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DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Detection of potential pathogenic and drug resistant bacteria isolated from inanimate hospital environments in Operation Theaters and Intensive Care Units of Tikur Anbessa Specialized Hospital and ALERT Hospital in Addis Ababa, Ethiopia

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MSc thesis on:

Detection of potential pathogenic and drug resistant bacteria isolated from inanimate hospital environments in OTs and ICUs of TASH and ALERT Hospital in Addis Ababa, Ethiopia

By: Shemse Sebre Muktar

A thesis presented to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Medical Microbiology

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List of Abbreviation and Acronyms

AHRI	Armauer Hansen Research Institute
ALERT	All Africa Leprosy Rehabilitation and Training Center
AmpC	Ampicillin-hydrolyzing Cephalosporinases
ARG	Antibiotics Resistance Gene
ARS	Antibiotics Resistance strain
ATCC	American Type Culture Collection
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CLSI	Clinical & Laboratory Standards Institute
CONS	Coagulase negative *Staphylococci*
CRB	Carbapenems resistant bacteria
CTX-M	Cefotaxime degrading enzyme
DDST	Double Disk Synergy Test
DMIP	Department of Microbiology , Immunology and Parasitology
DNA	Deoxyribonucleic Acid
ESBLs	Extended-Spectrum β-Lactamases
ESKAPE	*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter*
GBS	*Group B streptococcus*
GNB	Gram-negative bacteria
GPB	Gram-positive bacteria
HAIs	Hospital associated infections
HCAIs	Health care-associated infections
HCW	Health Care Worker
ICU	Intensive care units
KPC	*Klebsiella pneumoniae* carbapenemase
LIA	Lysine iron agar
LPS	Lipopolysaccharide
MBLs	Metallo-β-lactamases
MDR	Multi Drug Resistance
MRSA	Methicillin-resistant *S. aureus*
MSA	Mannitol salt agar
OT	Operation theater
PBPs	Penicillin-binding proteins
PCR	Polymerase chain reaction
rpo B	β subunit of bacterial RNA polymerase
RT-PCR	Real time polymerase chain reaction
SCC Mec A	Staphylococcal cassette chromosome Mec A
SHV	sulfhydryl reagent variable
SIM	Sulphide Indole Motility Medium
Spa	Staphylococcal protein A
TASH	Tikur Anbessa Specialized Hospital
Tet A	Tetracycline gene
VISA	Vancomycin intermediate *S. aureus*
VRE	Vancomycin resistant *Enterococci*
VRS	Vancomycin resistant *S. aureus*

ABSTRACT

Background: The role of hospital environments especially those of the operation theaters (OTs) and intensive care units (ICUs) in the transmission of hospital associated pathogens and multidrug resistant (MDR) bacterial strains like Extended-Spectrum β-Lactamases (ESBLs), Methicillin-resistant *S. aureus* (MRSA) and Vancomycin resistant *Enterococci* (VRE) dissemination are critical and an essential element in the control of Health care-associated infections (HAIs) and emergence of resistance genes.

Objectives: The current study aimed to detect potential pathogenic and drug resistant bacteria from inanimate hospital environments in OTs and ICUs of the selected hospitals.

Methodology: A cross-sectional study was conducted on 280 hospital environmental samples in two different hospitals from June to September, 2018 G.C: Tikur Anbessa Specialized Hospital (TASH) (n=187) and All Africa Leprosy Rehabilitation and Training Hospital **(**ALERT) (n=93). Settle plate's method (Passive air sampling following 1/1/1 schedule) was used for air sample collection while swab method was used to collect samples from inanimate surfaces in the OTs and ICUs of the selected hospitals. A total of 257 environmental swabs and 23 air samples were collected from different sites of ICUs and OTs. All isolates/samples were identified by using routine bacterial culture, Gram staining and a panel of biochemical tests. For each identified bacteria antibiogram profiles were determined by the Kirby Bauer disk diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) guidelines. Double disk synergy test was used to confirm ESBL production while Modified Hodge test (MHT) was used to screen carbapenemases production. On the other hand, Cefoxitin /oxacillin discs were used to screen MRSA.

Results: Out of 280 swabs and settle plates, 227(81%) of samples were positive for bacterial contamination. A total of 282 bacteria were identified. Of these, the predominant bacteria identified from the environmental samples from OTs and ICUs were *S. aureus* (27.5% vs 9.6%), Coagulase negative *Staphylococcus* (CONS) (16% vs 2.8%) and *Acinetobacter* spp (2.5% vs 14.5%) respectively. The bacterial load on air was found beyond the standard limits. The most common bacterial contaminated sites were bed linens 37(13.1%), followed by environmental surfaces including (wall, floor, corridor and door knob) 35(12.4%) and beds 33(11.7%). Out of the 280 environmental samples 76(27.1%), 25(8.9%) and 7(2.5%) were MRSA, ESBL and Carbapenemase producer bacteria respectively. Most the identified bacteria showed considerable resistance to antibacterial agents. Of the total 282 identified bacteria, 158(56%) of the isolates were resistant to at least 3 antibiotics and 58 multi-drug resistance phenotypes were exhibited by the MDR isolates.

Conclusion: Hospital environment especially those of the operation theaters and intensive care units are highly contaminated with potential pathogenic bacteria. Bacterial isolates were highly resistant to commonly used antibiotics with high multi-drug resistance percentage. Therefore, well-designed infection prevention and control strategies should be in place for combating health care-associated infections and the consequences.

Key words: Extended-Spectrum β-Lactamases (ESBLs), Hospital acquired infection, Inanimate Hospital environments, Methicillin-resistant *S. aureus* (MRSA), Vancomycin resistant *Enterococci* (VRE).

1. CHAPTER I: Introduction

1.1. Background

Confined environments, where people spend most of their time, are characterized by a specific microbial community, the indoor Microbiome. Hospitals are one of the indoor environments where both infected and healthy people, group together. How microbial communities persist and change in indoor environments is of immense interest to public health (de Abreu *et al*., 2014; Mora *et al*., 2016). This is because hospital environment represents a new ecological place for medically important nosocomial pathogens, antibiotic-resistant microorganisms and reservoirs of resistance gene, which have been commonly, found on various surfaces (e.g. medical equipment, housekeeping surfaces, workplaces and lobby (furniture) within hospitals (Rozman and Turk, 2016).

Hospital environments are sources of reservoirs of collection of antibiotic-resistant bacteria and antimicrobial resistance genes (resistome). Bacterial cross-contamination plays an important role in health care-associated infections (HCAIs) and resistant strain dissemination. The relative importance of the known hospital bacterial reservoirs and resistance genes including (health care provider hands, patient, environment, and health care equipment) in this process is unknown (Loftus *et al*., 2012; Mora *et al.*, 2016).

Most indoor environments are connected to the natural environment due to the accession to ventilation, while some other habitats such as intensive care units (ICUs) and operating theaters (OTs) have a limited exposure to the environment and hence remain with less ventilation. The purpose for confinement in ICUs and OTs is to protect the susceptible patients in these units from HCAIs (Bakkali *et al*., 2015; Mora *et al*., 2016).

Health care-associated infections (HCAIs) such as bacteremia, pneumonia, urinary tract and skin or soft tissue infections are among the most frequent ones which are accompanied with complications occurring patients within ICUs. Such patients are at risk for HCAIs because of invasive medical procedures. Contamination of the ICUs environment occurs through acquisition of nosocomial pathogens by both patients and health care workers (HCWs) (Tajeddin *et al*., 2016).

Intensive care units (ICUs) and operation theaters (OTs) confined places need to be microbiologically monitored and controlled by microbial cleaning and disinfection. However, these measures apply constant selective pressures, which support microbes with resistance capacities against antibiotics or chemical and physical stresses. Such selective pressures facilitate the rise of survival specialists and multi-resistant strains (Morales *et al*., 2016).

1.2. Statement of the problem

Microbial contamination of the hospital environments has greatly contributed in precipitating the prevalence of health care-associated infections (HCAIs). Risk of acquiring pathogenic infections, in hospital environments is higher than in other environments, and the course of an infection is more often fatal (Weber *et al*., 2013; Morales *et al*., 2016). Approximately 60% of these infections involve antimicrobial-resistant bacteria such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species (ESKAPE) pathogens (Weber *et al*., 2013; Santajit and Indrawattana, 2016; Laudy *et al*., 2017). Resistance to antibiotics has been a particular problem over the last decades, increasingly hampering the treatment of hospital acquired infections (Mirhoseini *et al*., 2016).

The majority of the HCAIs are believed to be transmitted directly from patient to patient, but increasing evidence demonstrates that also the medical personnel as well as the clinical environment (i.e., surfaces and equipment) often are a source of infection (Morales *et al*., 2016). Hospital design and hygienic practices have been largely directed at controlling nosocomial pathogens and resistance strains contaminating air, hands, equipment, and surfaces (Otter *et al*., 2011). A better understanding of how bacterial cross-contamination occurs can provide the basis for the development of evidence-based preventive measures (Loftus *et al*., 2012).

In Ethiopia, studies reported high prevalence of health care-associated infections (HCAIs) mainly due to multi drug resistant pathogens including the countries' largest tertiary referral Hospital (Endalafer *et al*., 2011; Desta *et al*., 2016; Yallew *et al*., 2016). Emergence of multi-drug resistant strains in a hospital environment; particularly in developing countries, is an increasing problem which is an obstacle for management of health care-associated infections (Gebremariam and Declaro, 2014; Tesfaye *et al*., 2015; Solomon *et al.*, 2017; Engda *et al*., 2018).

There is a paucity of information on the bacteriological quality of wards of hospital environments in Ethiopia, and the few available ones reported high bacterial load and multidrug resistant (MDR) strains (Shiferaw *et al*., 2013; Solomon *et al*., 2017; Engda *et al*., 2018; Getachew *et al*., 2018). So a reassessment of the role played by contaminated surfaces in the transmission of potential pathogenic bacteria and MDR strain are critical (Otter *et al*., 2011).

Isolation of Extended-spectrum beta-lactamases (ESBLs) producing *Enterobacteriaceae,* Methicillin resistant *S. aureus* (MRSA), Carbapenem resistant *Enterobacteriaceae* (CRE) and vancomycin resistant *Enterococci* (VRE) from hospitalized patients has been reported (Desta *et al*., 2016; Eshetie *et al*., 2016; Legese *et al*., 2017; Ferede *et al*., 2018; Toru *et al*., 2018; Teklu *et al*., 2019) however, the influx of this organisms from inanimate hospital environments remains poorly studied from hospitals in Ethiopia including Tikur Anbessa Specialized and ALERT Hospitals. Therefore, the aim of this study was to detect potential pathogenic and drug resistant bacteria (MRSA, ESBLs, CRE and VRE) from inanimate hospital environments (fomites) in the environments of OTs and ICUs at Tikur Anbessa Specialized and ALERT Hospitals in Addis Ababa, Ethiopia.

1.3. Significance of the study

There is a strong link between hospital-associated bacterial communities and a hospital-acquired infection that needs investigation. Especially, an understanding of bacterial community structure in hospital environments will be critical for mapping the dissemination of antimicrobial resistance strain (ARS) and antimicrobial resistance genes (ARG).

Hence, the present research work provides information about the magnitude of prevalent resistant strains especially MRSA, ESBL, CRE and VRE in the hospital environments. In addition, it produces crucial findings which may pave the way for the prevention and control of this problem. Moreover, the findings from this study will contribute towards development of proper strategies for preventing and treating nosocomial infections by identifying the distribution of causative pathogens and the prevalence of antimicrobial resistance strains. Subsequently, the data obtained from this research can be used by concerned bodies to evaluate and update hospital infection control protocols and procedures in accordance to the generated scientific evidence from this study. Finally, the result of this study will be used as baseline information for those who would like to conduct further study.

1.4. Literature review

1.4.1. Microbiology of common potential pathogenic bacteria

1.4.1.1. *Staphylococcus aureus*

Staphylococcus aureus (*S. aureus*), are Gram-positive cocci that are both as commensal bacterium and a human pathogens. Approximately 30% of the human population is colonized with *S. aureus* (Tong *et al*., 2015). Infections caused by this microorganism can be acquired through both hospital and community settings and it is still one of the most common causes of HAIs. *S. aureus* is the most common cause of surgical wound infections and pneumonia, and the second most common cause of bacteraemia. Other infections caused by this pathogen include endocarditis, septicaemia, osteomyelitis, meningitis, skin infections, gastroenteritis, and toxic shock syndrome (Adwan *et al*., 2015; Tong *et al*., 2015).

1.4.1.2. *Enterococci spp*

Enterococci are Gram-positive cocci that are common inhabitants of the gastrointestinal tracts of animals and humans. While a core member of the microbiome, they are also capable of causing a variety of severe infections, most often among antibiotic-treated hospitalized patients with perturbed intestinal microbiota (Fiore *et al*., 2018). *Enterococci*, nowadays, represent the second leading cause of nosocomial urinary tract infections and the third leading cause of nosocomial bacteremia (Van Tyne *et al*., 2013).

1.4.1.3. *Acinetobacter baumannii*

The genus *Acinetobacter* is considered ubiquitous organisms. It is a Gram-negative, strictly aerobic, non-fermenting, non-fastidious, non-motile, catalase-positive and oxidase-negative. *A. baumannii* strains resistant to all known antibiotics (Thong *et al*., 2011). Acting in synergy with this emerging resistance profile is the creepy ability of *A. baumannii* to survive for prolonged periods throughout a hospital environment, thus potentiating its ability for nosocomial spread (Peleg *et al*., 2008). The major factors possibly contributing to the persistence of *A. baumannii* in the hospital environment, (a) resistance to major antimicrobial drugs, (b) resistance to desiccation, and (c) resistance to disinfectants (McConnell *et al*., 2012; Tajeddin *et al*., 2016). The organism commonly targets the most vulnerable hospitalized patients, those who are critically ill with openings in skin integrity and airway protection. Hospital acquired pneumonia is still the most common infection caused by this

organism. However, in more recent times, infections involving the central nervous system, skin and soft tissue, and bone have emerged as highly problematic for certain institutions (Peleg *et al*., 2008; Alvarez-Ortega *et al*., 2011).

1.4.1.4. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is Gram-negative that is ubiquitous in moist hospital environments. It is opportunistic pathogen capable of infecting humans with compromised immunity and causing severe lung disease (Alhazmi, 2015; Parcell *et al*., 2018). It is one of the leading pathogen associated with nosocomial infections (Kramer *et al*., 2006; Ensayef *et al*., 2009). It has enormous pathogenicity factors that are used to interfere with host defenses. Pathogenesis in *P. aeruginosa* facilitates adhesion, modulate or disrupt host cell pathways, and target the extracellular matrix (Alhazmi, 2015). The propensity of *P. aeruginosa* to form biofilms further protects it from antibiotics and the host immune system. *P. aeruginosa* is intrinsically resistant to a large number of antibiotics and can be acquired resistance to many others, making treatment difficult (Salm *et al*., 2016; Mbanga *et al*., 2018). *P. aeruginosa* provokes a potent inflammatory response during the infectious process (Alhazmi, 2015; Parcell *et al*., 2018). *P. aeruginosa* infections are also commonly found in burn patients, mechanically ventilated patients and cystic fibrosis (CF) patients (Thong *et al*., 2011).

1.4.1.5. *Klebsiella pneumoniae*

Klebsiella pneumoniae, a member of the family Enterobacteriaceae, is a rod-shaped, Gram-negative and lactose-fermenting bacillus. On agar media, it has a mucoid phenotype that is conferred by the polysaccharide capsule attached to the bacterial outer membrane (Thong *et al*., 2011; Li *et al*., 2014). Typical *K. pneumoniae* is an opportunistic pathogen that is widely found in the mouth, skin and intestines, as well as in hospital settings and medical devices. They are the causative agent of several types of infections in humans, including respiratory tract infections, urinary tract infections (UTIs), and bloodstream infections (Thong *et al*., 2011; Martin *et al*., 2018; Bengoechea and Sa Pessoa, 2019). Nosocomial infections caused by *K. pneumoniae* tend to be chronic due to the two major reasons: (a) ability of *K. pneumoniae* to form biofilms that protect the pathogen from attacks of the host immune responses and antibiotics; and (b) nosocomial isolates of *K. pneumoniae* often display multidrug-resistance phenotypes that are commonly caused by the presence of extended-spectrum β-lactamases or carbapenemases, making it difficult to choose appropriate antibiotics for treatment (Li *et al*., 2014; Bocanegra-Ibarias *et al*., 2017).

1.4.2. Inanimate hospital environment

Fomites are inanimate objects that can serve as vehicles for pathogens transfer (Maryam *et al*., 2014). Hospital environment can encompass patient zone and healthcare area. The patient zone includes the patient and his/her immediate surroundings. Based on Centers for Disease Control and Prevention (CDC) classification, medical and surgical instruments are categorized to critical, semi critical, and non-critical (CDC, 2003). Inanimate/abiotic environmental surfaces can be further divided into medical equipment surfaces (including beds, knobs or handles on hemodialysis machines, X-ray machines, instrument carts, monitors, stethoscopes, oxygen supply, suction buttons, ventilator and dental units); housekeeping surfaces (e.g., floors, walls, and tabletops); workplaces, including keyboards, telephones, computer mice; lobby (furniture) including chair, locker and trowels; hospital textiles including uniforms, bed linen and pajamas, as well as protective clothing of health care personnel; it also includes sinks and air (Ekrami *et al*., 2011; Galvin *et al*., 2012; de Abreu *et al*., 2014; Claro *et al*., 2014; Russotto *et al*., 2015; Chen *et al*., 2017).

Intensive care units (ICUs) are special departments in hospitals that provide intensive medical care for patients suffering from severe and life-threatening diseases or injuries. These units can be divided into several categories, including neonatal ICUs, pediatric ICUs, psychiatric ICUs, cardiac ICUs, medical ICUs, neurological ICUs, trauma ICUs, and surgical ICUs. ICUs are considered potential reservoirs for (opportunistic) pathogenic microbial strains (Russotto *et al*., 2015). These microorganisms may thrive on the medical equipment, in other patients, personnel, and the surrounding environment of the hospital (Russotto *et al*., 2015; Mora *et al*., 2016).

Operation theatres (OTs) are hospital wards where most surgical procedures are performed on patients which can be admitted to hospitals. Surgical procedures are part of medical treatment that calls for very sterile ecosystem round there (Mishra and Wadhai, 2016). An operation room has its personal infrastructure special from different devices of hospitals it consists of bed, OR-Lamp, Anesthesia machine, surgical trolley, sterile air system each of which has critical function in completing the surgical treatment (Mishra and Wadhai, 2016; Mora *et al*., 2016).

These areas are subjected to strict cleaning procedures such as sterilization, disinfection and removal of contaminants (e.g., dust and organic waste) (Al Laham, 2012; Najotra *et al*., 2017). Cleaning and maintenance schedules are implemented for each operation room according to the surgical procedures performed (Mora *et al*., 2016). Hospital surfaces are frequently contaminated with important healthcare-associated pathogens and played important role in the transmission of resistant bacteria like MRSA, ESBL, VRE, *C. difficile*, *Acinetobacter* spp., (Weber *et al*., 2013, Santajit and Indrawattana, 2016).

1.4.3. Role of contamination of hospital environment in transmission of HCAIs

Many studies in which samples were taken from the environment have demonstrated that multi-resistant bacteria are present in the hospital environment (Al Laham, 2012; Weber *et al*., 2013; Najotra *et al*., 2017). The frequency of environmental contamination with multi-resistant bacteria and resistance genes are highest for colonized or infected patients (Talon, 1999; Bakkali *et al.*, 2015).

Microorganisms resistant to antimicrobial agents can be spread from patient to patient in health care facilities, often via the contaminated hands of health care personnel, contaminated medical or surgical equipment, or the inanimate hospital environment general called as clonal transmission of antibiotic-resistant organisms involving the transmission of a single strain of the antibiotic resistant organism (Mulvey and Simor, 2009).

Hospital surfaces are frequently contaminated with important healthcare-associated pathogens (Table 1.1). Contact and airborne are the main routes of transmission for most infections (Mirhoseini *et al*., 2016). There was a similarity observed among the isolated strains of colonized and/or infected patients and the strains of the environment by molecular characterization (Oliveira-de-Souza *et al*., 2014).

Table 1.1: Potential pathogenic bacteria detected on inanimate and animate hospital environments

Microorganism	Locality/Sampling points	Reference
Clostridium difficile	Bed, chair backs, hand rails, bed rails, , sofas ,television, bulletin board, computer keyboards	(Faires *et al*., 2013)
Klebsiella pneumoniae	abiotic surfaces(sink, toilet, bed linen, table, floors), hands of health workers	(Dziri *et al*., 2016b)
Staphylococcus aureus	air, bed, mattress cover, bathroom floor, bed linen, floors, door handles	(Asoh *et al*., 2005; Ekrami *et al*., 2011; Claro *et al*., 2014)
Acinetobacter baumannii	bed rails, sinks, tables, curtains, door handles	(McConnell *et al*., 2012; Tajeddin *et al*., 2016)
Pseudomonas aeruginosa	bed, tables, ward sinks and surgical equipment, shower, drug trolley, blood pressure cuff, laryngoscope, stethoscope, water	(Moya *et al.*, 2012; Parcell *et al*., 2017)
Escherichia coli	abiotic surfaces, hand of health workers	(Freeman *et al*., 2014; Dziri *et al.,* 2016a)
***Vancomycin resistant enterococci* (VRE)**	general areas in patients' rooms and toilets, light switch	(Rozman and Turk, 2016)

Contact with the contaminated environment by healthcare personnel is equally as likely as direct contact with a patient to lead to contamination of the healthcare provider's hands or gloves that may result in patient to patient transmission of nosocomial pathogens. Admission to a room previously occupied by a patient with MRSA, VRE, *Acinetobacter* spp, or *C. difficile* increases the risk for the subsequent patient admitted to the room to acquire the hospital associated pathogens (Rohr *et al*., 2009; Weber *et al*., 2013). Also fungi in particular *Aspergillus* species and *Candida albicans* can also contaminate the hospital environment and cause healthcare-associated infections (Kramer *et al*., 2006; Otter *et al*., 2011).

