Magnitude, diversity, and antibiogram of bacteria isolated from the selected wards of Arba Minch hospital, Arba Minch, Ethiopia

Aseer Manilal (aseermanilal@gmail.com)

Research

Keywords: Bacterial contamination, patient-care equipment, inanimate objects, Arba Minch Hospital, multi-drug resistance

Posted Date: February 19th, 2020

DOI: https://doi.org/10.21203/rs.2.23933/v1

Abstract

Background: Patient-care equipments and inanimate objects contaminated with bacteria are perilous to patients as well as to others who happen to get exposed, and is a persistent problem in developing countries like Ethiopia, it remains overlooked. Therefore, the present study aims to elucidate the magnitude of contamination, diversity and antimicrobial susceptibility pattern of bacteria associated with patient-care equipments and inanimate objects of selected wards of Arba Minch Hospital (AMH), Ethiopia.

Methods: A cross-sectional study on the diversity and drug susceptibility pattern of bacteria isolated from inanimate objects and patient-care equipments of three wards of Arba Minch hospital were done. Samples were inoculated into bacteriological media and were identified by biochemical characterization followed by antimicrobial susceptibility tests.

Results: Totally, 109 bacterial isolates were identified. The bacterial isolates were identified as *Staphylococcus aureus,* Coagulase-negative *Staphylococcus* (CNS), *Acinetobacter* sp., *Klebsiella* sp., *Citrobacter* sp., *Enterobacter* sp., *Salmonella* sp., *E. coli,* and *Serratia* sp. The surgical ward displayed the utmost contamination rate. Antibiogram of Gram-positive cocci revealed that *S. aureus* and CNS manifest higher resistance to both penicillin and trimethoprim-sulfamethoxazole. Regarding the Gram-negative bacilli, isolates of *Acinetobacter* showed 100% resistance to ceftriaxone and ampicillin. The overall prevalence of multi-drug resistant bacteria in this study was 57.7 %.

Conclusion Results revealed that the surgical ward, followed by paediatric and neonatal intensive care unit of AMH was heavily contaminated. Therefore, a stringent infection vigilance program comprising of routine sampling from the equipments and wards along with antimicrobial resistance surveillance and decontamination efforts must be instituted.

Introduction

Worldwide, HAI is considered a major public health challenge that causes a severe financial burden, potential disability and sometimes even death in hospital settings [1]. Even though the magnitude of this problem remains underestimated in developing countries, recently the prevalence of HAI is augmented beyond 20% [2]. A perusal of literature revealed that in Ethiopia there is a lack of documentation pertaining to all HAI and HAI associated mortality. However, the prevalence of HAI in two teaching hospitals in Ethiopia was 14.9% [3].

Nevertheless, any microorganism from endogenous or exogenous origin can cause an HAI, the most problematic one in terms of prevalence and treatment is bacteria [4]. Often acting as primary or secondary invaders, they could inflict a wide array of potentially fatal diseases. Reservoirs and sources of bacteria associated with HAI exist both in the inanimate and in the animate environments. It has been stated that both Gram-positive and Gram-negative bacteria are able to persist for months on dry inanimate surfaces, under humid and adverse conditions and serve as transmission points between healthcare workers and patients [5]. It is envisaged that up to 60% of surfaces in the patient environment are contaminated with HAI causing organisms. Indeed, those bacteria could easily enter into susceptible patients after contact with fomites in the surroundings (e.g., bedrails, emergency carts, and trolleys) or by the usage of contaminated patient-care equipments (e.g., stethoscopes, sphygmomanometers). In the recent decade, due to the irrational and inappropriate usage of antibiotics, multi-drug resistant bacteria are gradually emerging in hospital setups [6]. Consequently, colonization by multi-drug resistant bacteria is considered as one of the predisposing factors for HAI and a daily challenge for the clinicians, particularly dealing with critically ill patients [6, 7]. Bacteria that frequently implicated as multi-drug resistant bacteria are ESKAPE pathogens and responsible for a substantial percentage of HAI in the current scenario [8]. However, the diversity of species and strains are dramatically widening.

Bacterial contaminations from exogenous inanimate origins such as patient-care equipments and fomites are considered as one of the probable causes of HAI [7, 9]. Proper use of disinfectants is essential for limiting the transmission of infectious pathogens. However, in developing countries like Ethiopia, the use of expensive techniques for cleaning, disinfection, and sterilization is not always possible, so the poorly decontaminated patient-care equipments and inanimate objects could serve as potential sources of infection.

Diversity, biomass and antibiotic susceptibility patterns of bacterial isolates may vary geographically and among various health care units of the same hospital at various periods of time. Therefore, periodic monitoring by bacteriological analysis of inanimate surfaces and patient-care equipments are inevitable to detect changing trends of types and counts of bacterial flora [10]. As bacterial pathogens are still playing a critical role in HAI in Ethiopia, it is imperative to know the location or institution based etiological and susceptibility profiles. A survey of the literature demonstrated that only a few studies have been done so far in Ethiopia to indicate the magnitude of bacterial contamination in patient-care equipments and inanimate objects [11–14]. Hitherto, no study pertaining to the bacterial contamination of patient-care equipments and inanimate objects in Arba Minch hospital is done. In this background, this work was initiated to elucidate the magnitude of contamination, etiological profile and antimicrobial susceptibility pattern of bacteria isolated from patient-care equipments and inanimate objects of selected wards in Arba Minch Hospital, Arba Minch, Ethiopia.

