1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

Health care workers are potential source of nosocomial infections, because hands and other medical devices such as electronic thermometers, blood pressure cuffs, stethoscopes, latex gloves, and white coats can transmit many pathogens (Uneke *et al.*, 2010).

The development of nosocomial infection may be due to multiple causes like development and persistence of multidrug resistant (MDR) bacteria, immunocompromised states of patients, and mechanical transmission of agents from one patient to another. Transmission of microorganisms through contaminated medical devices is always a possibility because of their contact with patient bodies (Gupta *et al.,* 2014).

Sterilization of equipment and the disinfection of medical devices before interventions are usually ignored. Among those equipment, it is found that stethoscopes might have a role in the transmission of microorganisms from patient to patient (Kilic *et al.,* 2011).

In developed countries, between 5% and 10% of patients acquire one or more infection during hospitalization at least 72 hours after admission (Lazzari *et al.*, 2004).

Stethoscopes acquire microorganisms after contact with a patient; these organisms must then survive on the object for at least several minutes and be transferred to the skin of a second patient during subsequent use (Longtin *et al.*, 2014).

There are increasing reports of the risk of transmitting antibiotic resistant microorganisms from one patient to another on stethoscopes. These antibiotic-resistant organisms are capable of initiating severe infections in a hospital environment and could require contact isolation and aggressive treatment to prevent the spread of the organisms. Examples of such antibiotic-resistant organisms are ceftazidime-resistant *Klebsiella pneumoniae*, vancomycin-resistant Enterococci, methicillin- resistant Staphylococci, ciprofloxin-resistant *Pseudomonas aeruginosa*, gentamicin-resistant *Pseudomonas aeruginosa* (Uneke *et al*., 2010).

Many types of pathogens have been isolated from stethoscope, these pathogens include vancomycin resistant *Enterococcus* spp., methicillin resistant and sensitive *Staphylococcus* spp., and multidrug resistant *Pseudomonas aeruginosa*, *Escherichia coli, Klebsiella* spp., and *Streptococcus* spp. *(*Shiferaw *et al.,* 2013).

1.2. Rationale

Stethoscopes harbor microorganisms after contact with a patient skin; these organisms might be transferred to the skin of a second patient during subsequent

use. The nosocomial infection is a serious issue and must prevented by determining the source that facilitated the transmission of an infectious agent to a patient, stethoscope might be one of the sources that cause nosocomial infection. Therefore screening and awareness about microorganisms in the stethoscopes is essential to minimize transmission of nosocomial infections.

Despite a wide use of stethoscopes in Sudanese hospitals, this topic remains untouched. This study is expected to highlight the problem of contamination of such an important device.

1.3. Objectives

1.3.1. General objective

To assess Gram-negative bacterial contamination on stethoscopes

1.3.2. Specific objectives

To determine the frequency of bacterial contamination of stethoscopes.

To determine the bacterial load on the stethoscopes.

To identify Gram-negative bacterial species on stethoscopes.

2. LITERATURE REVIEW

Nosocomial infections are a serious problem in all hospitals. Semmelweis in 1861 showed that bacteria were transmitted to patients by contaminated hands of healthcare workers (Jeske *et al.,* 2007).

A prevalence survey was conducted under auspices of the World Health Organization (WHO) on 55 hospitals from 14 countries representing 4 regions. This survey revealed that an average of 8.7% of hospitalized patients developed hospital acquired infections (Talaat *et al.,* 2006).

In developed countries, between 5% and 10% of patients acquire one or more infection during hospitalization at least 72 hours after admission (Lazzari *et al.*, 2004).

Hospital acquired infections are associated with high mortality and morbidity rate and excess health care cost. Survey studies conducted, reported that these rates decreased by keeping hospital infections under control (Kilic *et al.,* 2011).

Health care workers are potential source of nosocomial infections, because hands and other medical devices such as electronic thermometers, blood pressure cuffs, stethoscopes, latex gloves, and white coats can transmit many pathogens (Storm *et al.,* 2004; Kotsanas *et al.,* 2008; Uneke *et al.,* 2009).

It is generally recognized that most physicians and nurses do not disinfect their stethoscopes frequently; less than once a month, if at all. Most surveys supported this perception and revealed that 70% to 90% of the physicians did not disinfect systematically their stethoscope after every patient contact (Muniz *et al.,* 2012).

The most common mode of transmission of exogenous nosocomial pathogen is hand carriage and medical accessories that were used by doctors and other medical personal (Pandey *et al.*, 2010).

Stethoscopes harbor potential pathogens capable of surviving on their surfaces. Therefore, health professionals can act as a vector for transmission of disease (Gupta *et al.*, 2014).

Stethoscopes acquire microorganisms after contact with a patient; these organisms must then survive on the object for at least several minutes and be transferred to the skin of a second patient during subsequent use (Pittet *et al.,* 2012; Longtin *et al.*, 2014).

The cultivation of swabs taken from stethoscope membrane showed that these health care tools might play a role in spreading of microbial flora and other potential pathogenic microorganism such as methicillin resistant *Staphylococcus aureus* (Madar *et al.*, 2005).

The emergence of antimicrobial resistance and nosocomial outbreaks are most important issues that are associated with hospital acquired infections. Urinary

tract, lower respiratory tract, blood, and surgical wounds are most frequent types of nosocomial infections. The source of infectious agents and the route of transmission are important elements in transmission of infection in hospital settings (Teng *et al.*, 2009).

Stethoscopes have always been part of the physician's basic paraphernalia when examining patients. It has recently been shown to harbor various organisms on their diaphragm surfaces with coagulase negative staphylococci as the predominant isolate. Other organisms isolated were *Staphylococcus aureus*, *Corynebacterium* spp., *Bacillus* spp., *Neisseria* spp., alpha-hemolytic streptococci, *Micrococcus luteus*, *Enterococcus* spp., *Candida* spp., Gram-negative organisms and *Aspergillus* spp. (Francis *et al.*, 2000).

Antibiotic sensitivity assessment of organisms that were isolated from stethoscope showed that the bacterial isolates were resistant to nearly all the antibiotics tested. The bacterial isolates were, however, completely susceptible to gentamicin and ampicillin and showed significant susceptibility to ciprofloxacin and chloramphenicol (Uneke *et al.*, 2010).

Infection prevention protocols are effective in reducing the health care associated infection, the use of isopropyl alcohol found to be effective in reducing contamination of stethoscope and other medical devices (Nelson *et al.*, 2013).

