PREVALENCE OF MULTIDRUG RESISTANT BACTERIA AMONG CLINICAL ISOLATES AT CENTRAL PATHOLOGY LABORATORY, MUHIMBILI NATIONAL HOSPITAL

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By

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A Dissertation submitted in (Partial) Fulfilment of the Requirements for the Degree of Master of Science (Microbiology and Immunology) of Muhimbili University of Health and Allied Sciences
October, 2021

CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination by Muhimbili University of Health and Allied Sciences a dissertation entitled; **"Prevalence of multidrug resistant bacteria among clinical isolates at a central pathology laboratory, Muhimbili National Hospital'',** in (partial) fulfillment of the requirements for the degree of Master of Science in Microbiology Immunology of Muhimbili University of Health and Allied Sciences.

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(Supervisor)

Date:_____

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Ms. Upendo Kibwana

(Co-Supervisor)

Date:_____

DECLARATION AND COPYRIGHT

I, **Khamis Chum Khamis,** declare that this **dissertation** is my original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature…… **Date**……..

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To the Almighty are the glory, honour and praise to the GOD, the creator of the Universe and everything in it, for his love which enabled me to complete this scientific task.

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DEDICATION

This dissertation is dedicated to my beloved mother Fatma Yussuf and my wives Amina and Ashirun, may this work bring them a happy and successful life.

ABSTRACT

Background: Antimicrobial-resistant is reported to evolve dramatically despite adopting several strategies. An increasing rate of antimicrobial resistance bacteria in resource-limited countries like Tanzania brings both health care costs and mortality. Antibiotic susceptibility testing plays a key role, in attaining proper choice of antimicrobial, and eventually improved treatment outcomes. The ability of the laboratory to identify and reporting of AMR is critical for control of its spread and for improving AMR stewardship.

Aim: This study aims at determining the prevalence of multidrug-resistant bacteria among clinical isolates at the central pathology laboratory, Muhimbili Nation Hospital

Method: This was a laboratory-based cross-sectional study, conducted from March 2021 to June 2021 at Muhimbili National Hospital (MNH). All specimens processed during a study period were followed to the final identification of bacteria. Isolates were identified using conventional methods and AST was performed following CLSI guidelines. ESBL and MRSA were detected using the double disk method and cefoxitin disk, respectively. MIC for ESBL-PE and MRSA to commonly used antibiotics were detected using the broth dilution method. Data were analysed by Statistical Package for Social Sciences (IBM SPSS) version 23.0.

Results: A total of 3549 samples were received and processed at CPL from March to July 2021, and 363(10.2%) clinical isolates were isolated. Out of 363 isolates recovered, 131 were *S. aureus* and 232 were Gram-negative bacteria. The majority of bacteria were highly resistant to commonly used antibiotics. The overall, prevalence of multi-drug resistance was 45%. MDR was more common in Gram-positive bacteria (57%) compared to gram-negative (44%) P-value = 0.007. The overall prevalence of ESBL among Gram-negative bacteria was 35%, (81/232); while 50.3% (66/131) *S. aureus* was MRSA.

MRSA was significantly resistant to gentamicin, trimethoprim-sulfamethoxazole and ciprofloxacin. MRSA resistance to clindamycin using MIC was low compared by disk diffusion (27.4%) vs (47.5%), p-value = 0.0405. There was a difference in MRSA resistance

rates to ciprofloxacin on using MIC (52%) and disk diffusion (59%), but not statistically significant.

Among ESBL producing isolates rates of resistance to ciprofloxacin (80.3%) and gentamycin (72.7%) was significantly high, p-value = 0.007. There was a significant difference in ESBL resistance rates to ciprofloxacin (89%) using Disk diffusion and MIC (33%) p-value= 0.001.

Conclusion

This study revealed majority of bacteria were resistant to multiple routinely prescribed antibiotics, and almost half of bacteria were MDR. Nevertheless, the study found, high prevalence of MRSA and ESBL producers among isolates at CPL.

Recommendations

The observed high proportion of MDR pathogen including ESBL PE and MRSA in our setting call for the need for a clinical microbiology laboratory to enforce the policy for regular screening and reporting for MRSA and ESBL PE.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AST	Antimicrobial Susceptibility Testing
BMC	Bugando Medical Centre
BSI	Blood Stream Infection
CPL	Central Pathology Laboratory
CLSI	Clinical and Laboratory Standard Institute
DDT	Disk Diffusion Test
ESBL	Extended Spectrum Beta-lactamase
ESBL PE	Extended-Spectrum Beta-Lactamase Producing Enterobacteriaceae
ICU	Intensive Care Unit
MBC	Minimum Bactericidal Concentration
MDR	Multi Drugs Resistance
MDRO	Multidrug Resistant Organisms
MIC	Minimum Inhibitory Concentration
MICU	Medical Intensive Care Unit
MNH	Muhimbili National Hospital
MRSA	Methicillin Resistant *Staphylococcus aureus*
NICU	Neonatal Intensive Care Unit
UTI	Urinary Tract Infection

DEFINITION OF TERMS

MDR: Defined as resistance to at least one antibiotic in three or more antimicrobial classes.

MRSA: Is a strain of *S. aureus* which is resistant to beta-lactam antibiotics

ESBL PE: Are Gram-negative bacteria that can produce ESBL enzymes that confer resistance to different antibiotics including first, second, third and fourth generation cephalosporin as well as aztreonam

Antimicrobial susceptibility patterns: Is testing done to assure the susceptibility to drugs of choice for particular infections or to detect resistance in individual bacterial isolates.

Antimicrobial resistance: Ability of microorganisms to resist the effects of medication that once could successfully treat the microbe.

Antimicrobial susceptibility patterns: Is testing done to assure the susceptibility to drugs of choice for particular infections or to detect resistance in individual bacterial isolates.

Minimum Inhibitory Concentration: Is the lowest concentration of antibiotic that will inhibit the visible growth of organism after overnight incubation

Clinical isolates: Are those bacteria isolated from patient's samples and identified as a true pathogen of the disease

MIC_{50}: Is the MIC value at which 50% of the isolates in a test population are inhibited; it is equivalent to the median MIC value.

MIC_{90}: Is the MIC value at which 90% of the strains within a test population are inhibited

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Multidrug resistant pathogen causing higher morbidity and mortality infection with costly treatment and prolong Hospital stays that result in greater burden on our Health systems(1–3). Globally the prevalence of Multidrug resistant bacteria varies worldwide(4), with reports from North America, South America, Europe and Asia, the MDR ranges from 10% to 50% among the bacteria isolates from different sites of infection (5–7).

In Africa, Multidrug resistant (MDR) has reached a critical point with significant risk in developing countries, where infectious diseases burden is high (55% to 75%), and treatment practices are relatively poor. Studies showed that the prevalence of ESBL-producing bacteria has reached 25–50% in some tropical and subtropical regions, and baseline carriage in the healthy population is 20–40%, thus rendering these regions endemic for ESBL(8–10).

In Tanzania, several studies have revealed an increase in MDR with the peak level seen in both MRSA and ESBL producing pathogens across the country. A study done at Bugando Medical Centre hospital between 20016 and 2019 has shown an increasing rate from 25 to 50% and 16 to 44% for extended-spectrum beta-lactamases producing *Escherichia coli* and Methicillin resistant *Staphylococcus aureus* respectively (1,11–13)**.**

Following this, World Health Organization (WHO) has declared AMR a public health threat and has urged different countries to develop an action plan to combat the problem (14). AMR is reported to be largely attributed to two groups of bacteria: extended-spectrum β-lactamase (ESBL) producing bacteria and Methicillin Resistance *Staphylococcus aureus* (MRSA). The worldwide spread of extended-spectrum β-lactamase (ESBL)-producing bacteria, particularly *Escherichia coli* and *Klebsiella pneumoniae* is of critical concern for the development of therapies against multidrug-resistant bacteria (15).

These resistant ESBL producing organisms have enzymes termed ESBL which confer additional ability to hydrolyze the β-lactam ring of cefotaxime, ceftazidime or aztreonam and render them ineffective for treatment (16).

MR*SA* is a strain of *S. aureus* which is resistant to beta-lactam antibiotics resulting in treatment challenges, increased morbidity and mortality. It was first discovered in 1961 and recently it found resistant to methicillin, amoxicillin, penicillin, oxacillin and other common antibiotics known as cephalosporins (17).

Due to the evolution of resistance to the existing antimicrobial agents, methods for antimicrobial susceptibility testing and novel antimicrobial agents have been extensively discovered. Although the disk diffusion method is the most commonly used technique in clinical microbiology laboratories (18), several studies have reported MIC to be the gold standard method for determining antimicrobial susceptibility of organisms (19,20) and so can be used as a confirmatory test to unusual resistance obtained from other methods (21).

Minimum Inhibitory Concentration is the lowest concentration of the assayed antimicrobial agent that inhibits the visible growth of the microorganism tested. It is usually expressed in mg/mL or mg/L and the universally accepted antibiotic concentrations range used for determining MIC are in doubling dilution steps and ranges down from 1 mg/L (20,21). The sensitivity and specificity of this method are relatively higher compared to the diffusion method (22). The MIC also provides the actual concentration of agents that will inhibit the growth of organisms quantitatively in-vitro and be used in vivo by the clinician to adjusting the dose concentration based on ranges on susceptible dose dependents (23) and increase the scope of use of available antibiotics.

