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Burden of Multi Drug Resistance (MDR) Bacteria and Factors Affecting the Level of Contamination in Medical Equipments (Stethoscope, Otoscope and Endoscope) Among Health Care Workers (HCW) at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

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This is to certify that the thesis is prepared by Zenebe Gebreyohannes,entitled: Burden of Multi Drug Resistance (MDR) Bacteria and Factors Affecting the Level of Contamination in Medical Equipments (Stethoscope, Otoscope and Endoscope) Among Health Care Workers (HCW) at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Submitted in partial fulfillment the requirement for Master of Science in Clinical Laboratory Science (Diagnostic and Public Health Microbiology) complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining committee:

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List of Abbreviations

AST	Antimicrobial Sensitivity Test
CLSI	Clinical and Laboratory Standard Institute
Co_2	Carbon dioxide
CoNS	Coagulase Negative Staphylococci
DRERC	Department of Research and Ethical Review Committee
DST	Drug Susceptibility Testing
GP	General Practitioners
HCAI	Health Care Associated Infections
HCW	Health Care Workers
HO	Health Officer
MDR	Multidrug Resistance
MRSA	Methicillin Resistant Staphylococcus Aureus
ICU	Intensive Care Unit
IRB	Institution review board
SPHMMC	St. Paulo's Hospital Millennium Medical College
SPSS	Stastical Package for Social Science
Pas	Physician Assistants
USIN	Unique survey identification number
WHO	World Health Organization

Abstract

Background: In health care facilities medical equipment like stethoscope owned by health care providers can be colonized by different microbial agents and could play major role as vehicles for the spreading of nosocomial infections.

Objective: To assess the burden of MDR bacteria and factors affecting the level of contamination in Medical Equipments (Stethoscope, Otoscope and Endoscope) Among Health Care Workers (HCWs) at St. Paul's Hospital Millennium Medical College.

Methods: A cross sectional study was conducted at St. Paul Hospital Millennium Medical College from November 2017-April 2018 on selected medical devices used by Health Care Workers (HCWs) to test the bacterial colonization. After obtained consent form Health Care Workers, 187 Stethoscopes, 8 Otoscope and 6 Endoscope were swabbed by rubbing the diaphragm of the Stethoscope, tips of the Otoscope and the tubes of the endoscope using sterile applicator stick moistened with sterile normal saline. The swab was quickly placed in a screwed sterile glass tube and transported to the Microbiology Laboratory for culture and identification immediately after collection and all pure isolates were tested for anti microbial sensitivity test using disc diffusion. SPSS version 20 used to analyze the data and p value <0.05 was considered as statically significant.

Results: Of the 187 Stethoscope swabbed 157(84%) were contaminated. The contamination levels of Stethoscopes of General practitioner, Residents, and Medical students of internship were (100%, 87.5%, and 83.2%) respectively. Seventy five percent of Otoscope tips were colonized with different bacteria. Among the total isolates *S.aureus and CoNS* showed the highest resistance to Penicillin G. Methicillin resistance *S.aureus* and MRCoNS were 20.3% and 37.7% respectively.

Conclusion: Most of isolates were resistance to multiple classes of antimicrobial agents. Even though cleaning stethoscope is not labor intensive and does not required much time, cleaning habits of the Health Care Workers were low. Therefore careful handling of stethoscope and other medical equipment is critical to minimize spreading of hospital acquired infections.

Key words: Medical instruments; Bacteria contamination; Multi drug resistance; Disinfection

1. Introduction

1.1. Background

According to World Health Organization (WHO) an infection is considered a health care associated infection (HCAI) if it is occurring in a patient during the process of care in a hospital or other healthcare facility which was not present or incubating at the time of admission, this includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility. It is estimated that more than 1.4 million people worldwide are suffering from infections acquired in hospitals (1).

In general, nosocomial infection is the infection that was not at the patients at the beginning of hospitalization it HCAI. This infections presents after 72 hour of hospitalization and causes more mortality and high economical costs (2).

The transmission of nosocomial infections in healthcare settings is a major public health problem worldwide. There is abundant evidence that health care workers are potential sources of HCAIs, which they can transmit to patients via their contaminated hands and from non-critical medical devices like stethoscope used by health workers for patient care have also been implicated in the transmission of HCAIs (3).

Nosocomial infections especially those involving resistant microorganisms, represent one of the challenging problems of modern medicine. Healthcare providers play an important role in the transmission of these infections. Both pathogenic and nonpathogenic bacteria commonly colonize medical items like white coats and stethoscopes which can plays a role as a vectors for transmission of infections by HCWs (4).

It is well known that hospital environment is a reservoir of wide varieties of microorganisms. Several strains of pathogenic bacteria have been frequently reported colonizing medical equipments like Stethoscopes. These pathogens include superbugs like Vancomycin resistant *Enterococcus spp*., Methicillin resistant and sensitive Staphylococcus species and Multidrug resistant, *P. aeruginosa, E. coli, Klebsiella spp*. And *Streptococcus spp*. 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. The contamination of

stethoscope particularly the diaphragm is reported mainly due to lack of regular disinfection before and after examining each patient (5).

Staphylococcus aureus (S.aureus) is gram-positive cocci and is normally found on the skin, as well the respiratory tract of humans. This bacterium is one of the most common causes of nosocomial infections which are an important pathogen due to a combination of toxin-mediated Virulence, invasiveness and antibiotic resistance nature of the organism. A wide range of diseases, including endocarditis, osteomyelitis, toxic-shock syndrome, pneumonia, food poisoning and carbuncles are caused by this organism (6) more over Meticillin-resistant *S.aureus* (MRSA) is the principal micro-organism responsible for causing infections in the hospital and healthcare setting (7).

The word stethoscope comes from the Greek words Stethos, meaning chest, and Skopein, meaning to explore which is the common instrument to all doctors. The invention of the stethoscope in 1816 by the French physician, Rene! Laennec, allowed for full examination of the thorax for the first time (8).

The stethoscope is a popular instrument used by health care providers to evaluate the lung, heart, and abdominal sounds of their patients and it has been reported to be potential vector for nosocomial infections in various part of the world because this medical devices has more frequency of contact with patients skin directly (9).

Antimicrobial resistance occurs when bacteria change in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection. Thus the bacteria survive and continue to multiply causing more harm. Widespread use of antibiotics promotes the spread of antibiotic resistance (10).

In the present day nosocomial infection is the major problem of the world which affected both developing and developed countries. Infection transmission in the hospital environment remains significant hazard for hospitalized patient and health workers are a potential source of these infections. There are increasing reports of the tremendous risk of transmitting antibiotic resistance bacteria from one patient to another from Stethoscope .Stethoscope used in hospital by medical doctors, medical students and other health practitioners for assessing patient's health have been reported as potential vector for transmitting infection in the hospital environment in various part of the world (11).

1.2. Statement of the problem

Transmission of infections on contaminated medical devices is also possible and outbreaks of hospital-acquired infections have been linked to medical devices such as stethoscopes ,electronic thermometers, blood pressure cuffs (12).

Unless there is proper disinfecting habit of the health care providers to their stethoscope the pathogens has an ability to attach and establish themselves on the diaphragm of stethoscopes makes possible the transmission of pathogens from person to person because the head of the stethoscope is usually placed in contact with a patient's skin. The skin surface may be broken or open due to a variety of injuries, including surgical incision, weeping dermatitis, infected lesion, rash, abrasion, laceration, puncture wound, needle sticks, open and infected wounds, topical irritation, micro-cuts, and skin breakdown. During contact of the stethoscope diaphragm with the skin of the patients the pathogenic microorganism will get an opportunity to enter (1).

Despite the high risk of HCAI transmission by stethoscopes it has been reported that sanitation of stethoscopes is one of the most neglected practices of health workers in many health care settings and due to many cases patient safety guidelines do not adequately address proper stethoscope usage and maintenance for the prevention of disease transmission (3).

In the present days nosocomial infections constitute a major problem globally with major social, economic, moral, and personal effects that increase morbidity and mortality of hospitalized patients. And this were supported by different studies conducted in different parts of the world indicated that medical equipment of HCWs are potential sources of nosocomial infections (13).

Several studies have demonstrated that stethoscope membranes harbor bacteria, including Methicillin Resistant *S.aureus*(MRSA) and vancomycin-resistant *Enterococci* (14) special care and awareness should be created otherwise this resistance strains could cause a major health problems world widely .

Some studies estimated that HCAI is to be 5% to 10% in developed countries and 25% in developing countries (15).And many Studies from developed countries have shown that a majority of doctors do not clean or disinfect their stethoscopes regularly. Although developing

countries have the highest burdens of communicable diseases. The negligence , limited awareness and low habit of cleaning of stethoscope by the HCWs increase the burden of HCAI especially in developing countries (16).

The contamination of stethoscope particularly the diaphragm is reported mainly due to lack of regular disinfection before and after examining each patient. A study reported that, 45% of general practitioners disinfect their stethoscope once a year or never and 35% disinfect their stethoscope monthly (5).

Even though health care workers believed that infection transmission occurs via stethoscopes and other related medical devices but the habit of disinfecting these medical devices is very poor this shows their practical aspect is neglected due to many reasons and could contribute for HAI (14).

Although there are some studies in Ethiopia, it is important to see change in microbial atiologies and their antimicrobial drug resistance testing. Hence we planned to assess the burden of MDR bacteria and their AST patterns at Saint Paul Hospital millennium medical college.

1.3. Rationale of the study

In many health care settings, patient safety guidelines do not adequately address for proper usage and maintenance of medical equipments like stethoscope for the prevention of disease transmission hence the main aim of this research paper is to provide scientific base lines that could aid in the development of intervention programs and guidelines for proper usage and maintenance of medical equipments in order to prevent HAI.

