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Bacteriological Assessment of Stethoscope Used by Health Care Personnel in Attat Hospital, Snnp, Gurage Zone, Ethiopia

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Abstract: The stethoscope has always been an important element of a physician's toolkit when it comes to examining patients. The widespread use of stethoscopes by health-care workers for patient examinations makes them a potential source of nosocomial infection transmission. The goal of this study was to see if stethoscopes used by different health-care professionals in Attat Hospital may transmit bacteria. From April to June 2018, a cross-sectional study was done in the molecular laboratory of Wolkite University's department of biotechnology and biology. A total of 26 stethoscopes from health workers who had direct contact with patients were gathered during the study period. The sample was obtained using a sterile cotton-tipped applicator saturated in a sterile solution of physiologic saline (0.85% sodium chloride) to swab the whole surface of the stethoscope's diaphragm and then inoculated into macconkey agar, tryptone soya agar, and blood agar medium. 18 (69.2%) stethoscopes out of total collected stethoscopes had bacterial growth, and 12 bacterial isolates were selected and characterized to genus level. Isolates include staphylococcus aureus (37.5%), coagulase negative staphylococci (28.12%), Streptococcus sp. (21.88%), and Bacillus sp. (12.5%). All isolates were susceptible to the co-trimoxazole and ciprofloxacine, while resistant to cifoxitine. They showed intermediate growth against vancomycine. All except streptococcus were found resistant against penicillin. Both S. aureus and CoNS were sensitive to the chloramphenicol; Streptococcus was intermediate while bacillus was resistant to the chloramphenicol. All stethoscopes (42.2%) that had never been cleaned and were last cleaned a week ago were severely contaminated, while those washed multiple times a day and cleansed between each patient before the examination of the patient had lower levels of contamination (27%).

Keywords: Isolates, Nosocomial Infection, Stethoscope

1. Introduction

1.1. Background of the Study

Nosocomial infections have existed since the beginning of hospitals, and they continue to be a significant public health issue even in the modern era of antibiotics. When infections become clinically obvious during hospitalization (at least 72 hours after admission), they are classified as nosocomial [21]. Such infections are caused by a variety of factors, including the emergence and persistence of multi drug-resistant bacteria, patients' compromised immune systems, and mechanical transmission of microorganisms, all of which result in high morbidity and mortality, prolonged hospitalization, increased antibiotic use, and increased costs [12]. According to studies, these infections occurred in 5% to 10% of all hospitalizations in Europe and North America, and in more than 40% of hospitalizations in Asia, Latin America, and Sub-Saharan Africa [34].

According to [32], more than 1.4 million people globally are infected with illnesses acquired in hospitals at any given time, and health-care personnel are possible sources of these infections. Because many infections can be spread through the hands, all health-care professionals must wash their hands before and after each patient encounter [14, 35]. Diseases can be transmitted through contaminated medical devices, and outbreaks of hospital-acquired infections have been connected to electronic thermometers, blood pressure cuffs,

stethoscopes, latex gloves, masks, neckties, pens, badges, and lanyards, white coats, computers, and keyboards [31].

The sterilization and disinfection of intrusive equipment and devices prior to interventions are frequently overlooked. Stethoscopes are the most commonly utilized medical devices by health care personnel to examine the health of patients among those equipments. As a result, they frequently come into touch with a large number of patients and have been identified as potential nosocomial infection vectors in various regions of the world [26, 31, 27].

According to a similar report from Jimma University Specialized Hospital, bacterial contamination of the stethoscope is significant and could be a vector for illness transfer between patients and health care staff [29]. Pathogens can adhere and establish themselves on the diaphragms of stethoscopes after contact with contaminated skin, and then be conveyed to other patients if the stethoscope is not cleansed [18].

There are also more cases of antibiotic-resistant bacteria being transmitted from one patient to another via stethoscopes [31, 9, 20, 12]. In a hospital setting, these antibiotic-resistant organisms are capable of causing serious infections, necessitating contact isolation and rigorous treatment to limit the spread of the organisms [12]. Ceftazidime-resistant *Klebsiella pneumonia*, vancomycin-resistant *enterococc*i, methicillin-resistant *staphylococc*i, ciprofloxin-resistant *Pseudomonas aeruginosa*, gentamicin-resistant *Pseudomonas aeruginosa,* and penicillin-resistant *pneumococci* are examples of antibiotic-resistant organisms [16, 10, 22].

Infection transmission in hospitals (nosocomial infections) is a major issue caused by contaminated medical equipment and health-care workers (HCWs). Medical devices that have not been adequately sterilized/disinfected may spread bacteria from one patient to the next. Due to rising morbidity and cost burden, health-care-acquired infections are becoming a major concern not only for doctors, but also for patients, and stethoscope disinfection is still not a widely accepted practice among most health-care workers.

