CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Contaminaed surfaces may play an important role in transmission of healthcare associated pathogens and act as reservoirs for bacterial pathogens (Aly *et al.,* 2008). Vancomycin-resistant enterococci and *Staphylococcus aureus* were frequently acquired on hands after contact with contaminated surfaces in patients' rooms. Similarly Boyce *et al*., (2002) demonstrated that nurses frequently acquired methicillin-resistant *S. aureus* (MRSA) on gloves after touching surfaces near colonized patients. In a medical intensive care unit, Hayden *et al*.,(2006) found that enforcing routine environmental cleaning measures was associated with decreased VRE contamination on surfaces and on the hands of healthcare cross-transmission (Hayden *et al*., 2006).

The risk of nosocomial infection depends on the ability of the pathogens to remain viable on a surface, the rate at which contaminated surfaces are touched by patients and health care workers, the context in which the patient is exposed, and the levels of contamination that result in transmission to patients (Perry *et al*., 2012). Numerous clinical studies have proven that soft surfaces such as privacy curtains are contaminated by pathogenic bacteria including multi drug resistant organisms (Neely *et al.*, 2000). All of these pathogens have been demonstrated to persist in the environment for hours to days (and in some cases months), to frequently contaminate the surface environment and medical equipments in the rooms of colonized or infected patients, to transiently

colonize the hands of healthcare personnel (HCP), to be associated with person-to-person transmission via the hands of HCP, and to cause outbreaks in which environmental transmission was deemed to play a role (Otter, 2011). Furthermore, hospitalization in a room in which the previous patient had been colonized or infected with MRSA, VRE, *Clostridium difficile*, multidrug-resistant *Acinetobacter* sp, or multidrug-resistant *Pseudomonas* has been shown to be a risk factor for colonization or infection with the same pathogen for the next patient admitted to the room (Floyd *et al.*, 2014).

Recent evidence sheds light on sub-optimal current practices for instance, in regards to laundering 37% of hospital facilities launder privacy curtains only when they are visibly soiled, this does not meet minimum requirments as should be launder after dialy use (Bearman *et al*., 2014).

Exposure to pathogens on contaminated curtains can occur through direct contact or indirectly through air borne particle spread (Gaspard *et al.,* 2009).

The survival of pathogens on divider curtains is poorly understood and not well documented. However, it is believed that MRSA can survive up to nine days on curtains in certain conditions (Huang *et al* ., 2006).

Infection control procedures play an important part in all clinical settings to prevent and reduce the rate of cross infection, scrupulous hand washing by health care staff before and after contact with patients and before any procedure is reported the single most important infection control measure (Corcoran *et al*., 2000).

However, there are various items that are touched after hand washing and prior to patient contact (e.g. clinical surfaces and/or cubic curtains) that could be contaminated by micro organisms. Therefore, the potential for cross infection increases with frequent contact with cubic curtains (Brit, 2000).

Studies have shown that pathogens such as MRSA, VRE, and gram-negative bacilli are transmitted from privacy curtains that separate patient care areas in hospitals to healthcare-professionals (HCPs) and patients (Boyce *et al.*, 2002).

Furthermore, HCPs touch privacy curtains before, during, and after performing patient care. Given that studies have shown the transfer of bacteria from curtains to HCPs' gloves, contaminated curtains are potential vehicles for the transmission of infection (Trillis *et al.*, 2008).

Privacy curtains are infrequently changed and difficult to clean. In many institutions, curtains may hang for weeks and are often changed only when visibly soiled. Privacy curtains are rapidly contaminated, a previous study found that 92.3% of curtains had evidence of contamination within 1 week (Ohi *et al.*, 2008).

1.2. Rationale

Hospitals curtains that surround patients' beds to give privacy could provide a source of transmission of health care associated pathogens for several reasons; They are commonly touched by patients and healthcare workers, in many institutions they are cleaned or changed infrequently and finally health care

workers may be less likely to disinfect their hands after contact with inanimate objects than after direct contact with patients (Bhalla *et al.*, 2004).

1.3. Objectives

1.3.1. General objective

To determine antibiogram of bacteria isolated from hospital curtains.

1.3.2. Specific objectives

A. To re-identify bacterial isolates recovered from hospital curtains.

B. To perform susceptibility test against reidentified isolates against selected antibiotics.

CHAPTER TWO

LITREATURE REVIEW

 Each cubicle curtain was 249 cm length and 245 cm width with a 12.5 cm distance from the ground. The curtains were made of 60% polyester and 40% cotton, drawn around an overhead track completely enclosing the treatment cubicle for patient privacy pre and post treatment (Ria *et al.,* 2010).

2.1. Nosocomial Infection

The term nosocomial comes from the Greek word *nosokomeian*, nosos meaning disease and *komeian* meaning hospital. Nosocomial infection is thus any infection causing illness that was not present, or in its incubation period, during the time of admission and includes those infections, which occur after 48 hours of admission to the hospital (Joyce *et al*., 2000). These infections are a significant hazard in health care facilities, exacting a tremendous toll and causing increased morbidity, mortality and increased length of hospital stay and health care costs. Hospitals bring together uniquely vulnerable hosts in special units as in ICUs, which bacteriologically are very hostile environments, containing a wide selection of pathogenic, antibiotic resistant organisms with which the patient becomes colonized (David *et al*., 2001). The National Nosocomial Infection Surveillance System (NNIS) reports a rate of 14.1 nosocomial infections per 1000 patients (Lodha *et al*., 2001).

2.2. Antibiotics

The antibiotics are substances produced by living organisms. They inhibit the metabolism and/or growth of other microorganism. Antibiotics may be produced naturally or by synthesis (Maarteens *et al*., 2011).

2.2.1. Mode of actions and mechanisms of bacterial resistance

Antibiotic activity is due to the inhibition of biochemical pathways that are involved in the biosynthesis of essential components of the bacterial cell. The three main bacterial targets of antibiotic agents are cell wall, protein, and nucleic acid biosynthesis. Various mechanisms neutralizing the action of antibiotic agents have developed in bacteria. The most wide spread antibiotic resistance mechanisms are enzymatic drug inactivation, modification or replacement of the drug target, active drug target, active drug efflux, and reduced drug uptake (Peterson and Hayword, 2002).

Bacterial resistance was present before antibiotics were used, this intrinsic innate ability of bacterial species to resist the activity of a particular antibiotic agent is inherent structural or functional characteristic. Acquired bacterial antibiotic resistance is a result of a genetic change. Which occurs in the presence or absence of antibiotic (Guardabassi and Courvalin, 2006).

This genetic change can be the result of mutation horizontal exchange of genetic material via transformation, transduction or conjugation. These genetic events occur in the presence or absence of antimicrobial, however antimicrobial therapy exerts a selective effect and subsequent competitive

effect which, when followed by a bacterial genetic transfer, contribute antimicrobial resistance (Fluruya and lowy, 2006).

2.2.2. properties of antibiotics

2.2.2.1. Potency

This is the amount of antibacterial active agent in a test substance, determined by mean of a bioassay, usually expressed in microgram per milligram (µg/mg) of the test substance (Levinson, 2010).

2.2.2.2. Concentration

This is the amount of antibacterial agent in a defined volume of liquid, preferably expressed as mg/liter rather than (mg/ml) or in a defined mass of solid, usually expressed as mg/g or mg/kg (Levinson, 2010).