Materials And Methods

Study area, design and period

This study was carried out at the Arba Minch Hospital, Arba Minch province which is situated in the south of Addis Ababa, Ethiopia. The study protocol was approved by the ethical review board of Arba Minch University, College of Medicine and Health Sciences. The hospital comprises of 300 beds in 11 service area. A large number of individuals from the surrounding area and nearby zones are visiting as inpatients and outpatients. A cross-sectional study was conducted to identify the magnitude of contamination, etiological profile and drug susceptibility patterns of bacteria isolated from inanimate objects and medical equipments of selected wards of AMH between May and June 2018.

Sampling technique

The hospital wards were selected purposively (convenient method). Patient-care equipments and inanimate objects were selected randomly by lottery method from the lists of equipments that obtained from the head office of selected wards in AMH. Samples were collected four hours after the last time of the cleaning process from the purposively selected wards such as NICU, surgical, and paediatric. The objects chosen for the sampling are shown in table I.

Specimen collection and processing

Sample collections were performed on the selected patient-care equipments and inanimate objects. A swab soaked in sterile normal saline was used to procure samples from the representative area (high-frequency patient's touch sites) of the patient-care equipments and inanimate objects. Afterward, swabs were placed in sterile labeled sleeves and transported at room temperature and stored at 5 °C until processing on the same day.

Isolation and identification of bacterial isolates

The isolation and identification of bacterial isolates were done at the Microbiology and Parasitology Laboratory, Department of Medical Laboratory Science, College of Medicine and Health Sciences, Arba Minch University, Arba Minch. All the samples were plated out on various isolation media including MacConkey agar, blood agar, and mannitol salt agar. The inoculated plates were incubated face-up for 24 hours at 37 °C. Following incubation, plates were inspected for bacterial growth. Pure cultures of respective bacterial isolates were subsequently subjected to species identification and confirmation. Biochemical, morphological and physiological characteristics of isolated bacteria were ascertained by adopting standard laboratory methods including Gram staining, colonial morphology on different media, growth on selective media, lactose and mannitol fermentation, H_2S production, catalase, oxidase, coagulase, indole, citrate utilization, and urease tests as stated elsewhere [15]. Corresponding American Type Culture Collection strains were used as reference standards to validate the biochemical identification of isolated bacteria.

Antimicrobial susceptibility testing

Kirby–Bauer disc diffusion method was followed for determining the antibiotic sensitivity profile of each isolate [16]. Fourteen commercially available antibiotic discs (Himedia®, Mumbai) were used for the determination of the antibiogram. Briefly, inoculums were prepared by aseptically collecting the test organisms with a sterile wire loop and then suspending in normal saline. The density of suspension was determined by comparison with the opacity standard on McFarland 0.5 barium sulfate solution. The bacterial suspensions were inoculated onto the Mueller-Hinton agar plates to attain an approximate lawn concentration of 1.5×10^6 CFU/cm^2. Five antibiotic discs were dispensed equidistant pentagonally in each Petri-dish. After 24 hours of incubation at 37 °C, the diameter of the zones of inhibition around the discs were measured and categorized as sensitive, intermediate, and resistant according to the standardized table described in CLSI [17]. The following antibiotic discs in respective concentrations (in μg mL^{-1}) [penicillin (10 IU), tetracycline [30 μg], cefoxitin [30 μg] gentamicin [10 μg], erythromycin (15 μg), ciprofloxacin [5 μg], norfloxacin [10 μg], clindamycin [30 μg], chloramphenicol [30 μg], amikacin (30 μg), trimethoprim-sulfamethoxazole, [1.25/23.75 μg], ceftriaxone [30 μg], ampicillin [10 μg] and amoxicillin [20 μg] were used to determine the antibiogram. Bacteria showing resistance to three classes (and above) of antibiotics were considered as multiple drug-resistant.

Results

Magnitude of bacterial contamination

In total, 99 swab specimens were collected from the diverse inanimate objects and patient-care equipments corresponding to three wards such as surgical (n = 34), paediatric (n = 32) and NICU (n = 33). Results of microbial inspection revealed that, of the 99 swab specimens collected, 71 (71.7%) showed bacterial contaminations such as 26 (76.4%) from surgical, 22 (66.6%) from paediatric and 23 (71.8%) from NICU (Table II). Among the three wards studied, the surgical ward displayed the utmost contamination rate followed by paediatric and NICU. It was found that 18 of 99 (18.5%) inanimate objects showed poly bacterial growth, while 81(81.8%) had pure growth.