2. Literature Reviews

A cross-sectional study conducted by Smith et al. in university of Texas Houston 159 (80%) of the 200 stethoscopes surveyed were contaminated with microorganisms. The majority of organisms that were isolated were gram-positive bacteria, primarily Staphylococcus species. 55% of the Staphylococcus species that were isolated, among this 17% was MRSA (17).

Ayatollahi AA *et al*. Conducted a cross sectional study on the prevalence of gram-negative Bacilli in the environment and the equipments of hospitals 770 samples were collected using sterile swab.Of the total samples 249 samples (32.33%) were contaminated with Gram-negative bacilli with the number of contaminations were *Enterobacter aerogenes* (37.75%). Among the medical equipments and surfaces, the highest level of contamination was observed in laryngoscope and its blades (10.44%) (18).

Kilic IH. *et al* conducted a cross-sectional study in Turkey in four distinct hospitals from 121 stethoscopes used by health-care personnel working 90(76%) Out of the 121 stethoscopes, were contaminated with microorganisms and of the 121 health-care persons, only 61 regularly cleaned their stethoscopes by using alcohol, batticon and various disinfectant substances (19).

A study conducted in, California total of 61 stethoscopes that belonged to physicians and PAs were cultured and all of the stethoscopes were found to be contaminated. The most commonly identified microorganisms were *Coagulase-negative Staphylococcus* (*CoNS*), *Micrococcus spp, Bacillus spp, Coryne bacterium spp, and Streptococcus spp. CoNS* was identified on 93.4% of the stethoscopes sampled (9).

A study was conducted on the bacterial contamination, disinfection of stethoscopes and knowledge gap among health care personnel's in India from 100 stethoscopes cultured 52% were found to be contaminated with bacteria. Among the isolates, *Bacillus subtilis* (36.84%) was prevalent organism followed by *Acinetobacter spp*(17.11%), *CoNS* (14.47%), *Micrococcus Spp* (10.53%), *S.aureus* (6.58%), *Pseudomonas spp* (6.58%), *Diphtheroids* (6.58%), *E.coli* (1.32%) and (40%) of *S.aureus* were found to be MRSA (20).

A cross sectional study conducted on Laryngoscope Handles from 40 samples sent for culture, 30 (75%) were positive for bacterial contamination. Of these positive cultures, 25 (62.5%) *CoNS*,

7(17.5%) *Bacillus spp.*3(7.5%) α-hemolytic *Streptococcus spp*. and one each (2.5%) of *Enterococcus spp., S.aureus,* and *Corynebacterium spp*. No Vancomycin-Resistant *Enterococci,* MRSA, or gram-negative rods were detected (21).

A Study done in AL Madinah Almonawwarah Saudi Arabia by Maqsud AND.et al the overall prevalence of bacterial growth in the studied stethoscopes was 30% among this 26.7% was *CoNS* and the remain3.3% *S.aureus* (22).

A study conducted in Nigeria on Bacterial contamination of stethoscopes used by health workers of the 107 stethoscopes surveyed, 84 (79%) were contaminated with bacteria; 59 (81%) of the contaminated stethoscopes belonged to physicians and 25 (74%) were from other health workers. Isolates included *S.aureus* (54%), *Pseudomonas Spp (*19%), *E.faecalis* (14%) and *E. coli* (13%) (12).

According to the study conducted by Grecia SC et al. 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 (23)

A cross sectional study conducted in Ethiopia by Shiferaw T et al. at Jimma university specialized hospital of 176 stethoscopes examined, 151 (85.8%) were considerably contaminated. From 151 (85.8%) contaminated stethoscope diaphragms *CoNS* species was the most frequent isolate (40.2%) among gram-positive isolates; followed by *S. aureus* (30.9%) and *Bacillus* spp (5.5%).From Gram negative isolates, *Klebsiella spp*. (4.7%) were the most common isolates, followed by *Citrobacter spp.* (4.3%), *Salmonella spp*. (3.5%), *Proteus spp*. (3.5%),*Enterobacter spp.* (3.1%),*P. aeruginosa* (1.2%) and *E. coli*(0.8%) (24).

Another study conducted at Black Lion Specialized hospital By Dabsu R et al 60.7% of the stethoscopes were colonized by microbial. Among the isolates *CoNS* accounted for 75.7% followed by *S.aureus* (18.9%) (25).

Even though infection transmission through medical devices like stethoscope can be preventable by decontaminating between every patients the study conducted in elsewhere showed 35% of the participants never clean their stethoscope (26).

3. Objective of the study

3.1. General objective

- To assess the burden of MDR bacteria and factors affecting the level of contamination in Medical Equipments (Stethoscope, Otoscope and Endoscope) Among Health Care Workers at SPHMMC.

3.2. Specific objectives

- To identify the level of bacteria contaminants from medical equipments.
- To determine the antibiotic resistance pattern of the isolated bacteria from medical equipments.
- To asses associated factors.

4. Hypothesis

The antimicrobial susceptibility pattern of bacterial isolate from stethoscope at SPHMMC could be as high as with previous similar study conducted in Jimma university specialized hospital, Ethiopia (24).The contamination level of Otoscope will be also similar to the study conducted at Tikur Anbesa Specialized hospital (25).

5. Methods and Materials

5.1. Study area

The study was conducted at SPHMMC, Addis Ababa, Ethiopia. The Hospital was established in 1968 by the late Emperor Haileselassie with the help of German evangelical church which is governed by a board under the Federal Ministry of Health. The college has more than 2800 clinical, academic and administrative and support staffs that provide medical specialty services to patients who are referred from all over the country, teaching medicine and nursing students and doing basic and applied researches. While the inpatient capacity is more than 700 beds, the hospital sees an average of 1200 emergency and outpatient clients daily.

5.2. Study design and study period

Hospital based cross sectional study was conducted at SPHMMC from November 2017-April 2018, Addis Ababa, Ethiopia.

5.3. Source population

The source population includes all health care workers working at SPHMMC.

5.4. Study population

Health care personnel accompanied with stethoscopes and use otoscope, laryngoscope and colonoscopy devices for diagnosis. The professionals include-clinical specialist, resident medical students, general practitioners (GP), Nurses, health officer (HO) and medical internship students attached in the hospital and these professionals who are working in different wards namely:-out patient department, pediatrics ward, medical ward, surgical ward, operating room, gynecology ward and emergency departments, transplant unit, maternity ward, NICU and adult ICU.

5.5. Inclusion and exclusion criteria

5.5.1. The inclusion criteria

- All professional health workers who full fill the selection criteria (having stethoscope in hand, working at assigned department), willing and cooperative to participate in the study were included.

5.5.2. Exclusion criteria

- Medical students who are attached on clinical I and clinical II.
- Nursing students who are attached for practical sessions in the hospital.

5.6. Study Variables

5.6.1. Dependent variables

- Rate of bacteria isolated from health care workers stethoscope and other medical apparatus
- Antibiotic resistance pattern of isolated bacteria

5.6.2. Independent variables

- Age, Sex, experience, Frequency of disinfection, Type of disinfectant used, Occupation

5.7. Sampling technique and sample size determination

5.7.1. Sampling technique

Convenient sampling technique was used to select the medical equipments used by HCWs working at different departments. Without any prior we went to those departments for collection of samples. The health workers present at that time with their own stethoscope in hand were included in this study after getting consent form.

5.7.2. Sample size determination

The required sample size for this study was calculated based on the study in Jimma University Specialized Hospital with the prevalence of 85.8% (24).

Sample Size is determined by the following formula: $n = Z^2_{\alpha/2} P (1- P)/ d^2$

Where:

P -is the estimated proportion.

Z- reflects the confidence interval; we will use 95 % confidence interval so the value of zα/2 will be 1.96 - d is the margin of error, here it is 0.05

α -is the level of error one is willing to tolerate.

- Stethoscope n = $\frac{1.96^2 \text{x } 0.858\ (1 - 0.858)}{0.05^2}$ =187

- Convenient sampling technique was used to Otoscope and endoscope devices because of small number of these devices availability in the hospital.

5.8. Data collection and laboratory processes

5.8.1. Data collection

A self-administered questionnaire employed to collect information about the socio demographic characteristics (age, sex, profession, experience and level of education, type of disinfectant used etc) and the health workers included in this study asked to answer the self-administered questionnaire after informed consent signed.

5.8.2. Sample Collection

Sterile cotton swab socked with sterile normal saline (0.9%w/v) was used to collect sample from diaphragm of stethoscope, tips of Otoscope and Endoscope. The swab was quickly placed in a screwed sterile glass tube and transported to the Microbiology Laboratory for culture and identification immediately after collection.

5.8.3. Laboratory processes.

5.8.3.1. Inoculation and identification of bacteria

All the collected samples were inoculated on to Blood agar, Chocolate agar and MacConkey agar plates and incubated at 35- 37±0.5°C. Blood and Chocolate agar plates were incubated at 5% Co_2 jar on the other hand MacConkey was incubated aerobically. Growth was observed at 24 and 48 hours of incubation. Grams staining were also done to characterize colony morphology. For gram positive isolated bacteria catalase and coagulase test was performed while for Gram negative bacteria Conventional biochemical test like, citrate, indole, oxidase, urea, TSI and motility will carried out to authenticate their identity was performed.