2.2.2.3. Pharmacokinetics and pharmacodynamics

Pharmacokinetics is the study of drug concentrations over time, in different body compartments, after a given dose of an antibiotic. Pharmacodynamics is the study of the relationship between pharmacokinetic parameters and the magnitude and time course of the response of the pathogen (EUCAST, 2000).

Antibiotic-resistant bacteria are implicated in an increasing amount of hospitalized patient infections worldwide. Among patients diagnosed with an infection, antibiotic resistance is associated with an increased length of hospital stay, health care costs, and patient morbidity, and mortality. Improved hand hygiene, environmental cleaning, and isolation of patients carrying pathogenic

bacteria are the main methods for tackling the problem. Despite clear evidence that hygiene improves surgical outcomes, there remains considerable controversy over whether or not contaminated environmental surfaces contribute to transmission of health care associated pathogens (Boyce *et al.*, 2002).

2.3. Previous Studies

Contamination of curtains may be more significant during outbreaks. For example, a study has shown a high level of contamination in curtains during an outbreak of carbapenemase-producing *Acinetobacter baumanii* in the United Kingdom (Das *et al.*, 2002).

A study confirmed that significant number of hospital curtains are contaminated with MRSA and that while current hospital protocol is to change and launder curtains quarterly, regular provision of clean hospital curtains is however, logistically difficult (Dancer, 2007).

In India, 220 isolates were obtained from hospital curtains, out of which 205 (93.2%) were bacteria and 15 (6.8%) were fungi. Among the bacteria, 158 (71.8%) were Gram negative bacilli and 47 (21.4%) were Gram positive cocci. *Klebsiella* species (16.4%), *Pseudomona*s species (13.6%), *Escherichia coli* (11.8%), *Enterobacter* species (11.4%), *Acinetobacte*r species (10%), *Citrobacter* species (5.9%) and other non fermenters (2.7%) were the predominant Gram negative pathogens. Among the Gram positive organisms, the major pathogens were *S. aureus* (12.3%), Coagulase negative *Staphylococci*

(5.5%), *Enterococcus* species (2.7%) and *Streptococcus* species (0.9%). The present study showed an alarming increase in the prevalence of multidrug resistant strains, ESBL producers and MRSA strains. Least sensitivity of all isolates was seen for Ampicillin, Gentamicin, and Cephalosporins. ESBL production was seen among 27 (81.8%) *Klebsiella* species, 19 (73.1%) *E. coli* and 15 (60%) *Enterobacter* sp. However, all the isolates were susceptible to β-lactam-βlactamase inhibitor combinations. All the 27 isolates of *S. aureus* were sensitive to Vancomycin and Teicoplanin. Only 5 (1.85%) isolates were sensitive to Penicillin. Out of 27 isolates, 8 isolates (29.6%) were Methicillin sensitive and 19 isolates (70.4%) were Methicillin resistant strains (Saritha *et al.*, 2010).

A study done in southern Nigeria, total of 504 samples from different curtains were sampled between the months of May and November 2009. *Proteus* sp (70.24%), *Pseudomonas* sp (59.13%), *Bacillus* sp (58.33%), *E. coli* (58.33%), *Campylobacter* sp (45.63%), *Klebsiella* sp (35.12%), *Bacillus* sp (86.51%), *Pseudomonas* sp (71.23%), *Aeromonas* sp (52.58%), *Salmonella* sp (47.02%). All isolates were resistant to Gentamicin, Chloramphenicol and Amikacin, while low resistance values were recorded in Erythromycin (25%) and Nalidixic acid (37.50%) (Ikpeme *et al.,* 2009).

In Aga Khan University Hospital, Nairobi, Kenya. A total of 90 specimens were obtained from the hospital curtains. There were 68 bacteria isolates comprising *Pseudomonas* 30 (44%), *Staphylococcus* 18 (27%), *Proteus* 12 (18%), and 8 (12%) *Klebsiella* sp. The antibiotic discs used were Ofloxacin ,

Gentamicin, Amoxycilin, Cotrimazole, Erythromycin, Chloramphenicol, Cloxacilin, Nalidixic acid, Tetracycline, Augmentin and Ceftazidine. Nearly all the organisms were most sensitive to Ofloxacin followed by Gentamicin. *S. aureus* sensitivity was not tested against Ofloxacin in this particular study. *S. aureus* was sensitive to a number of antibiotics including Augmentin, Chloramphenicol, Erythromycin and Cloxacilin. *Pseudomonas* was found to be most sensitive to Gentamicin, followed by *Klebsiella* and *Proteus* species with equal sensitivity. It was most sensitive to three antibiotics, namely Gentamicin, Augmentin and Chloramphenicol, in equal proportion, followed by Erythromycin and was less sensitive to Cloxacilin and Amoxycillin. *Pseudomonas*, *Klebsiella* and *Proteus* species showed resistance to varieties of antibiotics such as Amoxycilin, Cotrimoxazole, Tetracycline, Augmentin, Cloxacilin and Erythromycin (Adoga *et al*., 2010).

In Ekiti state in Nigeria Destitute MedicalCentre, 200 sample are collected from curtains of different medical wards, 37% of *E. coli*, 19% of *Klebsiella* sp, 13% of *Pseudomonas* sp, 15% of *Serratia* sp, 8% of *Staphylococcus* sp, 7% of *Enterococcus* sp and only 1% of *Salmonella* sp. The pattern of resistance of the bacterial isolates were Ceftazidime (87.7%), Cefuroxime (82.2%), Gentamicin (89.0%), Cefotaxime (84.9%), Ofloxacin (90.4%), Amoxicillin (95.9%), Augmentin (93.2%), Nitrofurantoin (93.2%) for the Gram negative bacteria, while, the percentage of resistant Gram positive isolates is as follows: Tetracycline (84.6%), Sulfamethoxazone (61.5%), Erythromycin (84.6%),

Fusidic acid (69.2%), Gentamicin (61.5%), Clindamycin (30.8%), Penicillin (100%) and Trimethropin (92.3%) (Odeyemi *et al.*, 2011).

In South Africa, Steve Biko Academic Hospital on October 2007 done a culture survy, samples are taken from 97 curtains from different wards, isolated bacteria included *Pseudomonas sp* (89.5%), *S. aureus* (11.8%) and *Serratia marcescens* (2.9%), the antibiogram showed that all isolates were resistant to Nitrofurantoin and Ceftazidime, and senstive to Imipenem and different reactions to Amoxicillin, Cotrimoxazole, Ciprofloxacin, Tetracycline and Gentamicin (Afolabi *et al.*, 2007).

Das *et al.,* (2002) reported a multiple antibiotic resistant *A. baumanii* that was first isolated from a patient in the general intensive care unit of a tertiary-referral university teaching hospital in Birmingham. Similar strains were subsequently isolated from 12 other patients, including those on another intensive care unit within the hospital. Environmental screening revealed the presence of the multiple resistant *Acinetobacter* species on fomites and bed linen. The major source appeared to be the curtains surrounding patients' beds. Typing by pulsed field gel electrophoresis demonstrated indistinguishable isolates (Das *et al.*, 2002).