The ability of contaminated surfaces contributing to the transmission of nosocomial pathogens relies on several determinants including the frequency at which surfaces are contaminated, if levels of contamination are sufficiently high to result in transmission, and the ability of pathogens to remain viable on various surfaces (Faires *et al*., 2013).

For many healthcare-associated pathogens the degree of hospital surface contamination is closely correlated with the risk of transmission (Freeman *et al*., 2014). Pathogen transfer from an affected patient to a susceptible host occurs most commonly by direct contact but also by contaminated medical equipment, hospital surfaces and less commonly water and air can be either directly or indirectly involved in the transmission pathway (Figure 1.1) (Kramer *et al*., 2006; Otter *et al*., 2011; Mirhoseini *et al*., 2016).

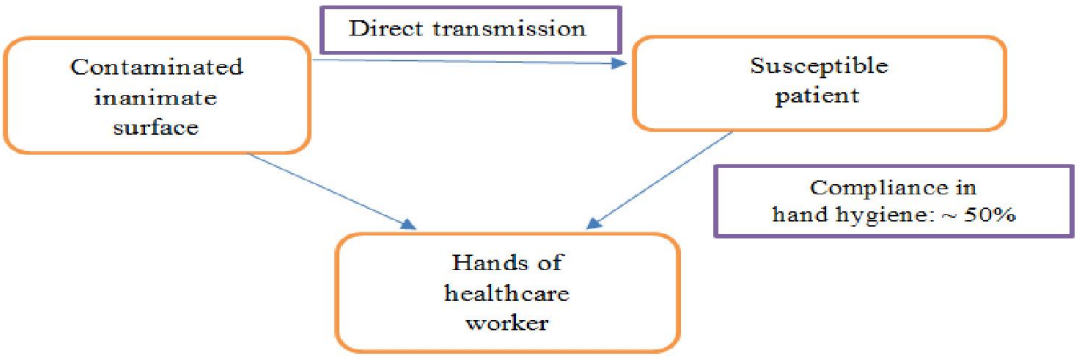


Figure 1.1: Common modes of transmission of hospital associated pathogens from inanimate surfaces to susceptible patients (Kramer *et al*., 2006).

1.4.4. Survival of potential pathogenic bacteria in the hospital environments

Many bacteria survive on dry surfaces for longer time (Table 1. 2). Most Gram-positive bacteria, such as: *Enterococcus* spp. (including VRE), *S. aureus* (including MRSA), or *Clostridium difficile*, survive for hours to days to months on dry surfaces. Many Gram-negative species, such as *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *P. aeruginosa*, *Serratia marcescens*, or *Shigella* spp can survive on inanimate surfaces even for months. Those were responsible for most nosocomial infections (Talon, 1999; Kramer *et al*., 2006; Rohr *et al*., 2009). The duration of bacterial survival depends not only on the bacterium concerned, but also on the nature of the contaminated surface (Talon, 1999). The longer a nosocomial pathogen persists on a surface, the longer it may be a source of transmission and thus endanger a susceptible patient or healthcare worker (Kramer *et al*., 2006).

Table 1.2: Persistence of potential pathogenic bacteria on inanimate surfaces

(Talon, 1999; Chemaly *et al*., 2014)

Organism	**Survival time (range)**
Gram-negative	
Acinetobacter spp.	3 days to 11 months
Escherichia coli	1.5 hours – 16 months
Klebsiella spp.	2 hours to > 30 months
Pseudomonas aeruginosa	6 hours – 16 months; on dry floor: 5 weeks
Serratia marcescens	3 days – 2 months; on dry floor: 5 weeks
CRE	19 days
Gram-positive	
Enterococcus spp. including VRE and VSE	5 days to >46 months
Staphylococcus aureus, including MRSA	7 days to >12 months
Clostridium difficile	>5 months (spores)

1.4.5. Mechanism of Antibiotic Resistance and their Genetic basis

There are many mechanisms that bacteria exhibit to protect themselves from antibiotics (Chroma and Kolar, 2010). These resistance mechanisms can be biochemical and genetic aspects (Džidić *et al*., 2008) (Table 1.3). Resistance of pathogens to drugs is determined by the presence of specific genes and / or mutations (Li *et al*., 2018).

Resistance to antibiotics basically can occur by (1) modification of a drug target results in the inability of the drug to bind to its biological target thus rendering the drug unable to kill the bacteria, (2) active efflux results in the intracellular dilution of drugs making the extruded drugs unavailable for their inhibitory action or (3) prevent cellular entry of drug into the inside of the bacterial cell; and (4) enzymatic inactivation of the drug results from the metabolic degradation of the drug into a form that is rendered ineffective in inhibiting bacterial growth (Wright, 2011; Kumar and Varela, 2013).

Table 1.3: Biochemical aspect of antibiotic resistance mechanisms

Mechanisms of resistance	**Description**	**Resistance gene**	**Reference**
Target modification	Alteration in the primary site of action can arise from mutations at the target gene resulting in altered target structure.	**rpoB[1],** Altered penicillin binding proteins (**MecA genes[2])**	(Davies and Davies, 2010)
Enzymatic inactivation of drug	Antibiotics are inactivated by enzymatic hydrolysis, group transfer and redox process	Gene β- lactamases (bla), by *Enterobacteriaceae,* amino glycoside modifying enzymes	(Kumar, 2017)
Efflux pumps	Trans-membrane transport proteins, used for exporting specific metabolites and xenobiotic toxic substances out of the cell	**tetA gene** in *E. coli* (tetracycline gene)	(Džidić *et al*., 2008)
Change in membrane permeability	Intrinsic ability to restrict the entry of small molecules	*P. aeruginosa* Lipopolysaccharide (LPS)	(Kumar, 2017)

1. *Mycobacterium tuberculosis* has arisen due to mutations in rpo B, encoding a β-subunit of RNA polymerase, a target site for rifampicin;
2. MecA genes encoding methicillin resistance in *S. aureus* resulting in production of altered PBP2a.

1.4.5.1. Genetic aspect of antibiotic resistance mechanisms

Genetic aspect of antibiotic resistance mechanisms can be classified as intrinsic resistance and acquired resistance (Kumar, 2017). Pathogens being initially drug resistant represents intrinsic (natural or de novo) characteristic feature of an organism, which allows bacteria to tolerate the encountered antibiotic (Wright, 2010). Acquired bacterial antibiotic resistance can result from a mutation of cellular genes or both the acquisition of foreign resistance genes. This type of resistance is much more important and significant for clinical aspect because of the possible spread of resistant genes through a sensitive microbial population (Chroma and Kolar, 2010). There are two main ways of acquiring antibiotic resistance: a) through mutation in different chromosomal loci and b) through horizontal gene transfer (i.e. acquisition of resistance genes from other microorganisms) such as bacterial conjugative plasmids, transposable elements and integron systems (Džidić *et al*., 2008; Chroma and Kolar, 2010).

1.4.5.2. Enzymatic Drug inactivation mechanisms

Enzymatic drug inactivation mechanism mediated by hydrolysis process result in the production of β-lactamases that hydrolyze the β-lactam ring of β-lactam antibiotics (Kumar, 2017). Beta-lactams are the common used antibiotics and include the penicillins, cephalosporins, monobactams and carbapenems (Cag *et al*., 2016). They all share a common beta-lactam ring and act similarly by binding to and inactivating the penicillin-binding proteins (PBPs), which are responsible for the formation of the bacterial cell wall (Meletis, 2016).

The genes encoding β-lactamases (bla) are either on the chromosome (e.g. AmpC β-lactamase) or on mobile genetic elements like plasmids (TEM-1 β-lactamase, SHV-1 (sulfhydryl variable active site), CTX-M (cefotaxime degrading enzyme) and transposons or can occur as a part of integrons located in these transferable elements (Chroma and Kolar, 2010; Kumar, 2017).

In Gram-negative bacteria, β-lactamase production remains the most important mechanism of resistance to β-lactam antibiotics. One group of these enzymes called extended-spectrum β-lactamases (ESBLs). TEM-1, TEM-2, SHV-1 evolved through time and hydrolyze a broad range of extended spectrum cephalosporins and produces ESBLs. TEM, SHV and CTX-M are the major ESBLs (Chroma and Kolar, 2010; Kumar, 2017).

ESBLs hydrolyze a wide range of cephalosporins including the oxyimino group of cephalosporins such as ceftriaxone, ceftazidime, cefotaxime and the monobactam drugs such as aztreonam, but do not hydrolyze cephamycins and carbapenems (Laudy *et al*., 2017, Kumar, 2017). In addition, ESBLs derived from OXA-type β-lactamase confer resistance to cloxacillin and oxacillin antibiotics and are referred to as OXA-type ESBLs. OXA types are not inhibited by clavulanates or tazobactam (June *et al*., 2014). Currently, more than 300 ESBL types have been discovered worldwide, and these have evolved from TEM, SHV and CTX-M types with 150, 88 and 69 variants, respectively by point mutations in hospital environments and community settings (Dropa *et al*., 2009, Ghafourian *et al*., 2015). β-lactamases are widespread among hospital associated *Enterobacteriaceae* such as *E. coli* and *Klebsiella pneumoniae* and some Gram-positive bacteria (Chroma and Kolar, 2010; Kumar, 2017) (Table 1.4).

Table 1.4: Common hospital associated bacteria and resistance strain by mechanisms of β-lactamases and target alteration

Types of β-lactamases	Description	locations	Examples	Reference
TEM	Hydrolyzing penicillin TEM-1 and TEM-2 are don't produce ESBLs, due to point mutation	Plasmid	*E. coli* and *K. pneumoniae*	(Ghafourian *et al*., 2015)
SHV	Hydrolyzing cefotaxime, point mutation	Plasmid	*K. pneumoniae*	(Ghafourian *et al*., 2015)
CTX-M (derived from *Klyuvera* spp)	Preferentially hydrolyze cefotaxime rather than ceftazidime and are better inhibited by tazobactam than by sulbactam.	Chromosome and plasmid	*Enterobacteriaceae*	(Dallenne *et al.*, 2010; Park *et al.*, 2012)
OXA (oxacillinases)	confer resistance to cloxacillin and oxacillin antibiotics	Plasmids & or transposons	*P. aeruginosa, A. baumannii, Enterobacteriaceae*	(June *etal*., 2014; Meletis, 2016)
AmpC β-lactamase (blaCMY-2, bla DHA).	Constitutively expressed enzymes, Cross-resistance to penicillins and cephalosporins.	Chromosome and plasmid	*P. aeruginosa, A. baumannii*	(Moya *et al*., 2012; Sadeghi *et al*., 2016)
Carbapenemases (class A KPC, class B metallo-blactamases, such as IMP, VIM, and NDM , class D OXA enzymes (OXA-23 and OXA-48))	They confer resistance to the Carbapenems (e.g. imipenem, meropenem)	plasmids or transposons	*K. pneumoniae, P. aeruginosa & A. baumannii*	(Meletis, 2016; Altamimi *et al*., 2017)
***mecA* gene**	Codes for the production of an altered penicillin-binding protein (PBP2a), not effectively bind beta-lactam antibiotics.	staphylococcal cassette chromosome *Mec* (SCC*mec*)	Methicillin resistant *S. aureus* (MRSA)	(Adwan *et al*., 2015; Cag *et al*., 2016)
***Van* A or *Van* B genes**	Synthesis of modified cell-wall precursors that do not bind glycopeptides.	plasmid-associated gene	Vancomycin-resistant *Enterococcus* (VRE)	(Loomba *et al*., 2010)

1.4.6. Frequency of hospital associated bacteria and MDR strain

Nosocomial infections are caused by a variety of organisms, including bacteria, fungi, viruses, parasites, and other agents. Infections can be derived from exogenous or endogenous sources and are transferred by either direct or indirect contact between patients, healthcare workers, contaminated objects, visitors, or even various environmental sources. Hospital surfaces are frequently contaminated with important healthcare-associated pathogens and played important role in the transmission of resistant bacteria like MRSA, ESBL, VRE, *C. difficile*, *Acinetobacter* spp (Weber *et al*., 2013; Santajit and Indrawattana, 2016).

It has been estimated that the source of pathogens causing HAI in the ICUs was the patients' endogenous flora, 40–60%; cross-infection via the hands of personnel, 20–40%; antibiotic driven changes in flora, 20–25%; and contamination of the hospital environment, 20% (Weber *et al*., 2013).

The frequency of environmental contamination with ESBLs has been reported to reach 1% to 25% (Table 1.5). The frequency of environmental contamination with MRSA has been reported to reach 20% in the environment of MRSA carriers and inanimate environments (Table 1.6). Concerning VRE, up to 94% of the environmental surfaces around carriers have been shown to be contaminated. Moreover, environmental contamination with *Acinetobacter* species during outbreaks has been reported to vary between 3% and 50% (Weber *et al*., 2013).

Table 1.5: Frequency of Extended spectrum beta lactamases (ESBLs) producing bacteria in hospital environments

Organism	Source	Contamination rate	Predominant isolated bacteria	Reference
ESBL-EbNoEc	Abiotic surfaces and biotic	9.3% (28 /300)	ESBL-KP (n= 11), ESBL-*E. cloacae* (n=11)	(Dziri *et al*., 2016).
ESBL-E	Environmental surfaces	3.4% (38/1104)	ESBL-KP (n=37), ESBL-EC (n=2)	(Freeman *et al*.,2014)
ESBL-Klebsiella infected/carrier patients	Environmental samples(Bath, sink, bed& others)	4% (19/470)	ESBL-KP (n=16), ESBL-EC (n=2)	(Guet-Revillet *et al*., 2012)
ESBL-E	Surfaces(hand wash basins, floors& others)	3.1%(45/1436)	ESBL-KP (n=11), *Enterobacter* spp (n=12)	(Muzslay *et al*., 2017)
ESBL-E	Environments surfaces	3.2%(68 /998)	*P. aeruginosa* (n=17), ESBL-K P(n=12)	(Nurain *et al*., 2015)
ESBL-RGN	Inanimate surfaces(sinks, countertops, and bed rails)	5% (28 of 606)	*P. aeruginosa* (n=6), *E. cloacae* (n=6)	(D'Agata *et al*., 1999)
ESBL- EC	Abiotic /Biotic surfaces	3.7%(11/300)	ESBL- EC (n=11)	(Dziri *et al*., 2016b)
ESBL-E	Environments surfaces(OTs)	1.2%(3/243)	ESBL- EC (n=2), *Klebsiella* spp (n=1).	(Al Laham, 2012)
ESBL-E	Bed frames, bedside tables, door handlers, floors, sinks, waiting chairs, walls, and the waste water	14.8%(57/384)	ESBL-KP (n=24), ESBL-EC (n=20)	(Engda *et al*., 2018)
ESBL-E	Inanimate surfaces and medical equipment	25.9%(15/58)	ESBL-*Klebsiella* spp (n=9 (69.2%), EC (n= 6 (54.5%)	(Mbanga *et al*., 2018)
ESBL-E	Ventilator ,Bed, Bed linen, Nurse's station & others	1.5%(9/605)	ESBL-*Klebsiella* spp (n=6(51.5%), ESBL-EC (n=2(77.8%)	(Tajeddin *et al*., 2016)

ESBL: Extended spectrum beta lactamase; ESBL-EbNoEc: ESBL-producing non-*E. coli Enterobacteriaceae*, K P-*Klebsiella pneumoniae*; EC: *E. coli;* ESBL-E: *Enterobacteriaceae*; ESBL-RGN: resistant Gram-negative

Table 1.6: Frequency of Methicillin resistant *S. aureus* (MRSA) in hospital environments

Microorganism	Source/Locality/Sampling points	Contamination rate by MRSA	% *S. aureus* producing MRSA	Reference
MRSA	Medical instrument, hand washing samples	4.7% (57/1208)	60%(57/95)	(Ekrami *et al*., 2011)
MRSA	Air, Bulletin board, Chair back, End of bed, Over bed table	2.5% (6/236)	2.5%	(Faires *et al*., 2013)
MRSA	Surface material, medical instrument	11.8% (72/612)	11.8%	(Faires *et al*., 2012)
MRSA	Immediate patient environment (bed linen, wall, floor, air)	10.5% (105/1000)	10.5%	(Rohr *et al*., 2009)
MRSA	environmental surfaces (bed side , floors, door knobs, bed rails, faucets and others)	3.4% (7/196)	12.1% (7/58)	(Adwan *et al*., 2015)
MRSA	Ventilator, bed, bed linen, Nurse's station & others	4.8% (29/605)	60% (29/49)	(Tajeddin *et al*., 2016)
MRSA	Inanimate surfaces and medical equipment	5.2% (3/58)	60% (3/5)	(Mbanga *et al*., 2018)

MRSA: Methicillin resistant *S. aureus*

1.4.7. Assessment of environmental contamination: objective monitoring systems

To assess clinical surface hygiene in hospitals bacteriological standards is required. The first standard concerns any finding of a specific 'indicator' organism, the presence of which suggests a requirement for increased cleaning. Indicators would include MRSA, *Clostridium difficile*, VRE and various Gram-negative bacilli. The second standard concerns a quantitative aerobic colony count of, 5 cfu/m^3on frequent hand touch surfaces and during pre-operation sampling the microbial contamination of the air is always within the limit of 35 cfu/m^3(range: 2–27 cfu/m^3) for conventional operating theatres at rest (Dancer, 2004; Dallolio *et al*., 2018).

Five objective monitoring methods of environmental hygiene were included in the CDC tool kit to decrease patient to patient transmission of healthcare associated pathogens (HCAI) as a result of the contaminated surface environment: (1) direct practice observation of staff performance and compliance with protocols; (2) improved cleaning and disinfection of room surfaces; (3) 'no touch' methods for terminal room disinfection; (4)'self-disinfecting' surfaces; (5) swab and agar slide cultures, providing a quantitative assessment of viable microbial contamination (Guh and Carling, 2015).

1.4.6.1. Improving surface cleaning and disinfection

Multiple studies have demonstrated that less than 50% of hospital room surfaces are adequately cleaned and disinfected when chemical germicides are used. Interventions including improved education of environmental service workers, checklists to assure that all surfaces (usually by environmental service workers) and medical devices/equipment (usually by nursing) are cleaned and disinfected, and assessment of the cleanliness of the environment (e.g., fluorescent dye, ATPase) with immediate feedback to the environmental service worker have been demonstrated to improve the frequency of adequate cleaning in the range of 71–77% (Chemaly *et al*., 2014).

Multiple methods of assessing the adequacy of cleaning have been developed but the two most practiced are the use of a fluorescent dye (If cleaning is adequate no fluorescence is detected when the dotted object is exposed to black light but a fluorescent dot appears if cleaning is inadequate) and ATP bioluminescence (marker for microbial contamination viability). Improved surface cleaning and disinfection have focused on terminal cleaning (i.e., after the patient has been discharged) (Weber *et al*., 2013)**.**

1.4.6.2. 'No-touch' methods of surface disinfection

Ultraviolet light (UV) or hydrogen peroxide (e.g., aerosolized dry mist hydrogen peroxide, hydrogen peroxide vapor) has developed as room disinfectant that can decontaminate environmental surfaces and objects. These methods can only be used for terminal or discharge room decontamination (i.e., cannot be used for daily room decontamination) because the room must be emptied of people. UV irradiation has been used for the control of pathogenic microorganisms in a variety of applications, such as control of legionellosis, as well as disinfection of air, surfaces, and instruments. At certain wavelengths, UV light will break the molecular bonds in DNA, there by destroying the organism (Chemaly *et al*., 2014).

1.4.6.3. 'Self-disinfecting' surfaces

Self-disinfecting surfaces, such surfaces have also been developed to reduce the bio-burden on environmental surfaces called 'self-sanitizing,' and because microbial killing requires direct contact with the surface, the term 'contact killing' has also been used. Self-disinfecting surfaces can be created by impregnating or coating surfaces with heavy metals (e.g., silver or copper), germicides (e.g., triclosan), or miscellaneous methods (e.g., light-activated antimicrobials) have anti-infective activity (Chemaly *et al*., 2014; Russotto *et al*., 2015).

1.4.6.4. Swab and agar slide cultures/Microbiological Methods

Methods typically utilize swab cultures, in which a moistened sterile swab is used to sample a surface and then inoculate agar, often with broth enrichment. Swab cultures are easy to use and are often used to sample irregular surfaces, medical equipment, and health care workers' hands. Swab cultures are most often used to identify specific pathogens during epidemiologic investigation of an outbreak. Importantly, the use of aerobic culture (with or without enumerating colony counts) is the only method that can provide information about the viability of our pathogens of interest (e.g. MRSA, VRE) (Galvin *et al*., 2012; Guh and Carling, 2015).

2. CHAPTER II: Objectives of the study

2.1. General Objective

- The project work aimed to detect the colonization of inanimate hospital environments with potential pathogenic and drug resistant bacteria in selected hospitals in Addis Ababa, Ethiopia.

2.2. Specific Objectives

- To detect potential pathogenic bacterial profiles in hospital environments in TASH and ALERT hospital.
- To assess antimicrobial susceptibility pattern of the recovered potential pathogenic bacteria
- To detect MRSA, ESBL,VRE and CRE from inanimate hospital environments
- To determine rate of multi-drug resistant bacterial pathogens

3. CHAPTER III: Materials and Methods

3.1. Study area

The data were collected from operation theaters and intensive care units of TASH and ALERT Hospital, in Addis Ababa, Ethiopia. These Hospitals are located in Addis Ababa the capital city of Ethiopia with a population of 3,384,569. TASH is a tertiary hospital and major referral center for other hospitals in Ethiopia. TASH has 800 beds and provides care for approximately 370,000−400,000 patients per year. TASH is also the center of excellence for training of undergraduate and postgraduate students in different disciplines. The main operating theatres of TASH consists of 10 operating rooms, 1 recovery room, patient reception room, and staff changing room and there are four intensive care units made up of general ward, and wards each designated for patient isolation.

