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Bacterial profiles and antibiotic sensitivity pattern of isolates from medical devices and inanimate surfaces from ICU room at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia.

Principal investigator: **Mulugeta Gebremedhin (BSC, MSC Candidate)**

Advisors: **Dr. Abreham Tesfaye (MSc, PhD)**

Mr. Melese Hailu (BSc, MSc, PhD Candidate)

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This is to certify that the thesis prepared by Mulugeta Gebremedhin, entitled:

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External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____ Date _____

Advisor_____Signature _____ Date _____

Advisor_____Signature _____Date _____

Advisor_____Signature _____ Date _____

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Abbreviations

AAU	Addis Ababa University
AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
CHS	College of Health Science
CLSI	Clinical Laboratory Standard Institute
CONS	Coagulase- Negative Staphylococcus
DMLS	Department Medical Laboratory Science
ELISA	Enzyme-Linked Immune Sorbent Assay
ESBL	Extended Spectrum B-Lactamase
GNB	Gram Negative Bacteria
GPB	Gram Positive Bacteria
HAI	Hospital Acquired Infection
HCAI	Health care-associated infection
HCWs	Healthcare workers
ICU	Intensive Care Unit
IPC	Infection Prevention Control
ISO	International Organization for Standardization
MDR	Multi Drug Resistance
MICU	Medicine Intensive Care Unit
MRSA	Methicillin Resistant *Staphylococcus aureus*
NI	Nosocomial Infections
NICU:	Neonatal Intensive Care Units
PICU	Pediatric Intensive Care Unit
SICU	Surgery Intensive Care Unit
SPSS	Statistical Package for Social Science
USA	United States of America
VRE	Vancomycin Resistant Enterococcus
WHO	World Health Organization

Abstract

Background: Infections acquired in hospitals are a major public health concern around the world, especially when caused by multidrug-resistant (MDR) microorganisms. Bacteria, including MDR isolates, were found in critical care units near the patient or nearby environment (inanimate surfaces and equipment). Microorganisms may be transmitted indirectly via inanimate surfaces and equipment, which could play a substantial role in ICU-related infections.

Objective: To assess bacterial profile and antibiotic sensitivity pattern of isolates from medical devices and inanimate surfaces of ICU room at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia.

Methods: A hospital-based cross-sectional study was conducted from April to June 2021 in Yekatit 12 Hospital Medical College in Addis Ababa. Swabs were collected twice in 30 days from medical devices and inanimate surfaces of ICU room. A total of 136 samples were collected by swabbing hospital surfaces, and the bacterial isolates were identified using Gram's staining, standard culture, and biochemical tests. Besides, the respective antimicrobial sensitivity tests were checked for antibiotic susceptibility using the Kirby–Bauer disk diffusion. SPSS Version 25 was used to analyzed and present the results in terms of percentage and table.

Result Bacterial growth was found in 45 (33.1%) of the 136 samples tested. A total of 56 bacterial pathogens were recovered from all specimens processed during the study. Gram-positive bacteria accounted for 16 (28.6%), while Gram-negative bacteria accounted for 40 (71.4%). Among the Gram-positive isolates, Coagulase-negative *Staphylococci* 13(23.2%) were frequently isolated followed by *Bacillus* species 2(3.6%). *Klebsiella* species 21(37.5%) were most isolated of the Gram-negative rods. The range of resistance for Gram-positive and Gram-negative bacteria were from 87.5% – 100% and 30% – 100% respectively. From the study participants, 32(82.1%) clean surfaces of medical equipment and inanimate, and 33 (84.6%) cleaned the reusable equipment before storage or used on next patient.

Conclusion: In the ICU, the rate of bacterial contamination found is high and mostly resistance to different types of anti-microbial drugs were prescribed. The infection control and prevention unit in the hospital is better to adopt observation on a regular basis.

Keyword: Bacterial profile, non–critical medical equipment, inanimate surfaces, antibiotic sensitivity

1. Introduction

1.1 Background

Nosocomial infections, previously known as hospital-acquired infections, are infections recently acquired whilst the patient is receiving health care in the hospital or other healthcare facilities. It will occur within two to three days of admission or one month or after discharge from its complication, resulting in, substantial mortality, morbidity & incurred costs, as well as increasing hospital stay, [1]. The infection spread in multiple ways to susceptible people in the health care system, such as by health care workers, infected instruments, medical records, colonized inanimate surfaces, colonized patients, contaminated surfaces and equipment used on the prior patient and MDR isolate included. The appearance of bacteria on inanimate surfaces, and equipment is similar. It has been documented that there is regular VRE and MRSA contamination of surfaces in the USA, [2].

Contamination of ICU patients occurs by cross-transmission and distribution, occupancy rate, often subject to non-critical equipment, i.e., objects which come into contact with skin that is intact, that includes blood pressure cuffs, stethoscopes, and electrodes for pulse oximetry, ultrasound transducers and leads for electrocardiography, etc., [3]. The type of organisms, source and destination surfaces, humidity level, and size of inoculums are the determinant conditions that may affect the transfer of microorganisms from one surface to another and cross-contamination, but other factors such as hand hygiene compliance, nurse-staffing levels, frequency/number of colonized or infected patients also play a role in contamination and cross-transmission rate in the ICU, [4].

Furthermore, care-related risk factors that increase the risk of infection include intensive care stay, the presence of invasive medical devices, antimicrobial therapy, overcrowding and understaffing, ward layout, and contact with colonized/infected family members, visitors, or healthcare workers. The proximity of colonized neonates, as well as increased length of stay, failed to meet standards are several risk factors for nosocomial, [5].Bacteria are responsible for approximately 90 percent of hospital-acquired infections, while protozoan, fungi, viruses, and mycobacteria are less attributed to nosocomial infection, [6].The commonest causative agents bacterial pathogens have a minor role in ICU contamination, but clinically important gram-positive and negative comprise *Staphylococcus aureus*, coagulase-negative *staphylococci, Enterobacteriaceae*, *Enterococci,* as a

major causative agent which contribute to nosocomial infection. Emerged as multidrug-resistant pathogens and compound infection control the MDR pathogens like *Enterobacteria* and *Acinetobacter baumannii*,[7].

Hospital-acquired infections can be prevented by introducing appropriate control measures. Recognizing the pathways, underlying cross-transmission of pathogens. Defining which locations are infected more often and what is more widely reported, [8]. Besides, the existing CDC guidelines also prescribe disinfection with a low to moderate level disinfectant of surgical equipment surfaces, bedside equipment, and environmental surfaces (e.g., bed rails, bedside tables, carts, commodes, doorknobs, and faucet handles) to avoid transmission of healthcare-related diseases and surface decontamination procedures and hand-touch locations, [9].

The foundation of hand hygiene is to reduce the risk of cross-infection by practicing hand hygiene before and after interaction with potentially infected patient care devices and intrusive treatment for each patient. It is recommended to include instruction on hand hygiene and IPC protocols for new or relocated workers, re-activation of infection prevention committees, and allocation of the budget for infection prevention from the budget of health facilities and where assistance is given at the national and regional levels, the introduction of the active surveillance policy becomes successful,[10].

The use of antibiotics to treat bacterial infections has contributed to a decline in mortality and morbidity. But bacteria may develop resistance to antimicrobial agents. Microorganisms in natural body flora, which are susceptible to the given medication, appear to be suppressed, and resistant strains are picked and endemic in the hospital population, [11]. Overprescribing, suboptimal dose administration, insufficient time for treatment and misdiagnosis are risk factors for the development of resistance against antimicrobial agents Co-occurring diseases are also commonly recognized as risk factors for MDR to develop, [12].

1.2. Statement of the Problem

The majority of nosocomial infections are spread through direct contact with environmental surfaces or equipment. Several studies have reported that it might be a recognized cause of common-source outbreaks of infection that rise from contaminated surfaces in hospitals, [13]. Patients who are admitted to an intensive care unit for an extended period are at a higher risk of contracting an infection. Globally, the impact of HAI on hospitalized patients in the intensive care unit (ICU) is already significant. In developed countries, the prevalence of ICU acquired infections is estimated to be 5–10%, whereas, in developing countries, the prevalence is estimated to be 2–20 times higher, [14].

The issue of environmental contamination poses a significant challenge in the intensive care unit (ICU), where critically ill patients are predisposed to nosocomial infection due to a number of risk factors. The findings of a study conducted in ICU rooms in the United States of America revealed the relative contribution of various potential sources of ICU-acquired infections: 40–60% of the patient's endogenous flora, followed by cross-infection via HCWs' hands (20–40%), antibiotic-driven changes in flora 20–25% and other sources (including environmental contamination,(20%), [15].Since microbes form a biofilm, which helps to shelter harmful factors in the environment and attach it by reducing the efficacy of terminal cleaning procedures and bacteria to survive in the environment for a long period of time and provides increased resistance to commonly used disinfectants, infection control in the ICU has been a major problem, [16].