5.8.3. 2. Antimicrobial susceptibility testing (AST)

AST was performed for all isolates using the standard Kirby-Bauer methods recommended by Clinical and Laboratory Standard Institute (CLSI 2017) (27) briefly, the bacterial suspension was

prepared equivalent to the 0.5 McFarland standards and the suspension was swabbed on Muller-Hinton agar. Appropriate antibiotic disks were placed and incubated for 18-24 hours at 37^{o}C based on the organisms tested. Diameters of the zone of inhibition around the discs were measured to the nearest millimeter using a ruler and classified as sensitive, intermediate, and resistant. Bacterial isolates which are resistance to two or more classes of drugs will be considered as multidrug resistant (MDR) (28).The following antibiotic disks were used for AST testing of the isolates: For gram positive Amikacin (AK- 30µg), Gentamicin (CN-10µg), Cefoxitin (CXT-30µg), Clindamycin (CLN-2 µg), Cotrimoxazole (COT 1.25/23.75 µg), Chloramphenicol (CHL-30µg),Ciprofloxacin(CIP-5µg),Tetracycline(30µg), Doxycycline (Doxy-30 µg),Erythromycin (ERY-5 µg).),Penicillin(10 µg).For the gram negative bacteria we used Amikacin (AK- 30µg), Ampicillin (AMP-10 µg), Gentamicin (CN-10µg), Amoxycillin-Clavulanic acid (Aug-30 µg),), Ceftriaxone (CTR-30µg), Cefotaxime (CTX-30µg), Ceftazidime (CAZ-30µg), Chloramphenicol (CHL-30 µg), Ciprofloxacin (CIP-5µg), Cotrimoxazole (COT 1.25/23.75 µg), and Meropenem (Mero-10 µg).

5.9. Quality control

The prepared culture media were checked for sterility by incubating randomly the 2% of the prepared media for overnight and observed for the presence of any growth. The saline was also checked for sterility by inoculating on Blood agar. The sample tube was labeled with the same unique survey identification number (USIN) that is indicated in the health worker questionnaire.
Control strain organisms such as *S. aureus* (*ATCC 25923), E. coli (ATCC 25922) and P. aeruginosa (ATCC 27853).*Were used as quality control (27). Questionnaires used to collect demographic data which were incompletely filled were discarded and pretest was performed.

5.10. Data analyses

Data was encoded in Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) version 20 software. P value less than 0.05 was considered as statistically significant.

5.11. Ethical Consideration

Ethical clearance obtained from Department of Research and Ethical Review Committee (DRERC) of Medical laboratory Science, School of Allied Health Science, College of Health Science, Addis Ababa University and from institution review board (IRB) of SPHMMC research

directorate. The purpose of the study was clearly explained for each study participant and written signed informed consent was obtained from study participants prior to sampling. Participant's confidentiality was strictly maintained during the interview process, during data processing and report writing.

5.12. Dissemination of result

The finding of this study will be disseminated to Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences, SPHMMC administration and whenever necessary the researcher will presents the result of this study for professional associations and can also submit to peer reviewed journals for publication.

6. Results

6.1. Socio Demographic Data of Study Participants

A total of 187 health care workers (HCWs) were enrolled in this study from 14 different departments of the hospital .Majority HCWs were males (56.1%).The average age was 26.3 with a standard deviation of ±3.72. Majority of the HCWs were internships medical students 119(63.6%).Most of the HCWs had 1 year experience (72.7%) (Table1).

Table1: Socio demographic characteristics of study participants at SPHMMC from November 2017-April 2018.

Variables		Frequency (%)
Sex	Male	105(56.1)
	Female	82(43.9)
Age	≤24 yr	68(36.4)
	25-29 yr	93(49.7)
	≥30 yr	26(13.9)
Occupation	Intern	119(63.6)
	GP/Residents	33(17.6
	Nurse/HO	35(18.7)
Experience	≤1 yr	136(72.7)
	>1 yr	51(27.3)
Department	AICU/Surgery	19(10.2)
	Emergency	19(10.2)
	Gyn/LW	33(17.6)
	Internal medicine	43(23)
	Pedi ward	42(22.5)
	NICU	17(9.1)
	Other	14(7.5)

Key:yr-year, Ho-health officer,LW-labor ward,AICU-adult ICU,GYN-gyny ward,GP-general practionare,

6.2 Bacterial contamination and isolates versus owners of stethoscopes

Of the 187 stethoscope swabbed 157(84%) were contaminated. The entire stethoscope owned by the general practitioners were 9(100%) followed by Residents doctors 21/24(87.5%) were colonized. Except transplant ICU department the entire stethoscope collected were colonized with different degree of bacteria. The maximum isolation per stethoscope diaphragm was four species and the minimum isolate was one bacterial species. *CoNS* were the most frequent gram positive isolate (46.6%) and *Klepsiella spp.* (10.7%) was the most frequent gram negative isolate (Table2).

Table 2 Bacterial profile isolated from stethoscopes used at SPHMMC from December 2017-April 2018.

Isolated bacteria	No (%)
CoNS	122(46.6)
S.aureus	70(26.7)
Klebsiella Spp.	28(10.7)
Bacillus Spp.	14(5.3)
E.coli	14(5.3)
Acinetobacter Spp.	8(3.0)
ShigellaSpp.	2(0.8)
Proteuse Spp.	2(0.8)
Pseudomonas Spp.	1(0.4)
Providential Spp.	1(0.4)
Total	262(100)

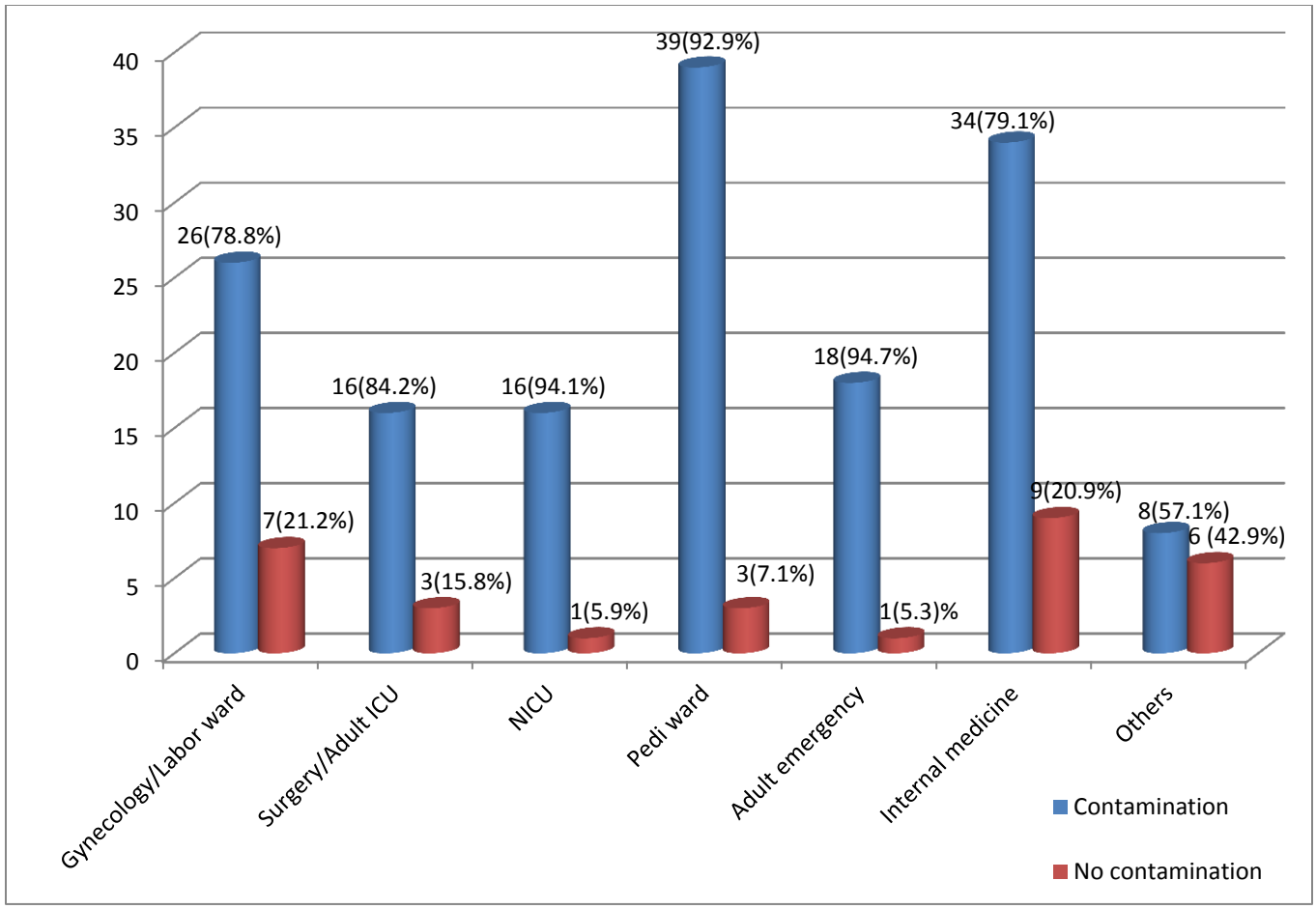

Figure 1 Rate of contamination versus stethoscope owners attending wards at SPHMMC from November 2017-April 2018.

High stethoscope contamination levels were observed at adult emergency and NICU.

6.3. Antimicrobial sensitivity patterns of the isolates

Among 262 isolates, 248 were tested against for different available antibiotics in the laboratory, based on the CLSI guidelines. Methicillin resistance level of *S.aureus and CoNS* was 20.3% and 37.7% respectively. *S.aureus* (88.7%) and *CoNS (*87.1%) showed the high resistance level for penicillin G.

Of the *S.aureus* the least resistance showed to Amikacin (15.1%), Ciprofloxacilin (23.2%) and followed by Clindamycin (23.9%) where as *CoNS* showed the least resistance to Ciprofloxacilin (18.6%) followed by 21.5% of Clindamycin and chloramphenicol.

From the gram negative isolates *Klebsiella spp* was the most dominant followed by *E.coli* and *Acinetobacter Spp*. *Klebsiella Spp* showed the highest sensitivity to Ciprofloxacilin (92.6%) where as the *E.coli* showed high sensitivity (95.7%) for both Meropenem and Amikacin.