A study that conducted at the University of Geneva Hospitals Switzerland compared between the contamination level of hands, stethoscope and other medical accessories showed that the contamination level of the diaphragm was lower than the contamination level of the fingertips (Longtin *et al.*, 2014).

Kilic *et al.*, (2011) reported that stethoscopes were contaminated with microorganisms (67%) and that 15 (16.3%) out of 92 had potential pathogens including methicillin sensitive *Staphylococcus aureus* (5), methicillin resistant *Staphylococcus aureus* (4), *Escherichia coli* (3), *Acinetobacter baumannii*, *Acinetobacter haemolyticus,* and *Enterococcus* spp.

The study of Marinella *et al.*, (1997) has shown that 100% of stethoscopes sampled from the health care workers at the University of Michigan Medical Center were contaminated with coagulase negative staphylococci and other bacteria.

In a study that carried out at the Conjunto Hospitalar de Sorocaba, a tertiary care hospital, samples were taken randomly from 300 stethoscopes used by medical staff, medical residents, medical students nurses, and nursing school students, and other sectors of the hospital. It showed that there was no significant association between the most predominant microorganisms and the professional category, or whether the user was under training or not. Of the 300 stethoscopes sampled, 87% were contaminated. Among the contaminated stethoscopes, 96% contaminated more than

one microorganism. The microorganisms isolated were *Staphylococcus aureus* (176), coagulase negative Staphylococci (153), yeasts (148), *Sarcina* (64), *Bacillus* spp. (45), *Streptococcus* spp. (7), *Acinetobacter* spp. (2), *Pseudomonas putida* (1) and *Klebsiella pneumoniae* (1) (Maluf *et al.,* 2002).

A study that conducted at Ebonyi State University Teaching Hospital and its training extension facility in southeastern Nigeria. After intervention, 89 health workers were requested to make available their stethoscopes for screening and all consented, giving a response rate of 100%. Of the 89 stethoscopes screened, *Staphylococcus aureus* (44.4%) and *Escherichia coli* (50%) were isolated (Uneke *et al.,* 2010).

Murguia *et al.,* (2014) examined 112 stethoscopes from 12 hospital departments at The General Regional Hospital of Leon institution in central Mexico, 58 contained organisms considered as skin contaminants with low pathogenic potential. Of the 48 remaining stethoscopes, 50 microorganisms with pathogenic potential were isolated, 3 of these were Gram-negative bacilli (*Klebsiella pneumoniae subsp. ozaenae*, *Acinetobacter baumannii*, and *Burkholderia cepacia*), 4 were *Enterococcus faecalis* and 43 were *Staphylococcus aureus*, 18 of which were identified as methicillin resistant *Staphylococcus aureus*.

Shiferaw *et al.,* (2013) concluded that 151 (85.8%) of 176 stethoscope were considerably contaminated and the rest 25 were not contaminated (14.2%). From the 151 contaminated stethoscope diaphragms, 256 bacterial strains were isolated.

Majority of the isolates (52%) were found to be potential pathogens, Coagulase-negative staphylococci were the most frequent isolates (40.2%) among Gram-positive bacteria followed by *Staphylococcus aureus* (30.9%) and *Bacillus* spp. (5.5%). Of the Gram-negative isolates, *Klebsiella* spp. (4.7%) the most common isolates followed by *Citrobacter* spp. (4.3%), *Salmonella* spp. (3.5%), *Proteus* spp. (3.5%), *Enterobacer* spp. (3.1%), *Pseudomonas aeruginosa* (1.2%), and *Escherichia coli* (0.8%).

Another study that was conducted at Ebonyi State University for medical students at Nigeria, 201 students participated in the study and the result showed higher proportion of contamination from male individuals above 40 years old and student who were married. The result showed that the highest colonization among stethoscope cleaned only with water (78.6%) or had never been washed and cleaned with any agents or even with water (89.9%), the examination of stethoscope showed high percentage of *Staphylococcus aureus* (32.8%) followed by *Pseudomonas aeruginosa* (24.2%), *Enterobacer faecalis* (9,9%), and *Escherichia coli* (5.6%) (Uneke *et al.,* 2009).

Bernard *et al.*, (2011) sampled and analyzed 355 stethoscopes; 78% of which were used by physicians, students, or nurses. The average stethoscope age was 4 years and its surface area was 3.5 cm^2. Stethoscopes were used 6 times per day in 53% of the cases. Questions about cleaning practices revealed that only 22% of users

regularly cleaned the membrane with liquid soap or 70% alcohol, and 11% of doctors warmed the membrane before auscultation with hand or laboratory coat. One hundred ninety-two stethoscopes (54%) were colonized with 20 CFUs per membrane, and 63 (18%) carried >100 CFU per membrane. Among the 355 stethoscopes, 234 had 2 different bacterial species, and up to 5 different bacterial species could be found on a membrane. Three hundred stethoscopes (85%) were colonized with nonpathogenic or weakly pathogenic bacteria, mainly coagulase-negative staphylococci (315), *Micrococcus luteus* (213), and *Bacillus* spp. (86). Potentially pathogenic bacteria were found on 31 stethoscopes (9%) and they were *Staphylococcus aureus* 15, *Acinetobacter* spp. 11, *Enterobacter* spp. 8, *Escherichia coli* 2, *Klebsiella* spp. 2, *Stenotrophomonas maltophilia* 2, there was a single isolate of methicillin-resistant *Staphylococcus aureus* and no multidrug-resistant strains were detected.

Pandey *et al.*, (2010) found that stethoscopes were colonized with various microorganisms. *Staphylococcus* spp. were the predominant isolates in (27.98%). Coagulase-negative Staphylococci were 15.50% and *Staphylococcus aureus* was 12.3%. Of the *Staphylococcus aureus* isolated, 7.3% were methicillin-resistant. *Escherichia coli* was the predominant bacteria among the Gram-negative bacterial flora (9.17%). The other were *Acinetobactor* spp. (4.58%), *Pseudomonas aeruginosa* (3.6%) and *Klebsiella* spp. (2.06%)

A study conducted by the Department of Microbiology in a tertiary care teaching hospital confirmed that stethoscopes used by healthcare workers were contaminated with pathogenic as well as nonpathogenic microorganisms which could be transmitted to consecutive patients. The pathogenic microorganisms included *Staphylococcus aureus*, *Acinetobacter* spp., *Citrobacter* spp., *Pseudomonas stutzeri*, *Bacillus* spp., and *Aspergillus fumigatus*. Among the potential pathogenic organisms, coagulase-negative staphylococci were isolated (Gupta *et al.*, 2014).