Furthermore, Gram-negative and Gram-positive bacteria can survive for months on dry inanimate surfaces under low temperature and humidity conditions, allowing for greater persistence in the environment, [17]. Nosocomial bacterial pathogens like MRSA, VRE, *Pseudomonas* spp., and *Acinetobacter* spp., are more stable in the hospital environment than fastidious organisms, like *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae* which are delicate, fastidious, and rapidly inactivated after excretion, Organisms from hospital objects are likely to be transmitted to healthcare workers, patients, and visitors, [18].

Antibiotic resistance continues to have a significant impact on HAIs in the ICU. The majority of HAIs are caused by multidrug-resistant microorganisms that enter the ICU via contaminated surfaces or devices or from patients. Furthermore, antibiotic resistance may be occult and silently

introduced into the ICU by a commensal member of patients' or health care personnel's microbiomes, and then transmitted, [19].

Non-critical medical equipment and inanimate surfaces have the capacity to harbor bacteria for an extended period of time and are available in contact with patients and medical personnel during disease management. Because these items come into contact with intact skin but not mucous membranes, they frequently receive insufficient attention from HCW and attendants,[12]. A Study conducted at ICU medical equipment and inanimate surfaces indicate that bacterial growth was identified in 88.5% of the swab specimens, Coagulase-Negative *Staphylococcus* 34.9%, *Staphylococcus aureus* 26.3%, *Citrobacter freundii* 9.2% and *Klebsiella pneumoniae* 8% were the most commonly isolated bacteria,[20].Inanimate surfaces in the vicinity of patients and highly-frequently touched surfaces within the hospital environment are reservoirs of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*, [21].

Despite seriousness of the problem, few studies were conducted on healthcare facilities and inanimate surfaces in the hospital, in Ethiopia and in Intensive Care Units. Therefore, this study was identifying the bacterial profile of the isolates from healthcare devices and inanimate surfaces from the ICU room in Yekatit 12 Medical College, Addis Ababa, Ethiopia, as well as the antibiotic sensitivity pattern.

1.3. Significance of the study

- The significance of this study is to sight the rate of bacterial contamination and antimicrobial pattern of non – critical medical equipment and inanimate surfaces within immediate patient surrounding that could be the potential source of health risk in ICUs.
- Evidence-based knowledge of the level of contamination in the hospital environment is vital for developing and implementing effective prevention and control measures for and other types of hospital acquired infections. Furthermore, the findings of the study may provide insight for health experts. This research is also helpful in identifying the drug sensitivity patterns of isolates to the most commonly used antibiotics in the area.

2. Literature Review

Bacterial contamination of inanimate hospital surfaces and equipment is thought to be a key contributor to the development of numerous nosocomial diseases across the world,[22]. A systemic review has estimated the prevalence of HAIs to be 7.6% in high income countries and 10.1% in low- and middle-income countries, [23]. However, high incidence (> 50%) of ICU acquired infections are reported as compared with other wards (10%), [24].

A growing body of evidence supports the contribution of inanimate surface and equipment contamination for transmission of pathogens to ICU patients,[15].Different hospitals have different bacterial profiles in their intensive care units, the only common factors seem to be that patients in intensive care units were more prone to bacterial infections given their compromised health state and that ICUs and NICUs have high bacterial contamination rates in inanimate surfaces and medical equipment,[25].

According to studies conducted in various ICUs, the most frequently isolated gram-negative organism was *E. coli* (12.08%), followed by *Acinetobacter (*8.75%), *Pseudomonas* (7.5%), and *Klebsiella* (6.25%), whereas among GPBs, the most common colonizers in the ICUs were *S. aureus* (15%) and CoNS (14.16%),[26]. One study was conducted at tertiary care hospital multidisciplinary intensive care units (ICUs) in Northern India. Of 100 stethoscopes, 56 have been found contaminated. Among the hospital areas, the highest contamination among stethoscopes being used by HCWs maximum contamination was observed in General medical wards 32 (71%) and emergency ward and labor ward14 (70%)) followed by Outpatient wards 6 (40%) and Intensive care units 4 (20%). Among 79% were positive bacteria growth observed from these, *Acinetobacter* was frequently isolated (39.2%), followed by *Klebsiella pneumonia* (27.8%), *S. aureus* (15%), *E. coli* 12% and *Enterococcus* (6%), [27].

A study done in Iraq revealed that out of 320 samples analyzed, from seventy different places in the ICU environment. Among the isolates obtained from these places, the most isolated were Gram-negative bacteria 44 (64.71%) followed by Gram-positive bacterial isolates 24 (35.29%). The highest rate of contamination had been found on the walls and the floors (19.11%). *Bacillus* spp. and *Enterobacter* spp. were the most commonly isolated bacteria in this study, [28].

A total of 160 samples were collected from the ICU environment in a study conducted in the Intensive Care Unit of a Tertiary Hospital in Incheon, Korea, of which 407 bacteria were identified

.The commonest isolated bacteria were Coagulase Negative *staphylococci* (CONS) (54.5%), followed by *Acinetobacter baumannii* (11.8%), *Pseudomonas aeruginosa* (8.1%), and *Enterococcus faecium* (5.9%), Keyboards (38 strains), bed linen sheets (average head, waist, and foot seats) (36 strains), bedside rails (33 strains), and curtains (27 strains) were the common isolation sites, [29]. A cross-sectional study conducted in Taiwan on contamination of patients' medical files showed that 90% percent of charts in the surgical ward and 72% in the ICU were contaminated with bacteria pathogens. The most isolated gram-positive bacteria were Coagulase-Negative *Staphylococcus*, which was the predominant isolate in both the ICU (44%) and the surgical ward (53.3%). Whereas the most isolated Gram-negative bacteria were *Klebsiella species* and *Acinetobacter* Spp., [30].

According to a study conducted in Saudi Arabia, 85.2% of patients' files in the ICU were found to be contaminated with pathogenic bacteria, while in surgical wards, 24.7% of patients' files were found to be contaminated. This study also revealed that *P. aeruginosa* was the most commonly isolated bacteria (32.3%) in the ICU, followed by *K. pneumoniae* (14.7%), *A. baumannii* (13.7%) and *S. marcescens* (0.9%), [31]. Another study conducted in medical hospitals of Shahid Beheshti University of Medical Sciences in Tehran, Iran reported that *K. pneumoniae* was the most frequent coliform bacteria isolated from the surface samples of medical equipment, ICU environment and HCWs' hands (1.8%). *E. coli* and *Enterococci*, were also observed in approximately 0.5% and 1.3% of the samples, respectively, [32].

The research was conducted in the Intensive Care Units of a Tertiary Hospital in the Zimbabwean city of Bulawayo, with 58 swabs collected from fomites and medical devices.50 (86.21%) were positive for bacterial contamination were recognized as the most prevalent isolated Coagulase-Negative *Staphylococci* (CONS) (20.31%) and followed by *Klebsiella* species (20.31%). A total of 51 (75%) of the 68 bacterial isolates were resistant to at least 3 antibiotics,[33].

Some reference hospitals in Assiut City, Egypt have carried out a study. Of the total 12863 samples collected from the ICU's Inanimate Surfaces and Equipment, 25.6 percent were bacterial-positive samples and the most commonly isolated were Coagulase-Negative *Staphylococci* (26 percent) and K. pneumonia (10.6 percent), [34].

The Windhoek Central Hospital, Windhoek, Namibia, conducted another study in the Neonatal Intensive Care Unit (NICU). Samples were collected from various environmental surfaces. Overall, surfaces swabbed, 52.8% showed bacterial growth. From this, the most frequently isolated bacteria were Coagulase-Negative *Staphylococci* (CONS) 14 (70%), followed by *Enterobacter* spp. (15%), *Pseudomonas aeruginosa* (5%), *Staphylococcus aureus* (5%) and *Acinetobacter* spp. (5%), Most of the organisms were isolated from wash basins (10%), bed linen (5%), bed trolley (5%), stethoscope (5), weighing scale (5%), saturation probe (5%), saturation on machine monitor (5%), CPAP line (5%) and humidifier (5%) respectively,[35].

In a Gezira State study in Sudan, 50 swab samples from 14 sites, including inanimate artifacts and hands of nurses, were collected. Coagulase-Negative *staphylococci* (30%), *Staphylococcus aureus* (20%), *Bacillus spp* (15%), and *Streptococcus spp* (4%). were identified as 85 isolates. Their data were: The Gram negatives were, however, the following: *Pseudomonas aeruginosa* (11%), *Klebsiella pneumoniae* (7%), *Proteus mirabilis* (5%) and *Enterobacter spp* (5 percent).The most contaminated sites were floor, monitors, oxygen masks, infusion stalls and the hands of nurses, [36].According to systematic review and meta-analysis done in Ethiopia, the pooled prevalence of bacterial contamination of inanimate surfaces and equipment was found to be 70%.Among the Gram-negative bacterial species, the prevalence of ampicillin-resistant *K. pneumoniae* was the highest 80% followed by *Citrobacter* species 78% ,[22].

