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COLLEGE OF HEALTH SCIENCES
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Magnitude and drug resistance profile of Extended Spectrum β-Lactamase (ESBL) producing gram negative bacteria from nasal swabs taken from health care workers and inanimate objects at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology).

June, 2019

ADDIS ABABA, ETHIOPIA

Addis Ababa University

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Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Asegedech Asmamaw, entitled: Magnitude and drug resistance profile of Extended Spectrum β-Lactamase (ESBL) producing gram negative bacteria from nasal swabs taken from health care workers and inanimate objects at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Acknowledgment

First of all I would like to express my deep gratitude to God who helped me in making this research. Then I would like to acknowledge Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University for giving me the opportunity to go through and finalize this research work and my MSc degree.

I would like to extend my gratitude and thanks to my advisors Kassu Desta and Dr. Yimtubeznash Woldeamanuel for their unreserved support, continuous guidance and encouragement and follow up throughout the course of my work.

Finally, I would like to express my thanks to Mequanint Mitiku and Bekelu Belama for their support during laboratory work.

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Abbreviations

AST Antibiotic Susceptibility Test

CLSI Clinical and Laboratory Standard Institute

CoNS Coagulase Negative Staphylococcus

ESBL Extended Spectrum Beta Lactamase

ESBL-PE Extended spectrum beta lactamase producing *enterobacteriaceae*

ECG Echocardiography

GNB Gram Negative Bacilli

HCW Health Care Worker

ICU Intensive Care Unit

MRSA Methicillin Resistance Staphylococcus Aureus

MRB Multidrug Resistant Bacteria

OT Operation Theatre

TASH Tikur Anbessa Specialized Hospital

VRE Vancomycin Resistant *Enterococci*

Abstract

Background: Hospital environment, inanimate objects and healthcare workers are likely to get colonized with diverse group of microbial agents. Infections caused by gram negative bacteria (GNB) are causing morbidity and mortality worldwide, gram negative bacteria which produce β-lactamase can result in multidrug resistance. The production of extended-spectrum β-lactamases (ESBLs) is an important mechanism which is responsible for the resistance to the third-generation cephalosporin**.**

Objective: The objective of this study was to determine the magnitude and drug resistance profile of ESBL producing gram negative bacteria isolated from nasal swabs taken from health care workers (HCWs) and inanimate objects at Tikur Anbessa Specialized Hospital.

Methods: Both prospective and retrospective cross sectional study were conducted from December 2018 to February 2019.Isolates from samples collected from an on-going PhD project were used for the purpose of the current study. The samples were taken from nasal swabs of health care workers and inanimate objects. About 105and 216 isolates were randomly selected from health care workers and inanimate objects respectively for further analysis. Biochemical tests for identification and antimicrobial susceptibility test was done by disc diffusion method. Screening of ESBLs was done using Cefotaxime (30ug, BD), Ceftazidime (30ug, NJ54, QB14), ceftriaxone (30ug BD) discs. A combined disk diffusion test was used as confirmatory test. The data were analyzed using SPSS software (version 20) and descriptive statistical tests were performed

Results: Out of 105 isolates, 5 (4.8%) isolates were identified as Extended Spectrum β--lactamases (ESBLs) producers**,** out of theses80% were *Klebsiella spp* (75% *Klebsiella rhinoscleromatis,* 25% *Klebsiella pneumoniae*) and 20% were *Entrobacter cloacae*. Three (60%) ESBL producing bacterial isolates were identified from Laboratory personnel and 2 (40%) were from Medical doctors. From environmental samples, 33/216 (15.3%) isolates were found to be ESBL producers based on the confirmatory test (combined disk method). Different ESBL producing gram negative bacteria, were isolated from the various inanimate objects of TASH including, *Klebsiella ozaenae*, *Klebsiella oxytoca, Klebsiella pneumoniae, Klebsiella rhinoscleromatis,Citrobacter_spp, Escherichia coli*, *Serriatia_spp* and *Acinetobacter_spp***.** ESBL

producing gram negative bacteria were found to be 100% resistant to ceftazidime and ceftriaxone.

Conclusion: It is worrisome to detect ESBL producing gram negative bacteria from the inanimate objects of TASH and the nasal cavity of some HCWs, calling for systematic screening of inanimate objects for ESBL and other multidrug resistant bacteria in the hospital. Furthermore, strengthening the infection prevention practice is vital to halt transmission of these noxious bugs.

Key words: ESBL, Inanimate objects, HCWS

Ethiopia

1. Introduction

1.1. Background

Gram-negative organisms can lose viability rapidly under dry conditions; as such the role of environmental reservoirs in the transmission of ESBL infection is often overlooked. But, most nosocomial pathogens can persist on inanimate surfaces for many weeks [1] and prolonged environmental survival of carbapenem-resistant *Klebsiella pneumonia* and ESBL-producing *Escherichia coli* has been demonstrated[2, 3].

Hospital environment, inanimate objects and healthcare workers are likely to get colonized with diverse group of microbial agents. The direct contact with infected and/or colonized patients as well as objects may lead to transmission, resulting into morbidity and mortality. The organisms from objects of frequent contact in hospital are likely to get transmitted to healthcare workers, patients and visitors [4].Microbial resistance through Extended Spectrum β-lactamase was firstly reported in the early 1980s in the Europe and subsequently in the United States soon after the introduction of third generation cephalosporins in clinical practice [5,6].

B-Lactams are a group of antibiotics acting on the cell wall of a bacterial cell. These include the penicillins, cephalosporins, carbapenems and monobactms. These bind to and inhibit the carboxypeptidases and transpeptidases. These are the cell wall synthesizing enzymes, also called the penicillin-binding proteins. As a result, there is weakening of the cell wall structure, leading to cell lysis [7], whereas Beta-lactamases are enzymes produced by the bacteria that inactivate β-lactam antibiotic by hydrolysis, which results ineffective compounds. ESBLs are one group of β –lactamases which able to hydrolyze and cause resistance against a wide range of novel β-lactams, including third-generation cephalosporins and monobactams [8].

Resistance mechanisms have been found for every class of antibiotic agents. The predominant mechanism for resistance to the β-lactam antibiotics in gram-negative bacteria is the production of β-lactamase [9]. The production of extended-spectrum β-lactamases (ESBLs) is an important mechanism which is responsible for the resistance to the third-generation cephalosporins during the last 2 decades [10].The common infections with ESBL-producing organisms include all the infections caused by gram negative organisms such as urinary tract or wound infection, peritonitis, cholangitis, intra-abdominal abscess, pneumonia and catheter-associated blood

stream infections. Resistance to multiple antibiotics makes these infections difficult to treat and results in poor outcomes for patients. ESBL-producing bacteria are a particular problem for patients in critical care units [11, 12].

An ESBL can cause resistance or reduced susceptibility to the oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and monobactams (aztreonam). However, it does not hydrolyze the cephamycins (e.g., cefoxitin and cefotetan), and the carbapenems (imipenem, meropenem) and their hydrolytic activity can be inhibited by several β-lactamase inhibitors such as clavulanic acid and tazobactam [7, 8, 13].

ESBL-producing organisms also exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic option. In addition, being resistant to β-lactam antibiotics, often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones [4, 7].An extended spectrum beta-lactamase production has been observed mostly in *Entrobacteriaceae,* and their resistance increased mainly due to the spreading of ESBL and the emergence of different genes [14, 15]. The most common ESBL producing *Entrobacteriaceae* are *Escherichia coli and Klebsiella pneumoniae,* being the main source of community and hospital acquired infections. However there are also other ESBL producer *Entrobacteriaceae* species which are clinically relevant [8, 14].

The bacteria that produce ESBL are spread in a similar manner to other multi-resistant organisms, via inadequately decontaminated hands of staff and indirectly via the environment (contaminated surfaces and equipment). They are capable of prolonged survival on wet surfaces and have been found colonizing taps and sink drains in wards [16].

The Clinical and Laboratory Standards Institute (CLSI) has proposed disk-diffusion methods for screening for ESBL production by *Klebsiella pneumoniae, K. oxytoca, Escherichia coli and Proteus mirabilis*. Laboratories using disk-diffusion methods for antibiotic susceptibility testing can screen for ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production. Cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disks are used. Since the affinity of ESBLs for different substrates is variable, the use of more than one of these agents for screening improves the sensitivity of detection [17].

1.2. Statement of the problem

Infections caused by Gram negative bacteria (GNB) are causing morbidity and mortality worldwide, and the most common contributing factor to β-lactam resistance is the production of β - lactamase enzymes [18]. The ESBLs cause resistance to broad-spectrum cephalosporins like ceftazidime, ceftriaxone and cefotaxime and aztreonam [8].

Currently multi drug resistance become emerging worldwide at an alarming rate among gram negatives, causing both community acquired and nosocomial infection. Hospitals provide a reservoir of microorganisms, many of which are multi-resistant to antibiotics [8, 13].The emergence of resistance to antimicrobial agents is a global public health problem especially in pathogens that cause nosocomial infections which contributed to the morbidity, mortality, increased health care costs resulting from treatment failures [19].

The enzymes of the β- lactamase group can hydrolyze the drug Monobactams, cephalosporins, penicillins, and carbapenems and they become microbiologically ineffective [8, 10]. In addition to this, ESBL producing bacteria also cause resistance to other classes of antibiotics such as aminoglycosides, cotrimoxazole, tetracycline and fluoroquinolones[4].Among *Enterobacteriaceae*, ESBLs are found mainly in *Klebsiella* spp and *Escherichia coli*, but have been also reported in other genera worldwide, such as *Citrobacter, Enterobacter, Morganella, Proteus, Providencia, Salmonella, Serratia* and *Pseudomonas*[13].

