

Confidentiality:

Personal information will not be required and any information that can lead to participant's personal identification will be treated in the strictest confidence and will only be available to the research investigators and supervisors. The only exceptions - and all of them are rare - would normally be:

4. personal information may be disclosed if required by law
5. the Human Research Ethics Committees of the University may exceptionally require personal data to respond to a formal complaint, or for a compliance audit
6. the South African Health Products Regulatory Authority (SAHPRA), which is the successor body to the South African Medicines Control Council (SAMCC), might conceivably require access to personal data, if conducting an investigation into a drug trial

This study has been submitted for approval by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg ("Committee"). A principal function of this Committee is to safeguard the rights and dignity of all human subjects who agree to participate in a research project and the integrity of the research.

If you have any concern over the way the study is being conducted, please contact the Chairperson of this Committee who is Professor Clement Penny, who may be contacted on telephone number 011 717 2301, or by e-mail on Clement.Penny@wits.ac.za. The telephone numbers for the Committee secretariat are 011 717 2700/1234 and the e-mail addresses are Zanele.Ndlovu@wits.ac.za and Rhulani.Mukansi@wits.ac.za

Thank you for reading this Study Information Sheet.

Contact details:

Dr Fikile Mabena, Principal Investigator, telephone no. 0825002178, or by e-mail at Fikile.Mabena@wits.ac.za,

Prof Sithembiso Velaphi, Supervisor, on telephone no. 011 9339430, or by e-mail at Sithembiso.Velaphi@wits.ac.za

Professor CB Penny, Chairperson of the Human Research Ethics Committee (Medical) at the University of Witwatersrand, on telephone no. 011 717 2301, or by e-mail at Clement.Penny@wits.ac.za.
Ms. Z Ndlovu or Mr Rhulani Mkansi, Committee Secretariat, telephone nos.: 011 717 2700 or 1234, or by e-mail at: Zanele.Ndlovu@wits.ac.za or Rhulani.Mkansi@wits.ac.za

Appendix E: Standard operating procedure for collection and processing of hand swabs

Standard operating procedure for the collection and processing of healthcare worker and parents' hand swabs for the study: *Microbiological Surveillance of Hospital environmental surfaces, Medical equipment, Healthcare Workers, Patients and patients' parents in a tertiary hospital in Soweto, South Africa.*

1)Purpose

To describe the procedure to be followed when collecting and processing hand swab specimens in order to determine the presence ESKAPE-C organisms

2)Scope

Specimen collection may be performed by doctors registered with the appropriate governing bodies, specimens may be processed by qualified technical staff.

3) Responsibility:

Microbiology registrars involved in the project are responsible for specimen collection. Technologists assigned to the project are responsible for processing and will be overseen by the Microbiology registrars.

4) Definitions:

- ESKAPE-C pathogens: Pathogens associated with nosocomial sepsis and drug resistance, namely:

Enterococcus faecium

Staphylococcus aureus

Klebsiella pneumonia

Acinetobacter baumannii

Pseudomonas aeruginosa

Enterobacter species

Candida auris

- CRE: carbapenem-resistant *Enterobacteriaceae*

5) Principles of the procedure:

Colonisation of healthcare providers and primary caregivers with ESKAPE-C organisms in a neonatal care setting may increase the risk of colonisation and therefore infection in neonates. Our aim is to determine rates of colonisation among caregivers and healthcare workers.

6) Environmental and safety control

6.1 Sample collection

During collection, research staff should don appropriate PPE:

Non-sterile gloves

Plastic aprons

Research staff should take all reasonable precautions to avoid contact with environmental hazards in the ward such as areas contaminated with biohazardous waste or sharp objects.

6.2 Sample processing

Appropriate Personal Protective Equipment should be worn by those processing samples including non-sterile gloves, goggles/visor and mask as well a clean lab coat at all times while in the lab.

All processing must take place in a SANAS accredited laboratory with appropriate safety measures in place including a designated health and safety officer.

7) Specimen collection and participant preparation:

Participants will be counselled and consent obtained prior to sampling. Participants will be free to withdraw participation at any point before or during sample collection.