The microbiology laboratory plays an important role in the recognition and surveillance of antimicrobial resistance, both within the hospital and in the community. The laboratory must provide high-quality diagnostic testing to correctly identify infections and accurate antimicrobial susceptibility testing to guide appropriate treatment. Delayed or incorrect laboratory diagnostic data frequently result in bad treatment outcomes. This study was aimed

at detecting MRSA and ESBL and make confirmation of antimicrobial resistance pattern of ESBL producing Enterobacteriaceae and MRSA among isolates by MIC methods.

1.2 Problem Statement

The increasing rate of antimicrobial resistance limits the empirical use of antimicrobial agents as treatment options for resistant pathogens (24). In our setting antimicrobial susceptibility tests is relied only on disk diffusion test, although MIC test are reported as a gold standard method (18,25). This method limits the spectrum use of routinely available drugs, since it is the qualitative measure of the activity of antimicrobial based on zone size of inhibition of bacteria growth, and result fall in three categorical values, whether sensitive, intermediate or resistance, the interpretation depends on zone size of inhibition (26). Due to considerable increase in resistance to the routinely available antibiotic, among the isolates in our tertiary hospital, especially the strains of MRSA and ESBL producing bacteria (2)**,** whereby most of the time they resist all available routinely prescribed antibiotics, the need for having a confirmatory test that will provide estimate concentration, of the tested antimicrobial agent and quantitatively measure antimicrobial activity against bacteria in vitro arises (19). **Some** studies report MIC to be the gold standard method for determining antimicrobial susceptibility of organisms (19,20) and so can be used as a confirmatory test to confirm unusual resistance obtained from other methods (21). Considering that there is no further antimicrobial test performed, for the resistant pathogen in our setting as a routine confirmatory test, it is important to determine one and thus raising the need to conduct this study(27).

1.3 Rationale

The findings obtained from this study aimed to reveal the burden of MRSA and ESBL -PE which sensitize continuous and regular screening of MRSA and ESBL in order to enable early detection which aid in generating early intervention programs to control transmission.

MIC values obtained help the clinician to improve treatment options against these infections, and provide evidence-based data on the importance of incorporating MIC testing as a confirmatory AST test for the resistant pathogens in our laboratories.

1.4 Research Questions

1. What is the proportion of ESBL-PE and MRSA among the clinical isolates of gram-negative rods and *S. aureus* at the clinical laboratory?
2. What is the antimicrobial resistance pattern of MRSA among *S. aureus* isolates?
3. What is the antimicrobial resistance pattern of gram-negative rods?
4. What is the MIC values of MRSA and ESBL-PE for routinely used antibiotics at the clinical laboratory?

1.5 Objectives

1.5.1 Broad Objective

Prevalence of multidrug resistance bacteria among clinical isolates at a central pathology laboratory**,** Muhimbili National Hospital.

1.5.2 Specific Objectives

1. To determine the proportion of ESBL-PE and MRSA among the clinical isolates of Gram-negative rods and *S. aureus* at the clinical laboratory.
2. To determine the antimicrobial resistance pattern of *S. aureus* isolated at the clinical laboratory.
3. To determine the antimicrobial resistance pattern of Gram-negative rods to commonly used antibiotics at MNH.
4. To determine the resistance pattern of MRSA and ESBL-PE to commonly used antibiotics by MIC at MNH.

1.5 Literature Review

1.5.1 Antimicrobial Resistance

Antimicrobial resistance is the ability of microbes to counteracts the effects of the antimicrobial agents that were previously effective against them leading to ineffective standard treatments, the persistence of infections and sometimes the spread of infections to other healthy individuals (15). The introduction of every antimicrobial agent into clinical practice has been followed by in-vitro detection of resistant strains of microorganisms that can multiply in the presence of drug concentrations higher than the concentrations in humans receiving therapeutic doses. Such resistance may either be a characteristic associated with the entire species or emerge in strains of a normally susceptible species through mutation or gene transfer (28,29) This challenge is increasing in resource-limited countries where culture and antimicrobial sensitivity are not routinely done in any settings and the choice of antimicrobials is limited (30). In Tanzania rate of resistance are increasing day to day with peak level seemed to be caused by both ESBL and MRSA (1,2). This study will provide the recent data on the prevalence of ESBL producing Enterobacteriaceae and MRSA among clinical isolates but also their susceptibility pattern by MIC method.

1.5.2 Magnitude of ESBL Producing Bacteria

ESBL producing bacteria poses global challenges and immediate action need to be taken against these potential pathogens (29,31). These multidrug resistance bacteria are found to cause a wide spectrum of infections in humans resulting in clinical and economic impacts on patients (31). In Dakar Senegal for instance, ESBL-E bloodstream infections show the highest frequency 44.5 %, followed by urinary tract infections 32.7 % and surgical site infections 11.8 % (31) on the other hand in Ethiopia study done among clinical specimens found ESBL prevalence of 57.7% whereby bloodstream infection shows the highest frequency 84.4%. A study done in Uganda, East Africa on surgical sites infection has reported an ESBL prevalence of 96.72 among GNR isolates (1). In Tanzania, ESBL producing bacteria has also been reported among isolates in various infections. In bloodstream infection studies done at a tertiary hospital has reported ESBL prevalence of 13.9%, 17.9% and 68% respectively

(1,12,32). Also studies done in surgical sites infection at different setting has shown ESBL prevalence of 79.3%, 50% (13,33). In some cases, ESBL producing bacteria have also been reported to be isolated from urinary tract infection (UTI) (16,34,35). Among these ESBL producing bacteria *Klebsiella pneumoniae* shows the highest prevalence followed by *Escherichia coli* (6,16,36,37). Less is known about the optional treatments for these potential pathogens to routinely available drugs since no study had been conducted focusing on detection of MIC values to routinely available drugs in our country.

1.5.3 Magnitude of MRSA

Methicillin-resistant *Staphylococcus aureus* (MRSA) has disseminated globally and become a leading cause of bacterial infections in both healthcare and community settings (17). Globally MRSA prevalence is ranging between 15% and 40% (17,38,39) which has been shown to vary greatly with geographical location whereby developing countries were reported to have high rate than developed countries (17). A study done in Bangladesh among clinical isolates has reported MRSA prevalence of 53.1%, whereby surgical wound swabs were highly reported with MRSA followed by pus from skin infections with the lowest prevalence found in aural swabs(40). The overall prevalence of MRSA in east Africa was 53.4% (38). In Tanzania, studies report an increase in the prevalence of multidrug resistance *S. aureus* colonization and infection, whereby at tertiary care hospital report of microbiological culture shows MRSA prevalence of 34.6% (37), another study done among patients attending regional hospital in Dar es Salam found that 24.7% were the career of MRSA (41). *Staphylococcus aureus* was commonly isolated from bloodstream infections (1,2) But also can be isolated in a variety of infections, a study done in Nepal among patients attending tertiary care hospitals has reported 35.5% of MRSA has recovered from Pus/wound swab (42). Moreover, MDR has been reported to cause surgical site infection among post-operative patients at regional hospitals in Uganda (43). In Tanzania studies done has found MRSA were isolated from nostrils, axillary blood, sputum and wounds (39,44).

Many studies conducted in our local setting shows MRSA has a wide spectrum of infections which are accommodated with a great challenge in treatment options. This study is aimed to provide useful data on treatment options to available routinely used drugs.

1.5.4 Multidrug Resistance

The emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health threat (45) Infections with MDROs can lead to inadequate or delayed antimicrobial therapy, and are associated with poorer patient outcomes as there are fewer, or even sometimes no, effective antimicrobial agents available for infections caused by these bacteria. Gram-positive and Gram-negative bacteria are both affected by the emergence and rise of antimicrobial resistance (1,13,16,46) .Study done in Ethiopia among fermentative Gram-negative bacilli isolates, showed 94.5% were MDR (46),In Tanzania recent study done on bloodstream infection reported prevalence of (70.5%) of the isolates were MDR (1), in addition, other studies done in a tertiary hospital in Tanzania among the clinical isolates found the prevalence of MDR to be 67.4% and 77.8% respectively (12,16).

1.5.5 Antimicrobial used to manage ESBL and MRSA

Several studies conducted to evaluate the susceptibility of ESBL and MRSA on different antibiotic including the empirical ones has fallen within the acceptable cut off value recommended by CLSI guidelines. In China, the assessment of in-vitro activity of different cefoperazone sulbactam against different MDR bacteria including ESBL *E.coli* and ESBL *Klebsiella pneumoniae* shows decreased MICs ranges of <64mg/l (47), On the other hand, it has been reported that ESBLs do not inactivate non-ß-lactam agents including ciprofloxacin, trimethoprim-sulfamethoxazole, and gentamicin (48), and for that Carbapenems, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole are all preferred treatment options for patients with uncomplicated infection caused by ESBL producer (48) nevertheless studies have reported that patients which do not have life-threatening MRSA infection Clindamycin, co-trimoxazole, fluoroquinolones or minocycline may be useful, and for serious infections caused by strains that are susceptible to rifampin, adding this agent to vancomycin or fluoroquinolone may contribute to improved outcomes (49). Also, there is a

study done in four geographic regions in America, Asia, West Africa, and East Africa to determine the MICs of 11 different antimicrobials against 284 bacterial enteropathogens by agar dilution methods found that Ciprofloxacin, levofloxacin, ceftriaxone, and azithromycin are highly sensitive in vitro against the ESBL producing Enterobacteriaceae with the MIC ranges of 0.0625ug/ml to 32ug/ml (50). In Tanzania the MIC ranges for routinely available antibiotic drugs against these MDR are not well known, this study aimed to find MIC values that will help to provide the treatment option on available routinely prescribed drugs.