A study which was conducted at North West Ethiopia in 2014 on hospital environments showed that out of 384 samples, 14.8% were ESBL producing *Entrobacteriaceae*, from these, 42.10% were *Klebsiella pneumoniae*, 35.09% *Escherichia coli* and 7.01% *Proteus mirabilis*. All ESBL producing *Entrobacteriaceae* were found to be resistant to ceftriaxone, ceftazidime, cefpirome, cefpodoxime and amoxicillin with Clavulanic acid [20].

Reports about ESBL producing gram negative bacteria from health care workers and inanimate objects at Tikur Anbessa Specialized Hospital are limited. Thus, this study was carried out to assess and determine the magnitude and drug resistance profile of Extended Spectrum β-Lactamase producing gram negative bacteria from nasal swabs taken from health care workers and inanimate objects at Tikur Anbessa Specialized Hospital.

1.3. Significance of the study

The problems which are associated with ESBLs include multidrug resistance, difficulty in detection and treatment, and increased mortality. Awareness and detection of these enzymes are necessary for optimal patient care. Therefore, this study will provide information on the magnitude of ESBL producing gram negative bacteria and there drug resistance profile. This study also helps for policy makers for better management of the hospital environment quality and to develop new infection control strategies. It also used as a reference for another researcher.

2. Literature review

According to the CDC report 2012, *Klebsiella oxytoca* is a primary health care associated pathogen acquired from environmental sources. During October 2006- March 2011, a total of 66 patients in a hospital in Toronto, Canada, acquired extended spectrum beta lactamase producing *K. oxytoca*. New cases continued to occur despite reinforcement of infection control practices, prevalence screening and contact precautions for colonized /infected patients. Cultures from hand washing sinks in the intensive care unit yielded *K. oxytoca*. No infection occurred after implantation of sink cleaning three times a day and sink drain modification [21].

According to the cross-sectional study conducted in France by Roux *et al.,* on contaminated sinks in intensive care units: an underestimated source of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the patient environment within 13 ICUs, including 185 sinks showed that, out of185 sink drain swabs, 57 (30.8%) yielded ESBLE. The contamination rate of sink drains varied between ICUs, from 0 to 81.8% of the samples. Two different ESBL isolates were obtained from each of three swabs such that a total of 60 ESBLE were isolated. Among the 60 ESBLE isolates, *Klebsiella* was the most numerous (33/60, 55.0%) with 29 *K. pneumonia* and four *K. oxytoca*. The *Enterobacter* genus was the second most numerous genus (18/60, 30.0%) with 16 *E. cloacae*, one *E. aerogenes* and one *E. asburiae*. In the seven ICUs contaminated sinks were identified, there was a complex pattern of sink drain contamination, with more than one species contaminating the sinks from the ICU [16].

Another cross-sectional study was conducted in Italy by Dore *et al.,* to show the microbial contamination of devices or surfaces located in the departments of Medicine and Surgery of an Italian University Hospital. Swabs were used to sample devices (keyboards, phones) and surfaces (door handles, water closed, light switches), at two different time-points. They were collected 189 swabs and the bacterial contamination prevalence was 42.9%, significantly higher in the Medicine than in the Surgery Department (51.6% vs 34%). A greater contamination was observed in water closet (22/36, 61.1%), phones (22/40, 55%), and keyboards of personal computers (18/36, 51.4%) [22].

Another prospective study conducted in India by Maheshwari *et al.,* to determine the prevalence of bacterial colonization among health care workers .The age range was between 20 to 65 years; 29 (41.4%) were males and 41 (58.6%) were females; 20 (28.6%) doctors, 20 (28.6%) nurses, 20 (28.6%) operation theatre (OT) technicians and 10 (14.2%) were laboratory technicians. Out of the 70 healthcare workers, 13 (18.6%) carried *Staphylococcus aureus*, of which 6 were MRSA and 15 (21.4%) carried ESBL producing gram negative bacteria. Among the 15 ESBL producers, 9 were *Klebsiella pneumoniae,* 5 were *Escherichia coli* and 1 was *proteus mirabilis* [23].

Another study was conducted in Nepal by Dharm *et al.,* by collecting 232 samples from various sites like surface of biometric attendance devices, elevator buttons, door handles, staircase railings, telephone sets and water taps, 219 bacterial isolates were recovered from 181 samples. The most common bacterial isolate was *Staphylococcus aureus* (44/219). *Acinetobacter* was the most common gram negative isolate followed by *E coli* and *Pseudomonas species*. Majority of *Acinetobacter species* 52.6% (10/19) and *E coli* 46.6% (7/15) were ESBL producers. All the isolates of *Acinetobacter species*, *E coli* and *Pseudomonas species* were susceptible to amikacin and imipenem [24].

In Iran a descriptive study was done by Ayatollahi *et al.,* to assess the prevalence of gram negative bacilli isolated from hospital's equipment and surfaces in western region of Golestan province in 2015.Out of 1210 devices which were examined, 352 positive microbial cultures (29.1%) contaminated with gram-negative bacilli where the highest bacterial contamination was related to *Enterobacter* (10.7%). Among different hospital wards the highest bacterial contamination was observed in ICU (19.32%) and among the various equipment, the highest contamination rate was related to laryngoscope blades (10.51%), phone (7.1%), ECG sensor device and monitoring interface (6.25%).Telephone handset (5.22%) and patients' bed (5.22%) [25].

A meta-analysis was performed to determine overall proportion estimate of ESBL-producing *Enterobacteriaceae* in East Africa hospitals. A total of 4076 bacterial isolates were included in the analysis. The overall pooled proportion of ESBL-producing *Enterobacteriaceae* among included surveys done in East African hospitals was found to be 0.42(95% CI: 0.34-0.50). Heterogeneity between countries' proportions in ESBL was significantly high (96.95 % and p

<0.001). The frequently detected genes encoding ESBL were CTX-M, TEM, SHV and OXA while the most infrequent reported genes were KPC and NDM. Finally they conclude that the available studies show a very wide variation in resistance due to ESBL between countries. This highlights a need for active surveillance systems which can help understand the actual epidemiology of ESBL, aid in formulating national or regional guidelines for proper screening of ESBL and support developing standardized approaches for managing patients colonized with ESBL [26].

A systemic review and meta-analysis was performed to assess the ESBL-PE clones circulating in humans, animals and the environment to provide evidence based insights for combating ESBL-PE using one heath approach. Systemic search from Medline/pubmed, Goggle Scholar and African journals online was carried out and retrieved nine articles based on phenotypic and genotypic detection of ESBL-PE between 2005 and 2006 in Tanzania. The overall prevalence of ESBL-PE in the three interfaces was 22.6% with the predominance of *Escherichia coli* strains (51.6%).The majority of ESBL-PE were resistance to the commonly used antimicrobials such as trimethoprim-sulfamethoxazole and tetracycline/doxycycline, 38%-35% were resistance to ciprofloxacin and all were sensitive to meropenem/Imipenem [27].

Another descriptive study was carried out by Gonsu K. H *et al.,* at the intensive care unit, neonatal care unit, surgical and obstetrical unit of the University Teaching Hospital of Cameroon, at 2008 to determine the prevalence of multi-drug resistant bacteria (MRB) carriage by health care workers. They collect different samples like nasal, rectal and hand swabs from a sampled staff involved in patient care in the targeted units and 264 different samples were collected in this study. Finally they were identified multidrug-resistant bacteria in 50 (18.9%) samples and phenotypes of MRB were identified, extended spectrum beta lactamase producing *Enterobacteriaceae* (ESBLE) constituted 22% with the species *Klebsiella pneumoniae*, *Enterobacter cloacae* and *E. coli* isolated [28].

In Algeria across sectional study was conducted by Manel *et al.,* to investigate and characterize *Enterobacteriaceae* strains isolated from different hospital surfaces and to determine the occurrence of Extended Spectrum Beta Lactamase (ESBL) producing *Enterobacteriaceae*. A total of 637 strains of *Enterobacteriaceae* were isolated between 2007 and 2012. The susceptibility study showed that the imipenem was the most active antibiotic against overall

enterobacteriaceae with resistance rate of 2.51%, however the high resistance rates were observed to ampicillin (98.11%) and cephalexin (78.65%). The prevalence of ESBL was 136 (21.35%) and ESBL-producing strains were *K. pneumonia* 66 (28.45 %), *E. coli* 31 (25.41%), *S. marcescens* 18 (19.15%), *K. oxytoca*9 (17.31%), *En .cloacae* 5 (5.43%), *C. freundii*04 (16*%)* and *P. mirabilis* 03 (15%) [29].

Another study also conducted in Madagascar by Micheel *et al.,* to assess the nasal occurrence of β-lactamase-producing *Enterobacteriaceae* both in patients in a hospital department of infectious diseases at admission and in healthy Madagascar students and health care workers. They collect nasal swabs from 681 students, 824 health care workers, and 169. Out of 1541 samples obtained from students and health care workers, gram-negative bacteria were grown from 816 samples and *enterobacterial* growth was observed on Brilliance ESBL agar for 37 study participants. Out of 169 samples from hospital patients obtained at admission, 126 showed gram-negative rod-shaped bacteria and 13 showed *Enterobacteriaceae* as well as 38 non fermentative rod-shaped bacteria on Brilliance ESBL agar [30].

According to the study conducted in Nigeria by Muhammad *et al.,* on distribution of potential nosocomial pathogens isolated from environments of the selected hospital in Sokoto, North Western Nigeria, 160 samples were collected from different items in four hospitals namely Sink, floor, bed, bed cover, toilet floor, bed pan and ward wall. A total of 258 bacteria were isolated and identified from the hospitals. The potential pathogenic bacteria isolated in this study were *Staphylococcus aureus* 78 (30.2%) and *Proteus vulgaris* 46 (17.8%), *Pseudomonas aeruginosa* 31(12.0%), *E. coli* 30 (11.6%), *Bacillus cereus* 30 (11.6%), *Klebsiella species* 13 (5%), *Salmonella species* 11 (4.3%), *Shigella species* 10 (4%) and *Proteus mirabilis* 9 (3.5%)[19].