The study conducted by Fitsum and his colleagues showed that the overall rate of bacterial contamination in two units of the hospital, the intensive care unit (75%) and medical wards (73.5%). Among Gram-positive bacteria, *S. aureus* was the most frequently isolated (35%), and regarding Gram-negative bacteria, the most frequently isolated *Klebsiella pneumonia* (12.4%) was followed by *E. coli* (7.3%), *Proteus spp*. (6.6%) and *Salmonella spp*. (5.8%), [37].Another study carried out by Darge and his colleagues in 2019 collecting samples from the ICU from various medical equipment and inanimate surfaces reported that the most commonly isolated bacteria were Coagulase-Negative *Staphylococcus* 53 (34.9%) followed by *S. aureus* 40 (26.3%). (8 percent). From the contaminated medical device, 32 (34.8%) CoNS, 23 (25%) *S. aureus*, and 13 (14.1%) *C. freundii*, while 28 (35.4%), 23 (29.1%) *S. aureus* and 5 (6.3%) *K. pneumonia* were the most common isolated bacteria from inanimate surface swab samples, [20].

Another study was conducted at TASH (Tikur Anbessa Specialized Hospital) and ALERT (All Africa Leprosy Rehabilitation and Training) by collecting 280 samples from the ICU, among which 282 bacteria were isolated. Frequently isolated bacteria from the inanimate surfaces of ICUs were *S. aures* (9.6%) followed by Coagulase-Negative *Staphylococcus* (CONS) (2.8%) and *Acinetobacter* Spp. (14.5 %.), [38].

3. Objectives

3.1. General objective

- To assess Bacterial profiles and antibiotic sensitivity pattern of isolates from medical devices and Inanimate Surfaces from ICU Room at Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To identify bacterial profiles of non-critical medical devices and inanimate surfaces in the immediate patient environment of the ICU Room.
- To determine the antimicrobial susceptibility pattern of isolated bacteria from non-critical medical devices and inanimate surfaces in the ICU Room's immediate patient surroundings.
- To assess infection prevention practices of health care workers in the ICU Room.

3.3. Hypothesis

H0: -There is no significant difference in bacterial profiles and antimicrobial susceptibility pattern of medical equipment and inanimate surfaces between the current studies and other studies in our country.

4. Materials and methods

4.1. Study design and Study Period

Hospital-based cross-sectional study was conducted from April to June, 2021.

4.2. Study area

The study was conducted in Addis Ababa, at Yekatit 12 hospital medical college. It is found near Sidest kilo, in Arada Sub-City of City Government of Addis Ababa. It was established in 1915 and its former name is Kedamawi Haile Selassie. The hospital began operations with a total of 25 beds and 37 health professionals with few facilities and has since grown to include 912 health professionals and 372 administrative staff, as well as around 370 beds and many departments that provide various services such as screening and diagnostics, specialized procedures, specialty and subspecialty clinics. It serves more than 5 million people in the catchment area. The hospital accepts the patients through a referral system and a private wing.

According to the data obtained from the hospital, currently, it is the only pioneer hospital under the city Administrative Health Bureau of Addis Ababa providing services for the high number of neonatal patients. Culture and drug susceptibility test services are also available in the hospital. According to current HIMS, PICU, NICU, and AICU i.e. MICU and SICU have a total of 42 beds and have around 165 admissions per month.

4.3. Population

4.3.1. Study Population

The study populations were all health personnel and non-critical equipment and inanimate objects suspected to harbor bacterial pathogens in ICUs room at Yekatit 12 HMC, AA, Ethiopia.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

-Non-critical equipment surrounding a patient

-Inanimate surfaces surrounding a patient

- Health professionals working in the intensive care unit

4.4.2. Exclusion criteria

- Non-functional Non-critical equipment
- New Non-critical equipment
- Health professionals not present at the time of study

4.5. Study variables

4.5.1. Dependent variables

- Bacterial profile
- Drug susceptibility pattern of isolates

4.5.2. Independent variables

- Infection prevention practices and control measures
- Non- critical medical devices
- Inanimate surfaces

4.6. Measurement and data collection

4.6.1. Sampling Method

All inanimate surfaces, non-critical medical equipment and health care workers found in ICU wards during the study period were included using a convenient sampling method**.**

4.6.2. Data collection procedure

Data collection Research-related information was collected from health care workers using questionnaires and observation checklists. This observational review and sample collection were carried out by qualified professionals. In addition, samples of inanimate surfaces and non-critical equipment within ICUs were collected to determine its bacterial profile and antibiotic susceptibility patterns. The measurement of bacterial infection in the ICUs was conducted using a swab process. The results of the laboratory investigation were collected using the provided formats which contain the details of the finding with AST results accordingly.

4.6.2.1. Sample collection

136 of Swabs were collected once in 15 days for one month of data collection period from all equipment's such as Pulse oximeter, blood pressure cuffs, stethoscopes, care trolleys, bedrails and computers, bedside tables, Incubator, weigh scale, bed sheet, blanket and manual suction.

The samples were taken while they were going about their daily activities and without the knowledge of health workers. Sterile swabs were moistened with 85% normal saline and then it was applied to high touching areas by observing from other areas and samples were collected by parallel spaced stripes, rotating them slightly, then on the same areas in perpendicular stripes according to ISO/DIS 14698-1. All the samples were labeled properly and immediately transported to the Microbiology Department of Yekatit 12 Hospital Medical College Laboratory for processing, [39].

4.6.2.2 Isolation, identification of bacteria and Interpretation of culture results

Blood agar (Oxoid, UK), MacConkey agar (Oxoid, UK), and Mannitol salt agar (MSA) were inoculated with the obtained swab samples, and the plates were incubated at 35°C for 18-24 hours. The inoculation plates were inspected after 24 hours of incubation, and bacterial isolates from culture-positive plates were identified at the species level using colony morphology, gram staining, and biochemical characteristics.

Gram stain, routine bacterial culture, and standard biochemical tests such as indole; triple sugar iron agar, urea utilization test, citrate utilization on Simon's citrate agar, motility test, mannitol fermentation, decarboxylation on lysine iron agar, and oxidase were used to identify Gram-negative bacteria. Gram positive bacteria, on the other hand, were identified using Gram stain, selective culture media, and various biochemical tests such as catalase, coagulase test, and salt tolerance test, while golden yellow colony color indicated Mannitol fermentation, which is a presumptive test for S. aureus, were performed and interpreted using laboratory SOPs.

4.6.2.3Antibiotic susceptibility testing (AST)

The susceptibility test for the isolates was investigated according to CLSI Guideline by using Muller-Hinton agar. The AST test was performed by using the Kirby Bauer test, which is a qualitative assay. Once isolated colonies were available from an organism that has been identified as a potential pathogen, the following steps followed; colonies selected, inoculums suspension prepared in saline, inoculums suspension standardized (turbidity measured by densitometer (0.5MF)), allowed a Mueller-Hinton Agar (MHA) plate to warm to room temperature so that any excess moisture was absorbed into the medium. Inoculated on the plate, antimicrobial disks added, plate incubated (16-18hours), inhibition zones measured. Then results were recorded according to

CLSI (2019), [40 and 41]. Antibiotics were tested for isolated Gram-negative and Gram-positive bacteria as listed below in the table-1 and table-2, respectively.

Antibiotics were tested for Gram-negative organisms (Oxoid)

Table 1: Antibiotics were tested for of Gram-negative bacterial pathogen

Antibiotic	Concentration(μg)	Antibiotic	Concentration(μg)	Antibiotic	Concentration (μg)
Ampicillin	10	Imipenem/ Meropenem	10	Doxycycline	30
Gentamycin	10	Cefotaxime	30	Cefuroxime	30
Augmentin	10/20	Ceftriaxone	30	Chloramphenicol	30
Norfloxacin	10	Cefepime	30	Cotrimoxazole	1.25/23.75
Tobramycin	10	Azithromycin	30	Cefixime	5
Amikacin	30	Ceftazidime	30	Ciprofloxacin	5

Antibiotics were tested for Gram-positive organisms (Oxoid)

Table 2: Showing antibiotics tested of Gram-positive bacterial pathogens

Antibiotics	Concentration(μg)	Antibiotics	Concentration(μg)	Antibiotics	Concentration(μg)
Penicillin	10	Ampicillin	10	Clindamycin	2
Ceftazidime	30	Erythromycin	15	Tobramycin	10
Vancomycin	30	Cotrimoxazole	1.25/23.75	Amoxicillin	10
Ceftriaxone	30	Gentamycin	10	Augmentin	10/20
Cefoxitin	30	Cefotaxime	30		
Cefuroxime	30	Azithromycin	30		

4.7. Data and laboratory Quality Assurance

The reliability of study findings was assured by implementing quality assurance methods throughout the whole process of data collection and laboratory work.

4.7.1 Pre-analytical

The processes of selecting appropriate tests, ordering, collecting, identifying and labeling (three label system i.e., PID, CODE, LSN), handling, and transporting biological samples and media preparation as per manufacturer instruction and laboratory Standard Operating Procedures (SOP) were strictly followed and checked media meet expiration date and quality control parameters per CLSI. Labeling containers, media, filling the forms were carried out per the standard guidelines. Aseptic techniques were implemented in all the steps of specimen collection and inoculation onto culture media to reduce contamination and all the process of pre-analytical steps were performed according to standard operating procedure.