1.5.6 Methods for detecting ESBL and MRSA

ESBL testing involves two important steps, the first is screening with an indicator cephalosporin which looks for resistance or diminished susceptibility, thus identifying isolates likely to be harbouring ESBL.(23) The second test for synergy between an oxyimino cephalosporin and clavulanate, distinguishing isolates with ESBL from those that are resistant for other reasons. ESBL screening the CLSI has proposed disk diffusion methods for screening of ESBL production by noting specific zone diameters which indicate a high level of suspicion for ESBL production cefpodoxime, ceftazidime, aztreonam, cefotaxime or ceftriaxone disk are used. Phenotypic confirmatory test for ESBL production involves the use of cephalosporin /clavulanate combination disk the CLSI advocates the use of cefotaxime(30ug) or ceftazidime disk with or without clavulanate (10ug).(23) Another method is double-disk synergy test in this test disk of third-generation cephalosporin and augmentin are kept 30mm apart, centre to centre on inoculated Mueller-Hinton agar a clear extension of the cephalosporin towards augmentin indicate positive for ESBL production,(2,23) other methods used are three-dimensional test, inhibitor potential disk diffusion test, cephalosporin/clavulanate combination disk on iso-sensitise agar, and disk approximation test.

MRSA detection is done by oxacillin and cefoxitin disk cefoxitin is a potent inducer of the *mecA* regulatory system recommended for detection of MR*SA* Oxacillin is also used to detect MR*SA*, however, Cefoxitin produced more accurate and reliable results (36,51).

1.5.7 Antimicrobial susceptibility test methods

A number of antimicrobial susceptibility testing (AST) methods are available to determine bacterial susceptibility to antimicrobials (25). Kirby Bauer Disk diffusion susceptibility testing methods are the most commonly used techniques in clinical microbiology laboratories (18), It is straightforward to perform, with low cost, and can be used as screening test against large numbers of isolates (25), however its provide qualitative results and has lower sensitivity for detection of resistance caused by MDR pathogen and it not suitable for slower growing fastidious organisms (52).

Broth and agar dilution methods, these determine the lowest concentration of the assayed antimicrobial that inhibits the visible growth of the bacterium being tested (MIC), The primary advantage of this method is the ability to obtain quantitative MIC values as well as the minimum bactericidal concentration (MBC) (52), it appear to be more reproducible and quantitative than agar disk diffusion (25), nevertheless it is the gold standard method for determining antimicrobial susceptibility of resistant organisms (19,20) and can be used as a confirmatory test to confirm unusual resistance obtained from other methods (21,53). A major disadvantage is that each antibiotic solution has to be prepared by hand hence its time consuming and labor intensive to perform(52).

Another method is Gradient Epsilon test (E -Test) is an extended version of the agar diffusion method, in this method predefined exponential gradient of antibiotic agent is applied to the bottom of a plastic strip, and the exact MIC of a drug that is necessary to stop bacterial growth is easily read on the strip (52). It is suitable for a variety of pathogens, ranging from rapid growing aerobic and anaerobic bacteria to slow-growing fastidious bacteria, and also can be used for finding heterogeneous resistance, or a bacterial strain that forms both susceptible and resistant colonies(52), However this method is more expensive than paper disk diffusion and broth macrodilution methods.

In Iran, the study was done on the consistency of disk diffusion and MIC tests in the diagnosis of antibiotic sensitivity of isolated organisms showed that Diffusion method about 52.5% of the isolates showed resistance to gentamycin while in the MIC method only 49.8% of the samples showed resistance (22). This difference reveals a higher accuracy of the MIC method

in determining the drug resistance, nevertheless the study done to evaluate MIC_{50} and MIC_{90} of ciprofloxacin and against enteropathogen reveal the highly active in-vitro activity of ciprofloxacin with the MIC_{90} of 0.125 and 0.5ug/ml respectively (50). Our clinical microbiology laboratories relied only on Disk diffusion methods for susceptibility testing, no studies have been reported on MIC values for routine antibiotics.

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Study Design

A laboratory-based descriptive cross-sectional study was conducted at Microbiology laboratory Central Pathology Laboratory MNH.

2.2 Study Site

The study conducted at Central Pathology Laboratory at Muhimbili Upanga, CPL is the largest laboratory which serves in the largest tertiary hospital in Tanzania, serving approximately Six million people from Dar es Salaam, and it provides services to approximately 1200 inpatients per week and approximately 1200 outpatients per day. CPL is also a training facility for the Muhimbili University of Health and Allied Sciences and is the main clinical diagnostic referral laboratory which is well equipped and is the one among accredited laboratory in the country practiced under 15189 ISO standard.

2.3 Study Population

Patients whose samples processed in CPL microbiology laboratory MNH with bacterial isolates obtained.

2.4 Study Duration

The study was conducted for four months starting from March to July 2021.

2.5 Selection Criteria

2.5.1 Inclusion Criteria

Staphylococcus aureus and Gram-negative rods isolated from patients with clinical syndrome during the study period (March to July 2021).

2.5.2 Exclusion Criteria

Unidentified bacterial isolates, contaminants, and fungi

2.6 Sample Size

The sample size for this study was calculated using the kish Leslie formula, Using the prevalence of 25.1% for ESBL obtained from a study done on microbiological cultures at tertiary hospitals in Tanzania, and 8.5% for MRSA obtained from a study done on MRSA among patients attending regional hospital in Tanzania (41). The sample size was determined by using the formula below,

$$n = \frac{Z^2\, p(100-p)}{d^2},$$

Whereby

Z= standard deviation of the normal distribution = 1.96(confidence level at 95%)

P= prevalence of ESBL pathogens 25s. 1% a study on the report of microbiological cultures at a tertiary hospital in Tanzania (2), and 8.5% MRSA pathogens based on the study on MRSA prevalence among patients attending regional hospital in Tanzania(41)

D= Tolerable error 5%

N= 289 for GNR and N=119 for *S. aureus*

2.7 Sampling Method

Convenient sampling was used to recruit the available isolates for this study. Every day new clinical identified isolates of GNR and *S. aureus* isolates was collected until the required sample size is attained.

2.8 Variables of the study

Samples, bacteria isolates, and antimicrobial susceptibility testing results.

2.9 Data Collection

A checklist was used for collecting both demographic and laboratory data. The isolates were collected from all clinical specimens processed at microbiology (Blood, Urine, body fluid Pus, Swabs etc) after being cultured and identified, the isolates were collected using Tryptose Soy Broth (TSB) (Oxoid Ltd) containing 20% glycerol and temporarily stored at − 20 °C in the laboratory. Then these isolates were tested for antimicrobial susceptibility pattern, perpendicular with the screening and detection for ESBL/MRSA, and those resist at least one antibiotic in three or more antimicrobial classes are considered MDR, Social demographic data which is age, sex, ward admitted physical address, and clinical information such as a history of illness, immune status, type of specimen was collected from laboratory request form and laboratory information system (GIVA) during data collection.

2.10 Laboratory Procedure

2.10.1 Antimicrobial susceptibility test

Antimicrobial susceptibility testing of all isolates was performed by the Kirby Bauer disk diffusion method. In this method, the inoculums were adjusted to the turbidity of a 0.5 McFarland standard and swabbed onto the surface of a Muller-Hinton agar plate. Antimicrobial disk such as Imipenem (10µg), meropenem (10µg), ceftazidime (30µg), ceftriaxone (30µg), gentamicin (10 µg), amoxicillin/clavulanic acid (20/10 µg), aztreonam (30 µg), trimethoprim-sulfamethoxazole 23.75 µg, and ciprofloxacin (5 µg) (Oxoid Ltd) was used for testing antimicrobial susceptibility of the GNR.

For *S.aureus* cefoxitin 30ug, erythromycin 15ug, trimethoprim-sulfamethoxazole 23.75 µg, ciprofloxacin 5ug, gentamycin 10ug, and clindamycin 2ug, were used. After putting the disks onto the inoculated plates, the plates were incubated at 37∘C for 24 hours. All susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI).MDR was defined as resistance to at least one antibiotic in three or more antimicrobial classes(45)

2.10.2 Phenotypic Detection of ESBL- Producing Enterobacteriaceae

2.10.2.1 ESBL Screening Test

Screening for ESBL was performed during antimicrobial susceptibility test, whereby ceftazidime and cefotaxime disk was placed into inoculated Muller Hinton Agar (MHA) plate, with test organism and incubated at 37 ℃ aerobically for 18h. The zones of inhibition were observed and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The isolates that showed an inhibition zone size of ≤ 22 mm with ceftazidime (30 μg) and/or ≤ 27mm with cefotaxime (30 μg) were considered as potential ESBL-producer (screening ESBL positive) and was shortlisted for confirmation for ESBLs production using Combination Disk Test as recommended by CLSI guideline (m100 s28)

2.10.2.2 Confirmation Test

ESBL detection was done by a combination disc diffusion test. In this test, a suspension of microorganisms was prepared and equilibrated to match 0.5 McFarland's standard. Then, a lawn culture on MHA was prepared, ceftazidime (30 μg) and ceftazidime + clavulanic acid (30/10 μg) and Cefotaxime (30 μg) and cefotaxime + clavulanic acid (30/10 μg) were placed and incubated at 37 ºC for 16 to 18 hours. if there was a zone size of ≥ 5 mm increase in diameter for either antibiotic tested in combination with clavulanate compared to the zone diameter of the antibiotic when tested alone, bacteria was confirmed as ESBL (54).