Despite the fact that stethoscopes are a possible vector for the transfer of health-care-associated illnesses and resistant bacteria, health-care professionals fail to disinfect them [31]. Swiping stethoscopes with alcohol pads is the current gold standard for stethoscope decontamination [3, 27]. To prevent nosocomial infections, medical devices such as stethoscopes should be tested for microbial colonization on a regular basis, and health care staff should be educated on proper cleaning procedures [7].

1.2. Objectives of the Study

1.2.1. General Objective

The study's overall goal is to establish the bacteriological agents responsible for stethoscope contamination in Attat Hospital, as well as to examine healthcare personnel's attitudes and knowledge about stethoscope hygiene behavior.

1.2.2. Specific Objectives

1) In order to identify and characterize bacterial isolates based on biochemical and morphological tests.
2) For determining a drug resistance profile of selective isolates.
3) To explore the behavior, attitudes and beliefs about stethoscope hygiene among healthcare personnel within the hospital's various clinical units.

1.3. Statement of Problem

Infection transmission in hospitals (nosocomial infections) is a major issue caused by contaminated medical equipment and health-care workers (HCWs). Medical devices that have not been adequately sterilized/disinfected may spread bacteria from one patient to the next. Because of rising morbidity and cost burden, health-care-acquired infections connected with stethoscopes are now a major concern for doctors as well as patients, and stethoscope disinfection is still not a widely accepted practice among most health-care workers. To our knowledge, there has only been one study on the function of stethoscopes in the transmission of nosocomial infections, and none has been conducted in Ethiopia's south. In Attat Hospital, a referral hospital serving people of Cheha Woreda and nearby communities in Southwest Ethiopia, so that it is important to look into the role of stethoscopes as potential fomites for possibly dangerous bacteria.

2. Literature Review

2.1. The Different Types of Nosocomial Infections

The CDC and the National Healthcare Safety Network (NHSN) divide health-care-associated infection sites into 13 primary kinds based on clinical and biological criteria, with roughly 50 potentially specific infection sites for surveillance. Surgical wound and other soft tissue infections, urinary tract infections (UTI), respiratory infections, gastroenteritis, and meningitis are the most frequent nosocomial diseases that can arise in a hospital setting [25]. However, with the increased use of invasive procedures for therapeutic and diagnostic purposes, cancer chemotherapy, immunotherapy, and advancements in organ transplantation, changes in the distribution of nosocomial infection sites can be observed over time.

2.2. Epidemiology of Nosocomial Infections

It is estimated that about 10% of hospital patients or more than 2-million hospitalized patients are annually suffering from hospital infection in the USA; and an estimated annual death rate is 20,000, which may reach even up to 88,000 deaths per year. Basic epidemiological patterns can be used to guide prevention and control actions in hospital-acquired infections. The virus that causes hospital infection has reservoirs, can be transferred in predictable ways, and needs a vulnerable host [33]. The inanimate environment, such as surgical instruments and the operating room, and the animate

environment, such as diseased or colonized health care staff, patients, and hospital visitors, could be reservoirs and sources of infection. Cross-infection from an endogenous flora present in the patient or auto-infection from an endogenous flora found in the patient are two possible modes of transmission for hospital acquired infection [4]. For example surgical site infection can be caused by an endogenous flora that trans-locate to a normally sterile site or when the sterile peritoneal cavity is contaminated by spillage from the gastrointestinal tract; and by an exogenous source of microbial contamination that comes from the surgical team, surgical instrument and the theatre environment.

Furthermore, aseptic procedures were not followed strictly by the majority of the nurses and physicians in several practice areas and are found to be significant for the transmission of the infection [24].

2.3. Nosocomial Infections: Sources and Transmission

Infections are caused by nosocomial microorganisms that can come from either endogenous or external sources. Hospital staff, other patients, visitors, food, water, fomites, urinary catheters, intravenous devices, respiratory apparatus, and other prosthesis are all examples of animate and inanimate sources of exogenous infections. Contact is the most common way for nosocomial illnesses to spread, generally directly but occasionally indirectly through bodily secretions. Air can also be a source of airborne nosocomial viruses that infect the respiratory tract (e.g., in droplet nuclei and aerosols). Food-borne and water-borne diseases can enter through the faeces-oral pathway. The oropharynx, gastrointestinal system, and urinary tract are the most prevalent reservoirs for nosocomial colonizers [26].

2.4. Nosocomial Infection Risk Factors

For a variety of reasons, hospitalized patients are at an unusually high risk of infection. Intrinsic and extrinsic factors are roughly classified into two categories. Intrinsic risk factors are those that are present in the patient as a result of the underlying disease. Patient care may contain extrinsic risk factors. Concurrent infections, prosthetic devices, surgery, immunosuppressive medications, broad-spectrum antibiotic therapy, and the emergence of multi drug-resistant organisms are some of the general predisposing factors that make patients prone to nosocomial infections. Other risk factors include the patient's age, length of stay in the hospital, underlying conditions such as diabetes, malignancies, or ward congestion. The length of hospital stay is the most important risk factor for contracting a nosocomial infection among the multiple risk variables [17].