On the other hand, ALERT is a medical facility in Addis Ababa, specializing in Hansen's disease, also known as "leprosy". It was originally the All Africa Leprosy Rehabilitation and Training Center (hence the acronym), but the official name is now expanded to include tuberculosis: All Africa Leprosy, Tuberculosis and Rehabilitation Training hospital. ALERT is currently a 240 bed hospital, which includes dermatology, ophthalmology, and surgery departments, also an orthopedic workshop, and a rehabilitation program. The main operating theatres consist of four OTs and one ICU unit. The OTs and ICU at these hospitals were included in the sample collection process. The criteria for selection of the study sites are based on the fact that these units are expected to be clean.

3.2. Study design and period

Institutional based cross-sectional study was conducted by collecting environmental samples from June to September, 2018.

3.3. Sample size and sampling technique

3.3.1. Sample size

The sample size (counting locations) was calculated according to the ISO 14644–2, which states that the numbers of counting locations are calculated according to the square root of the floor area (ISO, 2003). Since there was a chance of one microbe leaving one wards to another, there was an underlying correlation that was dealt with by using a design effect of 2 (DE= 2.0). Hence, overall minimum sample size =60 × 2 = 120 (Matinyi *et al*., 2018). A total of 257 swab and 23 air samples were collected from TSAH and ALERT hospital. Allotment

of sample size between the two hospitals was done arbitrarily, where 187 swabs and air samples were collected from TSAH based on large size and high patient load, while only 93 swab samples were collected from ALERT hospital due to low patient load and small number of confined habitat (Table 3.1).

Table 3.1: Sample locations and number of collected environmental samples

Hospital		Sites	Square root of floor area (average)	No of collected sample
TASH	**Operating theaters**	Emergency	4 M× 5 M= √20 ≈4	12
		Neurology	4 M× 5 M= √20 ≈4	15
		Endo-Renal	4 M× 5 M= √20 ≈4	18
		Gyn-obs	4 M× 5 M= √20 ≈4	20
		Pediatric	4 M× 5 M= √20 ≈4	21
		Cardio-Vascular	4 M× 5 M= √20 ≈4	17
		Gastro intestinal tract	4 M× 5 M= √20 ≈4	19
ALERT		Trauma	4 M× 5 M= √20 ≈4	16
		General	4 M× 5 M= √20 ≈4	18
		Major	4 M× 5 M= √20 ≈4	27
TASH	**Intensive care unit**	Surgical	4 M× 5 M= √20 ≈4	15
		Pediatric	4 M× 5 M= √20 ≈4	17
		Medical	4 M× 5 M= √20 ≈4	18
		Medical-Surgical	4 M× 5 M= √20 ≈4	15
ALERT		Trauma	4 M× 5 M= √20 ≈4	32
Total			**60 (locations)**	**280**

3.3.2. Sampling technique

Non probable convenient type of sampling technique was used to collect environmental swab and air samples.

3.4. Variables

3.4.1. Dependent Variable

Detection rate of potential pathogenic bacteria and antimicrobial susceptibility pattern (antimicrobial profiles of drug resistant bacteria (MRSA, ESBL, VRE and CRE)).

3.4.2. Independent Variable

Type of wards (Intensive care units (ICU) or Operation Theater (OT)), medical devices, various surfaces, inanimate objects such as sink, floor, operation bed, trowel, operation table, operation light, walls, equipment and air.

3.5. Data collection

3.5.1. Sampling locations

The samples were collected from five intensive care units (four from TASH and one from ALERT hospital including Surgical, Pediatric, Medical, Medical-Surgical and Trauma). A total of 10 operating theaters were examined (seven from TASH and three from ALERT including Emergency, Neurology, Endo-Renal, Obstetrics and gynaecology, Pediatrics, Cardio-Vascular, GIT, Trauma, General and Major).

The choice of sampling points were targeted the most frequently touched and most representative locations in each service and the sampling sites were categorized into groups: (1) commonly touched medical equipment's, including beds, monitors, OR-light, linens, ventilators, oxygen supply, suction buttons and intravenous pumps (2) workstation, including keyboards, computer mice; (3) environments, including floors, wall and corridors; (4) Lobby (furniture) including chair, table, lockers and trowels; (5) Sinks ; (6) Air (settle plate) based on literature reviews (Dancer, 2004; Messai *et al*., 2008; Chen *et al*., 2017; Dallolio *et al*., 2018).

3.5.2. Samples from surfaces and medical equipment (Swab method)

A sterile cotton swab stick was used to swab different types of inanimate hospital surfaces and medical equipment after cleaning in the morning in respective hospitals according to Claro *et al*., (2014). Swabs collected from the medical equipment and environmental surfaces were inserted aseptically into sterile test tubes that contained freshly prepared brain heart infusion broth (BHI) (Merck, Germany) (Ekrami *et al*., 2011; Galvin *et al*., 2012), and transported to laboratory within 2 hour. Analysis of swab samples was conducted at the post graduate Bacteriology laboratory of the Department of Microbiology, Immunology and Parasitology, Addis Ababa, University.

3.5.3. Air sampling (settle plate method)

Settle plate method was used for sampling of air from five operating theaters before the start of each surgical operation (pre-operation) in different areas of the theater after cleaning in the morning. Bacterial load of the air were performed by exposing 9 cm diameter sterile petri-dishes containing open 5% sheep's blood agar, Mannitol salt agar and MacConkey agar plates were placed at different points in the wards according to the 1/1/1scheme (at a height of 1m above the ground, 1m way from the walls of the rooms and for 1hour) (Pasquarella *et al*., 2000). During air sampling procedure, sterile gloves, surgical masks, and protective gowns were used to prevent contamination of the agar plates. Then, they were covered and transported to the laboratory within 2 hour for incubation at 35 °C for 18-24 hours. The resultant colonies were counted and results expressed in CFU/dm^2/h (Napoli *et al*., 2012). Colonies showing different appearances were isolated based on standard cultural and morphological methods, and purified into pure cultures and characterized to species level using biochemical methods (Garcia, 2010; Matinyi *et al*., 2018).

3.5.4. Analysis of Collected Swab Sample

The collected swab samples were incubated at 35°C for 18-24 hours. After 24 hours, sub-culturing was done by inoculating a loop full of broth culture into blood agar (Oxoid, UK), MacConkey agar (Oxoid, UK), Bile esculin agar, Mannitol salt agar (MSA) and Chromagar TM Strep B base plates (Chromagar microbiology, France) to identify common potential pathogenic bacteria. Aseptic streaking technique was strictly followed and the plates were incubated at 35°C for 18-24 hours. Differential and selective characteristics for each agar medium were recorded. The isolates were initially identified to the species level using standard cultural, morphological and biochemical methods described based on hand book of

Clinical Microbiology Procedures (Garcia, 2010). Gram-negative bacteria were identified by Gram stain, routine bacterial culture and standard biochemical tests like indole; triple sugar iron agar, urea, citrate, SIM medium, LIA, Malonate and oxidase. On the other hand, Gram-positive bacteria were identified by Gram stain, selective culture media and different biochemical tests including catalase, coagulase test and salt tolerance test whereas golden yellow colony color showed the fermentation of the Mannitol which is a presumptive test for *S. aureus* (Garcia, 2010; Badamchi *et al*., 2018).

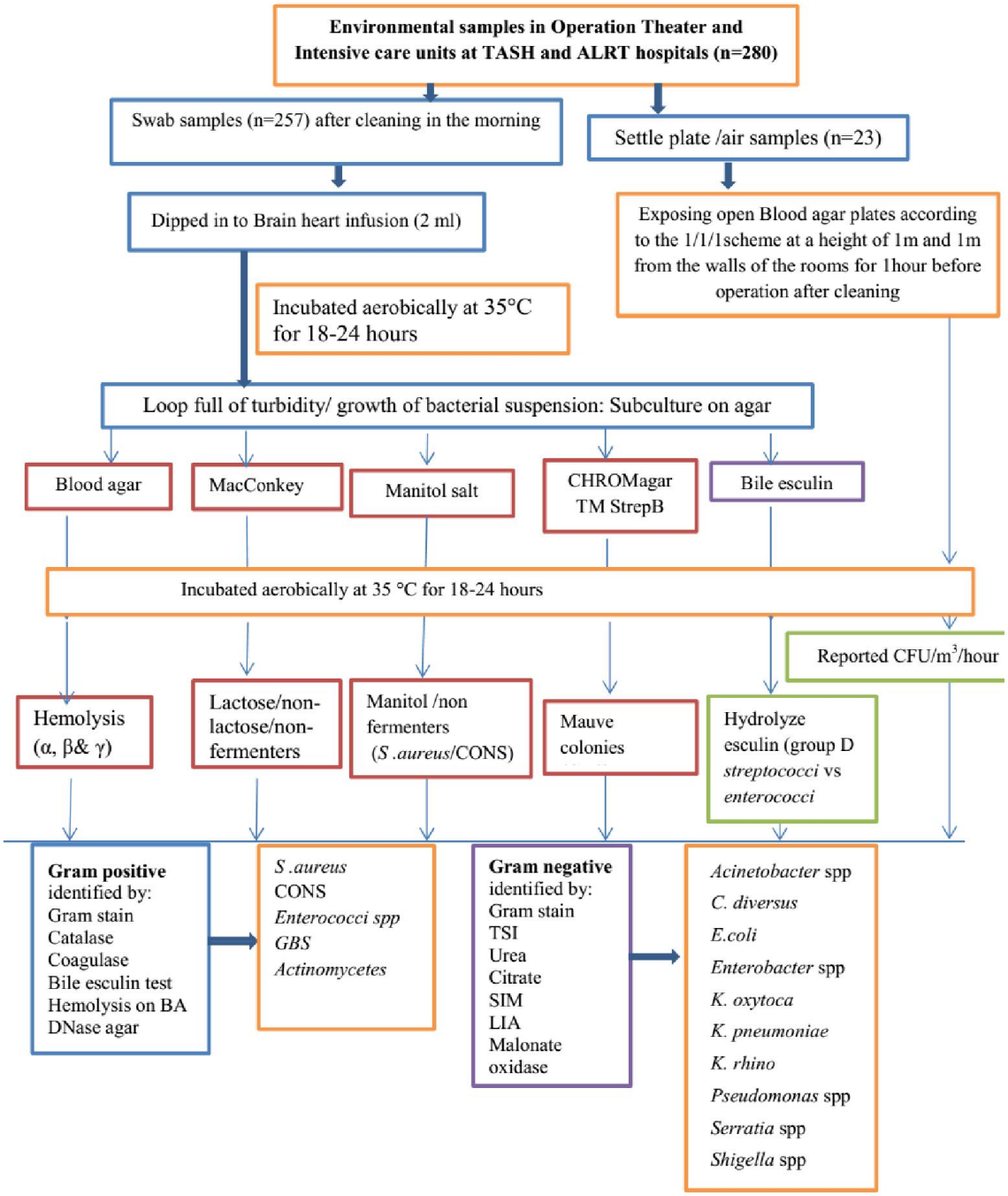


Figure 3.1: Flow chart of laboratory processing of environmental samples

3.5.5. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the potential pathogenic bacterial isolates were performed with 21 antibiotics (Oxoid, UK) based on the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA) (Oxoid, UK). An inoculum for each isolate was prepared by emulsifying colonies from an overnight pure culture in sterile normal saline (0.85%) in test tubes with the turbidity adjusted to 0.5 McFarland standards. The bacterial suspension was uniformly streaked on MHA plates using sterile swabs and left for 3 minutes prior to introduction of the antibiotics. For Gram-negative bacteria the following antibiotics were used (in µg/disk) ampicillin (10), amoxicillin and clavulanic acid (10/10), ceftriaxone (30), cefotaxime (30), ceftazidime (30), amikacin (30), gentamicin (10), ciprofloxacin (5), sulfamethoxazole-trimethoprim (1.25/23.75), cefoxitin (30), cefuroxime (30), cefepime (30), piperacillin-tazobactam (100/10), meropenem (10) and aztreonam (30) based on Clinical Laboratory Standards Institute (CLSI, 2018). For Gram-positive bacteria antibiotics (in µg/disk) penicillin (10 units), gentamicin (10), erythromycin (15), ciprofloxacin (5), doxycycline (30), vancomycin (30), cefoxitin (30), sulfamethoxazole-trimethoprim (1.25/23.75), clindamycin (2) and chloramphenicol (30) antibiotics were selected for susceptibility testing. Plates were incubated at 35 ◦C for 24 h, and the diameters of zone of inhibition were measured with Vernier caliper and results interpreted according to Clinical Laboratory Standards Institute (CLSI, 2018).

3.5.5.1. Detection of ESBL phenotype

Extended spectrum beta-lactamase producer Gram-negative isolates were tested using cefotaxime and ceftriaxone according to the CLSI standard using the modified Kirby Bauer method (Figure 3.2). Reduced susceptibility to cefotaxime (30 µg) and ceftriaxone (30 µg) with inhibition zone sizes ≤ 27 mm and ≤ 25 mm, respectively, was used as screening method for ESBL production; and double disk synergy test (DDST) using ceftazidime (30µg), cefotaxime (30µg) and co-amoxi/clavulanates (20/10 µg) was used to confirm ESBL production. A clear-cut enhancement of the inhibition in front of either ceftazidime and cefotaxime disks towards the clavulanic acid-containing disk (also called “champagne-cork” or “keyhole”) was interpreted as positive for ESBLs production. The combination disk (CD) method was also employed by placing disks with ceftazidime (30µg) and ceftazidime/clavulanic acid (30/10µg) on the surface of the inoculated agar. ESBL positive isolates were those in which the growth inhibition zone around the ceftazidime/clavulanic

acid disk was >5mm larger than the zone around ceftazidime alone as confirmatory test according CLSI guideline (CLSI, 2018).

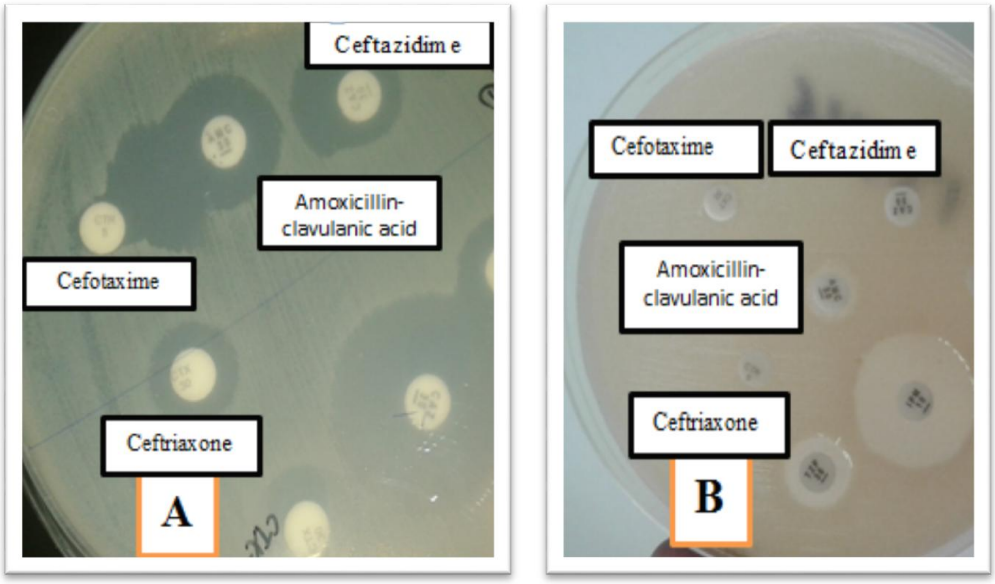


Figure 3.2: Double-disk synergy tests (DDST) for ESBL producing bacteria: from different environmental sample at TASH and ALERT hospital, 2018.

(A) **Positive result** (clear extension of the edge) and (B) **Negative result**

3.5.5.2. Phenotypic detection of carbapenem resistance

The selected carbapenems (imipenem, meropenem or ertapenem) resistant bacteria were screened for carbapenemase production by using Modified Hodge test (MHT) technique (Figure 3.3). An overnight culture of carbapenem susceptible strain of *E. coli* ATCC 25922 was adjusted to a turbidity of McFarland 0.5 and dilute 1:10 in saline or broth and streaked as a lawn over Mueller Hinton agar (MHA) plates and disks loaded with meropenem (10 µg) were placed in the center of the test area. Isolates to be tested along with a positive control (*K. pneumoniae* ATCC1705) and negative control (*K. pneumoniae* ATCC1706) were streaked as straight lines from the edge of the meropenem disk to the edge of the plate and allowed to dry for 3–10 minutes at room temperature and then incubated overnight at 35 ± 2 °C for 16–20 hours. The Hodge test is interpreted as positive by the presence of distortion (a clover leaf-like shape) of the inhibition zone based on (CLSI, 2018).

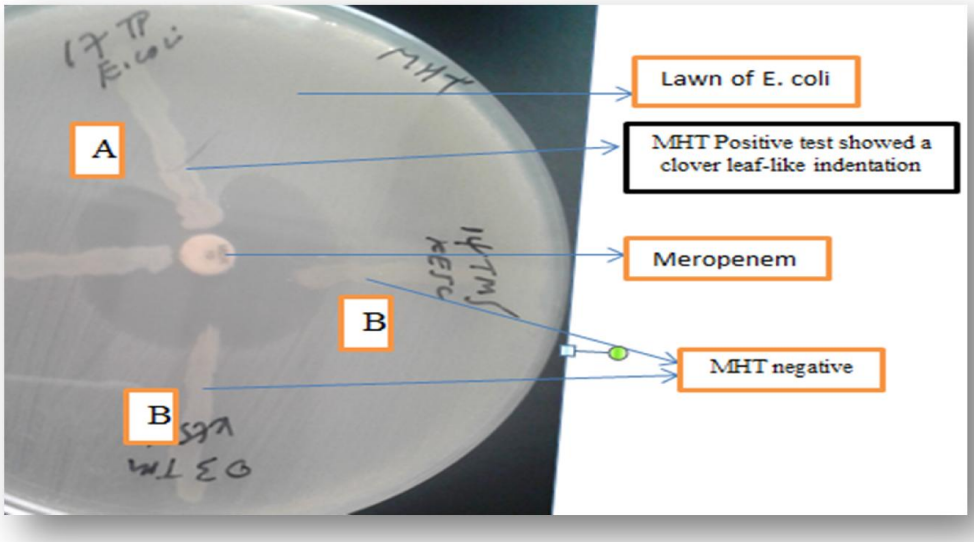


Figure 3.3: The MHT performed on a Muller Hinton Agar plate: from different environmental sample at TASH and ALERT hospital, 2018.

(A) **MHT positive result** (B) MHT **negative result**

3.5.5.3. Phenotypic detection of MRSA/MRCONS and VRE

All isolates identified as *S. aureus* and CONS were parallely subjected to antibiotic susceptibility testing to oxacillin (5 µg)/ cefoxitin (surrogate test for oxacillin) (30 µg) by disc diffusion test to detect phenotypic resistance to methicillin. Inoculated plates were incubated at 35^0C for 24 h. All isolates resistant to Oxacillin/ cefoxitin disc were considered to be presumptive MRSA and MRCONS. On the other hand, phenotypic vancomycin resistant *Enterococci* (VRE) were screened by vancomycin disk (30µg) (CLSI, 2018).

3.5.5.4. Determination of Multidrug-resistant (MDR), extensively drug-resistant (XDR) and pan drug-resistant bacteria (PDR)

MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories (Magiorakos *et al*., 2012).

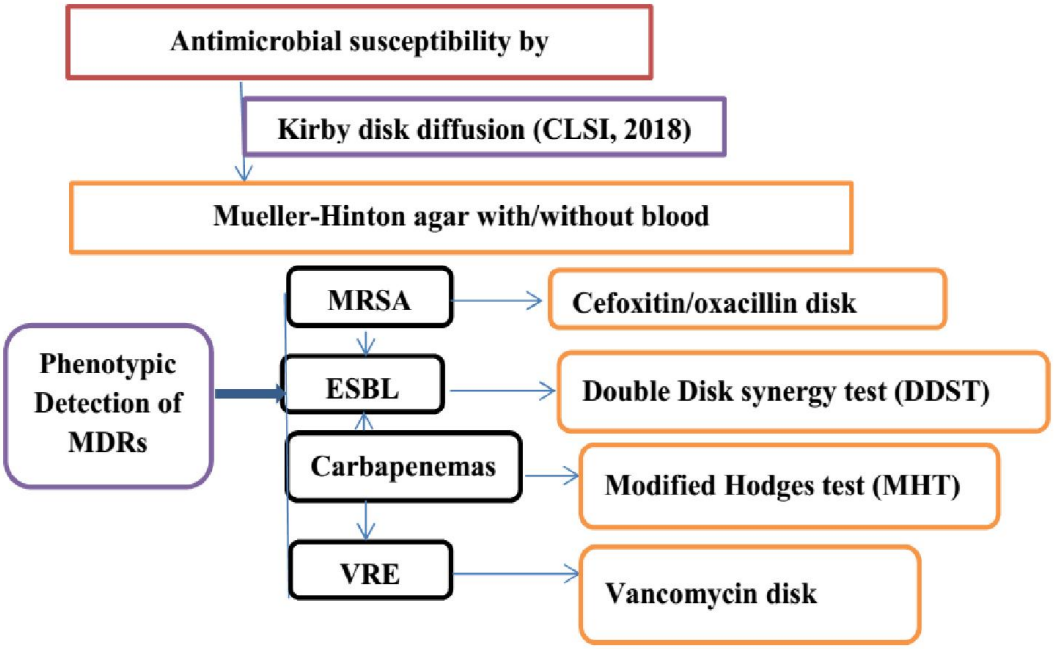


Figure 3.4: Flowchart of antimicrobial susceptibility pattern of potential pathogenic and phenotypic detection of MDR bacterial strain.