2.10.2.3 Detection of MRSA

Methicillin-Resistant *Staphylococcus aureus* (MRSA) was determined during antimicrobial susceptibility test, by the Kirby-Bauer disc diffusion method using cefoxitin disk (30μg), a suspension of microorganisms was prepared and equilibrated to match 0.5 McFarland's then inoculated to MHA followed by adding 30μg cefoxitin disk and incubated at 33 to 35^{o}C Where by *S.aureus* isolates with a zone of inhibition <-21mm phenotypically confirmed as MRSA positive. Following CLSI guidelines

2.10.3 MIC Determination

Minimum Inhibitory Concentration was performed by broth dilution method, the stocks solution of antibiotic was made from solution using the formula below

1. C = M/V (20)
2. C1V1 = C2V2

Where by C = Concentration, M= Mass, V= Volume

C1= Initial concentration of stock solution

V1= Initial volume of working solution (Volume of antibiotic)

C2 = Final concentration (concentration of working solution)

V2 = Final volume (volume of diluent)

Serial doubling dilution of antibiotic was made by using micropipette by taking 5ml of prepared solution of antibiotic and dispensed into a first tube containing 5mls broth medium, mix well and take 5ml from the first tube and dispensed to the second tube repeat the same until the last negative control tube, then 0.5ml of tested organism was added to the set of tubes except for the last remain as a negative control, and incubating overnight at 35^0C (20). Ciprofloxacin and Gentamycin were antimicrobial agents used for MIC determination of Gram-negative ESBL producing isolates, the dilution range used for ciprofloxacin was 0.004ug/ml – 128ug/ml, with a cutoff value of >4ug/ml, Gentamycin dilution range was 0.03 – 128ug/ml with a cutoff value of >16ug/ml. Clindamycin and ciprofloxacin were used for MIC determination of MRSA, The dilution range used for Clindamycin and Ciprofloxacin was 0.03 – 8ug/ml and 0.06 – 128ug/ml respectively and cut off value was >4ug/ml for both. Finally, the growth examination was carried out by looking at the turbidity of culture media. The MIC results were interpreted using the CLSI recommended guidelines.

2.10.4 Quality Control

All culture media were prepared according to the manufacturer's guidelines and were tested for performance and sterility. To standardize turbidity of the bacterial suspension for ESBL and MRSA tests, a 0.5 McFarland standard was used. As per CLSI, *Klebsiella pneumoniae*

(ATCC -700603) and *Escherichia coli* (ATCC-25922) were used as positive and negative control bacteria strains respectively for ESBL. Also MRSA standard *S. aureus* ATCC 25923/*S. aureus* ATCC 29213 were used as a negative control strain and *S. aureus* ATCC 700699 was used positive control (19,42) For MIC test standard *K. pneumoniae* ATCC 700603 was used as a positive control for ESBL (23) and *S. aureus* 12493 was used as positive for MRSA.

2.11 Data management and analysis

2.11.1 Data Management

Clinical information and laboratory results of isolates were cross-checked and coded before being entered into computer software. Data were edited, cleaned, entered and analysed using statistical package for social science (IBM-SPSS) version 23.0.

2.11.2 Data Analysis

Frequency distribution and two-way tables were used to summarize the data. A descriptive analysis on the proportion of MRSA and ESBL was presented using percentages. Also, the percentage was used to determine the rate of resistance of antimicrobial agents against isolated ESBL and MRSA. Chi-square was used for the comparison of variables. The results were of statistical significance when the p-value was <0.05.

2.12 Ethical Consideration

Ethical clearance was obtained from the Research and Publications Committee of the Muhimbili University of Health and Allied Sciences (MUHAS). Since the study did not involve contact with human subjects directly, no consent was sought from them. However, a waiver of informed consent was sought from MUHAS Research Ethics reviewer Board and Permission to conduct the study was obtained from the MNH administration.

2.13. Study limitation

Due to time and financial constraints, MIC test was performed for only two antibiotics per group of bacteria, also due to time 54 GNR isolates were not obtained to completing the sample size of 286 of the study.

CHAPTER THREE

3.0 RESULTS

3.1. Specimen received at CPL

A total of 3549 samples were received and processed at CPL from March to July 2021. The majority were blood 1204 (34%), followed by urine 889 (25%) and sputum 355(10%). General medical wards (25.4%) and outpatient departments (23.5%) contributed the highest proportion of samples, while the least samples came from Obstetrics & gynecology 1.8%.

Of the processed samples, 347 (9.7%), yielded clinical isolates, which were mostly recovered from pus (26.17%) and sputum (20.56%). The majority of isolates were recovered from samples collected at Medical ICU 15.96%, paediatric general 10.14%
and Neonatal ICU (10.16%) (Table 1).

Table 1: Distribution of total processed samples and positive culture collected during March to June 2021.

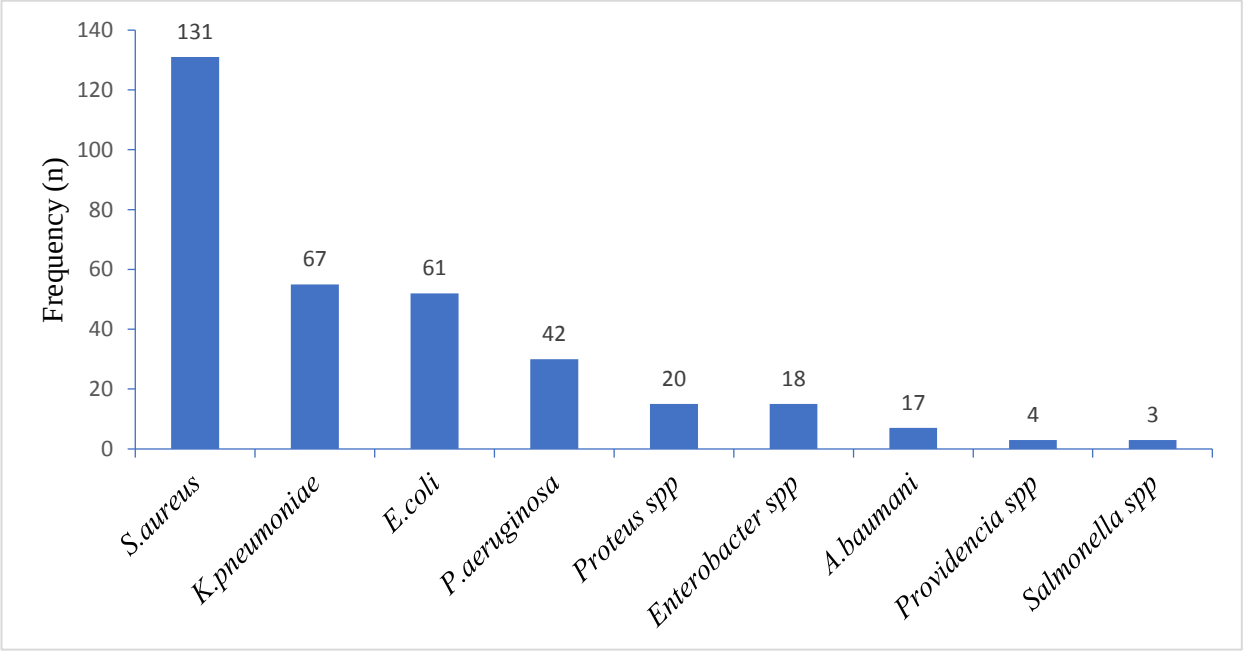
VARIABLES	N(samples received)	(%)	(n)culture positive	(%)
SAMPLES				
Blood	1204	34	**121**	**10.05**
Pus	298	8.3	**78**	**26.17**
Sputum	355	10	**73**	**20.56**
Urine	889	25	**51**	**5.73**
Fluid	387	11	**11**	**2.84**
Swabs	416	11.7	**13**	**3.13**
WARDS				
Medical general	902	25.4	**86**	**9.53**
Outpatients	834	23.5	**80**	**9.60**
Paediatric general	483	13.6	**49**	**10.14**
Surgery& Burn	370	10.4	**24**	**6.45**
Neonatal ICU	305	8.5	**31**	**10.16**
Urology & Nephrology	287	8.0	**26**	**9.06**
Medical ICU	213	6.0	**34**	**15.96**
Others	91	2.5	9	9.89
Obstetrics &Gyn	64	1.8	8	12.5

Others*: (Neurology, ENT, and Oncology), Swabs* (HVS, throat swab, stool, pus swabs)

3.2. Bacteria Isolates

Total of 363 bacteria isolates recovered from 347 samples. More than half 232 (64%) of the isolated pathogens were Gram-negative bacteria. Overall, *S. aureus* 131(36.1%), *Klebsiella spp* (18.4%) and *E. coli* 61 (16.8%) were the most frequently isolated bacteria (Figure 1).