2.5. Nosocomial Infection Agents

A large number of microorganisms are responsible for hospital infections and any microbe may have the capacity/ability to cause an infection in the hospitalized patients.

Nosocomial infections can be caused by a variety of microorganisms. The infecting organisms differ depending on the patient demographic, the health care setting, the facility, and the country.

2.5.1. Bacteria

These are the most commonly found nosocomial pathogens in hospitals. There is a distinction to be made between commensal bacteria found in the typical flora of healthy humans and pathogenic bacteria. These provide an important protective role by preventing harmful germs from colonizing the area. If the native host is harmed, some commensal bacteria may cause illness. Intravascular line infection is caused by cutaneous coagulase negative staphylococci, and urinary infection is caused by intestinal Escherichia coli.

Pathogenic bacteria have a higher pathogenic and, independent of host status, cause infections (sporadic or epidemic). Anaerobic Gram-positive rods (such as Clostridium) induce gangrene, for example.

Gram-positive bacteria: Staphylococcus aureus (a cutaneous bacterium that colonizes both hospital staff and patients' skin and nose) causes a wide range of lung, bone, heart, and bloodstream infections and is usually antibiotic-resistant; beta-haemolytic streptococci are also essential.

Gram-negative bacteria, such as E. coli, Proteus, Klebsiella, Enterobacter, and Serratiamarcescens, can colonize places where the host's defenses are impaired (catheter insertion, bladder catheter insertion, cannula insertion) and cause significant infections (surgical site, lung, bacteraemia, peritoneum infection). They could also be extremely resistant. Gram-negative bacteria, such as Pseudomonas spp., are frequently found in wet and damp environments. They may colonize hospitalized patients' gastrointestinal tracts [11, 30].

Other germs provide a distinct threat in hospitals. For example, Legionella species can cause pneumonia (sporadic or endemic) in people who inhale polluted water aerosols (air conditioning, showers, and therapeutic aerosols).

2.5.2. Viruses

Many viruses, including hepatitis B and C (transfusions, dialysis, injections, and endoscopy), respiratory syncytial virus (RSV), rotavirus, and enteroviruses, can be transmitted nosocomially (transmitted by hand-to-mouth contact and via the faecal-oral route). Other viruses that can be transferred include CMV, HIV, Ebola, influenza viruses, herpes simplex virus, and varicella-zoster virus.

2.5.3. Parasites and Fungi

Some parasites, such as Giardia lamblia, are easily spread between adults and children. Many fungi and parasites are opportunistic organisms that cause infections when the immune system is suppressed by antibiotics (Candida albicans, Aspergillus spp., Cryptococcus neoformans, Cryptosporidium). In immune-compromised patients, they are a primary source of systemic infections. Contamination of the environment by airborne organisms that originate in dust and dirt, such as Aspergillus spp., is also a worry,

particularly during hospital building. Sarcoptes scabies (scabies) is an ectoparasite that has caused outbreaks in health care on several occasions.

2.6. Diagnosis

The diagnosis and identification of hospital-acquired infection involves interpretation of clinical and laboratory findings. Clinically, a patient is assessed based on clinical sign and symptoms developed due to the infection. Pain, soreness, redness, localized swelling, and purulent discharge from the wound are symptoms of a superficial incision site infection. The patient developed a fever (>38°C), localized discomfort or tenderness, and purulent discharge from the incision if the infection was at a deep cut. Fever (>38°C), urgency, frequency, and dysuria were all reported in a patient with symptomatic UTI. However, patients under the age of one year may have hypothermia (37°C), apnea, bradycardia, lethargy, or vomiting.

The pathogen was isolated by urine culture, which was used to make the laboratory diagnoses. The amount and types of bacteria in the urine must be determined as part of the diagnostic process. If a mid-stream urine culture includes 105 organisms per ml and no more than two types of microbes, it is deemed positive.

2.7. Nosocomial Infection Prevention

It is the responsibility of all individuals and services providing health care to prevent nosocomial infections. In addition, everyone must work together to limit the risk of infection for both patients and employees. Although not all hospital infections are preventable, the majority of them can be. Surveillance of NIs is an important aspect of infection control, and it is widely recognized as a first step toward prevention around the world. Reduced health-care-associated infection rates, on the other hand, is dependent on a number of factors. Staff-related procedures, particularly hand hygiene, have recently received a lot of attention. Furthermore, there has been a growing understanding that environmental controls should be an important part of any overall plan for preventing healthcare-associated illnesses [2].

Hand washing is still the most critical action in infection prevention. Gloves, gowns, and masks have a role in avoiding infections, but they are frequently misused, resulting in unnecessary service expenses. Many people are visibly disturbed when their inadequate hygiene practices are revealed, and many are outraged when it is claimed that they could be disease vectors, spreading dangerous bacteria among their patients, complicating infection control efforts [2].