3.6. Quality Assurance

To ensure the quality of the result from different assays, internal quality assurance systems was in place for all laboratory procedures and double checking of the result was done. All the methods to be used were validated as fit for the purpose before use in the study and if an amendment and/or modification were made, the modifications were documented in the log books. Standard operating procedures (SOPs) were used for specific purpose for all laboratory procedures. Quality control strains of *Enterococcus faecalis* ATCC® 29212 *S. aureus* ATCC® 25923, *E. coli* ATCC® 2592, *K. pneumoniae* ATCC®1705 and *K. pneumoniae* ATCC®1706, kindly donated by Ethiopian public health institute (EPHI) and Armauer Hansen Research Institutes (AHRI), were used to confirm the result of antibiotics, media and to assess the quality of the general laboratory procedure (CLSI, 2018). The quality of the reagents, antibiotic disk and media used were checked regularly. The isolates were stored at−80 °C in a broth containing skimmed milk, tryptone, glucose and glycerol (STGG) at AHRI for further analysis.

3.7. Data Processing and Analysis

Data were entered, cleaned and analyzed using SPSS Statistical Software version 25 (IBM company, Comp.soft-sys.stat.spss.). Descriptive statistics such as frequencies and percentages were employed to report numerical summaries of findings. Patterns of quantitative values were presented using graph presentations and statistical tables. Associations between independent variables and the presence/absence of target organisms were computed using bi-variety analysis. Differences were considered statistically significant for P-value ≤ 0.05.

3.8. Ethical consideration

The project was ethically approved by the department of Microbiology, Immunology & Parasitology research ethics committee (DERC), College of Health Sciences, Addis Ababa University (Reference no DERC/17/18/02-G) and exempted from review by AHRI/ALERT Ethics committee (Reference no AH02122/0012/18). Official permission letter was obtained from TASH and ALERT hospital. All the data obtained from the research was kept confidential by keeping them locked in the office of the principal investigator.

3.9. Result dissemination plan

The thesis will be defended in Addis Ababa University and AHRI community. The study result will be submitted to Addis Ababa University, College of Health Science, Department of Microbiology, Immunology and Parasitology. The data will be made known to the scientific community through conference presentation and publication in reputable national and international journals. Major findings of the study will be communicated to concerned offices, health professionals and other stake holders. Infection control guidelines will be updated based on the findings with stakeholders.

3.10. Operational definitions

Nosocomial infections: defined as isolates within the first 48 hours after hospitalization result in acquisition of infections.

Hospital associated bacteria: Bacteria especially connected to hospital environment.

Cleaning schedule: whether the rooms/patient's room had been cleaned before or after environmental sampling.

MDR: defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories

Indoor air: the air inside the rooms of the selected wards

Settle plate or passive air sampling: Petri-dishes containing blood agar plates are left open to air for a given period of time. Microbes carried by inert particles fall onto the surface of the nutrient, with an average deposition rate of 0.46 cm/s being reported (Napoli *et al*., 2012).

4. CHAPTER IV: Results

4.1. Number of collected and culture positive environmental samples

During the four months study, a total of 280 samples (environmental swabs (n= 257) and settle plates (n=23) samples) were collected in the studied ICUs and OTs in the two hospitals. These samples were collected in TASH (n=187) and ALERT (n=93) (Table 4.1). Out of 280 swab and settle plate samples, 227(81%) samples were positive for bacterial growth. Out of 227 positive samples, a total of 282 bacterial isolates were obtained (Table 4.2).

Table 4.1: Number of collected environmental samples at TASH and ALERT hospital, 2018.

	Sample type, n (%)		
Hospital	**Settle plate**	**Surface swab**	**Total, n (%)**
TASH	23(8.2)	164(58.6)	187(66.8)
ALERT	0(0)	93(33.2)	93(33.2)
Total, n (%)	23(8.2)	257(91.8)	280(100)

Table 4.2: The total number of collected and culture positive environmental samples at TASH and ALERT hospital, 2018.

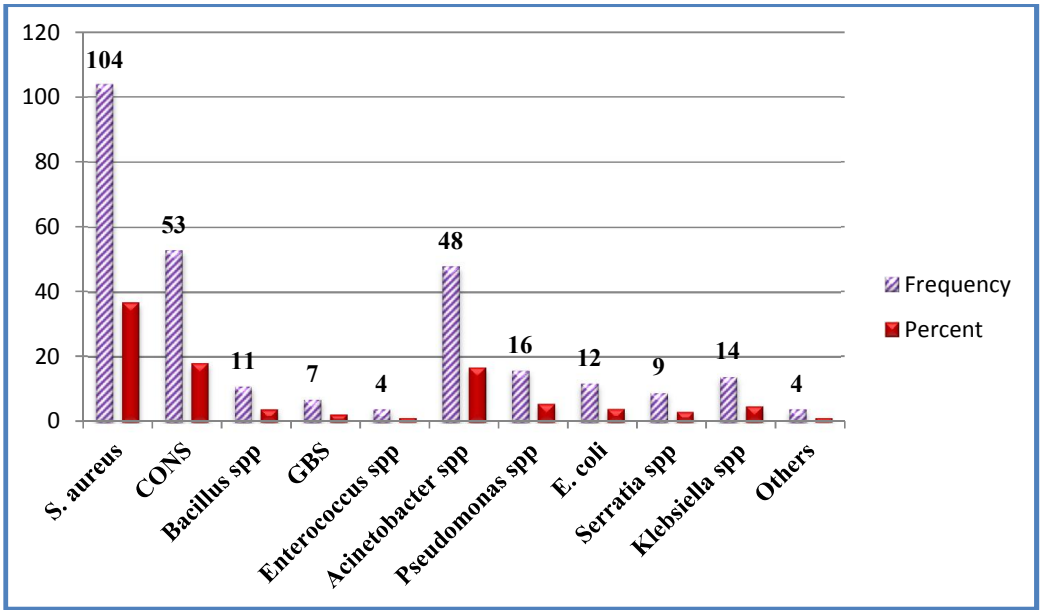
Name of hospitals		Sites	No of collected samples	Culture		No of bacteria isolated
				Positive	Negative	
TASH	Operation theaters	Emergency	12	12	0	12
		Neurology	15	13	2	13
		Endo-Renal	18	14	4	20
		Gynecology	20	16	4	16
		Pediatric	21	16	5	17
		Cardio-Vascular	17	16	1	17
		GIT	19	18	1	19
		Sub total	**122**	**105**	**17**	**114**
ALERT		Trauma	16	5	11	5
		General	18	18	0	22
		Major	27	21	6	21
		Sub total	**61**	**44**	**17**	**48**
TASH	Intensive care units	Surgical	15	15	0	16
		Pediatric	17	15	2	24
		Medical	18	14	4	22
		Medical-Surgical	15	13	2	30
		Sub total	**65**	**57**	**8**	**92**
ALERT		Trauma	32	21	11	28
		Sub total	**32**	**21**	**11**	**28**
Total			280	227(81%)	53(19%)	282

4.2. Distribution of potential pathogenic bacteria

Out of the 282 bacterial isolates, 179(63.5%) were Gram-positive and the rest Gram-negative bacteria. The majority of the bacteria were isolated from TASH which accounts for 73% (206/282) (Table 4.3). Among potential Gram-positive bacteria *S. aureus* (36.9%), CONS (18.8%) and *Bacillus* spp (4%) were the dominant isolates. Among Gram-negative bacteria *Acinetobacter* spp (17%), *Pseudomonas* spp (5.7%) and *E. coli* (4.2%) were the dominant isolates (Figure 4.1). Overall, *S.aureus* was the most frequently isolated bacteria 104 (36.9%) followed by CONS 53(18.8%) and *Acinetobacter* spp 48 (17%). *Citrobacter diversus, K. rhinoscleromatis* and *Shigella* spp were least isolated organisms with frequency as low as < 0.5% (Table 4.4).

Table 4.3: Distribution of Gram-positive and Gram-negative bacteria at TASH and ALERT hospital, 2018.

Gram reaction	Hospital		Total; n (%)
	TASH; n (%)	ALERT, n (%)	
Gram-positive	126(44.7)	53(18.8)	**179(63.5)**
Gram-negative	80(28.4)	23(8.2)	**103(36.5)**
Total	**206(73)**	**76(27)**	**282(100)**



Others: *Enterobacter* spp, *C. diversus*, *Shigella* spp

Figure 4.1: Frequency of potential pathogenic bacteria at TASH and ALERT, 2018.

Table 4.4: Frequency of potential pathogenic bacteria from the hospital environment at TASH and ALERT hospital, 2018.

Bacterial isolates	**Hospital**		
	TASH; n (%)	**ALERT; n (%)**	**Total; n (%)**
Gram negative	**80(28.4)**	**23(8.2)**	**103(36.5)**
Acinetobacter spp	39(13.8)	9(3.2)	48(17)
Pseudomonas spp	14(5)	2(0.7)	16(5.7)
E. coli	9(3.2)	3(1)	12(4.2)
Serratia spp	4(1.4)	5(1.8)	9(3.2)
Klebsiella pneumoniae	6(2.1)	2(0.7)	8(2.8)
Klebsiella oxytoca	4(1.4)	1(0.4)	5(1.8)
Enterobacter spp	2(0.7)	0(0)	2(0.7)
Klebsiella rhinoscleromatis	1(0.4)	0(0)	1(0.4)
Citrobacter diversus	0(0)	1(0.4)	1(0.4)
Shigella spp	1(0.4)	0(0)	1(0.4)
Gram positive	**126(44.7)**	**53(18.8)**	**179(63.5)**
S. aureus	82(29.1)	22(7.8)	104(36.9)
Coagulase negative *staphylococci* (CONS)	32(11.3)	21(7.4)	53(18.8)
Bacillus spp	6(2.1)	5(1.8)	11(4)
Group B *Streptococci* (GBS)	3(1)	4(1.4)	7(2.4)
Enterococcus spp	3(1)	1(0.4)	4(1.4)
Total	**206(73)**	**76(27)**	**282(100)**

4.3. Air-borne bacterial load among operating theatres

We compared the bacterial load as expressed by (mean ± SD) from the different OTs at TASH. The highest bacterial load was obtained from GIT (66.4±21.9), followed by gynecology theatre (37.66±16.9) and least bacterial load was recovered from Cardio-Vascular theatre that was (7.6±7) cfu/dm^2/h (Table 4.5).

Table 4.5: Total aerobic bacterial load from the selected operating theatres at TASH, 2018.

Operation theatres	**Mean ±sd(cfu/dm^2/h)**	**Total microbial count (cfu/dm^2/h) Standard (at rest)** (Pasquarella *et al*., 2000)		
		Optimal	Acceptable	Not acceptable
Gastro intestinal tract	66.4(21.9)	0–4	5–8	≥ 9
Gynecology	37.66(16.9)			
Pediatrics	24.6(15)			
Endo-Renal	22.25(19.8)			
Cardio-Vascular	7.6(7)			

4.4. Distribution of potential pathogenic bacteria across different ICUs

Out of 282 bacterial isolates, 120(42.6%) bacteria were identified from studied ICUs of hospital environments of the two hospitals (Table 4.6). The ICUs environment showed highest frequency of Gram-negative 77(64.2%) bacteria compared to Gram-positive 43(35.8%) bacteria. Most of the potential pathogenic bacteria in the ICUs were isolated from Medical-Surgical ICU (25%, 30/120) (Figure 4.2). The predominant isolated bacteria in this ICU were *S. aureus* (8.3%, 10/120) and *Acinetobacter* spp (8.3%, 10/120). The next mostly contaminated ICU was Trauma ICU (23.3%, 28/120). The major isolated bacteria in this ICU were *Acinetobacter* spp (7.5%, 9/120) and *S. aureus* (5%, 6/120). Pediatric ICU was mainly contaminated by *Acinetobacter* spp (25%, 6/24). Medical ICU was also mainly contaminated by *Acinetobacter* spp (5%, 10/120). Least contaminated ICU was Surgical ICU (13.3 %, 16/120).

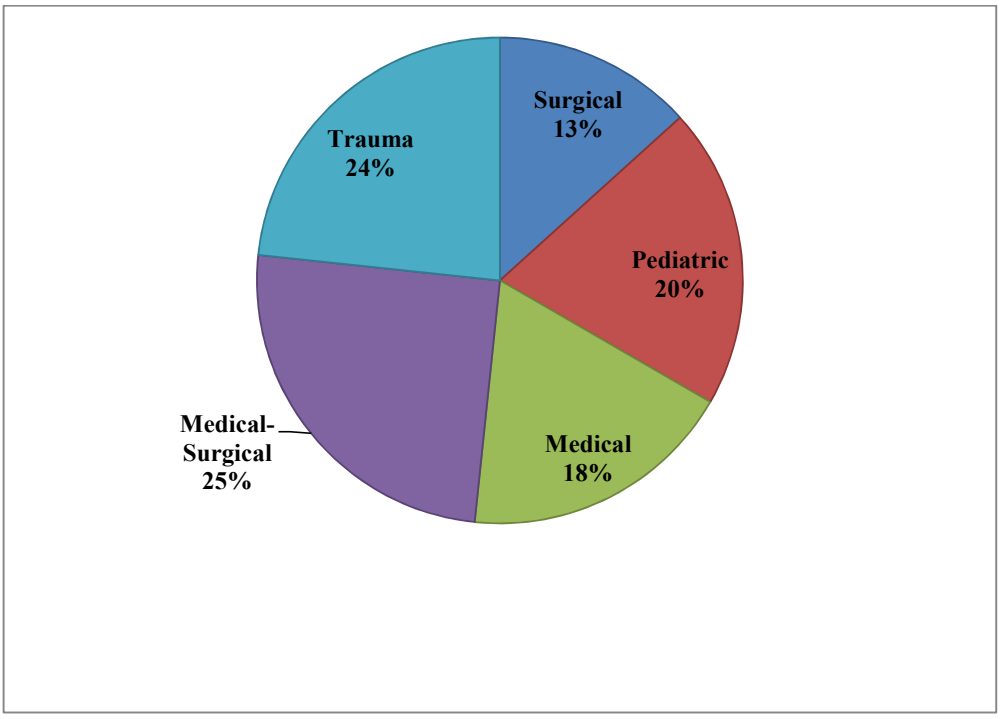


Figure 4.2: Distribution of potential pathogenic bacteria across different ICUs

Table 4.6: Frequency of potential pathogenic bacteria from the environment of ICUs at TASH and ALERT hospital, 2018.

Organism	Sites of environmental samples in the ICUs					Total N (%)
	TASH				ALERT	
	Surgical n (%)	Pediatric n (%)	Medical n (%)	Medical-Surgical n (%)	Trauma n (%)	
Gram negative	**10(8.3)**	**19(15.8)**	**15(12.5)**	**17(14.2)**	**16(13.3)**	**77(64.2)**
Acinetobacter spp	6 (5)	6 (5)	10 (8.3)	10 (8.3)	9 (7.5)	41(34.2)
E. coli	1(0.8)	4(3.3)	1(0.8)	3(2.5)	2(1.7)	11(9.2)
Pseudomonas spp	2(1.7)	5(4.2)	2(1.7)	0(0)	1(0.8)	10 (8.3)
K. pneumoniae	1(0.8)	2(1.7)	1(0.8)	1(0.8)	2(1.7)	7(5.8)
Klebsiella oxytoca	0(0)	2(1.7)	1(0.8)	1(0.8)	1(0.8)	5(4.2)
Citrobacter diversus	0 (0)	0 (0)	0(0)	0(0)	1(0.8)	1(0.8)
Enterobacter spp	0(0)	0(0)	0(0)	2(1.7)	0(0)	2(1.7)
Gram positive	**6(5)**	**5(4.2)**	**7(5.8)**	**13(10.8)**	**12(10)**	**43(35.8)**
S. aureus	4(3.3)	4(3.3)	3(2.5)	10(8.3)	6(5)	27(22.5)
CONS	1(0.8)	0 (0)	3(2.5)	1(0.8)	3(2.5)	8(6.7)
Enterococcus spp	0(0)	1(0.8)	1(0.8)	0(0)	1(0.8)	3(2.5)
GBS	0(0)	0(0)	0(0)	2(1.7)	1(0.8)	3(2.5)
Serratia spp	1(0.8)	0(0)	0(0)	0(0)	1(0.8)	2(1.7)
Total N (%)	**16(13.3)**	**24 (20)**	**22(18.3)**	**30(25)**	**28(23.3)**	**120(100)**

4.5. Distribution of potential pathogenic bacteria across different OTs

Out of the 282 bacterial isolates, 162(57.4%) bacteria were identified from the OTs of the two hospitals. The majority of the bacteria were isolated from the OTs of TASH. The OTs showed high frequency of Gram-positive 138(85.2%) compared to Gram-negative bacteria 24(14.8%). Most of the potential pathogenic bacteria in OTs were isolated from General OT (13.6%, 22/162) followed by Major OT (13%, 21/162) and Endo-Renal (12.4%, 20/162) (Figure 4.3). The predominant potential pathogenic bacteria isolated from the OTs were *S. aureus* (47.5%, 77/162), CONS (27.8%, 45/162), and *Acinetobacter* spp (4.3%, 7/162). The General OT was mainly contaminated by CONS (36.4%, 8/22) and *S. aureus* (31.8%, 7/22). The least contaminated OTs was trauma of ALERT hospital (3.1%, 5/162) (Table 4.7).

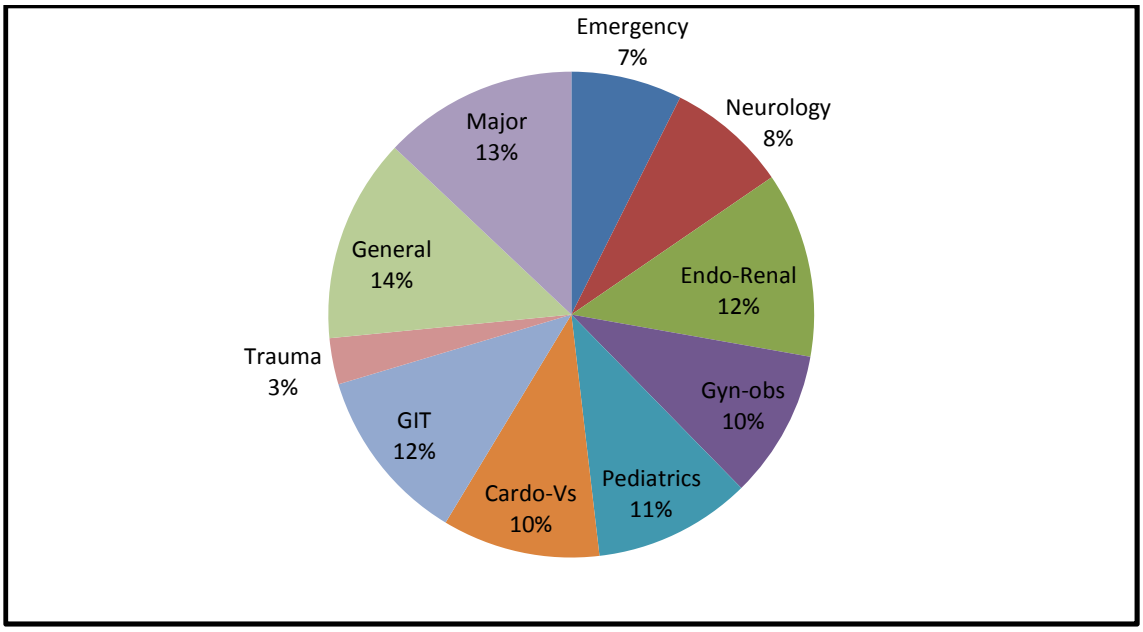


Figure 4.3: Distribution of potential pathogenic bacteria across different OTs

Table 4.7: Frequency of potential pathogenic bacteria isolated from the hospital environments of OTs at TASH and ALERT hospital, 2018.

Organism	Sites of environmental samples in the OTs										Total N (%)
	TASH							ALERT			
	Emergency (n=12)	Neurology (n=13)	Endo-Renal (n=20)	Gyn-obs (n=16)	Pediatrics (n=17)	Cardo-Vs (n=17)	GIT (n=19)	Trauma (n=5)	General (n=22)	Major (n=21)	
Gram positive	**12(7.4)**	**12(7.4)**	**9(5.6)**	**16(9.9)**	**16(9.9)**	**15(9.3)**	**16(9.9)**	**5(3.1)**	**20(12.3)**	**17(10.5)**	**138(85.2)**
S. aureus	8(66.7)	7(53.8)	9(45)	10(62.5)	9(52.9)	9(52.9)	9(47.4)	4(80)	7(31.8)	5(23.8)	77(47.5)
CONS	4(33.3)	2(15.4)	0(0)	5(31.3)	6(35.3)	6(35.3)	4(21)	0(0)	8(36.4)	10(47.6)	45(27.8)
Bacillus spp	0(0)	2(15.4)	0(0)	1(6.3)	0(0)	0(0)	3(15.8)	1(20)	2(9.1)	2(9.5)	11(6.2)
GBS	0(0)	1(7.7)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	3(13.6)	0(0)	4(2.5)
Enterococcus spp	0(0)	0(0)	0(0)	0(0)	1(5.9)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0.6)
Gram negative	**0(0)**	**1(0.6)**	**11(6.8)**	**0(0)**	**1(0.6)**	**2(1.2)**	**3(1.9)**	**0(0)**	**2(1.2)**	**4(2.5)**	**24(14.8)**
Acinetobacter spp	0(0)	0(0)	4(20)	0(0)	1(5.9)	1(5.9)	1(5.3)	0(0)	0(0)	0(0)	7(4.3)
Serratia spp	0(0)	0(0)	2(10)	0(0)	0(0)	1(5.9)	0(0)	0(0)	1(4.5)	3(14.3)	7(4.3)
Pseudomonas spp	0(0)	1(7.7)	2(10)	0(0)	0(0)	0(0)	2(10.5)	0(0)	1(4.5)	0(0)	6(3.7)
K. pneumoniae	0(0)	0(0)	1(5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0.6)
K. rhino	0(0)	0(0)	1(5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0.6)
E. coli	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(4.8)	1(0.6)
Shigella spp	0(0)	0(0)	1(5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(0.6)
Total N (%)	**12(7.4)**	**13(8)**	**20(12.3)**	**16(9.9)**	**17(10.5)**	**17(10.5)**	**19(11.7)**	**5(3.1)**	**22(13.6)**	**21(13)**	**162(100)**

4.6. Differences in distribution of bacteria between ICUs and OTs

In addition to the rate of bacterial load from OTs and ICUs, we also compared the yield between OTs and ICUs to identify areas which require emphasis in the mitigation of HAIs. Significant differences between Gram-positive and Gram-negative bacteria were observed between wards in OTs (48.9% vs 14.8%) and ICUs (14.5% vs 28%), respectively (p=0.000). The predominant bacterial isolates in the OTs and ICUs were *S. aureus* (27.5% vs 9.6%), CONS (16% vs 2.8%) and *Acinetobacter* spp (2.5% vs 14.5%), respectively (Figure 4.4).

