CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hospital acquired infection (Nosocomial infection) has been recognized for over a century as both a critical problem affecting the quality of health care and a leading cause of morbidity, mortality and increased health care cost (Bukharie*et al.,* 2004). Hospital environment is a reservoir of wide varieties of microorganisms (Schabrun and Chipchase, 2006).

Contaminated medical devices is possible source of transmission and outbreaks of hospital-acquired infections have been linked to devices such as stethoscopes, electronic thermometers, blood pressure cuffs, latex gloves, masks, neckties, pens, badges and lanyards, and white coats (Uneke*et al.,* 2010).

The first stethoscope invented by Rene TheophileHyacintheLaënnec (1781–1826) at the Necker-EnfantsMalades Hospital in Paris in 1816 to resolve the limitations of immediate auscultation. He was excited to discover that the heart sounds were clearly audible and this discovery later lead to the development of the first device specifically for this purpose (Roguin, 2006).

The stethoscope is commonly described as an instrument used by physician and other health professionals to hear the sounds made by heart, lungs or other various body organs, is used in hospitals by health care workers for assessing patient health, and have been reported as a potential vector for transmitting infections in the hospital environment in various parts of the world (ZulianiMaluf*et al.,* 2002).

The stethoscope is a tool healthcare providers use daily in the assessment of patients. Thus in a single day, the stethoscope may come in direct contact with multiple patients, clothing, and the environment. Following assessment, the stethoscope is typically placed in a laboratory coat pocket, draped around the neck, or suspended from a medication cart. The stethoscope is then taken to the next patient assessment without cleaning (Russell *et al.*, 2012). The device is directly contact with skin, following contact with infected skin, pathogens can attach and establish themselves on the diaphragms of stethoscopes and subsequently be transferred to other patients if the stethoscope is not disinfected (Uneke*et al.*, 2010).

Physicians should disinfect stethoscopes between one patient and the other, though unfortunately this good practice is not always implemented (Messina *et al.*, 2013). Medical equipments used in the non-critical care setting are less likely to have standard disinfection and cleaning protocols than equipments in the critical care setting. Thus medical care equipments are more likely to carry considerable number of pathogenic microorganisms (Cohen *et al.*, 1997). The contamination of stethoscope particularly the diaphragms reported mainly due to lack of regular disinfection (before and after examining each patient) (Schabrun and Chipchase, 2006).

Numerous studies in the past decade have reported the level of bacterial contamination on stethoscopes belonging to physicians and nurses (Nunez *et al.*, 2000), also there are increasing reports of the risk of transmitting antibiotic resistant microorganisms from one patient to another on stethoscopes (Uneke*et al.,* 2010). The large majority of the stethoscopes examined in different studies were contaminated: most with Gram-positive organisms, primarily *Staphyolococcus*specie (Wilkins *et al.,* 2007).The isolated organism include Gram-positive cocci, Gram-positive bacilli and Gram-negative bacilli (Bukharie*et al.*, 2004).

1.2. Rationale

A single stethoscope often used for all inpatients and outpatients. The universal and unavoidable use of the stethoscope and its direct contact with multiple patients makes it an important potential factor in the dissemination of microorganisms from one patient to another (Shiferaw*et al.,* 2013). Revisingliterature there is no reports about stethoscopes contamination in the Sudan. This study is about stethoscope contamination.

1.3. Objectives

1.3.1 General objectives

To assess phenotypically Gram positive bacterial contaminationon the stethoscope in some hospitals in Khartoum State.

1.3.2 Specific objectives

a. To determine the bacterial load.

b. To isolate Gram positive bacteria on stethoscopes and identify the isolates.

CHAPTER TWO
LITERATURE REVIEW

Nosocomial infections (NIs) result in significant financial and individual costs, with large numbers of patients acquiring infections annually (Schabrun and Chipchase, 2006). The patient-to-patient transmission of microorganisms is a major threat to hospitalized patients and causes significant morbidity and mortality. The present evidence indicates that health care workers' hands are the main route of cross-transmission and also small medical equipment, such as stethoscopes, may also contribute to the dissemination of microorganisms (Longtin*et al.,* 2009).

Healthcare equipment has been identified as a likely source of these infections, and research indicates that up to one-third of all NIs may be prevented by adequate cleaning of equipment. The risk of NI following contact with equipment is high. The included studies reported that 86.8% of all sampled equipment was contaminated, with 70% alcohol reducing the levels of contamination on equipment by 82.1%. Healthcare equipment is a significant source of NI. High levels of contamination are present on a wide range of healthcare equipment. However, the majority of contamination and hence any risk of acquiring a NI can be reduced substantially by regular cleaning of equipment with 70% alcohol (Schabrun and Chipchase, 2006).

In the past, stethoscopes have been shown to harbor potentially harmful bacteria. As early as 1972, stethoscopes were identified as a fomite on which bacteria are capable of surviving for varying duration of time (Russell *et al.,* 2012).

During auscultation stethoscope contamination is common; if the same stethoscope is used for the next patient without disinfection, it might bring risk of infection to the patient and may continuously impose the risk serially to all patients. Draping of stethoscopes around the neck is still a commonly seen practice, resulting in the risk of recontamination of the diaphragm of the stethoscope from the unclean earpieces, with normal flora and pathogenic bacterial strains harboring the ears of the Health Care workers (Shiferaw*et al.,* 2013).

Microorganisms are commonly found on stethoscope diaphragms. This includes Gram-positive cocci, Gram-positive bacilli, Gram-negative bacilli and fungi. It has also been shown that bacteria are quite capable of surviving up to 18 hours on this surface**.** Furthermore, it has been demonstrated experimentally that stethoscopes are able to transfer bacteria to human skin (Pimentel, 2006).

