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Isolation and characterizations of microbes associated with stethoscope from different health workers in Gondar university teaching hospital wards

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Abstract

Stethoscope has always been a part of the physicians' basic tool for examining patients. Universal use of stethoscope for examination of patients by health care personnel makes it a potential source for spread of nosocomial infection. This study was designed to assess both the potential for bacterial transmission by stethoscopes used by different health-care workers in Gondar university teaching hospital. A cross sectional study was conducted from April to June 2017 in university of Gondar department of biotechnology in cellular and molecular laboratory. During the study period there were a total of 128 stethoscopes from health professionals who had direct contact with patients are collected. Sample was collected by sterile cotton-tipped applicator moistened in a sterile solution of physiologic saline (0.85% sodium chloride) was used to swab the entire surface of the diaphragm of the stethoscope was inoculated into MacConkey agar, nutrient agar and blood agar media. Of the 115(89.8%) Stethoscopes out of total collected stethoscopes had bacterial growth and total 145 bacteria were isolated comprising of 4 different isolates. Isolates included staphylococcus epidermidis 45(31%) Staphylococcus aureus 40(27.5%), *Enterobacteria Proteus* 32(22%), Staphylococci 28(19.3%). All stethoscopes that had never been cleaned were contaminated while lower levels of contamination were found on those cleaned two week less before the survey. Motivation of health care providers to convert their knowledge to practice could be the next step to decrease the bacterial load significantly from the stethoscope which will automatically minimize cross-contamination and ensure improved patient safety in the hospital and design another options to use the instrument for physical examinations is important. Further study for molecular characterizations is another next step to identify the isolates in the level of species.

Keywords: nosocomial infection, stethoscope, bacterial Isolates

Introduction

It is estimated that at any one time more than 1.4 million people worldwide are suffering from infections acquired in hospitals (nosocomial infections) (Tikhomirov, 1987; Vincent, 2003) [41, 45]. Healthcare-associated infections occur worldwide and affect both developed and developing countries. Infections are considered nosocomial when they become clinically evident during hospitalization (at least 72 hours after admission) (Orrett *et al.*, 1998) [28]. In developed countries, between 5% and 10% of patients acquire one or more infections, and 15-40% of patients admitted to critical care are thought to be affected (Lazzari *et al.*, 2004; Klevens *et al.*, 2007) [23, 18].

Infection transmission in the hospital environment (nosocomial infection) remains a significant hazard for hospitalized patients, and health-care workers are potential sources of these infections. Many pathogens can be transmitted on the hands, which is a major reason that all health-care workers must wash their hands before and after seeing each patient (WHO 2009) [48, 49]. Transmission of infections on contaminated medical devices is also possible and outbreaks of hospital-acquired infections have been linked to devices such as electronic thermometers, blood pressure cuffs, stethoscopes, latex gloves, masks, neckties, pens, badges and lanyards, white coats, computers and keyboards (Unekeet *et al.*, 2008) [44].

Sterilization of invasive equipment and the disinfection of such kind of devices before the interventions are usually ignored. Among those equipment, stethoscopes are the most frequently used medical devices. Researchers showed that stethoscopes might have a role in the infestation of microorganisms from patient to patient (Jones *et al.*, 1995; Bernard *et al.*, 1999) [16, 4]. Stethoscopes are commonly used to assess the health of patients and have been reported

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to be potential vectors for nosocomial infections in various parts of the world (Uneke *et al.*, 2008; Youngster *et al.*, 2008; Zuliani-Maluf *et al.*, 2002; Schroeder *et al.*, 2009; Saloojee *et al.*, 2001) [44, 50, 51, 36, 33, 34]. Following contact with infected skin, pathogens can attach and establish themselves on the diaphragms of stethoscopes and subsequently be transferred to other patients if the stethoscope is not disinfected (Madar *et al.*, 2005) [24].