Pre-labelled and moistened swabs should be inspected by the collecting registrar prior to sample collection for contamination, correct labelling and suitability to task.

Participant will hold out both hands in front of them palms facing upward while the sampler swabs the palmer surface of both hands with the same swab including between the fingers. The participant will then turn their hand over and the sampler will swab the dorsal surface of the hand including between the fingers.

The swab will then be placed into Cary-Blair transport medium immediately after collection.

Swabs collected must be transported in sturdy, intact containers on the same day as collection to the site of processing.

Care must be taken to avoid contamination of the swab with environmental material.

8)Equipment and reagents:

Gram stain reagents

5% sheep blood agar

MacConkey agar

Mueller-Hinton agar

Baird parker agar

Cetrimide agar

Sabouraud dextrose agar

Antimicrobial impregnated disks

Inoculating loops

Bunsen burner

Microscope

Appropriate Personal Protective Equipment

Access to Vitek-2 automated identification system

	At Collection:	For Processing:
Equipment	PPE	Sterile scissors
	Polyester-tipped swab or Raylon-tipped swab with plastic/ aluminium shaft	Sterile forceps
	Appropriate transport medium	Sterile loops
	Plastic sleeve	Bunsen burner
	Ice pack (maintain temp. 2-4◦C)	35◦C ± 2◦C aerobic incubator
	Cooler box	Vortex Mixer
		VITEK-MS system VITEK® 2 automated system
Reagents and antimicrobials		Catalase rapid indole
		Oxidase
		PYR testing kit/Streptex kit
		5 ml Brain heart infusion broth (BHI)
		+/- vancomycin and Teicoplanin Etest
		MacConkey agar plate (MAC)
Agar plates		5% BAP+ Nalidixic acid+ Colistin agar plates (BNC)
		Sabouraud-dextrose-chloramphenicol agar
		CHROMagar Staph aureus

9) Procedural steps:

Swabs must be processed on the day that they are received, place each swab in an individual BHI broth medium and label the bottle with the specimen number. Incubate the BHI broth in ambient air at 35 degrees Celsius +/- 2 degrees Celsius. Observe BHI for visible turbidity at 24 and 48 hours.

Swabs may be rejected if they:

1)Are not accompanied by a valid consent form

2) Present a danger to those processing the specimen

3) Are obviously contaminated

BHI that is turbid at either 24 or 48 hours should be plated out onto MacConkey agar, 5% sheep blood agar, sabouraud dextrose agar, CHROMagar Staph aureus. Turbid broth should be vortexed for

30 seconds prior to inoculation. Plating should take place using 10µL wire loops with a flaming step between streaks in order to achieve single colonies.

BHI broth that remains clear will be recorded on the working card as 'no bacterial growth on BHI' and no further processing will be required.

Plates should be examined at 24 and 48 hours for growth of suspicious colonies.

Morphological features of target organisms:

Enterococcus faecium: Small off-white to grey round colonies with α-haemolysis or ϒ-haemolysis on 5% sheep blood agar.

Staphylococcus aureus: Small round black colonies on Baird parker with a surrounding zone of clearing.

Klebsiella pneumonia: Lactose fermenting on MacConkey agar, raised circular 2-3mm colonies with entire margins, often mucoid.

Acinetobacter baumannii: Small 1-2mm round convex colonies with an entire margin that are non-lactose fermenting but light pink in colour and opaque on MacConkey agar.

Pseudomonas aeruginosa: Non-lactose fermenting on MacConkey agar, flat spreading 2-3mm colonies with a wrinkled moist surface and a metallic sheen.

Enterobacter spp: Grey to off-white spreading round colonies with entire to undulate margins on 5% blood agar.

Candida auris: Large creamy whitish colonies on sabouraud dextrose agar

Suspicious colonies should be identified using Vitek-MS as per manufacturer's instructions and antimicrobial susceptibilities should be determined for ESKAPE-C. CLSI breakpoints and standards for antimicrobial susceptibility testing will be used. All ESCAPE organisms isolated should be put onto microbanks for -70-degree Celsius storage.

If isolates cannot be satisfactorily identified on Vitek-MS, VITEK-2 will be used for identification as well as antimicrobial susceptibility testing per manufacturer's instructions.