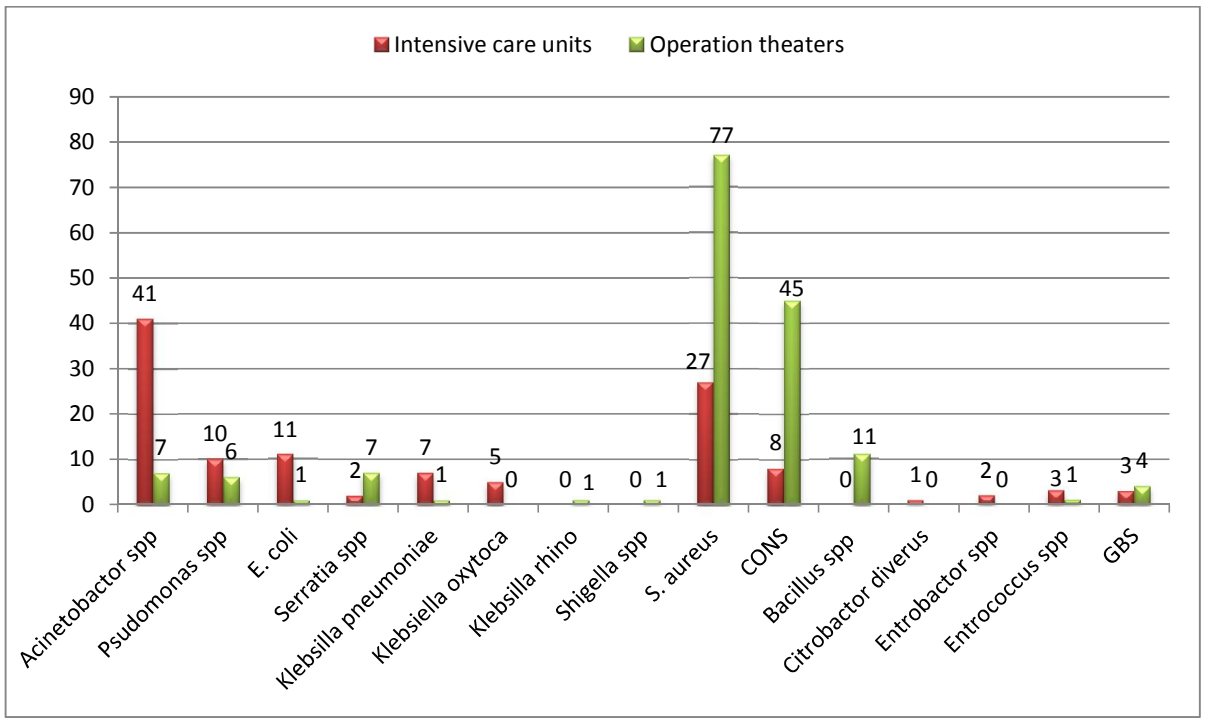


Figure 4.4: Frequency of potential pathogenic bacteria between ICUs and OTs at TASH and ALERT hospital, 2018.

4.7. Specific distribution of isolated bacteria in different environmental samples

Our results showed that the inanimate environments and air of the hospitals were greatly contaminated by different potential pathogenic bacteria. Multi-bacterial contaminations of the environmental samples were observed in sinks (60%), bed linens (48%), and ventilator and monitor each with 25%. The highest bacterial contaminated samples were taken from bed linens 37(13.1%), followed by environmental surfaces including (wall, floor, corridors and door knob) 35(12.4%) and bed 33(11.7%), while laparoscopy, oxygen cylinder, OR-Light and surgical trowels were least contaminated samples (Figure 4.5). Bed linens and beds were heavily contaminated by *Acinetobacter* spp (18.8% vs 20.8%), CONS (9.4% vs 5.7%) and *S. aureus* (7.7% vs 12.5%). Sinks were frequently contaminated by *Pseudomonas* spp (12.5%, 2/16), *S. aureus* (7.7%, 8/104) and *Acinetobacter* spp (4.2%, 2/48). Ventilators were frequently contaminated by *K. pneumoniae* (25%) followed by *E. coli* (16.7%) and *Acinetobacter* spp (8.3%). On other hand, air samples were frequently contaminated by *S. aureus* and CONS with 19(18.3%) and 4(7.5%), respectively (Table 4.8).

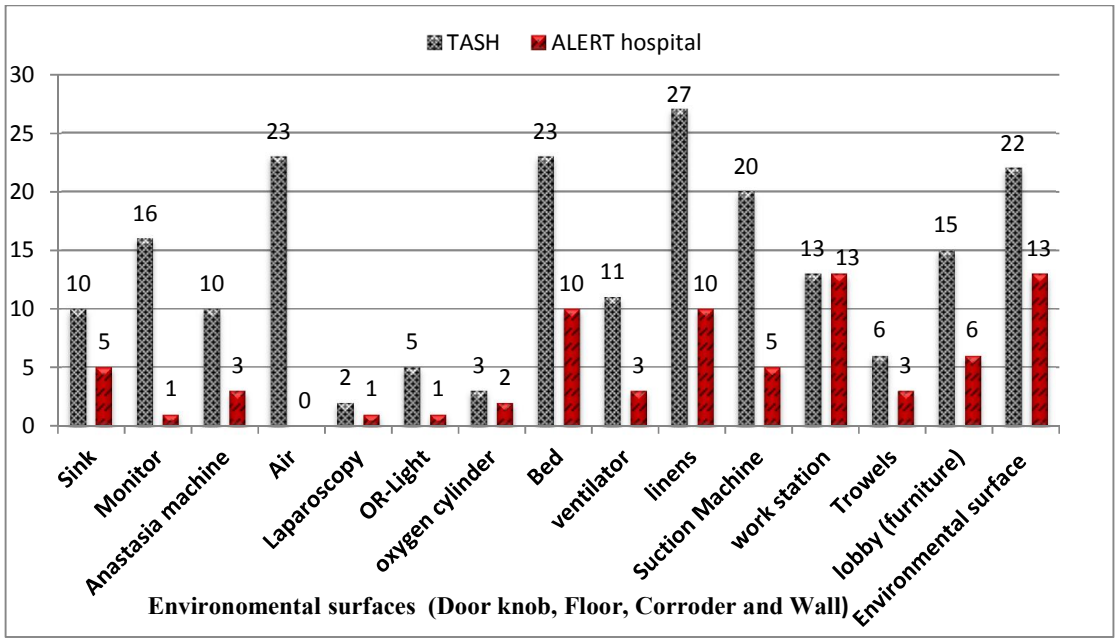


Figure 4.5: Frequency of bacterial contaminated environmental samples at TASH and ALERT hospital, 2018.

Table 4.8: Specific distribution of potential pathogenic bacteria in different environmental samples at TASH and ALERT hospital, 2018.

	Sites of environmental samples (n, %)											
Isolates	Bed Linens	Monitor	Anastasia machine	Bed	Sink	ventilator	Suction Machine	Work station	Lobby (furniture)	Environmental surface	Air	Others [b]
***Acinetobacter* spp**	9(18.8)	4(8.3)	1(2.1)	10(20.8)	2(4.2)	4(8.3)	5(10.4)	3(6.3)	6 (12.5)	4(8.3)	0(0)	0(0)
***Bacillus* spp**	0(0)	0(0)	2(18.2)	0(0)	0(0)	1(9.1)	1(9.1)	2(18.2)	1(9.1)	4(36.4)	0(0)	0(0)
CONS	5(9.4)	3(5.7)	3(5.7)	3(5.7)	0(0)	1 (1.9)	5(9.4)	6(11.3)	4(7.5)	11(20.8)	4(7.5)	8(15.1)
E. coli	3(25)	1(8.3)	0(0)	3(25)	1(8.3)	2(16.7)	1(8.3)	0(0)	1(8.3)	0(0)	0 (0)	0(0)
***Enterococcus* spp**	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(25)	1(25)	0(0)	1(25)	0 (0)	1(25)
GBS	1(14.3)	1(14.3)	0(0)	2(28.6)	0(0)	0(0)	0(0)	1(14.3)	0(0)	1(14.3)	0 (0)	1(14.3)
K. oxytoca	3(60)	0(0)	0(0)	0(0)	0(0)	1(20)	1(20)	0(0)	0(0)	0(0)	0 (0)	0(0)
K. pneumoniae	4(50)	0(0)	0(0)	1(12.5)	0(0)	2(25)	0(0)	0(0)	1(12.5)	0(0)	0 (0)	0(0)
***Pseudomonas* spp**	3(18.8)	1(6.3)	1(6.3)	1(6.3)	2(12.5)	1(6.3)	3(18.8)	0(0)	0(0)	2(12.5)	0 (0)	2(12.5)
S. aureus	8(7.7)	6(5.8)	5(4.8)	13(12.5)	8(7.7)	2(1.9)	8(7.7)	10(9.6)	7(6.7)	8 (7.7)	19(18.3)	10(9.6)
***Serratia* spp**	0(0)	0(0)	0(0)	0(0)	1(11.1)	1(11.1)	0(0)	3(33.3)	1(11.1)	3(33.3)	0 (0)	0(0)
Others [a]	1(16.7)	1(16.7)	1(16.7)	0(0)	1(16.7)	0(0)	0(0)	0(0)	0(0)	1(16.7)	0(0)	1(16.7)
Total=282	37(13.1)	17(6)	13(4.6)	33(11.7)	15(5.3)	14(5)	25(8.9)	26(9.2)	21(7.4)	35(12.4)	23(8.2)	23(8.2)

Others [a](*Shigella* spp, *Enterobacter* spp, *C. diversus* and *Klebsiella rhino*)
Others [b](Laparoscopy, OR-Light, oxygen cylinder, Trowels)

4.8. Antimicrobial susceptibility pattern of Gram-positive bacteria

The antimicrobial susceptibility patterns of the isolated Gram-positive bacteria were tested against eleven antibiotics from nine different antibiotic classes (Figure 4.6). The proportions of resistance among Gram-positive bacteria were high for penicillin (90.8%), oxacillin (60.5%), cefoxitin (60%) and erythromycin (55.1%). Low level of resistance was recorded among Gram-positive isolates for clindamycin (5.7%), chloramphenicol (12.6%), gentamicin (12.7%) and ciprofloxacin (12.9%). *S. aureus* showed the highest resistance level to penicillin (93.2%), followed by oxacillin (73.5%) and cefoxitin (59.1%). Clindamycin, with sensitivity of 95.1%, were the antibiotics with the highest activity against *S. aureus* (Table 4.9).

Out of 104 *S. aureus* and 53 CONS isolated from environmental samples 76(73.1%) and 16(30.2%) result in phenotypic methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant Coagulase negative *Staphylococcus* (MRCONS), respectively. On other hand, out of 104 *S. aureus* isolates, 20(19.3%) were vancomycin resistant *S. aureus* (VRSA). Moreover, out of 4 *Enterococcus* spp isolated from environmental samples, one (25%) *Enterococcus* spp was resistance to vancomycin (VRE).

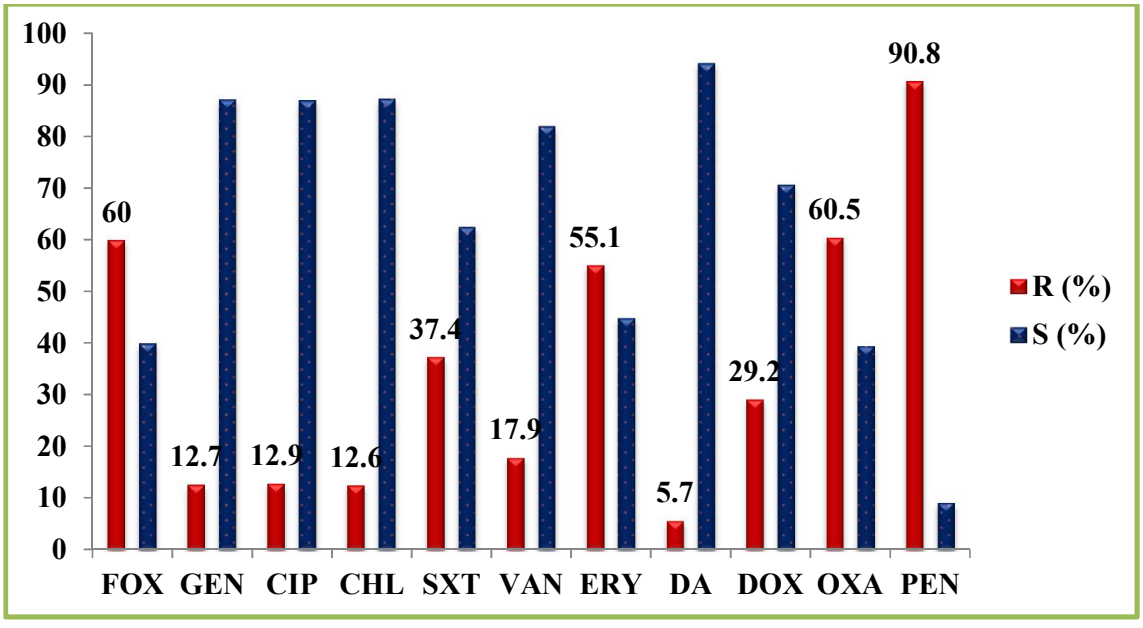


Figure 4.6: Antimicrobial susceptibility pattern of Gram-positive bacteria

Table 4.9: Antimicrobial susceptibility pattern of Gram-positive bacteria at TASH and ALERT hospital, 2018.

		Antimicrobial agent's n (%) (CLSI, 2018)										
Isolates	Ptn	FOX	GEN	CIP	CHL	SXT	VAN	ERY	DA	DOX	OXA	PEN
CONS	R	12(60)	3(13.6)	5(22.7)	4(18.2)	8(36.4)	NA	19(76)	1(3.7)	12(44.4)	16(30.2)	25(92.6)
	S	8(40)	19(86.4)	17(77.3)	18(81.8)	14(63.6)	NA	6(24)	26(96.3)	15(55.7)	37(69.8)	2(7.4)
Enterococcus	R	1(100)	0(0)	0(0)	2(100)	1(100)	1(25)	1(20)	2(50)	1(25)	4(100)	2(50)
spp	S	0(0)	1(100)	1(100)	0(0)	0(0)	3(75)	3(80)	2(50)	3(75)	0(0)	2(50)
GBS	R	2(66.7)	3(75)	0(0)	2(33.3)	3(100)	3(42.8)	5(71.4)	0(0)	3(42.9)	5(83.3)	5(71.4)
	S	1(33.3)	1(25)	4(100)	4(66.7)	0(0)	4(57.2)	2(28.6)	7(100)	4(57.1)	1(16.7)	2(28.6)
S. aureus	R	39(59.1)	7(9.6)	8(10.8)	6(7.4)	25(34.3)	20(19.3)	51(50)	5(4.9)	31(30.7)	76(73.1)	96(93.2)
	S	27(41)	68(90.4)	66(89.2)	75(92.6)	48(65.8)	NA	51(50)	97(95.1)	92(69.3)	28(26.9)	7(6.8)
Total	R	54(60)	13(12.7)	13(12.9)	14(12.6)	37(37.4)	24(77.4)	76(55.1)	8(5.7)	47(29.2)	101(60.5)	128(90.8)
	S	36(40)	89(87.3)	88(87.1)	97(87.4)	62(62.6)	7(22.6)	62(44.9)	132(94.3)	114(70.8)	66(39.5)	13(9.2)

n: Number of tested strains; R: Resistant; S: Sensitive; Ptn: Pattern; FOX: Cefoxitin; GEN: Gentamicin; CIP: Ciprofloxacin; CHL: Chloramphenicol; SXT: Trimethoprim-Sulfamethoxazole; VAN: Vancomycin; ERY: Erythromycin; DA: Clindamycin; DOX: Doxycycline; OXA: Oxacillin; PEN: Penicillin, NA: Not applicable

4.9. Antimicrobial susceptibility pattern of Gram-negative bacteria

The antimicrobial susceptibility pattern of the isolated Gram-negative bacteria was tested against 14 antibiotics from seven different classes (Figure 4.7). Most of the Gram-negative bacteria were resistant to most of the tested antibiotics as for example, ampicillin (97%), ceftazidime (89.3%), ceftriaxone (89%), aztreonam (88%), cefotaxime (81%), cefoxitin (74%) and amoxicillin and clavulanic acid (76.8%). Similarly, significant resistance level was also recorded for sulfamethoxazole-trimethoprim (68.3%), piperacillin-tazobactam (67%), cefepime (72.2%) and meropenem (51.4%). Low level of resistance was recorded for amikacin (28.2%), ciprofloxacin (37.8%) and gentamicin (45.1%). *Acinetobacter* spp showed the highest resistance level to almost all tested antibiotics including penicillin, cephalosporins, and carbapenems and monobactam groups of antibiotics including: ampicillin (100%), aztreonam (100%), ceftazidime (98%), cefotaxime (93.8%) and ceftriaxone (98%). Low resistance level was recorded to amikacin (41.6%) (Table 4.10).

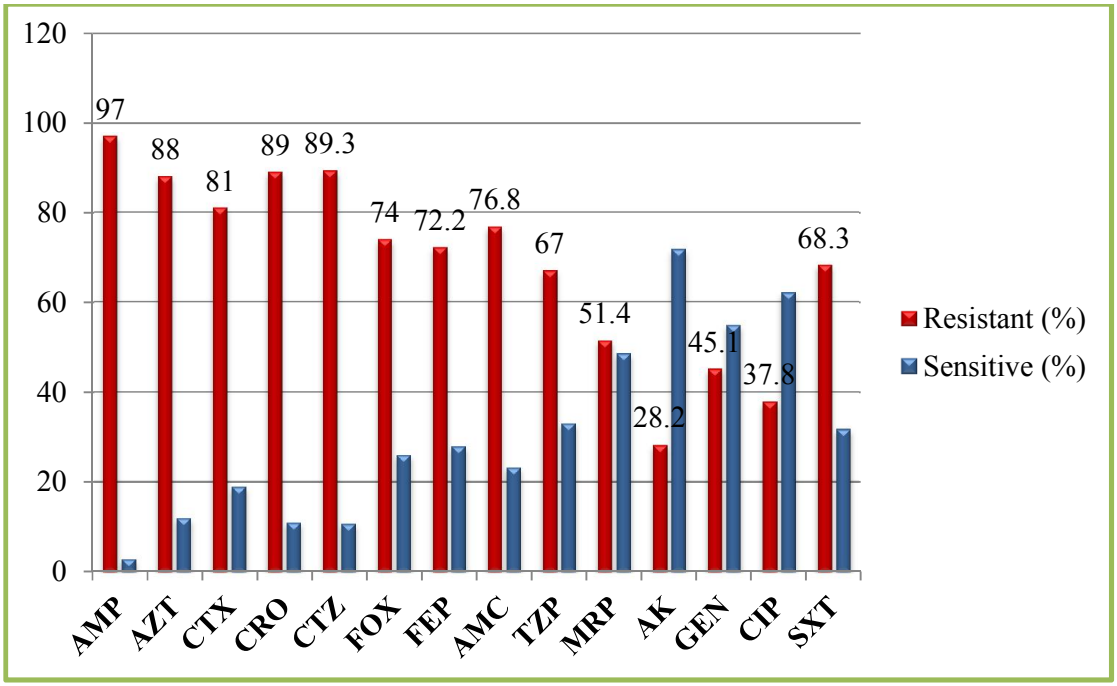


Figure 4.7: Antimicrobial susceptibility pattern of Gram-negative bacteria

Table 4.10: Antimicrobial susceptibility pattern of Gram-negative bacteria at TASH and ALERT hospital, 2018