4.7.2 Analytical

All materials, equipment and procedures were adequately controlled. The sterility and performance of culture media were tested and the media was checked by incubating overnight at 37^0c. Performance of MacConkey agar plate, blood agar plate, and MSA were tested using the control strains *E. coli* ATCC 25922, *P. mirabilis* ATCC 35659, *S. aureus* ATCC 25923, and *S. pneumonia* (patient strain), respectively. International control bacterial strains: *Escherichia coli* (ATCC 25922), *S. aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC27853), and *Klebsiella pneumonia* ATCC (700603) were used in controlling the potency of the drugs. For biochemical test media, bacteria with known positive and negative were inoculated into the media. Moreover, culture growth, biochemical test, and antimicrobial susceptibility test results were confirmed by specially trained experienced microbiologists working in the microbiology unit of the study site.

4.7.3 Post-analytical

All of the extracted information (filled with questionnaire and laboratory findings) was checked for legibility, completeness, consistency, and placed in a secure location. Cross-checking and data cleaning were done. During data cleaning and cross-checking, missing information was obtained by going back to the questionnaire and laboratory records. The data was stored on a CD as a backup. All laboratory isolates were stored as per the SOP of the study site.

4.8 Data analyses and interpretation

SPSS version 25.0 was used to analyze the work and to make inferences on the frequency of occurrence of the bacterial pathogens with non-critical equipment and inanimate surfaces and to show the resistance pattern to antibiotics. The finding was analyzed using descriptive statistics SPSS (the Statistical package for Social Sciences), and the results were explained by percentage and tables.

4.9 Data quality management

To ensure the data quality, one-day training on ethical issues on how to approach the respondent and how to administer the questionnaire was given to the data collectors and supervisors. A sample (10%) of the English version of the questionnaire was pre-test clarity, acceptability, and flow among the non-study subjects and functioning of Incubator and densitometer done. The data collection processes were checked by supervisors daily. The principal investigator conducted meetings with data collectors and supervision every day after the completion of the data to check data inconsistency and completeness. The systemic observation was carried out to control environmental sample sharing among the Intensive care unit. The principal investigator was controlling the overall activities.

4.10 Ethical considerations

Before data collection, ethical clearance or permission was obtained from Addis Ababa University, Department of Medical Laboratory Science. Then the formal support letter of cooperation was sent to Yekatit 12 hospital medical college**.**

4.11 Operational definitions

Multiple drug resistance (MDR): - In this study, it is defined as the resistance of bacteria to multiple antimicrobial agents, classes, or subclasses of antimicrobial agents, i.e. resistance to at least one antimicrobial drug in three or more antibiotics categories.

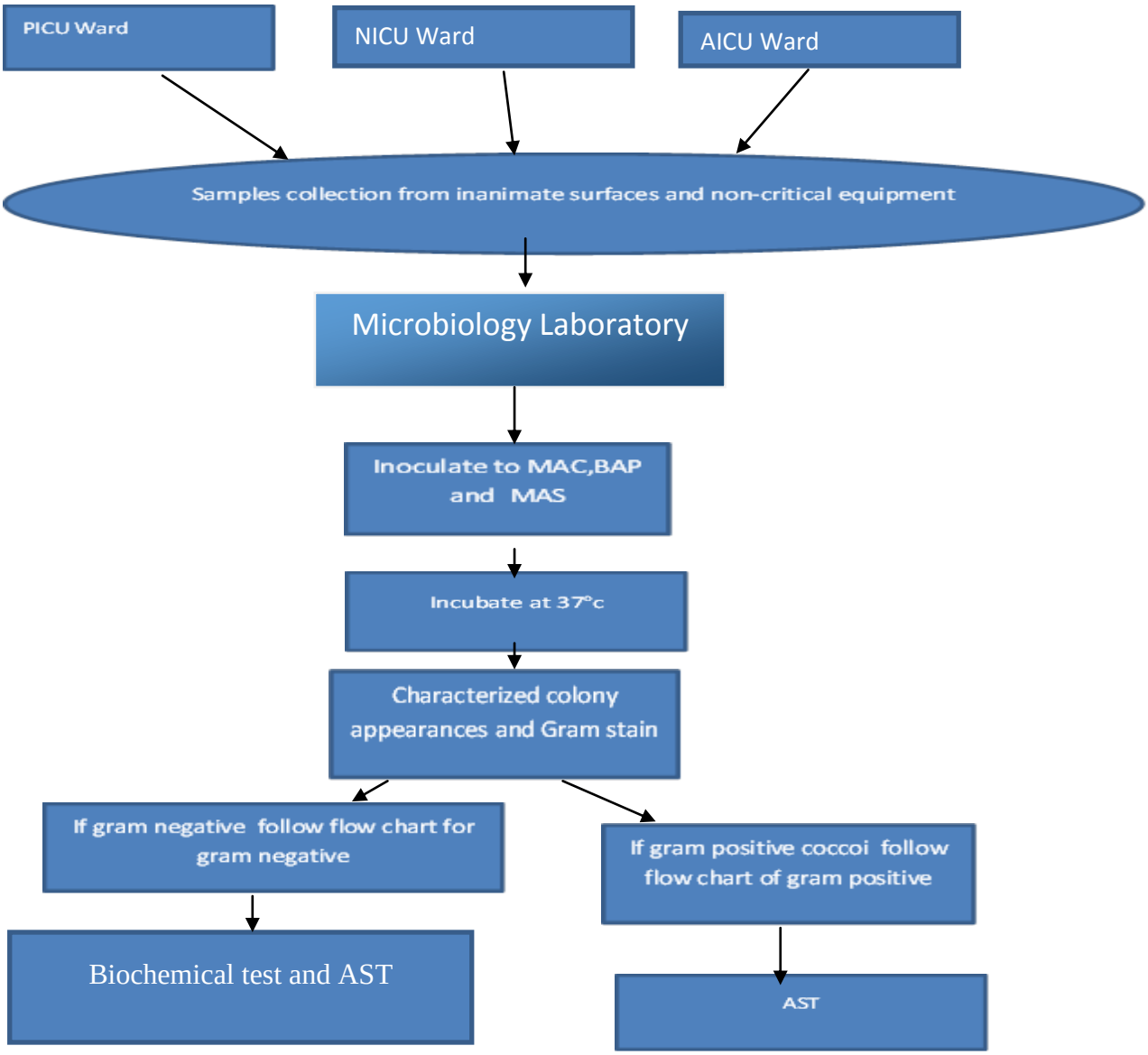
Non-Critical equipment: - are defined in the Spaulding classification system as those that come in contact with intact skin but not mucus membranes. They include such items as bedrails, patient furniture, blood pressure cuffs, crutches, and wheelchairs, [42].

Inanimate surface: - is a surface of the material used in the ICU for providing patient care such as bedside tables, mattresses, computers, and computer standing tables.

4.12 Work flow

A conceptual framework for isolation, identification and antimicrobial susceptibility testing

Figure 1. Flow chart for isolation of bacterial pathogens and antimicrobial susceptibility from non- critical equipment and inanimate surfaces in YHMC, Addis Ababa, 2021. Project management



5 RESULTS

5.7 Profile of bacterial isolates from non-critical medical equipment and inanimate surfaces

In the three ICUs, 136 specimens were collected from the ICU environment room by swabbing. Bacterial growth was shown in 45 (33.1%) of the total specimens. 12 specimens (26.7%) showed mixed bacterial growth whereas, 33 (73.3 %) had pure (single) bacterial growth. The remaining 91 (66.9%) revealed no growth of bacteria. A total of 56 bacterial pathogens were recovered in all specimens tested during the study. Among these, 16 (28.6%) were Gram-positive and 40 (71.4%) were Gram-negative bacteria. Among the Gram-positive isolates, Coagulase-Negative *Staphylococci* 13 (23.2%) were predominant followed by *Bacillus* species 2 (3.6%) and the Gram-negative isolates. (*Klebsiella* species 21 (37.5%) was found frequently and followed by *Acinetobacter* 10 (17.9%) and *Enterobacter* species (10.7%), while *Shigella* species, *E. coli* and *Citrobacter* were the least isolated with the prevalence of 1 (1.8%) respectively.

Table 3: Profiles of bacterial isolates identified in inanimate surfaces/medical equipment from ICU rooms in Yekatit 12 hospital, Addis Ababa, Ethiopia April 2021 to June 2021.

Bacterial isolates	Total	Percentage
Gram positive	16	28.6%
Coagulase Negative *Staphylococcus*	13	23.2%
Bacillus species	2	3.6%
Staphylococcus aureus	1	1.8%
Gram negative	40	71.4%
Klebsiella species	21	37.5%
Acinetobacter	10	*17.9%*
Enterobacter species	6	10.7%
Shigella species	1	1.8%
E.coli	1	1.8%
Citrobacter	1	1.8%

5.7.1 Bacterial isolates from non-critical medical equipment/inanimate surfaces in NICU

From the total 15 isolated bacteria in NICU, *Klebsiella* spp. 6 (40%) was the most often isolated organism, followed by *CoNS* and *Acinetobacter* spp. 3 (20%) respectively. Bacillus spp., *Shigella* spp., and *Enterobacter* spp. 1 (6. 7%) were shown to be the least often. The majority of bacteria identified on bed rails were isolated (Table 4).