Based on the study conducted in Uganda by Segujja *et al.,* on characterization and antimicrobial susceptibility patterns of isolates from ward fomites, out of 290 surfaces and instruments swabbed, 57.59% of them carried bacterial pathogens and the most common isolated pathogen was *Staphylococcus aureus*, 43 (25.75%), followed by *Klebsiella pneumonia* 35 (20.96%), *Escherichia coli* 31 (18.55%), *Pseudomonas aeruginosa*20 (11.98%), *Enterococcus faecalis*12 (7.19%), *Staphylococcus epidermidis*10 (5.98%), *Proteus mirabilis* 9 (5.39%), *Bacillus* spp. 4 (2.40%), and *Staphylococcus saprophyticus* 3 (1.80%). Among *enterobacteriaceae*, 16(21.33%) were identified as ESBL producers out of which 4/16 (25.00 %,) showed ESBL co-resistance [6].

A across sectional study was conducted in Egypt from April 2014 to September 2014 by Marwa *et al., to* estimate the prevalence and possible types of ESBLs among *Klebsiella pneumonia* and shows that out of 100 health care workers, there were 3 positive samples (3%), from 50 environmental samples there was 1 positive sample (2%) and from 50 instrumental samples, there were 1 sample (2%). All *K. pneumonia* isolates (100%) were found to be susceptible to imipenem followed by amikacine 85.7% then ciprofloxacin 71.4% and cefotaxime 71.4 %. Susceptibility was detected to *streptomycin* 28.5%, ceftazidime 57.1% and ceftriaxone 42.5.Resistances were detected to chloramphenicol 71.5% and rifampicin 71.5% as well. All *Klebsiella pneumoniae* isolates (100%) were resistant to ampicillin [31].

Another study in Egypt by Afifi *et al., reported that out of* 750 environmental samples and 227 environmental isolates, *K. pneumoniae*(n = 102) and *E. coli* (n = 125) were recovered from 750 samples of 6 different environmental surfaces at upper Egypt. ESBL production was observed in 80 isolates, for overall prevalence of 56.25% with a predominance of *K. pneumoniae* (45/80), followed by *E. coli*, with prevalence of 43.75% with a predominance of (35/80). The resistance rate was higher among ESBL producers than non ESBL producers. All *K. pneumoniae* and *E. coli* ESBL producers were found resistance (100%) to cephalothin, ampicillin, cefuroxime, cefotaxime, ceftriaxone and ceftazidime. The resistance rate was higher in aztreonam (93.3 and 100) and cefotaxime (95.5 and 91.42) then gentamicin (84.4 and 42.8) ciprofloxacin (77.7 and 68.5) followed by cotrimoxazole (46.6 and 60) of ESBL-producing K and E strains, respectively [32].

A hospital-based cross-sectional study was conducted by Moges *et al.,* at the University of Gondar Referral Hospital from January to June 2014 to assess and determine the rate of ESBLs producing *Enterobacteriaceae* and their antimicrobial susceptibility patterns in the hospital environment. Out of a total of 384 samples, 14.8% were ESBL producing *Enterobacteriaceae*, where 42.10% *Klebsiella pneumoniae*, 35.09% *Escherichia coli* and 7.01% *Proteus mirabilis* were the predominant isolates. The predominant species were *Klebsiella pneumonia* (42.10%), *Escherichia coli* (35.09%) and *Proteus mirabilis* (7.01%). The highest number of ESBL producing *Entrobacteriaceae* isolates were found from sinks (22.80%).All ESBL producing *Enterobacteriaceae* were found to be 100% resistant to cefpirome, cefpodoxime, ceftazidime, ceftriaxone, and amoxicillin with clavulanic acid. *Klebsiella pneumonia* was resistant to 91.7%

cotrimoxazole, 66.7% chloramphenicol, 45.8% norfloxacin, 45.8% ciprofloxacin, and 25.0% gentamicin. *Escherichia coli* was also resistant to chloramphenicol (55.0%), cotrimoxazole (35.0%), norfoxacin (25.0%), ciprofloxacin (30.0%), and gentamicin (20.0%) [20].

In Ethiopia a study done by Solomon *et al.,* at Wolaita Sodo university teaching and referral Hospital on antibiotic resistant airborne bacteria and their multidrug resistance pattern showed that antibiotic resistance range of 7.5 up to 87.5% was detected among gram-positive isolates of which CoNS showed a high level of resistance for chloramphenicol 68.1% and trimethoprim-sulfamethoxazole 66.7%. *S.aureus* isolates depicted 87.5% resistance to chloramphenicol and 28 (38.9%) were MRSA isolates. Eight isolates in this study were vancomycin-resistant *Enterococci* (VRE). Gram-negative bacterial isolates showed resistance ranging from 15.4–87% of which *Acinetobacter*-spp. showed the highest rate of resistance (>65%) for all antibiotics tested. Ampicillin and doxycycline resistant *E. coli* isolates 71.4 and 78.6% respectively were identified. Imipenem, ceftazidime, and ciprofloxacin resistance *P. aeruginosa* isolates with the rate of resistance, 38.5, 23.1 and 76.9% were also isolated respectively [33].

A cross-sectional study was conducted in Wolaita Sodo University Teaching and referral Hospital, Ethiopia by Solomon *et al* on Extended spectrum and metalo beta-lactamase producing airborne *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. A total number of 216 indoor air samples were collected from the delivery room, intensive care unit, and operation room and 43 *A. baumannii* isolates were identified (13 from delivery room, 21 from intensive care unit and 9 from operation room). Likewise 24 *P. aeruginosa* isolates were obtained (4 from delivery room, 13 from intensive care unit and 7 from operation room). Extended spectrum beta lactamase and metalo-beta lactamase production were observed in 24 (55.8%) and 13 (30.2%) isolates of *A. baumannii* respectively, whereas *P. aeruginosa* showed 15 (62.5%) extended spectrum beta lactamase and 9 (37.5%) metallo-beta lactamase production [34].

Generally ESBL are enzymes which inactivate the penicillin's, cephalosporin of the first, second and third generation antibiotics like cefotaxime, ceftazidime and monobactam. These enzymes are commonly found in *Klebsiella spp, pseudomonas aeruginosa* and *Escherichia coli*. Infections by ESBL producing organisms are a worldwide problem. There is a growing concern for the increasing antimicrobial resistance among the ESBL producing gram negative bacteria

3. Objectives

3.1. General objective

- To determine the magnitude and drug resistance profile of ESBL producing gram negative bacteria from nasal swab of health care workers and inanimate objects at Tikur Anbessa Specialized Hospital.

3.2. Specific objectives

- To determine the magnitude of ESBL producing gram negative bacteria from nasal swab of health care workers and inanimate objects.
- To determine the drug resistance pattern of ESBL producing gram negative bacteria

4. Materials and Methods

4.1 Study area

This study was conducted at Tikur Anbessa Specialized Hospital Addis Ababa Ethiopia, which is found in Lideta sub city from December 2018 to February 2019. The Hospital was built in 1933EC. It is the largest referral Hospital in the country and now the main teaching center for both clinical and preclinical training of most discipline. The hospital provides a tertiary level referral treatment and is open 24hours for emergency services. Tikur Anbessa Hospital offers diagnosis and treatment for approximately 370,000-400,000 patients a year. It has 800 beds, with 130 specialists, 50 non-teaching doctors. The emergency department sees around 80,000 patients a year.

4.2 Study design and period

Laboratory based study was conducted on stored isolates from an ongoing PhD project(Title: Burden of methicillin resistance –staphylococcus aureus and associated factors at TASH evidence from colonization of patients, health care workers- PhD candidate Kassu Desta). Socio demographic information such as sex, age, ward, professional category, year of service and marital status were also taken associated with the stored isolates. Further identification of the stored isolates, ESBL detection and determining the susceptibility pattern was conducted from December 2018 to February 2019.

4.3 Population

4.3.1 Source population

All health care workers and inanimate objects at Tikur Anbessa Specialized Hospital during the study period

4.3.2 Study population

The study populations for the PhD project that provided us the isolates were Tikur Anbessa Specialized Hospital health care workers who were available during working hours on their wards and different inanimate objects.

4.4 Inclusions and Exclusions criteria

4.4.1 Inclusions criteria

- ✓ Health care workers who gave their consent
- ✓ Inanimate objects in the hospital wards

4.4.2 Exclusion criteria

- ✓ Health care workers who do not give their consent
- ✓ Inanimate objects which are found in the administrative offices

4.5 Study variables

4.5.1 Dependent variables

- Magnitude of ESBL producing gram negative bacterial isolates
- Drug resistance pattern of ESBL producing gram negative bacterial isolates

4.5.2 Independent variables

- Age
- Gender
- Professional category
- Source of swabs (Ward)
- Marital status
- Year of service

4.6 Sample size calculation and sampling method

4.6.1 Sample size calculation

Two hundred ten isolates from health care workers were available and based on the available resource 105 isolates were taken using systemic random sampling (every two of it) from stored isolates and allocated proportionally among different Health care workers (Medical laboratory personnel, Pharmacy personnel, Anesthetist, Nurse, Radiologist, Medical Doctor, Physiotherapist) and were processed for further identification and antibiotic susceptibility test based on CLSI guidelines [17].

Table 1: Gram negative bacterial isolates selected based on proportional allocation for different health care workers at Tikur Anbessa Specialized Hospital from December 2018 to February 2019.