Isolates	Antimicrobial agent's n (%) (CLSI, 2018)														
	Ptn	AMP	AZT	CTX	CRO	CTZ	FOX	FEP	AMC	TZP	MRP	AK	GEN	CIP	SXT
Acinetobacter spp	R	48(100)	48(100)	45(93.8)	47(98)	48(100)	46(95.9)	43(89.6)	40(87.3)	42(87.5)	35(73)	20(41.6)	32(66.7)	24(50)	37(79.1)
	S	0(0)	0(0)	3(6.2)	1(2)	0(0)	2(4.3)	5(10.4)	6(13)	6(12.5)	13(27)	28(58.3)	16(33.3)	24(50)	11(22.9)
C. diversus	R	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
E. coli	R	12(100)	9(75)	9(65)	10(83.3)	10(83.3)	7(58.3)	9(75)	8(66.7)	7(58.3)	4(33.3)	3(25)	6(50)	5(41.7)	9(75)
	S	0(0)	3(25)	3(25)	2(16.7)	2(16.7)	5(41.7)	3(25)	4(33.3)	5(41.7)	8(66.7)	9(75)	6(50)	7(58.3)	3(25)
Enterobacter spp	R	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)	0(0)	1(50)	2(100)	2(100)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50)	2(100)	1(50)	0(0)	0(0)
K. oxytoca	R	5(100)	4(80)	4(80)	5(100)	4(80)	4(80)	4(80)	2(66.7)	3(60)	2(40)	1(20)	2(40)	3(60)	3(60)
	S	0(0)	1(20)	1(20)	0(0)	2(20)	1(20)	1(20)	1(33.3)	2(40)	3(60)	4(80)	3(60)	2(40)	2(40)
K. pneumoniae	R	7(87.5)	5(62.5)	4(50)	8(100)	7(87.5)	3(37.5)	6(75)	7(87.5)	4(50)	4(50)	4(50)	3(37.5)	2(25)	6(75)
	S	1(12.5)	3(37.5)	4(50)	0(0)	1(12.5)	5(62.5)	2(25)	1(12.5)	4(50)	4(50)	4(50)	5(62.5)	6(75)	2(25)
K. rhino	R	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
Pseudomonas spp	R	15(93.8)	15(93.8)	13(81.3)	14(88)	13(81)	13(81.2)	6(37.5)	10(62.5)	7(43.8)	5(37.6)	0(0)	2(12.5)	3(18.8)	9(56.3)
	S	1(6.3)	1(6.25)	3(18.8)	2(12.5)	3(18.8)	3(18.8)	10(62.5)	6(37.5)	9(56.3)	10(62.4)	16(100)	14(87.5)	13(81.3)	7(43.8)
Serratia spp	R	9(100)	5(55.6)	3(33.3)	3(33.3)	5(55.5)	1(11.1)	3(33.3)	5(55.5)	3(33.3)	1(11.1)	1(11.1)	0(0)	0(0)	2(25)
	S	0(0)	4(44.4)	6(66.7)	6(66.7)	4(44.4)	8(88.9)	6(66.7)	4(44.4)	6(66.7)	8(88.9)	8(88.9)	9(100)	9(100)	6(75)
Shigella spp	R	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	1(100)
	S	0(0)	0(0)	0(0)	0(0)	0(0)	1(100)	1(100)	0(0)	0(0)	1(100)	1(100)	1(100)	1(100)	0(0)
Total	R	**100(97)**	**91(88)**	**83(81)**	**92(89)**	**92(89.3)**	**76(74)**	**75(72.2)**	**76(76.8)**	**69(67)**	**53(51.4)**	**29(28.2)**	**46(45.1)**	**39(37.8)**	**69(68.3)**
	S	**3(2.9)**	**12(12)**	**20(19)**	**11(11)**	**11(10.7)**	**27(26)**	**29(27.9)**	**23(23.2)**	**34(33)**	**50(48.5)**	**74(71.8)**	**56(54.9)**	**64(62.1)**	**32(31.7)**

N: Number of tested strains; R: number of resistant strains to the tested antibiotic; S: Sensitive; %: percentage of resistance; Ptn: Pattern; AMP: ampicillin; AMC: Amoxicillin and clavulanic acid; CRO: ceftriaxone; CTX : cefotaxime; CTZ: ceftazidime; AK: Amikacin; GEN: Gentamicin; CIP: ciprofloxacin; SXT: Sulfamethoxazole + trimethoprim; FOX: Cefoxitin; CXM: Cefuroxime; FEP: Cefepime; TZP: Piperacillin-tazobactam; MRP: Meropenem; AZT: Aztreonam

4.10. ESBL producing bacteria isolated from the hospital environments

Phenotypic identification of ESBL producing isolates has been carried out using DDST screening method. Of the total 280 environmental samples, 25(8.9%) were ESBL producing bacteria. Out of 103 Gram-negative bacteria (both *Enterobacteriaceae* and non-*Enterobacteriaceae*) isolates, 25(24.3%) were identified as ESBL producers. These isolates consisted of 24 *Enterobacteriaceae* (*E. coli* (91.7%, 11/12), *Enterobacter* spp (100%, 2/2), *K. oxytoca* (80%, 4/5), *K. pneumoniae* (75%, 6/8) and *Serratia* spp (11.1%, 1/9) and 1 non-fermenting Gram-negative bacillus (*Pseudomonas* spp (6.3%, 1/16) (Figure 4.8). The highest number of ESBL producing Gram-negative bacteria were taken from bed linens 8(32%), ventilators 5(20%) and sink and bed, each 3(12%) (Table 4.11).

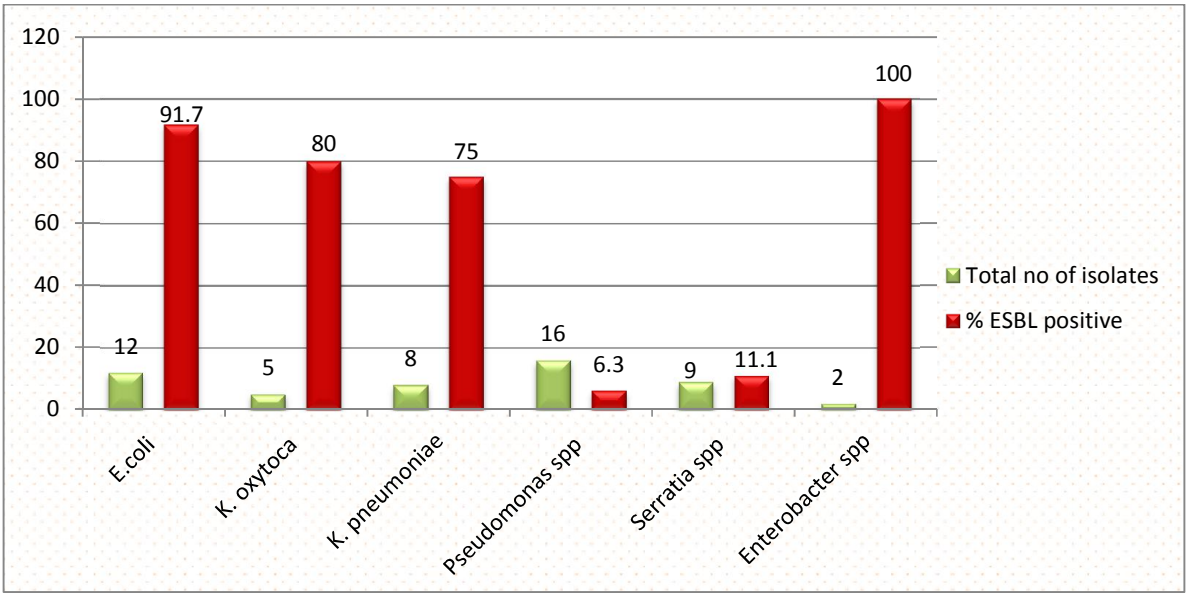


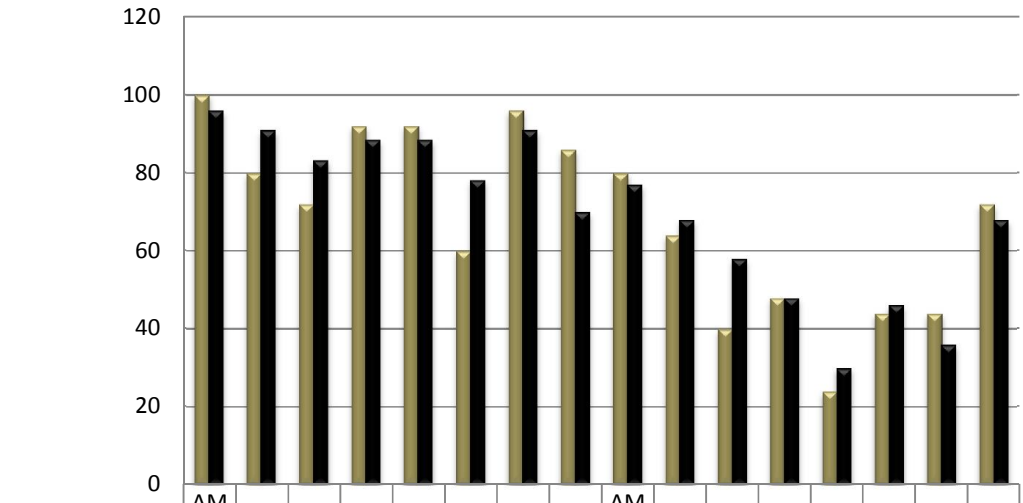
Figure 4.8: Frequency of ESBL producing Gram-negative bacteria at TASH and ALERT hospital, 2018.

Table 4.11: Distribution of ESBL producing bacteria isolated from the hospital environment at TASH and ALERT hospital, 2018.

Variables	ESBL, n (%)		X^2 (P-value)
	Positive	**Negative**	
Hospitals			
TASH	17(68)	63(80.8)	1.780(0.182)
ALERT	8(32)	15(19.2)	
Ward type			
ICUs	21(84)	58(74.4)	0.985(0.321)
OTs	4(16)	20(25.6)	
Type of Samples			
Swab	25(100)	78(100)	NA
Settle plate	0(0)	0(0)	
Sampling points			
Bed linens	8(32)	15(19.2)	13.451(0.407)
Ventilator	5(20)	6(7.7)	
Sink	3(12)	4(5.1)	
Bed	3(12)	12(15.4)	
Monitor	2(8)	5(6.4)	
lobby (furniture)	2(8)	7(9)	
Suction Machine	1(4)	9(11.5)	
Environmental surface	1(4)	9(11.5)	
Anastasia machine	0(0)	2(2.6)	
work station	0(0)	6(7.7)	
Oxygen cylinder	0(0)	3(3.8)	
Total	**25(24.3)**	**78(75.7)**	

4.11. Antimicrobial susceptibility pattern of ESBL and Non-ESBL bacteria

Extended spectrum β lactamase (ESBL) producing bacteria had significantly high resistance level to penicillin groups such as (ampicillin (100%) and cephalosporin groups (cefuroxime (96%), ceftazidime (92%), cefepime (86%), azetronome (80%), cefotaxime (72%) and cefoxitin (60%)). Low resistance level was recorded for amikacin (24%) and meropenem (40%). Non-ESBL isolates were 96, 91, 88.5 and 88.5 % resistance to ampicillin, aztreonam, ceftazidime and ceftriaxone, respectively. The most active drugs for non-ESBL-producing isolates were amikacin, ciprofloxacin and gentamycin with susceptibility results of 70, 64, and 53.8%, respectively (Figure 4.9).



	AMP	AZT	CXT	CRO	CTZ	FOX	CXM	FEP	AMC	TZP	MRP	TOB	AK	GEN	CIP	SXT
ESBLs Postive	100	80	72	92	92	60	96	86	80	64	40	48	24	44	44	72
ESBL Negative	96	91	83.3	88.5	88.5	78.2	91	70	77	68	58	48	30	46.2	36	68

Note: AMP: ampicillin; AMC: Amoxicillin and calvulanic acid; CRO: ceftriaxone; CTX : cefotaxime; CTZ: ceftazidime; AK: Amikacin; GEN: Gentamicin; CIP: ciprofloxacin; SXT: Sulfamethoxazole + trimethoprim; FOX: Cefoxitin; CXM: Cefuroxime; FEP: Cefepime

Figure 4.9: Resistance pattern of ESBL-positive and ESBL-negative bacteria at TASH and ALERT hospital, 2018.

4.12. Carbapenemase producing Gram-negative bacteria

In our study, 47 isolates were identified as meropenem resistant, and therefore further analyzed by Modified Hodge test (MHT) to screen for carbapenemase production. Out of the 280 environmental samples, 7(2.5%) of bacteria were positive for carbapenemase production by MHT. This isolates consisted of 4(50%) *K. pneumoniae*, 1(8.3%) *E. coli*, 1(11.1%) *Serratia* spp and 1(6.3%) *Pseudomonas* spp. Most of the isolates were collected from TASH (5(71.4%)) and mainly identified from ICUs 5 (71.4%). As for studied environmental samples the highest contamination rate were seen from ventilators 3(42.9%) followed by bed linens 2(28.6%) (Table 4.12).

Table 4.12: Distribution of carbapenemase producing Gram-negative bacteria isolated from the hospital environments at TASH and ALERT hospital, 2018.

Variables	MHT Positive, n (%)	MHT Negative, n (%)	X^2 (P-value)
Hospitals			
TASH	5(71.4)	75(78.1)	0.169(0.681)
ALERT	2(28.6)	21(21.9)	
Ward type			
ICUs	5(71.4)	74(77.1)	0.117(0.733)
OTs	2(28.6)	22(22.9)	
Type of Samples			
Swab	0(0)	7(2.7)	NA
Settle plate	0(0)	0(0)	
Sampling points			
Sink	1(14.3)	6(6.3)	18.301(0.146)
Monitor	0(0)	7(7.3)	
Anastasia machine	0(0)	2(2.1)	
Bed	0(0)	15(15.6)	
Bed linens	2(28.6)	21(21.9)	
Suction Machine	0(0)	10(10.4)	
Environmental surface	1(14.3)	9(9.4)	
Work station	0(0)	6(6.3)	
Ventilator	3(42.9)	8(8.3)	
Lobby (furniture)	0(0)	9(9.4)	
Oxygen cylinder	0(0)	3(3.1)	
Total	**7(6.8)**	**96(93.2)**	
Environmental surface (Door knob, Floor, Corroder and Wall)			

4.13. Distribution of Methicillin and Vancomycin resistant strains

Seventy-six of the 280 environmental samples (27.1%) contained MRSA as detected using oxacillin disk; whereas (16(5.7%)) and 1(0.4%) were MRCONS and VRE, respectively. Most 63(82.9%) of the MRSA were isolated from TASH; and between the hospital wards, special units presented with most contaminated items having statistically significant difference between those in OTs (52(68.4%)) and ICUs (24(31.6%)), p = 0.031. There was no significant association between hospitals and sampling points, and MRSA identification rate (p=0.096 and 0.38, respectively). As for contamination of hospital items, the highest contamination rate by MRSA were seen in beds, 12(15.8%), and air, 9(11.8%). Most of MRCONS were identified from monitors (3(18.8%)) followed by Anastasia machine and Suction machine each with 2(12.5%) (Table 4.13).

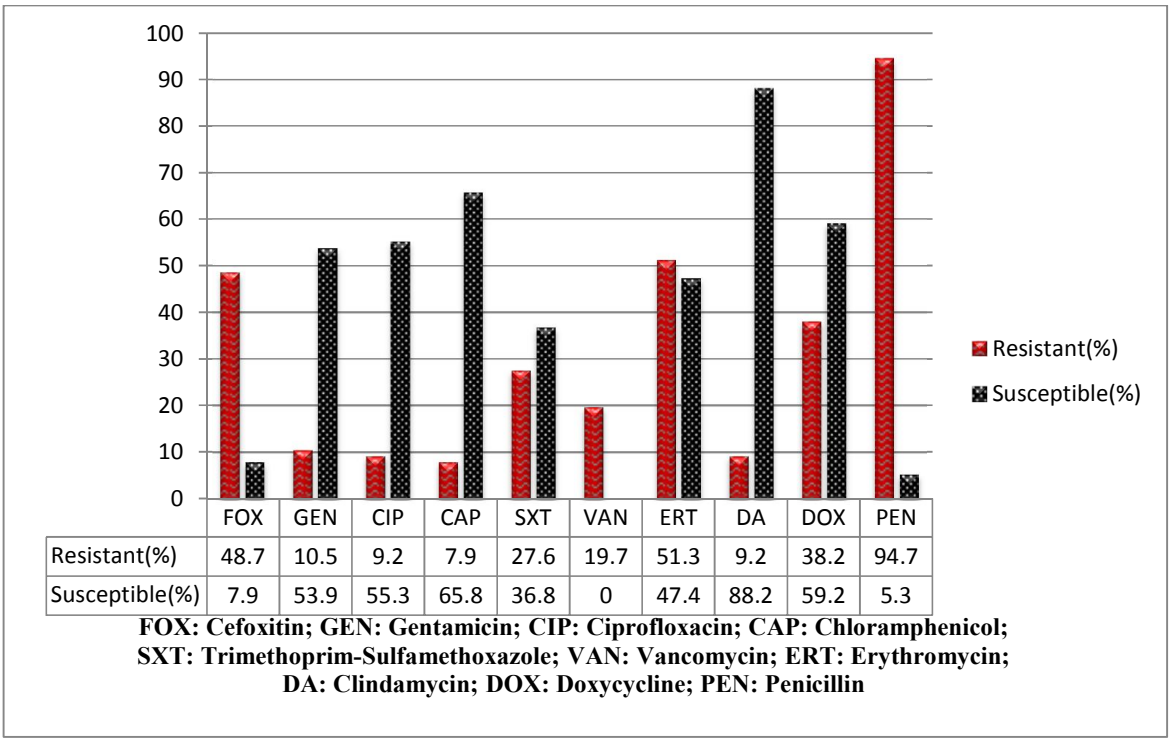
Table 4.13: Distribution of MRSA, MRCONS and VRE producing bacteria isolated from the hospital environment at TASH and ALERT hospital, 2018.

Variables	MRSA, n (%)		X² (P-value)	MRCONS, n (%)		X² (P-value)	VRE, n (%)		X² (P-value)
	POS	**NEG**		**POS**	**NEG**		**POS**	**NEG**	
Hospital									
TASH	63(82.9)	19(67.9)	2.7741 (0.096)	16(100)	9(81.8)	0.076	1(100)	2(66.7)	0.444 (0.505)
ALERT	13(17.1)	9(32.1)		0(0)	2(18.2)		0(0)	1(33.3)	
Ward type									
ICUs	24(31.6)	3(10.7)	**4.6342 (0.031)**	3(18.8)	0(0)	2.320 (0.128)	1(100)	2(66.7)	0.444 (0.505)
OTs	52(68.4)	25(89.3)		13(81.3)	11(100)		0(0)	1(33.3)	
Type of Sample									
Swabs	67(88.2)	18(64.3)	**7.8094 (0.005)**	15(93.8)	8(72.7)	2.283 (0.131)	1(100)	3(100)	NA
Settle plates	9(11.8)	10(35.7)		1(6.3)	3(27.3)		0(0)	0(0)	
Sampling point									
Sink	5(6.6)	3(10.7)	22.342 (0.172)	0(0)	0(0)	12.848 (0.38)	0(0)	0(0)	4.000 (0.261)
Monitor	6(7.9)	0(0)		3(18.8)	0(0)		0(0)	0(0)	
Anastasia machine	5(6.6)	0(0)		2(12.5)	1(9.1)		0(0)	0(0)	
Air	9(11.8)	10(35.7)		1(6.3)	3(27.3)		0(0)	0(0)	
Bed	12(15.8)	1(3.6)		1(6.3)	1(9.1)		0(0)	0(0)	
Bed linens	7(9.2)	1(3.6)		2(12.5)	0(0)		0(0)	0(0)	
Suction Machine	7(9.2)	1(3.6)		2(12.5)	0(0)		0(0)	1(33.3)	
Environmental surface	7(9.2)	1(3.6)		4(25)	2(18.2)		1(100)	0(0)	
work station	7(9.2)	3(10.7)		0(0)	0(0)		0(0)	1(33.3)	
Ventilator	1(1.3)	1(3.6)		0(0)	0(0)		0(0)	0(0)	
Lobby (furniture)	4(5.3)	3(10.7)		0(0)	2(18.2)		0(0)	0(0)	
Others	6(7.9)	4(14.3)		1(6.3)	2(18.2)		0(0)	1(33.3)	
Total	**76(73.1)**	**28(26.9)**		**16(59.3)**	**11(40.7)**		**1(25)**	**3(75)**	

MRSA: Methicillin resistant *S. aureus*; MRCONS: Methicillin resistant coagulase negative *staphylococci*; VRE: Vancomycin resistant *Enterococci* spp; Others (Laparoscopy, OR-Light, oxygen cylinder, Trowels); environmental surface ((for MRSA and MRCONS (Door knob (n=1,1), Floor (n=4,1), Corroder (n=1,1) and Wall (n=1,1), respectively; VRE (wall=n=1); NA=Not applicable)

4.14. Antimicrobial susceptibility pattern of MRSA in hospital environments

The resistant rates of MRSA for each tested antibiotics has been presented (figure 4.10); and therefore, high resistant rates were observed to penicillin (94.1%), erythromycin (51.3%) and cefoxitin (48.7%). In contrast, low levels of resistant rate were observed to chloramphenicol (7.9%), clindamycin (9.2%), and ciprofloxacin (9.2%).



	FOX	GEN	CIP	CAP	SXT	VAN	ERT	DA	DOX	PEN
Resistant(%)	48.7	10.5	9.2	7.9	27.6	19.7	51.3	9.2	38.2	94.7
Susceptible(%)	7.9	53.9	55.3	65.8	36.8	0	47.4	88.2	59.2	5.3

FOX: Cefoxitin; GEN: Gentamicin; CIP: Ciprofloxacin; CAP: Chloramphenicol; SXT: Trimethoprim-Sulfamethoxazole; VAN: Vancomycin; ERT: Erythromycin; DA: Clindamycin; DOX: Doxycycline; PEN: Penicillin

Figure 4.10: Antimicrobial susceptibility pattern of MRSA in hospital environments at TASH and ALERT, 2018.

4.15. Distribution of MDR phenotypes

Of the total 282 bacterial strains, 158(56%) of bacterial isolates were resistant to at least 3 antibiotics and Fifty-eight multi-drug resistance phenotypes were detected by the MDR isolates**.** Multidrug resistant (MDR) phenotypes were detected among 45.8% (82/179) of Gram-positive bacteria and 73.8% (76/103) of Gram-negative bacteria. The predominant MDR phenotype among Gram-positive bacteria was observed in MRSA (61/104, 58.6%) followed by GBS (4/7, 57.1%), *Enterococcus* spp (2/4, 50%) and CONS (15/53, 28.3%). The most common MDR combinations found by MRSA was against 7 classes of antibiotics (Table 4.14).The predominant MDR phenotype among Gram-negative bacteria was observed in *Acinetobacter* spp (45/48, 93.8%) followed by *E. coli* (11/12, 91%), *Klebsiella* spp (10/14, 71.4%), *Enterobacter* spp (1/2, 50%), *Serratia* spp (3/9, 33%) and *Pseudomonas* spp (5/16, 31.3%). The most common MDR combinations found by *Acinetobacter* spp was against 8 classes of antibiotics (Table 4.15).

Table 4.14: Multidrug-resistance profiles of Gram-positive isolates from hospital environments at TASH and ALERT hospitals, 2018.