Figure 1: Bacterial Isolates from positive culture growth



3.3 Antimicrobial Resistance patterns of bacteria isolates at CPL

Disc diffusion test was primarily used for susceptibility test. Enterobacteriaceae displayed high rates of resistance to multiple routinely prescribed antibiotics including cotrimoxazole (SXT) (85%) gentamycin (58%) and ciprofloxacin. (63%) More than half of *E.coli* isolates were resistant to ciprofloxacin and gentamycin (63%) and (58%) respectively, (35%) of isolated Enterobacteriaceae were ESBL producers 81/232. *S. aureus* was highly resistant to Penicillin (96%), erythromycin (82%) and cotrimoxazole (SXT) (82%). (Table 2).

Table 2: Antimicrobial Resistance pattern of bacteria isolates at CPL

	N	%Resistance												
Bacteria		SXT	CN	CIP	AMC	CAZ	CTX	AK	ME	FEP	E	DA	FOX	P
S. aureus	131	82	37	44	NA	NA	NA	NA	NA	NA	82	35	50	96
K. pneumoniae	67	74	58	52	47	49	45	33	23	20	NA	NA	NA	NA
E. coli	61	**85**	**58**	**63**	60	61	65	33	27	42	NA	NA	NA	NA
Pseudomonas spp	42	83	40	38	47	33	57	17	30	33	NA	NA	NA	NA
Acinetobacter spp	17	71	43	57	43	57	0	0	28	28	NA	NA	NA	NA
Proteus spp	20	87	67	80	60	60	60	13	13	40	NA	NA	NA	NA
Enterobacter spp	18	73	53	53	66	33	53	20	6.7	40	NA	NA	NA	NA
Providencia spp	4	100	33	33	33	0	67	0	33	33	NA	NA	NA	NA
Salmonella spp	3	67	33	0	0	0	33	0	33	0	NA	NA	NA	NA

Key: SXT- trimethoprim-sulfamethoxazole, CN- gentamicin, CIP- ciprofloxacin AMC- amoxicillin-clavulanic acid CAZ- ceftazidime, CTX- cefotaxime, AK- amikacin, ME- meropenem, FEP- Cefepime, E – erythromycin, DA- clindamycin, FOX- cefoxitin P- penicillin, NA- Not applicable.

3.4 Proportion of MRSA among *S.aureus* isolated at CPL

Out of 131 isolated *S.aureus* during the study period, 50.3% (66/131) were MRSA. The majority of isolated MRSA was recovered from blood samples 49(68.06%) followed by pus 9(33.3%) and the least was isolated from other samples 1(14.29%). 77.7% of *S.aureus* isolates from ICU units were MRSA, and 62.5% of *S.aureus* isolates from Surgery and Burn unit were MRSA, while only (44.4%) of the *S.aureus* isolates from Medical, and paediatric general wards were MRSA. MRSA proportion was significantly higher among *S.aureus* isolated from blood samples (68.06%) than other samples (p value= 0.000). (Table 3).

Table 3: Distribution of MRSA by wards and sample types

Ward	**N (131)**	**MRSA n (%)**	**P-value**
Out-Patient	41	18 (43.90)	0.119
ICU units	18	14 (77.78)	
Surg and Burn	8	5 (62.50)	
Pediatric General	18	8 (44.44)	
Medical General	36	16 (44.44)	
Others	10	5 (50)	
Sample type			**0.000**
Blood	72	49(68.06)	
Sputum	25	7 (28)	
Pus	27	9 (33.3)	
Others	7	1 (14.29)	

P-value is for comparison of MRSA proportion between one sample to another, and wards to another wards.

Other wards ***** - Neurology, Urology, OBGY, ENT, and Oncology, ICU units* Med and Neonatal ICU, swabs*-(HVS, throat swabs and pus swabs) other samples* Body fluids, Urine and Swabs.

3.5 Antimicrobial susceptibility profile of MRSA among *S.aureus* isolates

Antibiotic susceptibility test was performed on 131 *S.aureus* isolates using six selected antibiotics. Both MRSA and non-MRSA isolates were significantly more resistant to penicillin (96.2%), and trimethoprime (SXT) (82%) (P-value= 0.001). MRSA isolates were more resistant to routinely prescribed drugs including gentamycin 59.1% and ciprofloxacin 56.1% compared to non-MRSA isolates (15.4%) and (32.8%) respectively. Nevertheless majority (97%) of MRSA isolates were MDR compared with non-MRSA isolates (18.5%) (P-value= 0.001). The overall MDR among *S.aureus* isolates was (57%).(Table 4)

Table 4: Antimicrobial susceptibility profile of MRSA among *S.aureus* Isolates at MNH (n=131)

Antibiotics	% Resistance	MRSA (n %)	NON-MRSA (n %)	P-Value
Erythromycin	108(82.4)	58(87.6)	50(76.2)	0.040
Clindamycin	46(35.1)	31(47)	15(23.1)	0.002
Gentamycin	49(37.4)	39(59.1)	10(15.4)	0.005
Ciprofloxacin	58(44.3)	37(56.1)	21(32.8)	0.003
Trimethoprim/SXT	107(82)	59(89.4)	48(73.5)	0.001
Penicillin	126(96.2)	66(100)	60(92.3)	0.001
MDR	75(57.7)	63(97)	12(18.5)	0.001

*P value is for a comparison of resistance among MRSA with that among nonMRSA isolates.

3.6 Minimum inhibitory concentration values of clindamycin and ciprofloxacin among MRSA isolates at MNH

Minimum inhibitory concentration values were determined for 66 MRSA isolates using two antibiotic disks, clindamycin and ciprofloxacin. There was a difference in resistance rates for clindamycin using two different methods. The rate of resistance to clindamycin was significantly lower by (20%), 27.4% by the MIC method compared to the disk diffusion method at 47.05% (P-value= 0.040). There was a slight difference in resistance rates for ciprofloxacin between the two methods, 52.9% for MIC and 58.8% in the disk diffusion method. (Table 5).

MIC_{50} and MIC_{90} for ciprofloxacin and clindamycin were determined, whereby MIC_{50} for clindamycin against tested MRSA isolates were found to be 2ug/ml and MIC_{90} was found to be 32ug/ml (Cutoff value $\geq$ 4ug/ml). MIC_{50} for ciprofloxacin against MRSA isolates was found to be 4ug/ml and MIC_{90} was 32ug/ml (MIC Cutoff value $\geq$4ug/ml) (Table6).

Table 5: Antimicrobial Resistance Rate of MRSA isolates in MIC and Disk diffusion

Drugs	DD N (%)	MIC N (%)	P-Value
Clindamycin	31(46.97)	14(21.21)	0.0405
Ciprofloxacin	37(56.06)	27(40.91)	0.357

DD- Disk diffusion; MIC- Minimum inhibition concentration

Table 6: MIC_{50} and MIC_{90} among MRSA isolates

Drugs	MIC_{50}	MIC_{90}	Cutoff Value
Clindamycin	2ug/ml	32ug/ml	≥4ug/ml
Ciprofloxacin	4ug/ml	32ug/ml	≥4ug/ml

MIC_{50} = concentration required to inhibit 50% of isolates tested

MIC_{90} = concentration required to inhibit 90% of tested isolates.

Cutoff value = Reference value used to categorise organism as susceptible/ resistantant sated by CLSI standard

3.7 Proportion of ESBL producing Enterobacteriaceae among gram-negative isolated at CPL

All 232 Gram-negative isolates were screened for ESBL production, where by 81 (35.1%) were phenotypically confirmed as ESBL producing Enterobacteriaceae. Of the Gram negative isolates recovered *E.coli* 36 (59.0%) *and Klebsiella pneumoniae* 28 (41.8%), showed higher proportion of ESBL-PE. A higher proportion of ESBL producing Enterobacteriaceae was found significantly among isolates recovered from urine samples 30(47.6%) (P-value=0.022), followed by blood 21(37.5%), and pus 18(35.3%). Nonetheless, a higher proportion (42.6%), and (42.9%) of ESBL producing Enterobacteriaceae, were recovered from ICU Units,

Surgery and Burn unit respectively, and least proportion (25.4%), of ESBL- PE was found from Medical general wards (Table 7).

Table 7: Distribution of ESBL- PE among GNR by wards, sample type and isolates

Variable	N (232)	ESBL n (%)	P-value
Sample type			0.0229
Blood	56	21(37.5)	
Urine	63	30(47.6)	
Sputum	49	9(18.4)	
Pus	51	18(35.3)	
Others	13	3(23.1)	
Ward			0.427
ICU units	47	20(42.6)	
Medical general	63	16(25.4)	
Out Patients	39	14(35.9)	
Pediatric General	32	12(37.5)	
Others	30	10(33.3)	
Sugary & Burn	21	9(42.9)	
Isolates			0.116
K.pneumoniae	67	28(41.8)	
E.coli	61	36(59.0)	
Pseudomonas spp	42	0(0.0)	
Acinetobacter spp	17	0(0.0)	
Proteus Spp	20	11(55)	
Enterobacter spp	18	6(33.3)	
Providencia	4	0(0.00)	
Salmonella spp	3	0(0.00)	

P- value is for comparison of ESBL production between one sample type to another, wards to wards and between one isolate to another

Others***** - Neurology, Urology, ENT, OBGY and Oncology, ICU units*medical and neonatal ICU, Other samples*-(Body fluids, HVS, throat swabs and pus swabs)

3.8 Antimicrobial Resistance pattern of ESBL producing isolates among Gram-negative rods

Antimicrobial susceptibility test was performed for 232 Gram-negative isolates to determine their antimicrobial susceptibility pattern. A high rate of resistance to cotrimoxazole (SXT) was observed to both ESBL and non-ESBL producing isolates (78.0%) and (65.0%) respectively. Amikacin and meropenem were found to be highly susceptible antimicrobial agents against both ESBL-PE and non ESBL producing isolates with the resistance rate of (19.7%) and (17.6%) respectively. ESBL producing isolates showed high rates of resistance to ciprofloxacin 80.3% and gentamycin (72.7%) compared to non-ESBL producing isolates 23.8% and 25.8% respectively (p-value = 0.001). MDR was more common among ESBL producing isolates (82%) than non ESBL producing isolates (7%) (P-value= 0.001). Overall MDR was 44.4% among Gram negative isolates tested (Table 8).

