ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Multidrug-resistant bacterial profiles of inanimate objects at Zewditu Memorial Hospital, Addis Ababa, Ethiopia

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

February, 2025

Addis Ababa, Ethiopia

Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

This is to certify that the thesis prepared by Hiwot Lidet Yosef, entitled: **Multidrug-resistant bacterial profiles of inanimate objects at Zewditu Memorial 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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Addis Ababa University

College of Health Sciences

Department of Medical Laboratory Sciences

MULTIDRUG-RESISTANT BACTERIAL PROFILES OF INANIMATE OBJECTS AT ZEWDITU MEMORIAL HOSPITAL, ADDIS ABABA, ETHIOPIA.

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Acknowledgments

I would like to thank the Addis Ababa University Female Scholarship Program for accepting me into this program and the Department of Medical Laboratory Sciences for the opportunity to conduct this research.

My deepest gratitude also extends to Zewditu Memorial Hospital staff and administrators. I am grateful to Tikur Anbessa specialized teaching Hospital microbiology laboratory staff for their technical support and encouragement and allowed me to use available facilities. I would like to give my deepest gratitude to my advisors Dr. Melese Hailu and Mrs**.** Meron Yohannes for their continuous support, constructive feedback, and priceless advices.

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Abbreviations/Acronyms

- CDC----------Centers for Disease Control and Prevention
- CLSI----------Clinical Laboratory Standard Institute
- CONS----------Coagulase-Negative *Staphylococcus*
- ESBL----------Extended Spectrum B-Lactamase
- GNB----------Gram-Negative bacteria
- GPB----------Gram-Positive bacteria
- HCWs----------Healthcare workers
- HCAI----------Health care-associated infection
- HAI----------Hospital Acquired Infection
- ISO----------International Organization for Standardization
- MAC----------MacConkey
- MRSA----------Methicillin Resistant *Staphylococcus aureus*
- MDR----------Multi Drug Resistance
- MHA----------Mueller Hinton agar
- ICUs----------Intensive Care Units
- NIs----------Nosocomial Infections
- OR----------Operation Room
- SPSS----------Statistical Package for Social Science
- TASH----------Tikur Anbessa Specialized Hospital
- WHO----------World Health Organization

ABSTRACT

Introduction: Nosocomial pathogens cause serious nosocomial infections and are acquired in healthcare settings within a few days of patient admission. The transfer of bacteria from inanimate surfaces to patients is a major factor in the spread of HAIs.

Methods: A cross-sectional investigation was done at Zewditu Memorial Hospital from June to November 2024. A total of 204 inanimate objects situated at operational rooms and intensive care units were swabbed. All of the specimens were cultivated onto MacConkey as well as blood agar. Each positive sample was evaluated using colony structure, gram-staining plus biochemical assays. Susceptibility to antimicrobial agents test was done by Kirby–Bauer disk diffusion technique. ESBL producers were confirmed by the Double disc Synergy and combination disc test while carbapenemase producers was checked using the Modified Carbapenem inactivation method. MRSA was identified using the Cefoxitin Disk Diffusion Test. All tests were done according to the guidelines of CLSI 34th edition 2024.

Result: Out of the 204 swabbed samples, 77.45% (n=158/204) showed bacterial growth with an overall count of 171 bacteria isolates. Among them Gram-positive bacterial agent were 71.3% (n=122/171) and Gram negative bacteria were 28.6% (n=49/171). *CoNS* 46.1% (n=79/171) and *Bacillus* spp. 21.6% (n=37/171) were the most prevalent isolates identified. Out of the total isolates 55 were identified as pathogenic based on their ability to cause disease and selected for antibiotic resistance testing. Gram negative bacteria showed high resistance to ampicillin (67.3%), amoxicillin and clavulanic acid (61.2%), ciprofloxacin (63.2%), sulfamethoxazole-trimethoprim (63.2%), cefepime (57.1%), and piperacillin-tazobactam (55.1%). Similarly gram positive bacteria showed high resistance to azithromycin (100%), penicillin (100%), clindamycin (100%), and erythromycin (100%). Multidrug resistance was observed in 61.8% (34/55) of the isolates. The total incidence of ESBL as well as carbapenemase-producer bacteria from the suspected isolates was 26.5% (n=13/49) and 12.2% (n=6/49) respectively while the prevalence of MRSA was 50% (n=3/6).

Conclusion: The study reveals a significant presence of multidrug-resistant bacteria in hospital settings and inanimate objects, highlighting the need for effective infection precautions and control strategies.

Keywords: Multidrug-resistant, Operational Rooms, Intensive Care Units, Inanimate Environments, Nosocomial Infections.

1. Background

1.1. Introduction

Nosocomial pathogens are microorganisms that cause hospital-acquired infections (HAIs) and are obtained in healthcare settings (1). Healthcare-associated infections pose a significant risk to both the patient's and the public's health, which is linked with higher rates of morbidity, extended hospital stays, and death (2). Poor infection prevention and hygienic practice, inadequate equipment sterilization, and the advent of resistant bacterial strains are all contributing factors to the spread of hospital-acquired infections (HAIs) (3). Contaminants from inanimate objects and surrounding surfaces in intensive care units (ICUs) and operation rooms (ORs) lead to increased patient mortality and morbidity because patients found in these wards are more prone to infections (2).

The ability of bacteria to successfully spread to humans from inanimate objects depends on several factors, such as the type of microorganism, the size of the inoculum, the environment's temperature and humidity, the object's porosity, the existence of organic matter, the microorganism's ability to form biofilms, and the prevalence of infection control procedures (4). Bacteria, both gram-positive and gram-negative, have been isolated from inanimate surfaces. They can endure for several months on arid surfaces, but their longevity increases in humid and colder environments (2).

Bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Proteus* spp., *Pseudomonas aeruginosa*, and *Enterococcus* spp., are major cause of healthcare-acquired infections. These pathogens have been widely reported in hospital settings across the globe as potentially deadly bacteria (5, 6).

Gram-negative bacteria (GNB) are a serious worldwide health concern, Because they produce beta-lactamase and carbapenemase, which contribute to antimicrobial resistance (7). Bacteria that produce the extended-spectrum beta-lactamase (ESBL) enzyme are responsible for more than 19% of nosocomial infections (8). Penicillin, monobactam, and third-generation cephalosporins can all be hydrolyzed by the ESBL enzyme, whereas carbapenemase and

metallo-beta-lactamase can break down medications called carbapenems (9). In most studies common bacteria that produce extended-spectrum β-lactamases (ESBLs) and carbapenemases in nosocomial infections are *Escherichia coli*, *Klebsiella pneumonia*, *P. aeruginosa* and *E. cloacae*.

The emergence of novel beta-lactamases among gram-negative bacteria that are able to break down cephalosporins and carbapenems is a serious cause for concern. Such organism infections raise treatment costs, hospital stays, morbidity, and mortality rates (10).

Penicillin-resistant *pneumococci,* MRSA as well as resistant to vancomycin *Staphylococcus aureus* are the most prevalent drug-resistant gram-positive bacteria that are appearing in healthcare settings (5). Another bacterium connected to healthcare-associated infections (HAIs) and environmental contamination is *Acinetobacter baumannii* (11).

The most frequent hospital acquired infections (HAIs) obtained from intensive care units (ICUs) and operation rooms (ORs) include bloodstream, urinary tract, surgical site, and respiratory infections (3). The absence of proper cleanliness, routine, supplementary infection prevention measures, control procedures, and safe medical surroundings all increase this risk (12). The primary focus of hospital design and hygiene measures has been to manage nosocomial pathogens and resistant strains that can contaminate surfaces, hands, equipment, and air. A deeper comprehension of the mechanisms of bacterial contamination can serve as the foundation for the creation of evidence-based prevention strategies (13).

Particularly when considering underdeveloped nations, there is a dearth of information regarding the degree and kind of contamination as well as the microbiological profile of frequently utilized medical instrument as well as inanimate surfaces in hospitals. Therefore continuous research should be done on this area to prevent the problem.

1.2. Statement of the problem

Healthcare-acquired infections obtained in intensive care units (ICUs) and operation rooms (ORs) are important because they have the potential to significantly affect morbidity and mortality rates, economic ramifications, lengthens hospital stays, raises healthcare costs, promotes the growth of bacteria resistant to numerous antibiotics, and decreases the likelihood that other patients will receive treatment (14).

Nosocomial infections can range in severity from moderate to severe and have an incidence of 5–10% with a prevalence rate to be between 3.0 and 20.7%. Hospital infection management techniques can prevent 33 percent of nosocomial infections (15). One of the main causes of severe sepsis and septic shock is hospital-acquired bloodstream infection, which carries a high risk of morbidity, mortality, and treatment expenses (16). Research has demonstrated that the incidence of nosocomial infections that occur at intensive care units (ICUs) could reach up to 15% of patients (17).

Hospital locations have shown to be favorable in spreading infections because of the appropriate pathogen-host environment association that currently exists. Nosocomial infections encountered in intensive care units (ICUs) and operation rooms (ORs) represent a problem of immense worldwide proportion (14). Approximations from different nations indicate that a considerable proportion of hospitalized patients at any one time get infections that weren't present or incubating at the time of admission (18). However, in hospital environments, contaminated surfaces which aren't always the best for microbial survival and growth may still contribute to the spread of infection because surfaces near patients' surroundings are frequently touched at high frequencies, which makes it possible for infectious agents to spread from one living person to another (4).

Moreover, it is becoming more and more clear that hospital-acquired illnesses like these significantly increase morbidity and financial burden. International organizations have all made commendable efforts to check these infections after realizing how important they are to global health (19). Nonetheless, nosocomial infections continue to be a significant issue for hospitals

even after around thirty years of monitoring and management of these diseases in hospitalized patients worldwide (18).

Sometimes methicillin-resistant *Staphylococcus aureus* and other nosocomial infections cannot be completely eliminated by commonly used sterilizing procedures. Determining the most regularly contaminated places and the most commonly found bacteria can be crucial for promoting interventions and aiding in infection control efforts (4).