Diversity and biomass of bacteria

Totally, 109 bacterial isolates were retrieved and tentatively identified in different culture media. Among the diverse bacterial isolates, 80.7% (n = 88) comprised Gram-positive cocci and the remaining 19.2% (n = 21) were Gram-negative bacilli (Table III). Based on the colony morphology, culture, biochemical characteristics and subsequent comparison with previous reports, the isolates were identified and sorted into nine species including Gram-negative bacilli such as Acinetobacter sp., E. coli, Klebsiella sp., Enterobacter sp., Citrobacter sp., Serratia sp., Salmonella sp. and Gram-positive cocci such as S. aureus and CNS. Among the Gram-positive cocci identified, notably, CNS 46 (52.2%) was the predominant bacteria followed by S. aureus 42 (47.7%). In the case of Gram-negative bacilli, the most common isolate was Acinetobacter sp. representing 28.5%, followed by Klebsiella sp., 23.8%.

It was found that bacteria isolated from each ward showed variation in species diversity and biomass (Table III). The Gram-positive cocci, CNS and S. aureus were isolated from all the wards. However, the Gram-negative bacteria, Acinetobacter sp. and Klebsiella sp. were exclusively retrieved from the surgical and paediatric wards. Isolates of Enterobacter sp. and Salmonella sp. were recovered from the surgical ward and NICU. Meanwhile, E. coli and Citrobacter sp. were predominately isolated from the paediatric ward. Finally, the Serratia sp. was recovered from NICU. Bacteria retrieved and identified from the diverse patient-care equipments and inanimate objects in each ward showed variations in species diversity and biomass. Among the different inanimate objects analyzed in three wards, beds and tables showed the highest contamination. Twenty-nine Gram-positive and seven Gram-negative bacteria were recovered from the beds and tables. In the case of different patient-care equipments, stethoscopes showed the highest contamination, from which five Gram-positive and one Gram-negative bacteria were retrieved.

Results of the microbiological analysis revealed that among the three wards, the most contaminated one was the surgical ward. It demonstrated the highest number of isolates both from the inanimate objects and patient-care equipments (n = 41) which corresponds to 33(37.7%) Gram-positive and 8 (38.0%) Gram-negative bacteria. Notably, isolates of S. aureus (n = 17) and Acinetobacter sp. (n = 5) were found to be the predominant Gram-positive and Gram-negative bacteria respectively. The heavily contaminated inanimate objects in the surgical ward were tables and beds. Totally, twenty-five Gram-positive and 5 Gram-negative bacteria were isolated. Gram-negative species comprising Klebsiella sp., Salmonella sp. Enterobacter sp. and Acinetobacter sp. were identified. However, the patient-care equipments such as stethoscopes and sphygmomanometers showed minimum contamination with 5 Gram-positive and 1 Gram-negative bacterium (Acinetobacter sp.) (Table III). The second most contaminated was the paediatric ward. From the paediatric ward, 38 isolates comprising 29 (32.9%) Gram-positive and 9 (42.8%) Gram-negative bacteria were isolated. Particularly, the isolates of CNS (15) and Klebsiella sp. (4) respectively were found to be the major Gram-positive and Gram-negative bacteria that were retrieved. In the paediatric ward, the highest contamination was associated with inanimate objects. Twenty-seven Gram-positive and nine Gram-negative bacteria were isolated. Gram-negative species comprising Klebsiella sp., Citrobacter sp. Enterobacter sp., E. coli, and Acinetobacter sp. were identified. Among the inanimate objects, the bed was found to be the most contaminated one. Thirteen Gram-positive and three Gram-negative bacteria were isolated from beds. The number of bacteria isolated from patient-care equipments was minimal. NICU showed the least species diversity and biomass of bacterial isolates. Of the total 30 isolates, 26 (29.5%) corresponds to Gram-positive and the rest 4 (19.04%) were Gram-negative bacteria. Notably, the isolates of CNS (n = 15) and Enterobacter sp. (n = 2) were the predominant Gram-positive and Gram-negative bacteria. Like in the case of other wards, inanimate objects were found to be heavily contaminated compared to patient-care equipments. Totally 19 bacteria were isolated from inanimate objects. The heavily contaminated inanimate object in NICU was beds. Contamination in patient-care equipments was moderate in NICU. Among the different patient-care equipments, baby incubators were observed to be heavily contaminated (Table III). Notably, in all the three wards, fomites such as tables, bed- frames, medical charts and patient-care equipments like stethoscopes, ambu bags, suction machines, sphygmomanometers and baby incubators were contaminated with Gram-positive bacteria particularly the species of genus Staphylococcus (Table IV).

Antibiogram profiles

The antibiotic sensitivity profile of all isolates was inspected using fourteen antibiotics. The antibiogram of Gram-positive and Gram-negative bacteria are given in table IV and V. The isolated bacteria showed a broad spectrum of variations in the susceptibility/resistance patterns to all the antibiotics tested. It was observed that Gram-positive cocci were highly sensitive to most of the antibiotics tested. The highest degree of susceptibility was shown by S. aureus against eight antibiotics and the range was 61.9–95.2%.