Providencia species was the rare bacteria we encountered. This was found from a resident doctor during his visiting in internal medicine department. And this bacteria was 100% sensitive to (CN, CHL, CAZ, SXT, AK, AUG and Mero).

Table 3: Resistances *Pattern* of Bacterial Isolates from Stethoscope Diaphragm to Commonly Used Antibiotic at SPHMMC from December 2017-April 2018.

Antibiotic used	**Resistance patterns of isolates from stethoscope at SPHMMC**								
	S.aureus (n=70)	*CoNS* (n=122)	*Klepsiella* spp(n=28)	*Acinetobacterspp*(n=8)	*Proteus(n=2)*	*Pseudomonasspp(*n=1)	*E.coli* (n=14)	*Shigella* (n=1)	*Providencia spp* (n=1)
AK(30 µg)	11(15.1)	33(27)	12(42.9)	6(75)	0	0	2(14.3)	-	0
Amp(10 µg)	-	-	27(96.4)	8(10)	2(100)	-	12(80)	1(100)	1(100)
Aug(20/10)	-	-	26(92.9)	-	2(100)	-	8(57.1)	-	0
CAZ(30µg)	-	-	19(70.4)	6(75)	0	1(100)	6(42.9)	-	0
CIP(5 µg)	16(23.2)	22(18)	2(7.4)	4(50)	0	0	4(26.7)	-	0
CN(10 µg)	32(45.7)	47(38.5)	16(57.1)	8(100)	0	1(100)	4(26.7)	1(100)	0
CHL(30 µg)	18(26.1)	26(21.3)	14(50)	-	1(50)	-	3(18.8)	1(100)	0
CTR(30 µg)	-	-	3(11.5)	3(37.5)	0	-	3(18.8)	-	0
CTX(30 µg)	-	-	16(57.1)	3(37.5)	1(50)	-	4(28.6)	-	0
CXT(30 µg)	14(20.3)	46(37.7)	-	-	-	-	-	-	-
CLN(2 µg)	17(23.9)	26(21.3)	-	-	-	-	-	-	-
Dox(30 µg)	45(66.2)	51(41.8)	-	-	-	-	-	-	-

ERY(15 µg)	43(62.3)	51(41.8)	-	-	-	-	-	-	-
Mero(10 µg)	-	-	7(25)	4(50)	0	0	2(14.3)	-	0
P(10 µg)	62(88.7)	105(86.1)	-	-	-	-	-	-	-
TE(30 µg)	47(70.1)	71(58.2)	-	-	-	-	-	-	-
SXT(25 µg)	32(45.7)	68(55.7)	17(65.4)	6(75)	1(50)	-	6(46.2)	-	1(100)

Key: - (not done), 0(100% sensitive)

Most isolates were resistance to many antibiotic agents available in our laboratory. From the gram positive cocci both *S.aureus* and *CONS* were resistance to ten different antibiotic agents and with minimum resistance of five, six respectively. From the gram negative *Acinetobacter spp* and *klebsiella spp* shows a maximum resistance level were and *E.coli* shows a minimum resistance level.

Table 4 MDR Patterns of Bacterial Isolates from Stethoscope Diaphragm in SPHMMC from December 2017-April 2018.

Bacterial isolate	Quantity	Resistance to the Antibiotic
S.aureus	Max.	10 (Ak,CN,CHL,CLN,DOX,TET,ERY,P,SXT)
	Mini.	5 (P,SXT,DOX,TET,ERY)
CoNS	Max.	10 (AK,ERY,TET,DOX,SXT,P,CHL,CIP,CN,CXT)
	Mini.	6 (P, ERY, TET, DOXY, SXT, CN)
E.coli	Max.	6 (CHL,CN,Amp,Aug,CTX,CAZ)
	Mini.	2 (Amp,Aug)
Klebsiella spp	Max	9 (SXT,CIP,CHL,CN,AMC,Amp,Aug,CTX,CAZ)
	Mini.	5 (SXT,AMP,Aug,CTX,CAZ)
Acinetobacter spp	Max.	9 (SXT,CIP,CN,AK,Amp,Mero,CAZ,CTX,CTR)
	Mini	5 (SXT,CIP,CN,Amp,CAZ)

6.4. Factors Associated with Colonization level of Selected Medical Device

In this study the contamination level of stethoscope in both sex and experience level of our study participants shows almost equal.

From this study participants 159(85%) of the HCWS believe that stethoscope could carry microorganisms and that might be the source of HAI for visiting patients in health care facilities as well as for the health care providers, but only 19.3% clean their stethoscope after and before seeing each patient. working in pedantic ward, adult emergency, adult ICU and surgical ward is more likely to have contamination level than working in other departments and this is statically significant ($p<0.05$) (Table 5).

Table 5: Factors Associated with Bacterial Colonization Level of Selected Medical Device at SPHMMC from December 2017-April 2018.

Variables		Frequency (%)	Growth		P-value	COR 95% CI	P-value	AOR 95% CI
			Yes (%)	No (%)				
Sex	Male	105(56.1)	88(83.8)	17(16.2)	0.950	0.975(0.44,2.14)	-	-
	Female	82(43.9)	69(84.1)	13(15.9)	1.000			
Age	≤24yr	68(36.4)	56(82.4)	12(17.6)	0.239	0.389(0.08,1.87)	-	-
	25-29yr	93(49.7)	77(82.8)	16(17.2)	0.245	0.401(0.08,1.87)	-	-
	≥30yr	26(13.9)	24(92.3)	2(7.7)	1.00			
Experience	≤1yr	136(72.7)	113(83.1)	23(16.9)	0.593	0.782(0.313,1.95)	-	-
	>1yr	51(27.3)	44(86.3)	7(13.7)	1.000	6.457	-	-
Stethoscope last cleaned	≤ 1week	102(54.6)	83(81.4)	19(18.6)	1.000			
	2-4week	6(3.2)	6(100)	0	0.345	0.637(0.25,1.63)	-	-
	≥5week	24(12.8)	20(83.3)	4(16.7)	0.999			
	Never	55(29.4)	48(87.3)	7(12.7)	0.643	0.729(0.19,2.77)	-	-
Occupation	Medical students	119(63.6)	99(83.2)	20(16.8)	0.663	1.238(0.48,3.22)	-	-
	GP/Resident	33(17.7)	30(90.9)	3(9.1)	0.215	2.500(0.588,10.63)	-	-
	HO/Nurse	35(18.7)	28(80)	7(20)	1.000			
Believes	Yes	159(85)	132(83)	27(17)	1.00			
Stethoscope as fomite	No	28(15)	25(89.3)	3(10.7)	0.409	1.705(0.48,6.05)	-	-
IP training	Yes	84(44.9)	70(83.3)	14(16.7)	1.000			
	No	103(55.1)	87(84.5)	16(15.5)	0.834	1.087(0.49,2.38)	-	-
Department units	GYN/Labor ward	33(17.6)	26(78.8)	7(21.2)	0.136	2.786(0.724,10.72)	0.131	5.67(0.59,53.81)
	AICU/Surgery	19(10.2)	16(84.2)	3(15.8)	0.095	4.000(0.788,20.32)	0.040	11.33(1.12,114.70)
	NICU	17(9.1)	16(94.1)	1(5.9)	0.033	12.000(1.23,117.41)	0.051	18.92(0.98,363.96)
	Pedi ward	42(22.5)	39(92.9)	3(7.1)	0.005	9.750 (1.99,251.09)	0.012	22.36(1.99,251.09
	Emergency	19(10.2)	18(94.7)	1(5.3)	0.025	13.500(1.39,131.32)	0.033	19.05(1.27,285.09)
	Internal medicine	43(23)	34(79.1)	9(20.9)	0.113	2.833(0.78,10.28)	0.079	7.01(0.80,61.52)
	Other	14(7.5)	8(57.1)	6(42.9)	1.000			
Borrowing stethoscope	Yes	153(81.8)	128(83.7)	25(16.3)	.814	0.883(0.31,2.50)	-	-
	No	34(18.2)	29(85.3)	5(14.7)	1.000			
Cleaning before/after	Yes	39(20.9)	33(84.6)	6(15.4)	1.000			
	No	148(79.1)	124(83.8)	24(16.2)	0.900	0.939(0.36,2.49)	-	-
$H_2o$and soap reduce bacteria	Yes	114(61)	94(82.5)	20(17.5)	0.486	1.340(0.59,3.05)	-	-
	No	73(39)	63(86.3)	10(13.7)	1.000			
70% alcohol reduce bacteria effectively	Yes	175(93.6)	146(83.4)	29(16.6)	1.000			
	No	12(6.4)	11(91.7)	1(8.3)	0.463	2.19(0.27,17.59)	-	-

Key: COR-crude odd ratio, AOR-adjusted odd ration, I-Confidence interval, yr-year,IP-infecrion prevention,GP-general practionare,

Eight Otoscope's tips were swabbed for microbiological contamination. Of these six (75%) were colonized with bacteria and the most contaminant were *CoNS* .100%, 50% of the colonoscope and laryngoscope were contaminated respectively (Table 6).

Table 6.Types of Bacteria Isolated from Medical Devices at SPHMMC from December 2017-April 2018.

Bacteria isolates	Otoscope(n=8)	Colonoscopy(n=4)	Laryngoscope(n=2)
CoNS	4(50%)	3(33.4%)	-
S.aureus	3(37.5%)	1(11.1%)	1(50%)
Klepsiella spp	-	1(11.1%)	-
E.coli	-	1(11.1%)	1(50%)
Proteus spp	-	1(11.1%)	-
Moraxella spp	1(12.5%)	-	-
Pseudomonas spp	-	1(11.1%)	-
Bacillus spp	-	1(11.1%)	-

Key: n (Number), spp (Species), - (Not done),

The isolates from Otoscope shows high sensitivity pattern for Amikacin. *Moraxella spp* was the only gram negative isolate (Table 7).