There are increasing reports of the risk of transmitting antibiotic resistant microorganisms from one patient to another on stethoscopes (Uneke *et al.*, 2008; Fenelon *et al.*, 2009; Merlin *et al.*, 2009) [44, 9, 10, 26]. These antibiotic-resistant organisms are capable of initiating severe infections in a hospital environment and could require contact isolation and aggressive treatment to prevent the spread of the organisms (Gupta *et al.*, 2004) [13]. Examples of such antibiotic-resistant organisms are ceftazidime-resistant *Klebsiella pneumoniae*, vancomycin-resistant enterococci, *methicillin resistant* staphylococci, Ciprofloxacin-resistant *Pseudomonas aeruginosa*, gentamicin-resistant *P. aeruginosa*, and penicillin-resistant pneumococci (Gastmeier *et al.*, 2003; Kerr *et al.*, 2002; Lange *et al.*, 200; Parmar *et al.*, 2004) [11, 17, 29, 30].

Also antibiotic resistant microorganisms may be transmitted from one patient to another through medical devices (Fenelon *et al.*, 2009) [10]. Though clinicians are instructed about bacterial colonization and the importance of maintaining clean medical instruments, these devices may not be thought of a potential source of HAI (Wilkins *et al.*, 2007). The use of 70% isopropyl alcohol is found to be effective in reducing contamination of stethoscopes and other medical equipment than other agents like detergents (Alothman *et al.*, 2009; Parmar *et al.*, 2004; Nolson *et al.*, 2006) [3, 29, 30, 27].

The transmission of infections in the hospital (nosocomial infections) from contaminated medical equipment and health-care workers (HCWs) is a major problem. Medical devices, if not sterilized/disinfected properly, may transmit microorganisms from one patient to the other. However at present there is no enough scientific report available in Ethiopia on stethoscopes as health care associated infection transmitter. Nowadays health service acquired infections is not only a great challenge for doctors but also for the patients due to increased morbidity and economic burden and disinfection of stethoscopes is still not an established and accepted practice among most of the health care personnel. The outcome of this study may be helpful to develop a good culture of stethoscopes disinfections before and after the examinations of the patients at health service place. Moreover, this study will help to document information on different microbial distributions in different ward of the sample collections area with antibiotic susceptibility information.

Materials and Methods

This study was conducted from April to June, 2017, at university of Gondar, Department of biotechnology, cellular & microbial laboratory. Gondar is a historical town located 739 km far from Addis Ababa to the northwest of Ethiopia, North Gondar zone, Amhara region, North of Bahir Dar town and Lake Tana (the largest lake in Ethiopia) and South West of Semen Mountains. The town has 12°36'N latitude and 37°28'E longitude with an elevation of 2133 meters above sea level. According to 2008 Ethiopian statistical agency report, Gondar town has 231,977 total populations. A total of 115 stethoscopes from health professionals who had direct contact with patients were collected. From these individuals 80 were

from doctors and 35 were from nurse and other health workers who were volunteers to participate in this study and were included.

Stethoscope of doctors, nurse and others were randomly sampled by taking written and oral consents from all the participants included in this study.

The samples were collected aseptically using sterile cotton tipped applicators which was immersed in 0.85% sterilized normal saline solution (NSS) which used to swab the entire surface of the diaphragm of the stethoscope and the resulting specimen was cultured for bacteria within 2 hours.

The swabs were inoculated directly onto blood agar and MacConkey agar and incubated aerobically at 37°C for 24 hours before being examined for bacterial growth according to standard methods Cheesbrough M (2000) [5]. When three or more colony forming units (CFU) were obtained on a plate, the organism was regarded as a bacterial contaminant.

Colony characterization such as configuration, margin, elevation, opacity, pigment and shape were investigated microscopically and by direct observation of the 24 hrs old colony on the nutrient agar and MacConkey agar plate (Duncan, 2005) [7].

Gram staining: This was carried out by using standard techniques with a step-wise application of crystal violet solution, iodine solution, ethanol (95%) and Safranin solution as described in Harley and Prescott (2002) [14].

Based on the gram reactions obtained further identification of bacteria was made by a series of biochemical tests. Gram-negative bacteria were identified by using triple sugar iron agar, indole, Simon's citrate agar, lysine iron agar, urea, mannitol, and motility. Catalase and coagulase were used to identify Gram-positive bacteria.

Presence or absence of changes in the media was recorded as positive and negative, respectively, and the results were interpreted as per the information provided by Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994) [15] used for identification of bacterial isolates.

Catalase test: Thick emulsions of each bacteria isolates were prepared on a clean slide. Three drops of 3% hydrogen peroxide were added on each of the slides. Formation of bubbles was observed as positive result (Adetunji *et al.*, 2012).

Starch hydrolysis: This test was carried out by dividing starch agar plate into four equal sectors using a marker. After labeling the organism's name, the test organisms were spot inoculated and incubated for 24 h (Harley and Prescott, 2002) [14]. Zone of hydrolysis of starch was detected as a brownish clear zone in a blue black background after flooding the starch agar plate with iodine solution. The presence of zone of hydrolysis on the plate indicated the ability of the test organism to metabolize starch.

Urea hydrolysis: Urease test was carried out by preparing urea broth containing phenol red as pH indicator. After inoculating the broth with the test isolate and incubating the culture for 24h, color change of the broth from red to pink was observed and recorded as a positive result for urease test (Harley and Prescott, 2002) [14].

Gas production using Triple sugar iron test (TSI): Gas production was detected using TSI agar slants which are prepared from a mixture of agar, a pH-sensitive dye (phenol

red1%) lactose1%, sucrose1%, glucose, sodium thiosulfate and ferrous sulfate (Harley and Prescott, 2002; Sharma, 2007) [14, 38]. The bacterial isolate to be studied was inoculated both by streaking on slant and stabbing the butt. After incubating the inoculated TSI agar slant tubes for 24 hours, presence of H₂S, color change on the slant and in the butt were observed and interpreted according to Sharma (2007) [38]. Production of H₂S was indicated by the blackening of the TSI medium.

The antimicrobial susceptibility test of the isolates was performed according to the national committee for clinical laboratory standards (NCCLS) method using Kirby-Bauer disk diffusion test on Muller-Hinton agar. In short the isolated bacterium was suspended in a nutrient broth and incubated for 30 min to make it comparable with 0.5% McFarland standard. After incubation a sterile cotton swab was dipped in to the suspension and bacteria were inoculated on to the Muller-Hinton agar. Antibiotic discs were placed by using disc dispenser and the plate was incubated for 24 hrs. at 37°C. Results were interpreted after measuring the zone of inhibition and being compared with the standards. Escherichia coli ATCC 25922 and S. aureus ATCC 25923 were employed as strain of quality control for the antimicrobial susceptibility test.

Data were entered and analyzed using SPSS version 16 computer software, summarized in frequencies and percentages, and presented in tables and graphs. And the burden of bacteria and value less than 0.05 was considered as

statistically significant.

Results

A total of 128 stethoscopes, 80 from doctors, 35 from nurse and other health care workers were collected as shown on (Table-1). Of the 115 Stethoscopes out of total collected stethoscopes had growth and total 145 bacteria were isolated comprising of four different species.

Table 1: Total number of contaminated stethoscopes.

Professions	Day	Total No. of contaminants stethoscopes
Doctors	1	34±5.03 ^a
	2	30±5.03 ^a
	3	31±5.03 ^b
Nurse	1	22±0.55 ^a
	2	10±0.55 ^b
	3	12±0.55 ^b
Others	1	2±1.00 ^b
	2	3±1.00 ^a
	3	1±1.00 ^b

Specific values in the table are means of triplicate determinations. These values with different superscripts within the column were significantly different at ($P < 0.05$).

Stethoscopes sampled from different ward showed different amount of the contamination

Table 2: Bacterial isolated were colony counted as the following

Wards	Doctors		Nurse & Others		Total	
	Number of Stethoscopes	No. colony	Number of Stethoscopes	No. colony	Number of Stethoscopes	No. colony
Emergency	30	23	12	20	42	43
Medical	18	21	8	10	26	31
Opera. room	13	21	6	8	19	29
Surgical	11	20	5	7	16	27
Pediatric room	8	10	4	5	12	15

Analysis of the study questionnaire revealed that bacterial contamination was related to the time the stethoscope was not cleaned prior to the survey (Table- 3) results showed that there was 46 (85.2%) bacterial colonization of stethoscopes

that had never been cleaned while the least contamination(8 (57.1%) was found on stethoscopes cleaned two week less before the survey.

Table 3: Stethoscope cleaning and contamination at different times were taken and evaluated as

Time	Doctors		Nurse & Others		Total	
	No. of microbes	% of microbes	No. of microbes	% of microbes	No. of microbes	% of microbes
<2 week ago	10	(10.5%)	4	(8%)	14	(9.7%)
3-4 week ago	14	(14.7%)	8	(16%)	22	(15.2%)
>5 week ago	28	(29.47%)	18	(36%)	46	(31.7%)
never	38	(40%)	20	(40%)	58	(40%)
total	95	(100%)	50	(100%)	145	(100%)

Specific values in the table are means of triplicate determinations. These values with different weeks (time) within the column were significantly different at ($P < 0.05$).

The isolated microorganisms were characterized by morphologically and biochemically as shown on (Table -4)

Table 4: Morphological and biochemical characteristics of the selected bacterial isolates

Sample code	Shape of bacteria	Colon Arrangement	Colonial pigmentation	Gram staining	Biochemical tests					
					Motility test	Catalase test	Citrate test	Urease	coagulase	TSI
Mw-01	Rod	pair	Colorless	+	+	-	+	-	+	+
Ew-01	Circular	Chain	Yellow	+	-	+	+	-	-	+
Ew-02	Cluster	pair	Colorless	+	-	+	+	-	-	+
Ew-05	Circular	pair	Yellow	+	-	+	+	-	-	+
Mw-02	Circular	pair	Colorless	+	+	-	+	-	+	+
OR-01	Circular	pair	Colorless	+	-	+	+	-	-	+

OR-02	Circular	pair	Colorless	+	-	+	+	-	-	+
Ew-03	Circular	pair	Colorless	+	-	+	+	-	-	+
Ew-04	Circular	pair	Colorless	+	+	+	+	-	-	+
Mw-03	Circular	pair	Colorless	+	+	-	+	-	-	+
Sw-01	Circular	pair	Yellow	+	+	-	+	-	+	+
OR-03	Circular	pair	Colorless	+	-	+	+	-	-	+
Sw-02	Circular	pair	Yellow	+	+	-	+	-	+	+
OR-04	Circular	pair	Colorless	+	-	+	+	-	-	+
Pw-04	Circular	pair	Colorless	-	+	-	+	-	-	+
Pw-03	Rod	pair	Colorless	-	+	-	+	+	-	+
Ew-06	Cluster	pair	Colorless	+	+	+	+	-	-	+
Pw-02	Rod	Chain	Colorless	-	+	-	+	+	-	+
Pw-01	Rod	pair	Colorless	+	-	+	+	+	-	+
Mw-04	Circular	pair	Colorless	-	+	-	+	-	-	+

Key: +=Positive, - = Negative, Mw=medical ward, Pw=pediatric ward, OR=operations room, Ew =emergency ward and Sw=Surgical ward

Table 5: Percentage of bacteria isolated from the Stethoscope from Different Health Workers in Gondar University Teaching Hospital wards

S. No	Bacterial isolates	Percentage
1.	<i>S. epidermidis</i>	45 (31%)
2.	<i>S. aureus</i>	40 (27.5%)
3.	<i>Enterobacteria. Proteus</i>	32 (22%)
4.	<i>Staphylococci</i>	28 (19.3%)

The Antibiotic sensitivity testing nature indicated that the bacterial isolates were resistant, intermediate and susceptible to most of the antibiotics assessed (Table-5) and (Figure-1).

Table 6: Antimicrobial sensitivity of bacterial isolates from stethoscopes.

Antibiotic discs	Concentration	Type of isolates		Zone of inhibition	
		<i>S. epidermidis</i>	<i>Enterobacteria. Proteus</i>	<i>S. aureus.</i>	Millimeter
kanamycin	30 Mcg	R	R	R	10mm
streptomycin	10 Mcg		S		50mm
erythromycin	15 Mcg	S		S	37.5mm
amoxicillin	25 Mcg	R	R	R	11mm
ciprofloxacin	5 Mcg	S	S	S	22mm
tetracycline	30 Mcg	R	I	R	12mm
Nalidixic acid	30 Mcg	R	R	R	9mm
chloramphenicol	30 Mcg	I	I	I	16mm
gentamycin	10 Mcg	S	S	S	18mm



Fig 1: Image of antibiotic test result for different microbial isolates with different types of antibiotics.

Discussion

The result of our study were demonstrated that as many as 89% of the stethoscopes out of 128 stethoscopes surveyed were contaminated by bacteria which is comparable to the observations of previous studies that found 71% to 100% of stethoscopes were colonized by various bacteria (Chigozie *et al.*, 2010) [6]. The stethoscopes used by doctors were more contaminated (62.5%) than those used by other health workers (27.3%) which is similar with the study conducted by Chigozie and others had reported that only physicians were more contaminated and higher bacterial load than other health care workers and the fact that physicians use stethoscopes more frequently than other health workers explain the higher rate of bacterial colonization (Marinella *et al.*, 1997; Chigozie *et al.*, 2010) [25, 6]. Although the difference was statistically

significant, the fact that doctors use stethoscopes more frequently than other health workers might explain the higher rate of bacterial contamination.

A total of 145 bacteria were isolated from contaminated stethoscope collected from five wards such as medical, emergency, surgical, operation room and pediatric ward. The mean bacterial isolates count per diaphragm of this study was quite higher in comparison to a study conducted by (Kuhu *et al.*, 2015) [20]. Stethoscopes sampled from the doctors of emergency ward showed maximum contamination in this study, whereas a study in Mumbai showed highest contamination of stethoscopes used in Medicine ward (Singh *et al.*, 2013) [39]. The doctors posted in emergency perhaps use stethoscopes more frequently than others this might be the explanation of higher rate of bacterial contamination in them.

Among the isolates, *staphylococcus epidermidis* 45(31%) was predominant one followed by *staphylococcus aureus* 40(27.5%), *Enterobacteria Proteus* 32(22%), *Staphylococci* 28(19.3%). Where as in studies in (Chigozie *et al.*, 2010) [6]. *Staphylococcus aureus* was the most common organism is of although we did not show that stethoscopes can transmit infections, we did show stethoscopes were contaminated with pathogenic bacteria and that poor stethoscope cleaning/disinfection practices were significantly associated with this contamination. In particular, all stethoscopes that had never been cleaned were contaminated while the lowest levels of contamination were seen with stethoscopes cleaned <2 week before the survey. As even short periods of contact between a patient's skin and the stethoscope can result in transfer of bacteria (Africa-Purino *et al.*, 200) there is a need for strategies to decrease bacterial contamination of stethoscopes.

It was interest to note that stethoscopes belonging to health workers who practiced hand hygiene were less likely to be contaminated than those belonging to individuals with poor hand hygiene. Failure to wash hands could facilitate the introduction of pathogens onto devices that the health workers use frequently, such as stethoscopes. The World Health Organization recently noted that hand hygiene is fundamental in ensuring patient safety and should be performed in a timely and effective manner in the process of care WHO (2009) [48, 49].

Strategies to minimize the transmission of infection from stethoscopes have been proposed, including the use of disposable stethoscopes, especially for clinical high-risk environments, and the use of a single use, silicone membrane over the stethoscope head to create a prophylactic barrier (Patent Storm, 2004) [31]. Although these strategies could minimize the risk of stethoscope transmission of infections, they are unaffordable to most health workers and health facilities in developing countries. Instead hospitals should develop more rigorous programs and protocols for stethoscope disinfection as a standard of care (Sengupta, 200). Strict adherence to stethoscope disinfection practices by health workers will minimize cross-contamination and ensure improved patient safety in hospitals.

Conclusions

Our results indicate that stethoscopes are frequently contaminated. Many of the microorganisms isolated from the stethoscopes in our study (e.g., *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterobacteria proteus*, and *Staphylococci*) were known to cause serious infections in hospitalized patient populations. Contaminated stethoscopes were found in all hospital service areas and among all types of medical personnel. The study also indicates an urgent need to alert and educate hospital staffs about the potential health risks associated with the medical devices. Hospitals should develop rigorous programs and protocols for disinfection of medical devices a standard for care or design other methods of utilizations for different instrument like stethoscopes. Motivation of health care providers to convert their skill and practice could be the next step to decrease the bacterial load significantly from the stethoscope which will automatically minimize cross-contamination and ensure improved patients safety in the hospital area.

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