Table 4: Profiles of bacterial isolates identified in inanimate surfaces/medical equipment in NICU in Yekatit 12 hospital medical college, Addis Ababa, Ethiopia April 2021 to June 2021.

Site	Number bacterial of isolates						Total
	Gram-positive		Gram-negative				
	CONs	Bacillus species	Acinetobacter species	Shigella species	Enterobacter species	Klebsiella species	
Pulse oximeter	-	-	-	-	-	1	**1**
Perfusor	1	-	-	-	-	-	**1**
Bed rails	1	1	-	-	-	1	**3**
Radiant warmer button	-	-	-	-	-	-	**-**
Medicine preparation trolley	-	-	-	-	-	-	**-**
Stethoscope (diaphragm)	-	-	-	-	-	-	**-**
Trolley	-	-	-	-	-	-	**-**
Incubator	-	-	-	-	-	-	**-**
Weighing scale	-	-	-	-	-	-	**-**
Glucometer	-	-	-	-	-	-	**-**
Bed sheet	-	-	1	-	-	-	**1**
Blanket	-	-	-	1	-	-	**1**
Mattress	-	-	-	-	-	1	**1**
Oxygen cylinder	-	-	-	-	-	-	**-**
Face mask	1	-	-	-	-	-	**1**
Ambu bag	-	-	-	-	-	-	**-**
Manual suction	-	-	1	-	1	-	**2**
Sink	-	-	1	-	-	1	**2**
Thermometer (NICU)	-	-	-	-	-	1	**1**
Oxygen saturation	-	-	-	-	-	1	**1**
Total	**3**	**1**	**3**	**1**	**1**	**6**	**15**

5.7.2 Bacterial isolates from non-critical medical equipment/inanimate surfaces in PICU

From the total 13 isolated bacteria in PICU, the most frequently isolated bacteria were *Klebsiella* spp.5(38.5%) followed by Coagulase Negative *Staphylococcus 4 (30.8%) and Acinetobacter* spp.2 (15.4%) while the least isolated bacteria were *bacillus* spp. With the incidence of 1(7.7%). Most bacteria isolated observed on a bed sheet (Table 5).

Table 5: Profiles of bacterial isolates identified in inanimate surfaces/medical equipment in PICU in Yekatit 12 hospital Medical College, Addis Ababa, Ethiopia April 2021 to June 2021.

Site	Number of bacterial isolates						Total
	Gram positive		Gram negative				
	CONs	Bacillus species	Acinetobacter species	Shigella species	Enterobacter species	Klebsiella species	
Ambu bag	1	-	-	-	-	-	**1**
Per fuser	-	-	-	-	-	-	**-**
Manual suction	-	-	-	-	-	-	**-**
Stethoscope (diaphragm)	-	-	-	-	-	-	**-**
Bed rails	1	-	1	-	-	-	**2**
Blanket	-	-	-	-	-	2	**2**
Bed sheet	1	-	-	-	-	2	**3**
Glucometer	-	-	-	-	-	-	**-**
Ventilator	-	-	-	-	-	-	**-**
BP apparatus	-	1	-	-	-	-	**1**
Medicine preparation trolley	-	-	-	-	-	-	**-**
Oxygen cylinder	-	-	-	-	-	-	**-**
Monitor	-	-	-	-	1	-	**1**
Sink	-	-	1	-	-	-	**1**
Bedside table	1	-	-	-	-	-	**1**
mattresses	-	-	-	-	-	1	**1**
Total	**4**	**1**	**2**		**1**	**5**	**13**

5.7.3 Bacterial isolates from non-critical medical equipment/inanimate surfaces in AICU

From the total 28 isolated bacteria in PICU, showed that *Klebsiella* spp. 10(35.7%) was the most frequently isolated followed by Coagulase-Negative *Staphylococcus 6* (21.4), *Acinetobacter* spp 5 (17.9%) and *Enterobacter* spp. 4(14.3) respectively. While *E. coli*. *Citrobacter*, *S. aureus* 1 (3.6%) were least identified respectively. Most bacteria isolated found on bed side table (Table 6).

Table 6: Profiles of bacterial isolates identified in inanimate surfaces/medical equipment in AICU in Yekatit 12 hospital, Addis Ababa, Ethiopia April 2021 to June 2021.

Site	**Number of bacterial isolates**									**Total**
	Gram positive			**Gram negative**						
	CONs	**Bacillus species**	**Staph. Aureus**	**Acinetobacter species**	**Shigella species**	**Enterobacter species**	**Klebsiella species**	**E. coli**	**Citrobacter**	
Ambu bag	-	-	-	-	-	-	-	-	-	-
Bed sheet	-	-	1	-	-	2	1	-	-	**4**
Bed rails	1	-	-	-	-	-	2	-	-	**3**
BP cuff	-	-	-	-	-	-	-	-	-	-
Electrical suction machine	-	-	-	1	-	-	2	-	1	**4**
Bedside table	3	-	-	3	-	1	-	-	-	**7**
Blanket	-	-	-	-	-	1	2	-	-	**3**
Drug tray	-	-	-	-	-	-	-	-	-	
Shower pan	-	-	-	-	-	-	1	1	-	**2**
Monitor	-	-	-	-	-	-	1	-	-	**1**
Sink	1	-	-	1			1	-	-	**3**
Ventilator	-	-	-	-	-	-	-	-	-	-
Mattress	-	-	-	-	-	-	-	-	-	-
Oxygen cylinder	-	-	-	-	-	-	-	-	-	-
Incubator	-	-	-	-	-	-	-	-	-	-
Weighing scale	-	-	-	-	-	-	-	-	-	-
Glucometer	-	-	-	-	-	-	-	-	-	-
Trolley	1	-	-	-	-	-	-	-	-	**1**
Total	**6**	**-**	**1**	**5**	**-**	**4**	**10**	**1**	**1**	**28**

5.8 Antimicrobial susceptibility pattern of Gram-positive bacteria

The antimicrobial susceptibility patterns of the isolated Gram-positive bacteria were tested against fourteen antibiotics from six different antibiotic classes. The proportions of resistance among Gram-positive bacteria were high.

CoNs and *S. aureus* demonstrated high level of resistance to most of the antibiotics except Chloramphenicol. Bacillus species were sensitive to Trimethoprim-Sulfamethoxazole, Ciprofloxacin and Chloramphenicol (Table 7).

Table 7: Antibiotics susceptibility profiles of gram-positive isolates from ICU Rooms of Yekatit 12 Hospitals in Addis Ababa, April 2021 to June 2021.

Bacterial isolates	Antimicrobial agent's n (%) and Concentration(µg)														
	Ptn	CAF (30)	PEN (10)	ERY (15)	SXT (1.25/ 23.75)	GEN (10)	CIP	AMP (10)	FOX (30)	AUG (10/20)	CEP	CEF (30)	CFO (30)	CFT (30)	CTZ (30)
CoNs (10)	R	0(0)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)	13(100)
	S	13(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Bacillus species (2)	R	0(0)	2(100)	2(100)	0(0)	2(100)	0(0)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	NA	2(100)
	S	2(100)	0(0)	0(0)	2(100)	0(0)	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	NA	0(0)
S. aureus (1)	R	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
	S	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total	R	0(0)	16(100)	16(100)	14(87.5)	16(100)	14(87.5)	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	14(100)	16(100)
	S	16(100)	0(0)	0(0)	2(12.5)	0(0)	2(12.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

S=Sensitive, R=Resistant, %=Percent, Ptn= Pattern, NA= Not applicable AMP=Ampicillin, CIP=Ciprofloxacin, PEN= Penicillin, AUG= Augmentin, CEP=Cefepime, CFO=Ceftriaxone, CAF=Chloramphenicol, FOX=Cefoxitin, GEM=Gentamicin, ERY=Erythromycin, CFT=Cefotaxime, CTZ= Ceftazidime CEF=Cefuroxime, SXT=Trimethoprim- sulfamethoxazole.

5.9 Antimicrobial susceptibility pattern of Gram-negative bacteria

The isolates' susceptibility profiles revealed various degrees of antibiotic resistance. The majority of the antibiotics examined were highly resistant against Gram-negative rods obtained from various sample sources. Multiple drug resistance to frequently used antibiotics was also widespread in gram-negative isolates in the study location All isolates of *E. coli*, *Citrobacter* spp. and *Enterobacter* spp. *Klebsiella* species *Acinetobacter*, *Shigella* species were 100% resistant to ampicillin. The overall resistance of gram-negative bacteria to ampicillin was 100%, followed by cephalothin 92.5%, cotrimoxazole 97.5%, Ceftazidime 92.5%, Ceftriaxone 95%, cefotaxime 95%, Augmentin 87.5 %, Ciprofloxacin 72.5%, Gentamicin 62.5%, Tobramycin 60%, Amikacin 55%, Chloramphenicol 50%, and meropenem 45%. The predominant isolate, *Klebsiella* species demonstrated a high level of resistance to most antibiotics except Chloramphenicol, meropenem, Tobramycin and Gentamycin (Table 8).

Table 8: Antibiotics susceptibility profiles of gram-negative isolates from ICU rooms of Yekatit 12 hospital in Addis Ababa, April 2021 to June 2021.