Professional category	Isolates available	Isolates analyzed	Rate(%)
Medical laboratory personnel	42	21	20.0
Pharmacy personnel	24	12	11.4
Anesthetist	12	6	5.7
Nurse	66	33	31.4
Radiologist	2	1	0.9
Medical Doctor	62	31	29.5
Physiotherapist	2	1	0.9
Total	210	105	100

For inanimate objects the sample size was calculated based on single sample size estimation as follows. The value of P is taken as 14.8% (0.148) from the study which is conducted by Moges *et al.,* on Prevalence and antimicrobial susceptibility patterns of extended spectrum beta-lactamase producing *Enterobacteriaceae* in the University of Gondar Referral Hospital environments, northwest Ethiopia [20].

$$n = (Z_{\alpha/2})^2 P(1-P)/d^2$$

Where: n=the sample size, $(Z_{\alpha/2})^2$ = at 95% Confidence interval Z value (α=0.05) =1.96, P= expected prevalence or proportion (0.148) and d= Margin of error at 5% (0.05%).

$$n = \frac{(1.96)^2\ 0.148\ (1-0.148)}{(0.05)^2} = \frac{193.772}{} = \mathbf{194}$$

- Since we have 216 stored isolates and it is almost near to the calculated sample size so, all isolates were analyzed for identification and susceptibility testing to be more representative.

❖ Total sample size from both nasal and environmental isolates were = 105+216=**321**

4.6.2 Sampling method

Isolates from nasal swab of heath care workers and different inanimate objects were taken using systemic random and convenient sampling techniques respectively from stored isolates.

4.7 Measurement and Data collection

4.7.1 Socio demographic data

Some socio-demographic data like (Sex, Age, Marital status, Ward type, Professional category, year of service) which were previously collected using data collection sheet were used in this study.

4.7.2 Sample collection

In the current study, stored isolates which were collected from different sources like nasal cavity of health care workers and swabs from inanimate objects (ICU tables, ICU sinks, ICU IV stands, ICU beds, Incubators, ICU pediatrics trolley, oxygen regulators, OR tables, OR beds, OR computers, OR doors, lift buttons, x-ray chairs and some other items) were used.

4.7.3 Culture and identification

Isolates were inoculated into MacConkey agar (Oxoid, BD) and ESBL chromo agar and incubated at 37 °C for 24–48 hrs and the growth were inspected for colony morphology and ESBL screening respectively. The colonies developed after 24 hours of culture were sub cultured into nutrient agar to obtain pure cultures. Then suspension was performed from purified colony to do biochemical identification and antimicrobial susceptibility tests. Finally identified colonies were stored using skim milk storage media that contain 15-20 % glycerol.

4.7.4. Drug Susceptibility testing

Antimicrobial susceptibility pattern of the isolate was determined by the disc diffusion method on Muller Hinton agar (Oxoid, BD) using 0.5 McFarland's as the turbidity standard as per 2018 CLSI guidelines [17]. The sensitivity of the isolates to third generation cephalosporins (3GC), ceftazidime, cefotaxime, ceftriaxone, each 30 µg/disc and other antibiotics such as ciprofloxacin(5 µg), piperacillin-tazobactam (100/10µg), amikacine (30 µg), gentamycin (10 µg), meropenem (30 µg), ampicillin (10 ug), chloramphenicol (30), Trimethoprim-sulfamethoxazole (1.25/23.75), cefoxitin(30ug), cefotetan(30ug), Cefepime (30ug), cefuroxime (30ug), Amoxaciliin clavulanate (20/10ug), were determined by the zone of inhibition size

recommended by the 2018 CLSI guidelines [17]. The zone of inhibition was measured to the nearest millimeter and isolate was reported as sensitive, intermediate and resistant according to the CLSI standard tables for each antibiotic. To screen out ESBL production based on the 2018 CLSI guideline, isolates with cefotaxime, ceftazidime and ceftriaxone zones of inhibition less than 27mm, 22mm and 25mm respectively were suspected to be ESBL producers [17].

4.7. 5. Extended spectrum beta-lactamase detection

4.7.5.1 Chromogenic agar medium (CHROMID™ ESBL)

The test was performed for rapid screening of extended-spectrum beta lactamase-producing *Enterobacteriaceae* (ESBL) with selective chromogenic media. HiCrome ESBL supplement, containing antibiotics such as ceftazidime, cefotaxime, ceftriaxone, aztreonam and fluconazole, is used to inhibit other contaminating microorganisms and non-ESBL-producing bacteria. CHROMID® ESBL provides results in 18-24 hours, those colonies which produce ESBL were confirmed using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid [35].

4.7.5.2 .Combined disk test (CDT)

A confirmatory test was performed for the detection of ESBL by combination-disk test using both cefotaxime and ceftazidime, alone and in combination with clavulanic acid, according to the 2018 CLSI guidelines [17]. In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated by using sterile cotton swab on the surface of a Mueller Hinton Agar plate. The cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart on the agar. Similarly, the ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg / 10 µg) disks were placed 20 mm apart. After incubating overnight at 37°C, A≥5mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone was considered as ESBL producer [17].

4.7.6 Quality Control

All data quality control tools (pre-analytical, analytical and post analytical stages of quality assurance that are incorporated in standard operating procedures (SOPS) of CLSI 2018 [17] were followed strictly. Culture Media were prepared based on the manufactures instruction then the sterility of culture media was checked by incubating 5% of the batch at 35–37 °C overnight and

observing bacterial growth. Those culture Media which showed growth were discarded and antibiotic discs potency was also checked. *Klebsiella pneumoniae* ATCC 700603, (ESBL-positive) , *Escherichia coli* ATCC 25922 (ESBL negative), were used for the quality control of the ESBL testing methods and *Pseudomonas aeruginosa* ATCC 27853 was also used for the quality control of the Kirby-Bauer disk diffusion methods and antibiotic disc potency.

4.8 Data quality assurance

Before inoculation stored isolates were checked for its appropriate storage and cross matched with its labeling on the log book sheet. Muller Hinton agar was prepared and dispensed according to the 2018 CLSI guideline to avoid any false ESBL reports. The expiry date of the antibiotics was also checked. Before entry to statistical tool for analysis, results were recorded appropriately on the log book sheet and data was cleaned and checked for completion before analysis.

4.9. Data analysis and interpretation

The obtained data were analyzed using SPSS software (version 20), ESBL carriage with sex, age, ward type and source of swabs were descriptively analyzed. Finally the results were presented on words, charts, graphs and tables.

4.10 Operational definitions

I. β-lactams:- are a group of antibiotics acting on the cell wall of a bacterial cell. These include the penicillins, cephalosporins, carbapenems and monobactms.

II. Extended spectrum beta lactamase (ESBL):-are enzymes that mediate resistance to most beta-lactam antibiotics, including penicillins, cephalosporins, and the monobactam, aztreonam.

III. Multidrug Resistance bacteria (MDR):-area bacteria that develop resistance to at least one agent in three or more different antimicrobial class [36].

IV. Pooled sample: Those samples collected from different inanimate objects are mixed and processed together.

4.11 Ethical Consideration

Ethical approval was obtained for using stored isolates from the Department of Research and Ethical Review Committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. We used stored isolates from an on-going PhD research project (My supervisor's project), which has Institutional and National ethical approval. In the current project, we don't have any direct contact with the study participants and we were using only coded and anonymized stored isolates along with some demographic factors of the health care workers.

4.12 Dissemination of the results

After conducting the research, results will be presented to the Department of Medical Laboratory Sciences, College of health Sciences, Addis Ababa University and Tikur Anbessa Specialized Hospital. The manuscript will be submitted to journal/s for publication.

5 Work flow

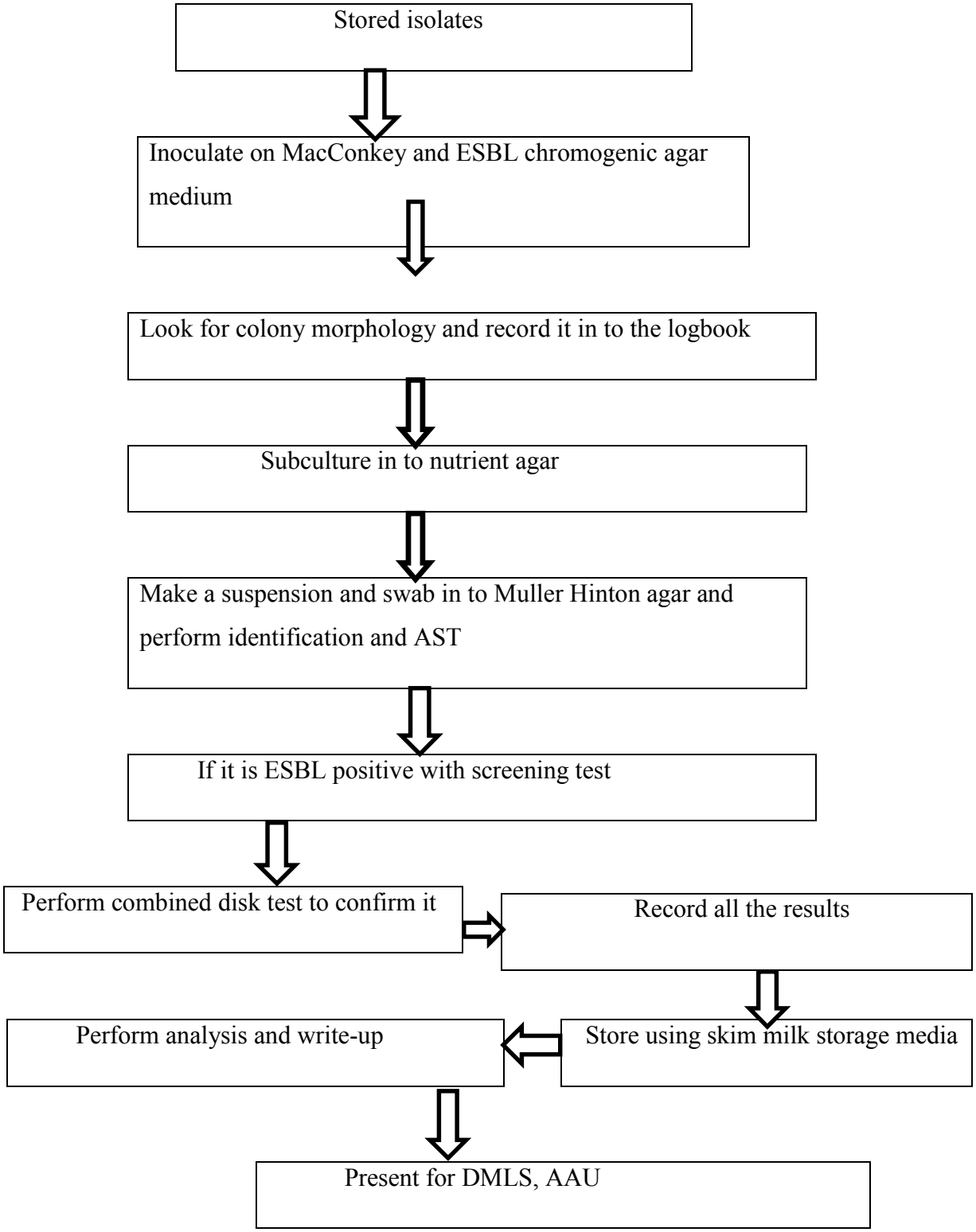

Figure 1:- Work flow

Table 2: List of biochemical tests [37]