Studies from Israel, Spain, and the United States reported contamination rates of 5 to 17% of ED health care providers' stethoscopes (Tang *et al.,* 2011). Wood *et al* (2007) reported 100% of cultured diaphragms were contaminated.

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.,* Alphahemolytic streptococci, *Micrococcus luteus, Enterococcus spp.* (Marie *et al* 2000). Also brief report done in USA show all of the stethoscopes were found to be contaminated. The most commonly identified microorganisms were coagulase-negative

Staphylocci(CoNS), *Micrococcus* spp., *Bacillus* spp., *Corynebacterium*spp., and *Streptococcus* spp. CoNS wasidentified on 93.4% of the stethoscopessampled, and *Micrococcus* specieson 63.9% of them (Wilkins *et al.,* 2007).Marinella*et al* (1997) showed in their study coagulase-negative staphylococci were presented on 100% of stethoscopes and *Staphylococcus aureus* on 38%.

Coagulase negative Staphylococciis a microorganism which frequently causes severe systemic infections, including catheter-associated and device-associated sepsis. Intact skin is an efficient barrier against most infective agents. However, small skin lesions are frequent and this route of exposure should not be underestimated. This is extremely important when treating patients with wounds or burns, or patients with catheters or tracheostomies (Bukharie*et al.,* 2004).

Marie *et al.,* (2000) study used a different methodology to show that stethoscopes can be a possible source of infection by showing that a clean stethoscope after coming in contact with patient's skin can harbor common normal skin flora like *Staphylococcus aureus.* The organism grew in 57% of the stethoscopes sampled. Although most patients might not be especially prone to infection after contact with contaminated stethoscopes, those with open wounds like patients with burns or with tracheostomies may be colonized leading to infection at a later time.

In Saudi Arabia a total of 100 stethoscopes were examined. The types of bacteria isolated from the stethoscopes are Gram positive bacilli 12% and *Staphylococcus epidermidis* 9%. There was, as expected, a predominance of microorganisms commonly found as cutaneous flora. Several other potentially pathogenic microorganisms were also isolated.

No methicillin-resistant staphylococci were isolated. Nurses and respiratory therapists cleaned their stethoscopes more often than doctors or medical students. None of the health personnel cleaned their stethoscopes after each patient. The results of their study demonstrate that stethoscopes that are utilized in clinical practice on a daily basis carry potentially pathogenic microorganisms. Since normal skin flora consists primarily of Gram-positive bacteria. The frequency of contamination of stethoscopes observed in this study is lower than the 70% to 100% reported in other studies. In this study, only 21% of the respondents regularly cleaned their stethoscopes. Nurses cleaned their stethoscopes more frequently than physicians and medical students. None of the health care workers cleaned their stethoscopes after use in every patient. Isopropyl alcohol has been shown to reduce bacterial colony counts when applied to the stethoscope diaphragm. Regular disinfection of stethoscopes or disposable cover should be used to minimize the possibility of spreading infectious agents in hospitalized patients. This is especially important today, since hospitals now care for more immune-compromised patients than in previous times and also there is increased resistance of bacteria to available antibiotics (Bukharie*et al.*, 2004).

In United States one hundred fifty-nine (80%) of the 200 stethoscopestested were contaminated with microorganisms. Eightyone(51%) of the 159 contaminated stethoscopes had twoor more microorganisms isolated. A total of 265 organisms were isolated from the 159 contaminated stethoscopes and an average of 1.67 (265 of 159) microorganisms were isolated from each contaminated stethoscope.Seventeen distinct species of microorganism were isolated. Gram-positive cocci constituted thegroup of organisms most frequently isolated (94%), followed by gram-positive bacilli (Smith *et al.*, 1996).

In Australia the 11 stethoscopes that were available, were distributed between 31 consultants, 2 registrars, a resident medical officer, and medical students were 51. Colonial growth of bacteria was found on all plates within the impressions made by the stethoscopes. Colony counts ranged from two coIonies to >15 colonies. The majority had less than 15 colonies. Most plates displayed a mixof coagulase-negative staphylcocci and *Micrococcus*species. No *Staphylococcusaureus*was isolated (Pimentel, 2006).

In Philippine Sixty-nine percent of the 90 stethoscopes were contaminated. Sixty-eight of these stethoscopes were owned personally, while 22 were unit or shared stethoscopes. Thirty- two (77.1%) of the 45 stethoscopes from the experimental group and 30 of 45 stethoscopes (66.7%) from the control group had bacterial growth after 48 to 72 hours of incubation. The most common isolate was *Staphylococcus* spp. at 77.4%. Majority (97%) of the respondents believed that stethoscopes are potential vectors of infection. However, only 34% of the respondents cleaned their stethoscopes more than once daily and only 33% had cleaned it within the past 24 hours. High workload and lack of awareness were cited as reasons for not adhering to stethoscope care recommendations. The post-intervention contamination rates were significantly lower in the experimental group compared to the control (11.4 % vs 44.2% for the control) (Grecia*et al.*, 2008).

In United Kingdom they assessed how often bedside stethoscopes in their intensive care unit were cleaned and whether they became colonized with potentially pathogenic bacteria. The 12 nurses attending the bed-spaces were questioned about frequency of stethoscope cleaning on the unit and the bedside stethoscopes were swabbed before and after cleaning to identify colonizing organisms. Twenty-two health care providers entering the unit were asked the same questions and had their personal stethoscopes swabbed. Out of 24 intensive care unit bedside stethoscopes tested, two diaphragms and five earpieces were colonized with pathogenic bacteria. MRSA cultured from one earpiece persisted after cleaning. Three out of the 22 personal stethoscope diaphragms and five earpieces were colonized with pathogens. After cleaning, two diaphragms and two earpieces were still colonized, demonstrating the importance of regular cleaning (Whittington *et al*., 2009).