It has been determined that gram-negative bacteria that are resistant to antibiotics and that produce carbapenemases or extended-spectrum beta-lactamases (ESBL) pose an immediate threat to the health of the world's population. The limited alternatives to treatment for infections caused by carbapenem-resistant and *Enterobacterales* resistant to third-generation cephalosporins have led the World Health Organization to place these microbes on the vital list of priorities for antimicrobial agent development (20). The spread of ESBL bacteria which includes ESBL *E. coli* into the environmental surfaces is the main cause of healthcare facilities problems, this indicates that the resistance to the growing global reliance and usage of β-lactam antibiotics (cephalosporins, penicillins, carbapenems, and monobactams) is the cause of illnesses linked to healthcare (21).

Data about the degree and kind of microbiological contamination of regularly used medical devices and inanimate surfaces in intensive care units (ICUs) and operation rooms (ORs) are not adequate, especially when considering developing nations. This study’s goal is to figure out multidrug-resistant bacterial profile of inanimate objects in hospital environments, including intensive care units (ICUs) and operation rooms (ORs) at Zewditu Memorial Hospital in Addis Ababa, Ethiopia.

1.3. Significance of the study

- This investigation identified types of bacteria found on inanimate hospital settings of operation rooms (ORs) as well as intensive care units (ICUs) at Zewditu Memorial Hospital in Addis Ababa, Ethiopia, plus their resistance to antibiotics of commonly prescribed medications. This gives information to physicians, nurses, and hospital management for appropriate antibiotic choices to treat patients who are suspected of hospital-acquired infection.
- This result is of great importance for the responsible bodies in improving the hospital's hygiene in order to prevent nosocomial infections from spreading among the patients. As well, this study helps patients to get better treatment without acquiring other complications during their stay in hospitals and to get quality treatment if any nosocomial infections have occurred among patients.
- This research also adds information to policymakers to put effective preventive measures because developing and implementing effective preventive and control strategies for hospital-acquired infections requires a scientific knowledge of the level of bacterial dissemination in the health-care setting.
- This study can aid as a baseline for further study.

2. Literature review

Given that hospital-acquired infections continue to be one of the major global public health concerns, the hospital environment is a potential reservoir for numerous pathogens that cause nosocomial infections. The cross-transmission of infections linked to healthcare is greatly endangered by bacterial contamination of inanimate objects and apparatus (22).

A study conducted in Dhaka by Sarah Shawly and colleagues used 16 samples to ascertain the prevalence, antibiotic susceptibility, and plasmid profile of bacteria isolated from hospital restroom door handles. The bacteria identified in this investigation included Staphylococcus species (37.21%), *Bacillus* species (18.6%), *E. coli* (16.28%), Fecal Coliform (13.95%), *Micrococcus* species (6.98%), *Pseudomonas* species (4.65%), and *Klebsiella* species (19%). Their study found that one of the surfaces with germs that can infect people is hospital door handles. Cleaning the door handles more frequently is necessary because everyone in the hospital uses them (23).

However, a research done in India tertiary healthcare facility on bacterial contaminants and their antimicrobial profile from hospital surfaces and equipment of various areas found that, out of 186 isolates, 56.99% were gram positive organisms and 43.01% were gram negative organisms. *Bacillus* spp. had the highest percentage of isolates (28.49%), followed by *Staphylococcus aureus* (12.90%). *Pseudomonas aeruginosa* was the most frequently isolated gram-negative bacterium (9.14%), followed by *Klebsiella* spp. (7.53%) and *Acinetobacter* spp. (7.53%) (24).

A study conducted in Brazil on sampled inanimate environmental surfaces and hospital equipment 32 microorganisms were isolated from the 22 contaminated samples, including 14 (43.8%) coagulase-negative *Staphylococcus*, 7 (21.9%) *Acinetobacter baumannii* complex, 3 (9.4%) *Enterobacter aerogenes*. Among the coagulase-negative *Staphylococcus*, 11 (78.6%) showed multidrug resistance to antimicrobials and three (42.9%) of the *Acinetobacter baumanni* complex isolates were extremely resistant (25).

According to a research done in a Baghdad hospital's ORs (operating room), the percentage of positive cultures was 4.0% in 2002 after it was 3.7% in 2001. According to the identification of

the bacterial isolates, *Pseudomonas aeruginosa* (30.4%) and *Staphylococcus epidermidis* (39.1%) were the most common isolates in 2001 and 2002, respectively, while coliform bacteria (62.5%) and *P. aeruginosa* (25.0%) were the most common isolates in 2002. There is a significant risk of infection in operation rooms, so cleanliness is crucial. Furthermore, their study demonstrated that the outcomes of bacterial profiles might fluctuate over time even within the same environment, indicating the need for ongoing research in this field (26).

In 2019 a study by Darge and his friends that collected specimen in intensive care units from a variety of medical devices and inanimate objects revealed that *S. aureus* (26.3%) and Coagulase-negative Staphylococcus (34.9%) were the most frequently isolated bacteria. The frequently identified bacteria from inanimate objects were 35.4%, 29.1% *S. aureus*, and 6.3% *K. pneumonia*, whereas 34.8% CoNS, 25% *S. aureus*, and 14.1% *C. freundii* were found in the contaminated medical device (27). But still, the collective prevalence of bacterial contamination of inanimate surfaces and equipment was higher, at 70%, in a study done by Teklehaimanot Kiros as well as his colleagues. This suggests that studying the inanimate surfaces of the hospital environment is extremely important in order to prevent nosocomial infection (22).

Maksum and colleagues' study in Fatmawati Hospital intensive care unit in Indonesia found that *Pseudomonas aeruginosa* accounted for 26.5% of all isolates, with *Klebsiella pneumoniae* at 15.3% and *Staphylococcus epidermidis* at 14.9% being the next most common isolates. The percentage of *P. aeruginosa* isolates that were resistant to cephalexin (95.3%), ceftriaxone (60.9%), and cefotaxime (64.1%) was high. When it came to treating *P. aeruginosa*, amikacin had the highest efficacy (84.4%), imipenem (81.2%) and meropenem (75.0%) came next. Resistance to *K. pneumonia* was found in ceftriaxone 75.7%, cephalexin 86.5%, cefpirome 73.0%, ceftazidime 73.0%, and cefotaxime 67.9% groups (28).

Based on a study investigated in Nigeria, of the 54 *Staphylococci* sp. that were isolated from 100 samples taken from various sources of facilities and equipment in the wards, 46.3% of the isolates showed total contamination of the facilities, environment, and equipment with door knobs showing the highest rate of contamination at 28.0%, followed by bed rails at 20.0%. This compares favorably to the employee stethoscope and cell phone percentages of 16.0% and

12.0%, respectively. While 98%, 64%, and 28% of the isolated bacteria were susceptible to streptomycin, vancomycin, and chloramphenicol, respectively, all isolated bacteria demonstrated 100% resistance to the used penicillin and oxacillin. Their study demonstrated the significance of identifying surfaces and equipment with higher bacterial profiles in order to help prevent infections. However, more research is needed to identify multidrug resistant bacteria and find more bacterial profiles (29).

On the other hand, a study done in four hospitals in Cameroon, 50.4% of the surface was contaminated with bacteria (30). Comparably, a research done by Enid et al. in 2022 demonstrates that the bacteria isolates that were found included *Staphylococcus aureus, Citrobacter hominis, Citrobacter* spp., and *Citrobacter freundii. Staphylococcus hominis* was the least common isolated bacterium (1.6%), while *Bacillus cereus* accounted for 49.1% of all isolates. The uro-endoscopy unit had the greatest number of isolates among the three units under examination, followed by the general surgical theater and the GI endoscopy. However, this research should look into the drug resistance of the established bacterial profiles (31).

In 2021 a research was conducted by Saikeerthana and his friends in india. The study includes the surveillance of hospital surfaces, including wards and ICUs, by taking swabs dipped in normal saline. Among those 65 positive cultures, 53.8% were isolated as *Pseudomonas aeruginosa*, 18% isolates were identified as *Klebsiella pneumonia*, 16% isolates were Methicillin Sensitive *Staphylococcus aureus* (MSSA), 6% were Coagulase-Negative *Staphylococcus*, 3% were Methicillin-Resistant *Staphylococcus aureus* (MRSA) and 1.6% were identified as *Enterococcus* species (32).

A study was conducted in 2019 on the antibiotic sensitivity and bacterial profile of the pathogens that were isolated from medical surfaces and equipment in a Nigerian hospital's children's emergency room. In this investigation, 70.3% of the specimens showed signs of bacterial growth. The most often cultured isolate was 53.4% *Staphylococcus aureus*, followed by Coagulase-negative *Staphylococcus* (18.8%), *Escherichia coli* (13.9%), and other bacteria. Of the isolates of *Staphylococcus aureus*, 25.9% contained MRSA. The gram-negative bacteria had a high level of

ampicillin resistance. Both ceftriaxone and ciprofloxacin were effective against all of the gram-negative isolates (33).

The majority of bacteria from 201 swab samples were recovered from the floor and thermometer, according to another study conducted at Mizan-Tepi University Teaching Hospital. These bacteria included 15.9% *E. coli*, 19.3% CoNS, 21.6% *S. aureus*, 11.4% *P. aeruginosa*, 14.8% *Klebsiella* species, 6.8% *Serratia* species, and 10.2% *Proteus* species. *Klebsiella* species (53.8%), *S. aureus* (79%), *Proteus* species (44.4%) and CoNS (47%) were the most multidrug resistant organisms. Just 6.45% of medical workers regularly clean their stethoscopes. Their study suggests that health professionals' knowledge of appropriate medical equipment disinfection is lacking. Therefore, responsible organizations should close this gap (34).

However, an investigation was undertaken in Ethiopia's Tikur Anbessa Specialized Teaching Hospital. 86% of the 164 swabbed samples in this investigation tested positive for bacterial growth. Coagulase-negative *Staphylococcus* 12.6% vs. 2.7%, *Acinetobacter baumannii* 3.8% vs. 17.5%, and *Staphylococci aureus* 23% vs. 11.5% were the most common bacteria found in OTs and ICUs, respectively. Of the products examined at the hospital, linens had the highest contamination rate (14.8%). The resistance levels of gram-positive bacteria (GPB) to cefoxitin (83.5%), penicillin (92.8%) and erythromycin (53.6%) were notably high. However, the highest levels of resistance to ampicillin (97.5%), ceftriaxone (91.3%), ceftazidime (91.3%), and aztreonam (90%) were found in Gram-negative bacteria (GNB). Nonetheless, a low level of resistance was noted for ciprofloxacin at 37.5% and amikacin at 25%. Methicillin-resistant *S. aureus* (MRSA) accounted for 85.7% of the 63 *S. aureus* isolates. Nevertheless, including wards other than those listed in their study can help us better understand the scope of bacterial profiles and prevent hospital-acquired diseases (13).