Lactose	Indole	Urea	Manitol	TSI H2S	Gas Glu	Citrate	Motlity	LDC	Malonate	Example
+	-	+	+	+	+	+	+	-		*Citrobacter spp*
		+	+	-	+	+	-	+	+	*K.pnemonia*
	+	+	+	-	+	+/-	-	+/-	-	*k.ozenae*
		+	+	-	+	+	-	+		*K.oxytoca*
		-	+	-	+	-	+/-	+/-		*E.coli*
-	+	+	-	+	+	+/-	+	-		*P.vulgaras*
		+	+	-	+	+	+	-		*Providencia spp*
	-	- +		+	-	-	+	-		*P.mirabilis*

TSI Triple sugar iron agar, LDC lysine decarboxylase agar, Glu Glucose

6. Results

6.1. Nasal carriage of ESBL bacteria by health care workers

6.1.1. Socio demographic data

In this study a total of 105 gram negative bacterial isolates were identified and further tested for antibiotic susceptibility pattern. The isolates were mainly from samples collected from nurses 33(31.1%) followed by medical doctors 31(29.2%). However, other health care workers (HCW) are also involved as depicted in Table 3. Majority of the participants were females 60(56.6%) resulting male to female ratio of 1:5. More than 61% (n=64) of the participants were single. Majority (31.4%) of the participants have 1-2 year of work experience. The ages of HCWs vary between 20 and 57 years.

Table 3: Socio demographic factors of heath care workers at Tikur Anbessa Specialized Hospital from December 2018 to February 2019.

Year of service as a health care worker	**Sex**	**Marital status**	**Current professional category**					**Total N (%)**
			Medical doctor **n=31**	Nurse **n=33**	Medical laboratory personnel **n=21**	Pharmacy personnel **n=12**	Other (Rad, Phy, An) **n=8**	**N=105**
1-2 yrs n=33	Male n=12	Single	3(100.0)	4(80.0)	1(100.0)	0(0.0)	2(100.0)	10(30.3)
		Married	0(0.0)	1(20.0)	0(0.0)	1(100.0)	0(0.0)	2(6.1)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Female n=21	Single	3(75.0)	11(0.0)	2(66.7))	0(0.0)	3(100.0)	19(57.6)
		Married	1(25.0)	0(0.0	1(33.3))	0(0.0)	0(0.0)	2(6.1)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
3-4 yrs n=26	Male n=12	Single	5(71.4)	0(0.0)	0(0.0)	2(50.0)	1(100.0)	8(30.8)
		Married	2(28.6)	0(0.0)	0(0.0)	2(50.0)	0(0.0)	4(15.4)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Female n=14	Single	0(0.0)	4(6.7)	0(0.0)	1(100.0)	1(100.0)	9(34.6)
		Married	0(0.0)	2(33.3)	0(0.0)	0(0.0)	0(0.0)	5(19.2)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
5-7yrs	Male n=11	Single	4(80.0))	0(0.0)	3(0.0)	0(0.0	0(0.0)	7(29.2)
		Married	1(20.0)	1(100.0)	0(0.0)	2(100.0)	(0.0)	4(16.7)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

n=24	Female n=13	Single	1(25.0))	0(0.0)	2(66.7)	0(0.0)	0(0.0)	4(16.7)
		Married	3(75.0))	6(100.0)	1(33.3)	0(0.0)	0(0.0)	9(37.5)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
8-10 yrs n=6	Male n=4	Single	1(100.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	2(33.3)
		Married	0(0.0)	0(0.0)	1(100.0)	1(50.0)	0(0.0)	2(33.3)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Female n=2	Single	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
		Married	0(0.0)	1(100.0)	1(0.0)	0(0.0)	0(0.0)	2(33.3)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
>10 yrs n=16	Male n=6	Single	1(100.0)	0(0.0)	150.0)	0(0.0)	0(0.0)	2(6.25)
		Married	0(0.0)	1(100.0)	1(50.0)	2(100.0)	0(0.0)	4(25.0)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	Female n=10	Single	0(0.0)	1(50.0)	1(14.3))	1(100.0)	0(0.0)	3(18.75)
		Married	0(0.0)	1(50.0)	6(85.7)	0(0.0)	0(0.0)	7(43.75)
		Divorced	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

The magnitude of ESBL producing bacteria in females is higher than males [4(80%) vs 1(20%] (Table 4).

Table 4: Age groups and sex wise details of heath care workers with ESBL producing bacteria at Tikur Anbessa Specialized Hospital from December 2018 to February 2019.

Age group (years)[38]	**ESBL (N, %)**		
	Male	**Female**	**Total**
<30	0(0.0)	2(100.0)	2(100.0)
30-39	1(50.0)	1(50.0)	2(100.0)
40-49	0(0.0)	1(100.0)	1(100.0)
>50	0(0.0)	0(0.0)	0(0.0)
Total	1(20.0)	4(80.0)	5(4.8)

6.1.2. Magnitude of ESBL producing bacteria from nasal swabs of health care workers

The present study showed that out of 105 isolates a total of 5 (4.8%) isolates were found to be Extended Spectrum β-Lactamase (ESBLs) producers**.** Eighty percent (80%) of ESBLs were produced by *Klebsiella spp* (75%*K. rhinoscleromatis,*25% *K.pneumoniae*) and 20% were *Entrobactercloacae* (Table 5).

Table 5: Magnitude of Extended Spectrum β- -lactamase production for each isolated organism from health care workers at Tikur Anbessa Specialized Hospital from December 2018 to February 2019.

Bacterial isolates	ESBL Production		
	Positive n=5(N, %)	**Negative n=100 (N, %)**	**Total (N, %)**
Klebsiella_spp **N=77**	4(80)	73(73)	77(73.3)
Entrobacter_spp **n=9**	1(20.0)	9 (9.0)	10(9.5)
Proteus_spp **n=1**	0(0.0)	1(1.0)	1(0.95)
Citrobacter_spp **n=7**	0(0.0)	7(7.0)	7(6.7)
E.coli **n=4**	0(0.0)	4(4.0)	4(3.8)
Morganella morganii **n=1**	0(0.0)	1(1.0)	1(0.95)
Serriatia –spp **n=4**	0(0.0)	4(4.0)	4(3.8)
Acinetobacter –spp **n=2**	0(0.0)	1(1.0)	1(0.95)
Total	**5(4.8)**	**100(95.2)**	**105(100.0)**

Majority of ESBL producing bacterial isolates were isolated from samples taken from Laboratory personnel 3 (60%), followed by Medical doctors 2 (40%). However all the other health care workers (Nurse, Pharmacy personnel, Radiologist, Physiotherapist and Anesthetist), were not having ESBL producing bacteria (Table 6).

Table 6: Distribution of ESBL by professional category at Tikur Anbessa Specialized Hospital from December 2018 to February 2019

ESBL	Current professional category					
	Medical doctors	**Nurse**	**Medical laboratory personnel**	**Pharmacy personnel**	**Others (Rad, Phy An)**	**Total**
Positive (N, %)	2(40.8)	0(0.0)	3(60.0)	0(0.0)	0(0.0)	5(4.8))
Negative (N, %)	29(29.0)	33(33.0)	18(18.0)	12(12.0)	8(8.0)	100 (95.2)
Total (N, %)	31(29.5)	33(31.4)	21(20.0)	12(11.4)	8(7.6)	105(100)

ESBL, Extended Spectrum Beta-Lactamase, Rad, Radiologist, Phy, Physiotherapist, An, Anesthetist

Health care workers who served for more than 3 years and above seems to have the chance of having ESBL bacteria in their nasal cavity (Table 7).

Table 7: Distribution of ESBL with year of service as a health care worker at Tikur Anbessa Specialized Hospital from December 2018 to February 2019

Year of service as a heath care worker	Extended spectrum β-Lactamase		
	Positive (N, %)	**Negative (N, %)**	**Total (N, %))**
1-2 yrs	0(0.0)	33(33.0)	33(31.4)
3-4 yrs	2(40.0)	24(24.0)	26(24.8)
5-7 Yrs	0(0.0)	24(24.0)	24(22.9)
8-10yrs	0(0.0)	6(6.0)	6(5.7)
>10 yrs	3(60.0)	13(13.0)	16(15.2)
Total(N, %)	5(100.0)	100(100.0)	105(100.0)

6.1.3. Antimicrobial susceptibility patterns of ESBL producing gram negative bacteria

All isolates which were positive for ESBL were found to be 100% resistant to cefotaxime and ceftriaxone. *Klebsiella pneumonia and Klebsiella rhinoscleromatis* were 100% resistant to

ampicillin, cefotaxime, ceftriaxone and ceftazidme. *Entrobacter spp* were resistant to cefotaxime, ceftriaxone and cotrimoxazole (Table 8).

Table 8:- Antimicrobial profile of the different ESBL-producing species from health care workers at Tikur Anbessa Specialized Hospital from December 2018 to February 2019.