The drug sensitivity manifested by S. aureus to clindamycin was 95.2% followed by 92.9% against chloramphenicol, 90.5% (ciprofloxacin), 80.9% (erythromycin), 76.1% (amikacin), 69% (ceftriaxone), 64.3% gentamicin and 61.9% (tetracycline). In the case of CNS, the corresponding values were 82.6% against clindamycin followed by 78.2% (amikacin), 73.9% (ciprofloxacin) and 63.4% (chloramphenicol). Pertaining to the resistant profiles, 100% of S. aureus was resistant to penicillin, but for the trimethoprim-sulfamethoxazole, the percentage was just 30.9%. Among the 42 S. aureus, 8 isolates with a zone of inhibition ≤ 21 mm in cefoxitin disc diffusion assay were extrapolated as Methicillin-Resistant S. aureus (MRSA). The percentage of MRSA among S. aureus was 19.1%. Isolates of CNS showed a higher degree of resistance, 78.3% to penicillin and a moderate degree, 43.4% to trimethoprim-sulfamethoxazole, 32.6% to tetracycline, 28.1% to both chloramphenicol and erythromycin. In this study, the multi-drug resistance was inferred as the resistance to three or more groups of antibiotics tested. Altogether, 47.7% of Gram-positive cocci were multi-drug resistant. Among them, 42.8% and 52.17% of S. aureus and CNS respectively were resistant to three (and above) antibiotics.

The susceptibility pattern of Gram-negative bacilli revealed that the predominant isolate, Acinetobacter sp. showed a lower range of sensitivity to the five antibiotics tested (16.7–33.3%). Only 16.7% of Acinetobacter isolates showed sensitivity to tetracycline, norfloxacin, and trimethoprim-sulfamethoxazole. Besides, 33.3% of the isolates were sensitive to gentamicin and ciprofloxacin. Invariably, all the isolates were extremely susceptible (100%) to amikacin. The antibiogram of Klebsiella sp. revealed that 100% of the isolates were susceptible to amikacin, whereas susceptibility to chloramphenicol was 80% only. This phenomenon was also observed in all the isolates of Citrobacter sp., which showed sensitivity to amikacin. However, only 33.3% of isolates were sensitive to chloramphenicol, ciprofloxacin, and norfloxacin. Isolates of Enterobacter sp. exhibited a lower degree of susceptibility in the range of 33.3 to 66.7% against five antibiotics tested. It was observed that 66.7% of the isolates were sensitive to amikacin and ceftriaxone. Regarding the Salmonella sp., 50% of the isolates were susceptible to both amikacin and ceftriaxone. The antibiotic sensitivity patterns of E. coli and Serratia sp. indicated that both the isolates were extremely susceptible (100%) to tetracycline, amikacin, ceftriaxone, chloramphenicol, ciprofloxacin, and norfloxacin.

Concerning the antibiogram of Gram-negative bacilli, it was found that all the isolates of Acinetobacter showed 100% resistance against ceftriaxone and ampicillin. Besides, 83.3% of the isolates were resistant to amoxicillin followed by tetracycline, chloramphenicol, ciprofloxacin, and trimethoprim-sulfamethoxazole (66.7%). Isolates of Klebsiella sp. showed notable resistance in the range of 80 to 100% against amoxicillin, ampicillin, and trimethoprim-sulfamethoxazole. Invariably, all the isolates of Citrobacter sp. showed maximum resistance (100%) to amoxicillin, ampicillin, and trimethoprim-sulfamethoxazole. This phenomenon also occurred in the case of Enterobacter sp. Salmonella sp., and E. coli. The resistance of Salmonella sp. to gentamicin, amikacin, ceftriaxone, chloramphenicol, and ciprofloxacin correspond to 50%. Species wise, the least diverse bacterial isolate, Serratia sp. were resistant to gentamicin, amoxicillin, ampicillin, and trimethoprim-sulfamethoxazole (100%). It can be noted that 100% of Gram-negative bacteria were found to be multi-drug resistant. Therefore, the overall prevalence of multi-drug resistant isolates in this study was found to be 57.7% (Table VI).

Discussion

Magnitude of bacterial contamination in different wards

Contaminated inanimate objects and patient-care equipments are considered as proven sources of infections since the bacteria can spread throughout the hospital wards in an epidemic fashion. Our results showed that potentially pathogenic bacteria are present in diverse samples collected from different wards, revealing that patient-care equipments and inanimate objects could be prominent sources of infections. Significant differences were found in the biomass and also in the diversity of bacterial isolates. The possible reason for this is the alterations in quality of ventilation system, the difference in cleaning processes applied, variations in traffic and occupancy rates in these wards [18]. Among the three wards studied, the surgical ward showed the highest degree of bacterial contamination followed by paediatric. The total number of bacteria isolated from the surgical ward was higher than that of other wards. Our results were in line with two earlier studies reported from Ethiopia [11, 19]. In these studies, investigators reported that the surgical ward showed the highest number of bacterial isolates. In contrast, a study from Nigeria noted that the operation/surgical room had the lowest number of bacterial contamination [18]. Besides, another study done in Nigeria reported that the orthopaedic ward encountered the highest number of isolates [20]. Results of our study also revealed that patient-care equipments and inanimate objects in the surgical ward are heavily contaminated by bacteria. Among the inanimate objects, tables were the most contaminated one followed by beds. This was in agreement with prior studies conducted in Nigeria and Ethiopia [11, 18]. The presence of bacterial isolates on inanimate objects can cause cross infections in surgical patients during postoperative care. In addition, among the patient-care equipments, sphygmomanometers were the most contaminated followed by stethoscopes. In other studies, almost the same pattern of bacterial contamination was observed in sphygmomanometers and stethoscopes [9, 11, 12, 21]. Contaminated patient-care equipments such as stethoscopes, sphygmomanometers, ambu bags, suction machines, and baby incubators can come in direct contact with patients thereby putting them at the risk of developing infections [22]. From our results, it is envisaged that surgical patients are at a higher risk of cross-infections. The second most contaminated spot in this study was the paediatric ward. Results are in concordance with an earlier report from Nigeria [20]. Patient-care equipments such as sphygmomanometer and stethoscope were contaminated only by the Gram-positive cocci, Staphylococcus. Besides, in comparison to our results, Getachew et al., [19] reported a much lower level of bacterial contamination in the NICU. In this ward, patient-care equipments such as ambu bags and suction machines were colonized with both Gram-positive and negative bacteria. However, stethoscopes and baby incubators were contaminated with only Gram-positive bacteria. The inanimate objects such as tables, beds, and medical charts were heavily contaminated with both Gram-positive and negative bacteria in all three wards. The magnitude contamination dealt within this study hospital may be due to the fault of cleaning personnel, products, or procedures. Housekeeping is of prime importance in reducing the risk of transmission of nosocomial bacterial pathogens to susceptible patients. Therefore, the periodic sterilization of patient-care equipments before usage and frequent disinfection of inanimate objects are extremely important.

Diversity of bacterial isolates

Results revealed that in all the three wards, different patient-care equipments and inanimate objects directly or indirectly associated with patients were heavily contaminated by diverse species of bacterial pathogens. Of the total isolates, Gram-positive cocci were the most dominant as compared to the Gram-negative bacteria and were comparable to a number of studies reported from Ethiopia and Nigeria [11, 13, 14, 19, 23]. Nevertheless, in contrary to our results, previous studies reported that Gram-negative bacilli are the predominant isolates in hospital settings [7, 18, 24]. Among the Gram-positive cocci, CNS 46 (52.2%) were the most frequently isolated bacteria from all the samples followed by S. aureus 42 (38.5%). This finding is in consonance with other studies that reported CNS as the major bacteria that colonized on inanimate and therapeutic equipments in hospital setups [11, 13, 25, 26, 27]. However, our results are not in agreement with other researchers who reported S. aureus as the most encountered isolates in inanimate and therapeutic equipments [12, 14, 23]. Furthermore, Chikere et al., [18] reported that S. epidermidis was the most frequently isolated Gram-positive cocci, from different wards. Isolation of Staphylococcus sp. from patient-care equipments and inanimate objects of all the three wards indicates their ubiquitous nature. This can be correlated to the fact that Staphylococci are members of the micro-biome of healthy as well as sick individuals. It can be dispersed widely through direct contact with contaminated inanimate objects or medical equipments or even by transient carriage on the hands of healthcare workers. Moreover, Staphylococcus sp. can grow as biofilms, enhancing its ability to glide over surfaces.

Due to their intrinsic resistance mechanism, Gram-negative bacilli are considered to cause most of the bacterial associated HAI [28]. Results of this study also proved that Gram-negative bacilli colonized in inanimate objects and patient-care equipments of all three wards. Species of Acinetobacter were the predominant isolate followed by Klebsiella sp., Citrobacter sp., Enterobacter sp., Salmonella sp., Serratia sp., and E. coli. The percentage and diversity of bacterial isolates observed in this work are in concordance with some of the previous studies [12, 14, 18, 23, 29]. Earlier it was reported that Acinetobacter sp. is the main Gram-negative bacilli isolated from therapeutic equipments of hospital settings [28, 30]. In contrast, some other studies described that E. coli was the most common Gram-negative bacteria isolated from medical equipments and hospital environment [14, 20, 23], whereas another study described that Enterobacter sp. was the dominant bacteria found followed by Serratia sp., Citrobacter sp., and Klebsiella sp. [32]. The second most predominant Gram-negative bacilli obtained were the Klebsiella sp., as in the case of a previous study done in Ethiopia itself [14]. In contrast, several studies from Ethiopia and Mexico reported that Klebsiella sp. was the major bacterial species isolated from the medical equipments and hospital environment [12, 21, 24]. In addition, a recent study from Ethiopia showed that Citrobacter sp. was the most commonly isolated bacteria followed by Klebsiella sp. [13]. The frequency of bacterial isolates such as Citrobacter sp. and Enterobacter sp. observed in this study was comparable to a certain extent with an earlier report from Ethiopia [11]. In our study, isolates of Salmonella sp., Serratia sp. and E. coli were rarely isolated. Prior studies from Ethiopia reported similar trends in the case of Salmonella, E. coli, and Serratia while examining the therapeutic equipments and inanimate objects [12, 14, 21]. The discrepancy in number and diversity of bacterial isolates in various studies could be due to the alterations in hygiene practices and environmental sanitation methods prevailing in hospital setups. It has been reported that Gram-negative bacilli such as Acinetobacter sp., E. coli, Klebsiella sp., and Serratia sp. can survive for months on dry surfaces, humid environments and adverse conditions [32]. Isolation of CNS, S. aureus, Klebsiella sp., E. coli, Enterobacter sp. and Acinetobacter sp. indicate a serious concern for a possible outbreak of nosocomial infections. Therefore, an infection control program for preventing, monitoring, and controlling the spread of these bacteria in the hospital facility is warranted.