Bacterial isolates	Antimicrobial agent's n (%) and Concentration(µg)														
	Ptn	**AMP (10)**	**GEN (10)**	**AUG (10/20)**	**AMK (30)**	**MER (10)**	**SXT (1.25/23.75)**	**CIP**	**CEZ (30)**	**CEPH**	**IMP (10)**	**CFO (30)**	**CRO (30)**	**CAF (30)**	**TOB (10)**
Klebsiella species (21)	R	21(100)	12(57.1)	19(90.4)	17(81)	8(38.1)	21(100)	19(90.5)	19(90.5)	20(95.2)	13(61.9)	21(100)	21(100)	7(33.3)	11(52.4)
	I	0(0)	0(0)	1(4.8)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(4.8)	0(0)
	S	0(0)	9(42.9)	1(4.8)	4(19)	13(61.9)	0(0)	2(9.5)	2(9.5)	1(4.8)	8(38.1)	0(0)	0(0)	13(61.9)	10(47.6)
Acinetobacter (10)	R	10(100)	5(50)	9(90)	3(30)	4(40)	9(90)	4(40)	9(90)	8(80)	4(40)	10(100)	9(90)	7(70)	5(50)
	I	0(0)	0(0)	1(10)	0(0)	0(0)	0(0)	0(0)	0(0)	1(10)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	5(50)	0(0)	7(70)	6(60)	1(10)	6(60)	1(10)	1(10)	6(60)	0(0)	1(10)	3(30)	5(50)
Enterobacter species (6)	R	6(100)	5(83.3)	5(83.3)	2(33.3)	4(66.7)	6(100)	4(66.7)	6(100)	6(100)	3(50)	5(83.3)	5(83.3)	5(83.3)	5(83.3)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	1(16.7)	1(16.7)	4(66.7)	2(33.3)	0(0)	2(33.3)	0(0)	0(0)	3(50)	1(16.7)	1(16.7)	1(16.7)	1(16.7)
Shigella species (1)	R	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
E. coli (1)	R	1(100)	1(100)	1(100)	0(100)	1(100)	1(100)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)
	I	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)
Citrobacter (1)	R	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)
	I	0(0)	0(0)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)
Total	R	40(100)	25(62.5)	35(87.5)	22(55)	18(45)	39(97.5)	29(72.5)	37(92.5)	37(92.5)	22(55)	38(95)	38(95)	20(50)	24(60)
	I	0(0)	0(0)	3(7.5)	0(0)	0(0)	0(0)	0(0)	0(0)	1(2.5)	0(0)	0(0)	0(0)	1(2.5)	0(0)
	S	0(0)	15(37.5)	2(5)	18(45)	22(55)	1(2.5)	11(27.5)	3(7.5)	2(5)	18(45)	2(5)	2(5)	19(47.5)	16(40)

S=Sensitive, R=Resistant, I=intermediate, %=Percent, Ptn= Pattern, AMP=Ampicillin, CIP=Ciprofloxacin, IMP=Imipenem, AUG= Augmentin, AMK=Amikacin, MER=Meropenem, CEZ= Ceftaz, CFO= Cefotaxime, CRO=Ceftriaxone, CAF=Chloramphenicol, GEM=Gentamicin, CEPH=Cephalothin, SXT=Trimethoprim- sulfamethoxazole, TOB=Tobramycin

5.10 General Infection Prevention Practice of HCWs.

A total of 39 healthcare workers were interviewed. From the total respondents' proportions of females were 20 (51.3%). The mean age of the participants was 29.87 (SD $\pm$ 6.2). Regarding their profession 23(59%) were nurses, 9(23%) were physicians and 7(18%) were interns.

According to the response of study participants, majority of them, 23(59%), had training on hand hygiene practice. Among the participants, 8 (20%) always followed all steps of proper hand washing. Besides, from the participants, 32(82.1%) clean surfaces of medical equipment and inanimate, and 33 (84.6%) cleaned the reusable equipment before storage or used on next patient (Table 9).

Table 9: Infection Prevention Practice of HCW in Yekatit 12 Hospitals Medical College in Addis Ababa, April 2021 to June 2021.

Variables	Category	Frequency (n)	Percent (%)
Gender	Male	19	48.7
	Female	20	51.3
Profession	Nurse	23	59
	Physician	9	23.1
	Other	7	17.9
Training on hand hygiene	Yes	23	59
	No	16	41
Alcohol-based Hand rub	Yes	32	82.1
	No	7	17.9
Cleaning hands in between providing services	Yes	33	84.6
	No	6	15.4
Follows all the steps of hand washing	Sometimes	16	41
	Frequently	15	38.5
	Always	8	20.5
Wear new gloves before contacting patient	Sometimes	10	25.6
	Frequently	14	35.9
	Always	15	38.5
Clean surfaces of medical equipment and inanimate	Yes	32	82.1
	No	7	17.9
Clean reusable medical equipment before storage	Yes	33	84.6
	No	6	15.4
Availability of written policies and procedures	Yes	33	84.6
	No	6	15.4
Type of sterilization	Physical	7	17.9
	Chemical	28	71.8
	Others	4	10.3

5.11 Observation results

A total of 39 healthcare workers were observed. Thirty-nine (100%) of the study participants applied proper hand hygiene after coming into contact with patients' blood, body fluids and contaminated surfaces. On the other hand, 2(5.1%) health professionals were not applying proper hand cleaning methods when moving from one patient to another.

Table 10: observation of Infection Prevention Practice of HCW in Yekatit 12 Hospitals Medical college in Addis Ababa, April 2021 to June 2021.

Variables	Category	Frequency (n)	Percent (%)
Gender	Male	19	48.7
	Female	20	51.3
Profession	Nurse	23	59
	Physician	9	23.1
	Other	7	17.9
Proper hand hygiene practice before contact with the patient or their immediate care environment	Yes	27	69.2
	No	12	30.8
Proper hand hygiene practice after contact with blood, body fluids or contaminated surfaces (even if gloves are worn)	Yes	39	100
	No	0	0
Proper hand hygiene practice when moving from one patient to another	Yes	2	5.1
	No	37	94.9
HCP wear face shield during procedures	Yes	24	61.5
	No	15	38.5
clean reusable equipment before to storage or use next patient (ex. Glucometer, blood pressure apparatus)	Yes	34	87.2
	No	5	12.8

6 DISCUSSION

6.7 Bacterial contamination of medical equipment and inanimate

In this study, the entire bacterial contamination of non-critical medical devices and inanimate surfaces were high. In addition of this, almost all isolates exhibited high resistances for various antibiotics tested. The most common isolates had a significant resistances profile showed against different antibiotics.

The present study somewhat in line the study conducted from Egypt [34] On the other hand, our finding is lower than another study was conducted Korea, Taiwan, Saudi Arabia, Zimbabwe, Namibia, Ethiopia and another Ethiopia[29,30,31,33,35,37 and 38].On the contrary, this finding is higher than the other study conducted from India,[27]. These variations might be indicated the level of health awareness of both, patients and health staff in different communities, method, type of media used, frequency decontamination of non-critical medical devices and inanimate surfaces and level of Infection practices with in the unit and type of disinfectant used.

The result of the present study showed that gram-negative bacteria were dominant over gram-positive bacteria. In contrast to this result, a finding from Iran revealed that gram-positive bacteria were predominant than gram-negative bacteria,[43]. Similarly; a study conducted in Ayder Specialized Comprehensive Hospital, Northern Ethiopia, showed that gram-positive bacteria were found to be higher than gram-negative bacteria, [20]. These disparities are may be due to the differences of the sites of swabs being taken from the environment of the hospitals as a whole or may be explained by the level of health awareness of both, patients and health staff in different communities.

In the current study, *Klebsiella spp* were the most isolated of all samples collected at NICU, PICU and AICU. This result was somewhat in line with a study conducted in a Zimbabwe,[33]. On the other hand, this finding is higher than the studies conducted from Iran [32].

On the contrary, this result contradicted studies conducted from Ethiopia, Korea, Taiwan, Egypt Namibia and Sudan [20,29,31,34,35 and36] respectively, which showed that Coagulase-negative *Staphylococci* are the most differentiating .The current study also inconsistent most of the

bacteria identified from a India were *E.coli* [26],Northern India *Acinetobacter* [27] , In Ethiopia *S. aureus* [37,38 &44] and Iraq *Bacillus* spp and *Enterobacter* spp.[28].The frequency variation of different types of bacteria is most likely due to differences in personal hygiene standards, treatment methods, geographical variation and sometimes departments in each hospital [45].

The most contaminated samples were taken from the bed rails 8 (14.3%) and bed sheets 8 (14.3%) This is consistent with other studies from Korea and Iran that highly contaminated samples were taken from bed linen and bed rails, [29 and 32]. These areas were frequently contacted by patients and health workers.

The present study, the most frequent site isolation was in beds 5.3%. this finding was similar to observed in Egypt, where this finding similar *Klebsiella spp* from several ICU were found more in beds,[46]. In contrast, no *Klebsiella spp* was isolated from Korea, Namibia and Sudan [29,36&37] This variation might be the media, method used and different infection prevention practice.