Bacterial isolates	Pattern	List of antibiotics								
		AMP (N, %)	AUG (N, %)	CFP (N, %)	CTX (N, %)	CTR (N, %)	CTT (N, %)	CXT (N, %)	CRX (N, %)	CAZ (N, %)
K. rhinoscleromatis	*S*	0(0.0)	2(66.7)	1(33.3)	0(0.0)	0(0.0)	1(33.3)	1(33.3)	1(33.3)	0(0.0)
n=3	**R**	3(100.0)	1(33.3)	2(66.7)	3(100.0)	3(100.0)	2(66.7)	2(66.7)	2(66.7)	3(100.0)
K.pneumoniae	**S**	0(0.0)	(10.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)
n=1	**R**	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0)
Entrobacter cloacae	**S**	1(100)	0(0.0)	0(0.0)	0(00.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)
n=1	**R**	0(0.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)

Bacterial isolates	Pattern	List of antibiotics							
		MEM (N, %)	GEN (N, %)	AMK (N, %)	CIP (N, %)	COT (N, %)	CHL (N, %)	PTZ (N, %)	TOB (N, %)
K. rhinoscleromatis	**S**	2(66.7)	3(100.0)	3(100.0)	3(100.0)	2(66.7)	3(100.0)	3(100.0)	3(100.0)
n=3	**R**	1(33.3)	-	0(0.0)	0(0.0)	1(33.3)	0(0.0)	0(0.0)	0(0.0)
K.pneumoniae	**S**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	0(0.0)
n=1	**R**	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)	-	1(100.0)
Entrobacter cloacae	**S**	0(0.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)
n=1	**R**	1(100.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)

AMP ampicillin, AUG amoxicillin with clavunic acid ,CFP cefpime, CTX cefotaxime, , CTR ceftriaxone,CTTcefotetan, CXT cefoxitin, CRX cefuroxime, CAZ ceftazidime , MEM meropenem, GEN gentamycin, AMK amikacine, CIP ciprofloxacin, COT sulfamethoxazole + trimethoprim (cotrimoxazole), CHL chloramphenicol, PTZ piperacillin-tazobactam, TOB tobramycin, S sensitive, , R resistancs. (-), susceptibility tests which do not have resistance or sensitive result

6.2. Isolates from environmental sample

6.2.1 Magnitude of gram negative bacteria in the hospital wards

A total of 216 gram negative bacteria were identified in the various ward area of TASH including from delivery room, intensive care unit, operation rooms, medical ward and Emergency departments. Among these *Klebsiella spp*, *Acinetobacter_spp* and *Citrobacter_spp* were the three most dominant gram negative bacteria accounting magnitude of 69 (31.9%), 38 (17.6%) and 26 (12.0%) respectively (Table 9).

Table 9: Magnitude of gram-negative bacterial contamination in different hospital wards at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia from December 2018 to February 2019.

Ward	**No. and types of gram negative bacteria**													**Number of contamination (%)**
	Klebsiella_spp	*Entrobacter_spp_*	*Proteus_spp*	*Providencia_spp*	*Citrobacter_spp*	*E.coli*	*Morganellamorganii*	*Shigella_spp*	*Salomnella_spp*	*Arizona*	*Serriatiaspp*	*Pseudomonas_spp*	*Acinetobacter_spp*	
Adult ICU	13	2	2	0	0	4	0	0	0	0	2	3	8	34 (15.7)
Neonatal ICU	26	2	4	0	8	2	0	0	1	2	2	0	9	56 (25.9)
Pediatrics ICU	3	0	2	0	5	0	1	1	0	1	0	0	0	13 (6.0)
Surgical ICU	0	0	1	0	0	0	1	0	0	0	0	0	0	2 (0.9)
Operation room	4	1	5	1	4	0	0	0	0	0	0	0	0	15 (6.9)
Medical ward	16	3	2	1	3	6	0	5	2	0	7	2	17	64 (29.6)
Delivery ward	2	1	0	0	5	0	0	1	1	0	3	0	2	15(6.9)
Emergency	3	0	1	0	0	1	0	1	1	0	0	0	0	7(3.2)
Hand rail	2	0	1	1	1	0	0	0	0	0	1	0	2	8(3.7)
Reception	0	0	1	0	0	1	0	0	0	0	0	0	0	2(0.9)
Total	69	9	19	3	26	14	2	8	5	3	15	5	38	216(100.0)

6.2.2. The magnitude of ESBL producing gram negative bacteria in the hospital environment

A total of 33 (15.3%) isolates were found to be Extended Spectrum β--Lactamases (ESBLs) producers in accordance with the confirmatory test (combined disk method). Figure 2 depicted atypical example from this study.

Figure 2:-Enhancement zones of inhibition by >5mm of discs containing ceftazidime+clavulanic acid and cefotaxime+clavulanicacid,compared with ceftazidime and cefotaxime alone.

The predominant ESBL producing species were *Klebsiella ozaenae* accounting24.2% (n=8) followed by *Acinetobacter-spp* 21.2% (n=7) and *Escherichia coli* 18.2% (n=6) (Table 10).

Table10: Profile of bacterial isolates which produce ESBL at Tikur Anbessa Specialized Hospital at Addis Ababa, Ethiopia, from December 2018 to February 2019.

	Bacterial isolates								
	Klebsiella_spp (N=69)				***Citrobacter _spp n=26***	***E.coli _spp n=14***	***Serriatia _spp n=15***	***Acinetobacter _spp n=38***	**Total**
ESBL (N, %)	***K.ozaenae n=21***	***K. rhinoscleromatis n=18***	***K.pneumoniae n=20***	***K.oxytoca n=10***					
Positive n=33	8 (24.2)	2(6.1)	3(9.1)	4(12.1)	1(3.0)	6(18.2)	2(6.1)	7(21.2)	33(15.3)
Negative n=183	13 (7.1)	16(8.7)	17(9.3)	6(3.3)	25(13.7)	8(4.4)	13(7.1)	31(16.9)	183(84.7)

According to these study among *Klebsiella spp*, ESBL production were very common among *Klebsiella ozaenae and Klebsiella oxytoca*, from 21 isolates, 8 (24.2%) and 10 isolates, 4(12.1%) were ESBL producers respectively. However, within *Klebsiella rhinoscleromatis* isolates, only 2out of 18 isolates were ESBL-producers, representing only 6.1% (Table 10).

Among the examined equipment and surfaces, the highest number of ESBL producing gram negative bacteria were found from chairs (15.2%), pooled sample of computer, office telephone hand and chairs (15.2%), sinks (12.1%), tables (9.1%), information desk (9.1%), desktop computer(9.1%) and less than 7% were found from monitor, incubator and bed. However no gram negative bacteria were isolated from oxygen regulator, door, lift button and air samples (Table 11).

Table 11:- Profile of ESBL producing gram negative bacteria with different source of sample with in the hospital environment at Tikur Anbessa Specialized Hospital, at Addis Ababa, Ethiopia, from December 2018 to February 2019.

	Source of sample and no. of isolates																
ESBL Results	Tables(**n=19**)	Sinks(**n=30**)	Monitors(**n=7**)	Incubators(**n=28**)	Beds(**n=14**)	Trollies (**n=8**)	Oxygen regulators(**n=2**)	Computers(**n=12**)	Door (**n=5**)	Chairs(**n=31**)	Pooled sample(**n=26**)	Hand rail (**n=8**)	Suction machine (**n=7**)	Lift button (**n=4**)	Information desk (**n=10**)	Air sample (**n=5**)	**Total**(%)(N=**216**)
Positive (%)	9.1	12.1	6.1	6.1	6.1	6.1	0.0	9.1	0.0	15.2	15.2	3.0	3.0	0.0	9.1	0.0	15.3
Negative (%)	8.7	14.2	2.7	14.2	6.6	3.3	1.1	4.9	2.7	14.2	11.5	3.8	3.3	2.2	3.8	2.7	84.7
Total (%)	8.8	13.9	3.2	13.0	6.5	3.7	0.9	5.6	2.3	14.4	12.0	3.7	3.2	1.9	4.6	2.3	100

6.2.3. ESBL producing gram negative bacteria from environmental samples stratified by ward types

Out of the total 216 isolates, 33(15.3%) of them were ESBL producing gram negative bacteria. Compared to other hospital wards the highest ESBL producing bacteria were isolated from medical wards and adult ICU whichaccounts16 (48.5%) and 7 (21.2%) respectively(table 12).

Table12: Magnitudeof ESBL producing gram negative bacteria from environmental samples stratified by ward specialty at Tikur Anbessa Specialized Hospital, Addis Ababa Ethiopia from December 2018 to February2019.

Ward specialty	ESBL producing gram negative bacteria			
	Positive		Negative	
	N	%	N	%
Adult ICU	7	21.2%	27	14.8%
Neonatal ICU	5	15.2%	51	27.9%
Pediatrics ICU	0	0.0%	13	7.1%
Surgical ICU	0	0.0%	2	1.1%
Operation room	0	0.0%	15	8.2%
Medical ward	16	48.5%	48	26.2%
Delivery ward	2	6.1%	13	7.1%
Emergency	2	6.1%	5	2.7%
Hand rail	1	3.0%	7	3.8%
Reception	0	0.0%	2	1.1%

6.2.4. Antimicrobial susceptibility patterns of ESBL producing gram negative bacteria from the hospital environment

Susceptibility test for 216 gram negative bacterial isolates from various environmental samples and medical equipment for selected antimicrobial agents was performed. In this study all ESBL producing gram negative bacteria were found to be 100% resistant to Ceftazidime and ceftriaxone.

ESBL producing *Klebsiella ozaenae* has a resistant rate of 25.0% to meropenem and 37.5% to amikacin. The most active antibiotics against *Klebsiella pneumonia* was amikacin, by having 100% susceptibility. About 71.4 % of *Acinetobacter _spp* was resistant to cefotaxime and 100% were susceptible to amikacin and meropinem (Table 13).