In Uganda, Kampala, in Mulago Hospital 300 samples were taken from different curtains, a total of 47 *Pseudomonas* sp, 52 *E. coli*, 20 *Aeromonas* sp, 67 *Klebsiella* sp, were isolated and tested for their susceptibility to 10 commonly used antibiotics: Ampicillin, Gentamicin, Streptomycin, Cotrimoxazole, Tetracycline, Colistinsulphate, Carbencillin, Cefioroxime,

Taroid, which showed that all of the *Pseudomonas* sp and *E. coli* were more widely resistant to the antibiotics, the level of susceptibility to Gentamicin is observed among the isolates (Ogbonna *et al.*, 2006).

A study done in UK in january 2008, Curtains were sampled using Chromogenic MRSA medium, a total of 200 ward curtains from patient bay areas were sampled over the course of one week. After anonymisation, the chromogenic MRSA agars were incubated and inspected for characteristic blue colonies by DNAse production, which found resistant to Cefoxitin, Susceptibility to Erythromycin, Tetracycline, Ciprofloxacin, Gentamicin, Trimethoprim, Rifampicin, Linezolid, Clindamycin, Mupirocin and Vancomycin. MRSA was recovered from 31/200 curtains (15.5%). Colony counts ranged from 1 to 13 cfu per plate (median 1 cfu, mean 2.5 cfu). The predominant antibiogram (resistance to Cefoxitin, Ciprofloxacin, Erythromycin and Trimethoprim) accounted for 20/31 strains. This is the antibiogram of the majority of clinical MRSA isolates seen in the hospitals (mostly EMRSA-15). Of the remainder, resistance to Tetracycline (N ¼ 7), Gentamicin (N ¼ 4) and Mupirocin (low-level) (N ¼ 1) was identified, in addition to Cefoxitin resistance Ciprofloxacin susceptibility was found in three MRSA strains (Kalkus *et al.*, 2008).

Wilks *et al.*, (2006) reported a recent outbreak of multidrug-resistant *Acinetobacter* infection, with environmental contamination found on curtains, laryngoscope blades, patient lifting equipment, door handles, mops, and keyboards. Medical equipment has been implicated, emphasizing the need for

special attention to disinfection of shared items. The predominant antibiogram resistance to Carabenems and Cephalosporins and intermediate susceptibility to Tobramycin, Amikacin, Polymyxin B (colistin) and Tigecyclin.

Another study done in Ethiopia, Besegah Teaching General Hospital, 350 sample were taken from curtains from different wards. A total of 72 (20.5%) of *Bacillus* sp*, 35 (10%) of *Proteus* sp, 46 (13.1%) of *Staphylococcus* sp, 11 (3.1%) of *Micrococcus* sp*, 28 (8%) of *Klebsiella* sp, 9 (2.5%) of *Arthrobacter* sp were isolated and tested for their susceptibility. All isolates were susceptible to Amikacin, Gentamicin and Pefloxacin, Ofloxacin with the exception of *Bacillus* and *Pseudomonas* were resistant to Norfloxacin. The bacterial isolates were generally resistant to Ampicillin and Rifampicin. In general, over 70% of all bacterial isolates were susceptible to Ofloxacin, Pefloxacin, Norfloxacin, Ciprofloxacin, Cotrimoxazole, Gentamicin and Amikacin. Over 50% were sensitive to Cephalothin, Novobiocin, Chloramphenicol, Nalidixic acid, Streptomycin and Erythromycin. At least 20% of bacterial isolates demonstrated susceptibility to Ampicillin, Tetracycline and Rifampicin. The level of resistance shown by bacterial isolates to specific antibiotic is as follows: Ampicillin 66.7%, Rifampicin 66.7%, Tetracycline 53.3%, Cephalothin 46.7%, Erythromycin 46.7%, Novobiocin 40%, Chloramphenicol 33.3%, Nalidixic acid 33.3%, Streptomycin 33.3%, Cotrimoxazole 26.7%, Norfloxacin 13.3%, Ciprofloxacin 6.7%, Ofloxacin 6.7%, Amikacin 0%, Gentamicin 0% and Pefloxacin 0% (Gideon *et al*., 2008).

In Chad, Abougoudam Adventist Hospital, a total of 82 *E.coli* isolates, 31 *K. pneumonia* and 20 *Ps. aeruginosa* were isolated from the hospital curtains. The antimicrobial susceptibility of the isolates to different antibiotics showed that percentage susceptibility of *E. coli, K. pneumonia* and *Ps. aeruginosa* isolates to the tested antibiotics were 83.33%, 50% and 66.67%. *E. coli* isolates were completely resistant to Ampicillin, Ofloxacin and Clindamycin and susceptible to Ciprofloxacin, Erythromycin and Gentamicin (Iroha *et al.*, 2013).

In a teaching hospital in Nigeria. Exactly 35 samples were taken from the A&E ward curtains of Ahmadu Bello University Teaching Hospital, Zaria between the months of July and October 2010. Twenty three (65.7%) isolates were obtained, the ratio of Gram positive to Gram negative organisms was 12 to 11. The bacteria isolated were *S. aureus* (21.7%), *S. epidermidis* (8.7%), *Streptococcus* sp (8.7%), *Bacillus* sp (13.0%), *Escherichia coli* (26.1%), *Pseudomonas* sp (8.7%) and *Klebsiella* sp (13.0%). The isolated bacteria showed varying susceptibility pattern to the antibiotics used and were all susceptible to Erythromycin and Streptomycin. *Streptococcus* sp were most sensitivity (100%) to Ciprofloxacin, Septrin, Erythromycin, Streptomycin and Ampiclox. All the isolated *E. coli* were susceptible to Gentamicin, Cephalexin and Chloramphenicol while all the isolated *Pseudomonas* sp were susceptible to Gentamcin and Ofloxacine. Similarly, all the *Klebsiella* sp isolates were susceptible to Gentamicin, Ceftriazone, Chloramphenicol and Ampicillin (Amino *et al.*, 2010).

In Baghdad, a total of 54 out of 67 (80.59%) of curtains swabs showed growth of *Ps. aeruginosa* (48.14%), *K. pneumonia* (31.48%), *S. aureus* (27.77%), *A. baumanii* (14.81%), *E. coli* (7.40%). All bacterial isolates were tested against 19 antibiotics. *Ps. aeruginosa* was the most drug-resistant pathogen of bacterial isolates tested. It showed resistance to third generation cephalosporin of 100% and 88.96% for Cefotaxime Ceftriaxone, whereas moderate resistant to combination of this generation of Ceftazidime / Clavulanic acid (46.15), and more sensitive to Cephalosporin, Ceftobiprole, Imipenem, and Meropenem, and was resistant to Ciprofloxacin, Norfloxacin, Levofloxacin, and Gemifloxacin, Cephalosporin third and fourth-generation, with resistance of 100%, 88.46%, and 76.92 for Cefotaxime, Ceftriaxone, and Cefepime, respectively, whereas sensitive to Cephalosporin fifth-generation, Ceftobiprole. *K. pneumonia* showed sensitivity to cephalosporin third generation combination, as Ceftazidime / Clavulanic acid, and Cefoperazone / Sulbactam, Imipenem, and Meropenem, and resistant to Ciprofloxacin (70.58%), and Norfloxacin (70.58%), but more sensitive to Levofloxacin (41.17%), and Gemifloxacin (11.76%). *S. aureus* was highly resistant to Amoxicillin (100%), Piperacillin (93.33%), and Carpenicillin (86.66%), Cefotaxime, and Ceftriaxone (93.33%), Cephalosporin, Cefepime (53.33%), Ciprofloxacin (53.33%), and Norfloxacin (46.66%), whereas and totally resistant to Vancomycin. *A. baumanii* was resistant to Cefotaxime (87.5%), Ceftriaxone (87.5%), and Cefepime (62.5%) with no resistance to Imipenem, Meropenem compared with *Ps. aeruginosa*, *K. pneumoniae*, or *S. aureus* and their

resistance to Fluoroquinolone was less than *Ps. aeruginosa*, *K. pneumoniae*, and had mild resistance to new generation Flouroquinolone, Levofloxacin (12.5%), and Gemifloxacin (12.5%) (Sadik, 2012).