Table 7: Antibiotic Resistance Patterns Isolated from Medical devices at SPHMMC from November 2017-April 2018.

Antibiotic used	Otoscope (n=8)			Colonoscope (n=4)						Laryngoscope(n=2)	
	S.aureus (n=3)	*CoNS* (n=4)	*MoraxellaSpp* (n=1)	*S.aureus* (n=1)	*CoNS* (n=3)	*E.coli*(n=1)	*KelebsiellaSpp* (n=1)	*Pseudomonas spp* (n=1)	*Bacillusspp* (n=1)	*Proteuse spp* (n=1)	*S.aureus* (n=1)	*E.coli* (n=1)
AK(30µg)	0	1(25)	0	0	1(33.3)	1	0	1	-	0	0	0
AMP(10µ)	-	-	1	-	-	1	1	-	-	1	-	1
AUG(30µg	-	-	1	-	-	0	1	1	-	1	-	1
CAZ(30µ)	-	-	1	-	-	1	1	1	-	1	-	1
CIP(5µg)	1(33.3)	4(50)	0	1	0	0	0	0	0	-	0	0
GN(10µg)	1(33.3)	1(25)	0	1	1(33.3)	0	0	0	-	0	0	1
CHL(30µ)	0	1(25)	0	0	1(33.3)	0	1	-	-	1	0	0
CTR(30µ)	-	-	0	-	-	0	1	-	-	1	0	0
CTX(30µ)	-	-	1	-	-	0	1	-	-	1	0	0
CXT(30µ)	1(33.3)	1(25)	-	1	0	-	0	0	-	0	-	1
CLD(2µg)	2(66.7)	1(12.5)	-	1	0	-	-	-	-	-	0	-
DOX(30µ)	2(66.7)	3(75)	-	0	3(100)	-	-	-	-	-	1	-
ERY(5µg)	2(66.7)	3(75)	-	1	3(100)	-	-	-	-	-	1	-
Mero(10µ)	-	-	0	-	-	0	0	0	-	0	-	0
P(10 µg)	2(66.7)	3(75)	-	0	3(100)	-	-	-	-	-	0	-
TTC(30µg)	3(100)	4(100)	-	0	3(100)	-	-	-	-	-	1	-
SXT(25µg)	2(66.7)	3(75)	-	1		1	1	-	-	0	1	1

Key: n (Number), spp (Species), - (Not done), 1-(100% resistance), 0-(100% sensitive)

Of 83 stethoscope disinfected by 70% alcohol, 78.3% were colonized by bacteria.27 (14.4%) HCWs use another disinfectant rather than 70% alcohol which showed high contamination level

The main reason of Health Care Workers for not disinfecting their stethoscope regularly was high work load 84(44.9%), but it was not statically Significant (p>0.05) (table 8).

Table 8: Possible Hindrance of Disinfecting Stethoscope versus Contamination Level of Medical Devices at SPHMMC from December 2017-April 2018.

Hindrance disinfecting stethoscope	**Frequency (%)**	**Contamination** No (%)	
		Yes	**No**
No cleaning agent	13(7)	12(92.3)	1(7.7)
High work load	84(44.9)	72(85.7)	12(14.3)
Lack of awareness	56(29.9)	44(78.6)	12(21.4)
Forgetfulness	34(18.2)	29(85.3)	5(14.7)
Total	187(100)	157(84)	30(16)

7. Discussion

This study focused on the bacterial contamination and multidrug resistance level of medical devices and health care provider's way of maintaining stethoscope cleaning for preventing the spreading of HAI.

Bacterial contamination level of medical devices is high and there is an increase report of the risk of transmitting MDR microorganism from patient to patient specially stethoscope are the commonest medical device used to assess the health of patients and have been reported to be potential vector for nosocomial infections in various part of the world (12).

In this current study the level of bacterial colonization of medical devices was found to be 84%. And magnitude of bacterial colonization of medical devices significantly associated with the Pedi ward and Adult emergency. Being in these departments showed 22 and 19 times more likely to have higher contamination level respectively. The type of the patient, environmental hygiene and knowledge of the health care to disinfect medical devices could contribute for this variation.

This study showed higher contamination than a study conducted in Milan Italy by Carducci A *et al* (68%) (29) And had in lined result with study conducted in Turkey by Kilic IH. *et al* (76%)(19). This difference may be due to knowledge gap and commitment to disinfect medical equipments for prevention of infections.

In India a study conducted in 2016 by Surase P *et al* the bacterial contamination level of stethoscopes were 91% (15) which is similar to this study but similar study was also conducted in India showed 52% contamination level (20) which is less compare to both studies. This could be due to lack of awareness and health facility set ups and the health facility policy of handling of medical equipments for infection prevention.

This study is also in line with studies conducted in Nigeria (80.1%, 79%) (11, 12) But the study conducted by Bernard L et al (85%) (30) Showed higher bacterial contamination level compare to the studies mentioned above. This difference may be due to different reasons like cleaning frequency of health care providers and difference in self motivation to disinfect their devices.

A study conducted in Sudan total contamination level of Stethoscope was 89.5 % (31) which was comparable to this study.

A study conducted by Shiferaw T *et al* (24) at Jimma specialized showed (85.8%) of the stethoscope was colonized with different bacteria which is similar to this study but the study conducted by Dabsu R. *et al* (25) and Worku T *et al* (32) at Tikur Anbesa Specialized Hospital and Mizan-Tepi University teaching hospital showed (60.3%, 25%) respectively which is less compare to this result. This difference could be due to sample size and the type of disinfectant they use. Also educational back ground and participating in infection prevention training and gaining information by self –learning attributed to difference.

Korkmaz H *et al* (33) conducted a study on cross contamination of otoscope showed 41.5% of contamination levels which is less compare to this study (75%). pseudomonas species was the only gram negative bacteria documented in this mentioned study where as we only documented one *morexella species* this difference of contamination level could be frequency of cleaning as well as sanitation level of working environments.

Another study conducted in korea by Ku CH *et al* (34) documented 66.7% of otoscope were contaminated which is similar to this study and the most isolate was *s.aureus* (83%) which is higher compare to this study's *s.aureus* isolated (37.5%).This difference might be due to these bacteria are a known normal flora of superficial part of the body. Another study conducted at black lion specialized hospital (25) showed (83%) of the otoscope were contaminated with different type of bacteria which are almost similar to this study.

Even though we collected only two samples from laryngoscope one (50%) of the equipment were contaminated with *S.aureus* and *E.coli spp.* This study in line with the study conducted in South Africa which was 57.3% of the laryngoscope was contaminated (35). However this study showed less contamination compared to study conducted by Call TR,*et al* (75%) (21).This difference might be because of sample size and decontamination policy between countries.

Studies showed that female has more tendencies to clean their equipments due to this male health care worker's stethoscope are more contaminated than female was reported by Kilic I.H *et al* (19). Although in this study the contamination level in both sexes was similar (83.8%, 84.1%) respectively this is supported by study conducted by Pal K *et al* (20).

This study showed cleaning stethoscope before and after seeing each patient was (20.9%) which is supported by the previous study conducted by Grecia SC *et al* ,Uneke CJ et al and Worku T *et*

al which was 30% ,15%,22% (23,12,32) respectively. But this study showed higher frequency of cleaning stethoscope than previously conducted study in Jimma University specialized hospital(24).

A study conducted by Aftab HB. *et al* in Pakistan shows 298(83.9%) of the study participants believed that fomites like stethoscope can be potentially act as carrier for hospital acquired infection (36) which is comparable with this study results 159(85%) .
As far as we are developing countries due to many reasons the antibiotic resistance level is high this could be due to non adherence to treatment strategies and drug quality can favor for emergence of multi drug resistance bacteria.

In this study prominent isolate from stethoscope was *CoNS* which account 65.2% followed by *S.aureus* (37.4%) which is similar to the prominent isolated from Jimma and Black lion hospitals (24,25). However the prominent isolated from Mizan Tepi hospital was *Klebsiella spp* (32). The above mentioned study also found *Citrobacter,salmonella* and *serratia spp* this variation in bacterial isolate type could be due to environmental sanitation.

In this study MDR bacterium was observed among the isolates. The MDR level was common in both gram positive and Gram negative bacterial isolates. From the gram positive *S.aureus and CoNS* showed high resistance to multiple classes of antibiotics where as *Klebsiella spp* and *Acinetobacter spp* showed the highest MDR level from the gram negative. This showed the MDR level of both gram positive and gram negative less compare to study conducted elsewhere (12) but it is higher compare to other studies conducted in Ethiopia (24,25).
The prevalence of MRSA in this study was 20.3% which is in line with the study conducted by Smith et al in Texas Houston (17%) (17). Also this study showed similarity with study conducted by Shiferaw T *et al* (26.6%) (24). But it is less compare to the study conducted by Sengupta et al and Mark A *et al* MRSA isolate were documented as (69.76%, 32%) (37,38) respectively. Whereas study conducted by Padey A *et al* (39) showed 7.3% which is less compare to the above mentioned studies. This difference might be due to geographical difference and sample size.

In this study Ciprofloxacin was the most effective antibiotic against all contaminant. This in line with studies conducted by Uneke CJ *et al*, Worku T *et al* and SMM Fageer (12, 25,40). From this study *S.aureus* showed 76.8%, 37.7% and 73.9% sensitivity level to Ciprofloxacin,

Erythromycin and chloramphenicol respectively. In contrast according to Uneke CJ *et al* this isolate showed 100% sensitivity to Ciprofloxacin and 100% resistance to Erythromycin and chloramphenicol and also the study conducted by SMM Frageer (40) *S.aureus* 100% sensitive to Ciprofloxacin, Erythromycin.