MDR phenotype (Gram +ve)	Frequency, n (%)	MDR phenotype (Gram +ve)	Frequency, n (%)
***S. aureus* (MRSA)**		**GBS**	
PEN,OXA,SXT,AZM,VAN,GEN,CIP	2(1.9)	PEN,VAN,AZM,DOX,CHL,SXT,GEN	1(14.3)
PEN,OXA,SXT,AZM,DOX,,VAN	11(10.6)	PEN,VAN,AZM,CHL,SXT,GEN	1(14.3)
PEN,OXA,SXT,AZM,DOX,DA	2(1.9)	PEN,AZM,DOX	1(14.3)
PEN,OXA,SXT,DOX,GEN,CIP	2(1.9)	PEN,AZM,GEN	1(14.3)
PEN,SXT,AZM,DOX,CIP	1(1)	**Total**	4/7(57.1)
PEN,OXA,SXT,AZM,DA	2(1.9)	**CONS**	
PEN,OXA,SXT,DOX,VAN	3(2.9)	PEN,OXA,DOX	4(7.5)
PEN,OXA,SXT,AZM,DOX	2(1.9)	PEN,OXA,AZM,DOX	3(5.7)
PEN,OXA,SXT,AZM	7(6.7)	PEN,OXA,AZM	2(3.8)
PEN,OXA,AZM,DOX	3(2.9)	PEN,OXA,GEN,AZM,CIP	2(3.8)
PEN,OXA,VAN	6(5.8)	PEN,OXA,AZM,DOX,CIP	3(5.7)
PEN,OXA,SXT	4(3.8)	PEN,GEN,AZM	1(1.9)
PEN,OXA,DOX	6(5.8)	Total	15/53(28.3)
PEN,FOX,OXA	1(1)	***Enterococcus* spp**	
PEN,OXA,AZM	5(4.8)	PEN,VAN,ERY,TE,CIP,CHL,SXT	1(25)
PEN,SXT,VAN	1(1)	PEN,ERY,SXT	1(25)
PEN,AZM,DOX	1(1)	**Total**	2/4(50)
AZM,VAN,DA	1(1)		
PEN,SXT,AZM	1(1)		
Total	61/104(58.6)		

Table 4.15: Multidrug-resistance profiles of Gram-negative isolates from hospital environments at TASH and ALERT hospital, 2018.

MDR phenotype	**Frequency, n (%)**	**MDR phenotype**	**Frequency, n (%)**
***Acinetobacter* spp**		***Enterobacter spp***	
CTX,FOX,FEP,GEN,MRP,TZP,CIP, SXT	23(48.9)	AMP,AMC,FEP,FOX,MRE,CIP, SXT,CHL	1(50)
CTX,FOX,FEP,GEN,MRP,TZP	2(4.4)	Total	1/2(50)
CTX,FOX,FEP,MRP,TZP,STX	4(8.9)		
CTX,FOX,FEP,GEN,MRP,TZP,SXT	6(13.3)	***Klebsiella spp***	
CTX,FOX,FEP,GEN,TZP,STX	2(4.4)	AMP,FOX,FEP,AMC,MER, CIP,SXT,GEN	3(21.4)
CTX,FOX,FEP,TZP,SXT	1(2.2)	AMP,FOX,FEP,AMC,MER,SXT	2(14.3)
CTX,FOX,FEP	3(6.7)	AMP,FEP,AMC,CIP,SXT,GEN	1(7.1)
CTX,FOX,FEP,MRP,TZP	1(2.2)	AMP,FOX,FEP,AMC,CIP,SXT,C HL	1(7.1)
CTX,FOX,FEP,GEN,MRP,TZP,CIP	1(2.2)	AMP,FEP,AMC,SXT	1(7.1)
CTX,FOX,FEP,TZP	1(2.2)	AMP,FEP,SXT,GEN,CHL	1(7.1)
CTX,FOX,STX	1(2.2)	AMP,AMC,MER	1(7.1)
Total	45/48(93.8)	Total	10/14(71.4)
***Pseudomonas* spp**		***E. coli***	
TZP,CAZ,FEP,MRP,GEN,CIP,SXT	2(12.5)	AMP,AMC,FEP,FOX,AZM,GEN, CIP,SXT	4(33.3)
TZP,CAZ,FEP,SXT,CHL	1(6.3)	AMP,AMC,FEP,AZM,GEN,SXT	1(8.3)
CAZ,MRP,SXT,CHL	1(6.3)	AMP,AMC,FOX,AZM,CIP	1(8.3)
TZP,CAZ,MRP	1(6.3)	AMP,AMC,FOX,AZM	1(8.3)
Total	5/16(31.3)	AMP,FEP,AZM,SXT	1(8.3)
***Serratia* spp**		AMP,FOX,GEN,SXT	1(8.3)
AMP,FEP,FOX,AZM,SXT	1(11.1)	AMP,FEP,AZM,SXT	1(8.3)
AMP,AMC,FEP,MRE	1(11.1)	AMP,AMC,AZM	1(8.3)
AMP,AMC,SXT	1(11.1)	Total	11/12(91.7)
Total	3/9(33)		

AMP: ampicillin; AMC: amoxicillin-clavulanic acid; CRO: ceftriaxone; CTX : cefotaxime; CTZ: ceftazidime; AK: amikacin; GEN: gentamicin; CIP: ciprofloxacin; SXT: sulfamethoxazole + trimethoprim; FOX: Cefoxitin; CXM: cefuroxime; FEP: cefepime; TZP: piperacillin-tazobactam; MRP: meropenem; AZT: aztreonam; VAN: vancomycin

5. CHAPTER V: Discussions

5.1. Culture positive environmental samples

Although ICUs and the OTs are expected to be sterile, often times they are found to be reservoirs of common nosocomial bacterial pathogens and multidrug resistant bacterial strains. In the present study, out of 280 fomites, medical devices and air samples from swabs and settle plates, 227(81.1%) were positive for bacterial contamination. Our result agreed with other reports where bacterial contamination was found to be (86.2%) from hospital environments in a study from Zimbabwe (Mbanga *et al*., 2018) and (96.3%) from Morocco (Lalami *et al*., 2016). In contrast to our result, lower bacterial contaminations were observed from the normally clean hospital environments in studies conducted elsewhere; 24.7% in Gaza Strip (Al Laham, 2012), 29.7% in Sudan (Nurain *et al*., 2015), 44.2% in Uganda (Sserwadda *et al*., 2018), 3.7% during 2001 and 4.0% during 2002 in Iraq (Ensayef *et al*., 2009), (39.4%) in Nigeria (Hammuel *et al*., 2014), and (39.6%) in Bahir Dar, Northwest Ethiopia (Getachew *et al*., 2018). Differences in hand hygiene, ventilation system, sterilisation and disinfection techniques could account for the discrepancies (Weber *et al*., 2013; Chemaly *et al*., 2014; Mora *et al*., 2016).

Higher levels of bacterial contamination observed in our study could be attributed primarily to the use of ineffective disinfectants during surface cleaning, and inadequate uses of standard precautions such as hand hygiene and contact precautions, as well as migration of the organisms through air flow or other means. Moreover, the ventlation system could be in place to reduce air born microbs (Dallolio *et al*., 2018).

Infrequent cleaning of inanimate surfaces and medical equipments could also contribute to poor microbial quality of the hospital surfaces (Weber *et al*., 2013; Santajit and Indrawattana, 2016; Mbanga *et al*., 2018). This situation is accredited to hospitals unwillingness to put funds into contamination control like the ventilation systems, lack of information about the level of contamination and effectiveness of commonly used disinfectants, and inappropriate waste controls.

5.2. Distribution of Gram-positive and Gram-negative bacteria

The results of our study showed that contamination of the inanimate environments and air by varied groups of bacteria, including both Gram-positive (63.5%) and Gram-negative (36.5%) bacteria. Comparable to our results, frequency of Gram-positive bacteria constituted the leading inhabited bacteria compared to Gram-negative bacteria from inanimate hospital environment from different countries, for example: in Iran (60.7% vs 39.3%) (Tajeddin *et al*., 2016), in Nigeria (52.2% vs 47.8%) (Maryam *et al*., 2014), at Gondar in Ethiopia (60.5% vs 39.5%) (Alemu *et al*., 2015) and at Northwest, Ethiopia (81.6% vs 18.4%) (Getachew *et al*., 2018). The dominance of Gram- positive over Gram-negative bacteria could be explained by the fact that Gram-positive bacteria, being devoid of lipid-dominant desiccation prone outer membrane, have natural ability to retain their viability on abiotic hospital environments for several days to months (Chemaly *et al*., 2014; Tajeddin *et al.*, 2016).

In contrast to our results several authors from different countries reported that Gram-negative bacteria were predominant than Gram-positive ones: for example, from Zimbabwe (66.2% vs 33.82%, respectively) (Mbanga *et al*., 2018), from Gaza Strip (51.6% vs 48.4%, respectively) (Al Laham, 2012) and from Morocco (73.3% vs 26.7%, respectively) (Lalami *et al*., 2016). These variations may be due to different sampling times (endemic vs outbreak situations), the presence of colonized and/or infected patients during sampling, sampling in different hospital sites (OTs vs ICUs) and the use of different sampling techniques and culture methodologies (Rohr *et al*., 2009; Ekrami *et al*., 2011; Faires *et al*., 2012; Faires *et al*., 2013).

5.3. Frequency of specific potential pathogenic bacteria in hospital environments

In our study the predominant isolated potential pathogenic bacteria from environmental samples were *S. aureus* 104 (36.9%), CONS 53(18.8%) and *Acinetobacter* spp 48(17%). This isolates were considered as the potential pathogenic bacteria that result in hospital acquired infections (HAIs) and indicators of inadequate clinical surface hygiene in hospital environments (Dancer, 2004; Fagade *et al*., 2010; Pinon *et al.*, 2013; Lax and Gilbert, 2015; Nurain *et al*., 2015; Li *et al*., 2018). These bacteria were also resistant to common disinfectant methods and hence spread easily in the environment, which enables them to colonize and infect the patients receiving health care service at the facility (Atata *et al.*, 2013; Tajeddin *et al*., 2016; Mirhoseini *et al*., 2016). Similarly, studies showed that *S. aureus* and CONS were the most frequent isolated bacteria in different countires: for examples, Nigeria (Maryam *et al*., 2014), Zaria, Nigeria (Hammuel *et al*., 2014) and Ethiopia (Mengistu *et al*., 2016).

Our results were contradicted to studies conducted in India where the most prevalent isolated bacteria were shown to be *Bacillus* species followed by *Micrococcus* (Kiranmai and Madhavi, 2016).

Most of the potential pathogenic bacteria in operation theatres were isolated from General Surgery OT (13.6%, 22/162) in ALERT hospital, which is in line with studies conducted by (Najotra *et al*., 2017; Matinyi *et al*., 2018) and is most probably due to highest patient load in the General Surgery OT.

5.4. Distribution of isolated bacteria in different environmental samples

The highest bacterial contaminated samples were taken from bed linens (37(13.1%)), environmental surface including (wall, floor, corridors and door knob) (35(12.4%)) and bed (33(11.7%)). This is in concordance with other studies from Ethiopia and abroad that the most contaminated samples were taken from bed linens, bed, door knob and sinks (Ekrami *et al*., 2011; Hammuel *et al*., 2014; Bakkali *et al*., 2015; Tajeddin *et al*., 2016; Sserwadda *et al*., 2018). These sites were frequently touched by patients and healthcare workers (Bouchra *et al*., 2017). Bed linens and bed were mainly contaminated by *Acinetobacter* spp (18.8% and 20.8%), CONS (9.4% and 5.7%), and *S. aureus* (7.7% and 12.5%), respectively. Comparable results were obtained in beds and bed linens samples in studies conducted in Iran (*S. aureus* (10.9% and 14.7%), CONS (19.5% and 3.2%), *Acinetobacter* spp (12.5% and 35.3%), respectively (Tajeddin *et al*., 2016); door knob/handle and bed were the predominant contaminated samples by *S. aureus* (9.5% and 11.1%, respectively) in studies conducted in Nigeria (Hammuel *et al*., 2014). The sources of such contaminations could be cross-contamination from a patient's flora, health care workers hand, contaminated storage cart, or contamination during the washing process especially the bed linens (Rohr *etal*., 2009; Faires *et al*., 2013; Pinon *et al*., 2013).

Bath basins are also commonly used in the care of patients in ICUs, operation theaters and regular patient wards. It could be reservoirs for common hospital-acquired pathogens (Marchaim *et al*., 2012).

In our study, sinks were frequently contaminated with *Pseudomonas* spp (12.5%, 2/16). These findings are consistent with several reports that hospital associated outbreaks in critical care wards occur largely due to the opportunistic pathogen like *Pseudomonas* spp-contaminated sinks (Hammuel *et al*., 2014; Salm *et al*., 2016; Mbanga *et al*., 2018).

This could be explained by the moist hospital environments that enhanced persistence of the bacteria and the ability of these bacteria to form biofilms in water, sinks, toilets, showers and drains (Kramer *et al*., 2006; Parcell *et al*., 2018). Moreover, acquisition of multiple virulence determinants and intrinsic resistance to commonly used antibiotics and disinfectants may result in the persistence of *Pseudomonas* spp (Ensayef *et al*., 2009; Parcell *et al*., 2018).

Bloodstream infection and ventilator-associated pneumonia are associated with device contamination such as central venous catheters, urinary catheters, and ventilators especially in the intensive care units (Sui *et al*., 2012). In our study, ventilators were frequently contaminated by *K. pneumoniae* (25%, 2/8), *E. coli* (16.7%, 2/12) and *Acinetobacter* spp (8.3%, 4/48). Comparable results were found in studies conducted in Iran, that ventilators were mainly colonized with *K. pneumoniae* (54.4%, 6/11). Respiratory medical equipment especially ventilators linked to main source of a nosocomial outbreak (Tajeddin *et al.*, 2016). Source of contamination of ventilators by *K. pneumoniae* might be due to the aspiration of secretions from the oropharynx of colonized patients' while staff hands acted as the transmission vehicle (Hu *et al*., 2016; Martin *et al*., 2018).

5.5. Bacterial Load from Air

The air quality in hospitals is of a great concern because of the presence of airborne bacteria that may cause surgical site infections (SSIs) and other nosocomial infections (Dancer, 2004; Mirhoseini *et al*., 2016; Najotra *et al*., 2017; Dallolio *et al.*, 2018). To assess clinical surface hygiene in hospitals bacteriological standards is required. The first standard concerns any finding of a specific 'indicator' organism, the presence of which suggests a requirement for increased cleaning. Indicators would include MRSA, *Clostridium difficile*, VRE and various Gram-negative bacilli. The second standard concerns a quantitative aerobic colony count of, 5 cfu/m^3on frequent hand touch surfaces and during pre- operation sampling the microbial contamination of the air is always within the limit of 35 cfu/m^3 (range: 2–27 cfu/m^3) for conventional operating theatres at rest (Dancer, 2004; Kiranmai and Madhavi, 2016; Dallolio *et al*., 2018).

In our study, all the five theatres had their mean colony counts exceeding the acceptable limit that was 5cfu/dm^2/h according to Fisher's Index of Microbial air contamination (Matinyi *et al*., 2018). Gastro intestinal tract theatre had the highest aerobic bacterial load (66.4±21.9 cfu/dm^2/h) followed by gynecology theatre (37.66±16.9 cfu/dm^2/h). The high aerobic bacterial load in the Gastro intestinal tract and Gynecology theatre are an indicator for fecal contamination and also poor hand hygiene (Ekrami *et al*., 2011; Dallolio *et al*., 2018). On the other hand, high aerobic bacterial load in Gynecology theatre could be attributed to huge gynecological and obstetric patient in flow. A much lower bacterial load (6 CFU/m^3) was recorded in Gynecology theatre from India by (Kiranmai and Madhavi, 2016).

In this study, we also identified, *S. aureus* isolates 19(18.3%) to be the most common operation theater air born contaminants followed by the commensal organism CONS 4(7.5%). The sources of the contamination could be either endogenously from normal skin flora of patients or exogenously from surgical staff (Ensayef *et al*., 2009; Najotra *et al*., 2017). This is in concordance with other studies from Ethiopia and abroad (Genet *et al*., 2011; Faires *et al*., 2013; Gebremariam *et al*., 2014; Mengistu *et al*., 2016; Najotra *et al*., 2017; Matinyi *et al*., 2018). Out of 23 settle plate samples cultured in this study, 9(39.1%) were contaminated with MRSA in the studied operating theaters. This rate was higher than reported from other studies in Ontario, Canada, (6.7%) (Faires *et al*., 2013) and in Germany, (4%) (Rohr *et al*., 2009). It is possible that air containing MRSA from nearby surfaces, the ventilation system, the disposal of linens, or an individual colonized with MRSA may have introduced MRSA in the operating theaters (Faires *et al*., 2012; Mirhoseini *et al*., 2016; Rohr *et al*., 2009).

5.6. Antimicrobial susceptibility pattern of the isolates

Our results showed high rates of drug resistance among the bacterial isolates. Most of the Gram-negative bacteria were resistant to most of the tested antibiotics such as penicillin group (ampicillin (97%)), cephalosporin groups (ceftazidime (89.3%), ceftriaxone (89%), aztreonam (88%), cefotaxime (81%), cefoxitin (74%)), and β-lactamase inhibitors (amoxicillin and clavulanic acid (68.7%)). Because these represent the antibiotics most frequently used in practice, serious problems can be encountered while prescribing those antibiotics. Establishing guidelines for prescribing antibiotics then becomes a necessity.

Our results were comparable with studies conducted elsewhere such as from Gaza, Palestine (ceftriaxone 60.53 % to 100%, ceftazidime 59.26% to 100% and cefotaxime 54.54% to 100%) (Al Laham, 2012); Morocco (ampicillin 100%, amoxicillin and clavulanic acid 87% to100%, ceftriaxone 60% to 95% and ceftazidime 60% to 100%) (Bakkali *et al*., 2015). The neighboring country, Sudan, also had high resistance rate for (ampicillin 50% to 100%, cefotaxime 100%, ceftazidime 100% and ceftriaxone 100%) (Nurain *et al*., 2015). Increased resistance to β-lactams antibiotics is due to the selective pressure exerted by the antibiotics (Davies and Davies, 2010; Blair *et al*., 2015).

On the other hand, low resistance level was recorded to non-beta-lactam antimicrobials such as, aminoglycoside group (amikacin (28.2%)) and fluoroquinolones group (ciprofloxacin (37.8%)). Comparable results were recorded from studies conducted in: Sudan (amikacin (23.5%) and ciprofloxacin (42.7%)) (Nurain *et al*., 2015); and in Morocco (amikacin (14.5%)) (Bakkali *et al*., 2015); in Gaza, Palestine (amikacin (6.1%) and ciprofloxacin (27.3%) (Al Laham, 2012).

5.7. Distribution of ESBL producing Gram-negative bacteria

In Gram-negative bacteria, β-lactamase production remains the most important mechanism of resistance to β-lactam group of antibiotics and has become a global challenge in infection control. One group of these enzymes is called extended-spectrum β-lactamases (ESBLs) (Chroma and Kolar, 2010; Bukhari *et al*., 2016; Kumar, 2017). Despite the recent worldwide spread of ESBL in *K. pneumoniae* and *E. coli* isolates from hospital-acquired infections, their dissemination has been little studied especially on inanimate hospital environments in Ethiopia.

In our study, ESBL producing bacteria were identified in 25 of 280 (8.9%) environmental samples. Our results were comparable with studies conducted in Tunisia (9.3%) (Dziri *et al*., 2016a), Israel (9%) (D'Agata *et al*., 1999) and Gonder, Ethiopia (14.8%) (Engda *et al*., 2018). On the other hand, lower than our results were recorded from environmental samples in different countries such as Tunisia (4%) (Guet-Revillet *et al*., 2012)**,** and UK (3.1%) (Muzslay *et al*., 2017). Moreover, our finding is lower than studies conducted from Pakistan (33%) (Bukhari *et al*., 2016).

Out of 103 Gram-negative bacterial isolates, 25(24.3%) were identified as ESBL producers. Comparable results were obtained on clinical isolates in Adama Teaching Hospital, Ethiopia (25%) (Mulisa *et al*., 2016); higher than our results were recorded from clinical isolates in Addis Ababa, Ethiopia (57.7%) (Shiferaw *et al*., 2019) and TASH, Ethiopia (51.2%) (Desta *et al*., 2016).

The most prevalent ESBL producer detected by DDST in our study was *E. coli* (92%). Similar findings were recorded with studies done in Ethiopia from clinical isolates (100%) (Legese *et al*., 2017). Higher than those of studies conducted in Gaza, Palestine (40%) (Al Laham, 2012), Zimbabwe (54.6%) (Mbanga *et al*., 2018) and Algeria (25%) (Debabza *et al*., 2014).

The next predominant ESBL producing organism was *K. pneumoniae* (75%) which is in line with a studies on clinical isolates in Ethiopia (84.2%) (Legese *et al*., 2017), Zimbabwe (70%) (Mbanga *et al*., 2018). Our result was higher than those of studies conducted in Iran (51.5%) (Tajeddin *et al*., 2016), Gaza, Palestine (20%) (Al Laham, 2012), Algeria (28.4%) (Debabza *et al*., 2014), and Sudan (54.5%) (Nurain *et al*., 2015). This could be explained by presence of colonized/infected patients in the hospital environments, or failure of routine cleaning and disinfection practices (Freeman *et al*., 2014; Guet-Revillet *et al*., 2012).

In this study, bed linens, ventilators, sinks and beds were associated with high rates of ESBL positivity detected strains 32%, 20% and each with 12% respectively. The identification of ESBL producing bacteria on linens, sink, bed and environmental surface is consistent with reports from the literatures by (D'Agata *et al*., 1999; Guet-Revillet *et al*., 2012; Debabza *et al*., 2014; Muzslay *et al*., 2017; Engda *et al*., 2018). It is generally assumed that ESBL producing Gram-negative bacteria require moist or damp sites for enhanced longevity (D'Agata *et al*., 1999; Debabza *et al*., 2014; Muzslay *et al*., 2017).