In Ghana, Divine Mercy Hospital, 90 samples obtained from hospital curtains. The proportion of microorganisms obtained from the samples shows *Bacillus* sp 33%, *Staphylococcus* sp 13%, *Streptococcus* sp 13%, *Pseudomonas* sp 7%, *Klebsiella* sp 7% and *Proteus* sp 6%. The antimicrobial susceptibility and distribution showed that *Bacillus* was 100% resistant to Augmentin and Amoxicillin, *Streptococcus* sp grossly resisted all the antibiotics used, from Gram negative bacterial isolates, *Klebsiella* sp was 100% resistant to Amikacin and Ampicilin-Sulbactam while *Proteus* sp was 100% resistant to all the antibiotics. *Bacillus* sp and *Staphylococcus* sp were found susceptible to Co-trimoxazole. The Gram negative bacteria identified in this study were susceptible to Ceftrazone and Imipenem. Gross resistance to Amikacin and Ampicilin-Sulbactam exhibited by *Klebsiella, Proteus and Pseudomonas* sp was observed in this study. *Klebsiella* sp was found susceptible to Norfloxacin. However, *Proteus* and *Pseudomonas* sp were resistant to Norfloxacin, *Proteus* sp found in this study was resistant to all the Gram negative-specific antibiotic (Olayiwola *et al.*, 2014).

In Tanzania, Temeke hospital, isolated bacterial organisms were analyzed for their susceptibility profiles against 25 different antibiotics, and about 58% of curtains samples (n=270) were contaminated with different microorganisms, with counts varying from 2 colony-forming unit (CFU) to 2.4×10^4 CFU. In

total, 37 different bacterial species were isolated, and the major isolates included *E. coli* (7.5%), *S. aureus* (7.5%), *Proteus* sp (14.2%), *Yersinia enterocolitica* (6.7%), and *Pseudomonas* sp (16.3%). The isolated organisms were highly resistant to Cefazolin (83.5%), Cefoxitin (69.2%), Ampicillin (66.4%), and Cefuroxime (66.2%). Intermediate resistance was observed against Gentamicin (10.6%), Cefepime (13.4%), Ceftriaxone (27.6%), and Cefotaxime (29.9%), Levofloxacin (0.7%), Ceftazidime (2.2%), Meropenem (3%), and Ciprofloxacin (3.7%) were the most active antibiotics against all the microorganisms, with all recording less than 5% resistance. Multiple drug resistance was very common, and 78% of the organisms were resistant to three or more antibiotics. Multiple drug resistance profiles defined as resistance to three or more antibiotics in this study were also observed. Of the 134 organisms isolated, 108 (80.6%) were multiple drug-resistant. The resistance ranged from three to 20 antibiotics at a time, with higher resistance to 13 (10.4%), followed by 9, 4, and 3 (8.2%) antibiotics at a time. None of the isolates was resistant to all the 25 antibiotics used while 18 (13.4%) were susceptible to all the antibiotics used (Samie *et al*., 2012).

This study was carried out in the Department of Microbiology, Dr. S.N. Medical College, Jodhpur, to detect prevalence and antibiogram of Microorganism in various curtains sample collected from Mahatma Gandhi Hospital. Samples were collected from different wards such as Intensive care unit (ICU), Critical care unit (CCU), surgical ward, Post Surgical ward, and Orthopaedic ward. *S. aureus,* Coagulase Negative *Staphylococcus,*

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Micrococcus sp, and Gram positive bacilli grown in all wards. Whereas *Klebsiella* sp was isolated from ICU B, CCU II and Post-operative Ward and *E. coli* was isolated from IICU, post operative ward and surgical ward only. Maximum number of microorganisms isolated from ICU A was Coagulase Negative *Staphylococcus* (33.85%) and minimum number of microorganism was *Micrococcus* (12.25%). While in ICU B Coagulase Negative *Staphylococcus* (48.87%) was found maximally and minimum isolates are *Klebsiella* (3.03%) and in IICU *S. aureus,* Coagulase Negative *Staphylococcus, Micrococcus sp* and Gram positive bacilli grown in all wards. Whereas *Klebsiella* sp was isolated from ICU B, CCU II and Postoperative, *E. coli* was isolated from IICU, postoperative ward and surgical ward only. Maximum number of microorganisms isolated from ICU A was Coagulase Negative *Staphylococcus* (33.85%) and minimum number of microorganism was *Micrococcus* (12.25%). While in ICU B Coagulase Negative *Staphylococcus* (48.87%) was found maximally & minimum isolates are *Klebsiella* (3.03%) and in IICU maximally and minimum isolates are *E. coli* (0.97%). CCU (I) *S.* aureus (30.09%) was found maximally & minimum isolates are Coagulase Negative *Staphylococcus* (20.5%). While in CCU (II) GPB (30.08%) was found maximally and minimum isolates are *Klebsiella* (1.09%). Postsurgical *Micrococcus* (44.76 %) was found maximally and minimum isolates were *Klebsiella* and *E. coli* (1.75%). While in Postoperative (F) GPB (34.43 %) was found maximally and minimum isolates were Coagulase Negative *Staphylococcus* (19.81%). Orthopaedic (M) *S. aureus* (32.19 %) was found

maximally & minimum isolates were Coagulase Negative *Staphylococcus* (11.36%). While in Orthopaedic (F), *S. aureus* (31.13%) was found maximally & minimum isolates are coagulase negative *Staphylococcus* (1.1%). Surgical GPB (25.55 %) was found maximally and minimum isolates were *E. coli* (3.33%). Antimicrobial sensitivity testing was done by commonly used antimicrobial agents, *Staphylococci* are mostly sensitive to most of the commonly used antibiotics like Azithromycin, Amoxyclave, Ampicillin, Cefotaxime, Ceftazidime, Ceftriaxone, Levofloxacin, Vancomycin. On other hand multidrug resistance was higher in ICU and CCU in compare to other ward. Coagulase negative *Staphylococcus* and *Micrococcus* were also sensitive for mostly used antibiotics. *Klebsiella* sensitivity pattern varied from 100% for Gentamicin, Imipenem, Tobramycin to (33.33%) for Ciprofloxacin and Ceftriaxone. For *E. coli* (100%) for Imipenem, Tobramycin and (33.33%) to Gentamicin (Laxmi *et al.*, 2014).