3. Materials and Method

3.1. Study Area and Period

The current research was carried out at the Attat Specialized Hospital, which is located 175 kilometers southwest of Addis Ababa, 17 kilometers from the town of Wolkite on the road to Hosanna in the Cheha Woreda of the Gurage Zone, SNNPRs, Ethiopia. Since its establishment in 1969, the hospital was managed and run by Medical Mission Sisters under the Eparchy of Emdibir. According to medical mission sisters, the hospital has ten wards (0-9) providing extensive integrated health services for more than 800,000 people with in and out of the operational area. Between April and June 2018, samples were evaluated at Wolkite University's Department of Biotechnology and Biology laboratory, which is part of the College of Natural and Computational Science.

3.2. Study Design

A cross-sectional descriptive study was conducted using a structured questionnaire and specimen assessment from the stethoscope of Attat Hospital healthcare workers.

3.3. Study Population

All healthcare personnel (doctors, nurses, health officers and students) having their stethoscope on-hand during data collection constitute the source population for the study. According to the report from the medical director of Attat hospital, they had around 50 stethoscopes for 10 wards, which were used as the sampling frame. A proportional sample size was determined for each department (including inpatients and OPD) and participants were selected using a simple random sampling.

3.4. Sample Size

There are 26 stethoscopes required for sample collection from different wards and from different professionals.

3.5. Selection Criteria

Healthcare personnel who were willing to give informed consent and the study included anyone who had their personal stethoscope on hand at the time of data collection. The study did not include those who:

1) Do not have a stethoscope on hand during data collection.
2) Already participated in the study while working in another ward.
3) Refuse to give informed consent.

3.6. Data and Sample Collection

The investigators went to any inpatient or outpatient department for data and sample collection without any prior notice. Then, after obtaining consent, self-administered questionnaires were utilized to gather socio-demographic data characteristics (gender, profession and experience) of participants, use of stethoscopes, stethoscope cleaning habits, and perceived barriers to cleaning. Swam samples for bacteriological and antibiotic resistance profiling were taken from the diaphragmatic section of stethoscopes using a sterile cotton-tipped applicator bathed in sterile normal saline (0.85% w/v).

The obtained swabs were immediately placed in Amies

transport media, and samples were sent to the laboratory in an ice box with proper and comprehensive labeling, along with the questionnaire.

3.7. Bacterial Pathogen Isolation, Enumeration, and Identification

The material was inoculated in duplicate on Blood agar, Tryptone soya agar, and MacConkey agar and incubated aerobically at 37°C for 48 hours after gentle mixing. The media were inspected for bacterial growth after incubation, and the total number of colonies for each sample was tallied. Significant growth was defined as a colony count of more than 20cfu/diaphragm [29], and the stethoscope was deemed contaminated. In tryptone soy broth and agar slant, representative colonies from contaminated stethoscopes were purified and stored. Following normal bacteriological techniques, the isolates were identified to the genus and species level.

Based on colony characteristics (appearance, size, and color), cell morphology, Gram reactions and KOH test obtained further identification of bacteria was made by a series of biochemical tests [5, 13]. Mannitol salt agar and blood agar plates were used to cultivate Gram-positive cocci. Following that, catalase and tube coagulase tests were performed. Staphylococcus aureus was identified in isolates that passed all three tests. Isolates tested negative for tube coagulase was considered coagulase-negative staphylococci (CoNS). b Catalase negative gram positive cocci were cultured on blood agar and pattern of hemolysis (alpha, beta, and gamma) was observed.

3.8. Antibiotic Sensitivity Test

The following antibiotics were used to determine the anti biogram of the isolates: Penicillin (10µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Cefoxitin (30µg), Cotrimaxozole (25µg), and Vancomycine (30 µg). The antibiotic discs were selected based on availability and current use in health facilities of Ethiopia. Direct colony suspension of the test organism in sterile saline solution were prepared, the turbidity of the inoculate was adjusted to a 0.5 McFarland standard (1.5x108CFU/ml). A new sterile cotton-tipped swab dipped in the suspension was used to wipe the surface of Mueller Hinton agar plates within 15 minutes of inoculum formation. Then, within 15 minutes of inoculating the MHA plate with sterile forceps, a set of 6 standard antibiotic discs was applied. MHA plates were incubated at 37°C for 18-24 hours, and the diameter of each antimicrobial disc's zone of inhibition was determined [8, 15].

3.9. Data Analysis

The SPSS v23 computer software was used to enter and evaluate the data. Categorical variables were displayed in tables and bar graph, with frequencies and percentages summarized. A statistically significant difference was defined as a P-value of less than 0.05.

4. Results

4.1. Study Participants' Socio-Demographic Profiles

A total of 26 stethoscopes were tested for bacterial contamination by four separate professionals from different hospital wards. The Medical ward (IPG & OPD) (8), Gynecology ward (6), Surgical ward (IPD & OPD) (5), Pediatrics ward (IPD & OPD) (3), Emergency ward (2), Neonatal (1), and Delivery ward (1) were among the 10 wards where these health professionals worked (1).