Hye Jin Shi et al.'s 2020 study on inanimate surfaces found that, from cultures of 160 samples, 407 bacteria of 38 species were isolated; of these, 109 (26.8%) being gram-negative and 298 (73.2%) remained gram-positive. The common isolation sites were keyboards, bed linen sheets, bedside rails, and curtains. The common bacteria isolated were coagulase-negative *staphylococci* 54.5%, *Enterococcus faecium* 5.9%, *Pseudomonas aeruginosa* 8.1%, and *Acinetobacter*

baumannii 11.8%. A total of 60 multidrug-resistant strains were isolated. There were multidrug-resistant *Acinetobacter baumannii* (n=32), multidrug-resistant *Pseudomonas aeruginosa* (n=2), vancomycin-resistant *Enterococcus* (n=20), and carbapenem-resistant *Enterobacteriaceae* (n=6). Healthcare professionals will be better able to treat infections brought on by these microbes thanks to the study's identification of those multidrug-resistant bacteria (35).

3. Objectives

3.1. General objective

- To assess multidrug-resistant bacterial profiles of inanimate objects at Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To identify bacterial profiles of inanimate surfaces from operation rooms and intensive care units.
- To determine the antibiotics resistance pattern of identified bacteria from inanimate surfaces.
- To determine the magnitude of ESBLs and carbapenemase-producing gram-negative bacteria from inanimate surfaces.
- To determine the prevalence of MRSA from inanimate surfaces.

4. Methods and Materials

4.1. Study Area

This research was carried out in Zewditu Memorial Hospital in Addis Ababa. Zewditu Memorial Hospital is centered in Kirkos sub-city of Addis Ababa, Ethiopia. As data from the institution indicate they have 231 beds, 11 wards and 3 Emergency wards. The hospital provides care for approximately 115,102 patients annually. The specimens were gathered from two intensive care units (ICUs) including adult and neonatal intensive care units. As a total three operating rooms were checked including major OR, gynecology and pediatrics neurology (CDC operation rooms), caesarian section units (delivery units).

4.2. Study design and Study Period

From June 05 to November 13 2024, a hospital-based cross-sectional investigation was carried out in the operating rooms (ORs) and intensive care units (ICUs) at Zewditu Memorial Hospital in Addis Ababa, Ethiopia.

4.2.1. Source of population

The source of the populations included all inanimate surfaces and equipment suspected to harbor bacterial pathogens and could be the source of nosocomial infections at the medical facility.

4.2.2. Study population

The study of the populations included inanimate surfaces and equipment suspected to harbor bacterial pathogens at operation rooms and ICUs of Zewditu Memorial Hospital, Addis Ababa, Ethiopia.

4.3. Inclusion and Exclusion criteria

4.3.1. Inclusion criteria

- Ward (operation rooms plus intensive care unit wards)

- Type of surface swabbed (floors, walls, tables, bed linen, bed rails, beds, IV stands, pulse oximeter, operation room light, ventilators, suction machines, phototherapy machines. anesthesia machine, oxygen supply and patient monitors).

4.3.2. Exclusion criteria

- Non-functional equipment found in ICUs and ORs.

- Uninstalled equipment ICUs and ORs.

4.4. Study variables

4.4.1. Dependent variables

- Magnitude of bacterial-profile
- Multidrug resistance level of isolated bacteria
- Magnitude of ESBL and carbapenemase-producer bacteria.
- Magnitude of MRSA

4.4.2. Independent variables

- Ward (operation rooms and intensive care unit wards)
- Type of surface swabbed (floors, walls, corridors, tables, bed linen, bed rails, beds, IV stands, Pulse oximeter, operation room light, ventilators, suction machines, phototherapy machines. anesthesia machine, oxygen supply, and patient monitors.)

4.5. Data collection

4.5.1. Sampling method

Selected inanimate objects and crucial healthcare supplies found in intensive care units and operation rooms that have frequent contact with patients as well as health care professionals in the study time were selected using a convenient sampling approach**.**

4.5.2. Sample size determination

The number of samples was identified using the single population proportion formula and the prevalence of p=86% from a previous study conducted by Shemse et al. at Tikur Anbessa Specialized Hospital TASH(13).

- The sample number computation formula is; $\mathbf{n = z^2 p(1-p)/d^2}$
- Where, n represents the sample size,
- *Z* indicates the degree of confidence (95% confidence; z = 1.96)
- *P* represents predicted prevalence or proportion, (In this study *P* was taken from Shemse et al. study in TASH, which was *P*= 0.86).
- *d* represents precision (in proportion of one; if 5%, *d* = 0.05).

$n = 1.96^2\ 0.86(1-0.86)/0.05^2$

- n= 185+ 10% contingency
- **n=204**

4.5.3. Data collection procedure

4.5.3.1. Sample collection

In overall 204 swabs were collected from selected equipment and environments in the operation rooms and intensive care units in the morning after cleaning was done. All swab samples were collected while staffs were starting their daily activities. Sterile swabs were dampened using 85% normal saline and administered to high-touching areas after observation. All samples were collected in parallel spaced stripes, slightly rotated, and subsequently in perpendicular stripes in accordance with ISO/DIS 14698-1. Each sample were collected then transported to a leak-proof container with tryptone soya broth as a transport media. Samples were collected and immediately delivered to Tikur Anbessa Specialized Hospital's Microbiology Laboratory for bacterial culture and processing.

4.5.3.2. Bacterial culture, identification, and interpretation of culture results

Swabs were inoculated onto MacConkey agar and Blood agar and aerobically incubated for 18-24 hours at 37 degrees Celsius. Isolates of bacteria on culture-positive dishes were determined based on colony appearance, gram staining, and biochemical properties. Triple sugar iron agar, urea utilization test, citrate utilization, indole, mannitol fermentation, decarboxylation on lysine iron agar, and oxidase test were among the biochemical tests applied to determine gram-negative bacteria. Gram-positive bacteria, on the contrary were determined via gram stain, catalase, and coagulase tests, which were then interpreted using laboratory standard operating procedures.

4.5.3.3. Antibiotic susceptibility testing

The antimicrobial susceptibility testing was tested as CLSI 34th edition guidelines by utilizing Muller-Hinton agar following the standard procedure. After placing selected antibiotics on MHA plate swabbed with tested bacteria, the plate was incubated for 16-18 hours. The area of inhibition was measured and interpreted in accordance with the CLSI 34th edition (2024). Antibiotics used for testing gram negative bacteria were amikacin, amoxicillin-clavulanic acid (AMC), gentamycin, ampicillin, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, cefepime, Ertapenem, imipenem/meropenem, trimethoprim-sulfamethoxazole (SXT) and piperacillin-tazobactam. Gram-positive bacteria were tested against gentamycin, ciprofloxacin, cefoxitin, trimethoprim-sulfamethoxazole (SXT), penicillin, azithromycin clindamycin, erythromycin, and tetracycline.

4.5.3.4. Phenotypic detection of ESBLs, carbapenemase producers, and MRSA

1. **Phenotypic detection of ESBLs**

1.1. Screening for ESBLs producing isolate

Potential producers of extended-spectrum antibiotic resistance (ESBLs) are strains that exhibit reduced susceptibility to ceftazidime, ceftriaxone and/or cefotaxime resistance based on CLSI recommendations (36). Those strains were deemed to be possible producers of ESBLs and were

subjected to additional investigation to confirm ESBL production using the double disk synergy test and combination disc test

1.2. Phenotypic confirmation with double disc synergy test (DDST)

The demonstration of synergy between clavulanate and the indicator cephalosporin(s) to which the isolate was initially found to be resistant is necessary for the confirmation of ESBL production. A disc containing amoxicillin-clavulanate (20 μg/10 μg) (augmentin) and a 30-μg disc containing each third-generation cephalosporin test antibiotic were compared for synergy. The discs were positioned 20 mm apart from one another on a mueller-hinton agar plate that had been swabbed with the test isolate. A clear enlargement of the margin of the cephalosporin inhibition area near the augmentin disc was regarded as a positive ESBL test result (37).

1.3. Phenotypic confirmation with combination disc test

Ceftazidime (30 μg) disks were used alone and in conjunction with clavulanic acid (30 μg/10 μg) to confirm the existence of ESBLs. A zone diameter ≥5 mm increase with ceftazidime/clavulanate disks than in Ceftazidime disks were found to produce ESBL(36).

1. Phenotypic detection of carbapenemase producer

1.1. Screening for carbapenemase producer

Organisms were subjected to a carbapenem resistance screening using Meropenem 10 μg disks and the Kirby Bauer disk diffusion method. Bacteria that shows resistant or intermediate for Meropenem 10 μg was further analyzed for the production of carbapenemase by phenotypic modified carbapenem inactivation method (38).

1.2. Phenotypic confirmation with modified carbapenem inactivation method (mCIM)

Modified Carbapenem inactivation method (mCIM) was used for carbapenemase production detection. Briefly, the method consists of suspending a 1 μL loop of test organisms into 2 mL of trypticase soy broth. A 10 μg meropenem disc was added to the prepared suspension and incubated for 4 hours at 35 °C. A suspension of *E. coli* ATCC 25922 calibrated to 0.5 McFarland

was prepared and inoculated on a mueller hinton agar plate. After 4 hours, the meropenem disc was removed and inserted on an MHA plate previously inoculated with the indicator strain *E. coli* ATCC 25922. The plates were then incubated for 18–24 h at 35 °C, and the plates were analyzed based on the clinical and laboratory standards institute's recommendations (38).

3. Phenotypic detection of MRSA

3.1. Cefoxitin disk diffusion test

The isolate was initially grown on a mueller-hinton agar plate using a 30-µg cefoxitin disk and a 0.5 McFarland standard suspension. Every plate was incubated aerobically for the entire night at 37°C. Zones smaller than 22 mm was classified as MRSA, while zones larger than 22 mm were designated as sensitive (36).