In Mozambique, 160 swab samples were collected from Maputo hospital curtains. The total percentage prevalence of *S. aureus* was (50.80%), *Ps. aeruginosa* (28.60%) and *E. coli* (20.60%). Out of (20.60%) of *E. coli* isolates (7.7%) were found to be *E. coli* O157:H7. *S. aureus* isolates were highly resistant to Amipcillin and Cefoxitin, *Ps. aeruginosa* and *E. coli* were resistant to Tetracycline. The multiple antibiotic resistance indexes of the pathogens were more than 0.2. Among the isolates*, S. aureus* showed more multidrug resistance (31.30%) and *E. coli* had the least multidrug. 100% susceptibility of *S. aureus* to Vancomycin, Linezolid, Gentamicin. The antimicrobial profile of

S. aureus showed that (25.0%) of the isolates were resistant to Cefoxitin, and Methicillin. The (0.0%) of the antimicrobial resistance profile of *E. coli* to Gentamicin, Cefoxitin, Ceftazidime and Chloramphenicol, resistant to Tetracycline (46.2%) and Ampicillin (7.7%). In this study Gentamicin, Cefoxitin, Ceftazidime and Chloramphenicol were the most active antibiotics against *E. coli*. The multidrug resistance of *Ps. aeruginosa* was (11.10%), *E. coli* (7.70%), *S. aureus* (31.30%) (Chrinius *et al*., 2014).

In Quetta city, One hundred and twenty five (125) samples were collected from hospital curtains, 110 (88%) showed highly pathogenic bacterial load, in which the most organism was *E. coli* 36 (28.8%), followed by *Enterobacter* 35 (28%), *Klebsiella* 24 (19.2%), *Pseudomonas* 10 (08%), and *Salmonella* 5 (4%). All pathogens in this study expressed a high level of resistance to Tetracycline, Gentamicin, Sulphamethaxazole, Piperacillin, Ampicillin, Augmentin and Imipenem. Only 15 (12%) samples were pathogens free. *K. pneumoniae* showed sensitivity zone 17mm to Imipenem and Meropenem (Mohammad *et al*., 2014).

In Bangladesh, atotal of 185 samples were taken from hospital curtains, the isolated microorganisms were *Pseudomonas* sp (32%), *Klebsiella* sp (25%), *Staphylococcus* sp (53%) and *E. coli (12%). Pseudomonas* were found to be completely resistant against Ampicillin 10 μg, Ciprofloxacin 5 μg, Amoxicillin 10μg, and moderately resistant against Gentamicin 10 μg. Like the *Pseudomonas* isolates, the Staphylococcal isolates also exhibited 100% resistance against Ampicillin 10 μg and Namoxicillin 10 μg, and such a

complete resistance was also scored against Piperacillin 10 µg. Besides, a moderate drug resistance attribute of *Staphylococcus* sp was noticed against Ciprofloxacin 5 µg and Gentamicin 10 µg. *Klebsiella* isolates were found to be moderately resistant against Nalidixic acid 30µg, Ciprofloxacin 5 µg, Ceftriazone 30 µg, Chloramphenicol 10µg and Erythromycin 15 µg. *E. coli* isolates were exhibited moderate degree of drug-resistance against Ceftriazone 30 µg, and Nalidixic acid 30 µg, Ciprofloxacin 5 µg, Chloramphenicol 10µg, Trimethoprim-Sulfamethoxazole 25 µg (Kaniz *et al.*, 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a laboratory-based study.

3.1.2. Study area

The practical part was carried out in the Research Laboratory, Sudan University of Science and Technology.

3.1.3. Study duration

Study was conducted during the period from March to June 2015.

3.2. Bacterial isolates

Bacterial isolate were obtained from the Research Laboratory, SUST. The isolates were checked for purity and then re-identified by conventional bacteriological methods.

3.2.1. Purification of isolates

The isolates were streaked on nutrient agar and incubated over night at 37°C. At the end of incubation period, a discrete colony was picked up and checked for purity under microscope, and then stored in Bijou bottle containing nutrient agar slant for further investigations.

3.3. Re –identification of the isolates

Re-identification of bacterial isolates was done macroscopically by colonial morphology, microscopically by Gram stain and Biochemically

3.3.1. Grams stain

The Gram stain reaction was used to help identify pathogens in specimens and culture by their Gram reaction (Gram- positive or Gram –negative). Gram-positive bacteria stain dark purple with crystal violet and were not decolorized by alcohol and Gram –negative bacteria stain red because after being stained with crystal violet decolorized by alcohol. The smears were fixed by dry heat and then covered with crystal violet for 30-60 seconds the stain was rapidly washed by tap water and tipped off the slide. The stained smear was then covered with iodine for 30-60 seconds, iodine washed off and the smear was decolorized with alcohol and immediately washed with clean water. Safranin was added to the smear for 30-60 seconds the red stain was then washed off with tap water and smear was subsequently air dried and microscopically examined using high resolution objective power (Cheesbrough, 2006).

3.3.2. Biochemical tests

3.3.2.1. Catalase test

Catalase enzyme acts as catalyst in hydrogen peroxide to oxygen and water. This test is used to differentiate Staphylococci from Streptococci. 2-3 ml of 3% hydrogen peroxide poured into a test tube. A sterile wooden stick used to

remove a good growth of the tested organism and immersed into the hydrogen peroxide solution. Immediate active bubbling indicated as positive result (Cheesbrough, 2000).

3.3.2.2. Coagulase test

Coagulase is an enzyme that causes plasma to clot by converting fibrinogen to fibrin when bacteria incubated with plasma. This test used to differentiate coagulase positive *Staphylococcus aureus* from coagulase negative Staphylococci. Drop of normal saline was added on each end of a slide, a colony of tested organism in each of the drop was mixed to make a thick suspension, a loopful of plasma was added to the suspension and mixed gently. Positive result clumping within 10 seconds (Cheesbrough, 2000).

3.3.2.3. Deoxyribonuclease (DNAse) test (DNA hydrolysis)

DNase enzyme hydrolyzes deoxyribonucleic acid DNA. This test was used to differentiate *Staphylococcus aureus* which produce DNase enzyme from other Staphylocci. The organism inoculated by using sterile loop on a medium which containing DNA and incubated at 37°C over night. After the period of incubation the surface of the plate was covered by 1 mol/l hydrochloric acid solution. The excess acid was tipped off. Positive result clearing around the colonies (Cheesbrough, 2000).

3.3.2.4. Mannitol fermentation test

A useful selective medium for *Staphylococcus aureus* which ferments Monnitol produce acid which convert the color of medium from pink to yellow. The tested organism inoculated by sterile loop and incubated at 37˚C overnight. After the period of incubation *Staphylococcus aureus* produced yellow colonies with yellow zones (cheesbrough, 2000).

3.3.2.5. Novobiocin susceptibility test

The mechanism of novobiocin-resistance includes inhibition of cell wall synthesis. The novobiocin disk was used to differentiate *Staphylococcus saprophyticus* from other coagulase-negative staphylococci. A suspension of the tested organism was prepared in normal saline equal to McFarland 0.5×10^6 standard. Sterile swab inoculated in Mueller Hinton agar and streaked over the entire agar surface, the agar surface allowed to dry, and by sterile forceps the disk was applied to the agar surface and incubated at 37˚C overnight. After incubation period the diameter of the zone of inhibition was measured using metric ruler. Sensitive zone of inhibition equal or greater than 16 mm (*S. epidermidis*), resistant zone of inhibition less than 16 mm (*S. saprophyticus*) (Collee *et al.*, 1996).