The study included an equal number of males (13) and females (13), where the majority (10, 39%) were doctors, followed by nurses (7, 27%), medical students (5, 19%), and health officers (4, 15%). Most of the participant's years of experience were less than 2 years (11, 42%), followed by 2-5 years (9, 34), and 5-10 years and >10 years (3, 12% each) as shown in (Table 1).

Table 1. *Health-care personnel's socio-demographic features at Attat Hospital, Wolkite.*

	GENDER		PROFESSION		YEARS OF EXPERIENCE						Total (%)
	Male	Female	Doctor	Nurse	HO	Student	<2	2-5	5-10	>10	
MEDICAL	3	5	4	2	2	-	5	2	-	1	8 (31)
GYNECOLOGY	3	3	3	1	-	2	2	3	-	1	6 (23)
SURGICAL	3	2	1	1	-	3	2	1	1	1	5 (19)
PEDIATRICS	2	1	1	2	-	-	1	1	1	-	3 (11)
EMERGENCY	2	-	1	-	1	-	1	1	-	-	2 (8)
NEONATAL	-	1	-	-	1	-	-	-	1	-	1 (4)
DELIVERY	-	1	-	1	--	-	-	1	-	-	1 (4)
TOTAL (%)	13 (50)	13 (50)	10 (39)	7 (27)	4 (15)	5 (19)	11 (42)	9 (34)	3 (12)	3 (12)	100%

4.2. Stethoscopes: Knowledge, Attitudes & Practices (KAP) Survey

Of 26 stethoscopes studied, almost one third (29%) of the owners reported that the last time they cleaned their stethoscope was last week. Other responded cannot recall (20%), never (17%), today (17%) and yesterday (17%). When asked how often they clean their stethoscope, the majority of respondents (23%) said once daily, followed by an equal number of respondents (19%) who said every patient, once weekly, or numerous times a day. Fewer (12% and 8%) responded rarely if ever and never, respectively. The agents they used for cleaning their stethoscopes were alcohol wipes (92%) and antiseptic wipes (8%). No significant relation was identified between the use of disinfectants and

bacterial contamination.

With regards to the ideal frequency of cleaning, 69% responded that cleaning before and after every patient would be the best approach to keeping the stethoscope clean. 81% of the participants believe that stethoscopes could transmit infectious agents. For 19% of participants, cleaning their stethoscopes at the start and end of the day was sufficient, while 12% had no notion of the appropriate frequency of cleaning. Forgetfulness (46%), lack of time (18%) (18%) were identified as the major barrier of cleaning stethoscopes. Concern for damage and lack of supplies were the other barriers to cleaning.

4.3. Bacterial Contamination of Stethoscope Diaphragm

After two days of incubation, the diaphragms of all stethoscopes examined from ten wards revealed varying degrees of bacterial contamination. 18 (69.2%) of the 26 stethoscopes tested were heavily contaminated (>20 CFUs/diaphragm), while the other (30.8%) were not.

From 18 contaminated stethoscopes, 11 (61%) were from females health care personnel and 7 (39%) were from males. In terms of profession, the most frequently contaminated stethoscopes were those used by doctors (6, 33%) and nurses (6, 33%), followed by medical students and health officers. While analyzing the proportion, the majority of stethoscopes are used in Medical (6, 33%), Gynecology (4, 22%) Contamination was found in the Surgical ward (4, 22%). On stethoscope diaphragms from emergency, pediatrics, delivery, and neonatal wards, there was significantly less contamination (Table 2).

Table 2. *Level of bacterial contamination in terms of gender, profession, experience and ward.*

		UNCONTAMINATED	CONTAMINATED	Total (%)
		Count (%)		
SEX	Men	6 (75)	7 (39)	13 (50)
	Woman	2 (25)	11 (61)	13 (50)
PROFFESSION	Doctor	4 (50)	6 (33)	10 (39)
	Nurse	1 (12.5)	6 (33)	7 (27)
	Student	2 (25)	3 (17)	5 (19)
	Ho	1 (12.5)	3 (17)	4 (15)
EXPERIANCE	<2Yrs	4 (50)	7 (39)	11 (42)
	2-5Yrs	3 (38)	6 (33)	9 (34)
	5-10Yrs	0	3 (17)	3 (12)
	>10Yrs	1 (12)	2 (11)	3 (12)
WARD	Medical	2 (25)	6 (33)	8 (31)
	Gynecology	2 (25)	4 (22)	6 (23)
	Surgical	1 (12.5)	4 (22)	5 (19)
	Pediatrics	2 (25)	1 (6)	3 (11)
	Emergency	1 (12.5)	1 (6)	2 (8)
	Neonatal	0	1 (6)	1 (4)
	Delivery	0	1 (6)	1 (4)

4.4. Phenotypic Characteristics of Bacterial Isolates

Based on colony appearance, size, and color, 13 (72%) of the contaminated stethoscopes showed a single, uniform colony growth, while the rest (5, 28%) had polymicrobal growth. Of these, all colonies identified were gram-positive organisms, while no gram-negative bacteria were observed. A total of twenty-five representative bacterial colonies were primarily selected, of which twelve distinct isolates were purified and preserved for further investigation.

The selected isolates had cultural characteristics that were similar and different, and they were identified at genus level based on their phenotypic and biochemical characteristics, as well as hemolysis on blood agar and mannitol salt agar for fermentation analysis. The results are presented in Table 3 and Figure 1. Among the isolates identified, Staphylococcus aureus constitutes 38.5%. Coagulase negative Staphylococcus, Streptoco*ccus* sp., and *Bacillus* sp. constitute 26.8%, 23.2% and 11.5% of the respective bacterial isolates (Figure 1).

Table 3. *The selected bacterial isolates' morphological and biochemical properties.*

PARAMETERS	ISOLATES OF BACTERIA			
	Staphylococcus aureus	Coagulase negative staphylococci (CoNS)	*Streptococcus* sp.	*Bacillus* sp.
Colony shape	Irregular clusters	Irregular clusters	Chain	Rod
Colonial pigmentation	Slightly larger (3mm), yellow, smooth, raised colonies	Smaller (1mm), greyish white, smooth, raised colonies	White, Glistening/mucoid	White, dry, flat irregular colonies
Staining by Gram	Positive	Positive	Positive	Positive
Test for catalase	Postive	Positive	Negative	Positive
Test for coagulase	Positive	Negative	Negative	Negative
Test for Oxidase	Negative	Negative	Negative	Negative
Growth on MSA	Growth+mannitol fermentation	Growth without mannitol fermentation	NG	NG
Hemolysis on Blood agar	Beta hemolysis	Non hemolytic	Beta hemolysis	Non hemolytic

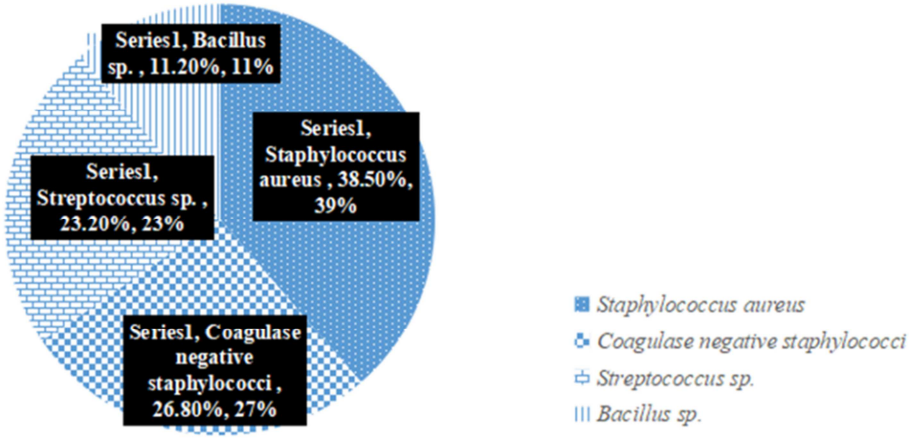

Figure 1. *Bacterial profile isolated from stethoscope diaphragm.*

For the isolation and identification of staphylococcus aureus from stethoscope samples that had previously grown in blood agar and tryptone soya agar, mannitol salt agar (MSA) was utilized as a selective and differential medium. This media was selective for staphylococcus aureus, which ferments mannitol and produces yellow colonies with a yellow zone around the colony; non-mannitol fermented bacteria remain red to pink and colorless in the medium (Figure 3). The majority of the isolates (38.5%) were show yellow zone in the medium and were identified as S. aureus.