Table 8: Antimicrobial resistance pattern of ESBL producing isolates among Gram-negative rods n=232

Antibiotics	Resistance n (%)	ESBL Producing n=81	Non-ESBL Producing n=151	P-value
Trimethoprim(SXT)	161(71.5)	63(78.0%)	98(65.0%)	0.065
Gentamycin	97(48.0)	58(72.0%)	39(25.8%)	0.001
Ciprofloxacin	101(52.1)	65(80.3%)	36(23.8%)	0.001
Amoxylin/Clav	106(50.5)	54(66.7%)	52(34.4%)	0.001
Ceftazidime	85(48.3)	69(86.0%)	16(10.5%)	0.001
Amikacin	39(19.7)	24(30.0%)	15(9.9%)	0.001
Cefotaxime	103(53.5)	68(84.0%)	35(23.1%)	0.001
Meropenem	35(17.9)	22(27.3%)	13(8.6%)	0.001
Cefepime	59(28.0)	32(39.4%)	27(18.0%)	0.002
MDR	78(44.4)	67(82%)	11(7.2%)	0.001

3.9 Minimum inhibitory concentration values of ciprofloxacin and gentamicin against ESBL producing isolates

Eighty-one, ESBL producing isolates were tested for MIC value by using ciprofloxacin and gentamycin. The present study found the least rate of resistance against ciprofloxacin (33%) among ESBL producing Isolates tested in MIC methods than the Disk diffusion method (89%). (P-value= 0.001) (Table 9).

Nonetheless, our study determines MIC_{50} and MIC_{90} for ciprofloxacin and gentamycin against ESBL-PE. Half of ESBL producing isolates tested in this study were inhibited by 2ug/ml (MIC_{50}) of ciprofloxacin, and MIC_{90} of ciprofloxacin against tested ESBL PE isolates was 32ug/ml (cutoff value $\geq$ 4ug/ml). However, gentamycin was the least active antimicrobial agent with the MIC50 of 32ug/ml and MIC_{90} was 64ug/ml (cutoff value $\geq$ 16ug/ml) (Table10).

Table 9: Resistance rate of ESBL isolates in MIC and Disk diffusion against gentamycin and ciprofloxacin

Drugs	DD n (%)	MIC n (%)	P-Value
Gentamycin n (%)	59(72.8)	53(65.4)	0.001
Ciprofloxacin n (%)	65(80.2)	27(33)	0.001

DD- Disk diffusion; MIC- Minimum inhibition concentration

Table 10: MIC_{50} and MIC_{90} values for ESBL producing Isolates against gentamycin and ciprofloxacin

Drugs	MIC_{50}	MIC_{90}	Cutoff Value
Gentamycin	32ug/ml	64ug/ml	>16ugl/ml
Ciprofloxacin	2ug/ml	32ug/ml	>4ugl/ml

MIC_{50} = concentration required to inhibit 50% of isolates tested

MIC_{90} = concentration required to inhibit 90% of tested isolates

Cutoff value = Reference value used to categorise organism as susceptible/ resistantant

CHAPTER FOUR

4.0 DISCUSSION

4.1 Positive Yield

In this present study, a total of 3549 samples received and processed at CPL. Only 347(10%) yield clinical isolates. Pus and Sputum contribute about one fifth of positive culture each (20.56% and 26.17%) respectively. This can be justified by nature of these samples they are collected from non sterile site and most of them carry more than one isolates. Also this study showed urine and fluid samples yielded less than ten percent with (5.73%) and (3.13%) respectively .The low yield of urine and body fluids samples are also justified by nature of these samples, they are collected from sterile sites and most of the time they yield only one isolate (55,56). The prevalence of blood culture positive in this study was 10.05%, this concurs with a recent study done by Manyahi *et al.* in the same setting which reports the prevalence of 11.4%. However, a slight difference was observed with other previous studies done in northwestern Tanzania by J. Seni et al. which report the prevalence of 14.2% and Moyo et al. which report the prevalence of 13.4% (1,12,51). The observed difference is because Moyo *et al* included coagulase-negative *Staphylococcus* as the true pathogens in the analysis, which were not considered in our study. With regard to urinary tract infection, in this analysis, 5.73% culture positive, this finding is lower compared to previous study done at Bugando Medical center which report prevalence of 27.3% ,the observed difference could be explained by difference in study design used, the previous study was retrospective analysis of microbiological cultures while our study was descriptive cross sectional study (2) .

4.2 Common bacteria isolated

In this present study the most frequently isolated organisms were, *S. aureus* (36.1%), *Klebsiella pneumonia* 67(18.4%) and *E. coli* 61 (16.8%). These findings concur with other previous studies done in Developing countries that found the majority of isolates were *S.aureus, K.pneumoniae and E.coli*(7,9,25).

As in previous studies , *E. coli* were the most frequently isolates from urine samples 42 (66.6%) while *Pseudomonas auroginosa* and *K.pneumoniae* were commonly recovered from Pus and Sputum samples (39.7%,67.1%) respectively. This finding is in agreement with the report on microbiological cultures at tertiary Hospital in Tanzania and another study done on ESBL Production and MDR among enterobactereacea isolated in Ethiopia (2,10).

In addition *S. aureus* was the predominant species isolated from blood samples (56.5%). These findings are similar to a study conducted at BMC Tanzania by Moremi *et.al.* which found (61%) of *S.aureus* recovered from blood specimen (2). However, our findings differ from the study done at a tertiary hospital in Dar es Salam by Manyahi *et al* which reported majority (74%) of isolates from blood was gram-negative bacteria (1). This may be caused by the shifting trend of bloodstream infection from gram-negative to gram-positive bacteria(51).

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4.3 Antimicrobial Susceptibility profile of isolates recovered at MNH

Antimicrobial susceptibility test was performed for 363 bacterial isolates to determine susceptibility patterns. Among the isolates tested in this study, S. *aureus* was commonly resistant to 96%, 82% and 82% to penicillin, erythromycin and cotrimoxazole (SXT) respectively. Overall (57%) of *S. aureus* were MDR. This finding is higher compared with a previous study done in Bugando medical centre by Moremi *et al* which report an MDR prevalence of (35.9%) (2). The observed difference alerts the increase of MDR pathogen in our setting, the overuse of antibiotics and self-prescription practices contribute to this situation(2,16).

In addition to that, 50.3 % of *S. aureus* were MRSA and the majority (97%) were MDR compared to non-MRSA (18.5%). This finding concurs with a previous study done by Joachim *et .al.* which report the prevalence of (95%) MDR among MRSA isolates(41).

Nonetheless, this study found more than half of *E. coli* isolates were resistant to ciprofloxacin (63%) and gentamycin (58%), and 53.6% of Enterobacteriaceae were ESBL producers. This study demonstrates high rates of resistance of ESBL producing isolates to non-beta lactam

antibiotics including gentamycin (72.7%), cotrimoxazole SXT (78.8%) and ciprofloxacin (80.3%) compared to non-ESBL producers (25.7%,65.8%, and 23.7%) respectively. As reported in the previous study done at a tertiary hospital in Tanzania by Moremi N. *et al* reported a 66.7% resistance rate to non-beta lactam including ciprofloxacin and 66% to 100% resistant to ampicillin and cotrimoxazole respectively (2,12). The rate of resistance to ciprofloxacin and cotrimoxazole is greatly enhanced by self-prescription practices in the community(2). Nonetheless, this observation may be explained by the fact that ESBL is plasmid-mediated enzymes that are transferable between one bacterium to another and such transferable plasmids also code for resistance determinants to antimicrobial agents other than beta-lactams (16). The majority (82%) of ESBL producers were MDR compared to (7%) non ESBL producing isolates. These findings agreed with a previous study done by Moyo *et al* that reported 82.4% of ESBL producing isolates were MDR (16).**The finding of this present study call for surveillance programs to control transmission rate in order to combat MDR pathogen in our setting.**

4.4 Proportion of ESBL producing *Enterobacteriaceae* among gram-negative rods

In this study, the overall prevalence of ESBL producing isolates was (53%) which is comparable to finding from the study done by Manyahi *et.al*. at a tertiary hospital in Dar es salam showed (45.2%) however it is slightly higher compared with the study done by Moremi *et al.* at a tertiary hospital in Mwanza that showed (35.9%) (2,16). Nonetheless, ESBL producing Enterobacteriaceae was found significantly among isolates recovered from urine samples 30(37.4%). A similar finding was observed by previous studies done in India by Basirekha *et al* which reported 48% of ESBL producing isolates were recovered from urine samples. Another study done in Ethiopia by Sirak Bisset *et al* reported a prevalence of 49.2% among ESBL producing GNR were recovered from a urine sample. Nonetheless, Moyo *et al*. in Tanzania reported that 54.4% of ESBL isolates were uropathogens (6,16,35).