According to the finding of the study done at Jimma University Specialized hospital *Proteus Spp* was 100% resistance to Ampicillin which is similar with this study. This mentioned study also showed high penicillin resistance of *CoNS* and *S.aureus* were documented as 87.4% and 75.9% respectively (24).even though this study showed similarity with the penicillin resistance of *CoNS* (86.1%) however the *S.aureus* resistance level for this antibiotic were higher compared to the above mentioned study which is documented as 88.7% .

8. Limitation of the study

- Small sample size used for both laryngoscope and Colonoscopy devices.
- Senior physicians were not included because of their availability and refusing to be part of this study.
- This study was done in a single hospital.

9. Conclusion and recommendation

9.1. Conclusion

Most of isolates were resistance to multiple classes of antimicrobial. Even though cleaning stethoscope is not labor intensive and does not required much time, cleaning habits of the Health Care Workers were low. Therefore careful handling of stethoscope and other medical equipment is critical to minimize spreading of hospital acquired infections.

9.2 Recommendation

Careful handling of stethoscope and other medical equipments is critical to maximize hygienic and reduction of contamination. Regular educational programs are needed to strengthen awareness on regular disinfection of stethoscope with suitable disinfectant. Further study will be needed to asses and evaluate the frequency and efficacy of the cleaning agents.

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.11. Annexes

11.1. Annex I. General information sheet for the study participants

Date_____EC_/_____GC

Introduction

Hello dear participants!

My name is Zenebe G/yohannes and I am MSC student of Addis Ababa University, School of Medical laboratory Sciences. I am doing a research entitled with "Burden of multi drug resistance (MDR) bacteria and factors affecting the level of contamination in medical equipments (stethoscope, otoscope and endoscope) among health care workers (HCW) at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia."

Purpose of the study

The objective of this study is to assess the burden of multi drug resistance (MDR) bacteria and factors affecting the level of contamination in medical equipments (stethoscope, otoscope and endoscope) among health care workers (HCW) at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia**.**

Duration:

The duration of this study depend upon the availability of study subjects. It might take about three months or more.

Risk associated with the specimen collection:

The risk associated with the sample collection is free of any risk and associated factors.

Procedure of the study

If you agree to participate in the study, sample will be collected from the diaphragm of the stethoscope and handle of endoscope and tip of otoscope with sterile swab moistened in normal saline by principal investigator.

Confidentiality

All records will be kept strictly confidential. Participants identifications will not be collected for the study purpose instead code number will be used. Personal identifying information will not be shared outside of the study and it will not be used in any of the publications.

Benefit of the study

The benefit of this study is to create awareness on nosocomial infections caused by medical devices like stethoscope this result will give hint to the health worker to keep their medical devices clear. And there is no any payment or direct benefit for participating.

Withdrawal rights

Your participation in this study is purely voluntary and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable. You are free to refuse to participate in the study or you can withdraw your consent at any time, without giving reasons and this will not involve any penalty or loss of benefits.

If you are not comfortable please feel free to stop it at any level of the study. I appreciate your cooperation to a great extent.

If you have any question regarding to this study, the address of the principal investigator and the school is:

Principal Investigator: **Zenebe G/yohannes**

Tel: +251-913294377

Email: zenebezoha@gmail.com

AAU College of health sciences school of allied health science department of medical laboratory science

Tel: +251-112755170

I would like to thank you for your time. Are you willing to participate in the study?

☐ Yes, I am willing to participate in the study.

☐ No, I do not wish to participate in the study.

Name_____signiture_____Date

11.1. Annex II: General information sheet for the study participants in Amharic

አጠቃላይመረጃ

አዲስ አበባ ዩንቨርሲቲ የድህረ ምረቃ ት/ቤት የላብራቶሪ ሳይንስ ትምህርት ክፍል

በጥናቱ የሚሳተፉ የህክምና ባለሙያዎች የፈቃድ መጠየቂያ እና መቀበያ ፎርም/ሺት/

መግቢያ፡

ሰላም እንደ ምን አሉ?

ስሜ ዘነበ ገ/ዮሀንስ እባላለሁ:: የአ.አ.ዩ. የላበራቶሪ ሳይንስ ትምህርት ክፍል የማስተርስ ዲግሪ ተማሪ ነኝ በአሁኑ ሰአት በ ቅ/ጳ/ሚ/ሚ/ሆ በህክምና መገልገያ መሳሪያዎቸ ላይ የሚገኙ ተህዋስ እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት ምን ያህል እንደሆነ ለማወቅ ጥናት እያካሄድኩ ነው፡፡

የጥናቱ ዋና አላማ፡

የጥናቱ አላማ በህክምና መገልገያ መሳሪያዎች ላይ የሚገኙ ተህዋስ እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት ምን ያህል እንደሆነ ለማውቅ ነው፡፡

የጥናቱ ጊዜ፡

የ ጥናቱ ጊዜ በሚገኙ የናሙና መጠን የሚወሰን ሲሆን 3 ወር እና ከዛም በላይ ሊወስድ ይችላል፡፡

ሊከሰቱ ስለሚችሉ ስጋቶችና የምቾት መጓደሎች፡

ለጥናቱ በሚወሰደዉ ናሙና ምክንያት የተለየ ችግር አይከሰትም፡፡የሚያሰጋ ምንም ነገር የለዉም ምክንያቱም የጥናቱ ናሙና አወሳሰድ ጊዚ የማይፈጅ ስለሆነ ይሄ ነው የሚባል ችግር የሚያስከትል ወይም የሚያሰጋ ነገር የለውም፡፡

<u>የጥናቱ ሂደት</u>

እርስዎ በጥናቱ ላይ ለመሳተፍ ፍቃደኛ ከሆኑ ከ ስቴቶስኮፕህ / ሽ ፣ ከአቶስኮፕህ / ሽ ፣ ከ ኢንዶስኮፕህ / ሽ በንጹህ ጥጥ ከነዚህ መሳሪያዎች ላይ ናሙናዎችን ጠርገን እንወስዳለን።

የጥናቱ ሚስጢራዊነቱ፡

የሚሰጡት መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡በስም አይጻፋም የዚህ ኮድ መፍቻ በፋይል ተቆልፎ የሚቀመጥ ሲሆን የተፈቀደለት ሰው ብቻ ፋይሉን ማየት ይችላል፡፡ከዚህ ጥናት በሚወጡ ዘገባዎች ወይንም የህትመት ውጤቶች ላይ ስምም ወይም ሌላ የእርስዎን ማንነት የሚገልጽ መረጃ አይኖርም፡፡ ኮምፒውተር ላይ ያሉ መርጃዎችም ስጢራዊነታቸው የተጠበቀ

ሲሆን በወረቀት ያሉ መረጃዎችም ደህንነቱ በሚጠበቅ ቦታ የሚቆለፉና የተፈቀደለት ሰው ብቻ ሊያያቸው እንዲችል ተደርጎ ይጠበቃሉ፡፡

የሚሰገኘው ጥቅም፡

በጥናቱ በመሳተፈዎ ምንም አይነት ክፍያ አይጠየቁም ወይም የሚያገኙት ገንዘብ አይኖርም፡፡

ከጥናቱ ስለማቋረጥ፡

በጥናቱየሚሳተፉትፈቃደኛ ከሆኑ ብቻ ነው፡፡ስለዚህ መሳተፍ አለመሳተፍ ከጀመሩ በኋላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ከሆነ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡በጥናቱ መሳተፍ ወይም አለመሳተፍ አገልግልት ላይ ምንም አይነት ጥቅምም ሆነ ጉዳት አይኖረውም፡፡ጊዜዎትን መስዕዋትአድርገው ስለ ተባበሩኝ ከልብ አመሰግናለሁ፡፡

ስለ ጥናቱ ሕጋዊነት ለመጠየቅ ከፈለጉ፡

ይህንን ጥናት አስመልክቶ ጥያቄ ካለዎት ወይም የጥናቱ የመጨረሻ ዉጤት ምን እንደሆነ ለማዉቅ ከፈለጉ በሚከተለዉ አድራሻ ሊያገኙን ይችላሉ፡፡

ጥናቱ አሰከያጅ፡ _ዘነበ ገ/ዮሃንስ

ሰ.ቁ፡-09-13-29-43-77

ኢ.ሜል : zenebezoha@gmail.com

11.2. Annex II: Informed consent

I under signed to confirm that, as I give consent to participate after a clear understanding of the objectives and conditions of the study & with recognition of my right to withdraw from the study if I change my mind. I …….do interestingly give consent to Mr zenebe G/yohannes to include me in the study entitled with Burden of MDR bacteria and. The proposal has been explained to me in the language I understand.

ID number of Participant _____

Participant's signature: _____

Name of data collector: _____

Signature of data collector: _____

Date: _____

11.3. Annex III: Informed consent in Amharic version

የተሳታፊው ስም…….ስለ ጥናቱ አስፈላጊ የሆኑትን መረጃዎች አንብቤ ወይም ተነቦልኝ ተረድቻለው አላማውም በቅ/ጳ/ሚ/ሚ/ሆ በህክምና መገልገያ መሳሪያዎች ላይ የሚገኙ ተህዋስ እና የተህዋስያኑ መድሃኒት የመቋቋም ያለዉን ስርጭት ምን ያህል እንደ ሆነ ለማወቅ ነው፡፡

እኔ…….የተባልኩ የተጠየኩትን ጥያቄ ለመመለስ እና ናሙና ለተመራማሪዉ ለመስጠት ፈቃደኝነቴን ገልጫለሁ ::

የተሳታፊው ፊርማ…….. ቀን……..

