SUSCEPTIBILITY PATTERN OF CLINICAL BACTERIAL ISOLATES AND PLASMID CURING OF RESISTANT BACTERIA

BY

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FACULTY OF LIFE SCIENCES

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A PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, FACULTYOF LIFE SCIENCES UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA. IN PARTIAL FULFILLMENT FOR THE AWARD OF BACHELOR OF SCIENCEB.sc (Hons), MICROBIOLOGY

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CERTIFICATION

This is to certify that this project work was carried out by EGUASA EVELYN ANGELA (miss)in the Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin Cityunder my supervision.

PROF. E. I. ATUANYA **Date**

(Project Supervisor).

APPROVAL

This project work is accepted in partial fulfillment for the award of Bachelor of Science, B. Sc (Hons) in the Department of Microbiology, University of Benin, Benin City.

PROF. S. E. OMONIGHO **DATE**

(Head of Department)

DEDICATION

To Almighty God for his infinite mercies, unmerited graces and love towards me and my parents Mr. and Mrs. Eguasa for their support and show of love.

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ABSTRACT

The control of infectious diseases is badly endangered by the rise in the number of microorganisms that are resistant to antimicrobial agents. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death. The antibiotics susceptibility pattern of clinical isolates obtained from University of Benin Teaching Hospital was determined by Kirby Bauer disc diffusion method. The bacteria isolates obtained were S. *aureus,* S. *epidermidis,* S. *hominis,* E. *coli* and K. *pneumonia*. All test isolates were susceptible to Levofloxacin and Chloramphenicol. *Corynebacterium jeikeium* showed resistance to Streptomycin and Amoxicillin, K. *pneumonia* showed resistance to Norfloxacin. All S. *epidermidis,* showed no resistance to any of the antibiotics tested likewise E. *coli* and S. *aureus.* The multiple antibiotics resistance index of bacteria isolates revealed *Corynebacterium jeikeium* had an index of 0.2 which is the limit, K. *pneumonia* had an index of 0.1 and other were zero indicating these isolates do not pose threat to public health as they are not multidrug resistant and as such, plasmid curing was not carried out on these bacteria isolates.

CHAPTER ONE

INTRODUCTION

The control of infectious diseases is badly endangered by the rise in the number of microorganisms that are resistant to antimicrobial agents. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic.

The primary cause of antibiotic resistance is genetic mutation in bacteria (Dromigny and Perrier-Gros-Claude, 2003).Inappropriate and irrational use of antimicrobial medicines provides favourable conditions for resistant microorganisms to emerge, spread and persist. The greater the duration of exposure of the antibiotic, the greater the risk of the development of resistance, irrespective of the severity of the need for the antibiotic. As resistance towards antibiotics becomes more common a greater need for alternative treatments arises. However, despite a push for new antibiotic therapies there has been a continued decline in the number of newly approved drugs (Zuccato*et al.* 2005).

Antibiotic resistance therefore poses a significant problem and it is therefore necessary to constantly create an awareness that the right antibiotics must be prescribed as well as the mechanism of resistance and the role of plasmids in drug resistance (Iroha *et al*., 2013; Lee *et al*., 2018). Plasmids are small DNA molecules that are found within the cell, the cells are physically separated from chromosomal DNA but they have the ability to coexist with the host. Plasmids are extrachromosomal mobile elements that are genetic which are found in bacteria, they contribute to antibiotic resistance, virulence, gene gain between species through horizontal gene

transfer by conjugate and non-conjugate mechanisms (Schief and Wensmk,1999; Dasmeh*et al*., 2015). Plasmids found in different bacteria vary from each other because some of the plasmids are stable and thus can be maintained from one generation to another during cell division into daughter cell. During the cell division process the cell receives one or more plasmid copy (Tevors, 1986).

Although bacterial resistance is different from eukaryotic resistance in many respects there are common sensitive points, such as transporter protein mediated efflux pump systems. In this respect the mechanism of resistance in bacteria, protozoa and tumour cells is similar and therefore it may be possible to overcome it in a similar way. Hence, this report reviews the mechanisms by which the antiplasmid activity of heterocyclic compound is expressed in plasmid carrying bacteria and the reversal of drug resistance by these bacteria by elimination of plasmids containing antibiotic resistant genes.

AIM AND OBJECTIVES

This study is aimed profiling plasmids of resistant bacteria and curing of resistance with acridine orange.

Specific Objectives Include;

1. Identification of clinical bacteria pathogens
2. Determination of antibiotics susceptibility pattern before curing
3. Curing of plasmid with acridine orange
4. Determination of antibiotics susceptibility pattern after plasmid curing
5. Determination of MAR-Index

CHAPTER TWO

LITERATURE REVIEW

2.1 Background study

Healthcare associated infections are a problem globally (Mbim*et al*. 2016). The widespread use of antibiotics continues to influence this menace giving rise to antibiotic-resistant bacteria in the hospital setting and, in the environment, (Bereket *et al*. 2012). The environment of the hospital is an obvious important focus for the selection and spread of multi-resistant bacteria and a possible direct source of nosocomial infections (Russotto *et al*. 2015). Antibiotic resistance has been described as one of the greatest global threats of the 21st century (Conly and Johnston, 2005).

Although it was recognized soon after antibiotics were first introduced, the impact was mitigated initially by the development and use of newer agents. However, very few new classes of antibiotics have been developed since the late 1960s, and development has stalled in recent years. For some organisms, particularly Gram-negative bacteria such as *Acinetobacter baumannii, Pseudomonas aeruginosa* and some Enterobacteriaceae (e.g., *Escherichia coli* and *Klebsiella* spp.), antibiotics have had a profound positive impact on the management of infectious diseases (e.g., community acquired pneumonia due to *Streptococcus pneumoniae*), but they have also become an essential component of all aspects of modern healthcare. No advance in medical or surgical practice would remain successful if patients developing infections could not be treated reliably.

Developments in chemotherapy for malignancy and organ transplantation make patients more vulnerable to infection, due to immune-suppression. These medical advances would be less meaningful if a patient survived the initial therapy, only to succumb to infection later. Potentially,

if antibiotic resistance became more widespread and infection became more difficult to treat, patients might, as just one example, choose to live with their disabilities rather than undergo joint replacement surgery. Unlike any other class of drugs used in clinical practice, antibiotics act on a third party, the microorganism, rather than just the patient. Microorganisms are transferred readily between individuals, and thus resistance emerging in one patient or the environment can soon affect another.

Tackling antibiotic resistance is therefore of great public health importance and must not be underestimated. Antibiotic resistance is truly a One Health issue and needs to be monitored and controlled not only in human medicine, but also in animal husbandry, agriculture and aquaculture. The term ‘antibiotic’ is used throughout to mean an agent with activity against microorganisms, rather than the more general term ‘antimicrobial’. Also, although antibiotic resistance is important in the treatment of infections due to viruses, fungi and protozoa, this discussion is limited to bacterial infections. Resistance to an antibiotic occurs when a microorganism is able to grow or survive in the presence of a concentration of antibiotic that is usually sufficient to inhibit or kill organisms of the same species.

The terms ‘susceptible’ and ‘resistant’ relating to antibiotics are usually used in clinical practice to infer the likely success or failure of treatment. Resistance is more likely when the concentration required to inhibit or kill microorganisms exceeds that achievable in a patient. Microorganisms can be either intrinsically resistant to an antibiotic or develop resistance following exposure to that antibiotic (acquired resistance). Resistance can develop as a result of mutation or direct transfer of genes encoding a resistance mechanism. Transfer of resistance genes can occur by a variety of mechanisms including conjugation (transfer of genes carried on

plasmids, which are also known as mobile genetic elements), transformation (direct transfer of naked DNA) or transduction (transfer of similar DNA by bacteriophage) (Livermore, 2004).