4.6. Data and laboratory quality assurance

The trustworthiness of the research results was ensured by employing quality assurance measures throughout the data gathering and laboratory work processes.

4.6.1. Pre-analytical

The procedures for choosing the right tests, placing the order, gathering, sorting, labeling, handling, and shipping biological samples, and preparing the media in accordance with manufacturer manuals and laboratory Standard Operating Procedures (SOP) was all closely adhered to. It was also verified that the media met quality control standards set forth by CLSI 34th edition and that the expiration date was met. Labeling media, containers, and completing forms was done in accordance with the prescribed procedures. To minimize contamination aseptic techniques was used throughout the sample collection and inoculation processes onto culture media. Additionally, all pre-analytical procedures were carried out in compliance with the TASH microbiology laboratory standard operating procedures and CLSI 34th edition guidelines.

4.6.2. Analytical

All supplies, procedures, and equipment were properly managed. The sterility and performance of culture media were examined, and the media was incubated overnight at 37^0 degrees Celsius. The performance of the MacConkey agar plate and blood agar plate was evaluated by applying the control strains *E. coli* ATCC 25922, *P. mirabilis* ATCC 35659, *S. aureus* ATCC 25923, and *S. pneumonia* (patient strain), respectively. The medications' efficacy was tested using international control bacterial strains such as *Escherichia coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC27853). Bacteria known to be positive and negative were added to biochemical test media. Additionally, the microbiology unit employs professional microbiologists with specialized training confirmed the results of culture growth, biochemical tests, and antibiotic susceptibility tests.

4.6.3. Post-analytical

All extracted information (laboratory findings) was reviewed for legibility, completeness, and consistency before being stored securely. Cross-checking and data cleansing were completed. The data was saved on a CD as an extra. All isolated bacteria were kept in accordance with the laboratory's standard operating procedures.

4.7. Data analyses and interpretation

The work was analyzed using SPSS version 20.0 to show the incidence of bacterial infections on inanimate surfaces as well as the antibiotic resistance level. The findings were evaluated via descriptive statistics SPSS (the Statistical Package for Social Sciences), and the findings were described using tables, graphs, charts, and text.

4.8. Operational definitions

Multiple drug resistance (MDR): - It is described as a bacterium resistance to at least one antibiotic drug in three or more classes of antibiotics.

Extensively drug resistance (XDR):- is characterized as the inability of bacterial isolates to remain susceptible to more than two antimicrobial categories, or the inability to respond to at least one agent in any of the remaining antimicrobial categories.

Inanimate surface: - is the surface of a material and equipment used for patient care in the operation rooms and intensive care unit.

4.9. Ethical considerations

Before beginning data collection, Addis Ababa University, the Department of Medical Laboratory Science, and the Addis Ababa Health Bureau all provided ethical approval with reference number MLS/165/24 and A/A/H/10432/227 respectively. Then, an official letter of cooperation was delivered to Zewditu Memorial Hospital and Tikur Anbessa Specialized Hospital.

5. Results

5.1. Prevalence of bacterial isolates from inanimate surfaces and medical equipment at ICUs and ORs

5.1.1. Frequency of bacterial isolates

In the current study, a total of 204 swab samples were gathered from different inanimate objects located in different operation rooms (ORs) (n=113) and ICUs (n=91) of Zewditu Memorial Hospital. Among all cultured samples, the overall prevalence of bacteria was found to be 77.4% (n=158/204). We identified a total of 171 bacterial isolates, with double isolates from 12 culture-positive samples among all swabs taken from inanimate objects.

Out of the 171 bacterial isolates, 71.3% (n=122/171) were gram-positive bacteria while gram-negative bacteria accounted for 28.7% (n=49/171) (Figure 1 and Figure 2). *S. aureus* 4.9% (n=6/122), *CoNS* 64.7% (n=79/122), and *Bacillus* spp. 30.3% (37/122) were gram-positive bacteria identified in this study. Among the gram-negative bacteria, *Acinetobacter spp.* 16.3% (n=8/49), *Pseudomonas aeruginosa* 36.7% (n=18/49), and *E. coli* 14.2% (n=7/49) were the predominant isolates identified. Overall, *CoNS* 46.1% (n=79/171) was the bacteria that was isolated the most often, followed by *Bacillus* spp. 21.6% (n=37/171) and *Pseudomonas aeruginosa* 10.5% (n=18/49).

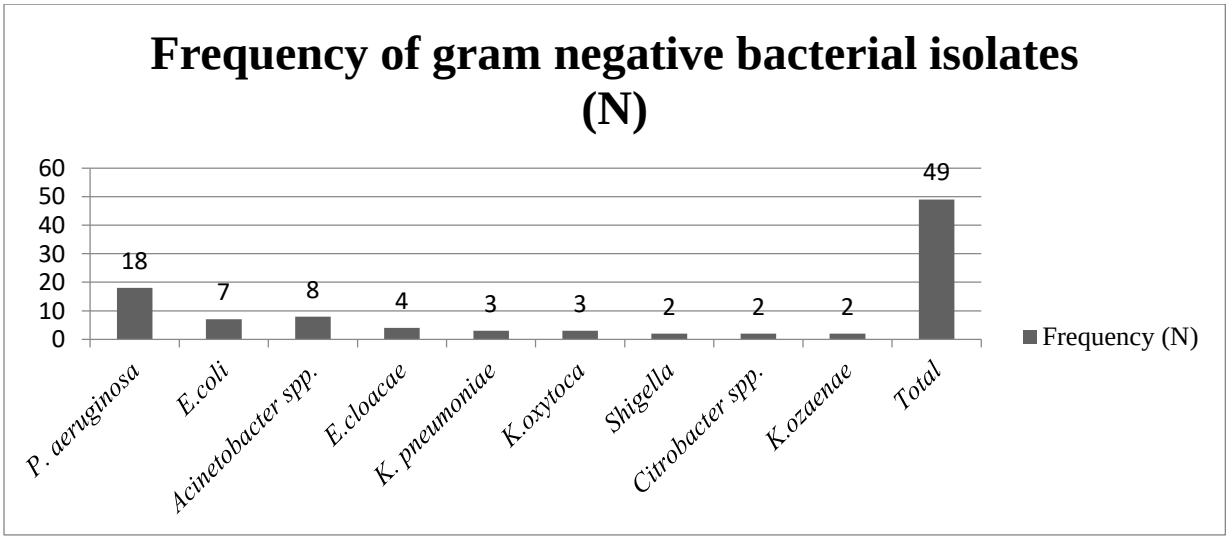

Figure 1. Gram negative bacteria isolated from environmental surfaces and medical equipment at Zewditu memorial hospital from June-November 2024.

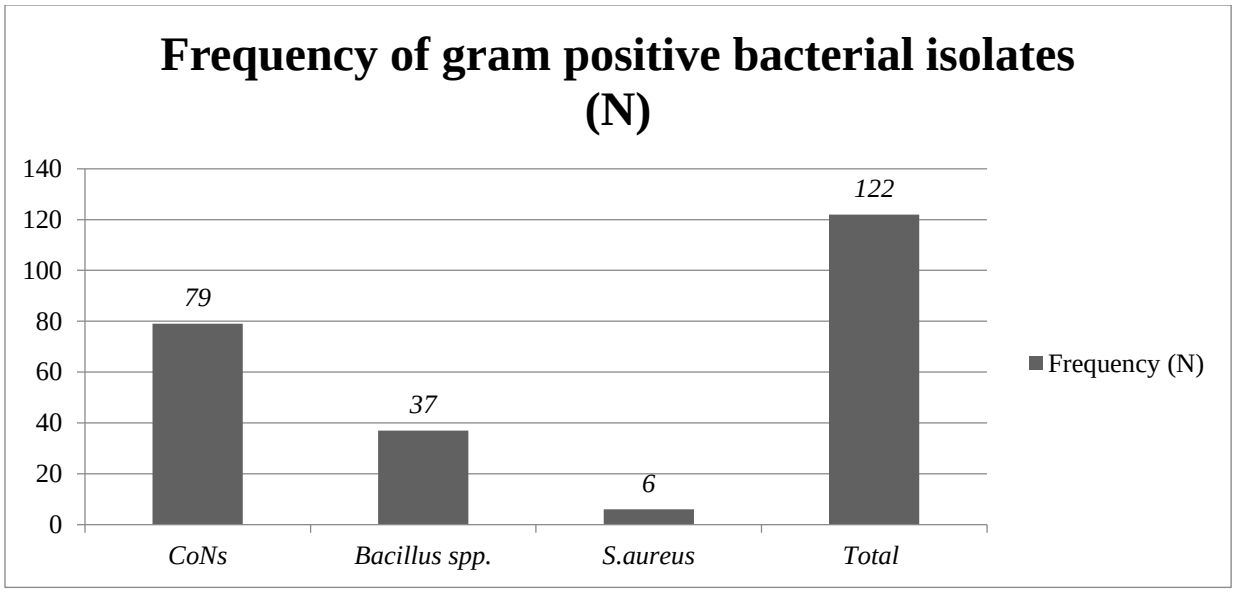

Figure 2. Gram Positive bacteria isolated from environmental surfaces and medical equipment at Zewditu Memorial Hospital from June-November 2024.

5.1.2. Distribution of bacterial isolates between intensive care units and operation rooms

In intensive care units (ICUs), the majority of the possible bacterial infections were isolated that accounted 50.8% (n=87/171). There have been gram-positive and gram-negative bacteria differed amongst wards in operation rooms (ORs) 71.4% (n=60/84) vs 28.5% (n=24/84) and intensive care units (ICUs) 71.2% (n=62/87) vs 28.7% (n=25/87) respectively. The intensive care units (ICUs) were mainly contaminated with GPB 71.2% (n=62/87), of which the most common ones are *CoNs* accounted for 52.8% (46/87) then *Bacillus spp.* with 12.6% (11/87) rate of isolation. Most of the bacteria in intensive care units (ICUs) were isolated from the neonatal intensive care unit (NICU) 51.7% (n=45/87). The major pathogens in this intensive care unit were *CoNs* from gram positive bacteria and *P. aeruginosa* from gram negative bacteria, each with an isolation rate of 51.1% (n=23/45) and 13.3% (n=6/45). The operation rooms (ORs) were mainly contaminated by gram positive bacteria, 71.4% (n=60/84) where the major were *CoNS*, 39.2% (n=33/84), and *Bacillus* spp., 30.9% (n=26/84) (Table 1).