Antibiogram

In the present study, the pattern of antibiotic resistance of bacterial isolates against fourteen antibiotics was explored. Most of these antibiotics were routinely prescribed for the management of diverse bacterial diseases in the study area. It is corroborated that ESKAPE pathogens are responsible for a substantial percentage of HAI in the recent decade. However, we could identify only four species belonging to the ESKAPE pathogens. It includes Gram-positive cocci, S. aureus, Gram-negative bacilli, Acinetobacter sp., Klebsiella sp., and Enterobacter sp. In the present study, the predominant Gram-negative bacilli, Acinetobacter sp. displayed the highest resistance to two commonly used antibiotics, such as ceftriaxone and ampicillin and are more or less comparable to the results of a prior study [30]. Especially, the isolates of Acinetobacter were found to be susceptible to amikacin. It has been reported that Acinetobacter emerged as an important nosocomial pathogen because it can survive as saprophytes in the environment and are impervious to commonly used antibiotics [33]. The second predominant Gram-negative bacilli, Klebsiella sp. showed resistance to trimethoprim-sulfamethoxazole, ampicillin, and amoxicillin. A similar trend of resistance was observed in the studies conducted from other regions of Ethiopia [12, 14]. Besides, susceptibility patterns exhibited by the isolates of Klebsiella sp. to amikacin and chloramphenicol were by and large similar to the results of earlier works [13, 19]. Apart from Klebsiella sp., other Gram-negative bacilli such as Citrobacter sp., Salmonella sp., Enterobacter sp., E.coli, and Serratia sp., exhibited appreciable resistance against the same antibiotics. A similar trend of resistance pattern has already been documented by various studies [12, 13, 14, 19]. In addition, the isolates of Salmonella sp. exhibited resistance to four antibiotics such as trimethoprim-sulfamethoxazole, ampicillin, amoxicillin, and tetracycline. In a similar fashion, Weldegebreal et al., [12] had already reported that Salmonella sp. isolated from patient-care equipments is resistant to tetracycline. However, the resistance exhibited against trimethoprim-sulfamethoxazole and amoxicillin were not in concurrence with the same study [12]. Bacterial isolates, E. coli, and Serratia sp. exhibited a similar trend of susceptibility to six antibiotics such as tetracycline, amikacin, ceftriaxone, chloramphenicol, ciprofloxacin, and norfloxacin. The susceptibility pattern of E. coli was consonant with the results of recent studies done in Ethiopia [12, 13]. Also, our results are in accordance with an earlier study that reported that Serratia spp. is highly susceptible to ceftriaxone and ciprofloxacin [14]. In the case of Gram-positive bacilli, the most predominant isolate, CNS showed resistance to both penicillin and trimethoprim-sulfamethoxazole to a greater extent. Similar phenomena were observed in a number of studies reported from various regions of Ethiopia [12, 13, 14]. It can be noted that isolates of CNS were highly susceptible to clindamycin and amikacin. In contrast, a previous study from Ethiopia itself reported that CNS is least susceptible to clindamycin [19]. Isolates of S. aureus showed high resistance to penicillin followed by trimethoprim-sulfamethoxazole which was already documented in some of the previous studies [12, 13, 14, 19, 21]. On the other hand, most of the isolates were sensitive to clindamycin, chloramphenicol, and ciprofloxacin [19]. The percentage of MRSA observed in this study is well-nigh comparable to the results of a prior study [19], whereas extremely lower than an earlier study [14]. Multiple antibiotics resistance was detected in 57.7% of the isolates which was well-nigh comparable to the results of a previous study [14], however, lower than the results of an earlier study done in Ethiopia [19]. The high frequency of multi-drug resistant bacteria observed in this study might have emerged as a result of inappropriate and frequent usages of antibiotics per patient per ward. It is envisaged that bacteria carrying the resistant genes can multiply rapidly and share their resistance with other microbes persisting in the hospital environ. Reducing the prevalence of multi-resistant isolates in a facility is crucial in preventing the emergence of specific nosocomial pathogens. It is high time to develop policies to monitor the periodic surveillance of pathogens, by giving proper guidance on the judicious selection of antibiotics, along with the implementation of remedial measures upon the detection of antibiotic-resistant bacteria.