In this study, the bedside table had a positive growth result with bacterial species. These findings were consistent with a study done in tertiary care center Saudi Arabia where the most common isolated organisms from the bed-side table were Coagulase-negative *Staphylococci* (CoNS), [31]. Similarly, a finding from Ethiopia, revealed that bedside was contaminated predominantly with CoNs [22]. In contrast the study was conducted in Egypt were isolated *Klebsiella spp.* [46] This variation might be this site frequently touch by health worker, attendants and patient hand and poor infection practice.

The present study showed that electronic suction was one of the highly contaminated equipment in the three ICU rooms. Similarly, a study done in Nigeria showed that a high contamination rate was recorded in AICU (*Klebsiella spp,* [47] and also in line with study done in Egypt [46]. On the other hand, *Klebsiella spp* not isolated were the study conducted in Nigeria [48].

The hand of health care professionals and rigorous attention to simple hand hygiene, as it acts as a vector for cross-transmission, colonized/infected patients, and poor cleaning procedures of contaminated inanimate surfaces are all possible causes for this high contamination incidence [49].

6.8 Antimicrobial susceptibility pattern of isolated bacteria

The results of antimicrobial susceptibility testing showed the various percentage of resistance among the bacterial isolates from the environment of ICU rooms. Resistance to bacterial antimicrobials results from the interaction of inappropriate antibiotic use or the lack of a medication selection guideline [47].

The results of this study were consistent to that of other studies, such as from Arba Minch, Ethiopia, where a rate of S. aureus sensitivity to chloramphenicol was shown. On the same study the resistance pattern to erythromycin, trimethoprim–sulfamethoxazole, ciprofloxacin, and gentamicin, on the other hand, was greater than in present studies. [50].

On the contrary, the result of the present study is higher than the findings reported from Mekelle, Ethiopia, which showed that CoNS were found resistant to penicillin 35(53.3%), erythromycin 30(50%) and amoxicillin–clavulanic acid 38(62.7%) [20].

The resistance level of gram-positive bacteria towards chloramphenicol in this study was low. From Southern Mozambique revealed that Gram-positive isolated cocci strains had a resistance of 26.3% to chloramphenicol [51].

This study is in line with the results of a study carried out in Windhoek, Namibia where high resistance of CoNS to Penicillin (100%) and Cefoxitin (100%) were obtained [35].

On the other hand; the resistance of CoNS to antimicrobials was higher than other studies conducted in Ethiopia Penicillin (92.9 %), Ampicillin (93.2%), Cefoxitin (78.6 %), Erythromycin (50%) and Augmentin (62.7%)) [12, 37], Zimbabwe (Erythromycin (64.71%) and Ampicillin (82.35%)) [34] and India (Augmentin (83.33%), Cefuroxime (80.95%) and Penicillin (54.76%)), [26].

In the present study, CoNS were sensitive to Chloramphenicol (100%). Somewhat similar, studies conducted in Bulawayo, Zimbabwe (82.35 %) [33] and Ethiopia (72.8 %) [20]. Conversely, a study from Jimma, Ethiopia showed that CoNS (57.3 %) were resistant to Chloramphenicol [52].

Variations in geographic areas, hospital environmental conditions, inappropriate antimicrobial drug administration, and self-medication practices could all contribute to the observed drug-resistant patterns [20].

This study is somewhat in line with findings from a study done in Iran which reported that *Klebsiella* spp. was resistant against Ampicillin (88.9%), Gentamicin (63.6%) and Amoxicillin–clavulanic (81.8%) [32]. In the same study area, lower level of resistances against Ciprofloxacin (45.5%) and Imipenem (45.5%) were identified. Conversely, a study done in India revealed that *Klebsiella* spp. were found to be highly sensitive to Amikacin (76.47%) [26].

These results are comparable with a study reported by Kakuru et al. [32], which showed that *S. aureus* were resistant to Cefoxitin (100%). On the other hand, in the same study, *S. aureus* had sensitivity pattern to Erythromycin (100%), Gentamycin (100%) and Ciprofloxacin (100%). Conversely, other study conducted in Kiwoko Hospital also reported 90.7% of *S. aureus* sensitivity to Cefoxitin [50]. Similarly, a study conducted at Ayder Comprehensive Specialized Hospital in Ethiopia's northern area found that Ampicillin and cefoxitin resistance rates were 89.1 percent and 73.9 percent, respectively [20].

The present study showed complete resistance of *Acinetobacter spp*s to cefotaxime and ampicillin. These findings are in line with the findings from a study done in Ethiopia, in which *Acinetobacter* isolates were resistant to Ampicillin [38]. However, the results of our study do not agree with a study conducted in Namibia which reported 100% sensitivity to Ampicillin, [35].

The results obtained in this study for the antimicrobial susceptibility patterns of *Enterobacter* species are consistent with those reported by Arth ND et al. in which 100% and 50% sensitivity to Trimethoprim-Sulfamethoxazole and Imipenem was observed respectively,[26].
Similar findings were also reported in a study done in Namibia, which showed high resistance to Ceftazidime (100%) [35]. However, our findings contradict those of a similar study, where there is sensitivity to ciprofloxacin (100%). Likewise; a study conducted in Tehran, Iran, found that *Enterobacter* species are highly sensitive to Ciprofloxacin (100%), [32].

The results obtained in this study for the antimicrobial susceptibility patterns of *E. coli* showed are consistent with those reported in a study by Arth ND et al. [26], which found 100% resistance to Augmentin and Amikacin. Likewise; E. Tajeddin et al. reported high resistances to Augmentin (100%), Amikacin (100%) and Ciprofloxacin (100%), [32].

The present findings are also consistent with those reported by Shemse et al., which found high resistance to Ampicillin, [38]. These results, on the other hand, do not agree with those findings

reported by Arth ND et al.] that showed 100% sensitivity of *E. coli* to Trimethoprim-Sulfamethoxazole,[26]. In this study, antimicrobial susceptibility patterns of Bacillus species findings contradict a study conducted in Gezira, Sudan, which found a high sensitivity to Gentamycin (100%) [36].

The results obtained in this study for the antimicrobial susceptibility patterns of *Citrobacter* species were in line with the results of a study done in Ethiopia, which showed high sensitivity to Cefotaxime (100%) Conversely; in the same study, *Citrobacter* spp. were sensitive to Ceftriaxone (100%), Chloramphenicol (100%) and Gentamicin (100%). [20]. This results also agree with the results of a study done in Addis Ababa, Ethiopia, which showed high resistance to Ceftriaxone (100%). In the same study, *Citrobacter* species had sensitivity pattern to Meropenem (100%), and Amikacin (100%), [38]. on the other hand, the current study does not agree with results of a study done in Ethiopia, which showed high sensitivity of *Citrobacter* spp. to Ampicillin, (100%), Cefotaxime (100%), Augmentin (100%), Gentamicin (100%), Ciprofloxacin (100%) and Trimethoprim- sulfamethoxazole (100%) [38].

On the other hand, a low resistance level was recorded to Chloramphenicol. Similarly, findings from southern Mozambique revealed that Gram-positive isolated cocci strains had a resistance of 26.3% to Chloramphenicol [51]. MDR gram-negative and positive bacteria have increased dramatically in hospitals, particularly in intensive care units (ICU). Antibiotic overuse, increased antibiotic resistance, and noncompliance with infection control techniques are all current issues in ICUs that are predisposing to the emergence and spread of HCAI [53].

6.9 Practices of infection prevention

Infection prevention and control programs in hospitals increase patient safety and quality of care while also minimizing the socioeconomic and psychological impacts of infectious diseases on patients and health systems [54]. Infection prevention in Ethiopian healthcare settings is a nationwide endeavor involving the adoption of recommended infection prevention techniques in all aspects of patient care. Hand hygiene, injection safety, and hospital waste management are examples of infection prevention techniques [55].

This study assessed important information regarding the infection prevention practice of health professionals working in the NICU, PICU and AICU in Yekatit 12 hospital medical college, Addis Ababa. According to the respondents, majority of them, 23(59%), had training on hand hygiene practice which is higher than study findings in southeast Ethiopian hospitals, 36.8% [50], and an Egyptian hospital, 57.3% [56]. On the other hand, the current study contradicts with studies done in governmental healthcare facilities, Addis Ababa, 66.1% [57], and in Shenen Gibe hospital, 68.08% [58]. These disparities might be due to variation in type and number of healthcare facilities included in these studies, the difference in healthcare worker infection prevention training and sample size discrepancy [54].

Limitation of the Study

The study was restricted to the ICU rooms of the hospital. Similarly, during observational subjective error can occur. Because of the sensitive question of collecting samples from the hands of ICU medical personnel, samples have not been collected from their hands.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

In the ICU, the rate of bacterial contamination found is high and mostly resistance to different types of Anti-microbial drugs were prescribed.

7.2 Recommendations

- The infection control and prevention unit in the hospital better to adopt observation on regular basis, and through cleaning of non –critical medical devices and inanimate objects surfaces before and after use, and follow basic steps of hand washing is an important part of infection prevention.
- Testing the efficacy of disinfectant which is used in the ICUs.