However our finding is slightly higher compared to a study done in Mwanza tertiary by *Stephen E* Mshana, *et al* showed 29.2% of ESBL isolates recovered from urine samples. This

difference may be contributed due to the technique used for detection, whereby in the study done by Mshana *et al* disc approximation method was used to confirm ESBL production. The present study also reveals a high proportion (24.7%)of ESBL producing isolates among samples received from ICU units, the high proportion of ESBL observed in ICU could be attributed to longer hospital staying and invasive procedures that increase the risk of infections by multidrug-resistant pathogens to admitted patients of ICU units .**This finding alerts the microbiology laboratory on proper screening, detection and reporting of these pathogens which will enable early detection and control its transmission.**

4.5 Minimum inhibitory concentration values of ciprofloxacin and gentamicin against ESBL isolates

The present study reveals 80% of tested ESBL producing Enterobacteriaceae isolates were resistant to ciprofloxacin in the disk diffusion method in contrary to MIC method whereby only 33% of tested ESBL producing isolates were found resistant to ciprofloxacin. **This justified reliability and effectiveness of the MIC method in identifying resistance patterns for multidrug-resistant pathogen**(50). Ciprofloxacin found effective against half of tested ESBL producing isolates in which MIC_{50} was 2ug/ml. However, these findings were two times higher compared with the previous study done in India by Dupont Gomi *et al* which reported lower MIC_{50} values of 0.125u/ml to ciprofloxacin against ESBL PE (50). The possible explanation of the variation of these findings may be due to the difference in MIC method used, the study done in India used the agar dilution method, while this study used the broth dilution method (50). Nonetheless our study determine the MIC_{50} and MIC_{90} for MDR isolates only, but the previous study involve non MDR enteropathogen isolates.

4.6 Proportion of MRSA among isolated *S.aureus*

Our study found MRSA prevalence of 50.3% among *S. aureus* isolated from different clinical samples. These findings concur with the findings from previous studies done at regional hospitals by Agricola Joachim *et al,* who reported MRSA prevalence of 50% among patients

diagnosed with acute illness, as well as another study done at a tertiary hospital in Dar es salam by Manyahi *et al* that reported MRSA prevalence of 40% (1,41).

In this study, we also found majority 68.1% of MRSA were isolated from blood samples. Furthermore, a higher proportion of MRSA isolate was recovered from medical ICU and neonatal ICU samples. These findings are similar to the previous study done by Moremi *et al* that reported the majority of 61% of MRSA isolates were recovered from pediatric ICU and neonatal ICU, also MRSA isolates were most frequently recovered from blood samples.(2)

4.7 Antimicrobial Resistance of MRSA among *S.aureus* Isolates at MNH

Our study found a high rate of resistance to erythromycin (72.5%) and penicillin (96.2%) among both MRSA and non-MRSA. Similar findings were observed from a previous study done by Alfred *et al* which report a resistance rate of (76.2%) and (100%) erythromycin and penicillin respectively (41). The contribution to these findings can be because these antibiotics are easy accessed and mostly prescribed in primary health care for the treatment of various infections and hence overuse of these drugs results in the observed resistance (38). Nevertheless least rate of resistance against clindamycin showed to both MRSA and non-MRSA isolates with a resistance rate of only 22.1%.

4.8 Minimum inhibitory concentration values of clindamycin and ciprofloxacin among MRSA isolates at MNH

In this present study, the susceptibility pattern of MRSA isolates, by MIC methods using clindamycin and ciprofloxacin showed a low resistance rate (27.4%) of MRSA against clindamycin by MIC method than Disk diffusion methods (47.05%). These findings are similar to the previous study done in Nepal by Raghabendra Adhikari *et al*. which showed the resistance rate of 35.9% MRSA isolates against clindamycin (42). The finding signifies that the MIC method is the gold standard method for the determination of resistance patterns among Multidrug resistance isolates including MRSA over the disk diffusion method and for that it can be used as one among the tool to for increasing treatment option on available drugs in our setting (58).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study revealed high prevalence of MRSA and ESBL producing *Enterobacteriaceae* among isolates at CPL and majority of these pathogen were recovered from blood and urine. In the present study Multidrug resistant were higher among S. *aureus* isolates than Gram negative isolates tested, and majority of these bacteria were found to be resistant to routinely prescribed antibiotics including gentamycin and ciprofloxacin. Nonetheless the present study found difference in resistance rate between two methods used, where by the least rate of resistance against available antibiotic were observed in MIC test than disk diffusion test.

5.2 Recommendations

The observed high proportion of MDR pathogen including ESBL PE and MRSA in our setting warrant the need for a clinical microbiology laboratory to enforce the policy for regular screening and reporting for MRSA and ESBL PE. If enforced, it will help in the early detection that helps in generating intervention programs to control transmission.

The findings support the need for surveillance of infections to establish the source and transmission pathway.

Nevertheless the study found the least rate of resistance to routinely used antibiotics in the MIC method while high resistance in the DD method this emphasize the need to perform MIC test to those antibiotics that can be adjusted the dose (increase /decrease) at certain level without causing toxic effect to patients. MIC test will provide the use of some available drugs,that formally found resisted by disc diffusion test, and reduce MDR rate by confirming the true resistant in our setting.

REFERENCES

1. Manyahi J, Kibwana U, Mgimba E, Majigo M. Multi-drug resistant bacteria predict mortality in bloodstream infection in a tertiary setting in Tanzania. PLoS One. 2020;15(3):1–11.

2. Moremi N, Claus H, Mshana SE. Antimicrobial resistance pattern : a report of microbiological cultures at a tertiary hospital in Tanzania. BMC Infect Dis. 2016;7(January 2017):10–7.

3. Nelson E, Kayega J, Seni J, Mushi MF, Kidenya BR, Hokororo A, et al. Evaluation of existence and transmission of extended spectrum beta lactamase producing bacteria from post-delivery women to neonates at Bugando Medical Center , Mwanza-Tanzania. 2014;1–6.

4. Thenmozhi S, Moorthy K, Sureshkumar BT, Suresh M. Antibiotic Resistance Mechanism of ESBL Producing Enterobacteriaceae in Clinical Field : A Review. 2014;2(3):207–26.

5. Neill JO'. Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations The Review on Antimicrobial Resistance Chaired. 2014;(December).

6. Ababa A. Multidrug-resistant profile and prevalence of extended spectrum β -lactamase and carbapenemase production in fermentative Gram-negative bacilli recovered from patients and specimens referred to National Reference. 2019;1–13.

7. Khawcharoenporn T, Vasoo S, Singh K. Urinary Tract Infections due to Multidrug-Resistant Enterobacteriaceae: Prevalence and Risk Factors in a Chicago Emergency Department. Emerg Med Int. 2013;2013:1–7.

8. Gidabayda J, Philemon R, Abdallah M, Saajan A, Temu T, Kunjumu I, et al. Prevalence, Aetiology, and Antimicrobial Susceptibility Patterns of Urinary Tract

Infection Amongst Children Admitted at Kilimanjaro Christian Medical Centre, Moshi, Tanzania. East African Heal Res J. 2017;1(1):53–61.

9. Ndihokubwayo JB, Yahaya AA, Desta AT, Ki-zerbo G. Antimicrobial resistance in the African Region : Issues , challenges and actions proposed. Microbiol J Infect Dis. 2016;(16).

10. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. production and multi-drug resistance among Enterobacteriaceae isolated in Addis. Microbiol J Infect Dis. 2019;4:1–12.

11. Moyo SJ, Aboud S, Kasubi M, Maselle SY. Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at muhimbili national hospital in Tanzania. Tanzan J Health Res. 2010;12(4):236–40.

12. Seni J, Mwakyoma AA, Mashuda F, Marando R, Ahmed M, Devinney R, et al. Deciphering risk factors for blood stream infections, bacteria species and antimicrobial resistance profiles among children under five years of age in North-Western Tanzania: A multicentre study in a cascade of referral health care system. BMC Pediatr. 2019;19(1):1–11.

13. Manyahi J, Matee MI, Majigo M, Moyo S, Mshana SE, Lyamuya EF. Predominance of multi-drug resistant bacterial pathogens causing surgical site infections in Muhimbili national hospital, Tanzania. BMC Res Notes. 2014;7(1):1–7.

14. Seni J. Antimicrobial resistance. Microbiol J Infect Dis. 2014;8:16, 23.

15. Frost I, Van Boeckel TP, Pires J, Craig J, Laxminarayan R. Global geographic trends in antimicrobial resistance: the role of international travel. J Travel Med. 2019 Dec 23;26(8):1–13.

16. Moyo SJ, Aboud S, Kasubi M, Lyamuya EF, Maselle SY. Antimicrobial resistance among producers and non-producers of extended spectrum beta- lactamases in urinary

isolates at a tertiary Hospital in Tanzania. 2010;1–7:2–6.

17. Lee AS, Lencastre H De, Garau J, Kluytmans J, Malhotra-kumar S, Peschel A, et al. Methicillin-resistant Staphylococcus aureus. Nat Publ Gr. 2018;4(May):1–23.