3.3.2.6. Oxidase test

The oxidase test was used to determine the bacteria that produce certain cytochrome oxidase enzyme, which catalyze the transport of electron between the electron donors in the bacteria and redox dye (tetra methylP. Phenylene

diamine), the dye was reduced to deep purple color. Disc impregnated with reagent tetra methylP. Phenylene diamine dihydrochloride (TMPD) was used,. The Oxidase disc was placed on sterile petri dish, and colonies were picked up with a wood and smear made, deep purple color within 5-10 seconds indicated positive result (Collee *et al.*, 1996).

3.3.2.7. Fermentation of sugar, H₂S and gas production

The fermentation of sugar, production of hydrogen sulphide (H₂S) and gas production was carried out by using Kiligler iron agar, tubes inoculated with test organisms by using sterile straight loop, the button firstly stabbed, then the slope streaked. The tubes were closed by sterile cotton and incubated at 35-37˚C overnight. Yellow slope indicated lactose fermentation, yellow butt indicated glucose fermentation, red color indicated no fermentation, air bubbles indicated gas production and blacking in the media indicated H₂S production (Cheesbrough, 2000).

3.3.2.8. Urease test

The test was used to determine the ability of the organism to produce the enzyme urease, which hydrolyzed urea. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. The organism was cultured in a medium which contain urea and the indicator phenol red. The medium became alkaline and the color of the indicator was changed to pink-red. A slope of urea agar medium inculated with

test organism and examined for 24 hour of incubation. Change of the color to red indicated positive reaction (Cheesbrough, 2000).

3.3.2.9. Indole test

This test demonstrated the ability of bacteria to decompose the amino acid tryptophan to indole, which accumulated in the media. The Indole then tested by colorimetric reaction with p-diamethyle-aminobenzaldhyde. Tryptophan broth was inoculated with test organism and incubated for 24 hour at 37˚C. 5 ml of kovacs reagent added and shacked gently, a red color in the alcohol layer indicated positive reaction (Collee *et al*., 1996).

3.3.2.10. Motility test

This test used to test movement of bacteria by show turbidity after inoculums. The test was done by using semi-solid agar. 0.2-0.5% of agar was added into nutrient broth. In a semi-solid media motile bacteria (swarmed) and gave diffuse spreading growth that was easily recognized by naked eye, thus may be detected more easily than microscopically (hanging drop) method (Collee *et al*., 1996).

3.3.2.11. Citrate utilization test

This test based on the ability of an organism to use citrate as its only source of carbon, and tested for the ability of an organism to utilize citrate as sole carbon and energy source for growth and ammonium as sole source of nitrogen. Simmons citrate agar inoculated by the test organisms and incubated at 37˚C for 24 hour. A blue bright color indicated positive result (Collee *et al*., 1996).

3.4. Susceptibility of bacterial isolates to antibiotics

Bacterial isolates were subjected to antibiotic sensitivity analysis using the kirby Bauer disc diffusion method. The organisms' suspension was prepared from pure culture for each isolates and the turbidity of suspension was compared to McFarland turbidity standard. Mueller Hinton medium was used for disk diffusion test. The plate surface was inoculated by a swab that was impregnated in bacterial suspension standardized to match turbidity of the 0.5×10^6 McFarland turbidity standard, the plate was swabbed in three directions to insure complete distribution of the inoculums over entire plate, within 15 minute over inoculation the antimicrobial discs were applied then incubated at 37^oC aerobically. The discs were commercially available and contained several antibiotic: Ciprofloxacin, Amoxicillin, Gentamicin, Methicillin, Imipenem, Co-trimoxazole, Tetracycline, Novobiocin, Vancomycin and Azithromycin.

3.4.1. Application of antibiotic discs

Sterile forceps was use, 5 antibiotic discs were applied and evenly distributed on the inoculated plate. The plate was inverted and incubated aerobically at 37^oC, for 18-24 hours.

3.4.2. Reading of zones of inhibition

After overnight incubation the test plates were examined. The ruler was used on the underside of the plate, the diameter of each zone of inhibition was measured in mm. The end point of inhibition was where the growth started.

3.4.3. Interpretation of the results

The zone size of each antibiotic was measured. The susceptibility of isolates was reported according to the manufacture's standard zone size interpretative manual. Sensitive organisms were when the zone of inhibition was equal to or greater than the standard .

CHAPTER FOUR

RESULTS

Bacterial isolates (n=27) were obtained from the Research Laboratory (SUST). Biochemical test adopted for re-identification and their results were tabulated in table (1 and 2). These were *Bacillus sp* 11 (40.7%), *Ps. aeruginosa* 6 (22.2%), *S. aureus* 5 (18.5%), *K. pneumoniae* 4 (14.8%) and *S. epidermidis* 1 (3.7%).

Studies on assessment of antibiogram of the isolates to antibiotics revealed that all isolates were susceptible to Ciprofloxacin, susceptibility to other antibiotics range from 0.0% to 80%. Assessment of antibiogram of each isolate to same antibiotic was found as follows, all five isolates of *S. aureus* isolate were susceptible to Ciprofloxacin, Gentamicin, Tetracycline, Imipenem, Cotrimoxazole, Amoxicillin, Novobiocin and Vancomycin, three isolates susceptible to Azithromycin and two isolates were resistant, two isolates susceptible to Methicillin and three isolates were resistant.

The one isolate of *S. epidermidis* was susceptible to Ciprofloxacin, Vancomycin, Imipenem, Cotrimoxazole and Methicillin. The four isolates of *K. pneumonia* were susceptible to Ciprofloxacin, Azithromycin, Cotrimoxazole and Novobiocin, three isolates were susceptible to Gentamicin and one isolate was resistant, two isolates were susceptible to Amoxicillin, Vancomycin, Methicillin and Tetracycline and two isolates were resistant, one isolate susceptible to Imipenem and three isolates were resistant.

The six isolates of *Ps. aeruginosa* were susceptible to Ciprofloxacin, Azithromycin, Imipenem, Gentamicin and Vancomycin. Five isolates susceptible to Amoxicillin and one isolate was resistant, three isolates susceptible to Tetracycline and Novobiocin and three isolates were resistant, two isolates susceptible to Cotrimoxazole and four isolates were resistant. One isolate susceptible to Methicillin and five isolates were resistant. Table (3).

Percentage of antibiotic susceptibility (%) showed in (Table 4).

Antibiotic resistance showed in (Table 5) and Percentage of antibiotic resistance showed in (Table 6).