Table13:-Antimicrobial susceptibility pattern of ESBL producing *Enterobacteriaceae and Acinetobacter_spp* isolated from the hospital environment at Tikur Anbessa Specialized Hospital at Addis Ababa, Ethiopia from December 2018 to February 2019.

Bacterial isolates	**Pattern**	**List of antibiotics**								
		AMP (N, %)	**AUG (N, %)**	**CFP (N, %)**	**CTX (N, %)**	**CTR (N, %)**	**CTT (N, %)**	**CXT (N, %)**	**CRX (N, %)**	**CAZ (N, %)**
K.ozaenae(n=8)	**S**	1(12.5)	1(12.5)	2(25.0)	1(12.5)	0(0.0)	3(37.5)	5(62.5)	1(12.5)	0(0.0)
	R	7(87.5)	7(87.5)	6(75.5)	7(87.5)	8(100)	5(62.5)	3(37.5)	7(87.5)	8(100)
K.pneumoniae(	**S**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(66.7)	1(33.3)	1(33.3)	0(0.0)
n=3)	**R**	3(100)	3(100)	3(100)	3(100)	3(100)	1(33.0)	2(66.7)	2(66.7)	3(100)
K.oxytoca(n=4)	**S**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(50.0)	3(75.1)	0(0.0)	0(0.0)
	R	4(100)	4(100)	4(100)	4(100)	4(100)	2(50.0)	1(25.0)	4(100)	4(100)
K.rhinoscleromatis (n=2)	**S**	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)	1(50.0)	1(50.0)	0(0.0)
	R	2(100)	2(100)	2(100)	1(50.0)	2(100)	2(100)	1(50.0)	1(50.0)	2(100)
Citrobacter_Spp(n=1)	**S**	0(0.0)	1(100)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)
	R	1(100)	0(0.0)	0(0.0)	1(100)	1(0.0)	1(100)	0(0.0)	1(100)	1(100)
Escherichia coli(n=6)	**S**	1(16.7)	2(33.3)	2(33.3)	1(16.7)	0(0.0)	4(66.7)	4(66.7)	2(33.3)	0(0.0)
	R	5(83.3)	4(66.7)	4(66.7)	5(83.3)	6(100)	2(33.3)	2(33.3)	4(66.7)	6(100)
Serraitia_ spp(n=2)	**S**	-	1(50.0)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
	R	2(100)	1(50.0)	1(50.0)	2(0.0)	2(0.0)	2(100)	2(100)	2(100)	2(100)
Acinetobacter_spp n=7	**S**			6(85.7)	2(28.6)	0(0.0)				7(100)
	R			1(14.3)	5(71.4)	7(100)				0(0.0)

Bacterial isolates	**List of antibiotics**							
	Pattern	**MEM (N, %)**	**GEN (N, %)**	**AMK (N, %)**	**CIP (N, %)**	**COT (N, %)**	**CHL (N, %)**	**PTZ (N, %)**	**TOB (N, %)**
*K.ozaenae (*n=8)	**S**	6(75.0)	2(25.0)	5(62.5)	4(50.0)	2(25.0)	5(62.5)	3(37.5)	2(25.0)
	R	2(25.0)	6(75.0)	3(37.5)	4(50.0)	6(75.0)	3(37.5)	5(62.5)	6(75.0)
K.pneumoniae	**S**	1(33.3)	2(66.7)	3(100)	2(66.7)	0(0.0)	2(66.7)	1(33.3)	1(33.3)
*(*n=3)	**R**	2(66.7)	1(33.3)	0(0.0)	1(33.3)	3(100)	1(33.3)	2(66.7)	2(66.7)
*K.oxytoca(*n=4)	**S**	4(100)	1(25.0)	2(50.0)	2(50.0)	0(0.0)	2(50.0)	1(25.0)	0(0.0)
	R	0(0.0)	3(75.0)	2(50.0)	2(50.0)	4(100)	2(50.0)	3(75.0)	4(100)
K.rhinoscleromatis	**S**	1(50.0)	1(50.0)	1(50.0)	1(50.0)	0(0.0)	2(100)	2(100)	1(50.0)
*(*n=2)	**R**	1(50.0)	1(50.0)	1(50.0)	1(50.0)	2(100)	0(0.0)	0(0.0)	1(50.0)
Citrobacter_spp	**S**	1(100)	1(100)	1(100)	1(100)	0(0.0)	1(100)	1(100)	1(100)
(n=1)	**R**	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)	0(0.0)	0(0.0)
Escherichia-	**S**	6(100)	6(100)	6(100)	2(33.3)	4(66.7)	5(83.3)	3(50.0)	3(50.0)
coli(n=6)	**R**	-	0(0.0)	-	4(66.7)	2(33.3)	1(16.7)	3(50.0)	3(50.0)
Serraitia_spp(n=2)	**S**	1(50.0)	1(50.0)	2(100)	2(100)	1(50.0)	0(0.0)	0(0.0)	2(100)
	R	1(50.0)	1(50.0)	-	0(0.0)	1(50.0)	2(100)	2(100)	-
Acinetobacter_spp	**S**	7(100)	6(85.7)	7(100)	5(71.4)	4(57.1)		5(71.4)	7(100)
(n=7)	**R**	0(0.0)	1(14.3)	0(0.0)	2(28.6)	3(42.9)		2(28.6)	0(0.0)

AMP ampicillin, AUG amoxicillin with clavunic acid ,CFP cefpime, CTX cefotaxime, , CTR ceftriaxone,CTTcefotetan, CXT cefoxitin, CRX cefuroxime, CAZ ceftazidime , MEM meropenem, GEN gentamycin, AMK amikacine, CIP ciprofoxacin, COT sulfamethoxazole + trimethoprim (cotrimoxazole), CHL chloramphenicol, PTZ piperacillintazobactam, TOB tobramycin, S sensitive, , R resistance

7. Discussion

Health care associated infections are a major problem to all heath care systems worldwide. Health care workers (HCWs) are the major sources of MDR bacteria like methicillin resistant *Staphylococcus aureus* (MRSA) and extended spectrum β- lactamase producing bacteria (ESBLs) [23,28]. Although in healthy individuals nasal carriage is harmless, these carriers can be a risk for transmission of organisms to the community and other health care workers.

Out of 105 isolates of HCWs, 5 (4.8%) were ESBL producers and the highest rate of ESBL producing bacteria were from laboratory personnel 3 (60%).A study done by Maheshwarri *et al*, India (2014), also found that different nasal carriage of MDR organisms among different health professionals but maximum rate of isolation for ESBL was seen among nurses and minimum among the laboratory technicians in contrary to our study[23]. This can be because of the more frequent and close contact of laboratory personnel with different types of patient specimens as compared to other health care staffs. Moreover, the working condition may be different from ours.

In the present study only 4.8%of the total gram negative isolates were ESBL producers whereas higher rate of ESBL is reported by Maheshwarri *et al* (2014), 21.4%, Gonsu *et al* (2013), 22% rate of ESBL producers[23, 28]. On the other hand our finding is quite higher than a finding of 0.3 % by Micheel *et al.,*(2015) in Madagascar, 0% Kock *et al.,(*2016) in German [30,39].

In our study 80% of ESBLs were produced by *Klebsiella spp* (75% *Klebsiella rhinoscleromatis,* 25% *Klebsiella pneumoniae*) and 20% were by *Entrobacter cloacae.* Other studies also reported the same, Gonsu *et al,* Camroon*(2013),*Micheel *et al* ,Madagascar(2015)[28,30]. All ESBL positive isolates from HCWs were found to be 100% resistant to cefotaxime and ceftriaxone which is in line with a study by Gonsu *et al* who reported a resistance rate of cefotaxime (100%) and ceftriaxone (90 %) [28].

The occurrence of bacterial pathogens in hospital environment is associated with an increasing incidence of nosocomial infections. Medical equipments and open surfaces are suitable for colonization of microorganism and gram negative bacteria are the most common cause of hospital and community acquired infection [20].

In this study the prevalence of Extended Spectrum β-Lactamase in the hospital environment is 15.3% and the predominant isolates were *Klebsiella ozaenae* (24.2%) and *E.coli* (18.2%) which indicates an alarming level of contamination which is in line with a previous study finding in Ethiopia, (14.8%) [20], but lower than those studies conducted in Uganda(21.33%), France (31%), and upper Egypt (35.2%) [6, 16, 32].These variations might be due to the number of patients that attended each hospital, disease exposure and geographic differences among the study areas.

According to this study, medical wards (48.5%) had the highest and delivery ward (6.1%) had the lowest rate of ESBL contamination level which contradicts with other studies. In 2017, a study conducted by Ayatollahl *et al,* reported the intensive care unit (ICU)had the highest infection rate (33.1%) [25].The variations may be due to a number of factors including the ward conditions and performance of the HCWs. However, the results of our study indicated higher level of contamination in the intensive care unit (ICU) and medical ward compared with the delivery ward, which further highlights the need to develop programs for prevention of infections in these wards. Nevertheless, infections in the delivery ward should not be neglected, since this is also more susceptible to nosocomial infections especially for neonates in post-natal unit.

The highest ESBL contamination levels in inanimate objects were observed from chair (15.2%), pooled samples (15.2%) and Sink (12.1%).It is generally assumed that gram negative micro-organisms require moist or damp sites for enhanced longevity. Recent reports suggest that, *E. coli* and *Klebsiella*-spp. may survive more than a year in dry surroundings [2, 3].In Manel *et al* study, incubators (20.45%), floor areas (17.86%), sink (9.28%) had the highest incidents of contamination [29]. These differences may be due to differences in the type of health care activities and cleaning practices in the hospital.