የተመራማሪዉ ፊርማ…….ቀን……

11.4. Annex IV: English version of the questionnaire

The title of this study is Burden of MDR bacteria and factors affecting the level of contamination in medical equipments (stethoscope, otoscope and endoscope) among health care workers (HCW) at St. Paul's hospital millennium medical college, Addis Ababa, Ethiopia**.**

We are gratefully for your agreement to participate in this study. This standard questionnaire is prepared to collect the demographic data of Health care workers how do they disinfect and frequency of maintenance of their stethoscope and other medical devices. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore we respectfully ask you to give us the right answer.

Date_____/_____/_____

Candidate number/code _____

1. Age _____

2. Sex_____

3. Occupation _____--

4. Currently assigned/Department_____

5. Length of practice/experience in the hospital (number of years/months?
_____

6. Number of hours of patient contact/care (state number of hours)
   A. How many hours in 24 hour?_____
   B. How many hours in 1 week? _____

7. Have you ever taken training on infection prevention?

A Yes

B No

The questions from 8-17 are practice related please put X your answer for the questions with in the table in the space provided and you are allowed to choose only one.

		Choices				
	Questions	Never	Sometimes	Often	Almost always	Always
8	Do you use your own stethoscope when examining patients?(please choose one)					
9	Do you borrow a stethoscope when examining patients?(please choose one)					
10	Do you clean your stethoscope before seeing each patient?					
10	Do you clean your stethoscope after seeing each patient? (choose one)					

11	Do you wash your hands after seeing each patient?					
12	Do you clean your hands after touching patient's intact skin during examination?					
13	Estimate how often your co-workers clean their hands after examining a patient					
14	Estimate how often your co-workers clean their stethoscope after each patient use					

15. How many times do you clean your stethoscope? (Please answer one)
A _____x/day
B _____x/week
C _____x/month
D Never
E others please specify _____

16. When did you last clean your stethoscope? (Please answer one)
A_____hours ago (how many?)
B _____days ago (how many?)
C _____1 week ago
D_____2-4 weeks ago
E _____more than 1 month
F never
G others, please specify: _____

17. What agent do you use to clean your stethoscope?

A water only
B Alcohol_____%
C Soap and water
D dry cloth
E Others, please specify_____

Please put X your answer for the questions with in the table in the space provided and you are allowed to choose only one.

Questions		Strongly agree	Agree	Disagree	Strongly disagree	Idon't know
18	Cleaning **stethoscope diaphragm** with soap and water reduces bacterial count effectively **(knowledge)**					
19	Cleaning **stethoscope diaphragm** with 70% alcohol reduces bacterial count effectively**(Knowledge)**					
20	In your observation, disinfection after each **stethoscope** use is not an established practice by health care providers **(practice)**					
21	I believe that stethoscopes are potential vectors of infection. (**attitude)**					
22	I feel safe to use the **stethoscope** I'm using now, in its current state, to examine my family/relatives without the fear of making them ill (**attitude)**					

23. What do you think are the possible hindrances/barriers for a health care provider to comply with the stethoscope care recommendations? **(Please rank one from most likely reasons).**

A. No accessible of cleaning agent's in the hospital.
B. High workload
C. Insufficient knowledge of recommended guidelines
D. Lack of awareness of the impact of stethoscope cleaning on prevention of infection spread
E. Forgetfulness
F .Insufficient or lack of time

Please specify others: _____

I THANK YOU!!

11.5. Annex VI Questioners in Amharic version

በመጀመሪያ በጥናቱ ላይ ለመሳተፍ ፈቃደኛ ስለሆኑልን ምስጋናችን የላቀ ነው። ይህ መጠይቅ የተዘጋጀው የህክምና ባለሙያዎች በህክምና መሳሪያዎች ላይ ያላቸው የአጠቃቀም እና በነዚህ መሳሪያዎች ላይ ያላቸውን የንጽህና አያያዝ ለማዎቅ የተዘጋጀ መጠይቅ ነው።

እርስዎ የሚሰጡን መረጃ ሚስጢራዊነቱ የተጠበቀ ሲሆን ፥ የሚሰጡን መረጃ ለጥናቱ እጅግ ጠቃሚ በመሆኑ ትክክለኛ መረጃ ይሰጡን ዘንድ በትህትና እንጠይቃለን።

የተሳታፊው ስም _____

ስለጥናቱ አስፋላጊ የሆኑ መረጃዎችን አንብቤ ወይም ተነቦልኝ ተረድቻለሁ። እናም በህክምና መገልገያዎች መሳሪያዎች የሚገኙ በሽታ አምጭ ባክቴሪያዎች እና መዳሃኒት የመቋቋም ያላቸውን አቅም ማወቅ ሲሆን ይህን ተረድቼ አስፈላጊውን መረጃ እና ናሙና ለመስጠት ፈቃደኝነቴን አረጋግጣለሁ።

☐ ፈቃደኛ ነኝ።

☐ ፈቃደኛ አይደለሁም

ቀን_____/____/_____

ኮድ_____

1. ዕድሜ _____

2. ጾታ _____

3 ሙያ_____

4 የስራ ክፍልዎ_____

5. የስራ ልምድ _____

6. ከታካሚወች ጋር ያለዎት የንክኪ ግዜ

ሀ_ በ 24 ሰአት ውስጥ ለ _____ ሰአት

ለ_ በ ሳምንት ለ _____ሰአት

7. ተላላፊ በሽታዎች ላይ ስልጠና ወስደው ያውቃሉን ?

A. አዎ

B. አይ

		ምርጫዎች				
	ጥያቄዎቸ	በጭራሽ	አንዳንድ ጊዜ	በዙ ጊዜ	ሁል ጊዜ ማለት ይቻላል	ሁል ጊዜ
8	ታማሚዎችን ስታዩ የግል የልብ ምት ማዳመጫ መሳርያ ይጠቀማሉ ?					
9	ታማሚውን ለማየት የልብ ምት ማዳመጫ መሳርያ ተውሰው ይጠቀማሉ ?					
10	ታካሚዎችን ካዩ በውሃላ በምን ያህል ግዜ እጅዎትን ይታጠባሉ ?					

11	ለእያንዳዱ ታካሚ የልብ ምት ማዳመጫ መሳርያ ከተጠቀሙ በውኃላ ያጸዱጻል ?					
12	ታማሚዎችን ካዩ በውሃላ የቆዳ ንክኪ ቢኖሮዎት በምን ያህል ግዜ እጅዎትን ይታጠባሉ ?					
13	የስራ ባልደረባዎ የህክምና ባለሙያ ታማሚዎችን ካየ በውሃላ ለምን ያህል ግዜ እጁን ያጸዳል ብለው ያስባሉ ?					
14	የስራ ባልደረባዎ እያንዳዱን ታካሚዎችን ካየ በውኃላ ለምን ያህል ግዜ የልብ ምት ማዳመጫ መሳርያውን ያጸዱታል ብለው ያስባሉ ?					

15 በምን ያህል ግዜ የልብ ምት ማዳመጫ መሳርያህን / ሽን ያጸዱታል ?

ሀ_ _____ ጊዜ በቀን

ለ_ _____ ጊዜ በሳምንት

ሐ_ _____ ጊዜ በወር

መ_ በጭራሽ አጽድቻቸው አላውቅም

ሰ_ እባኮትን ሌላ ካለ ቢገልጹልን ----------

16. የልብ ምት ማዳመጫ መሳርያ ህን / ሽን ለመጨረሻ ጊዜ ያጸዱት መች ነበር ?

ሀ_ ከሰአታት በፊት

ለ_ ከ ቀናት በፊት

ሐ_ ከሳምንት በፊት

መ_ ከ 2-4 ሳምንት በፊት

ሰ_ ከወር በላይ

ረ_ በጭራሽ አላጸዳሁትም

ሸ_ እባክዎትን ሌላ ካላ ይግለጹልን _____

17. የልብ ምት ማዳመጫ መሳርያህን / ሽን በምን ያጸዱታል ?

ሀ_ በውሃ ብቻ

ለ_ በአልኮል በ _____ %

ሐ_ በውሃና በሳሙና

መ_ በደረቅ ጨርቅ

ሰ_ እባክዎትን ሌላ ካለ ይግለጹልን _____

እባኮት ከዚ በታች ባለው ሰንጠረዥ ዉስጥ ያለው ጥያቄዎች በማነበብ በተዘጋጀው ክፍት ላይ የ **X** ምልክት በማስቀመት የመልሱ::ከአንድ በላይ መልስ መስጠት የተከለከለ ነው::

	ጥያቄዎቹ	ምርጫዎች				
		በጣም እስማማለው	እስማማለው	አልስማማም	በጣም አልስማማም	ግንዛቢው የለኝም
18	የልብ ምት ማዳመጫ መሳርያን በውሃ ና በሳሙና ማቲዳት የባክቲርያ ቁጥር ይቀነሳል ::					
19	የልብ ምት ማዳመጫ መሳርያን በ 70% ማፅዳት የባክቲርያ ቁጥር ይቀነሳል ::					
20	በሚሰሩበት ሆስፒታል ዉስጥ በህክምና ሰጪ					

	ባለሞያዎች የልብ ምት ማዳመጫ መሳርያቸው ታማሚ ካዩበት በሓላ የማፅዳት ባህላቸው ወጥነት የለውም ማለት ይቻላል					
21	የልብ ምት ማዳመጫ መሳርያ ተላላፊ በሽታዎች ያስተላልፋል ብለው ያምናሉ					
22	በቅርብ ሳት እየተጠቀሙት ያለው የልብ ምት ማዳመጫ መሳርያ ለወዳጅ ሆነ ለቢተሰብ ቢጠቀሙት በስታ ላስተላልፍባቸው አልችልም እንዲሁም ስጋት የለኚም ብለው ያስባሉ?					