In Nigeria, a total of 107 stethoscopes surveyed, 84 (79%) were contaminated with bacteria; 59 (81%) of the contaminated stethoscopes belonged to physicians and 25 (19%) were from other health workers. Gram positive isolates included *Staphylococcus aureus*(54%) and *Enterococcus faecalis*(14%). All stethoscopes that had never been cleaned were contaminated while lower levels of contamination were found on those cleaned one week or less before the survey. Contamination was significantly higher on stethoscopes cleaned with only water (100%) compared to those cleaned with alcohol (49%). Significantly fewer (9%) stethoscopes from health workers who washed their hands after seeing each patient were contaminated when compared with the instruments (86%) of those who did not practice hand washing (Uneke*et al.,* 2010).

In Turkey samples were taken from the diaphragm of a total of 121 stethoscopes from four different hospitals. 90 of them were from doctors and the others from nurses. The included health care personnel in this study were 67 male and 54 female. 90 out of the one 121 stethoscopes, were contaminated with microorganisms (76%). We found bacterial and fungal contamination in 92 (76%) of the stethoscopes. 15 out of 92 (16.3%) had potential pathogens including methicillin sensitive *Staphylococcus aureus*, methicillin resistance *S. aureus*, and *Enterococcus* spp. of the 121 health-care persons, only 61 regularly cleaned their stethoscopes by using alcohol and various disinfectant substances (Kilic*et al.*, 2011).

In Ethiopia at Jimma University Specialized Hospital a total of 176 stethoscopes examined, 151 (85.8%) were considerably contaminated (>20 CFUs/diaphragm), and the rest 25 (14.2%) were not contaminated. The Frequency of contamination was 100% for stethoscopes from ICU and 96% for Medical ward. Almost all stethoscopes diaphragm collected showed different degree of bacterial contaminations. From 151 (85.8%) contaminated stethoscope diaphragms, a total of 256 bacterial strains were isolated. The maximum isolation per diaphragm was five species and the minimum was one bacterial species, with over all mean of 1.79 bacterial species per diaphragm. Majority (52%) of the isolates were found to be potential pathogens. Coagulase Negative Staphylococci species was the most frequent isolate (40.2%) among gram-positive isolates; followed by *S. aureus* (30.9%) and *Bacillus* species (5.5%) (Shiferaw*et al.*, 2013).

In Canada study examining stethoscope contamination. The results of this study demonstrate that the majority of stethoscopes used by physicians and nurses in the study area are contaminated with bacteria, most of which are common skin flora Coagulase negative Staphylococci (CoNS). Overall, bacterial growth was observed in specimens from 70 stethoscopes after 48 hours of incubation; 30 had no growth. Coagulase-negative staphylococci were cultured from 54 specimens. One specimen or 1% (95% CI 0–5.5%) was positive for *S. aureus*, but this was not MRSA (Tang *et al.,* 2011).

In Italy at Italian teaching hospital they analyzed 35 stethoscopes and other small hospital device, comparing their contamination in four hospital units. Before cleaning, many samples were positive for *Staphylococcus* species. After cleaning, CFUs decreased to zero in most comparisons. The first aid unit had the highest and intensive care the lowest contamination. The other device had higher total bacterial count at 22°C than stethoscopes. Their results showed that although the percentage of stethoscopes contaminated by bacteria was lower than of other device, mean and median CFU were higher in stethoscope samples. This finding is also significant because stethoscopes are much easier to clean. This apparent contradiction (lower contamination percentages but higher numbers of CFUs) could mean that while healthcare personnel are certainly aware of the need to clean/disinfect stethoscopes, the practice is sometimes neglected. In their study, 21/35 stethoscopes were positive for *Staphylococcus spp*., 10/35 were positive for MRSA, respectively. They also isolated several bacterial species that are often the cause of Hospital Acquired Infections (HAIs), such as *Enterococcus* spp. They also compared microbial contamination of stethoscopes of physicians/nurses and shared stethoscopes and found some differences. They recorded greater contamination on non-shared stethoscopes by *Staphylococcus* spp., and MRSA. Healthcare professionals presumably use their own stethoscopes more often than shared ones and do not clean them very often, so that they are more likely to be contaminated. Other reasons for greater contamination of personal stethoscopes could be that shared stethoscopes are subject to established hygiene practices and procedures while personal ones are less likely to be cleaned and disinfected by the owner (Messina *et al.,* 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study design

3.1.1. Type of study

This is descriptive cross sectional study conducted to evaluate the Bacterial load and Bacterial contamination with Gram- positive bacteria on stethoscopes used in some hospitals in Khartoum state.

3.1.2. Study area

The study was carried out in hospitals located in different localities in Khartoum State. The practical part of the study was done in the Research Laboratory of Sudan University of Science and Technology, College of Medical Laboratories Sciences.

3.1.3. Study duration

Study was conducted during the period from March to June 2014.

3.2. Sampling technique

Stethoscopes used by physician, nurses, medical students and other health worker investigated for contamination with Gram positive bacteria. Convenient sampling technique was used to select stethoscopes. After permission from hospital administrator the researcher entered the floor silently and then began collecting stethoscopes individually after getting informed consent from each participant. Stethoscopes that were omitted included those were not visible and not volunteered to be studied. Anonymity was maintained for all participants by substituting random numbers in place of names.