Processing should be recorded in a detailed manner on the working card and signed and dated at each step by the person processing.

Each finalised working card should be reviewed, signed and dated by a microbiology registrar to signify that the processing and result are acceptable.

9) Quality control

All reagents, equipment and media will be quality controlled as per the procedural laboratories standard practices. Reagents, equipment and media must be within their expiration date and in good working order.

Quality Control strains:	+	-
Catalase	*S. aureus* ATCC 25923	*Enterococcus faecalis ATCC 29212*
PYR	*Enterococcus faecalis ATCC 29212*	Streptococcus agalactiae ATCC10386

Rapid indole	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Oxidase	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922

Appendix F: Standard operating procedure for collection and processing of rectal and vaginal swabs

Standard operating procedure for the collection and processing of parents' rectal and vaginal swabs for the study: *Microbiological Surveillance of Hospital environmental surfaces, Medical equipment, Healthcare Workers, Patients and patients' parents in a tertiary hospital in Soweto, South Africa.*

1) Purpose:

To describe the procedure to be followed when collecting and processing vaginal and rectal specimens in order to determine the presence ESCAPE organisms

2)Scope:

Specimen collection may be performed by doctors registered with the appropriate governing bodies, specimens may be processed by qualified technical staff.

3) Responsibility:

Microbiology registrars involved in the project are responsible for specimen collection, technologists assigned to the project are responsible for processing and will be overseen by the Microbiology registrars.

4) Definitions:

- ESKAPE-C pathogens: Pathogens associated with nosocomial sepsis and drug resistance namely:

Enterococcus faecium

Staphylococcus aureus

Klebsiella pneumonia/Candida auris

Acinetobacter baumannii

Pseudomonas aeruginosa

Enterobacter species

- CRE: carbapenem-resistant *Enterobacteriaceae*.

5) Principles of the procedure:

Colonisation of healthcare providers and primary caregivers with ESCAPE organisms in a neonatal care setting may increase the risk of colonisation and therefore infection in neonates. Our aim is to determine rates of colonisation among caregivers and healthcare workers.

6) Environmental and safety control

6.1 Sample collection

During collection, research staff should wear non-sterile gloves.

Research staff should take all reasonable precautions to avoid contact with environmental hazards in the ward such as areas contaminated with biohazardous waste or sharp objects.

6.2 Sample processing

Appropriate Personal Protective Equipment should be worn by those processing samples including non-sterile gloves, goggles/visor and mask as well a clean lab coat at all times while in the lab.

All processing must take place in a SANAS accredited laboratory with appropriate safety measures in place including a designated health and safety officer.

6) Specimen collection and participant preparation:

All participants will sign a consent form prior to sampling. Patients will receive education regarding the purpose of the sampling as well as sample collection in the form of an educational video as well as discussions with the research staff.

6.1 Vaginal swab self-collection:

To take place in a private comfortable place

No need to shower/bathe prior to collection

Wash hands with soap and water prior to collection

Open swab package but do not touch the cotton side of the swab or put it down

Undress from the waist down and get into a comfortable position (leg up on toilet or squatting)

Insert the swab into the vagina about 5cm from the opening (to the mark)

Rotate the swab for 10 seconds

Remove the swab

Insert the swab into the gel and close the lid tightly

6.2 Rectal swab self-collection

To take place in a private comfortable place

No need to shower/bathe prior to collection

Wash hands with soap and water prior to collection

Undress from the waist down and get into a comfortable position (leg up on toilet or squatting)

Open swab package but do not touch the cotton side of the swab or put it down

Insert the swab into the anus 2-3cm from the opening (to the mark)

Rotate swab for 10 seconds

Remove the swab

Insert the swab into the gel and close the lid tightly

7)Equipment and reagents:

Gram stain reagents

5% sheep blood agar

MacConkey agar

Meuller-hinton agar

Baird parker agar

Cetrimide agar

Sabouraud dextrose agar

Antimicrobial impregnated disks

Vancomycin and teicoplanin Etests

Inoculating loops

Bunsen burner

Microscope

Appropriate Personal Protective Equipment

Access to Vitek-2 and Vitek-MS automated identification system

Resist-4 CRE Lateral flow assay

	At Collection:	**For Processing:**
Equipment	PPE(non-sterile gloves and plastic apron)	Sterile scissors
	Raylon-tipped swab with plastic/ aluminium shaft	Sterile forceps
	Cary-Blair transport medium	Sterile loops
	Plastic sleeve	Bunsen burner
	Ice pack (maintain temp. 2-4◦C)	35◦C ± 20C aerobic incubator
	Cooler box	Vortex Mixer
Reagents and antimicrobials	Nil	Catalase reagent Rapid indole Oxidase
		PYR
		5 ml Brain heart infusion broth(BHI)
		+/- vancomycin Etest
	Nil	MacConkey agar plate
		Blood+ Nalidixic acid+ Colistin agar plates
Agar plates		CHROMagar Staph aureus
		Sabouraud dextrose agar

8) Procedural steps:

8.1 Procedure for processing of swabs received:

Swabs must be processed on the day that they are received, place each swab in an individual BHI broth medium and label the bottle with the specimen number. Incubate the BHI broth in ambient air at 35 degrees Celsius +/- 2 degrees Celsius. Observe BHI for visible turbidity at 24 and 48 hours.

Swabs may be rejected if they:

1)Are not accompanied by a valid consent form

2) Present a danger to those processing the specimen

3) Are obviously contaminated

BHI that is turbid at either 24 or 48 hours should be plated out onto MacConkey agar, 5% sheep blood agar, sabouraud dextrose agar, Baird parker and cetrimide agar. Turbid broth should be vortexed for 30 seconds prior to inoculation. Plating should take place using 10µL wire loops with a flaming step between streaks in order to achieve single colonies.

BHI broth that remains clear will be recorded on the working card as 'no bacterial growth on BHI' and no further processing will be required.

Plates should be examined at 24 and 48 hours for growth of suspicious colonies.

Morphological features of target organisms:

Enterococcus faecium: Small off-white to grey round colonies with α-haemolysis or ϒ-haemolysis on 5% sheep blood agar. PYR positive.

Staphylococcus aureus: Small round black colonies on Baird parker with a surrounding zone of clearing.

Klebsiella pneumonia: Lactose fermenting on MacConkey agar, raised circular 2-3mm colonies with entire margins, often mucoid. Indole negative.

Acinetobacter baumannii: Small 1-2mm round convex colonies with an entire margin that are non-lactose fermenting but light pink in colour and opaque on MacConkey agar.

Pseudomonas aeruginosa: Non-lactose fermenting on MacConkey agar, flat spreading 2-3mm colonies with a wrinkled moist surface and a metallic sheen, oxidase Positive.

Enterobacter spp: Grey to off-white spreading round colonies with entire to undulate margins on 5% blood agar. Indole negative.

Candida auris: Large creamy whitish colonies on sabouraud dextrose agar

Suspicious colonies should be identified using Vitek-MS as per manufacturer's instructions and antimicrobial susceptibilities should be determined for ESKAPE-C isolates. CLSI breakpoints and standards for antimicrobial susceptibility testing will be used. All ESKAPE-C organisms isolated should be put onto microbanks for -70-degree Celsius storage.

If isolates cannot be satisfactorily identified on Vitek-MS, VITEK-2 will be used for identification as well as antimicrobial susceptibility testing per manufacturer's instructions

Processing should be recorded in a detailed manner on the working card and signed and dated at each step by the person processing.

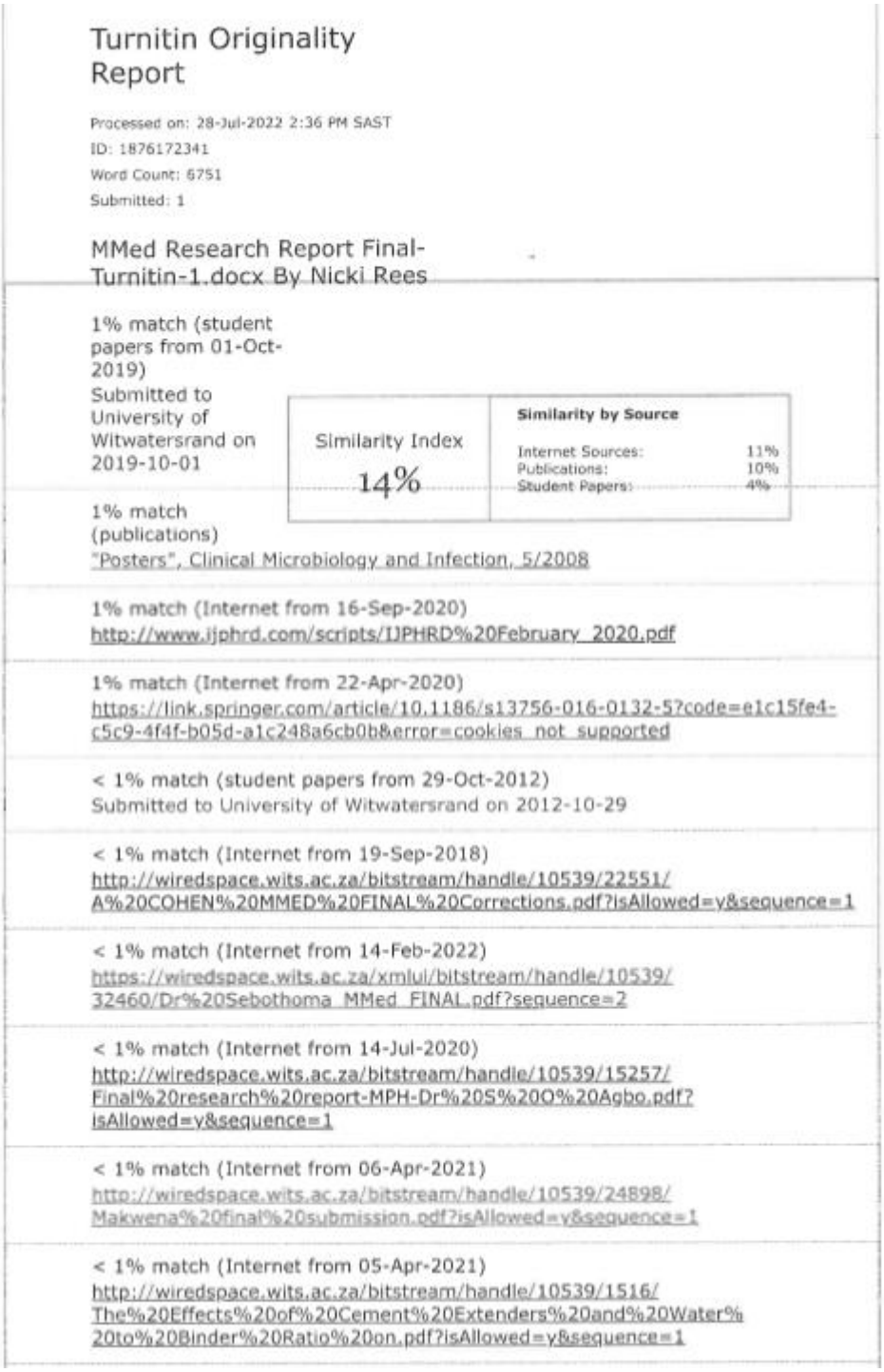
Each finalised working card should be reviewed, signed and dated by a clinical microbiology registrar to signify that the processing and results are acceptable.

9) Quality control

All reagents, equipment and media will be quality controlled as per the procedural laboratory's standard operating procedures. Reagents, equipment and media must be within their expiration date and in good working order.

Quality Control strains:	+	-
Catalase	*S. aureus* ATCC 25923	*Enterococcus faecalis ATCC 29212*
Aesculin bile agar/PYR	*Enterococcus faecalis ATCC 29212*	Streptococcus agalactiae ATCC10386
Rapid indole	*E. coli* ATCC 25922	*P. aeruginosa* ATCC 27853
Oxidase	*P. aeruginosa* ATCC 27853	*E. coli* ATCC 25922
DNAse/Latex agglutination	*S. aureus* ATCC 25923	*Staphylococcus epidermidis ATCC 12228*

Appendix G: Plagiarism/turn-it-in report cover page



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