23. የልብ ምት ማዳመጫ መሳርያቸው ዕንዳያፀዱ ምክንያት ምንድን ናቸው?

ሀ.ሆስፒታል ዉስጥ ማፅጃ ስለሌለ ነው

ለ. ስራ ስልሚበዛ ነው

ሐ.እውቀቱ ስሌለኝ ነው

መ. ግንዛቤ ስሌለኝ ነው

ሠ ጊዜ ስሌለኝ ነው

ረ ስለምረሳ ነው

ሠ.ሌላ ካለ ይግለጹልን

ጊዜ ሰጥተው ስለተባበሩን እናመሰግናለን!!!

11.6. Annex VII: Standard Operative Procedures

11.6.1. Specimen collection

Specimen will be collected from the diaphragm of the stethoscope, tip of the otoscope and the handle of endoscope using moisten sterile cotton swab, with (0.9% w/v) physiological saline. The swab will put quickly into its container and sealed with unique code number similar to the code number labeled on health worker's questioner and 1 drop of normal saline will be used to transport the collected sample to microbiology department of the hospital.

11.6.2. Culture media preparation

1. Weighing and dissolving of culture media
2. Sterilization and sterility testing
3. Addition of heat sensitive ingredients
4. PH testing of culture media
5. Dispensing of the culture media
6. Quality assurance of culture media
7. Storage of culture media

Prepare media made from dehydrated products in as damp-free an environment as possible. To prevent the risk of inhaling fine particles of dehydrated media, wear a dust mask while handling dehydrated media. Powder or use granulated media

- Wash the hands immediately after preparing a media.
- Once the ingredients are weighed, do not delay in making up the medium. Follow exactly the manufacturer's instructions.
- Use completely clean glassware, plastic or stainless steel equipment that has been rinsed in pure water. The container in which the medium is prepared should have a capacity of at least twice the volume of the medium being prepared.
- Use distilled water from a glass still.
- When heating is required to dissolve the medium, stir while heating and control the heat to prevent boiling and foaming which can be dangerous and damage the medium, Overheating a medium can alter its nutritional and gelling properties, and also its PH.
- Autoclave a medium only when the ingredients are completely dissolved. Always autoclave at the correct temperature and for the time specified.

- Dispense medium in bottles or tubes in amounts convenient for use. Know the length of time prepared media can be stored without deteriorating.

11.6.3. Dispensing sterile media into Petri dishes

1. Lay out the sterile Petri dishes on a level surface.
2. Mix the medium gently by rotating the flask or bottle. Avoid forming air bubbles. Flames sterilize the neck of the flask or bottle and pour 15–20 ml of medium into each dish (90–100 mm diameter). Air bubbles enter while pouring, rapidly flame the surface of the medium before gelling occurs. Rotate the dish on the surface of the bench to ensure an even layer of agar.
3. When the medium has gelled and cooled, stack the plates and seal them in plastic bags to prevent loss of moisture and reduce the risk of contamination. Do not leave the plates exposed to bright light especially sunlight.
4. Store at 2–8 O^C.

Note: Agar plates should be of an even depth (not less than 4 mm) and of a firm gel. The surface of the medium should be smooth and free from bubbles.

11.6.4. Inoculation to culture media

Immediately before inoculating a culture medium check the medium for visual contamination or any change in its appearance which may indicate deterioration of the medium, e.g. darkening in color. When inoculating, or seeding, culture media an aseptic (sterile) technique must be used. This will:

– prevent contamination of cultures and specimens,

– prevent infection of the laboratory worker and the environment.

Aseptic techniques

1. Flame sterilizes wire loops, straight wires, and metal forceps before and after use. Whenever possible, use a Bunsen burner with a protective tube to avoid particles being dispersed when flame sterilizing wire loops.
2. Flame the necks of specimen bottles, culture bottles, and tubes after removing and before replacing caps, bungs, or plugs.

3. When inoculating, do not let the tops or caps of bottles and tubes touch an unsterile surface. This can be avoided by holding the top or cap in the hand. Always use racks to hold tubes and bottles containing specimens or culture media.
4. Make slide preparations from specimens after inoculating the culture media.
5. Decontaminate the work bench before starting the day's work and after finishing.
6. Use a safety cabinet when working with hazardous pathogens.
7. Wear protective clothing; wash the hands after handling infected material.

11.6.5. Laboratory procedure for collection, transportation and culturing of the collected swab

1. Insert the tip of cotton swab in to normal saline and Rotate sterile cotton-tipped applicator on the side of the test tube containing to remove excess moisture.

2. Gently rotate the tip of cotton swab on the diaphragm of the stethoscope, tip of the otoscope and the handle of endoscope with sufficient pressure to collect bacteria from the surface.

3. Placing the swabs in to sterile test tubes having 1 ml of sterile normal saline solution.

4. Label the sample as soon as possible with the patient code number.

5. Transport the specimen to the laboratory at room temperature within 2 hour of collection

6. Inoculate in to Blood agar plate, MacConkey agar and chocolate agar aseptically.

7. Incubate the inoculated blood agar plate and chocolate agar at 35–37 ^{0}C in a carbon dioxide atmosphere (candle jar) and the MacConkey agar plate aerobically.

8. Examine and report the culture; if the cultures have growth, look for colony characteristics perform gram reaction and biochemical test and determine drug susceptibility pattern to the isolated organism.

II. **Laboratory procedure for Gram staining technique**

1. Labeling the slides clearly with patient code number using pencile

2. Making of smears by spread evenly covering an area about 15-20mm diameter on a slide.

3. Drying of smears after making smears, the slide should be left in a safe place to air-dry, protected from flies and dust.

4. Fix the dried smear by using heat.

5. Cover the fixed smear with crystal violet stain for 1 minute.

6. Rapidly wash off the stain with clean water. If the tap water is not clean, use filtered water or clean boiled rainwater.

7. Tip off all the water, and cover the smear with lugol's iodine for 1 minute.

8. Wash off the iodine with clean water.

9. Decolorize rapidly (15-30 seconds) with acetone alcohol. Wash immediately with clean water.

10. Cover the smear with neutral red or safranine stain for 1 minute.

11. Wash off the stain with clean water.

12. Wipe the back of the slide clean, and place in a draining rack for the smear to air-dry.

13. Examine the smear microscopically, first with the 40 X objective to check the staining and to see the distribution of materials and then with the oil-immersion objective to look for bacteria and cells.

Result

• Gram positive bacteria ----------dark purple

• Gram -negative bacteria ----------pale to dark red

III. Laboratory procedure for Biochemical testing

Biochemical tests for gram positive bacteria: Gram -positive cocci was identified based on Their gram reaction, catalase and coagulase tests results.

Catalase test

Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non catalase producing.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism will be tested for catalase production by bringing it into contact with hydrogen peroxide.

Bubbles of oxygen are released if the organism is a catalase producer.

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube

2. using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution

3. Look for immediate bubbling

4. Interpretation:

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test

Controls

Positive coagulase control: Staphylococcus aureus

Negative coagulase control: Escherichia coli

Coagulase test

This test is used to identify S. aureus which produces the enzyme coagulase

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

1. Place a drop of physiological saline on two separate slides

2. Emulsify the test organism in each of the drop to make thick suspension

3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds

4. Clumping within 10 secs . . . . . S. aureus

No clumping within 10 secs . . . . . No bound coagulase

Controls

Positive coagulase control: Staphylococcus aureus

Negative coagulase control: Escherichia coli

Biochemical test for gram negative bacteria:- Identification of gram negative bacteria will be based on their test result with a series of conventional biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth.3-4 colony of test organism in 5 ml nutrient broth.

2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, TSI, urea agar and motility medium.

3. Incubate at 35-37 ^{O}C for 18-24 hours.

4. Look for color change (turbidity for motility) of the medium.

5. Identify the test organism by considering the result of the biochemical tests.

11.6.6 .Antimicrobial susceptibility tests

11.6.6.1. Disc diffusion susceptibility tests

Disc diffusion techniques are used by most laboratories to test routinely for antimicrobial susceptibility. A disc of blotting paper is impregnated with a known volume and appropriate concentration of an antimicrobial, and this is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism. The antimicrobial diffuses from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism. Strains susceptible to the antimicrobial are inhibited at a distance from the disc where as resistant strains have smaller zones of inhibition or grow up to edge of the disc.

11.6.7. Laboratory procedure for Antimicrobial sensitivity testing

1. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.
2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper
3. using a sterile swab inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o} to ensure even distribution.
4. With the petri dish lid in place, allow 3–5 minutes (no longer than 15 minutes) for the surface of the agar to dry.
5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated plate ensure the discs are correctly placed.

Note: The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.

6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35^{o}C for16–18 hrs.

7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. By using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

8. Using the Interpretative Chart, interpret the zones sizes of each antimicrobial, reporting the organism as ‘Resistant’, ‘Intermediate’ and ‘Susceptible” (41).

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for the thesis have been duly acknowledged.

MSC candidate Zenebe G/yohannes (BSc)

Signature _____

Date of submission _____

Place: Addis Ababa, Ethiopia.

This research thesis has been submitted with our approval as advisors.

Name of advisor: **Kassu Desta (MSc, PhD fellow,)**

Signature _____

Place: Department of Medical Laboratory Sciences, Addis Ababa University

Date of submission_____/_____/_____

Name of advisor: **Semaria Solomon (MSC, Ass.prof)**

Signature _____

Place: SPHMMC

Date of submission_____/_____/_____

Name of advisor: **Rozina Ambachew (MSC)**

Signature _____

Place: SPHMMC

Date of submission_____/_____/_____