Genetic material, including antibiotic resistance genes, can spread very effectively between bacteria, even those of unrelated species. The efficiency and rate at which a resistant phenotype spreads within a previously susceptible species are unpredictable. For example, the staphylococcal β-lactamase gene is very successful in *Staphylococcus aureus*, with similar genes in *Haemophilus influenzae* and many Enterobacteriaceae, but they have never spread widely in enterococci. Fortunately, vancomycin resistance genes (e.g. vanA) found in enterococci remain rare in S. *aureus.*

2.2 Causes of antibiotics resistance in bacteria

Although antibiotic resistance can be considered to be an inevitable consequence of antibiotic use, injudicious use of antibiotics is a major factor facilitating the emergence of resistance worldwide. In many areas, the availability of antibiotics 'over the counter' or via the internet allows the non-prescriber to have free and unrestricted access to these agents. Once resistance has emerged, subsequent dissemination of resistant strains is facilitated by the selection pressure exerted by further antibiotic use, failure to adhere to infection control measures and by poor hygiene (notably in terms of hand hygiene, sanitary conditions and food preparation), which can occur both within and outside healthcare settings. Antibiotic resistance has significant costs to society in terms of increased mortality, morbidity, use of healthcare resources and time off work. Infections due to resistant microorganisms resulted in 25,000 deaths and cost €1.5 billion per year in hospital and societal costs in one report from the European Union(EU), Iceland and Norway. Another report suggested that nearly 23 000 people die each year as a direct result from infections due to resistant microorganisms acquired in US hospitals, with associated healthcare

costs >$20 billion (CDC, 2013).Not only does antibiotic resistance have a huge financial impact, but more worryingly, there is major concern about the lack of development of new antibiotics. During the past 25 years, only two new classes of antibiotics have been developed and introduced into clinical practice. These include the oxazolidinones (e.g. linezolid) and lipopeptides (e.g. daptomycin). Many of the other newer antibiotics are modifications of older drugs rather than new classes of agent. Daptomycin was actually discovered several decades ago, but it was not developed commercially because of concern about adverse effects. Both of these new classes of drugs have activity against Gram positive bacteria; new classes of compound with activity against Gram negative bacteria have not been found. This is partly due to failure of new drug discovery, failure to bring potential new agents to market and a restrictive regulatory environment. It has been estimated that it costs in excess of £0.5–£1 billion to develop and market a new antibiotic. The failure rate during development is also higher for antibiotics than for most other drugs, and the returns from antibiotic sales are low compared with other drugs because they are generally only used for short periods of time. In addition, because of concern about resistance, new agents are typically used sparingly and as a last resort. Worldwide, the availability of cheaper generic products potentiates this issue. As a result, developing new antibiotics is unattractive as a business model for the pharmaceutical industry, and many of the larger companies have withdrawn from this market.

2.2.1 Where does it happen?

Antibiotic resistance does not only develop in the hospital environment. As healthcare systems have evolved, there has been a blurring of boundaries between traditional healthcare facilities and the community, such that nursing and residential homes are now important reservoirs for resistant organisms in addition to outpatient settings such as dialysis and oncology day units

(Rooney *et al*. 2009).Modern travel networks have also made it easier for people and the resistant organisms they carry to spread rapidly across and between continents. Increasing resistance can result from proliferation of the resistant bacterium itself or by transfer of resistance genes from one bacterial species to another. However, the relative importance of these varies with organism and resistant mechanism. The recent increase in resistance to carbapenem antibiotics (e.g. meropenem) is an example of this. The New Delhi metallo-β-lactamase-1 (NDM-1) carbapenemase enzyme found in some Enterobacteriaceae was first detected in 2008 in Sweden from a patient transferred from the Indian subcontinent (Kumarasamy *et al*. 2010).Many of the NDM-1-positive patients seen in the UK have also had a recent history of travel to India or Pakistan, or had links with these countries. By 2011, however, it was established that not all patients infected with NDM-1 producing organisms had a history of contact with hospitals or any travel history (Johnson and Woodford, 2013).Environmental studies have shown that sewage samples and drinking water contained a variety of different organisms harbouring NDM-1 (e.g. *Shigella boydii* and *Vibrio cholerae*) suggesting transfer of mobile resistance elements between species (Walsh *et al*. 2011).Conversely, the spread of *Klebsiella pneumonia* carbapenemases (KPC) in the USA, Israel and Greece was associated with spread of a single clone of the organism [sequence type (ST); ST258](Johnson and Woodford, 2013).This single clone is responsible for the recent increase of carbapenemase producing Enterobacteriaceae reported in Greece. Thus, in this example, it is spread of a resistance mechanism within a particularly transmissible variant of a single organism that has led to increasing resistance, rather than rapid transfer of the resistance genes themselves. The spread of various β-lactamase enzymes capable of hydrolyzing cephalosporin antibiotics [such as the CTX-M extended spectrum β-lactamase (ESBL)] within a single clone of E. *coli* (ST131) is another example of this (Lau *et al.* 2008). It

was the emergence of these ESBL-producing Enterobacteriaceae that led to increased reliance on carbapenems for effective treatment of infections due to these, and hence an increase in selection pressure for resistance Meticillin resistance in S. *aureus* (MRSA) is seen in many different clones of S. *aureus*, and these each have specific geographical associations. For example, epidemic (E)-MRSA-15 and EMRSA-16 were the healthcare-adapted strains of MRSA causing problems in UK hospitals in the 1990s and 2000s but were less frequently seen in the USA, where different strains predominated. Antimicrobial resistance is related to the amount of antibiotic consumed. Studies from both community and hospital settings have demonstrated this (Costelloe *et al*. 2010).This may explain some of the differences in resistance prevalence between northern and southern European countries. The English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) recently examined this issue in England. It reported that the total number of patients with bloodstream infections in England increased each year from2010 to 2013,there were an increased number of bloodstream infections where antibiotic resistance was identified, antibiotic prescribing had increased year on year, the majority (80%) of antibiotic prescribing takes place in the community, there is considerable variation in both antibiotic resistance and antibiotic prescribing across England; frequently, areas with high prescribing also have high resistance.

2.3 Mechanisms of Antibiotics Resistance

2.3.1 The modifications

Modifications that happen in the drug-related receptor and the location of the target regions of the relation with the antibiotics are distinct, these can be complex enzymes and ribosomes (Prashanth *et al*. 2012). The most frequently identified resistance consistent with variations in the ribosomal target is in macrolide antibiotics (Shaikh *et al*. 2007).

2.3.2 Enzymatic inactivation of antibiotics

Most of the bacteria synthesize antibiotic degrading enzymes, the enzymatic inactivation mechanism is one ofthe most important antibiotics resistance mechanisms (Perez-Llarena and Bou, 2016).In this group, beta-lactamases, aminoglycosidase, chloramphenicol, and erythromycin modifying enzymes are the most popular examples (Sharkey and O'Neill, 2019).

2.3.3 Reduction of the inner and outer membrane permeability

This mechanism results from changes in the permeability of the internal and external membrane so that decreased drug uptake into the cell or rapidly ejected from the pump systems (Santajit and Indrawattana, 2016). Due to a decrease in membrane permeability as a result of porin mutations that may occur in proteins of resistant strains for example; a mutation in specific porins called OprD can cause resistance to carbapenem in *Pseudomonas aeruginosa* strain (Nikaido and Page, 2012). Reduction in outermembrane permeability can play an important role in quinolone resistance and aminoglycoside resistance (Li *et al*. 2012).

2.3.4 Active Pumps System

Resistance develops most commonly in the tetracycline group of antibiotics via the active pump systems. With an energy-dependent active pumping system, tetracyclines are thrown out and cannot concentrate within the cell (Li *et al*. 2020).

This mechanism of resistance is in plasmid and chromosomal control. Active pumping systems for example are effective in resisting quinolones, 14-memberedmacrolides, chloramphenicol and beta-lactams (Guo *et al*. 2020).

2.3.5 Use of alternative metabolic pathway

Unlike some of the target alterations in bacteria, the latest drug-susceptible pathway eliminates the need for objective development (Tan *et al*. 2020). Bacteria can prepare folic acidfrom the environment, rather than synthesizing folic acid so that it becomes resistant among sulfonamide and trimethoprim.

2.4 Resistance by Antibiotics Group Mechanisms

2.4.1 Beta-lactams Resistance

Antibiotics of beta-lactam are a wide class of antibiotics, including penicillins, cephalosporins, monobactams, and carbapenems. Synthesis of beta-lactamase enzymes is the most common resistance mechanism here (Zango *et al*. 2019).

2.4.1.1 Beta-lactamase Enzymes

At the molecular level, there are 4 groups (A, B, C, D) of beta-lactamase enzymes (Heinz *et al*. 2019). Beta-lactamases A, C, and Dthat deferent from B-class that function cool ester enzymes mediated, while the latest was need zinc ion as metalloenzyme (Walkty*et al*. 2020).

2.4.1.1.1Beta-lactamases Class A

These resistances occur in both Gram-positive and Gram-negative bacteria and mostly mediated by plasmid or transposon. Capable usually of being inducible (Walkty*et al*. 2020). This group includes the gram-negative bacteria TEM, SHV,ESBL. ESBL primarily occurs in E. *coli* and *Klebsiella pneumonia* (Lomovskaya*et al*. 2020).

2.4.1.1.2Beta-lactamases Class B

Bacteroides fragilis, observable species of *Aeromonas* and *Legionella*, enzymes that hydrolyze carbapenems, penicillin,and cephalosporins.

2.4.1.1.3Beta-lactamases Class C

Generally seen in Gram-negative bacteria and chromosome localized (Group I, AmpC, etc.) (Jacobs *et al*. 2019). This resistance mechanism is not inhibited by clavulanic acid and has aninducible characteristic so produced high levels the presence of beta-lactam antibiotics (Meini *et al*. 2015). Often known as Inducible Beta-Lactamases (IBL),theyfound in*Enterobacter cloacae*, *Citrobacter freundi*i, S*erratia marcescens*, and P. *aeruginosa*.

2.4.1.1.4Beta-lactamases Class D

These enzymes are induced by beta-lactamases antibiotics and produced in Gram-positive cocci such as *Staphylococcus aureus* so that degrade Oxacillin.

2.4.1.2 Modifications in Penicillin-Binding Proteins (PBP)

Penicillin-binding proteins (PBP) in peptidoglycan synthesis in the back responsible for the antibiotic target of beta-lactam, Carboxypeptidase PBPs, and the enzymes otranspeptidase PBP is the most common in gram-positive bacteria, due to changes in it, resistance results in Methicillin-resistant S. *aureus* (MRSA) is willing to take responsibility for methicillin resistance in strains, mecAgene, this gene results in PBP-2a synthesis enhancing betalactam antibiotic resistance (Kong *et al*. 2010). The modifications in S. *pneumoniae* in PBP 2b are responsible for the resistance to penicillin and cephalosporin (Fisher *et al*. 2016).

2.4.1.3 Modifications in Proteins of the membrane

Change in the porin channels in gram-negative bacteria, for example, P. *aeruginosa* with a devoted channel protein registered in OprD may evolve carbapenem resistance. Antibiotic accumulation can be prevented in the active pump systems cell. Consequently, the group of beta-lactams, tetracyclines, chloramphenicol, and quinolones can lead to resistance (Harkins *et al*. 2017).

2.4.2 Antibiotics Resistance of Aminoglycoside Group

2.4.2.1 Aminoglycosides Modifying Enzymes

The most important mechanism for the emergence of resistance to aminoglycosides in aerobic gram-negative bacteria is enzymatic inactivation. Enzyme modifying has a major role in resistance to aminoglycosides (Naha *et al*. 2020). These enzymes are often of plasmid or transposon origin, there are acetyltransferase and phosphotransferase in this group. Modified enzymes are responsible for the high extent of gentamicin resistance in enterococci (Gil-Gil *et al*. 2020).

2.4.2.2 Ribosomal target Modifications

This approach is crucial in Streptomycin resistance, the target of streptomycin is not connected to the ribosomal 30Ssubunit due to mutations in the ribosomal 30S, in enterococci, this kind of resistance to streptomycin is essential.

2.4.3 The resistance of Tetracyclines

2.4.3.1 Prevention of the absorption of drugs into cells andactive pump systems

Reduction of membrane permeability resulting from spontaneous chromosome mutations in bacteria as a resultof resistance development to prevent drug uptake (Das *et al*. 2020). The organisms also can develop Tetracyclines resistance depending on active pump systems (Bohm *et al.,* 2020).

2.4.3.2 Protection of Ribosome

The second significant mechanism which leads totetracycline resistance. With tetM, tetO, tetQ, tetS genes inhibit drug activity by modifying a cytoplasmic ribosome that binds to the tetracycline. These genes have been found in many genera like Campylobacter, Mycoplasma, Ureaplasma, and Bacteroides, for example. They are plasmid and chromosome origin (Poole, 2007).

2.4.4 The resistance of Macrolide, lincosamide, streptogramins (MLS) groups

Gram-negative bacteria are naturally resistant to MLS group antibiotics

2.4.4.1 Ribosomal Target Modification

This mechanism is most common in Gram-Positive bacteria, in the 50S ribosomal subunit, this is connected tothe drug with the 23S of the ribosome in rRNA-specific methylation of an adenine molecule has structural change and reduces the drug's binding to ribosomal RNA. The resistance is of a structural or inducible type Zhanel *et al*. 2020).

2.4.4.2 Inactivation of drug by Enzymatic activity

The bacterial cells having enzymes that play a critical role in resistance like Erythromycin and other Macrolide resistance.

2.4.5 The resistance of Chloramphenicol

The inactivation of the chloramphenicol acetyltransferase (CAT) by enzymes that acetylate the chloramphenicol antibiotic leads to resistance in bacteria produced by this enzyme. Reduced drug uptake in certain bacteria especially gram-negative can also be responsible for chloramphenicol resistance (Siibak, 2011).

2.4.6 The resistance of Quinolones

There are different mechanisms for quinolone resistance that including

2.4.6.1 Mutation modification of the target topoisomerase

Modifications in the target enzymes topoisomerases caused mainly by mutations that reduce the affinity of quinolones without compromising the enzyme function are the most common mechanism of acquired quinolone resistance and have already been reported in several bacterial species (Munita and Arias, 2016). resistance-related mutations are clustered in discrete regions of the enzyme subunits, calledregions determining quinolone resistance (QRDRs).

2.4.6.2 A decreased intake of drugs by reduced permeability or active efflux

Increased resistance to quinolones in gram-negative bacteriadue to variations in their outer membrane proteins so thatthey reduce the intake of drugs.

2.4.6.3 The target protection of topoisomerase with specific proteins

Target protection is provided by a family of small pentapeptide-repeat proteins, called Qnr proteins, which bind to the targets for topoisomerase and protect them from quinolone interaction (Bansal *et al*. 2017). A similar mechanism has developed in bacteria to protect topoisomerases frommicrocin, which are pentapeptide-repeat family proteins that are produced as a mechanism

of biological competition by certain bacteria and can kill susceptible bacteria by inhibiting their topoisomerases.

2.4.6.4 Inactivation of the drug

The most recently identified mechanism of resistance toquinolones was inactivation by drug modification. Acetylation is performed by a plasmid-encoded AACenzyme variant which, has the ability to acetylate some quinolone molecules in addition to aminoglycosides and have unsubstituted secondary amines such as ciprofloxacin and norfloxacin (Riuz*et al*. 2012).

2.4.7 Resistance of Rifampicin

The high-level resistance develops readily mainly due tochromosomal mutation in most bacteria so developed of stable changes that prevent binding in RNA polymerase (Sithole, 2015). Rifampicin should only be used in association withanother antibacterial drug since the mutation risk is high.Rifampicin resistance is not transferable, and otherantibacterials do not have cross-resistance.

2.4.8 Resistance of Sulfonamide and Trimethoprim

Sulfonamides are para-aminobenzoic acid analogs (PABA)and the dihydropteroate synthesis (DHPS)enzyme andtrimethoprim dihydrofolate reductase (DHFR) metabolic pathways inhibiting tetrahydrofolic acid synthesis in bacteria (Goldstein, 2014). Chromosomal and plasmid-mediated resistance to sulfonamides and trimethoprim. Bacterial expression of the DHPS sulfonamides low affinity plasmid comprising this case is the most commonly observed resistance to sulfonamide (Dowling *et al*. 2017).

2.5 Remedy to Antibiotics Resistance

Antibiotic resistance is an international concern. Broadly, interventions can be categorized into two main approaches. Firstly, there are strategies aimed at protecting the existing antibiotics and preventing the emergence and spread of further resistance. Then, there are strategies aimed at reinvigorating drug development and bringing new antibiotics to market. Alternatives to current antibiotic therapy also need to be assessed, either through the development of new drug classes or through the use of vaccines or other therapeutic strategies (Spellberg *et al*. 2013).

2.6 Strategies to protect antibiotics as a limited resource and prevent the emergence and spread of further resistance.

Globally, the resistance problem has been recognized for many years. Although there are many stakeholders in this issue, the World Health Organisation (WHO) has a global overview. The WHO has held meetings, consultations and workshops since 1971. The WHO's first World Health Assembly on antibiotic resistance was held in 1998 where member states were urged to take action. WHO also targets the veterinary and food sectors by publishing booklets on antibiotics for a food safety perspective, running national and sub-regional workshops and creating an advisory group on integrated surveillance. The World Health Assembly may be a forum through which international collaboration can be facilitated. Most countries have strategies that are based on governance, surveillance, infection prevention and control, regulation, international engagement, communication and research. Effective antibiotic stewardship is required globally, together with better diagnostic tests to identify or rule out infection quickly. Several international groups and societies have been established to tackle antibiotic resistance. One of the most prominent is Action on Antibiotic Resistance (ReAct), an independent international organization funded by the Swedish International Development Cooperation

Agency.It aims to raise awareness and stimulate action on antibiotic resistance. One example of an international awareness campaign is European Antibiotic Awareness Day, held annually on 18 November since 2008 under the auspices of the European Centre for Disease Control (ECDC).

In 2009, the British Society of Antibiotic Chemotherapy (BSAC) convened a working party to consider issues relating to the lack of antibiotic discovery and development. This became known as 'The Urgent Need' or 'TUN' report. It suggested increased funding to support antibiotic research and development and promoted the establishment of a BSAC Chair of Public Engagement in order to increase the public and political awareness of antibiotic resistance and promote dialogue. This has been taken forward under the banner of 'Antibiotic Action,' a UK led global initiative to ensure that we have effective antibiotics in the future. Initiatives with similar aims have been established in the USA under the auspices of the Centers for Disease Control and Prevention (CDC) and the Infectious Diseases Society of America (IDSA) (Boucher *et al*. 2009).In India, the Chennai Declaration aimed to tackle the challenge of antibiotic resistance in a developing nation (Ghafur *et al*. 2013).Until this, the authors of the declaration claimed that there were no functioning national antibiotic policies and no national policy to contain antimicrobial resistance in India. There were no restrictions in purchasing antibiotics and no standardized infection control practices. The first meeting laid out a roadmap for tackling antibiotic resistance. It managed to create awareness among policymakers and the highest authorities on the need of effective antibiotic policies in India. In Asia, the Asian Network for Surveillance of Resistant Pathogens (ANSORP) acts as a centre for research collaboration of infectious diseases and antibiotic resistance. More than 100 hospitals in 14 countries participate. The Australian Antimicrobial Resistance Prevention and Containment Steering Group have set out strategies and mandated Standard 3 of the National Safety and Quality Health Service

(NSQHS) Standards ‘Preventing and Controlling Healthcare Associated Infection’ in all Australian hospitals (Mossialos*et al*. 2015).The Central Asian and European Surveillance on Antibiotic Resistance (CEASAR) is a collaboration initiated in 2012. The first UK strategy against antimicrobial resistance was published over a decade ago and aimed to improve antibiotic prescribing practice and increase funding for drug discovery programmes and research. Some have argued that its impact was limited. In 2013, the Department of Health in England launched

a new Five-Year Antibiotic Resistance Strategy (2015– 2018).It was published as part of a One Health programme, which aimed to address antibiotic resistance in humans, animals, agriculture and the wider environment. Its main objectives were to improve the knowledge and understanding of antibiotic resistance, to conserve and steward the effectiveness of current antibiotics and stimulate the development of new agents, diagnostics and novel therapies. In the strategy

and her annual report, published in February 2013, the Chief Medical Officer in England recommended that antibiotic resistance be placed on the national risk register and seven key priorities were outlined:

1. Optimizing prescribing practices (i.e. antimicrobial stewardship),
2. Improving infection prevention and control,
3. Raising awareness and changing behaviour,
4. Improving the evidence base through research,
5. Development of new drugs/vaccines/other diagnostics and treatments,
6. Improving evidence base through surveillance,
7. Strengthening the UK and international collaboration.

Optimizing antibiotic prescribing has been targeted in both community and hospital settings. Antibiotic stewardship programmes aim to ensure the effective treatment of patients with infection whilst minimizing collateral damage from antimicrobial use (Dellit *et al*. 2007). They do this by optimizing antimicrobial selection, dosing, the route and duration of therapy to maximize clinical cure or prevention of infection while limiting unintended consequences (e.g. emergence of resistance, adverse drug events and costs). Education, audit, guidelines and policies, IV to oral conversion and appropriate de-escalation are all potential elements. These interventions to reduce excessive antibiotic prescribing in hospital inpatients can reduce antimicrobial resistance, hospital-acquired infections and can improve clinical outcomes (Davey *et al*. 2013). Antibiotic cycling or rotating (i.e. the scheduled alternation of various classes of antibiotics) has also been studied, although its benefit is still debated. The goal of antibiotic cycling or rotation is a sustainable decline or stabilization in antimicrobial resistance through successive, prospective alterations in antibiotic selection pressures that prevent the selection of specific resistance mechanisms. Wiesch *et al*.(2014) recently performed a meta-analysis of 46 clinical studies addressing the effect of cycling on nosocomial infections; 11 met their selection criteria (Wiesch*et al*. 2014).They concluded that cycling may be useful in some circumstances, though too long cycling periods could bedetrimental. In the UK, a number of tools are available to support antimicrobial stewardship in primary care. They include 'Target Antibiotics Responsibly, Guidance and Educational Tool' (TARGET), available on the Royal College of General Practitioners website. Another is the 'Stemming the Tide of Antibiotic Resistance' (STAR), an educational programme that includes resources for clinicians to share during public consultation. In 2007, the Health Protection Agency established a multiagency collaboration to improve antimicrobial prescribing in primary care. From this, epidemiological data collections

and primary care directed guidelines were produced (e.g. antibiotic and diagnostic guidance on urinary tract infection). The 'Start smart then focus' programme is an antibiotic stewardship initiative from the UK directed at secondary care. This programme was introduced in England in 2011 but was updated in 2015 as an evidence-based toolkit for hospitals and explains the importance of antimicrobial stewardship for treatment and prophylaxis.New techniques have been developed to aid the diagnosis of infection and/or resistance earlier than conventional culture and sensitivity testing. Biomarkers such as C reactive protein (CRP) or procalcitonin can potentially reduce unnecessary antibiotic use (Schuetz *et al*. 2012).Molecular methods such as polymerase chain reaction(PCR) have allowed earlier detection of MRSA strains and also rifampicin resistance in M. tuberculosis (Drobniewski*et al*. 2000).Multiplex gene detection PCR assays and next generation sequencing are other methods that are being utilized to achieve earlier detection of antibiotic resistance (Greatorex *et al*. 2014).Identification of cultured bacteriathrough mass spectrometry (e.g. by Matrix-Assisted Laser Desorption Ionization Time of Flight (MALDIToF)) has reduced the time to identification of organisms compared with conventional biochemical means. Automated susceptibility testing also has the potential to deliver results more quickly.

2.7 Reinvigorating drug development pathways and bringing new antibiotics into market

The need for new antibiotics was illustrated in the TUN report. Among the aspects that need addressing is the failure of new drug discovery (described above).In addition, increasing levels of bureaucracy and lack of clarity within regulatory frameworks and variation in the clinical trials process in different countries hinder the development of new agents. Several antimicrobials have failed to reach the market at this final hurdle. Lack of international harmonization,

continual changes to processes and ineffective pathways for dialogue between organizations, industry and regulators are all significant deterrents to the research and development of new antibiotics.

However, it is clear that there is now political engagement with this issue and many initiatives are now ongoing around the world. In 2003, the IDSA launched the 'Bad Bugs, No Drugs' campaign with recommendations to Congress, the Food & Drug Administration and the National Institute for Allergies & Infectious Diseases (Boucher *et al*. 2009).In 2009, the EU, under the presidency of the Swedish Government, launched the 'Innovative Incentives for Effective Antibacterials' programme (Mossialos *et al*. 2015).In 2010, the IDSA produced a report entitled 'The 10 × 20 Initiative: Pursuing a Global Commitment to Develop 10 New Antibacterial Drugs by 2020'.This initiative aspires to develop 10 new antibiotic agents by 2020.A number of novel approaches to reinvigorate antibiotic development have been proposed. One area being widely discussed in the concept of 'delinking', where the pharmaceutical company revenue from antibiotics is not directly dependent on absolute sales of these agents. Public–private partnerships could beset up to mitigate the up-front costs of drug discovery. Pathogen-targeted approaches could be developed to optimize efficacy against a single pathogen/resistance mechanism. Orphan drug legislation could help address the issue of needing large numbers of patients in clinical trials, thus shortening the length of a trial. Other examples laid out in the English Chief Medical Officer's Annual Report to foster research and development of new drugs include research-related tax incentives, patent buyouts, health impact funds and funding of translational research. The WHO held a summit in 2011 entitled 'No action today, no cure tomorrow'. In Europe, the 'New Drugs 4 Bad Bugs' initiative is a series of programmes that were set up by the Innovative Medicines Initiative (IMI) in the EU and was designed to directly address some of the scientific

challenges associated with antibacterial drug discovery and development. One element of this is the DRIVE-AB programme, which aims to produce and review economic models for the various ways that funding of antibiotic drug development could be undertaken. In the USA, the Generating Antibiotics Incentives Now (GAIN) Act was enacted in 2012. This provided a pay-out at the end of the development process with 5 years of guaranteed market exclusivity and priority review for antibiotics that target certain qualifying pathogens. The President's Council of Advisors on Science and Technology (PCAST) reported about antimicrobial resistance in September 2014and, in the UK, the Prime Minister asked Dr Jim O'Neill, an economist, to chair a review of the economic impact of antibiotic resistance and consider possible solutions. It is due to produce a final report in 2016, but the initial findings have been published and include a proposal to set up a global antimicrobial resistance innovation fund to boost the number of early research ideas, ensuring that existing drugs are used appropriately, improving the use of diagnostics wherever they can make a difference, attracting and retaining a high calibre skills base and modernizing the surveillance of drug resistance globally (O'Neill, 2015).

2.8 Bacteria Plasmids

Bacterial plasmids have a major impact on metabolic function. Lactose fermentation of *E. coli* or hemolysin B transporter expressed by the plasmids that carry these respective genes could be readily obviated by heterocyclic compounds that readily bind to plasmid DNA. These compounds could also reverse the resistance to antibiotics of *E. coli*, *Enterobacter*, *Proteus*, *Staphylococcus* and *Yersinia* strains by eliminating plasmids. However, the frequency and extent of this effect was significantly less than might have been expected based on a complex interaction with plasmid DNA. The effects of heterocyclic compounds on the plasmids responsible for the virulence of *Yersinia* and *A. tumefaciens*, or on nodulation, nitrogen fixation

of *Rhizobia* accounted for the elimination of 0.1 to 1.0 % of plasmids present in the populations studied. Bacterial plasmids can be eliminated from bacterial species grown as pure or mixed bacterial cultures in the presence of sub-inhibitory concentrations of non-mutagenic heterocyclic compounds.

The antiplasmid action of the compounds depends on the chemical structure of amphiphillic compounds having a planarring system with substitution in the L-molecular region. A symmetrical p-electron conjugation at the highest occupied molecular orbitals favours the antiplasmid effect. The antiplasmid effect of heterocyclic compounds is expressed differentially in accordance with the structural form of th eDNA to which they bind. In this manner "extrachromosomal" plasmid DNA that exists in a superhelical state binds more compound than its linear or open-circular form; and least to the chromosomal DNA of the bacterium, that carries the plasmid. It can also be noted that these compounds are not mutagenic and their antiplasmid effects correlate with the energy of HOMO-orbitals. Plasmid elimination is considered also to take place in ecosystems containing numerous bacterial species. This opens up a new perspective in rational drug design against bacterial plasmids. The inhibition of conjugational transfer of antibiotic resistance plasmid can be exploited to reduce the spread of antibiotic resistance plasmid in the ecosystem. Inhibition of plasmid replication at various stages, as shown in the "rolling circle" model (replication, partition, conjugal transfer) may also be the theoretical basis for the elimination of bacterial virulence in the case of plasmid mediated pathogenicity and antibiotic resistance.

2.9 Importance of Plasmids

Membrane transporters can be encoded by genes localized on the chromosomes and on plasmids such as haemolysin and tetracycline transporters (Rosenberg *et al*. 2000). The multidrug membrane transporters are classified into two main groups such as ABC transporters and proton pump systems based on energetic requirements and the second class is sub-divided into a large number of subclasses. Some transporters such as the tetracycline efflux protein mediate the extrusion of the particular antibiotic. This active efflux is important in ensuring a significant level of resistance to tetracycline or other antibiotics. In contrast the *tet*-transporters are multidrug-transporters, which confer resistance against a wide variety of structurally unrelated compounds (Lewis *et al*. 1997; Baranova *et al*. 1997). The mdr-transporters can be inhibited a wide variety of compounds such as uncouplers and calcium antagonists. The proton motive force utilizing efflux pumps is sensitive to compounds that dissipate the proton gradient inthe membrane. These pumps mediate the efflux of xenobiotics in a coupled exchange with protons. The majority of multidrug transporters use ATP as the energy to pump the antibiotics out of the cells, while the second largest groups of transporters utilize the transmembrane proton gradient to drive the antibiotics or other xenobiotics out of the cells. Several subclasses belong into this group (Paulsen *et al*. 1996; Brown *et al*. 1999). Experiments suggested that drug resistance by bacteria and cancer cells can be achieved in various ways, however the inhibition of efflux pump systems is the most promising mechanism in this respect because the intracellular concentration of antibiotics is enhanced in all individual cells of the population simultaneously and at much lower concentration of resistance modifier compounds than is needed for plasmid elimination. This resistance modifying effect is apparently independent from the antibacterial or cytotoxic effects.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Ethical Approval

Ethical approval with reference number (RP/REC/2021/338) was obtained from the Edo State Health Research Ethics Committee

3.2 Collection of Samples

A total of 50 swabs were obtained from the hospital environment of the University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria and were aseptically manipulated using standard microbiological techniques.

3.3 Media Preparation and Sterilization

All media used were prepared following manufacturer's specification. Sterilization of glassware and other autoclavable materials was done at 121°C at 15psi for 15 min. Work surfaces was sterilized using 3% bleach.

3.4 Identification and Characterization of Isolates

3.4.1 Cultural Characterization

The cultural characteristics of the bacterial isolates were observed on nutrient agar plates. The characteristics observed included size, shape, transparency, elevation, margin and color.

3.4.2 Morphological Characterization

3.4.2.1 Gram Staining

Gram staining was done to differentiate the Gram-positive and Gram-negative isolates and the procedure was carried out according to Cheesbrough, 2009. A smear was prepared and heat fixed on the slide, then crystal violet (primary stain) was applied on the fixed smear for 60 sec, the stain was then washed with distilled water, Gram's iodine was added onto the smear for 60 sec,

it was poured off and rinsed with distilled water, a few drops of decolorizer (95% ethyl alcohol) was added and washed with distilled water to remove the alcohol, finally Safranin (secondary stain) was added for 30 sec and washed with distilled water, the smear was then allowed to air dry. After drying the slide, it was mounted under the microscope and observed.

3.4.3 Biochemical characterization

3.4.3.1 Catalase production test

Using a sterilized inoculating loop, a small amount of culture from the nutrient agar Petri plates was picked and a sterile dropping pipette was then used to place a drop of 3% hydrogen peroxide on the smear. The fluid on the smear was observed for the appearance of gas bubbles as this is an indication of presence of catalase. Effervescence of gas indicates the presence of Gram-positive bacteria and this is as a result of the hydrogen peroxide been broken down into oxygen and water.

3.4.3.2 Indole test

Indole test helps to determine the ability of bacterial species to convert tryptophan into Indole. Pure isolated bacterial colony was smeared on a Whatman paper, five drops of Kovac's Indole reagent was added to the Whatman paper. A positive result is indicated by the presence of red or red-violet coloration after 10 sec.

3.4.3.3 Oxidase test

This test is used to rapidly identify Gram-negative bacteria, in the presence of the enzyme cytochrome oxidase the bacteria is able to convert N, N-dimethyl-p-phenylenediamine oxalate and α-naphthol to indophenol blue. To carry out this test, few drops of 1% aqueous solution of tetra methyl-p-phenylenediamine hydrogen chloride (oxidase reagent) was added to a piece of Whatman filter paper and smeared with a few colonies obtained from the pure culture broth. A positive result is indicated by the presence of a purple color and a negative result appears yellow.

3.4.3.4 Citrate utilization test

This test detects the ability of an organism to use citrate as the sole source of carbon and energy. Isolates was picked up using a sterilized wire loop and inoculated onto Simmons citrate agar (SCA) and incubated overnight at 37°C, a citrate positive organism changes color from green to blue.

3.4.3.5 Coagulase Test

This test was carried out because of the presence of positive cluster cocci. A drop of normal saline was put on a slide; colony of the test organism was emulsified in the saline. A drop of human plasma was added and mixed gently. After about 10seconds, it was observed for clotting. The presence of agglutination means a positive result, no agglutination means a negative result (Tortora *et al.,* 2013).

3.4.3.6 Urease production test

To determine the ability of a bacterium to produce urease, an enzyme that breaks down urea to release ammonia, the test is done with the bacteria been inoculated onto urea agar base (UAB) supplemented with urea in slants and incubated for 24h at 37^{o}C. A color change of the colorless agar to pink indicates a positive reaction.

3.4.4 Sugar fermentation test

This test is used in identification of bacteria isolates that are able to grow in the presence of glucose, sucrose and lactose. The bacteria isolates were inoculated into test tubes with triple sugar iron agar (TSI) and incubated at 37°C and the observation was taken 24h after inoculation.

3.4.5 Motility test

This test is used to determine if the isolate is motile or not. Motility agar with the following composition; Glucose: 20 g, Agar: 2 g, Distilled water: 1 l was prepared, dispensed in 15 ml

amounts into test tubes and sterilized at 121^0C for 15 minutes. On cooling, the semi-solid agar was stab inoculated with the pure culture of the isolate using a straight platinum wire and incubated at 28% ± 2^0C for 24 hr. Spreading growth of the isolate out of the line of stab that renders the medium slightly opaque indicate a positive result. A negative result is indicated by growth confined to the stab.

3.5 Antibiotics Susceptibility Test

Bacteria isolates were subjected to antibiotics sensitivity test using the Kirby Bauer disc diffusion method on Muller Hinton agar (Oxoid, UK) plates. The antibiotics used were OFX-Ofloxacin, PEF-Reflacine, CPX-Ciprofloxacin, AU-Augmentin, CN-Gentamycin, S-Streptomycin,CEP-Ceporex, NA-Nalidixic acid, SXT-Septrin, PN-Amplicin, NB-Norfloxacin, AML-Amoxyl, E-Erythromycin, CH-Chloramphenicol, APX-Ampiclox,LEV-Levofloxacin,AM-Ampicillin, FOX-Cefoxitin, AMX-Amoxicillin and CAZ-Ceftazidime

3.6 Plasmid Curing

Plasmid curing was carried out on the resistant isolates. This was carried out following methods previously described by (Raghada *et al*. 2013). Briefly, 10ml of each bacterial culture inoculated into peptone water and incubated for 24hrs was introduced into a set of 8 test tubes, respectively. The test tubes were compared with McFarland's standard. Ethidium bromide and or acridine orange in various concentrations of 0, 25, 50, 100, 200, 400, and 600 µl/ml were then introduced accordingly into the test tubes and incubated for 24 hrs at 37ºC to determine the sub-lethal concentrations of ethidium bromide and or acridine orange. After 24hrs of incubation, 1ml aliquot from each test tube was inoculated onto nutrient agar plates and incubated, after which colonies were selected and inoculated onto freshly prepared Muller Hinton agar plates. Then, antibiotic discs of prior resistance were aseptically introduced into the plates, ensuring that the

discs made appropriate contact with the surface of the agar. These were incubated for 24hrs at 37ºC after which plates were examined for cured colonies.

3.7 Multiple Antibiotic Resistance (MAR) index

MAR index is a handy tool used in assessing and identifying health risk factors caused by isolates either from high or low use of antibiotics. The MAR index is calculated by dividing the number of resistant isolates by the total number of antibiotics used.

3.8 Data Analysis

Data generated were managed and analyzed with descriptive statistics and Chi-square tests using SPSS version 21 while the level of significance was set at 0.05 (95%).

CHAPTER FOUR

RESULT

Table 4.1 shows the cultural, morphological and biochemical characteristics of clinical bacteria isolates. The result revealed that S. *aureus,* S. *epidermidis,* S. *hominis,* E. *coli* and K. *pneumonia* were the various isolates obtained from University of Benin Teaching Hospital (UBTH).

Table 4.2 and 4.3 shows the antibiogram of bacteria isolates. The result revealed that all isolates were susceptible to Levofloxacin and Chloramphenicol. *Corynebacterium jeikeium*showed resistance to Streptomycin and Amoxicillin, K. *pneumonia* showed resistance to NB. All S. *epidermidis,* showed no resistance to any of the antibiotics tested likewise E. *coli* and S. *aureus.*

Table 4.4 shows the multiple antibiotics resistance index of bacteria isolates. The result revealed *Corynebacterium jeikeium* had and index of 0.2 which is the limit, K. *pneumonia* had an index of 0.1 and other were zero indicating these isolates do not pose threat to public health as they are not multidrug resistant and as such, plasmid curing was not carried out on these bacteria isolates.

Table 4.1: Cultural, morphological and biochemical characteristics of bacteria isolates

	EveI	**EveII**	**EveIII**	**EveIV**	**PreI**	**PreII**	**PreIII**	**PreIV**	**DebI**	**DebII**	**DebIII**	**DebIV**
Morphological characteristics												
Shape	Cocci	Cocci	Short rod	Cocci	Cocci	Rod	Rod	Cocci	Cocci	Cocci	Rod	Rod
Cell arrangement	Cluster	Cluster	Cluster	Cluster	Cluster	Singly	Singly	Cluster	Cluster	Cluster	Singly	Singly
Gram stain	+	+	+	+	+	-	-	+	+	+	-	-
Cultural characteristics												
Size	2mm	1mm	Pinpoint	1mm	2mm	3mm	3mm	1mm	2mm	1mm	3mm	3mm
Shape	Round	Round	Club-like	Round	Round	Round	Round	Round	Round	Round	Round	Round
Elevation	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised	Raised
Margin	Smooth	Smooth	Rough	Smooth entire	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Color	Yellow	White	Greyish white	White	Yellow	Greyish white	Greyish white	White	Yellow	White	Greyish white	Greyish white
Biochemical test												
Catalase	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	+	+	+	-	-	+	+	-	+	-	+
Mannitol	+	+	+	-	+	+	+	+	+	+	+	+
Motility	-	-	-	-	-	-	+	-	-	-	-	+
Coagulase	+	-	-	-	+		-	-	+	-	-	-
Citrate	+	-	+	-	+	+	-	-	+	-	+	-
TSI												
H_2s^-	-	+	-	+	-	-	-	+	-	+	-	-
Gas	-	+	+	+	-	+	+	+	-	+	+	+
Sucrose	+	+	-	+	+	+	+	+	+	+	+	+
Lactose	+	+	-	+	+	+	+	+	+	+	+	+

Glucose	+	+	-	+	+	+	+	+	+	+	+	+
Haemolysis	B	-		-	B	Y	-	-	B	-	Y	-
Putative Identity	S. *aureus*	S.*epidermidis*	*Corynebacterium jeikeium*	S. h*ominis*	S. *aureus*	K. *pneumonia*	E. *coli*	S.*epidermidis*	S. *aureus*	S.*epidermidis*	K. *pneumonia*	E. *coli*

Table 4.2: Antibiotics susceptibility pattern of bacterial isolates

	CN	LEV	E	CPX	CH	NB	S	AMX	RD	APX
S. *epidermidis*	15	25	20	25	19	15	17	20	22	19
S. *epidermidis*	25	25	25	25	23	20	23	18	25	22
Corynebacterium jeikeium	25	25	28	25	20	17	10	11	23	15
S. *epidermidis*	23	21	25	25	21	19	23	25	22	17
K. *pneumonia*	24	20	19	25	21	19	23	25	22	17
S. *aureus*	20	25	19	25	21	20	19	24	20	20
S. *hominis*	20	22	25	23	20	21	20	22	22	19
E. *coli*	16	21	18	20	20	23	20	21	20	22
S. *aureus*	20	22	20	17	18	18	21	19	25	15
K. *pneumonia*	20	20	15	18	21	12	20	20	18	20
S. *aureus*	20	21	20	25	22	18	16	20	21	17
S. *hominis*	19	25	25	25	25	16	20	23	24	16
E. *coli*	12	20	24	20	23	22	20	23	16	24

Key <14=Resistant (R), 14-17=Intermediate (I) and >17=Susceptible (S)

CN=Gentamycin, LEV=Levofloxacin, E=Erythromycin, CPX=Ciprofloxacin, CH=Chloramphenicol, NB=Norfloxacin, S=Streptomycin, AMX=Amoxicillin, RD=Rifampicin, and APX=Ampiclox

Table 4.3: Antibiotics susceptibility pattern of bacterial isolates

	CN	LEV	E	CPX	CH	NB	S	AMX	RD	APX
S. *epidermidis*	I	S	S	S	S	I	I	S	S	S
	S	S	S	S	S	S	S	S	S	S
S. *epidermidis*										
Corynebacterium jeikeium	S	S	S	S	S	I	R	R	S	I
S. *epidermidis*	S	S	S	S	S	S	S	S	S	I
K. *pneumonia*	S	S	S	S	S	S	S	S	S	I
S. *aureus*	S	S	S	S	S	S	S	S	S	S
S. *hominis*	S	S	S	S	S	S	S	S	S	S
E. *coli*	I	S	S	S	S	S	S	S	S	S
S. *aureus*	S	S	S	I	S	S	S	S	S	I
K. *pneumonia*	S	S	I	S	S	R	S	S	S	S
S. *aureus*	S	S	S	S	S	S	I	S	S	I
S. *hominis*	S	S	S	S	S	I	S	S	S	I
E. *coli*	R	S	S	S	S	S	S	S	I	S

Key <14=Resistant (R), 14-17=Intermediate (I) and >17=Susceptible (S)

CN=Gentamycin, LEV=Levofloxacin, E=Erythromycin, CPX=Ciprofloxacin, CH=Chloramphenicol, NB=Norfloxacin, S=Streptomycin, AMX=Amoxicillin, RD=Rifampicin, and APX=Ampiclox

Table 4.4: Multiple Antibiotics Resistance (MAR) Index of bacteria isolates

Isolates	No. of Resistant	No. of antibiotics	Resistant antibiotics	MAR-Index
S. *epidermidis*	0	10	-	0.0
S. *epidermidis*	0	10	-	0.0
Corynebacterium jeikeium	2	10	S and AMX	0.2
S. *epidermidis*	0	10	-	0.0
K. *pneumonia*	0	10	-	0.0
S. *aureus*	0	10	-	0.0
S. *hominis*	0	10	-	0.0
E. *coli*	0	10	-	0.0
S. *aureus*	0	10	-	0.0
K. *pneumonia*	1	10	NB	0.1
S. *aureus*	0	10	-	0.0
S. *hominis*	0	10	-	0.0
E. *coli*	0	10	-	0.0

CHAPTER FIVE

DISCUSSION

The control of infectious diseases is badly endangered by the rise in the number of microorganisms that are resistant to antimicrobial agents. This is because infections caused by resistant microorganisms often fail to respond to conventional treatment, resulting in prolonged illness and greater risk of death. Increased in antimicrobial resistance has made it necessary for the generation of an up-to-date information on antibiotic resistance patterns of bacterial isolate in order to determine appropriate empirical therapy. Findings in this research have shown that S. *aureus,* S. *epidermidis,* S. *hominis,* E. *coli* and K. *pneumonia* were present in clinical samples obtained from University of Benin Teaching Hospital (UBTH). These pathogens have also been reported by other researchers (Amsalu *et al.,* 2016) who reported that the most common bacteria isolated were E. *coli*, S. *aureus* and *Klebsiella* spp. which is in line with this study. The isolation rate of E. *coli* as well as other pathogens in this study is in conformity with previous studies conducted (Alemu *et al*., 2012; Kabew*et al*., 2013)

With regard to the antimicrobial resistance pattern of isolates the results revealed that all isolates were susceptible to Levofloxacin and Chloramphenicol. *Corynebacterium jeikeium* showed resistance to Streptomycin and Amoxicillin, K. *pneumonia* showed resistance to Norfloxacin. All S. *epidermidis,* showed no resistance to any of the antibiotics tested likewise E. *coli* and S. *aureus.* Thess findings however showed contrast to findings of Alemu *et al*., (2012); Muluye*et al*., (2013); Abejew and Denboba, (2014) who reported high resistance rate by *Klebsiella* spp. to gentamicin, chloramphenicol, cotrimoxazole and ampicillin, S. *aureus* being resistant to ampicillin and penicillin, Esayas*et al*., (2014) reported resistance to ceftriaxone and amoxicillin clavunilic acid.

Multiple antibiotics resistance index of bacteria isolates in this study showed that *Corynebacterium jeikeium* had an index of 0.2 which is the limit, K. *pneumonia* had an index of 0.1 and other were zero indicating these isolates do not pose threat to public health as they are not multidrug resistant and as such, plasmid curing was not carried out on these bacteria isolates. Contrary to this study, Amsalu *et al.,* (2016) observed multi-drug resistance in isolated bacteria. This marked difference in resistance pattern of bacterial isolates in this study might be due to the difference in operational definition of multidrug resistance and also the class of antibiotics used for testing. Nevertheless, this study has some limitations in light that the nature of the study the result may not represent the general population live in the study area.

CONCLUSION

Despite the hype in the increase of drug resistance among bacteria isolates, findings in this study have shown that clinical isolates could still be susceptible to a plethora of conventional antibiotics. So many researchers have reported the presence of multidrug resistant bacteria in clinical samples which may be due to the choice of antibiotics used for testing and treatment. Hence, before carrying out susceptibility test and further treatment with any antibiotic's regimen, proper diagnose should be carried out and susceptibility testing should be pathogen specific.

REFERENCES

Bansal, S., Bajaj, P., Pandey, S. and Tandon, V. (2017). Topoisomerases: Resistance versus sensitivity, how far we can go. *Medicinal research reviews* **37**(2):404-438.

Baranova, N.N. and Neyfakh, A.A. (1997). Antimicrobial Agents. *Antimicrobial Agents and Chemotherapy* **41**:1396-1398.

Bereket W, Hemalatha K, Getenet B, Wondwossen T, Solomon A, Zeynudin S, Kannan S. (2012). Update on bacterial nosocomial infections. *European Review for Medical and Pharmacological Science* **16**:1039-1044.

Böhm, M. E., Razavi, M., Marathe, N. P., Flach, C. F. and Larsson, D. J. (2020). Discovery of a novel integron-borne aminoglycoside resistance gene present in clinical pathogens by screening environmental bacterial communities. *Microbiome* **8**(1):1-11.

Brown, M.H., Paulsen, I.T. and Skuarry, R.A. (1999).Molecular microbiology and its application. *Molecular Microbiology* **31:**394-395

Cantón, R., Akóva, M. and Carmeli, Y. (2012). Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clinical Microbiology andinfection* **18**:413–31.

Costelloe, C., Metcalfe, C. and Lovering, A. (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *British Medical Journal* **340**:2096-2117.

Das, B., Verma, J., Kumar, P., Ghosh, A. and Ramamurthy, T. (2020). Antibiotic resistance in *Vibrio cholerae*: understanding the ecology of resistance genes and mechanisms. *Vaccine* **38**: 83-92.

Dasmeh, M., Baserisalehi, M. and Emami, A. (2015). Plasmid Curing Assay in Clinical Isolates of Antibiotic Resistant *Acinetobacterbaumannii*. *Microbiology Journal* **5**: 43 – 48.

Davey, P., Brown, E. and Fenelon, L. (2005) Interventions to improve antibiotic prescribing practices for hospital inpatients. *Clinical Infectious Diseases* **48**(1):1–12.

De Oliveira, D. M., Forde, B. M., Kidd, T. J., Harris, P. N., Schembri, M. A., Beatson, S. A. and Walker, M. J. 2020. Antimicrobial Resistance in ESKAPE Pathogens.*Clinical Microbiology Reviews* **33**(3):17-19.

Dellit, T.H., Owens, R.C. and McGowan, J.E. (2007). Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America Guidelines for Developing an Institutional Program to Enhance Antimicrobial Stewardship. *Clinical Infectious Diseases* **44**: 159–177.

Drobniewski, F.A., Watterson, S.A. and Wilson, S.M. (2000). A clinical, microbiological and economic analysis of a nationalUK service for the rapid molecular diagnosis of tuberculosis and rifampicin resistance in *Mycobacterium tuberculosis*. *Journal of Medical Microbiology* **49**:271–8.

Dromigny, J.A. and Perrier-Gros-Claude, J.D. (2013).Antimicrobial resistance of Salmonella enterica serotype Typhi in Dakar, Senegal. *Clinical Infectious Diseases* **37**:465-466.

Elizabeth, N.,Mbim, I., Clement, I.,Mboto, I. and Bassey, E. (2016). A Review of Nosocomial Infections in Sub-Saharan Africa. *British Microbiology Research Journal* **15**(1): 1-11.

Fernández-Villa, D., Aguilar, M. R., and Rojo, L. (2019). Folic Acid Antagonists: Antimicrobial and Immunomodulating Mechanisms and Applications. *International Journal of Molecular Sciences* **20**(20):4996-5046.

Fisher, J. F. and Mosbashery, A.S. (2016) B-Lactam Resistance Mechanisms: Gram-Positive Bacteria and*Mycobacteriumtuberculosis*. *Cold Spring Harbor Perspectives in Medicine* **6**(5): 34-87.

Gil-Gil, T., Martínez, J. L. and Blanco, P. (2020). Mechanisms of antimicrobial resistance in *Stenotrophomonas maltophilia*: A review of current knowledge. *Expert Review of Anti-infective Therapy* **18**(4):335-347.

Goldstein, B. P. (2014). Resistance to rifampicin: A review. *The Journal of antibiotics* **67**(9):625-630

Greatorex,J., Ellington, M.J. and Köser, C.U. (2014). Newmethods for identifying infectious diseases. *British Medical Bulletin* **112**:27–35.

Guo, R., Li, K., Qin, J., Niu, S. and Hong, W. (2020). Development of Polycationic Micelles as an Efficient Delivery System of Antibiotics Overcoming Biological Barriers to Reverse Multidrug Resistance in *Escherichia coli*. *Nanoscale* **22**:20-78.

Harkins, C. P., Pichon, B., Doumith, M., Parkhill, J., Westh, H., Tomasz, A., de Lencastre, H., Bentley, S. D., Kearns, A. M. and Holden, M. (2017). Methicillinresistant *Staphylococcusaureus* emerged long before the introduction of methicillin into clinical practice. *Genome Biology* **18**(1):130.

Heinz, E., Ejaz, H., Scott, J. B., Wang, N., Gujaran, S., Pickard, D. and Strugnell, R. A. (2019). Resistance mechanisms and population structure of highly drug resistant Klebsiella in Pakistan during the introduction of the carbapenemase NDM-1. *Scientific Reports* **9**(1):1-13.

Heln, G. H., Talbot, J.S. Bradley, J.E. Edwards, G.J., Lous, B., Rice, S. and John, B. (2013).The Chennai declaration: A roadmap to tackle the challenge of antimicrobial resistance. *Indian Journal of Cancer* **50**(1):71-73.

Iroha, I., Nwakaeze, E., Ejikeugwu, C., Oji, A. and UduIbiam, E. (2013) Frequency and antibiogram of uropathogens isolated from urine samples of HIV infected patients on antiretroviral therapy. *American Journal of BioScience* **1**: 50-53.

Jacobs, M. R., Abdelhamed, A. M., Good, C. E., Rhoads, D. D., Hujer, K. M., Hujer, A. M. and Kreiswirth, B.N. (2019). ARGONAUT-I: activity of cefiderocol (S-649266), a siderophore cephalosporin, against Gram-negative bacteria, including carbapenem-resistant nonfermenters and Enterobacteriaceae with defined extended-spectrum -lactamases and carbapenemases. *Antimicrobial Agents and Chemotherapy* **63**(1):1801-1818.

Johnson, A.P. and Woodford, N. (2013). Global spread of antibiotic resistance: the example of New Delhi metallo-β-lactamase (NDM)-mediated carbapenem resistance. *Journal of Medical Microbiology* **62**:499–513.

Kabir, J.P.S., Gill, S., Singh, H.S., Rissam, S., Todi, B.M., Hegde, P.(2011). Antibiotics: mode of action and mechanisms of resistance. *National library of Medicine*. Formatex Research Center: Badajoz, Spain. 536-545pp.

Kong, K. F., Schneper, L. and Mathee, K. (2010). Betalactam antibiotics: from antibiosis to resistance and bacteriology. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica* **118**(1):1-36.

Kumarasamy, K.K., Toleman, M.A. andWalsh, T.R. (2010). Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: A molecular, biological, and epidemiological study. *The Lancet Infectious Diseases* **10**:597–602.

Lau, S.H., Kaufmann, M.E. and Livermore, D.M. (2008). UK epidemic Escherichia coli strains A-E, with CTX-M-15 beta-lactamase, all belong to the international O25: H4-ST131 clone. *Journal ofAntimicrobialChemotherapy* **62**: 1241–1244.

Lee, D.S., Lee, S.J. and Cho, H.S. (2018). Community Acquired Urinary Tract Infection by *Escherichiacoli* in the Era of Antibiotic Resistance. *BioMedical Resource International* **34**:23-67.

Lewis, K., Hooper, D.C. and Ouellette, M. (1997). Cyclosporine analog inhibit *in vitro* growth of *Crytosporodium parvum*. *American Society for Microbiology News* **63**:605-610.

Li, H., Luo, Y. F., Williams, B. J., Blackwell, T. S. and Xie, C.M. (2012). Structure and function of OprD protein in *Pseudomonas aeruginosa*: from antibiotic resistance to novel therapies. *International Journal of Medical Microbiology* **302**(2):63-68.

Li, T., Liu, C., Lu, J., Gaurav, G. K. and Chen, W. (2020). Determination of how tetracycline influences nitrogen removal performance, community structure, and functional genes of biofilm systems. *Journal of the Taiwan Institute of Chemical Engineers* **106**:99-109.

Lomovskaya, O., Nelson, K., Rubio-Aparicio, D., Tsivkovski, R., Sun, D. and Dudley, M. N. (2020). Impact of intrinsic resistance mechanisms on potency of QPX7728, a new ultrabroad-spectrum beta-lactamase inhibitor of serine and metallo-betalactamases in Enterobacteriaceae, *Pseudomonas aeruginosa,* and *Acinetobacter baumannii. Antimicrobial Agents and Chemotherapy* **64**(6):50.

Mbim, E. N., Mboto, C. I. and Agbo, B. E. (2016). A Review of Nosocomial Infections in Sub-Saharan Africa. *British Microbiology Research Journal* **15**(1): 1-11.

Meini, M. R., Llarrull, L. I. and Vila, A. J. (2015). Overcoming differences: the catalytic mechanism of metallo- -lactamases. *FEBS letters* **589**(22):3419- 3432

Munita, J. M. and Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Virulence mechanisms of bacterial pathogens*. Springer publishers. USA. 481-511pp.

Naha, A., Miryala, S. K., Debroy, R., Ramaiah, S. and Anbarasu, A. (2020). Elucidating the multi-drug resistance mechanism of Enterococcus faecalis V583: A gene interaction network analysis. *Gene* **144**:704.

Nikaido, H. and Pagès, J. M. (2012). Broad-specificity efflux pumps and their role in multidrug resistance of Gram-negative bacteria. *FEMS Microbiology Reviews* **36**(2):340-363.

O'Neill, J. (2015).The Review on Antimicrobial Resistance. Tackling a Global Health Crisis: Initial Steps. *PLOS* **60**:23-45.

Paulsen, I.T., Brown, M.H. and Skurray, R.A. (1996).Proton dependent multidrug efflux systems. *Microbiology Reviews* **60:**575-608.

Pérez-Llarena, F. J., and Bou, G. 2016. Proteomics as a tool for studying bacterial virulence and antimicrobial resistance. *Frontiers in Microbiology* **7**:410.

PiaAbel, Z., Wiesch, R. K., So¨ren, A., Wolfgang, V. and Sebastian, B. (2014). Cycling Empirical Antibiotic Therapy in Hospitals: MetaAnalysis and Models. *PLOS Pathogens* **10**(6): 1-13.

Poole, K. (2007). Efflux pumps as antimicrobial resistance mechanisms. *Annals of Medicine* **39**(3):162-176.

Prashanth, K., Vasanth, T., Saranathan, R., Makki, A. R. and Pagal, S. (2012). Antibiotic resistance, biofilms and quorum sensing in *Acinetobacter* species. *Journal of Antimicrobial Chemotherapy* **33**:179-212.

Rooney, P.J., O'Leary, M.C. and Loughrey, A.C. (2009). Nursing homes as a reservoir of extended-spectrum beta-lactamase (ESBL)-producing ciprofloxacin-resistant *Escherichiacoli*. *Journal of Antimicrobial Chemotherapy* **64**:635–641.

Rosenberg, E.M., Ma, D. and Nikaido, H. (2000). The Rosenberg self-esteem scale: its dimensionality, stability and personality correlates in Estonian. *Journal of Bacteriology* **182**:754-756.

Ruiz, J., Pons, M. J. and Gomes, C. (2012). Transferable mechanisms of quinolone resistance. *International Journal of Antimicrobial Agents* **40**(3):196-203.

Russotto, V., Cortegiani, A., Raineri, S. M., Iozzo, P., Gregoretti, C. and Giarratano, A. (2015). What is the risk of acquiring bacteria from prior intensive care unit bed occupants? *Critical Care* **10**:21-55.

Schleif, R.F. and Wensimk, P. (1981). Methods in molecular biology application. *Practical Methods in Molecular Biology* **10**: 4612-5956.

Schuetz, P., Briel, M. and Christ-Crain, M. (2012). Procalcitonin to guide initiation and duration of antibiotic treatment in acute respiratory infections: an individual patient data meta-analysis. *Clinical Infectious Diseases* **55**:651-662.

Shaikh, S. A., Jain, T., Sandhu, G., Latha, N. and Jayaram, B. (2007). From drug target to leads-sketching a physicochemical pathway for lead molecule design in silico. *Current Pharmaceutical Design* **13**(34):3454- 3470.

Sharkey, L. K., and O'Neill, A. J. (2019). Molecular Mechanisms of Antibiotic Resistance Part II. *Bacterial Resistance to Antibiotics from Molecules to Man*. USA. 27-50pp.

Sithole, B. (2015). Peptide-antibiotic conjugates as novel developments for increased antimicrobial transport and efficiency. *Antibiotics Application*. University of York. USA. 96-144pp.

Spellberg, B., Bartlett, J.G. and Gilbert, D.N. (2013). The future of antibiotics and resistance. The *New England Journal of Medicine* **368**:299–302.

Tan, J., Tay, J., Hedrick, J. and Yang, Y. Y. (2020). Synthetic macromolecules as therapeutics that overcome resistance in cancer and microbial infection. *Biomaterials* **45**: 23-67.

Tevors, J.T. (1986). Plasmid curing in bacteria. *Fems Microbiology Letters* **32**: 149-157.

Tortora, G. J., Funke, B. R. and Christine, L. (2013). Coagulase test procedure. *Microbiologia.* Porto. 827pp.

Vincenzo, R., Andrea, C., Santi, M. R. and Antonino, G. (2015). Bacterial contamination of inanimate surfaces and equipment in the intensive care unit. *Journal of Intensive Care* **3**:54-57.

Walkty, A., Karlowsky, J. A., Baxter, M. R., Adam, H. J., Alexander, D., Bay, D. C. and Zhanel, G. G. (2020). Fosfomycin resistance mediated by fos genes remains rare among extended-spectrum betalactamase-producing Escherichia coli clinical isolates recovered from the urine of patients evaluated at Canadian hospitals (CANWARD, 2007 2017). *Diagnostic Microbiology and Infectious Disease* **96**(3):114-123.

Walsh, T.R., Weeks, J. and Livermore, D.M. (2011). Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *TheLancet Infectious Diseases* **11**:355–362.

Zango, U. U., Ibrahim, M. and Shawai, S. A. A. (2019). Lactam antibiotic drug resistance. *MOJ Drug Design Development and Therapy* **3**(2):52-58.

Zhanel, G. G., Esquivel, J., Zelenitsky, S., Lawrence, C. K., Adam, H. J., Golden, A. and Bay, D. (2020). Omadacycline: A Novel Oral and Intravenous Aminomethylcycline Antibiotic Agent. *Drugs* **70**:1-29.

Zuccato, E., Castiglioni, S. and Fanelli, R. (2005). Identification of the pharmaceuticals for human use contaminating the Italian aquatic environment. *Journal of Hazardous Materials* **122** (3):205-209.

APPENDIX I

STAINS AND REAGENTS

i. Crystal violet solution

crystal violet	2.0g
Ammonium oxalate	9.0g
Ethanol (absolute)	20.0ml
Distilled water	80.0ml

ii. Lugol's iodine

Iodine	1.0g
Potassium iodide	2.0g
Distilled water	300ml

Iii Decolorize Acetone

iv. Safranin

Dafranin	0.5g
Distilled water	100ml

v. Neutral red

1% acetic acid	1ml
Distilled	500ml

APPENDIX II

Media Composition

Nutrient agar

Formula	*gm/litre*
'Lab-Lemco' powder	1
Yeast extract	2
Peptone	5
Sodium chloride	5
Agar	15
pH 7.4 ± 0.2	

Simmon Citrate agar

Formula	*gm/litre*
Sodium chloride	5
Sodium citrate	2
Ammonium dihydrogen phosphate	1
Dipotassium phosphate	1
Magnesium sulphate	0.2
Bromothymol blue	0.08
Agar	15

Urea agar base

Formula	*gm/litre*
Urea	20
Sodium chloride	5
Monopotassium phosphate	2
Peptone	1
Dextrose	0.012
Phenol red	15
Agar	