5.7.1. Antimicrobial susceptibility pattern of ESBL producing bacteria

ESBL producing bacteria had significantly high resistance level to penicillin groups such as ampicillin (100%) and cephalosporin groups such as cefuroxime (96%), ceftazidime (92%), cefepime (86%), azetronome (80%) and cefotaxime (72%). Our results were comparable with studies conducted in Algeria ampicillin (98.1%) (Debabza *et al*., 2014) and Gonder, Ethiopia (cefpirome, cefpodoxime, ceftazidime, ceftriaxone and amoxicillin with clavulanic acid each with 100% resistant level) (Engda *et al*., 2018). This could be explained by lacks of screening and confirmatory test to detect resistant strain in the studied hospitals might also contribute to increases of ESBL producing bacteria in the hospital environments.

High resistance rate was also recorded form ESBL-producing isolates for non-beta-lactam antimicrobials such as, cotrimoxazole (72%), ciprofloxacin (44%) and gentamycin (44%).This was in close agreement with studies done in Gondar, Ethiopia (cotrimoxazole 64.91%, ciprofloxacin 56%) (Engda *et al*., 2018), in Addis Ababa, Ethiopia from clinical isolates, cotrimoxazole (77%), ciprofloxacin (46.3%) and gentamycin (43.4%) (Teklu *et al*., 2019); TASH, Ethiopia from clinical isolates, cotrimoxazole (83.6%) and gentamycin (70.9%) (Legese *et al*., 2017).The most active drugs for ESBL-producing isolates were amikacin (76%). Low resistance rate in our study could be explained by the scarcity in usage of amikacin in study setting.

5.8. Carbapenem resistant Gram-negative bacteria

In our study, 47(45.6%) of GNB were carbapenems resistant. This finding agreed with the findings of Hammuel *et al*., (2014) that occurrence of MDR is very common and mainly among Gram-negative bacteria. Comparable results also reported from studies conducted in Algeria where (48%) of GNB were carbapenem-resistant (Bouguenoun *et al*., 2016). The overall rate of Gram-negative isolates resistant to carbapenem in this study was 16.8% (47/280). In contrast to our result, lower carbapenem resistance rate was recorded on clinical isolates from TASH, Ethiopia 2% (5/267) (Desta *et al*., 2016). The presence of isolates producing carbapenemases in hospital environments is a major reservoir for resistance genes, which can be transmitted horizontally to other isolates (clinical or environmental) (Dziri *et al*., 2016a). In our study, highest carbapenemases production was recorded by *K. pneumoniae* (50%, 4/8), which is a well-known producer of biofilm (by type 1 fimbriae) (Bocanegra-Ibarias *et al*., 2017; Martin *et al*., 2018).

Lower carbapenemases production rate was recorded from clinical isolates in Addis Ababa, Ethiopia where *K. pneumoniae* (10.5%, 2/19) (Legese *et al*., 2017).

5.9. Distribution of MRSA from hospital environment

In our study MRSA were recovered in 76 of 280 (27.1%) environmental samples, as determined by the susceptibility testing to oxacillin. Resistance to oxacillin/cefoxitin by disc diffusion can be used for the detection of MRSA strains in routine testing because cefoxitin is a potential inducer of the system that regulates mecA gene (Hammuel *et al*., 2014; CLSI, 2018). It seems that hospital environments were possible sources of common nosocomial bacterial pathogens that are resistant to antibiotics used for patients (Tajeddin *et al*., 2016). In contrast to our result lower contamination rates were recorded in confined environments in Ontario, Canada (2.5%) (Faires *et al*., 2013), in another Canadian study (11.8%) (Faires *et al*., 2012), in Northern Palestine (3.6%) (Adwan *et al*., 2015) and in Northwest Ethiopia (5%) (Getachew *et al*., 2018).

A meta-analysis conducted from clinical specimen showed that overall estimation of MRSA prevalence in Ethiopia was 32.5% (Eshetie *et al*., 2016), which is in line with the rate obtained from our result. The MRSA detection rate from our study was lower than that reported from Germany (45%) (Rohr *et al*., 2009). These wide variations in the reported magnitude of MRSA contamination may be attributed to different study designs, including sampling times (endemic versus outbreak settings), the presence of colonized and/or infected patients during sampling, sampling in different hospital wards, sampling surfaces once versus multiple times, and the use of different sampling techniques and culture methodologies (Rohr *et al*., 2009; Faires *et al*., 2012; Faires *et al*., 2013).

In our study, highest contamination rate of MRSA were seen in bed 12(15.8%). Similarly, the most contaminated sites were beds in studies, such as in Canada (4.1%) (Faires *et al*., 2013), Germany (13%) (Rohr *et al*., 2009) and Tanzania (34%) (Nkuwi *et al*., 2018). The sources of such contaminations could be cross-contamination from patient's flora and health care workers' hand (Rohr *et al*., 2009; Faires *et al*., 2013; Pinon *et al*., 2013).

5.9.1. Antimicrobial susceptibility pattern of MRSA

In our study MRSA isolates were highly resistant to penicillin, erythromycin, tetracycline and vancomycin with resistance level 94.7, 51.3, 38.2 and 19.1%, respectively. Low levels of resistance rate were noted to chloramphenicol (7.9%), ciprofloxacin and clindamycin each with (9.2%). Similar resistance level was reported from Ethiopia by a Meta- analysis study for penicillin, erythromycin and tetracycline with a pooled resistance ratio of 99.1, 97.2 and 51.9 %, respectively. In contrast to our result low level of resistance rate were noted to vancomycin, 5.3% (Eshetie *et al*., 2016).

In the present study, 19.3% of *S. aureus* isolates were vancomycin resistant *S. aureus* (VRSA); and 25% of *Enterococcus* spp were VRE, despite its scarcity in the antibiotic usage. In contrast to our result high VRSA was reported in a study from Zimbabwe (40%) (Mbanga *et al*., 2018). The spread of MRSA and VRE from the hospital to the community, coupled with the emergence of VISA and VRSA, has become major concern among healthcare providers. It has been suggested that patients at risk for VRSA are co-infected or co-colonized with VRE and MRSA, which enables conjugative transfer of vanA gene from VRE to MRSA in a biofilm environment leading to a VRSA strain (Loomba *et al*., 2010; Tajeddin *et al*., 2016).

5.10. Distribution of MDR phenotypes

There are many potential pathogenic bacteria that are resistant to multiple antibiotic classes, and infections caused by multidrug resistant (MDR) organisms are limiting treatment options and compromising effective therapy (Džidić *et al*., 2008). Inanimate hospital surfaces have often been described as a potential reservoir for multidrug resistant strains (Zenati *et al*., 2017). Our results showed that 158(56%) of bacterial isolates were resistant to at least 3 antibiotics. This finding is lower than reports from Northwest Ethiopia (75%) (Getachew *et al*., 2018), Zimbabwe (75%) (Mbanga *et al*., 2018) and Iran (79.4%) (Tajeddin *et al*., 2016).

MDR phenotypes were detected among both Gram-positive and Gram-negative bacteria (45.8% vs 73.8%, respectively), which is consistent with reports from Iran (47.8% vs 83.1%, respectively) (Tajeddin *et al*., 2016). The persistent pressure of disinfectants on the microorganisms present in the environment may lead to the development of MDR strains (Chemaly *et al*., 2014).

The highest MDR phenotypes were observed from *Acinetobacter* spp (93.8%, 45/48), which is consistent with reports from Iran (Tajeddin *et al*., 2016) (94%). All isolates of *Acinetobacter* spp exhibited high resistance to β-lactams, but they did not produce any ESBLs enzymes and carbapenemase by DDST and MHT, respectively.

The exhibition of fact very high MDR by *Acinetobacter* spp may be due to the ability of these bacteria to form biofilms; resist desiccation on inanimate hospital environments; ability to adhere to colonize and invade human epithelial cells, its repertoire of antibiotic resistance mechanisms that are able to be promptly up-regulated as required; and the ability to form horizontal gene transfer and intrinsic resistance mechanisms (Alvarez-Ortega *et al*., 2011; Zenati *et al*., 2016). The MDR phenotypes were also detected in other bacterial species among Gram-positive bacteria such as *S. aureus*, *Enterococci* spp and GBS which accounted for 58.6%, 50%, and 57.1%, respectively and among Gram-negative bacteria such as *Klebsiella* spp and *E. coli*, accounting for, 71.4% and 91.7%, respectively.

6. CHAPTER VI: Strengths and Limitations

The strength of our study was that inanimate environments were simultaneously analyzed by phenotypic detection of MDR such as MRSA, ESBL, CRE and VRE from a single sample collection in one glance. On the other hand, the limitation of the study could be that anaerobic and/or fastidiously growing bacteria such as *C. difficile* were not identified in this study because the isolation of these species requires special procedures and equipment that were not available. Other microbial contaminants, such as molds and yeasts, which were beyond the scope of this study, were not investigated. Moreover, settle plate samples were not taken from all ICUs and OTs of the selected hospitals and samples were taken once in each unit as limitations of our study.

7. CHAPTER VII: Conclusions and Recommendations

7.1. Conclusions

We therefore, conclude that inanimate surfaces and air near immediate patient environment and commonly touched medical equipment within the hospital environment are reservoirs of potential pathogenic bacteria and MDRs. *S. aureus*, CONS and *Acinetobacter* spp form the majority of the environmental contaminants. OTs was dominated by Gram-positive isolates, but ICUs were dominated by Gram-negative isolates. Most of the potential pathogenic bacteria in operation theatres were isolated from General OT. The most common bacterial contaminated ICU was Medical-Surgical ICU. The air-borne bacterial load of operating theatres in TASH exceeds the acceptable limits and index in predicting post-operative infections. The identified bacterial isolates showed the same antibiotic susceptibility pattern like isolated from patients. Amikacin and ciprofloxacin showed the highest bacterial sensitivity for Gram-negative bacteria. On other hand, clindamycin and ciprofloxacin showed highest bacterial sensitivity for Gram-positive bacteria. New strategies and re-enforced hygienic rules could help minimizing the dissemination of potential pathogenic bacteria and MDRs in hospital environments.

7.2. Recommendations

- Environmental surface sampling should not be limited to times of outbreak; however, it should be part of a routine practice.
- The environment should be considered a significant factor in infection control practices, and resources should be directed to improving our understanding of the interaction of pathogen survival, disinfection, hand hygiene, and HAI risks.
- More stringent routine environmental decontamination practices in healthcare facilities with regular monitoring is necessary in the MDR organism's containment bundle.
- Architectural properties of the hospital environments (such as ventilation systems, temperature, pressures and humidity) should be measured
- The effectiveness of routinely used disinfectants needs to be evaluated regularly.
- Appropriate air filtration systems should be used and maintained within hospitals.
- Infection-control measures, appropriate antibiotic prescribing practices and avoidance of broad spectrum antibiotics can prevent long-term emergence of resistance and HAI.
- Reliable laboratory screening and detection of resistance strain and resistance gene can prevent long-term emergence of resistance.
- Molecular typing of these isolates in comparison to the isolates from clinical samples will helps us to better understand these correlations.
- Finally, we recommend future studies to investigate the prevalence of anaerobic and fastidious-growing bacteria and other microbial contaminants, such as fungi, in our hospital operating theaters and intensive care units.

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Annex: 1: Microbiological methods

1.1. Collection of environmental sample and Isolation and identification of bacteria

Swab methods were used to collect samples from hospital surfaces and other materials in operating theatres (OTs) and intensive care units (ICUs). Settle plate method was employed to collect air samples. After overnight incubation with BHI broth, loop full of bacterial suspension were inoculated onto blood agar, MacConkey agar, Bile esculin agar, CHROMagar TM StrepB base (CHROMagar microbiology, France), Manitol salt agar using an aseptic streaking technique. The inoculated plates were incubated aerobically at 35-37 ℃ for 18-24 hours. Bacterial identification was based on standard culture and biochemical characteristics of isolates. Gram-negative bacteria were identified by standard biochemical tests. Gram-positive bacteria were identified with the corresponding laboratory tests including Gram staining, catalase, coagulase and hemolysis pattern on 5% sheep blood agar (Oxoid, UK), and mauve color on Chroma agar for GBS identifications, manitol fermentation for *staphylococci* spp. Biochemical identification systems for *Enterobacteriaceae*, *staphylococci* and *Enterococci* were used to identify the bacterial isolates at the species level.

1.1. Samples from surfaces and medical equipment (Swab method) procedure

1. A sterile cotton swab stick was used to swab different types of inanimate hospital surfaces and medical equipment after the daytime cleaning
2. Swabs collected from the Medical equipment and environmental surfaces were inserted aseptically into sterile test tubes that contain freshly prepared brain heart infusion broth (BHI) (Merck, Germany)

1.2. Settle plate sample procedure

2. Bacterial load of the air were performed by exposing open Blood agar plates and Mannitol salt agar (MSA) according to the 1/1/1scheme (at a height of 1m and 1m from the walls of the rooms for 1hour).
3. Then, they were covered and transport to the laboratory for incubation at 35-37 °C for 18-24 hours.
4. The Bacterial air contamination was measured by counting the number of Colony Forming Unit (CFU) per cubic meter (CFU/ m^3) of air.

5. Colonies showing different appearances were isolated based on standard cultural, morphological and biochemical methods and purified into pure cultures and characterized to species level.

1.1.1. Biochemical testing procedures Identification of Gram-positive bacteria

Gram-positive cocci were identified based on their Gram reaction, catalase, coagulase and bile esculin tests results.

Catalase test: This test were used to differentiate *staphylococci* (**+ve**) from *streptococci* **(-ve).**

Procedure:

1. The test organism is taken using a sterile wooden stick and added on the slide.
2. A drop of 3% hydrogen peroxide is added to a slide and looked for immediate bubbling.
3. Interpretation: Active bubbling positive test and No release of bubbles negative test.

Coagulase test: This test was used to differentiate *staphylococcus aureus* from other staphylococcus spp.

Procedure

1. A drop of physiological saline is placed on two separate slides.
2. The test organism was emulsified in each of the drop to make suspension.
3. One drop of plasma is added to one of the suspensions and mix gently. It is looked for clumping of the organism within 10 seconds.
4. Interpretation: Clumping within 10 seconds *S. aureus*, No clumping within 10 seconds indicates Coagulase negative *staphylococcus* (CONS).

Bile Esculin test: To isolate and identify bacteria able to hydrolyze esculin in the presence of bile. Commonly used for presumptive identification of group D *streptococci* and *Enterococci*, all of which are positive. This includes opportunistic pathogens such as *Enterococcus faecalis*, *Enterococcus faecium*, and *Streptococcus bovis*.

Tube Test:

1. Using sterile loop, pick one or two colonies from an 18-24 hours culture.
2. Inoculate onto the surface of slant of bile esculin medium with an S-shaped motion.
3. Incubate the inoculated tube at 35-37°C for 24 hours.
4. Observe the result as blackening of more than half of the agar slant, if the medium containing ferric ammonium citrate is used considered as positive result.

DNase agar, a differential medium is used to test the ability of an organism to produce deoxyribonuclease or DNase. This medium is pale green in color because of DNA-methyl green (indicator) complex (Note: Methyl green is a cation which binds to the negatively-charged DNA). It also contains nutrients for the bacteria. If the organism that grows in the medium produces Deoxyribonuclease, it breaks down DNA into smaller fragments. When the DNA is broken down, it no longer binds to the methyl green, and green color fades and the colony is surrounded by a colorless zone.

Requirements:

Media: DNase Agar or DNase agar with Methyl green indicator.

Reagent: Hydrochloric acid (1mol/L) only when DNase agar without indicator is used

Others: Inoculating loop, Bunsen burner

Procedure of DNase (DNA hydrolysis test)

1. Dry the surface of agar plates before use. Each plate may be divided into sections by drawing lines on the bottom of the plate.
2. Inoculate the test agar medium: There are two types of inoculation that can be done.

Spot Inoculation:

1. Touch a colony of the organism under test with a loop and inoculate it onto a small area of the DNase test agar plate, in the middle of one of the marked sections to form a thick plaque of growth 5-10 mm in diameter after incubation.
2. Incubate the plate at 37°C for 18-24hr.

Band or line streak inoculation

1. Use a heavy inoculum and draw a line 3-4 cm long from the rim to the centre of the DNase test agar plate
2. Incubate the plate at 37°C for 18-24hr.

When using DNase agar without the indicator,

(1). Flood the plate with 1N Hydrochloric Acid. (2). Leave the plate to stand for a few minutes to allow the reagent to absorb into the plate. Decant excess hydrochloric acid and then examine the plate within 5 minutes against a dark background.

Chromagar™ StrepB: For isolation and differentiation of *Streptococcus agalactiae* (GBS)

Typical appearance of microorganisms: Group B *Streptococcus* –› Mauve, Other Microorganism→ blue, colourless or inhibited

1.1.2. Identification of Gram-negative bacteria was identified based on their test result with a series of biochemical tests.

Procedure:

1. A suspension of the test organism is prepared with nutrient broth by adding 3 -4 colony
   of test organism in 5 ml nutrient broth.
2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, triple sugar iron agar, lysine decarboxylase agar, urea agar and SIM medium.
3. Incubated at 35-37oc for 18-24 hours.
4. Looked for color change (turbidity or motility) of the medium.
5. Then, the test organisms were identified by considering the results of the biochemical tests.

1.2. Antibiotic susceptibility testing

Antimicrobial susceptibility testing of isolated bacteria were determined by the disk diffusion method (Kirby-Bauer) according to the Clinical and Laboratory Standards Institute (CLSI) recommendations, using Mueller-Hinton medium (CLSI, 2018). Briefly, single isolate colonies were selected and inoculated in sterile normal saline (0.85 g/l) until its turbidity is comparable to 0.5 McFarland turbidity standards. Then the plates were inoculated with each broth culture and left to dry at room temperature before the application of antibiotic discs. The plates were incubated at 35-37 °C for 18-24 hours. Zone of inhibition for each

antimicrobial agent is interpreted, reporting the organism as resistant, intermediate or susceptible.

For Gram-negative bacteria panels of antibiotics were used such as, ampicillin, ampicillin-sulbactam, amoxicillin, amoxicillin-clavulanic acid, piperacillin, cefotaxime, ceftriaxone, cefoxitin, ceftazidime, imipenem, gentamycin, tobramycin, amikacin, ciprofloxacin and trimethoprim-sulfamethoxazole., the following panel were used such as; penicillin, ampicillin, oxacillin, vancomycin, erythromycin, clindamycin, gentamycin, cefoxitin, amikacin, doxycycline, ciprofloxacin and trimethoprim-sulfamethoxazole were used for Gram-positive bacteria.

Procedure for antibiotic susceptibility testing

1. A suspension of the test organism is prepared by emulsifying 4-5 colony of the organism in a small volume of sterile normal saline broth.
2. The turbidity of suspension is matched with turbidity standard.
3. With a sterile swab sample is taken from the suspension (the swab squeezed against the side of the test tube to remove the excess fluid).
4. The inoculums are spread evenly over the Muller-Hinton agar plate with the swab.
5. Using a sterile forceps, the antimicrobial disc is placed on the inoculated plate and incubated at 35-37° c for 18-24 hours.
6. The test is read after checking that the bacterial growth is neither heavy nor light. The radius of the inhibition zone is measured.
7. The reaction of the test organism to each antibiotics interpret as sensitive, intermediate, or resistance as per the (CLSI, 2018) guideline.

1.2.1. Screening for ESBL-producer isolates

Extended spectrum beta-lactamase producer Gram-negative isolates were tested using cefotaxime and ceftriaxone according to the CLSI standard using the modified Kirby Bauer method. Reduced susceptibility to cefotaxime (30 µg) and ceftriaxone (30 µg) with zone sizes ≤27 mm and ≤ 25 mm respectively were used as screening method for ESBL production. Double disk synergy test using ceftazidime (30µg), cefotaxime (30µg) and co-amoxi/clavulanates (20/10 µg) was used to confirm ESBL production.

1.2.2. Phenotypic detection of carbapenemase production by MHT

Carbapenemase an enzyme that hydrolyses a group of antibiotics called carbapenems. Among phenotypic tests, MHT is a relatively easy and simple test to be performed in a laboratory.

Procedure:

1. A 0.5 McFarland dilution of the *Escherichia coli* ATCC 25922 in 5 ml of broth or saline was prepared.
2. A 1:10 dilution was streaked as lawn on to a Mueller Hinton agar plate
3. A 10 µg meropenem disk was placed in the center of the test area
4. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate
5. The plate was incubated overnight at 35 ± 2^{o}C in ambient air for 16-24 hours.
6. Quality control of the following organisms MHT Positive *Klebsiella pneumoniae* ATCC1705 and MHT negative *Klebsiella pneumoniae* ATCC1706 were run with each batch of the test
7. After 24 hours, MHT Positive test showed a cloverleaf-like indentation of the *Escherichia coli* 25922growing along the test organism growth streak within the disk diffusion zone.
8. MHT Negative test showed no growth of the *Escherichia coli* 25922 along the test organism growth streak within the disk diffusion.

1.2.3. Screening for methicillin resistance among staphylococci isolates

Methicillin-susceptible *Staphylococcus aureus* strains (MSSA) and methicillin-susceptible coagulase negative *staphylococci* (MS-CoNS) were differentiated from methicillin-resistant *Staphylococcus aureus* strains (MRSA) and methicillin-resistant coagulase negative *staphylococci* (MR-CoNS) according to the CLSI guidelines 2018 (CLSI, 2018). Oxacillin disc (1 µg)/Cefoxitin (30 µg) were used as screening method to test for methicillin resistance. Isolates that show growths around oxacillin disc were considered as methicillin resistance, while those that did not grow were considered as methicillin sensitive.

Annex: 2: Data collection form

1. Code number_____

2. Where the sample collected (OTs/ICUs)_____

3. Room equipment where sample taken_____

4. Type of disinfectant used daily in cleaning_____

5. Media used_____

6. Biochemical result_____

7. Gram reaction result_____

8. Organism isolated_____

9. Drug susceptibility pattern_____

    1. Sensitive_____
    2. Resistant_____
    3. Intermediate_____

10. Phenotypic result of resistance profile

10.1. ESBL_____

10.2. MRSA_____

10.3. VRE_____

10.4. CRE_____

Comments_____

Name of principal investigator_____

Signature _____ Date _____

Declaration

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

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Signature _____

Date _____

Place of submission: Addis Ababa University

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