In our study the common ESBL producing strains from the hospital environments were *Klebsiella spp* (24.6%),*Acinitobacter spp*(21.2%),*E.coli* (18.2%) .Similarly a study conducted in Algeria reported that *K. pneumoniae* (28.45 %), *E. coli* (25.41%), *S. marcescens*(19.15%), *K. oxytoca*(17.31%),*C. freundii*(16%) as ESBL producing strain [29].This results are consistent with the findings by Roux *et al*, *Klebsiella*(55.0%) with *pneumoniae*(87.8%)and K. *oxytoca*(12.2%) and by Affifi *et al* ,in upper Egypt reported that prevalence of 56.25% with a predominance of *K. pneumoniae* (45/80), followed by *E. coli* prevalence of 43.75% with a predominance of (35/80)[16,32]. The percentage of *Klebsiella pneumoniae* from environmental samples in this study was 9.1% which is relatively high compared with similar study in Egypt (2%) [31].

The resistance rate was higher among ESBL producers than non ESBL producers and all isolates of ESBL producing bacteria were 100% resistant to ceftazidime and ceftriaxone and this result is in line with a study by Engda *et al* in Ethiopia, however lower results were reported from Algeria, ceftazidime (37.83%), ceftriaxone (38.62%) and Upper Egypt, *Klebsiella pneumoniae* (95.5%) for cefotaxime and *Escherichia coli* (91.4%) for cefotaxime [29, 32].

The resistance level of ESBL producing bacteria for non β-lactam antibiotics are lower than to β-lactam antibiotics. Of all antimicrobials agent tested the carbapenems (meropenem) and amikacin had the highest activity against the ESBL producing organisms followed by gentamicin which is in line with other studies [39, 20]. In our finding, *Klebsiella Pneumoniae* was 33.3% resistant to gentamicin and ciprofloxacin. According to a study by Afifi *et al*, 2013 a higher resistance rate to gentamicin (84.4%) and ciprofloxacin (77.7%) were reported. *E.coli* was found to be one of the common ESBL producers and was 66.7% resistant to ciprofloxacin and amoxicillin clavulante. However it was 100 % sensitive for meropenem, gentamicin and amikacine. In contrary to our study, Kader *et al* found a lower resistance rate of ciprofloxacin (54%) and susceptibility of meropenem (93%) and amikacine (72.8%) [32, 40].

8. Conclusion and recommendation

8.1 Conclusion

The study showed a considerable contamination of hospital environments with ESBL producing gram negative bacteria. Similarly the nasal cavities of health care workers are found to be a carrier for these bacteria with high antibiotic resistance.

8.2 Recommendation

The finding of this study emphasizes the need

- ✓ For continuous surveillance in the hospital environment to detect the resistant strains
- ✓ Implementation of infection control measures to reduce the increasing burden of antibiotic resistance.
- ✓ It is recommended that along with conventional susceptibility test routine ESBL test could be useful.
- ✓ Performing molecular characterization is helpful to look the genomic pattern of the ESBL enzymes in the hospital environments.

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9. References

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10. Annexes

Annex I: Bacteriological culture method

Purpose: The purpose of using cultural techniques in microbiology is to diagnose and identify the presence of organisms which may by causing disease, and also to test the antimicrobial susceptibility of those isolated pathogens.

Procedure

1. Weigh the medium accurately using balance, tightly caped the bottle as soon as possible after removing and seal the cap of the container with adhesive tape.
2. Storing media in the coolest driest place available and almost out of sun light, once the ingredients are weighed, do not delay in making up the medium. Follow manufacturer' instruction for each media.
3. Use clean glassware, plastic or stainless steel equipment, 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.
4. Add the powder ingredients to distilled water and mix by rotating or stirring the flask do not shake a medium.
5. Stir while heating if heating is required to dissolve the medium and control the heat to prevent boiling which can be damage the medium.
6. Autoclave the medium when the ingredients are completely dissolved. Always autoclave at the correct temperature and for the time specified.
7. If we are interested to prepare tube medium, first dispense unsterilized media in to unsterilized tubes and sterilize it using autoclave.

Dispensing sterile media, in to sterile petri dish

1. Lay out the sterile Petri dishes on a level surface.
2. Mix the medium gently by rotating the flask or bottle. Flames sterilize the neck of flask or bottle and Pour 15-20 ml of medium in to each Petri dish (90-100mm diameter).
3. When the medium has gelled and cooled, stack the plates and seal them in plastic bags to prevent loss of moisture and reduce risk of contamination, store the plates.

Annex II: Laboratory procedure for biochemical testing of gram negative bacteria.

Purpose: Biochemical tests are used to differentiate different organisms based on their genus and species characteristics using a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with a nutrient broth. 3-4 colony of the test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to triple sugar iron agar, Citrate agar, lysine decarboxylase agar, manitol, urea agar, Malonate, Oxidase and motility medium.
3. Incubate at 37°C for 18-24 hours.
4. Look for color change (turbidity for motility test) of the medium.
5. Identify the test organism based on the result of all those tests.

Annex III: Laboratory procedure for antimicrobial sensitivity testing.

Procedure

1. Emulsify several colonies of similar appearance of test organism in small volume of nutrient broth**.**
2. Match the turbidity of the suspension against the turbidity standard.
3. With a sterile swab take sample from the suspension (squeeze the swab against the side of the tube to remove the excess fluid)
4. Swab the surface of the susceptibility testing agar. The plate is swabbed in three directions, rotating the plate approximately 60° to ensure even distribution.
5. Using a similar inoculation technique, inoculate an overnight broth culture of the control organism evenly across the upper and lower third of the plate.
6. Using a sterile forceps or needle, place the antimicrobial disc on the inoculated plate.
7. Inoculate the plate aerobically at 35-37°C for 18-24hours
8. Read the tests after checking that the bacterial growth of the test and control organism is neither too heavy nor too light.
9. Measure the radius of the inhibition zone; interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard.

Annex IV: Budget Justification

Table 14: Budget break down

I: Materials and reagent costs used in the study

Items	Unit	Unit size	Unit prize	Required amount	Total price
Culture medium					
MacConkey agar	Bottle	500g	1930	3by 500g	5790
Chromogenic agar medium	Bottle	500g	2500	3 by 500	7500
Muller Hinton agar	Bottle	500g	1850	3by 500g	5790
Nutrient agar	Bottle	500g	1890	2by 500g	3780
Skim milk storage media	Bottle	500g	2000	1by500	2000
Biochemical tests					
Urea (urease test)	Kg	500g	100	1by 500g	100
Urea 40% supplement	Bottle	5ml	250	10by 5ml	2500
Kovacs reagent(Indole)	Bottle	500g	650	1by 500g	650
Triple sugar iron agar(TSI)	Bottle	500g	1430	1by 500g	1430
Motility media	Bottle	500g	2620	1by 500g	2620
Simmons citrate	Bottle	500g	1700	1by500g	1700
LDC	Bottle	500g	1850	1by 500g	1850
Malonate	Bottle	500g	1750	1 by 500g	1750
Manitol	Bottle	500g	1800	1by500g	1800
Antimicrobial tests and antibiotic discs					
GN-Gentamicin	Ug	10	390	9 cartridge pack	3,510
CIP- Ciprofloxacin	Ug	5	390	9 Cartridge pack	3,510
AMP-Ampicillin	Ug	10	390	9 cartridge pack	3510
CAZ- Ceftazidime	Ug	30	400	9 cartridge pack	2,000

CHL- Chloramphenicol	Ug	30	390	9 cartridge pack	3,510
MEM- Meropenm	Ug	10	350	9 cartridge pack	3,150
FOX-Cefoxitin	Ug	30	390	9 cartridge pack	3,510
CTT-Cefotetan	Ug	30	390	9 cartridge pack	3510
PTZ- Piperacilin- tazobactam	Ug	100/10	390	9 cartridge pack	3,510
TOB- Tobramycin	Ug	30	350	9 cartridge pack	3,150
AMK- Amikacine	Ug	30	395	9 cartridge pack	3,555
CFP –Cefepime	Ug	30	375	9 cartridge pack	3,375
CRX- Cefuroxime	Ug	30	375	9 cartridge pack	3,375
COT-Trimethoprim-sulfamethoxazole	Ug	1.25/23.75	390	9 cartridge pack	3510
AMS-Ampicillin sulbactam	Ug	50	380	9 cartridge pack	3,420
AUG-Amoxicillin-clavulante	Ug	20/10	350	9 cartridge pack	3150
CTX –Cefotaxime	Ug	30	350	9 cartridge pack	3150
Subtotal 1					**95,665**

II: Stationary materials cost used in the study

Item	Unit	Unit size	Unit Price(Birr)	Amount required	Total cost (Birr)
Printing	Page	1	1.50	600	900
Copy	Page	1	1.00	300	300
Pen	Pieces	1	7.00	5	35
Pencil	Pieces	1	2.00	2	4
Parker	Pieces	1	20	6	120
RW CD	Pieces	1	25	6	150
Subtotal 2					**1,509**

III: Personnel cost

Category	Total price(Birr)
Transportation	3000.00
Assistant lab technical	2000.00
Subtotal 3	**5000.00**

IV: Total budget

- Total budget= Subtotal-1+Subtotal-2+Subtotal-3

95,665+1509+5000= 102,174

10% Contingency=10,217

Grand total= 112,391

V: Source of found: This project was supported from the funds available from the PhD project and AAU.

Assurance of principal investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of progress reports for all stakeholders of the research project.

Asegedech Asmamaw (PI)

Signature: _____ Date: _____

Note: If you have any question about this study, you should feel free to ask now or anytime throughout the study:

PI Address: Asegedech Asmamaw: Department of Medical Laboratory Sciences, Collage of Health Sciences, Addis Ababa, Ethiopia.

E-mail:asegeyesewzer@gmail.com Tell: 0923596235

Annex V: Declaration

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

M.Sc. candidate: Asegedech Asmamaw (BSc)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors:

Kassu Desta (BSc, MSc, PhD candidate, Associate professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Dr. Yimtubeznash Woldeamanuel (MD, MSc, PhD, Associate professor)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.