Table 1. Re-identification of Gram- positive bacterial isolates

Isolate code	Biochemical test			Identified Organism
	Catalase	Mannitol fermentation	DNase	
C11	Positive	positive	positive	*S. aureus*
C12	Positive	positive	positive	*S. aureus*
C13	Positive	positive	positive	*S. aureus*
C14	Positive	positive	positive	*S. aureus*
C15	Positive	positive	positive	*S. aureus*
C16	Positive	negative	negative	*S. epidermides*

Table 2. Re-identification of Gram- negative bacterial isolates

Isolate code	Biochemical test								Identified organism
	KIA				Urease	Indole	Citrate	Oxidase	
	S	B	G	H$_2$S					
C1	R	R	_	_	negative	negative	positive	positive	*Ps .aeruginosa*
C2	R	R	_	_	negative	negative	positive	positive	*Ps. aeruginosa*
C3	R	R	_	_	negative	negative	positive	positive	*Ps. aeruginosa*
C4	R	R	_	_	negative	negative	positive	positive	*Ps. aeruginosa*
C5	R	R	_	_	negative	negative	positive	positive	*Ps. aeruginosa*
C6	R	R	_	_	negative	negative	positive	positive	*Ps. aeruginosa*
C7	Y	Y	+	_	positive	negative	positive	negative	*K. pneumoniae*
C8	Y	Y	+	_	positive	negative	positive	negative	*K. pneumoniae*
C9	Y	Y	+	_	positive	negative	positive	negative	*K. pneumoiae*
C10	Y	Y	+	_	positive	negative	positive	negative	*K. pneumoniae*

Table 3. Susceptibility of bacterial isolates to antibiotics

Antibiotic	Susceptibility of			
	S. aureus (n=5)	*S. epidermidis* (n=1)	*K. pneumonae* (n=4)	*Ps.aeruginosa* (n=6)
CIP	5/5	1/1	4/4	6/6
VA	5/5	1/1	2/4	6/6
GEN	5/5	NT	3/4	6/6
TE	2/5	NT	2/4	3/6
IPM	5/5	1/1	1/4	6/6
AZM	3/5	NT	4/4	6/6
AMX	5/5	NT	2/4	5/6
COT	5/5	1/1	4/4	2/6
NV	5/5	NT	4/4	3/6
MET	2/5	1/1	2/4	1/6

Key: CIP=Ciprofloxacin, MET=Methicillin**,** TE=Tetracycline, IPM=Imipenem**,** GEN=Gentamicin, AZM=Azithromycin**,** AMX=Amoxicillin, COT=Cotrimoxazole**,** NV**=**Novobiocin, VA=Vancomycin, NT=Not Tested**.**

Table 4. Percentage of antibiotic susceptibility of bacterial isolates

Antibiotic	Susceptibility (%) of bacterial isolates			
	S.aureus (n=5)	*S. epidermidis* (n=1)	*K. pneumonie* (n=4)	*Ps.aeruginosa* (n=6)
Ciprofloxacin (CIP)	100	100	100	100
Amoxicillin (AMX)	100	NT	50	83.3
Gentamicin (GEN)	100	NT	75	100
Azithromycin (AZM)	60	NT	100	100
Methicillin (MET)	40	100	50	16.6
Imipenem (IMP)	100	100	25	100
Co-trimoxazole (COT)	100	100	100	33.3
Tetracycline (TE)	100	NT	50	50
Novobiocin (NV)	100	NT	100	50
Vancomycin (VA)	100	100	50	100

Table 5. Resistance of bacterial isolates to antibiotics

Antibiotic	Resistance of			
	S. aureus (n=5)	*S. epidermidis* (n=1)	*K. pneumonae* (n=4)	*Ps.aeruginosa* (n=6)
CIP	0/5	0/1	0/4	0/6
VA	0/5	0/1	2/4	0/6
GEN	0/5	NT	1/4	0/6
TE	0/5	NT	2/4	3/6
IPM	0/5	0/1	3/4	0/6
AZM	2/5	NT	0/4	0/6
AMX	0/5	NT	2/4	1/6
COT	0/5	0/1	0/4	4/6
NV	0/5	NT	0/4	3/6
MET	3/5	0/1	2/4	5/6

Table 6. Percentage of antibiotic resistance of bacterial isolates

Antibiotic	Susceptibility (%) of bacterial isolates			
	S.aureus (n=5)	*S. epidermidis* (n=1)	*K. pneumonie* (n=4)	*Ps.aeruginosa* (n=6)
Ciprofloxacin (CIP)	0.0	0.0	0.0	0.0
Amoxicillin (AMX)	0.0	NT	50	16.6
Gentamicin (GEN)	0.0	NT	25	0.0
Azithromycin (AZM)	40	NT	0.0	0.0
Methicillin (MET)	60	0.0	50	83.3
Imipenem (IMP)	0.0	0.0	75	0.0
Co-trimoxazole (COT)	0.0	0.0	0.0	66.6
Tetracycline (TE)	0.0	NT	50	50
Novobiocin (NV)	0.0	NT	0.0	50
Vancomycin (VA)	0.0	0.0	50	0.0

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Divider curtains are used in health care settings to surround patient's bed and provide privacy. However, studies implicated curtains may act as reservoirs for bacteria and a potential source of hospital acquired infection (Bhalla *et al*., 2004). This study was conducted to determine the antibiogram of potentially pathogenic bacteria isolated from hospital curtains to selected antibiotics.

The isolates included in this study were *Bacillus* sp, *Staphylococcus aureus*, *Staphylococcus epidermides*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa.* Antibiogram of *S. aureus* against Ciprofloxacin, Tetracycline, Gentamicin, Imipenem, Amoxicillin, Cotrimoxazole, Methicillin, Azithromycin, Novobiocin and Vancomycin is found similar to that reported by Chrinius *et al.,* (2014) in Mozambique who reported susceptibility (100%) to Vancomycin and Gentamicin, and in line with the result of Gideon *et al*., (2008) in Ethiopia who reported susceptibility (100%) to Gentamicin, Ciprofloxacin, Cotrimoxazole and Novobiocin, also in agreement with the result of Laxmi *et al*., (2014) in India who reported susceptibility (100%) to Amoxicillin and Vancomycin. The result obtained in this study is higher compared to Afolabi *et al.,* (2007) in South Africa who reported (84%) susceptibility to Ciprofloxacin, Tetracycline and Gentamicin and that found by Sadik, (2012) in Baghdad who reported (53.3%) susceptibility to Ciprofloxacin, Vancomycin and (0.0%) susceptibility to Amoxicillin.

Antibiogram of *K. pneumonia* against Ciprofloxacin, Tetracycline, Gentamicin, Imipenem, Amoxicillin, Cotrimoxazole, Methicillin, Azithromycin, Novobiocin and Vancomycin is found in agreement with the result of Gideon *et al*., (2008) in Ethiopia who reported susceptibility (100%) to Ciprofloxacin, Cotrimoxazole and Novobiocin, but lower than that of Laxmi *et al.,* (2014) in India who reported suscepiblity (100%) to Gentamicin and Imipenem. On the other hand is higher when compared with that of Mohammad *et al*., (2014) in Quetta city who reported susceptibility (0.0%) to Tetracycline, Gentamicin and Imipenem.

Antibiogram of *Ps. aeurgenosae* against Ciprofloxacin, Tetracycline, Gentamicin, Imipenem, Amoxicillin, Cotrimoxazole, Methicillin, Azithromycin, Novobiocin, and Vancomycin is found in agreement with the result of Afolabi *et al.,* (2007) in South Africa who reported (100%) susceptibility to Imipenem. This result is higher when compared with that of Kaniz *et al*., (2014) in Bangladesh who reported (0.0%) susceptibility to Ciprofloxacin, Gentamicin and Amoxicillin, and that found by Chrinius *et al.,* (2014) in Mozambique who reported (0.0%) susceptibility to Ciprofloxacin.