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Annexes

Annex I: Informed Consent

Information sheet and consent form is prepared for health workers who are participated in research project, a cross-sectional study to determine bacterial profile and antimicrobial pattern isolated from non – critical medical equipment and inanimate surfaces with in immediate patient surrounding and healthcare workers (HCWs) towards to infection and prevention practices in the ICUs Addis Ababa, Ethiopia, 2021.

Name of Principal investigator: Mulugeta G/Medhin

Name of the organization: Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Technology

Name of the Sponsor: Self

Please listen carefully and ask any questions about the study before you agree to join. You may ask questions at any time after joining the study. The investigator is a graduate student in Master of Science Degree in Clinical Laboratory Sciences (Diagnostic & Public Health Microbiology Track) from college of health science, Addis Ababa University.

Purpose of Research Project: - I am hopeful that this research will contribute to sight the rate of bacterial contamination and antimicrobial pattern of non – critical medical devices and inanimate surfaces within immediate patient surrounding that could be the potential source of health risk in ICUs.

Procedure to assess practice of health worker on infection prevention

You are invited to take part in this study. If you are willing to participate in this study, you need to understand and tick yes in the agreement form. Then after, you will receive the questionnaire from the data collector to give your response. You do not need to write your name on the questionnaire and all your responses and the results obtained will be maintained confidentially by using coding system whereby no one will have access to your response.

Risk/ Discomfort: - By participating in this research study, you may feel that it has some discomfort especially on wasting time about twenty-five minutes. I hope you will participate in the study for the sake of the benefit of the research result. There is no risk in participating in this research study.

Benefits: If you participate in this research study, there may not be direct benefit to you but your participation is likely to help us in assessing the Practice of health worker on infection prevention. Ultimately, this will help us to identify the gap and take the appropriate intervention by the authorized personnel.

Incentives: -You will not be given any incentive to take part in this study.

Confidentiality: - confidentiality maintain by investigator the collected information from this research study will be kept in locker in secured place, by a code number assigned to it.

Right to refuse or withdraw: You have full right to refuse from participating in this research. You can choose not to respond to some or all questions if you do not want to give your response.

Persons to contact: If you have any question to ask, please contact Mulugeta G/medhin

Tel: +251-9167497, Email = gmedhinmulugeta2@gmail.com

Annex-1 Observational check list

1. Hand hygiene performed correctly:	Practice Performed
a. Before contact with the patient or their immediate care environment (even if gloves are worn)	Yes □ No □ NA □
b. Before exiting the patient's room after touching the patient or the patient's (even if gloves are worn)	Yes □ No □ NA □
c. After contact with blood, body fluids or contaminated surfaces (even if gloves are worn)	Yes □ No □ NA □
d. Did staff member clean their hands in between providing one patient to another patient	Yes □ No □ NA □
2. PPE is correctly used	Yes □ No □ NA □
a. PPE were removed and discarded before exiting the patient's room or care area	Yes □ No □ NA □
b. Hand hygiene is performed immediately after removal of PPE	Yes □ No □ NA □
c. HCW wear gloves for potential contact with blood, body fluids, mucous membranes, non-intact skin, or contaminated equipment	Yes □ No □ NA □
d. up on entering the patient's room, did staff member perform hand hygiene and apply the appropriate PPE correctly for the level of isolation	Yes □ No □ NA □
e. Is their schedule for cleaning non- critical medical equipment after each activity	Yes □ No □ NA □
f. HCW do not wear the same glove for the care of more than one patient	Yes □ No □ NA □
g. HCP wear face shield during procedures that are likely to generate splashes or sprays of blood or other body fluids	Yes □ No □ NA □
3. Environmental Cleaning	Practice Performed
a. Environmental surfaces, with an important on surfaces in near to the patient and those that are frequently touched, are cleaned and then disinfected	Yes □ No □ NA □
b. Cleaners and disinfectants are used in accordance with manufacturer's instructions (e.g., dilution, storage, shelf-life, contact time)	Yes □ No □ NA □
4. Did staff member clean reusable equipment before to storage or use next patient (ex. Glucometer, blood pressure apparatus)	Yes □ No □ NA □
a. Reusable medical devices are cleaned, reprocessed (disinfection or sterilization) and maintained according to the manufacturer instructions.	Yes □ No □ NA □
b. Single-use devices are discarded after use and not used for more than one patient	Yes □ No □ NA □
c. Reprocessing area has a workflow pattern such that devices clearly flow from high contamination areas to clean/sterile areas (i.e., there is clear separation between soiled and clean workspaces)	Yes □ No □ NA □

Annex-II Data collection form

1. Location _____

2. Code number _____

3. Media used._____

4. Organism isolated._____

5. Antimicrobial susceptibility pattern

5.1 Sensitive _____

5.2 Resistance _____

5.3 Intermediate _____

6. Biochemical test_____

7. Gram stain result from culture _____

8. Other remarks _____

Antimicrobial susceptibility testing

isolated microorganism	Antimicrobial susceptibility result																
	Gentamicin	Ciprofloxacin	Ceftriaxone	Clindamycin	Amikacin	Cefoxitin	Ampicillin	Nalidixic Acid	Meropenem	Cefepime	Piperacillin tazobactam	Sulfamethoxazole-Trimethoprim	Oxacillin	Amoxicillin+ clavulanic acid	Penicillin	Erythromycin	Vancomycin	Tobramycin

Note -R-resistance, S-Sensitive, I-Intermediate

Annexe. III- Questionnaire (English version) Questionnaire

Questionnaire for Health Care Workers

Hello! My name is Mulugeta Gebremedhin. I'm studying for my second degree at Addis Ababa University. Now, I'm researching the bacterial profile and antibiotic sensitivity of the isolates from intensive unit care room environments in Yekatit 12 hospital medical college in Addis Ababa. You are in direct contact with the patient on daily work and this is why I am interested in your opinion on your infection preventive practices. Data collected will be used for research purposes only. I requisite you to complete it anonymously. We would appreciate it if you answer all the questions and answer them as honestly as possible.

Do you agree? □ yes □ no

code: _____.

Please circle on the number you select those best answers the question

Ser.no	Socio-Demographic	Response	Remark
1.	Sex	1. Male 2. Female	
2.	Age	___in years	
	Location	1. NICU 2. AICU 3. PICU	
3.	Profession	1. Nurse 2. Physician 3. student 5.Other (specify)_____	
4.	level of qualification	1. MSc 2. Dr. 3. BSc 4. Diploma 5. Other (specify) ____	
5.	Did you receive formal training on hand hygiene	1.Yes 2. No	
6.	Do you follow all five moments of hand hygiene	1.Sometimes 2.Frequently 3.Always	
7.	Do you follow all of the steps of hand washing, as stated in WHO guidelines (Six stage of hand washing)	1.Sometimes 2.Frequently 3.Always	
8.	Are you always wear new(fresh) gloves before patient contact	1.Sometimes 2.Frequently 3.Always	

9.	Do you have guidelines/protocols for hand hygiene in your unit	1.Yes 2. No	
10.	what is your view the quality of sanitation services provided in your unit	1.Bad 2.Good 3.Excellent	
11.	All the patient care equipment is sterilized properly in your unit	1. Disagree 2.Agree 3.Strongly disagree	
12.	The most effective way infection control	1.Handwashing 2.wearingGlove 3.wearingface shield 4.Using hand sanitizer	
13.	For how long the instrument soaked in the chlorine solution	1.10 minute 2.1hour 3. 24 hours	
14.	Do you regularly use an alcohol-based hand rub for hand hygiene?	1.yes 2.No	
15.	Do you clean your hands in between providing one patient to another patient	1. Yes 2. No	
16.	Do you Clean and disinfect surfaces of equipment and inanimate surfaces and allow for the appropriate contact time with the disinfectant?	1.Yes 2.No	
17.	If your answer yes, how often disinfects the equipment and inanimate surfaces?	1.Once a day 2.Twice time a day 3.Three time a day 4. four time a day 5. Other	
18.	Do you have schedule for cleaning non- critical medical equipment after each activity	1. Yes 2. No	
19.	Do you clean reusable equipment before to storage or use next patient (ex. Glucometer, blood pressure apparatus)	1. Yes 2. No	
20.	Do you have written policies and procedures for the appropriate cleaning and disinfection of equipment	1. Yes 2. No	
21.	Do you wear face shield during procedures that are likely to generate splashes or sprays of blood or other body fluids	1 Yes 2.No	
22.	What Type of disinfectant do you use?	1.denature alcohol 2. Bleach 3. Others	
23.	Which one concentration bleach suitable for disinfect inanimate surfaces and medical equipment	1. 1: 100 2. 1: 9 3. 1:5 4. 1:1	

24.	Prepare 0.1% bleach of 100ml from 5% bleach	- How much water is needed - How much bleach is needed	
25.	For how long you store the prepared bleach solution	1. 24hrs 2.2days 3.3days 4. 4days 5.other	
26.	what type of sterilizations used in your unit?	1. Physical 2. Chemical 3. Others (specify) _____	

Annex-IV 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: Mulugeta Gebremedhin(B.Sc.)

Signature: _____

Date of submission: _____

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

Advisor: Abreham Tesfaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Melese Hailu (MSc, PhD Candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.