The isolation of Gram-negative organisms poses a real risk of spreading potentially serious infections, especially in settings of intensive care departments. Gram-negative organisms were isolated from nine different samples (21%) including one isolates of *Acinetobacter baumannii* from Forty-three stethoscopes belonging to senior physicians, residents, interns and medical students at the paediatric ward (Youngster *et al.*, 2008).

3. MATERIALS AND METHODS

3.1. Study Design

3.1.1. Type of study

This is a descriptive cross-sectional study conducted to assess Gram-negative bacteria on stethoscopes in Khartoum state hospitals.

3.1.2. Study area

The study was conducted in Omdurman Military Hospital, Omdurman Teaching Hospital, Khartoum North Teaching Hospital and Ibrahim Malik Teaching Hospital.

3.1.3. Study duration

The study was conducted in the period from March to June 2014.

3.2. Laboratory procedure

The laboratory procedures such as sample collection, sample processing, culture, microscopic examination and conventional biochemical tests were used to determine colony count, isolation and identification of indicator organisms and selected pathogens.

3.2.1. Sampling technique and sample collection

3.2.1.1. Sampling technique

Two hundred stethoscope swabs were collected randomly from different types of stethoscopes.

3.2.1.2. Sample collection

The interior and exterior part of each diaphragm of stethoscope was swabbed with a sterile swab moistened in sterile normal saline before sampling. The swabs were immediately transferred into sterile containers contain 2ml normal saline to the laboratory for processing.

3.2.2. Bacterial load

Ten-fold serial dilutions of each sample was made using sterile normal saline as diluents.

a. nine milliliter of sterile normal saline were placed in four sterile glass test tubes 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}, from the well mixed sample using vortex 1ml was taken and added to the 9 ml in first tube after that serial dilution was made.

b. 1ml of the dilution from the second (10^{-2}), third (10^{-3}) and fourth(10^{-4}) was placed into sterile Petri dishes.

c. about 15 ml of molten clear nutrient agar was added to each plate with temperature 45 ^{o}C.

d. each plate was mixed well by moving it five times in a vertical, clockwise, horizontal and anticlockwise direction.

e. all plates were incubated at 37 ^{o}C, for 24 hr (Mackie and McCartney, 1998).

3.2.3. Calculation

All of the petri plates containing between 30 and 300 colonies were selected. Plates with more than 300 colonies was not counted and are designated too many to count (TMTC). Plates with fewer than 30 colonies were designated too few to count (TFTC). The colonies were calculated on each plate. The number of bacteria calculated as colony forming unit (CFU) per milliliter dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar (Mackie and McCartney, 1998).

The calculation formula as follow:

CFU× dilution factor×1/aliquot = CFU/ml

CFU/ml × 2 = CFU/diaphragm

Two means the volume by milliliter of total sample.

3.2.4. Identification of bacteria

Identification of bacteria was based on staining reaction, organism morphology, growth condition, colonial appearance on media, and biochemical characteristics.

3.2.4.1. Gram stain

3.2.4.1.1. Smear preparation

Using a wire loop sterilized by flame, the smears were prepared; the dried smears were fixed by passing three times over the flame

3.2.4.1.2. Staining procedure

a. The fixed smears were covered with crystal violet solution for one minute.

b. The smears were washed with tap water, and covered with gram Iodine solution for one minute.

c. The smears were washed with acetone alcohol solution for few seconds, then washed with tap water.

d. The smears were then covered with safranine solution for two minutes. washed with tap water, and allowed to dry.

e. The smears were examined under oil immersion lenses.

3.2.4.2. Biochemical tests

The organisms were finally identified using conventional biochemical tests.

3.2.4.2.1. Citrate utilization test

The measurement of this characteristic is important in identification of Entetobacteriaceae. Utilization of citrate by tested bacteria was detected in Simmons citrate medium by the production of alkaline byproducts. The medium

contained sodium citrate as sole source of carbon and ammonium phosphate as sole source of nitrogen. Bacteria that can use citrate can also extrat nitrogen from ammonium salt with production of ammonia. A well-isolated colony was picked from the surface of the medium and inoculated as a single streak on the slant surface of citrate agar tube. The tube was incubated at 35°C for 24 to 48 hours. A positive color was represented by the development of deep blue color within 24 to 48 hour (Koneman *et al.*, 2006).

3.2.4.2.2. Indole test

Indole production is an important characteristic in the identification of many species of microorganisms. Indole is one of metabolic degradation products of the amino acid tryptophan. The test is based on the formation of a red complex when indole reacts with aldehyde group of p-dimethylaminobenzaldehyde which is the active chemical in kovac reagent. Tryptophan broth was inoculated with the test organism and incubated at 37°C for 18 to 24 hour. At the end of incubation period few drops of kovac reagent were added, and the development of bright fuchsia color at the interface of the reagent and broth within seconds after adding the reagent was an indicator of presence of indole (Koneman *et al.*, 2006).

3.2.4.2.3. Oxidase test

The oxidase test is a test used to determine the bacteria that produce certain cytochrome oxidases enzyme. By using disks impregnated with a reagent such as tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD) which is a redox

indicator, the reagent turns dark-blue to maroon color when oxidized, and colorless when reduced. Oxidase-positive bacteria possess cytochrome oxidase or indophenol oxidase these both enzymes catalyze the transport of electrons from donor compounds (NADH) to electron acceptors (oxygen). The test reagent, TMPD acts as an artificial electron donor for the enzyme oxidase. The oxidized reagent forms the colored compound indophenol blue. The test was performed by using commercial disks impregnated with TMPD reagent**.** A pure colony was smeared on the disc with a sterile wooden stick. A positive reaction was indicated by developing deep blue color in 10 to 60 seconds (Koneman *et al.,* 2006).

3.2.4.2.4. Urease test

The test was used to determine the ability of organisms to produce the enzyme urease, which hydrolyzes urea. Hydrolysis of urea produces ammonia and Co_2, the formation of ammonia alkalinizes the medium and the pH shift was detected by the color change of phenol red from light orange to magenta which indicated a positive result. A well-isolated colony was picked from the surface of the medium and inoculated as single streak on the slant surface of Christensen's urea agar (Bailey and Scott's *et al.,* 2007).

3.2.4.2.5. Sugar fermentation and H_2S production

The fermentation of sugars, and production of hydrogen sulphide and gas was carried out by using kligler iron agar.

Kligler iron agar was inoculated using a straight wire, by stabbing the butt first and streaking the slope in the direction of removing the wire. After overnight incubation, the results were noted. Lactose fermentation was indicated by a yellow slope yellow butt. Red slope and a yellow butt was indicated glucose fermentation, blackening of the medium indicated H_2S production (Cheesbrough Monica, 2000)

3.3. Quality control

The quality of study was kept by preparing and using standard operational procedures for laboratory investigation and media preparation. Sample collection and processing were carried out using aseptic techniques. The samples were labeled properly, cultured and the bacterial count were determined by experienced laboratory personal. The performance and sterility test of prepared media were also checked.

3.4. Statistical analysis

The statistical analysis was performed using the statistical package for social sciences (SPSS).

4. RESULTS

Two hundred stethoscopes were processed for bacterial contamination, load of the bacteria and the type of contaminated bacteria.

Forty samples were collected from Omdurman Military Hospital, sixty samples from Omdurman Teaching Hospital, fifty from Khartoum North Teaching Hospital and fifty from Ibrahim Malik Teaching Hospital Khartoum (Figure 1).

The average of contamination in these hospitals is 126×10^4 CFU/diaphragm. The mean of contamination in Omdurman Military Hospital was 56×10^4 CFU/diaphragm. In Khartoum North Teaching Hospital 132×10^4 CFU/diaphragm, In Omdurman Teaching Hospital 150×10^4 CFU/diaphragm and 166×10^4 CFU/diaphragm in Ibrahim Malik Teaching Hospital (Table 1).

One hundred seventy nine (89.5%) of 200 stethoscopes tested were contaminated with microorganism (Table 2). Thirty-eight was identified as Gram-negative (19%) bacteria and the rest as Gram-positive bacteria (70.5%).

From thirty eight Gram-negative bacteria, 11 *klebsiella pneumoniae* (5.5%), 12 *pseudomonas aeruginosa* (6%), 10 *Escherichia col*i (5%), 5 *proteus* spp. (2.5%) was recovered (Table 6).

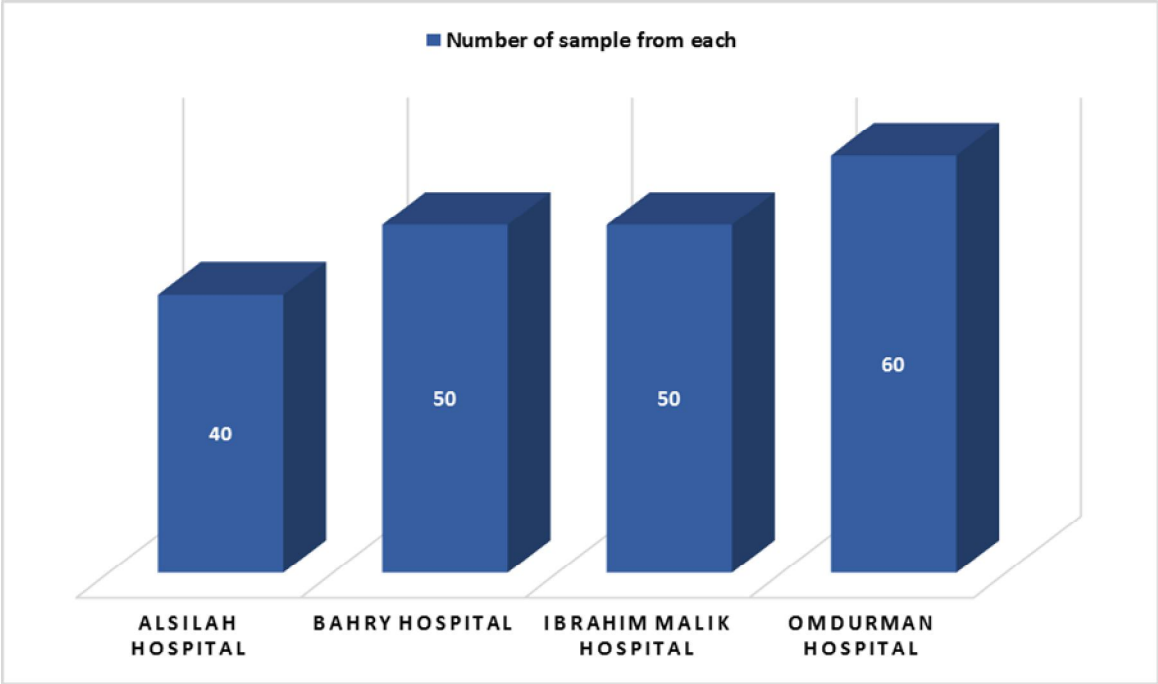

Figure 1. Shows the number of sample from each hospital.

Table 1. Average of contamination in Khartoum state hospitals

	Average of contamination		
	CFU/ml	CFU/diaphragm	CFU/cm²
Omdurman Military Hospital	28×10^4	56×10^4	4×10^4
Khartoum North Teaching Hospital	66×10^4	132×10^4	9×10^4
Ibrahim Malik Teaching Hospital	83×10^4	166×10^4	12×10^4
Omdurman Teaching Hospital	75×10^4	150×10^4	10×10^4
Total	63×10^4	126×10^4	8×10^4

Table 2. Number and percentage of growth.

Sample with	Number	Percentage
Positive growth	**179**	**89.5%**
Negative growth	**21**	**10.5%**
Total	**200**	**100%**

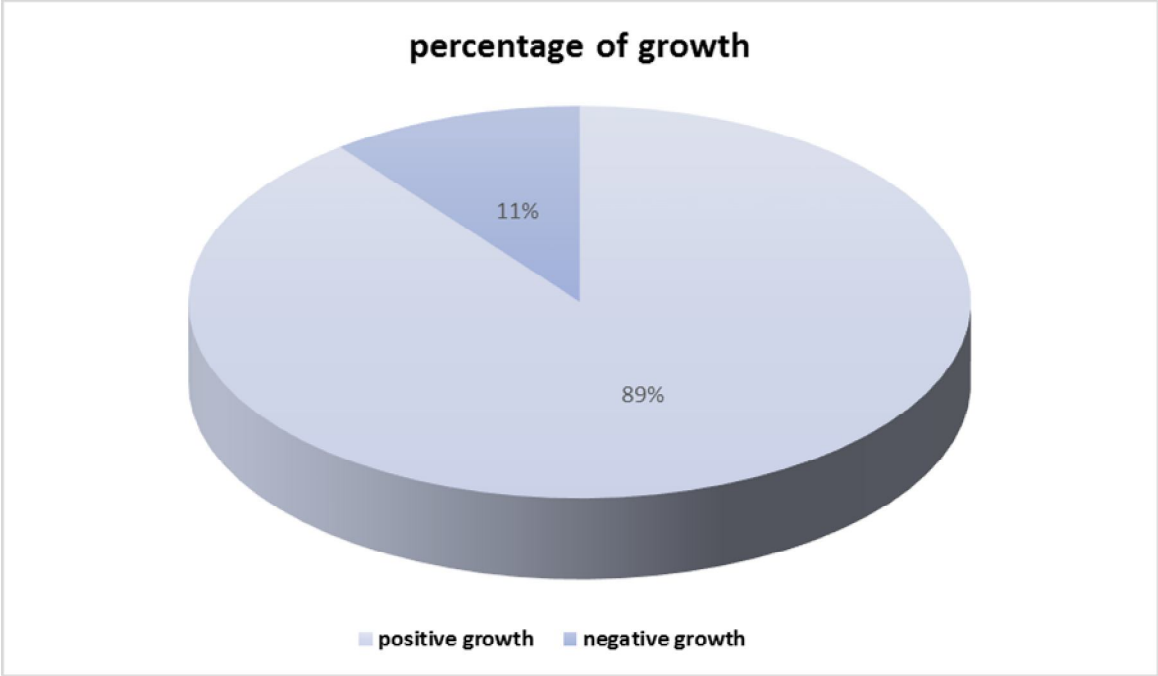

Figure 2. Shows the percentage of sample with positive growth

Table 3. Number and percentage of sample with positive growth from each hospital.

	Number of positive sample	**Percentage**
Omdurman Military Hospital	37	18.5%
Khartoum North Teaching Hospital	48	24%
Ibrahim Malik Teaching Hospital	45	22.5%
Omdurman Teaching Hospital	49	24.5%
Total	179	89.5%

Table 4. Number and percentage of sample with no growth from each hospital.

	Number of sample with no growth	**Percentage**
Omdurman Military Hospital	3	1.5%
Khartoum North Teaching Hospital	2	1%
Ibrahim Malik Teaching Hospital	5	2.5%
Omdurman Teaching Hospital	11	5.5%
Total	21	10.5%

Table 5. Number and percentage of Gram-negative bacteria in sample from each hospital.

	Number	Percentage
Omdurman Military Hospital	4	2%
Khartoum North Teaching Hospital	15	7.5%
Ibrahim Malik Teaching Hospital	9	4.5%
Omdurman Teaching Hospital	10	5
Total	38	19%

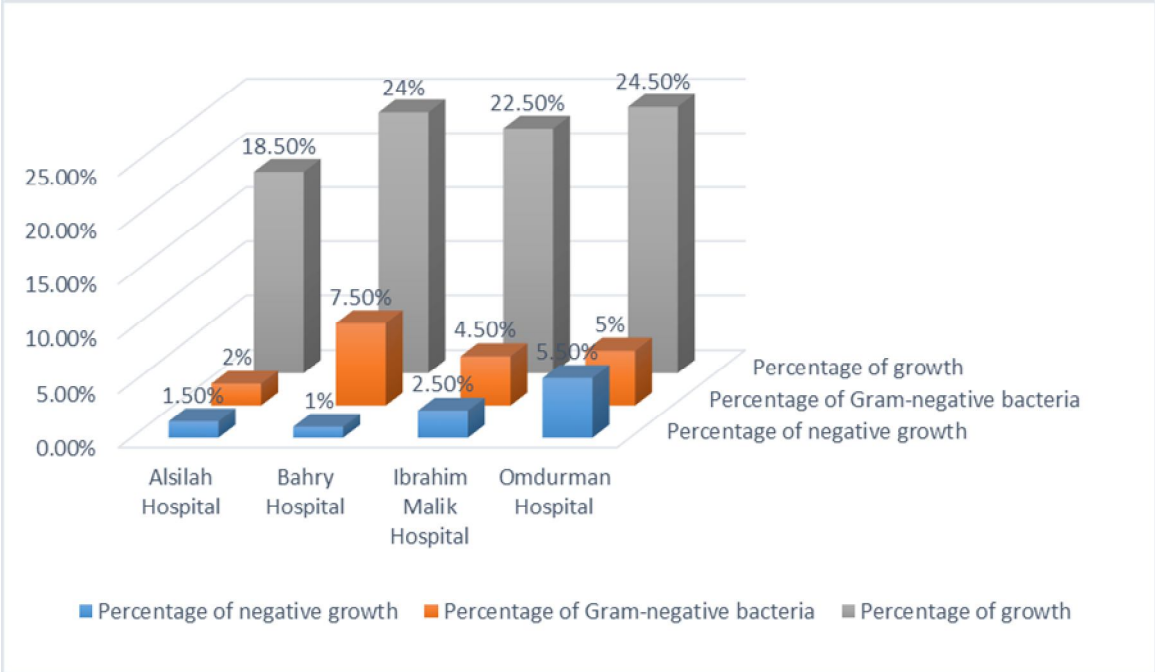

Figure 3. Shows percentage of Gram-negative bacteria, and samples with negative and positive growth from each hospital

Table 6. Number and Percentage of isolated bacteria

Bacterial isolates detected	Number	Percentages
*Escherichia col*i	10	5%
Klebsiella pneumoniae	11	5.5%
Proteus spp.	5	2.5%
Pseudomonas aeruginosa	12	6%

5. DISCUSSION

5.1. Discussion

Use of medical devices for management diseases may contribute in the development of hospital-acquired infections. Almost all health care workers (HCW) and medical students do not follow the standard protocol to prevent infections in using crucial medical equipment like stethoscopes.

Our study showed a low percentage (19%) of Gram-negative bacteria which is consistent with previous studies reported by Maluf *et al.,* (2002), Shiferaw *et al.,* (2013) and Gupta *et al.,* (2014). Since normal skin flora consists primarily of Gram-positive bacteria, it is not surprising that so few Gram-negative bacteria were isolated.

The result of this study revealed that as many as 89.5% of the stethoscopes were contaminated by bacteria which is comparable to the observations of previous studies by Marinella *et al.,* (1997), Youngster *et al.,* (2008), Kilic *et al.,* (2011) and Shiferaw *et al.,* (2013) who found 71% to 100% of stethoscopes were colonized by various bacteria. Which is not surprising since most of the doctors and nurses usually do not clean their stethoscope. Although most of the organisms isolated in these studies were considered nonpathogenic, a significant percentage of the isolates were potentially pathogenic. The implication of the findings is that stethoscopes may play an important role in the transmission of potential

pathogenic microorganisms, as well as in the spread of antibiotic-resistant strains in the hospital environment.

Furthermore; in this study the mean total bacterial count was 126×10^4 CFU/ diaphragm which is higher in comparison with previous study reported by Shiferaw *et al.,* (2013) (1.44×10^4 CFU/diaphragm), there could be a variety of reasons for the differences among them, possibly differences in hygiene practices.

Many of the microorganisms isolated from the stethoscopes in this study such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus* spp., are known to cause serious infections in hospitalized patient populations. Pandey *et al.,* (2010) agree with this observation.

Klebsiella pneumoniae was the predominant isolates in Gram-negative bacteria followed by *Escherichia coli* which is not consistent with Pandey and his colleagues who found that *Escherichia coli* the predominant one.

The spectrum of organisms isolated in this study was also reported in previous studies (Pandey *et al.,* (2010), Bernard *et al.,* (2011), Shiferaw *et al.,* (2013)) on bacteria isolated from stethoscopes.

Klebsiella pneumoniae, Pseudomonas aeruginosa, *Escherichia coli* and *Proteus* spp. were the common Gram-negative isolates in present and previous investigations of medical equipment and hospital environment; it is consistent with Uneke *et al.,* (2009).

Although the study did not show that stethoscopes can transmit infections, it show stethoscopes were contaminated with pathogenic bacteria and that poor stethoscope cleaning/disinfection practices were significantly associated with this contamination.

Strategies to minimize the transmission of infection from stethoscopes have been proposed, including the use of disposable stethoscopes, especially for clinical high-risk environments, and the use of a single-use silicone membrane over the stethoscope head to create a prophylactic barrier.

5.2. Conclusion

The study concluded that the stethoscopes are highly contaminated (89.5%) , and may be important in the spread of infectious agents.

5.3. Recommendations

1. Systematic disinfection of stethoscopes with 70% alcohol or liquid soap or the use of disposable covers should be recommended to minimize the chance of spreading infectious agents between hospitalized patients.

2. The practice of using disposable stethoscopes, especially for clinical high-risk environments, and the use of a single-use silicone membrane over the stethoscope head to create a prophylactic barrier should be adopted.

3. It is particularly interesting that stethoscopes designated for single room use might had fewer contaminated stethoscopes than other groups. The practice of using a single stethoscope in designated rooms should be supported.

4. Prospective studies to determine the benefit of regular disinfection are warranted. Further studies are required to validate the results of the present study.

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APPENDICES

Appendix 1

Reagents preparation:

a. Acetone alcohol

Equal volume of ethanol (95%) and acetone solution are mixing.

b. Crystal violet stain

Crystsl violet 20g in absolute ethanol 195ml, 9g of ammonium oxalate in 200ml of distilled water mixed together with stain solution, the volume completed with distilled water until one liter.

c. Gram iodine

Potassium iodide 20g and iodine10g dissolved in one liter distilled water.

d. Kovac reagent

P-Dimethylaminobenzaldehyde 10gm in amyl or isoamyl alcohol 150ml, the volume completed to 200 by adding concentrated HCL.

e. Normal saline (90% physiological saline)

9g salt in 70ml distilled water as the salt dissolve completely the volume complete to 100 ml.

f. Safranine solution

25g of safranine powder in 100ml of 95% ethanol alcohol.

Appendix 2

Media preparation:

a. **Indole medium**

Formula of tryptophan broth

Peptone or pancreatic digest of casein	2gm
Sodium chloride	0.5gm
Distell water	100 ml

Preparation

Dissolve the ingredients in water by heating.

Autoclave for 15 minutes at 121 ± 3°

Dispense in a test tubes

b. **Kligler iron agar**

Formula / Liter

Enzymatic Digest of Casein	10gm
Enzymatic Digest of Animal Tissue	10gm
Lactose	10gm

Dextrose	1gm
Ferric Ammonium Citrate	0.5gm
Sodium Chloride	5gm
Sodium Thiosulfate	0.5gm
Phenol Red	0.025gm
Agar	15gm

Preparation

52 grams of the medium in one liter of distilled water. Sterilize at 121º C (15lbs. pressure) for 15 minutes in autoclave. Cool and pour the media in a slanted position so to obtain butts of 1’5-2 cm. Depth.

c. Nutrient agar

15g nutrient agar powder in one liter of distilled water, then sterilized by autoclaving at121^{o}C for 15minutes. Cooled to about 50^{o}C and poured into sterile petri dishes in 15ml amount. The poured media left to solidify at room temperature.

d. Urea agar

Enzymatic Digest of Gelatin	1gm
Dextrose	1gm
Sodium chloride	5gm
Monopotassium Phosphate	2gm
Urea	20gm
Phenol red	0.012gm
Agar	15gm

Preparation:

29 g of the urea base in 100 mL of purified water until dissolved completely. Autoclave at 121°C for 15 minutes. Cool sterilized agar to 45 - 50°C and aseptically add the sterile Urea Agar Base. Then mixed thoroughly and dispense into sterile tubes in a slanted position

e. Simmons citrate agar

Formula per litter

Magnesium sulphate	0.2gm
Ammonium dihydrogen phosphate	1gm

Dipotassium phosphate	1gm
Sodium citrate	2gm
Sodium chloride	5gm
Bromothymol blue	0.08gm
Agar	15gm

Preparation

Suspend 24.28 grams in 1000 ml distilled water. Heat, to boiling, to dissolve the medium completely. Mix well and distribute in tubes or flasks. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Final pH is 6.8.

Appendix 3

Count			
Sample code	CFUL/ml	CFU/diaphragm	CFU/cm²
1	33×10^4	66×10^4	4.4×10^4
2	13×10^4	26×10^4	1.73×10^4
3	18×10^4	36×10^4	2.4×10^4
4	10×10^4	20×10^4	1.3×10^4
5	11×10^4	22×10^4	1.47×10^4
6	–	–	–
7	30×10^4	60×10^4	4×10^4
8	19×10^4	38×10^4	2.53×10^4
9	18×10^4	36×10^4	2.4×10^4
10	5×10^4	10×10^4	0.67×10^4
11	9×10^4	18×10^4	1.2×10^4
12	12×10^4	24×10^4	1.6×10^4
13	11×10^4	22×10^4	1.47×10^4
14	135×10^4	270×10^4	18×10^4
15	13×10^4	26×10^4	1.73×10^4
16	26×10^4	52×10^4	3.47×10^4
17	14×10^4	28×10^4	1.87×10^4
18	6×10^4	12×10^4	0.8×10^4
19	8×10^4	16×10^4	1.07×10^4
20	–	–	–

21	15×10^{4}	30×10^{4}	2×10^{4}
22	–	–	–
23	15×10^{4}	30×10^{4}	2×10^{4}
24	6×10^{4}	12×10^{4}	0.8×10^{4}
25	2×10^{4}	4×10^{4}	0.27×10^{4}
26	8×10^{4}	16×10^{4}	1.07×10^{4}
27	7×10^{4}	14×10^{4}	0.93×10^{4}
28	8×10^{4}	16×10^{4}	1.07×10^{4}
29	80×10^{4}	160×10^{4}	10.7×10^{4}
30	20×10^{4}	40×10^{4}	2.67×10^{4}
31	9×10^{4}	18×10^{4}	1.2×10^{4}
32	9×10^{4}	18×10^{4}	1.2×10^{4}
33	4×10^{4}	8×10^{4}	0.35×10^{4}
34	293×10^{4}	586×10^{4}	39.07×10^{4}
35	97×10^{4}	194×10^{4}	12.93×10^{4}
36	21×10^{4}	42×10^{4}	2.8×10^{4}
37	6×10^{4}	12×10^{4}	0.8×10^{4}
38	17×10^{4}	34×10^{4}	2.27×10^{4}
39	12×10^{4}	24×10^{4}	1.6×10^{4}
40	8×10^{4}	16×10^{4}	1.07×10^{4}
41	167×10^{4}	334×10^{4}	22.27×10^{4}
42	99×10^{4}	198×10^{4}	13.2×10^{4}
43	257×10^{4}	514×10^{4}	34.27×10^{4}

44	332×10^{4}	664×10^{4}	44.27×10^{4}
45	563×10^{4}	1126×10^{4}	75.07×10^{4}
46	218×10^{4}	436×10^{4}	29.07×10^{4}
47	189×10^{4}	378×10^{4}	25.2×10^{4}
48	153×10^{4}	306×10^{4}	20.4×10^{4}
49	74×10^{4}	94×10^{4}	6.27×10^{4}
50	22×10^{4}	44×10^{4}	2.93×10^{4}
51	50×10^{4}	100×10^{4}	6.67×10^{4}
52	27×10^{4}	54×10^{4}	3.6×10^{4}
53	6×10^{4}	12×10^{4}	0.8×10^{4}
54	–	–	–
55	6×10^{4}	12×10^{4}	0.8×10^{4}
56	9×10^{4}	18×10^{4}	1.2×10^{4}
57	5×10^{4}	10×10^{4}	0.67×10^{4}
58	4×10^{4}	8×10^{4}	0.53×10^{4}
59	3×10^{4}	6×10^{4}	0.4×10^{4}
60	5×10^{4}	10×10^{4}	0.67×10^{4}
61	7×10^{4}	14×10^{4}	0.93×10^{4}
62	35×10^{4}	70×10^{4}	4.67×10^{4}
63	22×10^{4}	44×10^{4}	2.93×10^{4}
64	1×10^{4}	2×10^{4}	0.13×10^{4}
65	2×10^{4}	4×10^{4}	0.27×10^{4}
66	1×10^{4}	2×10^{4}	0.13×10^{4}

67	1×10^{4}	2×10^{4}	0.13×10^{4}
68	42×10^{4}	84×10^{4}	5.6×10^{4}
69	73×10^{4}	146×10^{4}	9.73×10^{4}
70	320×10^{4}	640×10^{4}	42.67×10^{4}
71	20×10^{4}	40×10^{4}	2.67×10^{4}
72	28×10^{4}	56×10^{4}	3.37×10^{4}
73	30×10^{4}	60×10^{4}	4×10^{4}
74	52×10^{4}	104×10^{4}	6.93×10^{4}
75	37×10^{4}	74×10^{4}	4.93×10^{4}
76	17×10^{4}	34×10^{4}	2.67×10^{4}
77	19×10^{4}	38×10^{4}	2.53×10^{4}
78	21×10^{4}	42×10^{4}	2.8×10^{4}
79	2×10^{4}	4×10^{4}	0.27×10^{4}
80	18×10^{4}	36×10^{4}	2.4×10^{4}
81	53×10^{4}	106×10^{4}	7.07×10^{4}
82	43×10^{4}	86×10^{4}	5.73×10^{4}
83	2×10^{4}	4×10^{4}	0.27×10^{4}
84	–	–	–
85	65×10^{4}	130×10^{4}	8.67×10^{4}
86	31×10^{4}	62×10^{4}	4.13×10^{4}
87	17×10^{4}	34×10^{4}	2.27×10^{4}
88	5×10^{4}	10×10^{4}	0.67×10^{4}
89	6×10^{4}	12×10^{4}	0.8×10^{4}

90	18×10^{4}	36×10^{4}	2.4×10^{4}
91	37×10^{4}	74×10^{4}	4.93×10^{4}
92	67×10^{4}	134×10^{4}	8.93×10^{4}
93	28×10^{4}	56×10^{4}	3.73×10^{4}
94	39×10^{4}	78×10^{4}	5.2×10^{4}
95	1×10^{4}	2×10^{4}	0.13×10^{4}
96	61×10^{4}	122×10^{4}	8.13×10^{4}
97	26×10^{4}	52×10^{4}	3.47×10^{4}
98	–	–	–
99	138×10^{4}	276×10^{4}	18.4×10^{4}
100	47×10^{4}	94×10^{4}	6.27×10^{4}
101	71×10^{4}	142×10^{4}	9.74×10^{4}
102	3×10^{4}	6×10^{4}	0.4
103	–	–	–
104	21×10^{4}	42×10^{4}	2.8×10^{4}
105	–	–	–
106	15×10^{4}	30×10^{4}	2×10^{4}
107	9×10^{4}	18×10^{4}	1.2×10^{4}
108	32×10^{4}	64×10^{4}	4.27×10^{4}
109	43×10^{4}	86×10^{4}	5.73×10^{4}
110	11×10^{4}	22×10^{4}	1.47×10^{4}
111	163×10^{4}	326×10^{4}	21.73×10^{4}
112	106×10^{4}	212×10^{4}	14.13×10^{4}

113	30×10^{4}	60×10^{4}	4×10^{4}
114	21×10^{4}	42×10^{4}	2.8×10^{4}
115	173×10^{4}	346×10^{4}	23.07×10^{4}
116	31×10^{4}	62×10^{4}	4.13×10^{4}
117	122×10^{4}	244×10^{4}	16.27×10^{4}
118	–	–	–
119	156×10^{4}	312×10^{4}	18.5×10^{4}
120	–	–	–
121	70×10^{4}	140×10^{4}	9.3×10^{4}
122	176×10^{4}	352×10^{4}	23.47×10^{4}
123	34×10^{4}	68×10^{4}	4.53×10^{4}
124	31×10^{4}	62×10^{4}	4.13×10^{4}
125	–	–	–
126	27×10^{4}	54×10^{4}	3.6×10^{4}
127	12×10^{4}	24×10^{4}	1.6×10^{4}
128	–	–	–
129	58×10^{4}	116×10^{4}	7.73×10^{4}
130	190×10^{4}	380×10^{4}	25.3×10^{4}
131	46×10^{4}	92×10^{4}	6.13×10^{4}
132	31×10^{4}	62×10^{4}	4.13×10^{4}
133	23×10^{4}	46×10^{4}	3.07×10^{4}
134	100×10^{4}	200×10^{4}	13.3×10^{4}
135	119×10^{4}	238×10^{4}	15.87×10^{4}

136	33×10^{4}	66×10^{4}	4.4×10^{4}
137	65×10^{4}	130×10^{4}	8.67×10^{4}
138	–	–	–
139	76×10^{4}	152×10^{4}	10.13×10^{4}
140	115×10^{4}	230×10^{4}	15.3×10^{4}
141	–	–	–
142	219×10^{4}	438×10^{4}	29.2×10^{4}
143	–	–	–
144	28×10^{4}	56×10^{4}	3.73×10^{4}
145	140×10^{4}	280×10^{4}	18.67×10^{4}
146	76×10^{4}	152×10^{4}	10.13×10^{4}
147	250×10^{4}	500×10^{4}	33.33×10^{4}
148	190×10^{4}	380×10^{4}	25.33×10^{4}
149	105×10^{4}	210×10^{4}	14×10^{4}
150	–	–	–
151	27×10^{4}	54×10^{4}	3.6×10^{4}
152	17×10^{4}	34×10^{4}	2.67×10^{4}
153	43×10^{4}	86×10^{4}	5.73×10^{4}
154	–	–	–
155	27×10^{4}	54×10^{4}	3.6×10^{4}
156	35×10^{4}	70×10^{4}	70×10^{4}
157	121×10^{4}	242×10^{4}	16.13×10^{4}
158	22×10^{4}	44×10^{4}	2.93×10^{4}

159	37×10^{4}	74×10^{4}	4.93×10^{4}
160	–	–	–
161	258×10^{4}	516×10^{4}	3.44×10^{4}
162	291×10^{4}	382×10^{4}	25.47×10^{4}
163	192×10^{4}	384×10^{4}	25.6×10^{4}
164	245×10^{4}	490×10^{4}	32.67×10^{4}
165	229×10^{4}	458×10^{4}	30.53×10^{4}
166	78×10^{4}	156×10^{4}	10.4×10^{4}
167	297×10^{4}	594×10^{4}	39.6×10^{4}
168	121×10^{4}	242×10^{4}	16.13×10^{4}
169	207×10^{4}	414×10^{4}	27.6×10^{4}
170	47×10^{4}	94×10^{4}	6.27×10^{4}
171	–	–	–
172	17×10^{4}	34×10^{4}	2.27×10^{4}
173	86×10^{4}	172×10^{4}	11.47×10^{4}
174	77×10^{4}	154×10^{4}	10.27×10^{4}
175	38×10^{4}	76×10^{4}	5.07×10^{4}
176	53×10^{4}	106×10^{4}	7.07×10^{4}
177	87×10^{4}	172×10^{4}	11.47×10^{4}
178	122×10^{4}	422×10^{4}	28.13×10^{4}
179	37×10^{4}	74×10^{4}	4.93×10^{4}
180	35×10^{4}	70×10^{4}	4.67×10^{4}
181	183×10^{4}	366×10^{4}	24.4×10^{4}

182	188×10^{4}	376×10^{4}	25.07×10^{4}
183	12×10^{4}	24×10^{4}	1.6×10^{4}
184	61×10^{4}	122×10^{4}	8.13×10^{4}
185	–	–	–
186	37×10^{4}	74×10^{4}	4.93×10^{4}
187	12×10^{4}	24×10^{4}	1.6×10^{4}
188	63×10^{4}	126×10^{4}	8.4×10^{4}
189	18×10^{4}	36×10^{4}	2.8×10^{4}
190	1×10^{4}	2×10^{4}	0.13×10^{4}
191	79×10^{4}	158×10^{4}	10.53×10^{4}
192	–	–	–
193	25×10^{4}	50×10^{4}	3.33×10^{4}
194	32×10^{4}	64×10^{4}	2.20×10^{4}
195	3×10^{4}	6×10^{4}	0.4×10^{4}
196	21×10^{4}	42×10^{4}	2.8×10^{4}
197	13×10^{4}	26×10^{4}	1.73×10^{4}
198	15×10^{4}	30×10^{4}	2×10^{4}
199	74×10^{4}	148×10^{4}	9.87×10^{4}
200	67×10^{4}	134×10^{4}	8.93×10^{4}

Appendix 4

Sample code	Isolated organism
7	*Klepsiella*
8	*Pseudomonas aeruginosa*
35	*Escherichia coli*
38	*Klepsiella pneumonia*
44	*Pseudomonas aeruginosa*
55	*Proteus* spp.
58	*Escherichia coli*
63	*Proteus* spp.
64	*Proteus* spp.
66	*Pseudomonas aeruginosa*
67	*Klepsiella pneumonia*
69	*Escherichia coli*
71	*Escherichia coli*
77	*Pseudomonas aeruginosa*
78	*Pseudomonas aeruginosa*
79	*Escherichia coli*
80	*Escherichia coli*
88	*Klepsiella pneumonia*
89	*Proteus* spp.
92	*Pseudomonas aeruginosa*
94	*Klepsiella pneumonia*
114	*Escherichia coli*

121	*Escherichia coli*
132	*Klepsiella pneumonia*
133	*Klepsiella pneumonia*
137	*Pseudomonas aeruginosa*
139	*Escherichia coli*
140	*Escherichia coli*
146	*Pseudomonas aeruginosa*
148	*Pseudomonas aeruginosa*
156	*Pseudomonas aeruginosa*
157	*Klepsiella pneumonia*
158	*Klepsiella pneumonia*
159	*Pseudomonas aeruginosa*
175	*Klepsiella pneumonia*
178	*Klepsiella pneumonia*
180	*Pseudomonas aeruginosa*
181	*Proteus* spp.
182	*Proteus* spp.