18. Galani I, Kontopidou F, Souli M, Rekatsina PD, Koratzanis E, Deliolanis J, et al. Colistin susceptibility testing by Etest and disk diffusion methods. Int J Antimicrob Agents. 2018;31(5):434–9.

19. Testing S. M100 Performance Standards for Antimicrobial. 2020. 36,43.

20. Andrews JM. JAC Determination of minimum inhibitory concentrations. Diagnostic Microbiol Infect Dis Microbiol. 2011;7:5–16.

21. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity : A review $. J Pharm Anal. 2016;6(2):71–9.

22. Karami A, Mazloom Zadeh S, Rastin A, Karami A, Shiri P. The consistency of antibiotic resistance' results in two methods of disk diffusion and MIC in isolated organisms from patients with urinary tract infections. J Med Life. 2015;8(Spec Iss 4):282–6.

23. Dolinsky AL. M100 Performance Standards for Antimicrobial Susceptibility Testing. Vol. 8, Journal of Services Marketing. 2017. 27–39 p.

24. Al-hasan MN, Gould AP, Drennan C, Hill O, Ann J, Kohn J, et al. Journal of Global Antimicrobial Resistance Empirical fl uoroquinolones versus broad-spectrum beta-lactams for Gram-negative bloodstream infections in the absence of antimicrobial resistance risk factors. Integr Med Res. 2020;22:87–93.

25. Laboratory Methodologies for Bacterial Antimicrobial Susceptibility Testing. Microbiol J Infect Dis. 2012;7:1–11.

26. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. 2016;(December 2016):1–23.

27. Khamis khamis, Adelina G., Benson L. Central Pathology Laboratory Procedure for Antimicrobial Susceptibility testing and IQC. SOP for AST. Daresalam; 2020.

28. WHO. The Current Evidence for the Burden of Group A Streptococcal Diseases. WHO/FCH/CAH/0507. 2015;

29. WHO. Organization World Health. Global Strategy for Containment of Antimicrobial Resistance. World Heal Organ. 2018;12(1):105.

30. Bax R, Bywater R, Cornaglia G, Goossens H, Hunter P, Isham V, et al. Surveillance of antimicrobial resistance - What, how and whither? Clin Microbiol Infect. 2016;7(6):316–25.

31. Ndir A, Diop A, Ka R, Faye PM, Dia-Badiane NM, Ndoye B, et al. Infections caused by extended-spectrum beta-lactamases producing Enterobacteriaceae: Clinical and economic impact in patients hospitalized in 2 teaching hospitals in Dakar, Senegal. Antimicrob Resist Infect Control. 2016;5(1):1–8.

32. Hu YJ, Ogyu A, Cowling BJ, Fukudaa K, Panga H. Available evidence of antibiotic resistance from extended-spectrum ß-lactamase-producing enterobacteriaceae in paediatric patients in 20 countries: A systematic review and meta-analysis. Bull World Health Organ. 2019;97(7):486-501B.

33. Manyahi JP, Kibwana U, Sensa V, Yongolo S, Lyamuya E. Predictors of Surgical site infections among patients undergoing open urological surgery at a Tertiary hospital, Tanzania: A cross sectional study. BMC Infect Dis. 2019;7(2):101–3.

34. Mahony M, McMullan B, Brown J, Kennedy SE. Multidrug-resistant organisms in urinary tract infections in children. Pediatr Nephrol. 2020;35(9):1563–73.

35. Sasirekha B. Original article : Prevalence of ESBL , AMPC B - Lactamase and MRSA

among uropathogens and its antibiogram. Microbiol J Infect Dis. 2013;81–8.

36. Blomberg B, Manji KP, Urassa WK, Tamim BS, Mwakagile DS, Jureen R, et al. Antimicrobial resistance predicts death in Tanzanian children with bloodstream infections: a prospective cohort study. BMC Infect Dis. 2017;7(1):1–14.

37. Moremi N, Claus H, Vogel U, Mshana SE. The role of patients and healthcare workers Staphylococcus aureus nasal colonization in occurrence of surgical site infection among patients admitted in two centers in Tanzania. Antimicrob Resist Infect Control. 2019;8(1):1–7.

38. Wangai FK, Masika MM, Maritim MC, Seaton RA. Methicillin-resistant Staphylococcus aureus (MRSA) in East Africa : red alert or red herring ? 2019;1–10.

39. Mohamed AA, Aboud S, Sciences A. Methicillin-resistant staphylococcus aureus (MRSA) colonization among Intensive Care Unit (ICU) patients and health care workers at Muhimbili national hospital, Dar Es Salaam, Tanz... 2015;(October).

40. Rahman MM, Amin KB, Rahman SMM, Khair A, Rahman M, Hossain A, et al. Investigation of methicillin-resistant Staphylococcus aureus among clinical isolates from humans and animals by culture methods and multiplex PCR 11 Medical and Health Sciences 1103 Clinical Sciences 11 Medical and Health Sciences 1108 Medical Microbiolog. BMC Vet Res. 2018;14(1):1–6.

41. Joachim A, Moyo SJ, Nkinda L, Majigo M, Mmbaga E, Mbembati N. Prevalence of methicillin‐resistant Staphylococcus aureus carriage on admission among patients attending regional hospitals in Dar es Salaam , Tanzania. BMC Res Notes. 2017;5:1–7.

42. Adhikari R, Pant ND, Neupane S, Neupane M, Bhattarai R, Bhatta S, et al. Detection of Methicillin Resistant Staphylococcus aureus and Determination of Minimum Inhibitory Concentration of Vancomycin for Staphylococcus aureus Isolated from Pus/Wound Swab Samples of the Patients Attending a Tertiary Care Hospital in Kathmandu, Ne. Can J Infect Dis Med Microbiol. 2017;2017.

43. Hope D, Ampaire L, Oyet C, Muwanguzi E, Twizerimana H, Apecu RO. Antimicrobial resistance in pathogenic aerobic bacteria causing surgical site infections in Mbarara regional referral hospital, Southwestern Uganda. Sci Rep. 2019;9(1):1–10.

44. Kumburu HH, Sonda T, Leekitcharoenphon P, Van Zwetselaar M, Lukjancenko O, Alifrangis M, et al. Hospital Epidemiology of Methicillin-Resistant Staphylococcus aureus in a Tertiary Care Hospital in Moshi, Tanzania, as Determined by Whole Genome Sequencing. Biomed Res Int. 2018;2018(2018):12–6.

45. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.

46. Beyene D, Bitew A, Fantew S, Mihret A, Evans M. Multidrug-resistant profile and prevalence of extended spectrum β-lactamase and carbapenemase production in fermentative Gram-negative bacilli recovered from patients and specimens referred to National Reference Laboratory, Addis Ababa, Ethiopia. PLoS One. 2019;14(9):1–13.

47. Lai CC, Chen CC, Lu YC, Lin TP, Chuang YC, Tang HJ. Appropriate composites of cefoperazone– sulbactam against multidrug-resistant organisms. Infect Drug Resist. 2018;11:1441–5.

48. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America Antimicrobial Resistant Treatment Guidance: Gram-Negative Bacterial Infections. Clin Infect Dis. 2020;6(2020):1–10.

49. Baddour MM. Methicillin-Resistant Staphylococcus Aureus (MRSA). BMC Res Notes. 2010;6(2011):7–12.

50. Dupont HL, Gomi H, Jiang Z, Adachi JA, Ashley D, Lowe B, et al. In Vitro Antimicrobial Susceptibility Testing of Bacterial Enteropathogens Causing Traveler's Diarrhea in Four Geographic Regions In Vitro Antimicrobial Susceptibility Testing of

Bacterial Enteropathogens Causing Traveler's Diarrhea in Four Geographic. Am Soc Microbiol. 2011;45(1):2012–6.

51. Moyo S, Aboud S, Kasubi M, Maselle SY. Bacteria isolated from bloodstream infections at a tertiary hospital in Dar es Salaam, Tanzania - antimicrobial resistance of isolates. South African Med J. 2010;100(12):835–8.

52. Schumacher A, Vranken T, Malhotra A, Arts JJC, Habibovic P. In vitro antimicrobial susceptibility testing methods : agar dilution to 3D tissue-engineered models. 2018;187–208.

53. Zucchi PC. Invitro Antimicrobial Susceptibility Testing. Am Soc Microbiol. 2019;8:6–10.

54. Sonda T, Kumburu H, Zwetselaar M Van, Alifrangis M, Lund O, Kibiki G, et al. Meta-analysis of proportion estimates of producing Enterobacteriaceae in East Africa hospitals. Antimicrob Resist Infect Control. 2016;

55. Cymrot C. Textbook of Diagnostic Microbiology. Textbook of Diagnostic Microbiology. 2018. p. 106–12.

56. Sutherland T, Mpirimbanyi C, Nziyomaze E, Niyomugabo JP, Niyonsenga Z, Muvunyi CM, et al. Widespread antimicrobial resistance among bacterial infections in a Rwandan referral hospital. PLoS One. 2019;14(8):1–15.

57. Shrestha LB, Baral R, Poudel P, Khanal B. Clinical , etiological and antimicrobial susceptibility profile of pediatric urinary tract infections in a tertiary care hospital of Nepal. 2019;1–8.

58. Diederen B, Kampinga G, Le T, Overdevest I, Stals F, Voorn P, et al. disc diffusion. 2018;(July):2380–7.