Table 1. Distribution of bacteria among wards isolated from environmental surfaces and medical equipment at Zewditu memorial hospital from June-November 2024.

Bacterial Isolates	Adult ICUs N(%)	Neonatal ICUs N(%)	Major OR N(%)	CS OR N(%)	CDC OR N(%)	Total N(%)
CoNs	23(29.11)	23(29.11)	18(22.7)	12(15.18)	3(3.79)	79(46.1)
Bacillus spp.	5(13.5)	6(16.2)	15(40.5)	9(24.3)	2(5.4)	37(21.6)
S. aureus	3(50)	2(33.33)	0(0)	1(16.66)	0(0)	6(3.5)
P. aeruginosa	3(16.66)	5(27.77)	0(0)	0(0)	10(55.55)	18(10.5)
Klebsiella spp.	2(11.11)	4(22.2)	1(12.5)	0(0)	1(12.5)	8(4.67)
E.coli	2(28.5)	1(14.28)	1(14.28)	1(14.28)	3(42.8)	7(4.09)
Acinetobacter spp.	2(25)	0(0)	2(25)	4(50)	0(0)	8(4.67)
Citrobacter spp.	0(0)	1(50)	1(50)	0(0)	0(0)	2(1.16)
E. cloacae	1(25)	2(50)	0(0)	0(0)	1(25)	4(2.33)
Shigella spp.	1(50)	1(50)	0(0)	0(0)	0(0)	2(1.16)
Total	42	45	38	27	19	171(100)

*CS OR; (caesarean section operation room)

*CDC OR; (Gynecology & pediatrics neurology operation room)

5.1.3. Distribution of bacterial pathogens over different surfaces

The highest number of bacterial-contaminated samples was taken from environmental surfaces 14%, tables 12.8% and patient monitors 9.94%. Environmental surfaces were mostly contaminated with *Bacillus spp*., 37.5% (n=9/24) followed by *CoNs*, 29.1% (n=7/24). Tables used by health care workers in operation rooms and intensive care units were mainly contaminated with *CoNs* 45.4% (n=10/22). Patient monitors were mainly colonized by *CoNs* 47% (n=8/17), *bacillus spp.* 23.5% (n=4/17), and *Acinetobacter spp.* 11.7% (n=2/17) (Table 2).

Table 2A. Distribution of bacterial pathogens over different surfaces isolated from environmental surfaces and medical equipment at Zewditu memorial hospital from June-November 2024.

N (%)

Inanimate objects	***CoNs***	***Bacillus spp.***	***S. aureus***	***P. aeruginosa***	***Klebsiella spps.***	***E. coli***	***Acinetobacter spp.***	***Citrobacter spp.***	***E. cloacae***	***Shigella spp.***	**Total**
Anastasia Machine (7)	3(3.79)	1(2.7)	0(0)	1(5.5)	0(0)	1(14.28)	1(1.25)	0(0)	0(0)	0(0)	7(4.09)
Bed (11)	8(10.1)	1(2.7)	0(0)	0(0)	2(25)	0(0)	0(0)	0(0)	0(0)	0(0)	11(6.4)
Linens (15)	6(7.5)	4(10.8)	2(33.3)	1(5.5)	2(25)	0(0)	0(0)	0(0)	0(0)	0(0)	15(8.77)
Monitor (17)	8(10.1)	4(10.8)	0(0)	1(5.5)	0(0)	1(14.2)	2(25)	1(50)	0(0)	0(0)	17(9.94)
Suction Machi	3(3.7)	2(5.4)	0(0)	0(0)	0(0)	1(14.2)	0(0)	0(0)	1(25)	1(50)	8(4.67)

ne (8)

Table 3B. Distribution of bacterial pathogens over different surfaces isolated from environmental surfaces and medical equipment at Zewditu memorial hospital from June-November 2024.

Inanimate objects	*CoNs*	*Bacillus spp.*	*S. aureus*	*P. aeruginosa*	*Klebsiella spps.*	*E. coli*	*Acinetobacter spp.*	*Citrobacter spp.*	*E. cloacae*	*Shigella spp.*	Total
OR Table (6)	3(3.7)	3(8.1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	6(3.5)
Tables (22)	10(12.6)	4(10.8)	0(0)	5(27.77)	0(0)	3(42.8)	0(0)	0(0)	0(0)	0(0)	22(12.8)
Work Station (6)	3(3.79)	0(0)	0(0)	2(11.11)	1(12.5)	0(0)	0(0)	0(0)	0(0)	0(0)	6(3.5)
Oxygen Cylinder (12)	7(8.8)	3(8.1)	0(0)	1(5.5)	0(0)	1(14.28)	0(0)	0(0)	0(0)	0(0)	12(7.01)
Bed Trails (10)	6(7.59)	1(2.7)	1(16.66)	0(0)	1(5.555)	0(0)	1(12.5)	0(0)	0(0)	0(0)	10(5.8)
OR light (11)	5(6.3)	3(8.1)	0(0)	2(11.1)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	11(6.4)
IV Stand (12)	6(7.5)	1(2.7)	1(16.66)	3(16.66)	0(0)	0(0)	1(12.5)	0(0)	0(0)	0(0)	12(7.01)
Environmental surfaces (24)	7(8.8)	9(24.3)	2(33.33)	2(11.11)	1(5.555)	0(0)	0(0)	1(50)	1(25)	1(50)	24(14.03)
Others (10)	4(5.06)	1(2.7)	0(0)	0(0)	1(12.5)	0(0)	2(25)	0(0)	2(50)	0(0)	10(5.8)
Total	**79**	**37**	**6**	**18**	**8**	**7**	**8**	**2**	**4**	**2**	**171(100)**

*Others; (ventilators, phototherapy machines, Electro-surgical unit generators, pulse oximeter)
*Environmental surfaces; (floors, walls, door knobs)

5.2. Antibiotic resistance pattern

Among the total isolates 55 bacteria were selected for antibiotics resistance test because these bacteria are pathogenic and can cause disease. Those isolates were selected from gram negative and gram positive bacteria of the total isolates.

The majority of gram-negative bacteria revealed significantly high resistance to the majority of the tested antibiotics; for example, ampicillin 67.3%, amoxicillin and clavulanic acid 61.2%, ciprofloxacin 63.2%, sulfamethoxazole-trimethoprim 63.2%, cefepime 57.1% and piperacillin-tazobactam 55.1%. Similarly, significant resistance level was also found for amikacin 53.06%, chloramphenicol 53.06%, gentamicin 46.9%, ceftazidime 34.6%, ceftriaxone 30.6%, and ertapenem 32.6%. Resistance at low levels was noted for cefotaxime 16.3% and meropenem 12.2%. Antibiotics such as ampicillin 100%, gentamicin 100%, amoxicillin and clavulanic acid 100%, cefepime 100%, ciprofloxacin 100%, and sulfamethoxazole-trimethoprim 75% were among the antibiotics to which *E.cloacae* exhibited the highest level of resistance. *E.cloacae* showed a low level of resistance to ceftriaxone 0% and meropenem 0% (Table 3).

Table 3.Antimicrobial resistance pattern of GNB isolated from environmental surfaces and medical equipment at zewditu memorial hospital from June-November 202

Isolated bacteria	Ptn	Antibiotics N(%)													
		AMI	**AMC**	**GEN**	**AMP**	**CTX**	**CTZ**	**CTR**	**CMP**	**CIP**	**CPM**	**ERT**	**MEM**	**SXT**	**PPT**
Pseudomonas aeruginosa (18)	R	17 (94.4)	11(61.1)	9(50)	10(55.5)	2(11.1)	5(27.7)	3(16.6)	6(33.3)	6(33.3)	9(50)	7(38.8)	0(0)	12(66.6)	9(50)
	S	1(5.5)	7(38.88)	9(50)	8(44.4)	16(88.8)	13(72.2)	15(83.3)	12(66.6)	12(66.6)	9(50)	11(61.1)	18(100)	6(33.3)	9(50)
Escherichia coli (7)	R	3(42.8)	6(85.7)	3(42.8)	6(85.7)	2(28.5)	3(42.8)	2(28.5)	4(57.1)	6(85.7)	3(42.8)	2(28.5)	0(0)	4(57.1)	3(42.8)
	S	4(57.1)	1(14.28)	4(57.1)	1(14.28)	5(71.4)	4(57.1)	5(71.4)	3(42.8)	1(14.28)	4(57.1)	5(71.4)	7(100)	3(42.8)	4(57.1)
Enterobacter cloacae (4)	R	2(50)	4(100)	4(100)	4(100)	0(0)	2(50)	2(50)	3(75)	4(100)	4(100)	1(25)	0(0)	3(75)	2(50)
	S	2(50)	0(0)	0(0)	0(0)	4(100)	2(50)	2(50)	1(25)	0(0)	0(0)	3(75)	4(100)	1(25)	2(50)
Acinetobacter spp.(8)	R	1(12.5)	3(37.5)	2(25)	6(75)	3(37.5)	4(50)	4(50)	7(87.5)	3(37.5)	4(50)	2(25)	2(25)	4(50)	5(62.5)
	S	7(87.5)	5(62.5)	6(75)	2(25)	5(62.5)	4(50)	4(50)	1(12.5)	5(62.5)	4(50)	6(75)	6(75)	4(50)	3(37.5)
Citrobacter spp. (3)	R	1(50)	1(50)	1(50)	0(0)	0(0)	1(50)	1(50)	1(50)	2(100)	1(50)	0(0)	0(0)	1(50)	1(50)
	S	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)	1(50)	1(50)	0(0)	1(50)	2(100)	2(100)	1(50)	1(50)
K.pneumoniae (3)	R	0(0)	1(33.33)	0(0)	1(33.33)	0(0)	0(0)	0(0)	1(33.330)	3(100)	0(0)	0(0)	2(66.66)	0(0)	1(33.33)
	S	3(100)	2(66.66)	3(100)	2(66.66)	3(100)	3(100)	3(100)	2(66.66)	0(0)	3(100)	3(100)	1(33.3)	3(100)	2(66.66)
K. oxytoca (3)	R	1(33.33)	1(33.33)	1(33.33)	3(100)	1(33.33)	1(33.33)	2(66.66)	2(66.66)	3(100)	3(100)	1(33.33)	1(33.33)	3(100)	2(66.66)
	S	2(66.66)	2(66.66)	2(66.66)	0(0)	2(66.66)	2(66.66)	1(33.33)	1(33.33)	0(0)	0(0)	2(66.66)	2(66.66)	0(0)	1(33.33)
K.ozaenae(3)	R	1(50)	2(100)	2(100)	2(100)	0(0)	1(50)	1(50)	1(50)	2(100)	2(100)	1(50)	1(50)	2(100)	2(100)
	S	1(50)	0(0)	0(0)	0(0)	2(100)	1(50)	1(50)	1(50)	0(0)	0(0)	1(50)	1(50)	0(0)	0(0)
Shigella spp (3)	R	0(0)	1(50)	1(50)	1(50)	0(0)	0(0)	0(0)	1(50)	2(100)	2(100)	2(100)	0(0)	2(100)	2(100)
	S	2(100)	1(50)	1(50)	1(50)	2(100)	2(100)	2(100)	1(50)	0(0)	0(0)	0(0)	2(100)	0(0)	0(0)