3.3. Collection of samples

The samples collected following the method of (Unekee*t al.*, 2010) with some modification. The internal and external surface of each stethoscope diaphragm was swabbed with a sterile swab moistened by sterile normal saline. The cottony part of swab placed in 2 ml sterile normal saline. The tubes were then coded and placed upright in a box. This was repeated each sample.

3.4. Laboratory investigation

After collection samples were then taken to Sudan University of Science and Technology, College of Medical Laboratory Sciences, Research laboratory, where the medium was kept. The medium was prepared in the day of collection. Before inoculation each sample was applied to vortex and diluted in 4 tubes of sterile normal saline in serial (10 folds). The last 3 dilutions were inoculated, each dilution in 3 plates using pour plate method. Each plate was labeled. The cultures were incubated at 37°C overnight. At the end of incubation period plates were assessed for growth.

3.4.1. Bacterial load

Pour plate method was used to calculate the bacterial load. The number of colony forming unit(living bacteria in liquid culture) was counted. A measured amount of the suspension is mixed with molten agar medium in a Petri dish. After incubation, the number of colonies was counted. Counts of pure cultures should be made on plates inoculated to yield between 30 and 300 colonies.

-Procedure

Bacterial suspension was diluted in 10 fold serial dilutions. 9 ml of diluent (sterile normal saline) pipetted into each of several sterile test tubes. With sterile pipette 1 ml of suspension transferred into first tube of diluent. The first dilution mixed and 1 ml from it transferred into the next tube. The following dilutions were made in the same way by using fresh and sterile pipette for each. 1 ml of each dilution pipetted onto 3 petri dishes and 15 ml of clear nutrient agar poured into each dish, mixed and allowed to cooling. Then incubated at 37^{o}C overnight (Collee*et al.*, 1996).

- Calculation

Plates contained 30-300 cfu/ml in two plate or three per dilution were used in calculation.

Cfu/ml = average of cfu in 3 plates x dilution factor.

Cfu/diaphragm = cfu/ml x 2 (total sample = 2 ml).

Cfu/cm^2 = cfu/diaphragm / area of diaphragm.

(Cfu: colony forming unit).

3.4.2 Bacterial identification:

Identification of bacteria was done macroscopically by Colonial morphology, microscopically by Gram stain and Biochemically.

3.4.2.1 Gram stain:

Smear was prepared and fixed by heating. The fixed smear covered with crystal violet stain for 30-60 seconds. Rapidly washed off the stain with clean water. After that the smear covered with Lugol’s iodine for 30-60 second. The iodine washed off with clean water. Acetone- alcohol applied rapidly for decolorization and washed immediately with clean water. Lastly the smear covered with Safranine stain for 2 minutes then washed off with clean water. The back of slide cleaned and the smear left to dry. The smear was examined microscopically with 40x and 100x objectives (Cheesbrough, 2000).

3.4.2.2 Biochemical tests

3.4.2.2.1. Catalase test

Catalase enzyme acts as catalyst in hydrogen peroxide to oxygen water. This test is used to differentiate staphylococci from streptococci. Two to three ml of 3% hydrogen peroxide poured into a test tube. A sterile wooden stick usedto remove a good growth of the test organism and immerse it in the hydrogen peroxide solution. Immediate active bubbling indicated as positive result (Cheesbrough, 2000).

3.4.2.2.2.Coagulase test

Coagulase is an enzyme that causes clot to form when bacteria incubated with plasma. This test used to differentiate coagulase positive *Staphylococcus aureus* from coagulase negative staphylococci. In a small test tube 0.5 ml of dilute plasma placed with 5 drop of bacterial suspension. After mixed gently, incubated at 37^{o}C and up to 4 hours and examined for clot formation (Cheesbrough, 2000).

3.4.2.2.3. Deoxyribonucease (DNAse) test (DNA hydrolysis)

DNase enzyme hydrolyzes deoxyribonucleic acid DNA. This is used to differentiate *Staphylococcus aureus* which produce DNase enzyme from other staphylococci. Tested organism inoculated by using sterile loop or swab and incubated at 37^{o}C overnight. After incubation period the surface of the plate covered with 1mol/l hydrochloric acid solution and the excess acid tipped off. The positive result showed by clearing around the colonies within 5 minutes(Cheesbrough, 2000).

3.4.2.2.4. Mannitol fermentation

Test organism was inoculated into Mannitol Salt Agar, incubated at 37°C and then examined after 24 hours for mannitol fermentation. It was indicated by formation of yellow color around the growth(Cheesbrough, 2000).

3.4.2.2.5. Sugar fermentation test

Fermentation is a type of microbial metabolism in which bacteria breakdown organic compound to get energy. It results in various end product acid, gas, both acid and gas or other end products. Bacteria will be inoculated in different carbohydrate broths. The media used in fermentation test consisted of nutrient broth supplemented with fermentable carbohydrate and indicator. Tubes of various carbohydrate media were selected, labelled and inoculated with test organism aseptically. Tubes were incubated at 37^{o}C for 24-48 hours with non- inoculated tubes as control. Color changes were observed (Pommerville, 2005).

CHAPTER FOUR

RESULTS

A total of 200 stethoscopes from 4 hospitals selected in different localities in Khartoum State were examined for bacterial contamination.Among these 60 from Hospital A, 50 from Hospital B, 40 from Hospital C and 50 from Hospital D (Table 1).

One hundred seventy nine (179) (89.5%) were yielded bacterial growth, and the rest 21 (10.5%) were demonstrated no bacterial growth.The frequency of contamination of stethoscopes according to hospital was as 93.3% in Hospital A, 92% in Hospital B, 80% in Hospital C and 90% in Hospital D (Table 2).

The average of bacterial load in all contaminated stethoscopes was $105.1x10^4$cfu/ml, $210.2x10^4$cfu/diaphragm and $14.01x10^4$cfu/cm^2. The average of bacterial load in different hospital arranged in ascending from $52.4x10^4$cfu/ml, $104.8x10^4$cfu/diaphragm and $8.98x10^4$cfu/cm^2 in hospital B, $107.6x10^4$cfu/ml, $215.2x10^4$cfu/diaphragm and 14.35 10^4cfu/cm^2 in hospital C, $128.3x10^4$cfu/ml, $256.6x10^4$cfu/diaphragm and $17.11x10^4$cfu/cm^2 in hospital D to $132x10^4$cfu/ml, $264x10^4$cfu/diaphragm and $17.6x10^4$cfu/cm^2 in hospital A (Table 3).

From the contaminated stethoscopes, 179 bacterial strains were isolated. Majority of these isolates wereGram-positive bacteria 140 (78.2%).According to hospitals Gram-positive bacteria were 49 (87.5%) in hospital A, 32 (69.6%) in hospital B, 23 (71.9%) in hospital C and 36 (80%) in hospital D (Table 4).

The most frequent isolates of Gram-positive bacteria were coagulase negative staphylococci (CoNS) 84 (60%), followed by *Bacillus*species 43 (30.7%) and *Staphylococcus aureus* 13 (9.3%)(Table 5).

Staphylococcus epidermidis 38 (39%) was the most predominant staphylococci followed by *S. warneri* 16 (16.5%), *S. aureus* 14 (14.4%), *S. haemolyticus* 11 (11.3%), *S. hominis* 7 (7.2%), *S. lugdunensis* 6 (6.2%) and *S. saprophyticus* 5 (5.2%) (Table 6).

Table 1 shows distribution of samples.

Hospital name	No. of samples
A	60 (30%)
B	50 (25%)
C	40 (20%)
D	50 (25%)
Total	**200 (100%)**

Table 2 shows frequency of contamination.

Hospital name	Growth (%)	No growth (%)
A	56 (93.3%)	4 (6.7%)
B	46 (92%)	4 (8%)
C	32 (80%)	8 (20%)
D	45 (90%)	5 (10%)
Total	**179 (89.5%)**	**21(10.5%)**

Table 3 shows the average of bacterial load.

Hospital	Cfu/ml	Cfu/diaphragm	Cfu/cm^2
A	$132x10^4$	$264x10^4$	$17.6x10^4$
B	$52.4x10^4$	$104.8x10^4$	$8.98x10^4$
C	$107.6x10^4$	$215.2x10^4$	$14.35x10^4$
D	$128.3x10^4$	$256.6x10^4$	$17.11x10^4$
Total	**$105.1x10^4$**	**$210.2x10^4$**	**$14.01x10^4$**

- CFU colony forming unit.

Table 4 shows frequency of Gram- positive bacteria according to hospitals.

Hospital	Total isolates	Gram-positive (%)
A	56	49 (87.5%)
B	46	32 (69.6%)
C	32	23 (71.9%)
D	45	36 (80%)
Total	179	140 (78.2%)

Table 5 shows frequency of Gram-positive species.

Hospital	CoNS	*Bacillus*spp	*S.aureus*	Total
A	30 (61.22%)	11 (22.45%)	8 (16.33%)	49
B	19 (59.4%)	9 (28.1%)	4 (12.5%)	32
C	16 (69.6%)	6 (26.1%)	1 (4.3%)	23
D	18 (50%)	17 (47.2%)	1 (2.8%)	36
Total	84 (60%)	43 (30.7%)	13 (9.3%)	140 (100%)

Table 6 shows frequency of*Staphylococcus* species according to hospitals.

Staphylococci spp	Hospital A	Hospital B	Hospital C	Hospital D	Total
S. aureus	8 (57.1%)	4 (28.6%)	1 (7.15%)	1 (7.15%)	14 (14.4%)
S. epidermidis	17 (44.7%)	9 (23.7%)	6 (15.8%)	6 (15.8%)	38 (39.2%)
S. haemolyticus	4 (36.4%)	3 (27.3%)	3 (27.3%)	1 (9%)	11 (11.3%)
S. hominis	2 (28.6%)	2 (28.6%)	1 (14.3%)	2 (28.6%)	7 (7.2%)
S. saprophyticus	0	1 (20%)	3 (60%)	1 (20%)	5 (5.2%)
S. lugdunensis	1 (16.7%)	3 (50%)	1 (16.7%)	1 (16.7%)	6 (6.2%)
S. warneri	6 (37.5%)	1 (6.3%)	2 (12.5%)	7 (43.8%)	16 (16.5%)
Total	38 (39.1%)	23 (23.7%)	17 (17.5%)	19 (19.6%)	97 (100%)

CHAPTER FIVE

DISCUSSION

Stethoscope considered as most important device used in hospitals. However studies implicated stethoscopes as potential source of hospital acquired infection. This is the first Sudanese study examining stethoscope contamination. The results of this study demonstrate that the majority of swabbed stethoscopes were contaminated with bacteria, most of which are common skin flora coagulase negative staphylococci (CoNS).

High rate of stethoscope contamination 89.5% observed in this study indicate that stethoscopes may play a role in transmitting organisms in hospital environment.This rate in comparison with other previous study that reported in Philippine 69% (Grecia*et al.*, 2008), in Neigeria 79% (Uneke*et al.*, 2010), in Turkey 76% (Kilic*et al.*, 2011), in Ethiopia 85.8% (Shiferaw*et al.*, 2013) and in Canada 70% (Tang *et al.*, 2011) is relatively high. On other hand, in United States 100% contamination was reported by Marinella*et al.*, (1997) and Wood *et al.*, (2007).

In this study the average of bacterial load was 210.2x10^4cfu/diaphragm which is higher in comparison withShiferaw*et al.*, (2013)1.44x10^4cfu/diaphragm.

A total of 179 organism isolated from 179 stethoscopes 1 organism per stethoscope which was low in comparable with Shiferaw*et al.*, (2013) study.They found a total of 256 organisms from 151 stethoscopes with average 1.79 organism per stethoscope and Smith *et al.*, (1996) found a total of 265 organisms were isolated from the 159 contaminated stethoscopes with average of 1.67 organism per stethoscope.

The isolated Gram- positive bacteria were 140 (78.2%), this might be due to the direct contact of the stethoscope to human skin flora, which contains mostly Gram-positive bacteria. This result in line with the results ofShieraw*et al.,* (2013)who found Gram-positive isolates were more frequent (78.9%). The majority of normal skin flora is made up of Gram-positive organisms, making it likely that organisms transferred to healthcare equipment during patient contact are predominantly Gram positive.

The Gram- positive isolates included in this study were coagulase negative staphylococci followed by *Bacillus* species and *Staphylococcus aureus*. *Micrococcus* and Gram positive filaments were included in Shiferaw*et al.,* (2013) study. *Corynebacterium spp.,* Alpha-hemolytic streptococci, and *Enterococcus spp.*were reported by Marie *et al.,*(2000).

In the present study coagulase negative staphylococci was the most predominant isolate 84 (46.9%). This result in agreement with Shiferaw*et al.,* (2013) (40.2%), and in Wood *et al.,* (2007) study the CoNS isolated from all samples (100%). The most frequent *Staphylococcus* was *Staphylococcus epidermidis*38/179 (21.2%) which was higher as compared with (9%) which was reported by Bukharie*et al.,* (2004).

Staphylococcus aureus was 13/179 (7.8%) of total isolates which was lower in comparable with (30.9%) in Shiferaw*et al.,* (2013) study and (38%) in Marinella*et al.,* (1997) study. In other hand there were no *Staphylococcus aureus* isolate in Bukharie*et al.,* (2004) study. Although *S. aureus* is normal flora of human skin but its potential pathogen and also well documented fact that *S. aureus*is a primary causative agent of hospital acquired infection (Shiferaw*et al.,*2013).

Conclusion

-The stethoscope contamination was significant and potential pathogens were isolated from it. Stethoscope is potential vehicle in the transmission of infections between patients and healthcare workers.

-Stethoscope diaphragm should be disinfected before and after each patient contact. High rate of bacterial contamination was shown in this study.

-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. Although these strategies could minimize the risk of stethoscope transmission of infections, they are unaffordable to most health workers and health facilities in developing countries. Instead hospitals should develop more rigorous programs and protocols for stethoscope disinfection as a standard of care. Strict adherence to stethoscope disinfection practices by health workers will minimize cross-contamination and ensure improved patient safety in hospitals (Uneke*et al.*, 2010).

Recommendation

1. Recommendations for future studies include repeating this study in more hospitals, including those in different regions, teaching hospitals and smaller community hospitals. This would give a better picture of stethoscopes bacterial load and contamination.

2. More information should also be gathered qualitatively on stethoscopes cleaning patterns and hand washing.

3. Additionally, studying which types or brands of stethoscopes are more susceptible for harboring bacteria. A possible difference may exist between stethoscopes with a plastic diaphragm versus stethoscopes with a metal diaphragm. This may make one type more prone to bacterial carriage.

4. Studying the effect of regular, multiple cleaning with isopropyl alcohol on the integrity of the diaphragm.

5. Furthermore, more studies are needed to identify all isolates from stethoscopes and assess the antimicrobial susceptibility testing for isolates.

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Appendices

Appendix 1: shown bacterial load.

No.	Bacterial load		
	Cfu/ml	Cfu/diaphragm	Cfu/cm^2
1	33x10^4	66x10^4	4.4x10^4
2	13x10^4	26x10^4	1.73x10^4
3	18x10^4	36x10^4	2.4x10^4
4	10x10^4	20x10^4	1.3x10^4
5	11x10^4	22x10^4	1.47x10^4
6	-	-	-
7	30x10^4	60x10^4	4x10^4
8	19x10^4	38x10^4	2.53x10^4
9	18x10^4	36x10^4	2.4x10^4
10	5x10^4	10x10^4	0.67x10^4
11	9x10^4	18x10^4	1.2x10^4
12	12x10^4	24x10^4	1.6x10^4
13	11x10^4	22x10^4	1.47x10^4
14	135x10^4	270x10^4	18x10^4
15	13x10^4	26x10^4	1.73x10^4
16	26x10^4	52x10^4	3.47x10^4
17	14x10^4	28x10^4	1.87x10^4
18	6x10^4	12x10^4	0.8x10^4
19	8x10^4	16x10^4	1.07x10^4
20	-	-	-
21	15x10^4	30x10^4	2x10^4
22	-	-	-
23	15x10^4	30x10^4	2x10^4
24	6x10^4	12x10^4	0.8x10^4
25	2x10^4	4x10^4	0.27x10^4
26	8x10^4	16x10^4	1.07x10^4
27	7x10^4	14x10^4	0.93x10^4
28	8x10^4	16x10^4	1.07x10^4
29	80x10^4	160x10^4	10.7x10^4
30	20x10^4	40x10^4	2.67x10^4
31	9x10^4	18x10^4	1.2x10^4

32	$9x10^4$	$18x10^4$	$1.2x10^4$
33	$4x10^4$	$8x10^4$	$0.53x10^4$
34	$293x10^4$	$586x10^4$	$39.07x10^4$
35	$97x10^4$	$194x10^4$	$12.93x10^4$
36	$21x10^4$	$42x10^4$	$2.8x10^4$
37	$6x10^4$	$12x10^4$	$0.8x10^4$
38	$17x10^4$	$34x10^4$	$2.27x10^4$
39	$12x10^4$	$24x10^4$	$1.6x10^4$
40	$8x10^4$	$16x10^4$	$1.07x10^4$
41	$167x10^4$	$334x10^4$	$22.27x10^4$
42	$99x10^4$	$198x10^4$	$13.2x10^4$
43	$257x10^4$	$514x10^4$	$34.27x10^4$
44	$332x10^4$	$664x10^4$	$44.27x10^4$
45	$563x10^4$	$1126x10^4$	$75.07x10^4$
46	$218x10^4$	$436x10^4$	$29.07x10^4$
47	$189x10^4$	$378x10^4$	$25.2x10^4$
48	$153x10^4$	$306x10^4$	$20.4x10^4$
49	$47x10^4$	$94x10^4$	$6.27x10^4$
50	$22x10^4$	$44x10^4$	$2.93x10^4$
51	$50x10^4$	$100x10^4$	$6.67x10^4$
52	$27x10^4$	$54x10^4$	$3.6x10^4$
53	$6x10^4$	$12x10^4$	$0.8x10^4$
54	-	-	-
55	$6x10^4$	$12x10^4$	$0.8x10^4$
56	$9x10^4$	$18x10^4$	$1.2x10^4$
57	$5x10^4$	$10x10^4$	$0.67x10^4$
58	$4x10^4$	$8x10^4$	$0.53x10^4$
59	$3x10^4$	$6x10^4$	$0.4x10^4$
60	$5x10^4$	$10x10^4$	$0.67x10^4$
61	$7x10^4$	$14x10^4$	$0.93x10^4$
62	$35x10^4$	$70x10^4$	$4.67x10^4$
63	$22x10^4$	$44x10^4$	$2.93x10^4$
64	$1x10^4$	$2x10^4$	$0.13x10^4$
65	$2x10^4$	$4x10^4$	$0.27x10^4$
66	$1x10^4$	$2x10^4$	$0.13x10^4$
67	$1x10^4$	$2x10^4$	$0.13x10^4$
68	$42x10^4$	$84x10^4$	$5.6x10^4$

69	$73x10^4$	$146x10^4$	$9.73x10^4$
70	$320x10^4$	$640x10^4$	$42.67x10^4$
71	$20x10^4$	$40x10^4$	$2.67x10^4$
72	$28x10^4$	$56x10^4$	$3.73x10^4$
73	$30x10^4$	$60x10^4$	$4x10^4$
74	$52x10^4$	$104x10^4$	$6.93x10^4$
75	$37x10^4$	$74x10^4$	$4.93x10^4$
76	$17x10^4$	$34x10^4$	$2.67x10^4$
77	$19x10^4$	$38x10^4$	$2.53x10^4$
78	$21x10^4$	$42x10^4$	$2.8x10^4$
79	$2x10^4$	$4x10^4$	$0.27x10^4$
80	$18x10^4$	$36x10^4$	$2.4x10^4$
81	$53x10^4$	$106x10^4$	$7.07x10^4$
82	$43x10^4$	$86x10^4$	$5.73x10^4$
83	$2x10^4$	$4x10^4$	$0.27x10^4$
84	-	-	-
85	$65x10^4$	$130x10^4$	$8.67x10^4$
86	$31x10^4$	$62x10^4$	$4.13x10^4$
87	$17x10^4$	$34x10^4$	$2.27x10^4$
88	$5x10^4$	$10x10^4$	$0.67x10^4$
89	$6x10^4$	$12x10^4$	$0.8x10^4$
90	$18x10^4$	$36x10^4$	$2.4x10^4$
91	$37x10^4$	$74x10^4$	$4.93x10^4$
92	$67x10^4$	$134x10^4$	$8.93x10^4$
93	$28x10^4$	$56x10^4$	$3.73x10^4$
94	$39x10^4$	$78x10^4$	$5.2x10^4$
95	$1x10^4$	$2x10^4$	$0.13x10^4$
96	$61x10^4$	$122x10^4$	$8.13x10^4$
97	$26x10^4$	$52x10^4$	$3.47x10^4$
98	-	-	-
99	$138x10^4$	$276x10^4$	$18.4x10^4$
100	$47x10^4$	$94x10^4$	$6.27x10^4$
101	$71x10^4$	$142x10^4$	$9.47x10^4$
102	$3x10^4$	$6x10^4$	$0.4x10^4$
103	-	-	-
104	$21x10^4$	$42x10^4$	$2.8x10^4$

105	-	-	-
106	$15x10^4$	$30x10^4$	$2x10^4$
107	$9x10^4$	$18x10^4$	$1.2x10^4$
108	$32x10^4$	$64x10^4$	$4.27x10^4$
109	$43x10^4$	$86x10^4$	$5.73x10^4$
110	$11x10^4$	$22x10^4$	$1.47x10^4$
111	$163x10^4$	$326x10^4$	$21.73x10^4$
112	$106x10^4$	$212x10^4$	$14.13x10^4$
113	$30x10^4$	$60x10^4$	$4x10^4$
114	$21x10^4$	$42x10^4$	$2.8x10^4$
115	$173x10^4$	$346x10^4$	$23.07x10^4$
116	$31x10^4$	$62x10^4$	$4.13x10^4$
117	$122x10^4$	$244x10^4$	$16.27x10^4$
118	-	-	-
119	$156x10^4$	$312x10^4$	$312x10^4$
120	-	-	-
121	$70x10^4$	$140x10^4$	$9.3x10^4$
122	$176x10^4$	$352x10^4$	$23.47x10^4$
123	$34x10^4$	$68x10^4$	$4.53x10^4$
124	$31x10^4$	$62x10^4$	$4.13x10^4$
125	-	-	-
126	$27x10^4$	$54x10^4$	$3.6x10^4$
127	$12x10^4$	$24x10^4$	$1.6x10^4$
128	-	-	-
129	$58x10^4$	$116x10^4$	$7.73x10^4$
130	$190x10^4$	$380x10^4$	$25.3x10^4$
131	$46x10^4$	$92x10^4$	$6.13x10^4$
132	$31x10^4$	$62x10^4$	$4.13x10^4$
133	$23x10^4$	$46x10^4$	$3.07x10^4$
134	$100x10^4$	$200x10^4$	$13.3x10^4$
135	$119x10^4$	$238x10^4$	$15.87x10^4$
136	$33x10^4$	$66x10^4$	$4.4x10^4$
137	$65x10^4$	$130x10^4$	$8.67x10^4$
138	-	-	-
139	$76x10^4$	$152x10^4$	$10.13x10^4$
140	$115x10^4$	$230x10^4$	$15.3x10^4$

141	-	-	-
142	$219x10^4$	$438x10^4$	$29.2x10^4$
143		-	
144	$28x10^4$	$56x10^4$	$3.73x10^4$
145	$140x10^4$	$280x10^4$	$18.67x10^4$
146	$76x10^4$	$152x10^4$	$10.13x10^4$
147	$250x10^4$	$500x10^4$	$33.33x10^4$
148	$190x10^4$	$380x10^4$	$25.33x10^4$
149	$105x10^4$	$210x10^4$	$14x10^4$
150	-	-	-
151	$27x10^4$	$54x10^4$	$3.6x10^4$
152	$17xx10^4$	$34x10^4$	$2.67x10^4$
153	$43x10^4$	$86x10^4$	$5.73x10^4$
154	-	-	-
155	$27x10^4$	$54x10^4$	$3.6x10^4$
156	$35x10^4$	$70x10^4$	$70x10^4$
157	$121x10^4$	$242x10^4$	$16.13x10^4$
158	$22x10^4$	$44x10^4$	$2.93x10^4$
159	$37x10^4$	$74x10^4$	$4.93x10^4$
160	-	-	-
161	$258x10^4$	$516x10^4$	$34.4x10^4$
162	$291x10^4$	$382x10^4$	$25.47x10^4$
163	$192x10^4$	$384x10^4$	$25.6x10^4$
164	$245x10^4$	$490x10^4$	$32.67x10^4$
165	$229x10^4$	$458x10^4$	$30.53x10^4$
166	$78x10^4$	$156x10^4$	$10.4x10^4$
167	$297x10^4$	594	$39.6x10^4$
168	$121x10^4$	$242x10^4$	$16.13x10^4$
169	$207x10^4$	$414x10^4$	$27.6x10^4$
170	$47x10^4$	$94x10^4$	$6.27x10^4$
171	-	-	-
172	$17x10^4$	$34x10^4$	$2.27x10^4$
173	$86x10^4$	$172x10^4$	$11.47x10^4$
174	$77x10^4$	$154x10^4$	$10.27x10^4$
175	$38x10^4$	$76x10^4$	$5.07x10^4$
176	$53x10^4$	$106x10^4$	$7.07x10^4$

177	$87x10^4$	$172x10^4$	11.47
178	$122x10^4$	$422x10^4$	$28.13x10^4$
179	$37x10^4$	$74x10^4$	$4.93x10^4$
180	$35x10^4$	$70x10^4$	$4.67x10^4$
181	$183x10^4$	$366x10^4$	$24.4x10^4$
182	$188x10^4$	$376x10^4$	$25.07x10^4$
183	$12x10^4$	$24x10^4$	$1.6x10^4$
184	$61x10^4$	$122x10^4$	$8.13x10^4$
185	-	-	-
186	$37x10^4$	$74x10^4$	$4.93x10^4$
187	$12x10^4$	$24x10^4$	$1.6x10^4$
188	$63x10^4$	$126x10^4$	$8.4x10^4$
189	$18x10^4$	$36x10^4$	$2.4x10^4$
190	$1x10^4$	$2x10^4$	$.13x10^4$
191	$79x10^4$	$158x10^4$	$10.53x10^4$
192	-	-	-
193	$25x10^4$	$50x10^4$	$3.33x10^4$
194	$32x10^4$	$64x10^4$	$2.20x10^4$
195	$3x10^4$	$6x10^4$	$0.4x10^4$
196	$21x10^4$	$42x10^4$	$2.8x10^4$
197	$13x10^4$	$26x10^4$	$1.73x10^4$
198	$15x10^4$	$30x10^4$	$2x10^4$
199	$74x10^4$	$148x10^4$	$9.87x10^4$
200	$67x10^4$	$134x10^4$	8.93