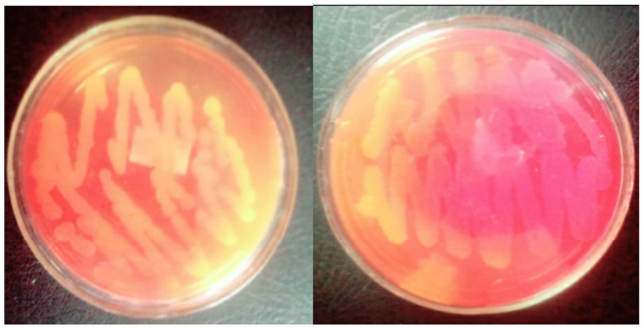

Figure 2. *Image of bacterial isolates that grown in mannitol salt agar.*

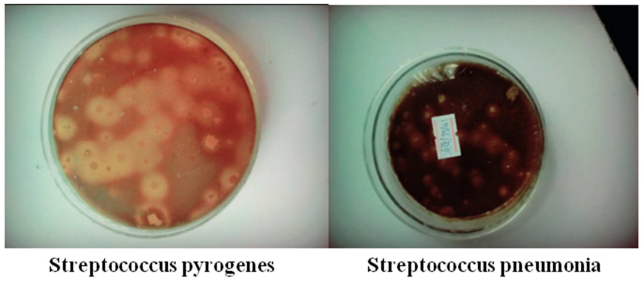

Figure 3. *Image of hemolytic streptococcus grown in blood agar.*

The action of bacterial hemolytic exotoxins on red blood cells was used to identify normal flora from pathogenic bacteria using a blood agar plate (BAP) as a bacterial growth medium. The isolates were described and identified as streptococcus species based on their hemolytic (Alpha hemolysis, Beta hemolysis, and Gamma hemolysis) patterns (Figure 4). Streptococcus pyrogenes was identified as the bacterium that caused beta hemolysis on blood agar. On BAP, alpha hemolysis indicated the growth of normal flora, while gamma hemolysis suggested that the growth on BAP had no effect on The agar's appearancs indicated that Streptococcus pyrogenes (13.2%) was identified from the isolates and streptococcus pneumonia (10%) as normal flora as it showed gamma hemolysis in BAP.

Table 4 shows bacterial colony counts by gender, profession, experiences and wards. The mean colony count of different wards was 109, where the highest (220) and lowest (36) were recorded in Delivery and Emergency ward, respectively. The data also showed a difference between female (151) and male (67) mean colony count, which showed a significant difference at $P<0.05$. Nurses had the greatest mean colony count (148), while doctors had the lowest (79). In contrast, a non-significant mean difference was found in respect with health care personnel's years of experience.

Table 4. *Bacterial colony counts from culture of stethoscope diaphragm surface.*

		MEAN BACTERIAL COUNT
GENDER	Male	67.7
	Female	150.8
PROFFESSION	Doctor	79.2
	Nurse	148.6
	Student	112.6
	Ho	111.3
EXPERIANCE	<2Yrs	111.1
	2-5Yrs	111.7
	5-10Yrs	74.3
	>10Yrs	130
WARD	Medical	135.6
	Gynecology	106.7
	Surgical	140
	Pediatrics	36.7
	Emergency	22.5000
	Neonatal	40
	Delivery	220

4.5. Patterns of Antimicrobial Susceptibility in Isolates

The isolates' antibiotic sensitivity pattern was examined for the following antibiotic discs: co-trimoxazole, chloramphenicol, penicillin, vancomycin, ciprofloxacin and cefoxitine. All isolates were susceptible to the co-trimoxazole

and ciprofloxacine, while resistant to cifoxitine. They showed intermediate growth against vancomycine. All except streptococcus were found resistant against penicillin. Both S. aureus and CoNS were sensitive to the chloramphenicol; Streptococcus was intermediate while bacillus was resistant to the chloramphenicol (Table 5 & Figure 4).

Table 5. *Bacterial isolates from stethoscopes were tested for antimicrobial sensitivity.*

ANTIBIOTIC DISCS	TYPE OF ISOLATES			
	S. aureus	**CoNS**	***Streptococcus* sp.**	***Bacillus* sp.**
Co-trimoxazole (25 µg)	S	S	S	S
Vancomycin (30 µg)	I	I	I	I
Cifoxitine (30 µg)	R	R	R	R
Penicillin (10 µg)	R	R	I	R
Ciprofloxacin (5 µg)	S	S	S	S
Chloramphenicol (30µg)	S	S	I	R

Key: R=Resistant; S=Susceptible; I=Intermediate.

ANTIBIOTIC DISCS	TYPE OF ISOLATES WITH THEIR DIAMETER (CM)			
	S. aureus	**CoNS**	***Streptococcus* sp.**	***Bacillus* sp.**
Co-trimoxazole (25 µg)	2.4	3.1	2.5	3.2
Penicillin (10 µg)	1.3	1.5	1.7	1.3
Cifoxitine (30 µg)	1.2	1.5	1.3	1.4
Vancomycin (30 µg)	1.67	1.7	1.7	1.9
Ciprofloxacin (5 µg)	3.5	2.9	2.8	3.2
Chloramphenicol (30µg)	3.6	3.00	1.7	1.3

Figure 4. *Antimicrobial sensitivity test result (inhibition zone and diameter measurement).*

5. Discussion

The stethoscope is a piece of medical equipment that is utilized by all health-care workers. Our research indicated that 69.2 percent of the stethoscopes surveyed were infected, which is similar to previous findings that found 71 percent to 100 percent of stethoscopes were colonized by different bacteria [7, 29].

Doctors' and nurses' stethoscopes were found to be more polluted (33 percent apiece) than those used by other health workers. This is similar to the study conducted by Chigozie et al. [7]. The fact that doctors and nurses use stethoscopes more frequently than other health care staff may explain the higher rate of bacterial colonization, even if the difference was not statistically significant [19, 7]. Nurses, on the other hand, had a greater mean microbial load (149) than medical students (113), and the lowest was recorded in doctors (79), which might be due to improved stethoscope cleaning habits in later cases.

In this investigation, a swab of stethoscopes taken from clinicians in the medical ward revealed the highest level of infection. Medical physicians may wear stethoscopes more frequently than others, which could explain why they have a greater prevalence of bacterial contamination.

A total of 25 colonies were isolated from 18 (69.2%) contaminated stethoscope diaphragms, although only 13 unique bacterial isolates were examined for further phenotypic characterization. Surprisingly, no gram-negative bacteria were found in any of the stethoscope diaphragms that were analyzed. Gram-positive bacteria were found in all of the isolates.

For bacterial growth and enumeration, blood agar, MacConkey agar, and tryptone soya agar media were utilized. Gram positive bacteria from four different species were recovered from both blood and tryptone soya agar. The largest number of bacterial isolates per diaphragm was three, while the lowest number was one. The majority of the isolates (40%) were identified as potential pathogens. In this study, Staphylococcus aeurus species was the most frequent isolate (38.5%) among the isolates followed by coagulase negative staphylococci, whereas in studies by Chigozie et al. [5, 7], Staphylococcus epidermis and enterobacteracea were the most common organisms isolated from stethoscopes. Co-trimoxazole and ciprofloxacine were determined to be effective against all gram-positive isolates based on their resistance profiles. Meanwhile, cifoxitine and penicillin were not. That is, While all isolates were susceptible to the co-trimoxazole and ciprofloxacine, they were resistant to cifoxitine. They showed intermediate growth against vancomycine. All except streptococcus were found resistant to penicillin. Both S. aureus and CoNS were sensitive to the chloramphenicol; Streptococcus was intermediate, while bacillus was resistant to the chloramphenicol.

In this study, a questionnaire was used to analyze participants' knowledge, attitudes, and practices regarding the role of stethoscopes as carriers of infectious organisms. We found that stethoscopes were contaminated with dangerous germs and that inadequate stethoscope cleaning and

disinfection techniques were related with high contamination. In particular, 34 stethoscopes cleaned on the same day as the data collection were uncontaminated, compared to 100 percent contamination in those who said they never or cannot recall. Because even brief contact between a patient's skin and the stethoscope can result in bacterial translocation [1], measures to reduce bacterial contamination through better stethoscope cleaning habits are needed.

Disposable stethoscopes, especially in clinical high-risk contexts, and the placement of a single-use silicone membrane over the stethoscope head to provide a prophylactic barrier have been proposed as ways to reduce infection transmission from stethoscopes [6, 23]. Although these measures could reduce the risk of infection transmission via stethoscope, they are out of reach for the majority of health workers and health facilities in developing nations, including Attat Hospital. Instead, hospitals should implement more stringent stethoscope disinfection programs and practices as a standard of care [28]. Health personnel who strictly follow stethoscope disinfection procedures will reduce cross-contamination and increase patient safety in hospitals.

6. Conclusion

The current study found a higher percentage of bacterial contamination on the stethoscope diaphragm, indicating that there is a risk of nosocomial pathogen transmission. Many of the bacteria found in our study's stethoscopes (e.g., Staphylococcus aureus, CoNs, S. epidermis, Streptococcus sp., S. pyrogene, and Bacillus subtilis) were known to cause serious infections in hospitalized patients. Staphylococcus and Bacillus species showed increased resistance to the drugs tested, but Streptococcus species did not. Infected stethoscopes were discovered in all parts of the hospital and among all types of medical professionals. The study also suggests that hospital employees should be alerted and educated about the potential health concerns linked to medical equipment.

To reduce infection transmission through stethoscopes, various techniques have been proposed, including the use of disposable stethoscopes, particularly in clinical high-risk areas, and the placement of a single-use silicone membrane over the stethoscope head to establish a prophylactic barrier. Although these measures could reduce the risk of infection transmission via stethoscope, they are out of reach for the majority of health workers and health facilities in developing nations, including Attat Hospital. Instead, hospitals should implement more stringent stethoscope disinfection programs and practices as a standard of care. Health personnel who strictly follow stethoscope disinfection procedures will reduce cross-contamination and increase patient safety in hospitals. Training and motivating health care providers to put their knowledge into practice could be the next step in drastically lowering the bacterial load from the stethoscope, which would immediately reduce cross-contamination and improve patient safety in the hospital setting.

7. The Study's Limitations

The sample size was tiny (26 people), and it came from only one hospital. The frequency with which the stethoscopes were used differed from one participant to the next. In this investigation, the colonization of stethoscopes was not linked to hospital-acquired illnesses. Other contaminants such as anaerobic bacteria, fungi, and viruses were not investigated. The length of time the stethoscope was in contact with the patient's skin or clothing was unknown. Bacterial identification was done using phenotype characterization, which is not as reliable as molecular approaches. The identification of such contaminating organisms and their role as nosocomial infections should be the focus of future research.

8. Recommendations

1) The four bacterial isolates need to be fully characterized using molecular techniques.
2) Further study is needed to identify other microbes from large enough sample sizes of different wards with their drug sensitivity tests.
3) Design instrument processing of stethoscopes like other health service instruments.
4) There is a need of training for health personnel to increase the culture of decontamination of their stethoscopes.

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