The rate of resistance to antibiotics among gram-positive bacteria was high for penicillin 100%, azithromycin 100%, clindamycin 100%, and erythromycin 100%. A minimal amount of resistance was noted for gentamicin 33.3%, sulfamethoxazole-trimethoprim 33.3%, and tetracycline 16.66%. With cefoxitin disk as a surrogate marker, 50% (n=3/6) of *S. aureus* isolates were described as MRSA (Table 4).

Table 4. Antimicrobial resistance pattern for gram positive bacteria isolated from environmental surfaces and medical equipment at Zewditu memorial hospital from June-November 2024.

Isolated bacteria	Ptn	Antibiotics N(%)								
		GEN	CIP	OXA	SXT	PEN	AZI	CLI	ERY	TET
S.aureus	R	2(33.33)	3(50)	3(50)	2(33.33)	6(100)	6(100)	6(100)	6(100)	1(16.66)
	S	4(66.66)	3(50)	3(50)	4(66.66)	0(0)	0(0)	0(0)	0(0)	5(83.33)

Among those 55 bacterial isolates selected for antimicrobial resistance test, multidrug resistance (bacterial resistance to at least one antibiotic drug in three or more classes of antibiotics) were recorded in 61.8% (34/55)of all bacterial isolates (Table 5).

Table 5. Multi drug resistance bacteria isolated from environmental surfaces and medical equipment at Zewditu memorial hospital from June-November 2024.

Gram negative bacteria	Isolated bacteria	R_0	R_1	R_2	R_3	R_4	R_5		MDR
		N (%)	N (%)	N (%)	N (%)	N (%)	N (%)		
	Pseudomonas aeruginosa (18)	5(27.77)	1(5.55)	1(5.55)	4(22.22)	0(0)	6(50)		10(55.55)
	Escherichia coli (7)	1(14.2)	0(0)	2(28.5)	1(14.2)	0(0)	3(42.8)		4(57.14)
	Enterobacter cloacae (4)	0(0)	0(0)	0(0)	1(25)	0(0)	3(75)		4(100)
	Acinetobacter spp. (8)	1(12.5)	1(12.5)	2(25)	1(12.5)	1(12.5)	2(25)		4(50)
	Citrobacter spp. (2)	0(0)	1(33.33)	0(0)	0(0)	0(0)	1(33.33)		1(33.33)
	K. pneumoniae (3)	0 (0)	1(33.33)	1 (33.33)	1(33.33)	0(0)	0(0)		1(33.33)
	K. oxytoca (3)	0 (0)	0 (0)	0 (0)	1(33.33)	1(33.33)	1(33.33)		3(100)
	K. ozanae (2)	0(0)	0(0)	0(0)	0(0)	1(33.33)	1(33.33)		2(66.66)
	Shigella spp. (2)	0(0)	0(0)	0(0)	1(33.33)	0(0)	1(33.33)		2(66.66)
Gram positive bacteria	**Isolated bacteria**	**R_0**	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**MDR**
		N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**
	S.aureus (6)	0(0)	0(0)	3(50)	1(16.66)	0(0)	1(16.66)	1(16.66)	3(50)

5.3. ESBL producing bacteria

Eighteen (18) isolates were suspected as ESBL producers from 49 gram-Negative bacterial isolates using the ESBL screening method. These isolates were *E. coli* 16.6% (n=3/18), *K. ozaena* 5.5% (n=1/18*), E. cloacae* 11.1% (n=2/18), *Citrobacter spp.* 5.5% (n=1/18), *K. oxytoca* 11.1% (n=2/18), *Acinetobacter spp.*22.2% (n=4/18), and *P.aeruginosa* 27.7% (n=5/18).

The total prevalence of ESBL-producer bacteria was 26.5% (n=13/49) using combination disc test method. *K. ozaenae* 100% (n=1/1), *E. coli* 66.6% (n= 2/3), *K. oxytoca* 50% (n=1/2), *Acinetobacter spp*. 50% (n=2/4), *E. cloacae* 50% (n=1/2), *Citrobacter spp*. 100% (n=1/1), and *P. aeruginosa* 80% (n=4/5) were positive for ESBL using this combination disc test (figure 3).

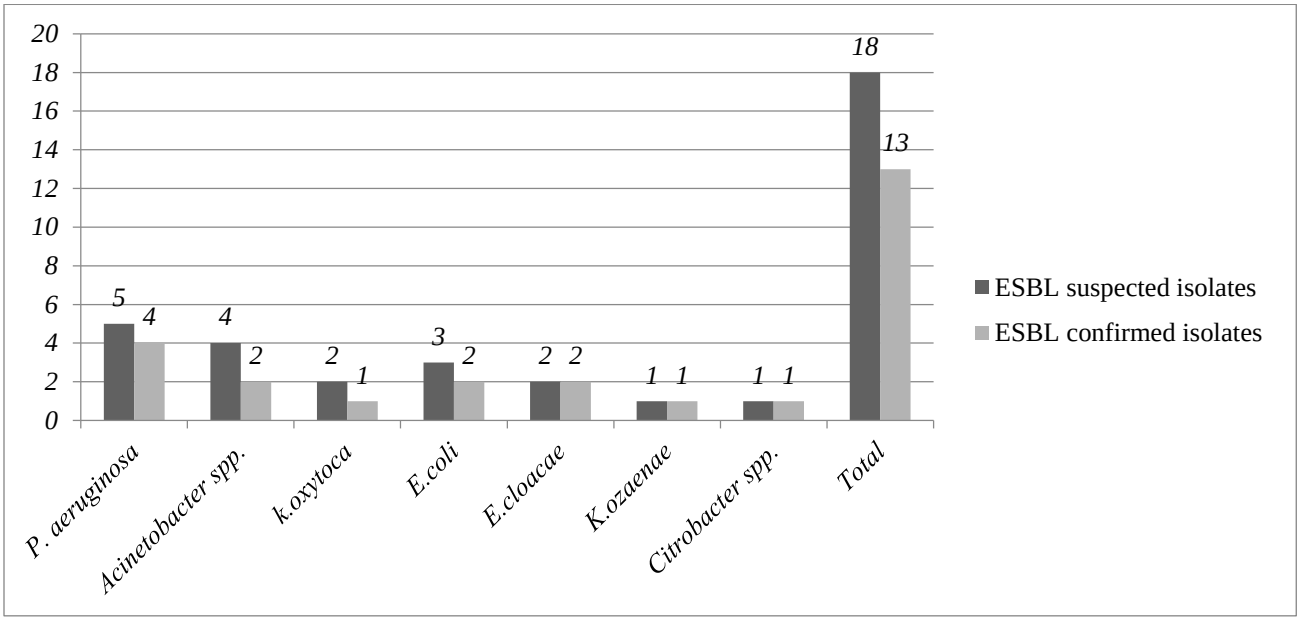

Figure 3. ESBL producers confirmed with combination disk method isolated from environmental surfaces and medical equipment at Zewditu memorial Hospital from June-November 2024.

The double disk synergy process, another phenotypic confirmatory method, was used to test all isolates (n=18) for the generation of ESBL. According to the double disk approach, 24.4% of ESBL cases (n=12/49) were confirmed. The following bacteria were found to be ESBL positive using this method: *E. coli* 33.33% (n=1/3), *K. ozaenae* 1/1 (100%), *K. oxytoca* 100% (n=1/1), *Acinetobacter spp.* 50% (n=2/4), *P. aeruginosa* 80% (n=4/5), *E. cloacae* 100% (n=2/2), and *Citrobacter spp.* 100% (n=1/1) (figure 4).

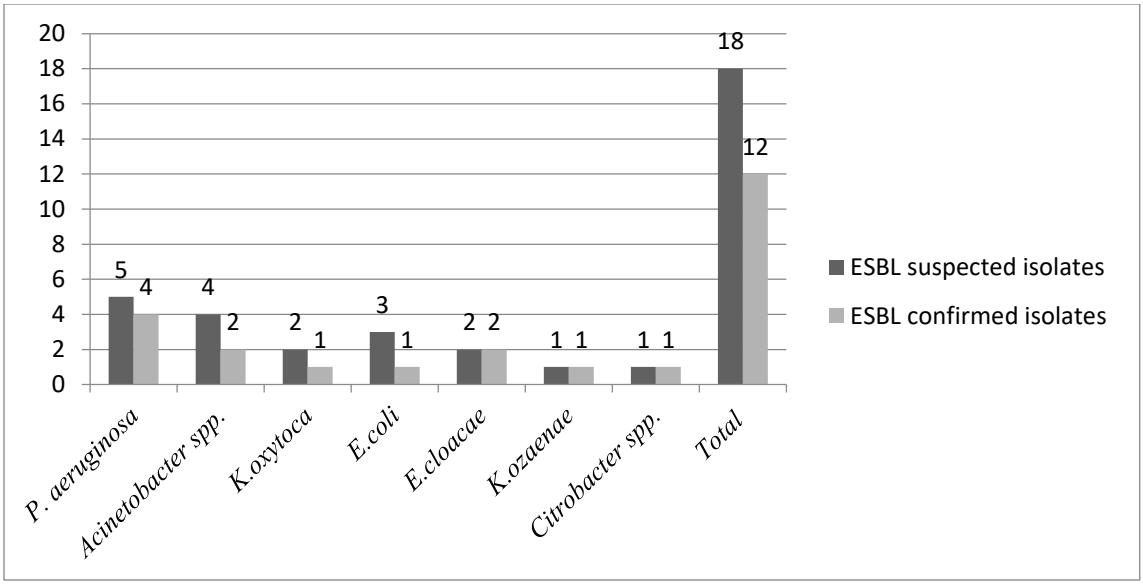

Figure 4. ESBL producers confirmed with Double disk synergy method isolated from environmental surfaces and medical equipment at Zewditu memorial Hospital from June-November 2024.