5.2. Conclusion

The study concluded that all isolates exhibited high susceptibility to Ciprofloxacin and high resistance to Methicillin.

5.3. Recommendations

1. Modified Kirby–Bauer method is recommended to be adopted for all microbiological laboratories to assess susceptibility of clinical isolates to different antibiotics.

2. Health-care workers should perform hand hygiene before and after contact with hospital curtains.

3. Periodical cleaning to hospital curtains every 2-3 days with specific antiseptic and disinfectant.

4. Further studies with a large number of bacterial isolates and advanced techniques are highly recommended to validate these results.

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APPENDICES

Appendix (1):

A) Culture media

Difco™ Nutrient Agar

Approximate formula * per Liter

Beef Extract ……………………………………………..............…3.0g

Peptone…………………………………………………......…..5.0g

Agar ……………………………………………………..……...15.0g

Difo ™ MacConkey Agar

Approximate formula * per Liter

Peptone……………………………………………………..…......20.0g

Lactose……………………………………………...…..…......10.0g

Bile Salts…………………………………………………..5.0g

Sodium Choloride…………………………………………........12.0g

Agar…………………………………………………..............12.0g

Nutrient Red ……………………………………………..…0.05g

Difco™ Manitol Salt Agar

Approximate formula * per Liter

Proteose Peptone
No.3……………………………………………………………10.0g

Beef Extract……………………………………………...1.0g

D-Manitol ……………………………………………………10.0g

Sodium Choloride…………………………………………75.0g

Agar…………………………………………………………..15.0g

Phenol Red……………………………………………………25.0g

Difco ™ Muller Hinton Agar

Approximate formula * per Liter

Beef Extract…………………………..…………………….……...2g

Acid Hydrolysate of Casein………………………………….…..…17.5g

Starch………………………………………………………….1.5g

Agar……………………………………………………...…17g

Final PH: 7.3 -+ o.1 at 25C

Kliglar iron agar (KIA)

 Lab-Lemco powder………………………………………..…...3.0g /l

Yeast extract ………………………………………..…….…3.0g/l

Peptone…………………………………………...……………....20.0g/l

Sodium chloride …………………………………………...5.0g/l

Lactose ………………………………………………....10.0g/l

Dextrose (glucose)……………………………………….....1.0g/l

Ferric citrate…………………………………………….…0.3g/l

Sodium thiosulphate …………………………………..…...0.3g/l

Phenol red …………………………………...………...0.05g/l

Agar ………………………………………….……………12.og/l

DNAse agar

Tryptose…………………………………………………………20g/l

Deoxyribonuclic acid………………………………..………2g/l

Sodium chloride ………………………………………………5g/l

Agar…………………………………….…………………12g/l

Christensen's urea agar

Glucose ……………………………………………..……………………..5g

Sodium chloride………………………………………………………..…5g

Potassium dihydrogen phosphate …………………………………..……..2g

Peptone …………………………………………………..…………….…1g

Agar………………………………………………………………………20g

Distilled water …………………………………………..………………1 liter

Simmons' citrate medium

Koser's medium …………………………………...…………………....1liter

Agar …………………………………………………..…………….…20g

Bromothymole blue, 0.2%...40ml

B) Preparation of reagents

1. Gram's Stain reagent

Crystal violet

Approximate formula *per liter

Crystal violet…………………………………………………….………20.0g

Ammonium oxalate………………………………………….…..9.0g

Ethanol, obsolute …………………………………………………..95ml

Distilled water …………………………………………………….……to 1 liter

Lugols Iodine

Approximate formula *per liter

Potassium iodine …………………………………..…………..…20.0g

Iodine ………………………………………………………………10.0g

Distilled water………………………………………………….……to 1 Liter

Acetone- alcohol decolorizer

Approximate formula* per liter

Acetone……………………………………………………………….…..500ml

Ethanol, absolute……………………………………………………………475ml

Distilled water…………………………………………………….…..25 ml

Saffranin

Approximate formula*per liter

Saffranin …………………………………………………………….....2.5g

95% ethanol…………………………………………………………..10 ml

Distilled water………………………………………………...…to 100ml

2. Physiological saline (8.5g/l)

Sodium chloride …………………………………………………..…...8.5g

Distilled water ……………………………………………...………..to 1 liter

3. Hydrochloric acid

Hydrochloric acid, concentrated ………………………………….....8.6ml

Distilled water ……………………………………………….………to 100ml

4. Kovac's reagent

Approximate formula*per liter

Amyle or isoamyle alcohol ……………………………………….…..15ml

P-Dimethyl-aminobenzaldehyde……………………………………..10g

Hydrochloric acid concentrated ………………………………..…..50ml

5. Hydrogen peroxide

H_2O_2 solution ……………………………………………………10 vol

Appendix 2: Diameter of inhibition zones of various discs of antibiotics against bacteria

Code of Isolate	CIP	AMX	GEN	AZM	MET	IPM	COT	TE	VA	NV
S 1	28	30	27	25	7 R	42	35	37	27	35
S 2	32	28	24	11 R	17	45	37	28	30	42
S 3	32	29	21	17R	6 R	45	40	30	24	31
S 4	35	34	25	34	15	50	35	30	29	37
S 5	28	37	29	30	9 R	42	31	32	33	34
S.e 1	28	NT	NT	NT	18	28	42	NT	23	NT
K 1	34	17	26	28	16	21	29	24	24	39
K 2	35	12R	28	23	5 R	12R	27	20	21	32
K 3	40	10R	11R	25	6 R	10R	18	12R	16	35
K 4	37	20	20	31	15	7R	21	9 R	11R	31
Ps.1	30	25	25	19	8 R	45	23	25	22	22
Ps.2	43	11 R	20	25	15	34	9 R	15	30	27
Ps.3	38	18	21	32	5 R	30	8 R	12 R	25	31
Ps.4	32	21	21	34	6 R	40	10R	11R	33	17R
Ps.5	35	23	30	27	5 R	28	9 R	27	28	11R
Ps.6	39	27	27	21	7R	31	21	9R	31	15R

Key:

S = *Staphylococcus aureus*

S.e = *Staphylococcus epidermidis*

K.p = *Klebsiella pneumoniae*

Ps = *Pseudomonas aeruginosa*

R= resistant

NT= not tested

Appendix 3: Himedia zone size Interpretation

Antimicrobial Agent	Disc Content	Interpretation Criteria		
		Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Ciprofloxacin (CIP)	5 mcg	21	16-20	15
Tetracycline (TE)	30mcg	19	15-18	14
Cotrimoxazole (COT)	25mcg	16	11-15	10
Amoxicillin (AMX)	30mcg	18	14-17	13
Azithromycin (AZM)	15mcg	28	14-17	13
Novobiocin (NV)	30mcg	22	18-21	17
Imipenem (IMP)	10mcg	16	14-15	13
Methicillin (MET)	5mcg	14	10-13	9
Gentamicin (GEN)	10mcg	15	13-14	12
Vancomycin (VA)	30mcg	17	15-16	14