HAI can be caused by any kind of microorganisms includes those that are surviving in the hospital environment for a prolonged period of time and hence develop resistance to antibiotics and disinfectants posing a great concern in the present scenario. Therefore, the medical fraternity must urgently address this issue. A periodic analysis of antibiotic susceptibility patterns of bacteria sourced from the hospital environment could provide insight into the resistance profile, eventually helping to develop stringent strategies to circumvent the problem.

Limitations of the study

The limitations of this study are the smaller sample size, the limited period of collection and the lack of advanced techniques for the identification of fastidious bacterial pathogens.

Conclusion

Results revealed that medical equipments and inanimate objects of the surgical ward, followed by paediatric and NICU were heavily contaminated with diverse species of bacterial pathogens. The most contaminated area was the surgical ward. The possible reason for this may be due to the understaffing, insufficient availability of cleaning materials, insufficient training, and high patient turnover prevailing in the hospital. It was found that the predominant bacteria responsible for the contamination of patient-care equipments and inanimate objects are S. aureus, CNS, Klebsiella sp. and Acinetobacter sp. Gram-positive Staphylococci were more frequently isolated from the surgical ward especially from inanimate objects such as beds and tables. Results of the antimicrobial susceptibility tests revealed that bacterial isolates are resistant to multiple antibiotics, indicating a high risk of nosocomial outbreaks with drug-resistant bacteria. This might be a reflection of inappropriate use of antibiotics or unavailability of a guideline regarding the selection of antibiotics for prophylaxis or empirical treatments. Therefore, stringent infection prevention and control program comprising of routine sampling from the patient-care equipments and inanimate objects of different wards along with antimicrobial resistance surveillance and decontamination efforts must be implemented.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

Not applicable

Competing interests

All authors declare that they have no competing interests.

Funding

This study was not funded by any funding agencies.

Authors' contributions

AM: Primary researcher conceived the idea for this study. MB, MM, KB, MS and GB participated in lab, conducted data analysis. AM, AA, MS drafted and finalized the manuscript for publication.

Acknowledgements

The authors are very grateful to Department of Medical Laboratory Science, College of Medicine and Health Sciences, Arba Minch University for their technical assistance.

References

1. Haque M, Sartelli M, McKimm J, Bakar MA. Health care-associated infections – An overview. Infect Drug Resist. 2018; 11: 2321–2333.
2. Pittet D, Allegranzi B, Storr J. The WHO clean care is safer care programme: field-testing to enhance sustainability and spread of hand hygiene improvements. J Infect Public Health 2008;1: 4–10.
3. Yallew WW, Takele AK, Yehuala FM. Point prevalence of hospital-acquired infections in two teaching hospitals of Amhara region in Ethiopia. Drug Health Patient Saf, 2016; 8: 71–76.

4. Khan HA, Baig FK, Mehboob R. *Nosocomial infections*: *Epidemiology*, *prevention*, *control* and *surveillance*. Asian Pac J Trop Biomed. 2017; ***7***:*478–482*.
5. Kramer A, Schwebke I, Kampf G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC* Infect Dis. 2006; 6:130.
6. Wang M, Wei H, Zhao Y, Shang L, Di L, Lyu C, Liu J. Analysis of multidrug-resistant bacteria in 3223 patients with hospital-acquired infections from a tertiary general hospital in China. Bosn J Basic Med Sci. 2019; 19: 86–93.
7. Mbim EN, Mboto CI, Edet UO. Plasmid profile analysis and curing of multidrug resistant bacteria isolated from two hospital environments in Calabar Metropolis, Nigeria. *Asian J Med* Health, 2016; 1:1–11.
8. French GL, Phillips I. Antimicrobial resistance in hospital flora and nosocomical infection. Hospital Epidemiology and infection control, Williams and Wilkins, Baltimore, MD (May hall,C. Ed), 1996; pp. 980-990.
9. Yusha'u M, Bukar A, Aliyu BS, Abdulkareem A. Bacterial Contamination of Some Hospital Equipments in Kano, Nigeria. Hamdard Med. 2012; 55: 3.
10. Sandle T. Environmental Monitoring Risk Assessment. J GXP Comp, 2006;10(2).
11. Gelaw, A., Gebre-Selassie, S., Tiruneh, M., Mathios, E., Yifru, S. Isolation of bacterial pathogens from patients with postoperative surgical site infection and possible sources of infection at university of Gondar hospital, northwest Ethiopia. J Environ Occup Sci; 2014;3,103-108.
12. Weldegebreal F, Admassu D, Meaza D, Asfaw M. Non-critical healthcare tools as a potential source of healthcare-acquired bacterial infections in eastern Ethiopia: A hospital-based cross-sectional study. SAGE Open Med. 2019. doi:10.1177/2050312118822627.
13. Darge A, Kahsay AG, Hailekiros H, Niguse S, Abdulkader M. Bacterial contamination and antimicrobial susceptibility patterns of intensive care units medical equipment and inanimate surfaces at Ayder Comprehensive Specialized Hospital, Mekelle, Northern Ethiopia. BMC Res Notes, 2019;12: 621.
14. Worku T, Derseh D, Kumalo A. Bacterial Profile and Antimicrobial Susceptibility Pattern of the Isolates from Stethoscope, Thermometer, and Inanimate Surfaces of Mizan-Tepi University Teaching Hospital, Southwest Ethiopia. Int J Microbiol. 2018, Article ID 9824251.
15. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey's manual of determinative bacteriology.9th Williams and Wikins co, Baltimore, 1994.
16. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45: 493-496.
17. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Wayne: CLSI, 2016.

18. Maina SM, Nyerere AK, Ngugi CW. Isolation of bacterial diversity present in medical waste and health care settings in hospitals in Kenya. Afr J Microbiol Res. 2018;12: 606-615.
19. Getachew H, Derbie A, Mekonnen S. Surfaces and Air Bacteriology of Selected Wards at a Referral Hospital, Northwest Ethiopia: A Cross-Sectional Study. Int J Microbiol. 2018, Article ID 6413179.
20. Chikere C, Omoni V, Chikere B. Distribution of potential nosocomial pathogens in a hospital environment. Afr J Biotechnol. 2008; 7: 3535-3539.
21. Shiferaw T, Beyene G, Kassa T, Sewunet T. Bacterial contamination, bacterial profile and antimicrobial susceptibility pattern of isolates from stethoscopes at Jimma University Specialized Hospital. Ann Clin Microbiol Antimicrob. 2013; 12: 39.
22. Willey JM, Sherwood LM, Woolverton CJ. Prescott, Harley and Klein's Microbiology, 7th Edn., Mc Graw Hill Companies, New York, USA, 2008.
23. Maryam A, Usman-Sani H, Usman MA. Characterization and determination of antibiotic susceptibility pattern of bacteria isolated from some fomites in a teaching hospital in northern Nigeria. Afr J Microbiol Res. 2014; 8: 814–818.
24. Garcia-Cruz CP, Aguilar MJN, Arroyo-Helguera OE. Fungal and Bacterial Contamination on Indoor Surfaces of a Hospital in Mexico. Jundishapur J of Microbiol. 2012;5: 460-464.
25. Teng SO, Lee WS, Ou TY, Hsieh YC, Lee WC, Lin YC. Bacterial contamination of patients' medical charts in a surgical ward and the intensive care unit: Impact on nosocomial infections. J Microbiol Immunol Infect. 2009;42: 86-91.
26. Abubakar AS, Barma MM, Balla HJ, Tanimu YS, Waru GB, Diba J. Spectrum of bacterial isolates among intensive care unit patients in a tertiary hospital in Northwestern Nigeria. Ind J Sci Res Tech, 2014;2: 42-47.
27. Ekrami AR, Kayedani A, Jahangir M, Kalantar E, Jalali M. Isolation of common aerobic bacterial pathogens from the environment of seven hospitals, Ahvaz, Iran. Jundishapur J Microbiol. 2011;4: 75-82.
28. Mehrad B, Clark NM, Zhanel GG, Lynch JP. Antimicrobial Resistance in Hospital-Acquired Gram-Negative Bacterial Infections. Chest, 2015;147: 1413–1421.
29. Bakkali MEL, Hmid K, Kari KE, Zouhdi M, Mzibri MEL, Laglaoui A. Characterization of Bacterial Strains and their Resistance Status in Hospital Environment. J Trop Dis. 2015; 4: 180. doi:10.4172/2329-891X.1000180
30. Kim LJ, Lee H, Kim H, Chang K. Isolation Frequency and Antimicrobial Susceptibility of Bacterial Pathogens Isolated from Physical Therapeutic Instruments in General Hospitals. J Phys Ther Sci. 2010;22: 61-67.
31. Ayatollahi AA, Amini A, Rahimi S, Takrami SR, Darsanaki RK, Nezhad MS. Prevalence of Gram-Negative Bacilli Isolated from the Equipment and Surfaces in Hospital Wards of Golestan Province, North of Iran. Eur J Microbiol Immunol (Bp), 2017; 7:261–266.

32. Moniri R, Heravi M. Evaluation of Bacterial Contamination in Medical Devices and Anti-Bacterial Resistance of Isolated Gram Negative Bacilli in Shahid Beheshti Hospital in Kashan, Iran, 2004. Feyz, 2006; 9:;55-50.
33. Lone R, Shah A, Kadri SM, Lone S, Faisal S. Nosocomial Multi-Drug-Resistant Acinetobacter Infections - Clinical Findings, Risk Factors and Demographic Characteristics. Bangladesh J Med Microbiol. 2009;3: 34-38.

Tables

Due to technical limitations, Tables 1 - 6 are only available for download from the Supplementary Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TablesDr.AMHAI.docx