5.3.1. Comparison of double disc synergy test with the combination disc test

According to the double disk approach, 66.66% of ESBL cases (n=12/18) were confirmed whereas using combination disc test 72.2% (n=13/18) of ESBL cases were confirmed to be ESBL producer. Just 7.69% (n=1/13) were negative, compared to 92.3% (n=12/13) who were positive using double disc synergy method out of the 100% (n=13/13) who were positive using the reference (combination disc test) method. Out of the bacteria suspected of having ESBL, only 33.3% (n=6/18) were verified to be ESBL negative using this double disc synergy test.

5.4. Carbapenemase-producing bacteria

From the 49 identified bacteria, 18.2% (n=6/49) of them showed intermediate or resistance pattern to imipenem and/or meropenem which were suspicious for carbapenemas production. They were confirmed phenotypically by using the Modified carbapenemase inactivation method (MCIM). *Acinetobacter spp.* 33.3% (n=2/6), *K. ozaenae* 16.6% (n=1/6), *K. oxytoca* 16.6% (n=1/6), *K. pneumoniae* 33.3% (n=2/6) were carbapenem resistance suspected isolates. From a total of 6 isolates which showed resistant or intermediate zone for meropenem, 100% (n=6/6) were positive for carbapenemase production by modified carbapenemase inactivation method (MCIM) test, having a general prevalence of 12.22% (n=6/49) from the total gram negative bacteria isolated (figure 5). The predominant carbapenemase producing organisms in this study

were *Klebsiella* species 8.16% (n=4/49) which were *K. pneumoniae* 66.6% (n=2/3), *K. ozaenae* 50% (n=1/2) and *K. oxytoca* 33.3% (n=1/3). The second carbapenemase-confirmed bacteria was *Acinetobacter spp*. 25% (n=2/8).

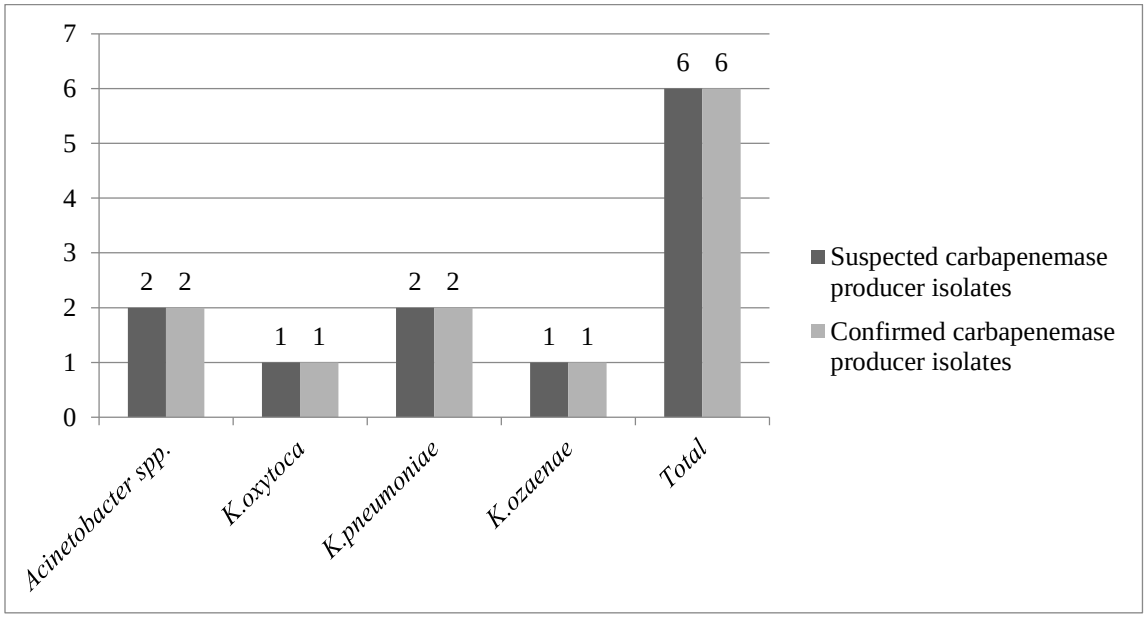

Figure 5. Carbapenemase producer confirmed with MCIM isolated from environmental surfaces and medical equipment at Zewditu memorial Hospital from June-November 2024.

5.5. Methicillin resistance *staphylococcus aureus* (MRSA)

Out of 6 *S. aureus*, 50% (n=3/6) were detected as MRSA using cefoxitin as a surrogate marker (figure 6). Those confirmed MRSA were found in adult intensive care unit (AICUs) 33.33% (n=1/3), neonatal intensive care unit (NICUs) 33.33% (n=1/3), and caesarian section operation room (CS OR) 33.33% (n=1/3).

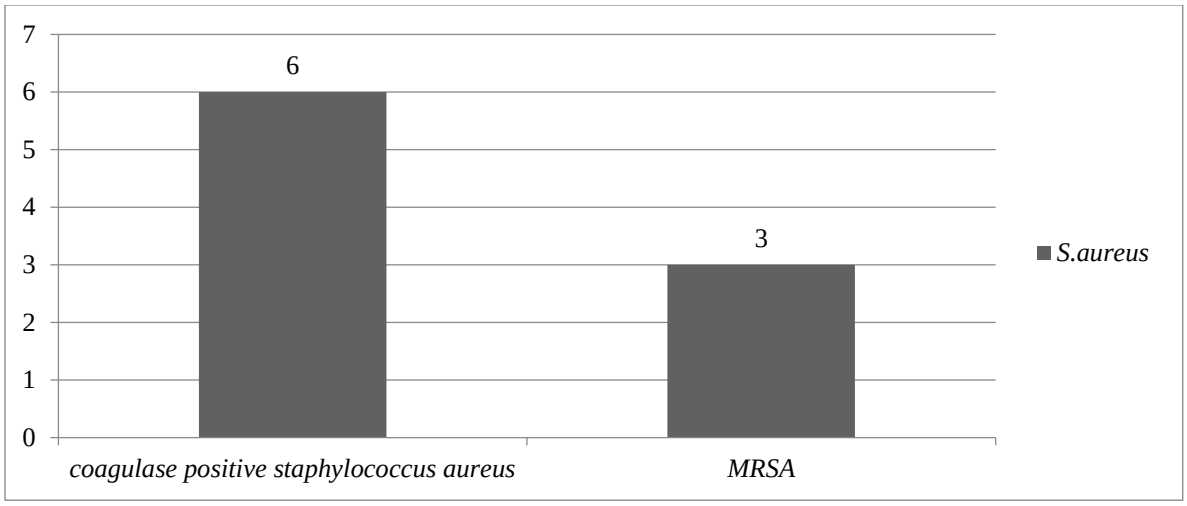

Figure 6. Methicillin Resistance *S. aureus* (MRSA) isolated from environmental surfaces and medical equipment at Zewditu Memorial Hospital from June-November 2024.

6. Discussion

In the current research, out of 204 environmental specimens from swabs, 158 (77.4%) were positive for bacterial growth which has been founded lower from studies close to Tikur Anbessa Hospital (86%) (13), a nearby hospital to the current study site, from Mekelle, Northern Ethiopia (88.5%) (27) and it was close from a study conducted in Nigeria (70.3%) (33). In contrast to our result, another study from Bahir Dar, Northwest Ethiopia (39.6%)(39) showed a lowered contamination rate. Other studies conducted from Cameroon (50.4%) (30), Nigeria (46.3.4%) (29), Baghdad hospitals (3.7%) (26), and India (43.3%) (32) also reported far minimal rate of surface contamination. These discrepancies may be due to differences in ventilation systems, hand hygiene, and sterilization and disinfection methods.

Utilization of ineffective disinfectants when cleaning surfaces, poor application of common precautions like hand washing and contact precautions, and the movement of the microorganism through airflow could be the main causes of the increased bacterial contamination levels seen in our analysis. Hospitals with inadequate waste controls, a lack of knowledge about the degree of contamination and the inefficiency of the commonest used disinfectants, and a reluctance to invest in contamination control measures like ventilation systems are all strongly associated with this situation (40).

The findings of our analysis indicated substantial contamination of inanimate objects by different gram-positive (71.3%) and gram-negative (28.7%) bacteria. Several authors from other studies have reported similar findings such as, in Ethiopia including Tikur Anbessa Hospital, which is situated close to our study site in Addis Ababa, (56.3% vs 43.7%)(13) and from other countries: Korea (73.2% vs 26.8%)(35), India (56.99% vs 43.01%)(24) and Nigeria (52.2% vs 47.8%)(41). The fact that these bacteria naturally sustain their survival in abiotic hospital circumstances for several days up to months due to the absence of the lipid-dominant, desiccation-prone outer membrane may account for gram positive bacteria's supremacy (13). Despite this, the growth and resistance patterns of gram-positive bacteria are quite diverse (42). However, contrary to our findings researches done in India (32), Indonesia(28) and Morocco(43) reported gram-negative bacteria as the major environmental isolates. Different sampling

times, utilization of various sampling approaches and culture methods, and changes in sampling items could all be contributing factors to these discrepancies.

Overall, in our study *CoNS* was the first frequently isolated bacteria (46.1%) and secondly *Bacillus spp.* (21.6%) and *P. aeroginosa* (10.5%) around the wards which is comparable with results from varied studies around the globe (24, 35). Even if *Coagulase-negative staphylococci* are normal human skin flora they have the potential to cause clinically significant infections of the bloodstream and other tissue sites (44). Immune impairment and the use of prosthetic materials, such as intravascular catheters, are risk factors for *CoNS* infection (45).

In this study, among the many surfaces and inanimate objects analyzed, the highest bacterial contaminated samples were yielded from tables, environmental surfaces, monitors, and bed linens which are consistent with findings from prior research conducted in Ethiopia and other countries.(13, 34, 46, 47). Environmental surfaces and tables were mainly contaminated by *CoNS* (29.1% and 45.45%), *bacillus* (37.5% and 18.18%), and *P. aeruginosa* (8.33% and 22.7%), respectively. Consistent findings were found on linens and beds samples from studies done in Mizan Tepi, Ethiopia (34). Cross-contamination from a patient's vegetation, healthcare personnel's hands, or contaminated patient and healthcare worker footwear could be the source of this type of contamination. In our study *Klebsiella spp.* were mainly found in adult intensive care units and neonatal intensive care units. Ventilator-associated pneumonia and bloodstream infection are caused by those bacteria. Consistent result was also documented from a research done in Iran (48).

The majority of Gram-negative bacteria in our study showed high resistance to most tested antibiotics, For instance, resistance rates were 67.3% for ampicillin, 61.2% for amoxicillin and clavulanic acid, 63.2% for ciprofloxacin, 63.2% for sulfamethoxazole-trimethoprim, 57.1% for cefepime, and 55.1% for piperacillin-tazobactam. This high resistance is likely due to the distinctive structure of Gram-negative bacteria. Our results are consistent with comparable resistance percentages found in past research projects carried out in Ethiopia and other African nations like Tikur Anbessa(13), Sudan(49), and Morocco(47). Higher resistance to β-lactams antibiotics is because of selective pressure exerted by the antibiotics (50). Because these analyzed antimicrobials are the most often used antibiotics in practice, prescribing them can

create significant challenges (13). In other way, a minimal resistance level was documented to antimicrobials like ceftriaxone 30.6%, meropenem 12.2%, and cefotaxime 16.3%. This is somehow consistent with findings from Sudan (49).

On other hand, gram positive bacteria showed high resistance to azithromycin 100%, penicillin 100%, clindamycin 100% and erythromycin 100%. Furthermore from *S. aureus* isolated in this study 50% were MRSA. In a similar way, an elevated resistance level was also documented from Ethiopia by a Meta-analysis study for erythromycin and penicillin with a pooled resistance level of 97.2% and 99.1%, respectively(51). Also, Tikur Anbessa Hospital found a comparable level of penicillin resistance which was 92.8%(13).

ESBL are produced by the nosocomial pathogens *E. coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter spp*. (52). Numerous factors, such as higher hospital expenses, length of stay, and fatality rates, make ESBL infections worrisome. Hospital-acquired infections have been found to be primarily caused by *Enterobacteriaceae* (53).

In our study the suspected isolates were phenotypically confirmed for ESBL using combination disk method and double disc synergy method. As a combination disc method the overall prevalence of ESBL bacteria were 26.5% (n=13/49). Our result was high compared to other study from Muzslay, M. et.al. work which was 3.1%(54), Tikur Anbessa hospital 15.3%, 9%, (55, 56) and 20.1% in Tanzania (57), these variations might be due to the difference in the number of patients that attended each hospital, sample size, the methodology used and geographic differences among the study areas.

In our study among the tested isolates *P.aeruginosa*, *Klebsiella spps*., *E.coli*, *Acinetobacter spp.* with ESBL phenotypes showed ESBL production. This is somehow in line to a study conducted in Tikur Anbessa hospital *Klebsiella spp*., followed by *Acinetobacter spp* . and *E.coli* (55). In Nepal, *Acinetobacter spp*., and *E.coli* were reported as the dominant ESBL producing bacteria(58) with some differences which could be due to differences in the overall prevalence of gram negative bacteria among countries and hygiene and general infection prevention measures.

The highest ESBL producing bacterial contamination was observed from environmental surfaces followed by oxygen cylinders. However, in another study the highest ESBL contamination

was observed from, chairs followed by pooled samples and Sinks (55). These differences may be due to differences in the cleaning practices of the hospitals.

In this study we compared double disc synergy methods with combination disc test for detection of ESBL Producing bacteria to know if it is the best suitable phenotypic method in Ethiopia context for application in routine bacteriology laboratory because combination disc test is expensive and cannot be done on routine bacteriology laboratory of our country. We have used statistical comparison to evaluate among the two methods.

92.3% (n=12/13) of the 100% (n=13) phenotypically verified ESBL producing isolates by CDT had an enhanced zone, as discovered by the double disk synergy method using any of the four disks with a disk spacing of 20 mm. This demonstrated DDST's significant sensitivity (92.3%) and specificity (83.3%), which can be used as a standard ESBL detection technique in hospital bacteriology laboratory's where technological resources and experience might be scarce.

Carbapenemase producer bacterial infections are a major source of morbidity and death. There have been reports of carbapenem resistance in various locations across the world, such as Korea(59). In the present study, we analyzed carbapenemase producing bacteria using modified carbapenemase inactivation method. Those isolates were *K. ozaenae*, *K.pneumoniae*, *K. oxytoca*, and *Acinetobacter spp*.. Carbapenemase producer bacterial isolates often develop resistance to other classes of antibiotics because of the transfer of genes or mutations in different genetic loci (60). In our study we found an overall 12.2% prevalence of carbapenemase producer bacteria. This finding was higher with the research done in Korea which showed 0.4% of Carbapenemase producer *enterobacteriaceae*(59). The higher prevalence in our study may be attributed due to the reality that geographical variation, sample size and hygienic practice differences.

Renowned nosocomial pathogens *S. aureus* and MRSA are linked to a number of clinical problems in intensive care units and operation rooms. In our study mostly ICUs were contaminated with *S. aureus.* Among the isolated *S. aureus* 50% of them were MRSA. the overall prevalence of MRSA was 2.45% which was very low compared to study done in Ethiopia 85.7% (13), manipal teaching hospital at Nepal which was 33.3%, 54.4% (40, 61) and 19.5% in Tanzania (62). This might be because of sample size difference, hygienic practice difference and geographical variation.

Compared to other locations, the surfaces of bed linens and bed trails produced the greatest number of *S. aureus* isolates. One of the surfaces that patients, visitors, and healthcare professionals touch the most is bed linens and bed trails. Intensive care units (ICUs) and operation rooms (ORs) patients are frequently at risk for nosocomial infections. MRSA and *S. aureus* contamination on these places raises the patients' probability of transmission and can cause pneumonia and septicemia.

The results of our study are crucial in raising knowledge on the infection control team and healthcare workers about bacterial agent contamination of the intensive care units (NICUs) and operating room (ORs) and its potential link to nosocomial infections.

Strength and limitations of the study

Strength

- We have met our objectives and assessed bacterial profiles and multidrug resistance level of pathogenic bacteria from inanimate surfaces.

Limitations

- Our research doesn’t include all wards found in Zewditu Memorial Hospital.
- The results may not be generalizable because this was a single center study.
- The relationship between nosocomial infections and contaminated objects or devices was not investigated by us.

7. Conclusion and recommendation

Coagulase negative staphylococcus aureus, Bacillus species, Acinetobacter species, staphylococcus aureus, and *Pseudomonas aeruginosa* were the commonest bacteria identified which are likely to cause healthcare-associated infections. The rate of the isolates' antimicrobial resistance profile increased in clean inanimate environments. The current investigation revealed a significant level of multidrug-resistant bacterial contamination of inanimate objects in hospital settings. The findings of this research highlight the necessity of strong infection prevention strategies and ongoing hospital environment surveillance to curb the spread of resistant bacteria in the hospital.

We suggested that in order to lower the possible danger of pathogenic bacteria and resistant strains spreading in the hospital environment, particular emphasis should be paid to infection prevention and control policy, antimicrobial resistance screening, appropriate cleaning practice, and clinical practices. This is particularly important for vulnerable patient populations, such as neonates and patients in intensive care units (ICUS) and operating rooms (ORs). Moreover, extensive research is required to evaluate the clonal link between clinical strains and the inanimate surfaces.

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Annexes

Annex I: Data collection form

1. Location _____
2. Code number _____
3. Culture results;
    - MacConkey result_____
    - Blood agar result_____
4. Gram stain result from culture _____
5. Biochemical tests;
    - Indole_____
    - triple sugar iron agar_____
    - urea utilization test_____
    - citrate utilization_____
    - mannitol fermentation_____
    - decarboxylation on lysine iron agar_____
    - oxidase_____
    - catalase_____
    - coagulase_____
6. Organism isolated._____
7. Antimicrobial susceptibility pattern (S-sensitive, I-intermediate, R-resistance)
    - Amikacin____
    - Amoxicillin-clavulanic acid (AMC)_
    - Gentamycin__
    - Ampicillin___
    - Cefotaxime___
    - Ceftazidime__
    - Ceftriaxone___
    - Chloramphenicol____
    - Ciprofloxacin_

- Cefepime ____
- Ertapenem ___
- Imipenem/Meropenem_____
- Trimethoprim-sulfamethoxazole (SXT) ____
- Piperacillin-tazobactam_____
- Gentamycin __
- Cefoxitin ____
- Penicillin ____
- Azithromycin_
- Clindamycin__
- Erythromycin_
- Tetracycline__

8. Phenotypic test for ESBL

- Screening test_____
- Double disc synergy test_____
- Combination disc test_____

9. Phenotypic test for Carbapenemase producer

- Screening test_____
- Modified carbapenemases inactivation test_____

10.Phenotypic confirmatory for MRSA_____

Annex II: 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: Hiwot Lidet (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors:

Dr. Melese Hailu (BSc, MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Mrs. Meron Yohannes (BSc, MSc, PhD Candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia