SCHOOL OF PUBLIC HEALTH

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ASSESSMENT OF THE ACTIVITY OF SELECTED ANTIBIOTICS AGAINST URINARY ISOLATES OF *Escherichia coli* AMONG SELECTED PHARMACEUTICAL INDUSTRY WORKERS IN ACCRA

BY

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DECLARATION

I, JOSEPH POKU ASUMADU hereby declare that, with the exception of cited literature, this study is the result of my own original research and has not been presented elsewhere either in part or in whole.

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DATE:	**DATE:**
JOSEPH POKU ASUMADU	**DR. JUDITH STEPHENS**
STUDENT	**SUPERVISOR**

DEDICATION

This work is dedicated to the Almighty God, my parents (Mr. and Mrs Amponsah), my siblings (Mary, Ann, Emmanuel, Joachim, Philomina and Margaret) and to the entire B. Pharm graduating class of 2015.

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ABSTRACT

Exposure to sub-therapeutic amounts of antibiotics has been identified to be a major contributory factor to the susceptibility patterns of microorganisms. Pharmaceutical industry workers are thus at risk of developing resistant strains to the antibiotics they produce through repeated and long exposure to sub-therapeutic doses of antibiotics in the course of their daily work. The study was done to assess the activity of Amoxicillin, Cefuroxime and Ciprofloxacin against urine isolates of *E. coli* among pharmaceutical industry workers in Accra. The study also sought to find an association between industrial exposure to antibiotics and susceptibility patterns of microorganisms to the antibiotics produced using the current station of the workers. The agar disc diffusion method was used to determine the susceptibility of the *E. coli* isolates to the selected antibiotics whiles the broth-microdilution method was used to determine the minimum inhibitory concentrations (MIC) of the antibiotics against the sensitive and intermediate *E. coli* strains. *E. coli* strains were identified and isolated from 20 out of the 101 urine samples taken from both production floor workers and non-production floor workers selected across three (3) pharmaceutical industries. All isolated strains of *E. coli* were resistant to Amoxicillin whiles 75% (15 of 20) of the isolated *E. coli* strains were resistant to Cefuroxime. 15% (3 of 20) of the isolated strains showed intermediate resistance whiles 10% (2 of 20) of the isolated strains were sensitive to Cefuroxime. 45% (9 of 20) of the *E. coli* urine isolates were resistant whiles 15% (3 of 20) of the isolated strains showed intermediate resistance to Ciprofloxacin. 40% (8 of 20) of the isolated strains were sensitive to Ciprofloxacin. However, there was significant association between industrial exposure to antibiotics and subsequent development of resistant strains of microorganisms to the antibiotics produced. The MIC for Cefuroxime ranged between 20–30 μg/mL with the latter occurring for the intermediate resistant strains whiles the MIC for Ciprofloxacin ranged between 3–5 μg/mL with the later occurring for the intermediate resistant strains.

Keywords: Antibiotic resistance, *Escherichia coli*, Pharmaceutical Industry workers

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

APIs	– Active Pharmaceutical Ingredients
BNF	– British National Formulary
CDC	– Centre for Disease Prevention and Control
DNA	– Deoxyribonucleic acid
GNDP	– Ghana National Drug Policy
LEKMA	– Ledzokuku–Krowor Municipal Assembly
MBC	– Minimum Bactericidal Concentration
MDR	– Multi drug resistant
MIC	– Minimum Inhibitory Concentration
mL	– Millilitre
MLC	– Minimum Lethal Concentration
mRNA	– Messenger Ribonucleic Acid
MRSA	– Methicillin Resistant *Staphylococcus aureus*
^oC	– Degree Celsius
^oF	– Degree Fahrenheit
PBPs	– Penicillin Binding Proteins
PPE	– Personal Protective Equipment
rRNA	– Ribosomal Ribonucleic acid
STG	– Standard Treatment Guidelines
µg	– Microgram
µL	- Microlitre
µm	– Micrometre

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

DEFINITION OF TERMS

Active Pharmaceutical ingredient – An active pharmaceutical ingredient is the compound in a pharmaceutical drug formulation that elicit the intended biological effect.

Antibiotic – A substance that inhibit the growth of microorganisms or kill microorganisms.

Bacteria – A large group of single cell microorganisms that usually cause infections and diseases in humans and animals.

Chelating Agent – A substance that coordinates with a metal to form a chelate, often used to trap and remove heavy metal ions and other compounds.

In vitro – Outside or occurring outside the body of biological systems or context.

Microorganism – A microscopic organism, especially a bacterium, virus or fungus.

Sub-therapeutic amount – An amount of a drug substance which is usually less than the amount required to elicit a therapeutic effect.

CHAPTER 1

INTRODUCTION

1.1 Background

Prior to the discovery of antibiotics, infectious diseases were the leading cause of morbidity and mortality. Treatment of infections was initially based primarily on medicinal folklore until the early moments of the 20th Century when certain ancient cultures used specially selected mould, plant materials and extracts as means of treatment. Serendipity surrounding the discovery of natural antibacterial agents produced by certain strains of bacteria and fungi was one of the greatest hopes in the practice of medicine and therapeutics. Alexander Fleming discovered one of the most important antibacterials, Penicillin from fungi (Landsberg, 1949). Other antibacterial agents such as sulfonamides (Prontosil) were subsequently discovered and the era of antibacterial agents was opened. In 1927, Selman Waksman, an American microbiologist described substances produced by microorganisms that antagonize the growth of other microorganisms in high dilution as antibiotics. This was the first and foremost instance when the term antibiotic was used. However, the advent of synthetic ways of producing antibacterial compounds has led to the revision of the initial definition of antibiotics (Hugo & Russell, 1998).

This therapeutic breakthrough and era however, has been jeopardized by the evolution of drug resistant microorganisms. Microorganisms have developed several mechanisms to become resistant to the actions of antibiotics. Several of these resistant mechanisms are influenced by humans and these arise from manufacturers, health practitioners and patients. The identified factors include exposure to sub-therapeutic and sub-standard amounts of the antibiotics and the lack of application of standard procedures such as isolation, culture and sensitivity testing before employing the antibiotics in the treatment of infections (Ventola, 2015). The prescription of long courses of antibiotic therapy, the use of antibiotics in self-limiting infections which do not require antibiotics, the overuse of antibiotics for prophylaxis before and after surgery and the sale of antibiotics over the counter to the general public particularly in developing countries are great contributing factors to the evolution of resistant strains of organisms (Kelesidis et al., 2007). The practice of using antibiotics in animal feed to prevent infections and promote growth in poultry and livestock farming results in the selection of resistant organisms in the animals contributing to the pool of resistant organisms in humans (Ventola, 2015). Also, the presence, sale and use of substandard or counterfeit drugs are major causes of resistance especially in developing countries (Kelesidis et al., 2007). In addition, the emergence of spurious antibiotics has become a major issue of global concern since the use of these low quality drugs results in adverse clinical outcomes contributing to the burden of disease and consequently to excess mortality and morbidity (Kelesidis et al., 2007). Substandard or counterfeit drugs usually contain lesser or sub-therapeutic amounts of the active pharmaceutical ingredients (APIs). Exposure to sub-therapeutic amounts of drugs to treat infections usually results in treatment failure as these agents are unable to kill the microorganisms. These microorganisms due to sensitization by the sub-therapeutic amounts of these antibiotics usually develop resistance towards these antibiotics. Pharmaceutical industry workers, by the nature of their job description, are usually exposed to sub-therapeutic amounts of antibiotics which causes them to develop resistant strains of bacteria towards the antibiotics they produce (Heron & Pickering, 2003). This is a major threat to their health.

The incidence of resistance led to the synthesis of newer and more potent antibiotics. However, synthesis of newer antibiotics has stalled due to less profit in the pharmaceutical market of antibiotics. The incidence of resistance has yielded and continue to yield detrimental consequences in healthcare globally, making infections difficult or sometimes impossible to treat at escalating cost.

A recent one is the spread of "untreatable" super gonorrhoea infection which is resistant to most antibiotics. Therefore, it is essential for these antibiotics to be periodically evaluated to determine the susceptibility patterns of microorganisms to these agents.

1.2 Problem statement

Antibiotic resistance is on the rise and threatens the effective treatment of an ever-increasing range of bacterial infections. The widespread of antibiotic resistance has called on the efforts of major stakeholders of health to work efficiently to minimize its progression. The World Health Organization during its annual World Health Assembly in 2014 warned that the world is heading towards post-antibiotic era where common infections and ailments that were once treatable and curable with antibiotics will once again claim lives due to resistance (WHO, 2014). Most industrial workers, by the nature of their job description, are exposed to a number of chemicals they work with and these chemicals in one way or the other have harmful and deterrent effects on their health. The effects from these harmful chemicals vary and is greatly influenced by the nature and type of chemical exposure. People in the pharmaceutical industries suffer from the harmful effects of the raw APIs they are exposed to. The major routes of exposure to these ingredients are mainly via the skin and inhalation. As a result of these exposures, they do not only suffer from the side effects of these ingredients but also develop resistant strains of microorganisms to majority of the antibiotics manufactured in such industries (Heron & Pickering, 2003; Holmström et al., 2003).

As bacteria begin to build resistance against the first line antibiotics coupled with the limited numbers of antibiotics available, more money will be required to treat infections, thus raising treatment costs significantly. Newer and more expensive generations of antibiotics will be required in the treatment of common infections and ailments or treatment will require extended dosage regimens of the already existing first-line options leading to a significant reduction in productivity as individuals will keep a relatively longer period away from their work post. These individuals can also serve as carriers for the resistant bacteria strains, thus serving as a means of spreading resistant bacteria. Resistance to the first-line antibiotics which includes Amoxicillin, Cefuroxime and Ciprofloxacin will require the nation to review its antibiotic regimens, thus requiring the importation of newer, expensive and reserved classes of antibiotics to treat common infections thereby putting an enormous cost on the state. Antibiotic resistance is also associated with significant morbidity and mortality. An example is the case that happened in the Korle-Bu teaching hospital in 2012 where the Children's Emergency ward had to be closed down due to an outbreak of Methicillin Resistant *Staphylococcus aureus* (MRSA) which resulted in the death of three children (Pesewu et al., 2014).

With these problems imminent, it has become imperative to find appropriate solutions so as to reduce the problems associated with antibiotic resistance significantly. Industrial exposure to raw APIs as a cause of developing resistant bacteria strains has to be studied and appropriate measures taken to reduce the exposure. Industrial exposure to the APIs can be reduced through a number of ways which includes provision of appropriate personal protective equipment, provision of appropriate chelating agents and reducing the length of stay at various stations through rotation of workers. Addressing the issue of industrial exposure to APIs will be a major step in reducing the rise in antibiotic resistance and also improving the quality of life of pharmaceutical industry workers.

1.3 Justification

The study sought to address the susceptibility patterns of *Escherichia coli* to Amoxicillin, Cefuroxime and Ciprofloxacin among pharmaceutical industrial workers. These people are at risk of developing resistant strains of microorganisms as a result of exposure to sub-therapeutic amounts of these antibiotics. Antibiotic resistance as an issue of global concern has to be addressed from all possible areas so as to reduce its progression. Pharmaceutical industrial workers, even though constitute a minority of the population are at a high risk of developing resistant strains of microorganisms particularly to the antibiotics they produce. The resistant microbial strains are transmitted upon transmission and infection which eventually increases the pool of the resistant microbes. Based on the susceptibility patterns, safer and more appropriate antibiotics can be for therapy during infection.

1.4 Research questions

1. What is the prevalence of urinary isolates of *Escherichia coli* among the selected pharmaceutical industry workers in Accra?
2. Are the isolated strains of *E. coli* from the urine samples sensitive or resistant to Amoxicillin, Cefuroxime and Ciprofloxacin?
3. Is there an association between industrial exposure to antibiotics (current station and duration at the current station) and susceptibility patterns of microorganisms to the antibiotics produced?

1.5 Objectives

1.5.1 General objective

To determine the activity of selected antimicrobial agents against isolates of *E. coli* from urine samples among selected pharmaceutical industry workers in Accra.

1.5.2 Specific objectives

- To determine the prevalence of *E. coli* strains among workers in selected pharmaceutical industries in Accra.
- To isolate *E. coli* from urine samples collected from pharmaceutical industry workers.

- To test for the activity of Amoxicillin, Cefuroxime and Ciprofloxacin against the isolated strains of *E. coli*.
- To determine the association between industrial exposure (current station and duration at current station) to antibiotics and susceptibility patterns of microorganisms to the antibiotics produced.

1.6 Conceptual framework

Antibiotic resistance is an issue of global concern and can arise from several factors. The major factors identified to contribute to antibiotic resistance from fig 1.1 include industrial factors, personal factors, microbial factors and health care and community factors. Industrial workers are exposed to antibiotics in a couple of ways which are through inhalation and deposition on the skin. They are thus exposed to sub-therapeutic amounts of the antibiotics which in–turn sensitize microorganisms to develop resistant mechanisms towards these agents. Other industrial factors identified to contribute to antibiotic resistance include unavailability and use of personal protective equipment and chelating agents. Ideally personnel involved in the production of pharmaceuticals such as antibiotics are to be given chelators after production so as to complex out the ingredients which in one way or the other end up in the body. The chelating agents thus reduce absorption of the drug by making it more water soluble to enhance excretion. Personal protective equipment on the other hand reduce exposure of the workers to the raw active pharmaceutical ingredients thus protecting the workers from the harmful effects of these ingredients which includes the risk of building resistant strains of microorganisms to the antibiotics they produce.

The personal factors identified to contribute to antibiotic resistance includes the health seeking behaviour, level of education and the health status of the individuals. Health seeking behaviour on the part of the individual can also influence the risk of building resistant strains of microorganisms. Frequent patronage of antibiotics coupled with non – compliance to the dosage regimens are also associated with the development of resistant strains to antibiotics. The level of education which influences individuals' knowledge about antibiotics can also influence the frequency of exposure to antibiotics as an individual who is more knowledgeable would want to protect himself from the harmful effects of these agents than another who is less knowledgeable. The reverse is also true. Also, the health status of the individual plays a key role in the development of resistant strains. Immunocompromised individuals, that is individuals with suppressed immune function, are usually unable to fight even self-limiting infections on their own and hence will almost always require antibiotic therapies. Over – exposure of antibiotics in these individuals sensitizes the microorganisms to build resistant strains leading to resistance to the antibiotics.

Health – care providers also contribute significantly to antibiotic resistance through a number of factors. Unnecessary prescription of antibiotics for conditions that do not even require antibiotic therapies, as in the case of self–limiting infections, sensitizes the microorganisms that constitute the normal flora of the body to develop resistant mechanism to counter the effects of these antibiotics thus putting individuals at risk of developing resistance of microorganisms to the antibiotics. Next is the influx of substandard and counterfeit antibiotics on the market of most developing countries which contributes significantly to development of resistance. Substandard and spurious drugs, on the other hand, usually contain a lesser amount of the API expected to be present and hence treatment of infections with such antibiotics usually results in treatment failure. The antibiotics do not kill the microorganisms but rather sensitize them leading to the development of resistance against these antibiotics.

The last factor influencing the development of resistance to antibiotics arises from the microorganisms themselves where they are able to develop certain mechanisms to inactivate the antibiotics rendering them ineffective. Others also undergo mutation in the genes which naturally results in resistant strains. This study focuses on the industrial factors that contribute to the development of antibiotic resistance.

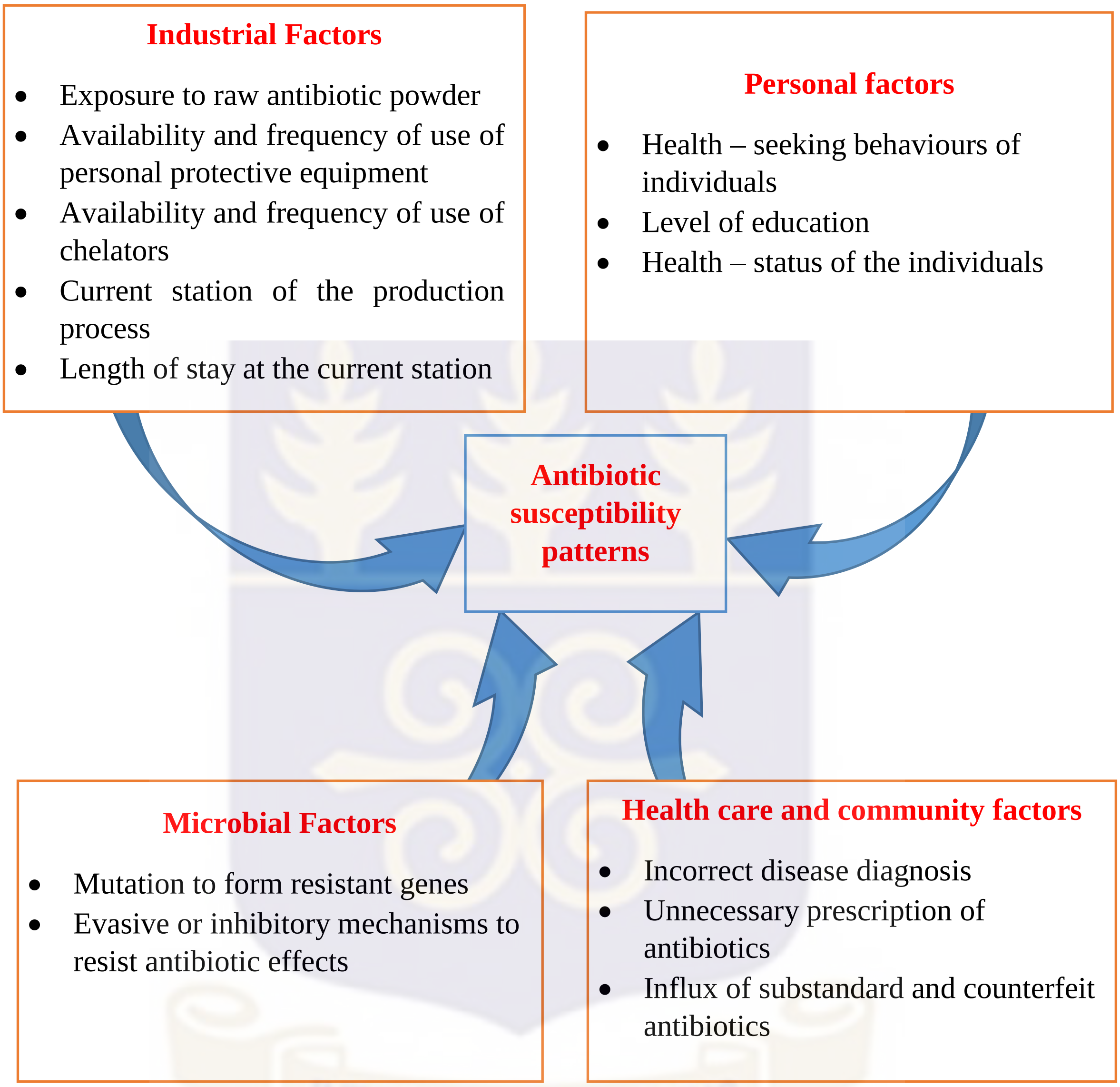


Fig 1.1: Conceptual framework showing the various factors that influence the antibiotic susceptibility patterns of microorganisms

CHAPTER 2

LITERATURE REVIEW

2.1 Definition of antibiotics

Antibiotics, were originally defined as substances produced by microorganisms which exhibited inhibitory actions on other microorganisms. However, this definition has been modified due to the synthesis of newer antibiotics to include substances which are produced by microorganisms or synthesized wholly or in part that inhibit the growth of other microorganisms. Penicillin as an antibiotic was produced by the fungi *Penicillium notatum* and *Penicillium chrysogenum* while antibiotics such as the sulphonamides and the 4-quinolones are produced wholly by synthetic means (Prescott, 2002).

2.2 Sources of antibiotics

Antibiotics can be obtained from three main sources namely biological, chemical synthetic and semi- synthetic origins. Antibiotics obtained from biological origin are usually derived from microorganisms where some metabolites they produce inhibit the growth of other microorganisms. Examples of antibiotics obtained from biological sources include bacitracin and polymyxin from some *Bacillus species*; streptomycin and tetracyclines from *Streptomyces species*; gentamycin from *Micromonospora purpurea*; griseofulvin and some penicillins and cephalosporins from certain species of *Penicillium* and *Acremonium* of the family Aspergillaceae; and monobactams from *Pseudomonas acidophila* and *Gluconobacter* species. However, most of the antibiotics currently in use are produced from *Streptomyces* species.

The second means through which antibiotics are produced is by chemical synthesis where chemical processes and procedures result in the production of compounds which can inhibit growth of microorganisms. Chloramphenicol is now produced mainly by synthetic process.

Sulphonamides and anti-tubercular drugs such as isoniazid and pyrazinamide are also produced through this method (Hugo & Russell, 1998).

The third source is by semi-synthetic means where portions of the antibiotic moieties are produced by biological means using the appropriate microorganisms in fermentation processes and the other part is produced via a synthetic chemical process. Many cephalosporins are produced in this way (Ryan & Ray, 2004).

2.3 Classification of antibiotics

Antibiotics can be classified in three categories according to the effect on the target species, the spectrum of the activity covered by the antibiotic and the mode of action of the antibiotic.

An antibiotic is classified as bactericidal if it exhibits lethal effects on the susceptible bacteria. Such antibiotics are usually preferred in the treatment of infection as they kill microorganisms thereby reducing the risk of possible re-infection and subsequent development of resistant strains. An antibiotic is classified as bacteriostatic if it merely inhibits the growth of the bacteria without necessarily killing them. These agents thus rely on the body's own immune functions to eradicate the microorganisms once they suppress their growth. For this reason, these agents are usually not preferred except in cases where the individual has got an uncompromised immune function to eradicate the microorganisms. This is because once the agent is removed, the microorganisms might grow again (Tortora et al., 2013).

An antibiotic is classified as narrow spectrum if it exhibits activity against either gram-negative or gram-positive bacteria only. These agents are usually used only when the causative organism for the infection is known. Narrow spectrum antibiotics hardly alter the normal bacterial flora of the body as they are specific for the causative microorganisms. However, selection of narrow spectrum antibiotics to treat an infection is very key as the antibiotic may miss the causative microorganisms.

An antibiotic is classified as broad spectrum if it exerts activity against both gram-negative and gram-positive bacteria. These agents are usually employed in the treatment of infections as in most cases, the causative organisms are not known. Due to the broad spectrum of activity affecting both gram negative and gram-positive bacteria, they alter the normal bacteria flora of the body. They are however, associated with the development of resistance as they target other bacteria other than the causative organisms. This, thus causes significant sensitization of the bacteria to the antibiotics thus causing the development of resistant strains (Pommerville & Alcamo, 2007).

Different antibiotics have different mechanisms of antagonizing bacterial existence hence are also classified based on their modes of action (Prescott, 2002).

2.4 Modes of action of antibiotics

Bacteria suffer from either the lethal or inhibitory effects of antibiotics via a number of different mechanisms of action on the bacterial cell. Some ways in which they exert their actions are as follows:

2.4.1 Inhibition of cell wall synthesis

Most prokaryotic bacteria have cell wall structures unique and different from that of most eukaryotic cells. The cell wall protects bacteria from the environment, as well as osmolysis. Some antibiotics target the unique cell wall structures of bacteria and inhibit synthesis. As a result, these antibiotics have high therapeutic indices as the structures affected are not found in eukaryotes. Examples of such antibiotics are penicillins, cephalosporins, vancomycin, and bacitracin. The cell wall of most bacteria is made up of peptidoglycan and these antibiotics exert their action by inhibiting the biosynthesis of peptidoglycan which is a very vital component of virtually all bacterial cells (Hugo & Russell, 1998). Synthesis of the peptidoglycan cell wall of bacteria involve several steps and the inhibitory mechanism may occur at any stage of the peptidoglycan biosynthesis. Beta lactam antibiotics such as penicillins and cephalosporins interfere by inhibiting transpeptidation processes by binding to the penicillin binding proteins. In this vain, the rigidity of the cell wall is compromised (Schaecter, 2009).

2.4.2 Disruption of cell membrane

These types of antibiotics act by selectively disrupting the phospholipid component of the cytoplasmic membrane. Hence the balance of ionic hydrophobic and hydrogen bonding interactions responsible for the stability of the membrane is compromised leading to leakage of contents of the cytoplasm or alterations in the metabolic functions of the membrane. Examples include naftidine which decreases ergosterol in the fungal cell membrane causing cell destruction. Polyenes such as amphotericin B and nystatin, imidazoles and triazoles all act by cell membrane disruption. Polymyxins act by increasing the permeability of the cell membrane thus causing leakage of the cell content (Schaecter, 2009; Talaro, 2002; Tortora et al., 2013).

2.4.3 Inhibition of protein biosynthesis

Another target for antibiotics is the bacterial ribosomes where they bind and block the various processes involved in the synthesis of new proteins such as elongation of the polypeptide chains. They also cause distortions to the site of attachment of mRNA, leading to mistranslation of codons and failure to produce the correct amino acid sequence in proteins. Chloramphenicol, tetracyclines, macrolides and aminoglycosides are examples of antibiotics in this category (Tille, 2014).

2.4.4 Interference with nucleic acid synthesis

This class of antibiotics act by inhibiting the enzymes involved in the synthesis of DNA. Rifampin acts by inhibiting the RNA polymerase enzyme thereby preventing the production of mRNA. Fluoroquinolones also act by binding and interfering with the DNA gyrase enzymes involved in the regulation of bacterial DNA supercoiling. This action leads to the inhibition of replication, repair, transcription and bacterial chromosome separation during division and other cell processes involving DNA. The newer fluoroquinolones are also known to inhibit topoisomerase IV, another enzyme that unwinds DNA during replication and as such make them bactericidal (Prescott, 2002).

2.5 Principles in antibiotic selection for therapy

Certain principles serve as guidelines which are essential and very useful when it comes to the selection of antibiotics for treatment of an infection. These principles help in choosing the most appropriate agent in the treatment of infections. These include susceptibility of the infecting organisms, host factors, pharmacological factors, adverse reactions, superinfection and chemoprophylaxis (Gilbert et al., 2014)

2.5.1 Susceptibility of infecting organisms

Selection of antibiotics for the treatment of infections should be dependent on the activity of the antibiotic against the possible infecting microorganisms. The susceptibility patterns of certain microorganisms to a particular anti-infective agent are predictable and hence, laboratory tests are not usually performed in the treatment of infections caused by these microorganisms. All strains of *Streptococcus pyogenes* have been identified to be susceptible to the actions of penicillin. However, the susceptibility patterns of most Gram-negative microorganisms associated with enteric infections are less established and hence successful treatment of such conditions requires laboratory procedures such as culture and sensitivity testing to enable safe prescriptions. Moreover, the selection of antibiotic with marked activity against the infective microorganisms during *in vitro* analysis for therapy may be compromised due to pharmacological basis or availability of more safer antibiotics (Gilbert et al., 2014).

2.5.2 Host factors

Factors associated with the host of the microorganisms play an important part in determining outcome of antibiotic usage. The immune response elicited by the host to the infective microorganisms depends on the factors such as the circulating and tissue phagocytes. Infections can progress rapidly in immune-compromised patients as a result of either an absolute or functional deficiency of phagocytic cells. Haematological malignancies, such as the acute leukaemias, results in impaired phagocytic function occurring both by the disease and by the use of potent cytotoxic drugs which destroy healthy, as well as malignant, white cells. Bactericidal agents are thus required in the treatment of infections in patients with impaired phagocyte function as bacteriostatic agents depend on the host phagocytic function to eliminate the bacteria (Moellering & Eliopoulos, 2000).

2.5.3 Parasite factors

Some microorganisms have the inherent ability to neutralise the antimicrobial effects of certain antibiotics which includes efflux pumps which is able to pump out the antibiotics out of the systems (as associated with some strains of *Pseudomonas*). Some microorganisms also possess enzymes such as penicillinases and cephalosporinases which are able to break down the structures of penicillins and cephalosporins respectively thereby neutralizing their antimicrobial effects (Schaecter, 2009)

2.5.4 Pharmacological factors

Clinical efficacy of antibiotics can only be achieved if satisfactory concentrations of the antibiotics reach the site of infection. The bioavailability of antibiotics is influenced by pharmacological factors such as absorption, distribution, metabolism and excretion. Oral agents are affected by gastrointestinal absorption across the micro-villi and may be impaired by factors such as drug-food interactions, drug-drug interactions (including chelation), or impaired gastrointestinal function either as a result of surgical resection or mal-absorptive states. Parenteral agents usually exhibit a faster onset of action as satisfactory concentrations are achieved at the site of infection within a shorter period. Unlike oral absorption, parenteral antibiotics are appropriate in patients who are vomiting or have undergone a recent surgery. Parenteral antibiotics are used to deliver the loading doses required to achieve peak plasma concentrations after which oral antibiotics may be used (Brunton et al., 1982). The excretion route of antibiotics is also an important pharmacological factor of the drug. The selection of an antibiotic for the treatment of urinary tract infection requires concentration of the antibiotic in the urine hence antibiotic with activity against the infective microorganism but does not concentrate in the urine cannot be used.

2.5.5 Adverse reactions

All chemotherapeutic agents have the potential to cause unintended adverse reactions which may vary in the degree of frequency and severity. They are usually dose-dependent and can be predicted in a patient with a history of hypersensitivity or a previous toxic reaction to a drug or its chemical analogues. However, the cause of many adverse events are idiosyncratic and therefore unpredictable. These manifestations such as hypersensitive reactions may be fatal or minor reversible reactions. Also, drug toxicity which is mainly dose-dependent may affect multiple organs or tissues. An example is the bone marrow suppression induced by chloramphenicol which can be corrected with a reduction in the dose or withdrawal of the drug (Brunton et al., 2008).

2.5.6 Superinfection

Anti-infective drugs, particularly broad-spectrum antibiotics, do not only affect the causative microorganism but also have an impact on the normal bacterial flora of the body. The use of broad-spectrum antibiotics may result in depletion of the normal bacterial flora which fight off invading microorganisms. This, thus results in the over growth of resistant microorganisms with subsequent superinfection such as the occurrence of oral or vaginal candidiasis in patients following the use of broad-spectrum antibiotics such as ampicillin or tetracycline. Pseudomembranous colitis is a severe form of superinfection which results from the overgrowth of toxin-producing strains of *Clostridium difficile* present in the bowel flora following the use of broad-spectrum antibiotics especially clindamycin. This condition is managed by drug withdrawal and oral vancomycin or metronidazole. Rarely, colectomy may be necessary for severe cases (Gilbert et al., 2014).

2.5.7 Chemoprophylaxis

Antibiotics are used as prophylaxis in the prevention of infections in patients at high risk of microbial invasion due to reduced immune function. Chemoprophylaxis is employed especially in the practice of surgery. Infection remains one of the most important complications of many surgical procedures, and the recognition that peri-operative antibiotics are effective and safe in preventing this complication has proved a major advancement in surgery. The principle underlying the chemoprophylactic use of antibiotics is the ability to predict the infecting microorganisms and the susceptibility of the microorganisms to antibiotics (Moellering & Eliopoulos, 2000)

2.6 Ideal properties of antibiotics

An antibiotic must possess certain properties in order to render them useful for clinical purposes. These properties enhance the bactericidal or bacteriostatic effects of antibiotics. An ideal antibiotic should possess several of the following properties. It should have high order of selective toxicity; therefore, it should be able to distinguish between the host cells and the cells of the causative microorganisms. It is thus required to be non-toxic to the host cells at concentrations required to kill the causative microorganisms (Olaniyi, 2005); they should not have any significant effect on the normal bacterial flora of the host (Hogg, 2002; Talaro, 2002); It should not cause any hypersensitivity reactions (Olaniyi, 2005); they should also be soluble in body fluids to ensure maximum bioavailability which will enable them to exert their effect by penetrating the body tissues. The compound must not be metabolized so quickly that it is excreted from the body before exerting the desired effects. If administered orally, it must not be de-activated by the acidic environment of the stomach and should be well absorbed in the small intestines (Hogg. 2002; Pommerville & Alcamo, 2007); it should be sufficiently stable to have a good shelf life, without special storage considerations (Hogg. 2002; Tortora et al., 2013) and its binding to serum protein should be of low order so as to increase bioavailability (Olaniyi, 2005).

2.7 Selected antibiotics

2.7.1 Amoxicillin

Amoxicillin belongs to the penicillin class of antibiotics. It is a derivative of ampicillin with better absorption than ampicillin following oral administration. It is a broad-spectrum antibiotic with activity against both gram-negative and gram-positive bacteria. It is however inactivated by penicillinases hence resistant to most of the penicillinase producing bacteria such as *Staphylococcus aureus* and *Escherichia coli*. Amoxicillin acts by binding to the penicillin binding proteins and inhibits cell wall synthesis in both gram-negative and gram-positive bacteria. It is used in the treatment of urinary tract infections, otitis media, bronchitis, sinusitis and low or moderate-severity community acquired pneumoniae (BNF, 2017).

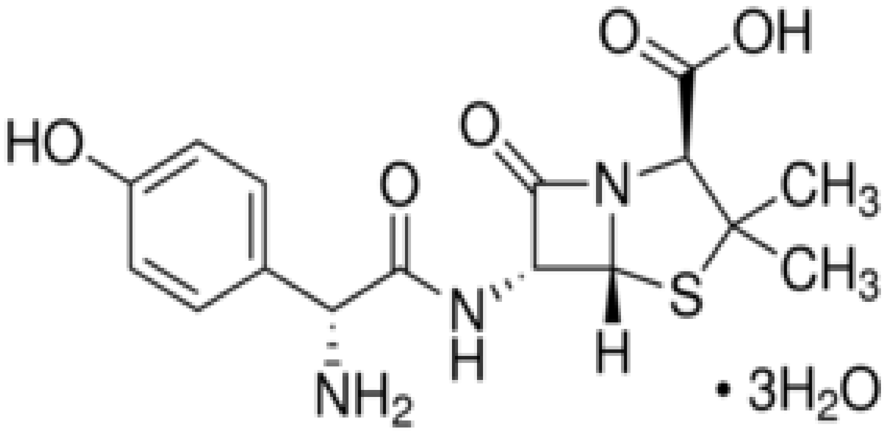


Fig 2.1: Amoxicillin Trihydrate structure (British Pharmacopoeia, 2013)

2.7.2 Ciprofloxacin

Ciprofloxacin belongs to a class of antibiotics known as fluoroquinolones. Ciprofloxacin is a broad–spectrum antibiotic with activity against both gram-negative and gram-positive bacteria. It has activity against gram-negative strains including *salmonella, shigella, campylobacter, neisseria and pseudomonas*. It however has moderate activity against gram-positive strains such as *Streptococcus pneumoniae* and *Enterococcus faecalis*. It is used in the treatment of respiratory tract infections, urinary tract infections, infections of the gastro-intestinal system (including typhoid fever), bone and joint infections, gonorrhoea and septicaemia caused by sensitive organisms (BNF, 2017). Ciprofloxacin acts by binding to and interfering with DNA gyrase enzymes involved in the regulation of bacterial DNA supercoiling, a process essential for DNA replication, recombination, thus inhibiting DNA synthesis (Tortora et al., 2013), Ciprofloxacin is one of the antibiotics with rapidly emerging resistance to most microorganisms and resistance can even occur in the course of treatment (Tortora et al., 2013).

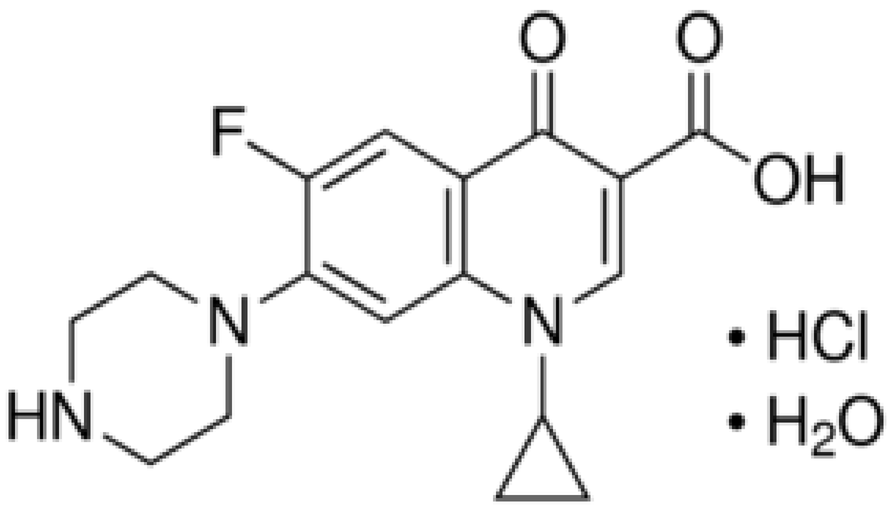


Fig 2.2: Ciprofloxacin Hydrochloride structure (British Pharmacopoeia, 2013)

2.7.3 Cefuroxime

Cefuroxime is a second-generation cephalosporin with marked activity against gram-negative bacteria and less susceptibility to inactivation by beta-lactamases. It has activity against *Haemophilus influenzae*. Cefuroxime acts by binding to penicillin-binding proteins (PBPs) such as transpeptidases, carboxypeptidases and endopeptidases inhibit cell wall synthesis in both gram-positive and gram-negative bacteria (Blumberg & Strominger, 1974). Cefuroxime is used for the treatment of urinary tract infections, respiratory tract infections, otitis media sinusitis and as a prophylaxis against *H. influenzae* (BNF, 2017).

Resistance to Cefuroxime can result from either reduced affinity of existing penicillin-binding protein components or the acquisition of a supplementary beta-lactam insensitive penicillin binding protein (Moosdeen, 1997).

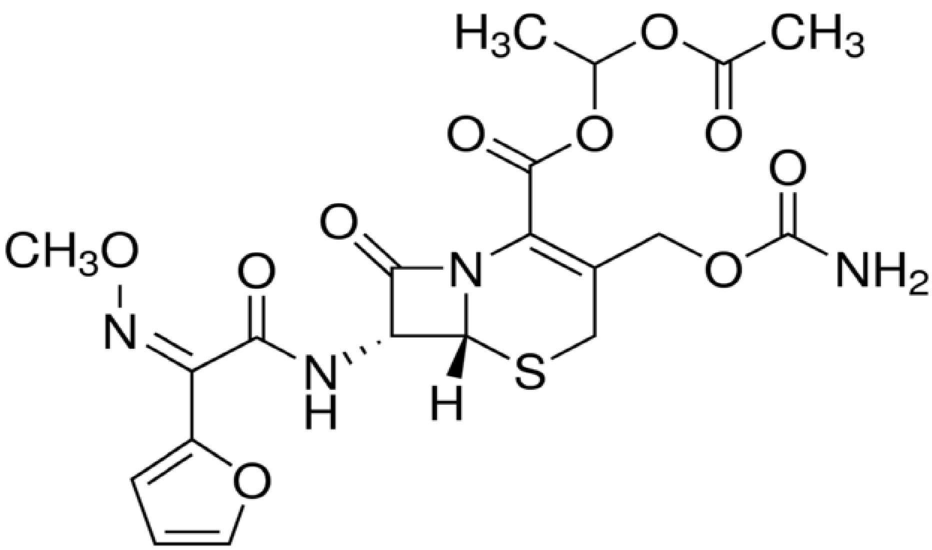


Fig 2.3: Cefuroxime Axetil structure (British Pharmacopoeia, 2013)

2.8 Antibiotic resistance

Over the years, resistant bacteria have evolved and these bacteria are able to grow and replicate in the presence of antibiotics they are supposed to be susceptible to. Resistance usually arises when bacteria are not inhibited by usually achievable systemic concentrations of an agent with normal dosage schedules and/or fall in the minimum inhibitory concentration ranges. In instances where a microorganism is resistant to multiple antibiotics or antibiotic classes, that pathogen is considered as being multi-drug resistant (MDR) (Talaro, 2002). Acquisition of resistance to one antibiotic can confer resistance to another antibiotic of the same class to which the microorganism is yet to be exposed to and this is referred to as cross-resistance (Schaecter, 2009).

2.8.1 Causes of antibiotic resistance

Certain practices on the part of both practitioners and individuals have grossly contributed to the increased evolution of resistant strains of bacteria. These include the use of multiple antibiotics where one could be sufficient in managing certain bacterial infections. The prescription of long courses of antibiotic therapy by practitioners, the use of antibiotics in self-limiting infections for which they are not needed, the overuse of antibiotics for prophylaxis before and after surgery and the sale of antibiotics over the counter to the general public particularly in developing countries are great contributing factors to the evolution of resistant strains of organisms. Exposure to sub-therapeutic amounts of antibiotics can cause sensitization of the microorganisms to the antibiotics thus leading to the development of resistance toward them. The practice of using antibiotics in animal feed to prevent infections and to promote growth has also resulted in the development of resistant organisms in the animals which can be transferred to humans thus contributing to the pool of resistant microorganisms in humans. The presence, sale and use of substandard or counterfeit drugs are major causes of resistance especially in developing countries (Kelesidis et al., 2007). However, in instances where the use of antibiotics is appropriate dose regimens are usually shorter than needed to eradicate the infection, thereby encouraging the survival of resistant strains of bacteria.

2.8.2 Mechanisms of antibiotic resistance

Antibiotic resistance may be intrinsic (natural) or acquired. Pathogens have several strategies for growing resistant to antimicrobials. Intrinsic resistance is inherent and best considered as insensitivity of the microorganism to the antibiotic as a result of physiological characteristics of the microorganism or the possession of a structural gene. For example, *E. coli* is resistant to various classes of antibiotics due to the presence of an external membrane which prevents permeability of the drug into the bacterial cells. *Pseudomonas aeruginosa* also possesses endogenous multidrug efflux pumps. These efflux pumps cause drug expulsion from the cell. These pumps are mostly nonspecific and hence can expel many different drugs. Organisms with this kind of drug resistance strategy often exhibit multi-drug resistance (Hugo & Russell, 1998). Some microorganisms also resist actions of antibiotics by chemical modifications to the drug structure. This is typically seen in the hydrolysis of beta lactam ring of penicillins and cephalosporins by the enzyme penicillinases and cephalosporinases.

Acquired resistance is the ability of a microorganism to resist the action of an antibiotic as a result of a genetic change or genetic acquisition of nucleic acids. Resistant bacteria have also developed means of transferring their resistant genes to other bacteria of the same or different species. Resistance can be acquired through vertical or horizontal transfer of the genetic materials. Resistant traits may be passed from parents to successive daughter cells during cell division in a process known as vertical transfer or chromosome-mediated resistance. vertical transfer occurs within organisms of the same species. Another mode is by horizontal transfer or plasmid-mediated resistance where genetic information is passed from a donor cell to another cell. Horizontal transfer can occur among microorganisms of different species. Plasmid-mediated resistance can be achieved in three different forms namely; conjugation, transduction and transformation (Novak et al., 2000).

Enterococci species develop resistance by altering the terminal D-alanine-D-alanine in their peptidoglycan to a D-alanine-D-lactate (Bush & Johnson, 2000). Resistance to macrolides by microorganisms is acquired through methylation of the adenine residue. Resistance to rifampin is achieved through mutations to the *rpoB* gene as seen in some strains of *Mycobacterium, Streptococcus pneumoniae, Staphylococcus aureus and Neisseria meningitides*. Resistance to fluoroquinolones is achieved through alterations of the targets which are the type II topoisomerases, DNA gyrase and topoisomerase IV. Another mechanism to fluoroquinolone resistance is achieved through acquisition of the plasmid-borne *qnr* (quinolone resistant gene) (Schaecter, 2008).

Nonetheless, some bacteria strains may also possess several mechanisms of resistance at the same time. The resistance can result from random mutations that provide a selective advantage or may also acquire resistance from its environment.

2.8.3 Antibiotic resistance in Ghana

In developing countries, particularly Ghana, increasing prevalences of resistance towards various antibiotic groups have been reported in several bacterial pathogens. A study conducted at the Komfo Anokye Teaching Hospital on isolated Gram-negative pathogens in urine samples reported that more than 20% of *E. coli* strains were resistant to ampicillin and co-trimoxazole. A similar study, also conducted at Komfo Anokye Teaching Hospital involving urinary tract infections among infants found all the 150 isolates (comprising *E. coli*, *Klebsiella*, *Proteus* and *Staphylococci*, the dorminant flora) to be resistant to ampicillin. They found that 77.8% of the isolates were susceptible to co-amoksiklav and 67% to nitrofurantoin, and only 11.1% of the isolates were susceptible to Co-trimoxazole (Adjei & Opoku, 2004).

In 2011, Newman et al. reported very high proportions of resistant bacteria strains isolated from various clinical specimens to be resistant to ampicillin (76%), tetracycline (82%), chloramphenicol (75%) and co-trimoxazole (73%) and lower proportions of resistance was reported for ceftriaxone (6.3%), Ciprofloxacin (11%) and amikacin (9.9%).

In January 2012, the Korle – Bu Teaching hospital children's block had to be closed down due to the outbreak of methicillin resistant *Staphylococcus aureus* which resulted in the death of three children (Pesewu et al., 2014).

A study conducted in Accra between 2006 to 2008 revealed that 13.7% of *E. coli* isolates were resistant to nalidixic acid, 52% of the *E. coli* strains isolated between 2006 and 2007 and 66.7% of the 2008 isolates were resistant to Ciprofloxacin (Namboodiri et al., 2011).

Ghana embraced the ReAct (action on Antibacterial resistance project) in 2010 in support of national efforts at containing the phenomenon on antimicrobial resistance in Ghana (GNDP 2010 & Newman et al., 2011). The phenomenon of antibiotic resistance is not only a national issue but also has regional and global dimensions and has serious financial, economic and clinical impact on the gains already made in health.

2.9 Antibiotic assays

A number of techniques are employed in determining the activity of antibiotics which include the diffusion method, the dilution method and the Epsilometer test (E-test). The effectiveness of an antibiotic can be assessed by its ability to kill or inhibit the growth of bacteria. For the diffusion method, the effectiveness is measured as a function of the zones of inhibition. The MIC is usually used to determine the effectiveness of an antibiotic in the dilution method but can also be done on solid media in diffusion method (Macias et al., 1994).

Selective media are used for primary isolation of specific types of microorganisms from samples containing several different species. They contain compounds that inhibit the growth of certain microorganisms whiles promoting the growth of other microorganisms. They; thus, hasten the isolation of microorganisms by suppressing growth of the unwanted microorganism and favouring the growth of the desired ones. An example is Mannitol salt agar and broth used for the identification and isolation of species of *Staphylococcus*. The high sodium chloride (NaCl) concentration in mannitol salt agar inhibits the growth of most human pathogens whiles promoting those of the *Staphylococcus* genus.

Differential media on the other hand are used to differentiate closely related organisms or groups of organisms. Certain dyes and chemicals present in the media allow growth patterns characteristic to certain microorganisms hence are used for selective identification and differentiation. They are employed in various fields to isolate microorganisms for laboratory exercises (Talaro, 2002). An example is MacConkey agar which is used to differentiate lactose fermenting *Enterobacteria* from non-lactose fermenting ones.

The agar diffusion method, also known as the Kirby-Bauer test is the most widely employed in the assay of various chemotherapeutic agents against microorganisms. A stabilized liquid agar medium is inoculated with a standardized amount of the microorganisms. poured into a sterile petri dish and allowed to solidify. Antibiotic disks containing known concentrations of the antibiotic are put on the solidified agar, allowed to stand for some time after which the plate is incubated at the required temperature for the required duration. The chemotherapeutic agents diffuse through the agar media during incubation and produce clear zones of inhibition in sensitive agar plates. The zones of inhibition produced are usually a function of the susceptibility patterns of the microorganism to the antibiotics. The larger the zone of inhibition the more susceptible the microbe is to the antibiotic. The reverse is true. By comparing the measured zones of inhibition to the standard table of the antibiotic at the test concentration, the organism can be reported as sensitive, intermediate or resistant. The procedure is however, affected by the solubility of the drug as drugs with poor solubility properties diffuse poorly producing smaller zones of inhibition even though may exhibit activity against the microorganism. However, the test is simple and inexpensive and is most often used when more sophisticated laboratory facilities are not available. A weakness of the diffusion method is that it does not determine whether a drug is bactericidal and not just bacteriostatic (Totora et al., 2013). Agar-well diffusion method is another form of the agar diffusion. With this method cups or wells are made in the solidified agar plates seeded with the microorganisms and different concentrations of the test antibiotic are administered into different cups. The petri dishes are incubated at the required temperature for the required duration after which clear zones of inhibitions are measured (Agyare et al., 2012). Mathematically, a plot of the square of the zones of inhibition against the natural logarithms of the antibiotic gives a linear regression with the zero intercept as the MIC. Figure 2.4 shows an agar plate with antibiotic disks and zones of inhibitions.

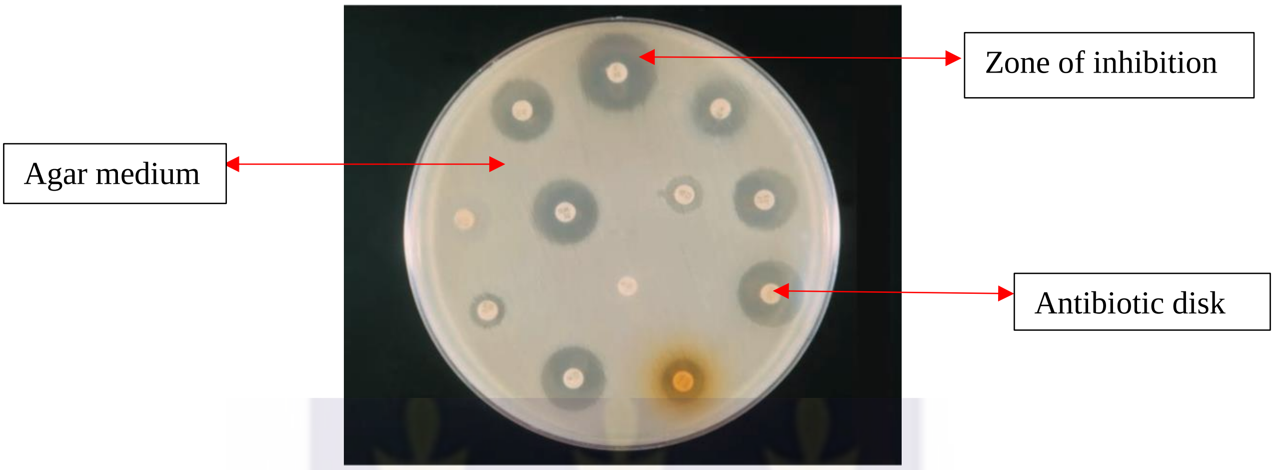


Fig 2.4: An agar plate showing clear zones of inhibition (Tortota et al., 2013)

The broth micro-dilution test is a useful and accurate laboratory procedure used for determining the MIC and the Minimum Bactericidal Concentration (MBC) of an antimicrobial drug. The procedure is usually carried out in the 96-well microtiter plate where each well of the plate is filled with broth media inoculated with the antibiotic and test organism. After the incubation period, MTT dye is added to the content of each well and the appearance of blue to purple colour is indicative of presence of microbial growth whiles the persistence of the unchanged yellow colour of MTT dye is indicative of the absence of bacterial growth. The MIC of the antimicrobial drug is thus recorded as the lowest concentration in the series at which no bacterial growth occurred. The broth micro-dilution method can also be used to differentiate between bacteriostatic and bactericidal agents and this can be done by inoculating the contents of the wells in broth or agar media free of the antibiotic. The appearance of microbial colonies following the incubation period depicts inhibition of growth, thus a bacteriostatic agent whiles the absence of microbial colonies depicts death of microorganisms thus a bactericidal agent. Determination of the MIC and MBC is important because it avoids the excessive or erroneous use of expensive antibiotics and minimizes the chance of toxic reactions associated with the use of larger doses of antibiotics (Tortora et al., 2013). Figure 2.5 shows a 96-well micro-titre plate where the presence of microbial growth changes the yellow colour of the MTT dye to blue while the yellow colour of the MTT dye persists in the absence of microbial growth.

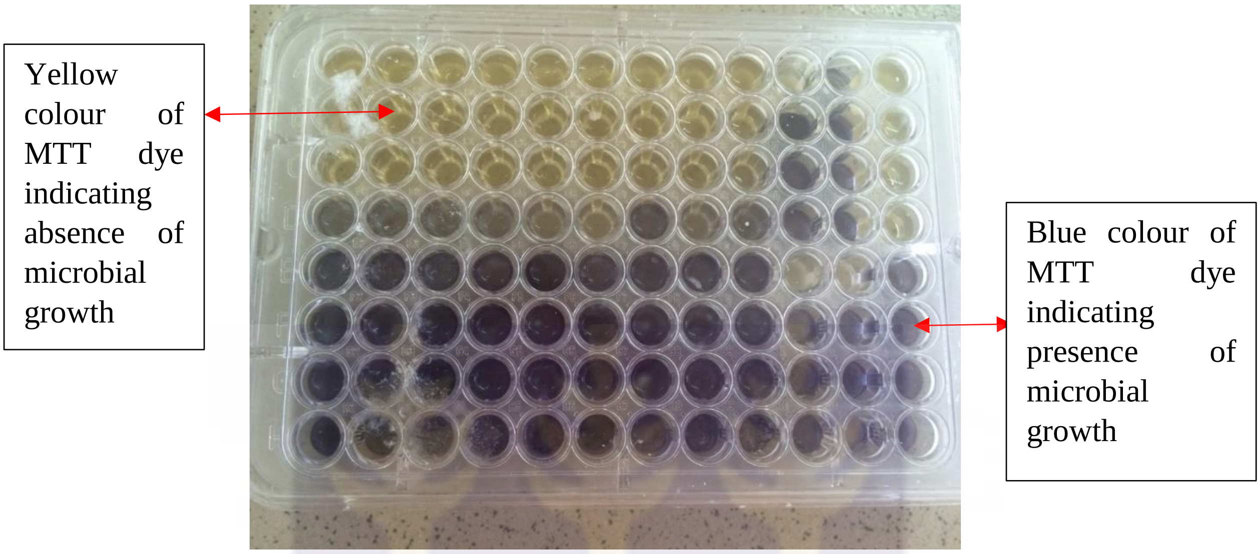


Fig 2.5: A 96-well micro-titre plate showing growth and no-growth for MIC determination (Talaro, 2002)

For the Epsilometer test, an E-test strip containing with gradient concentrations of the antibiotic is placed on the surface of an agar plate inoculated with the test organism. The antibiotic diffuses through the agar to produce an elliptical zone of inhibition after the incubation period. The MIC of the antibiotic is determined as the concentration at where the ellipse meets the test strip (Tortora et al., 2013).

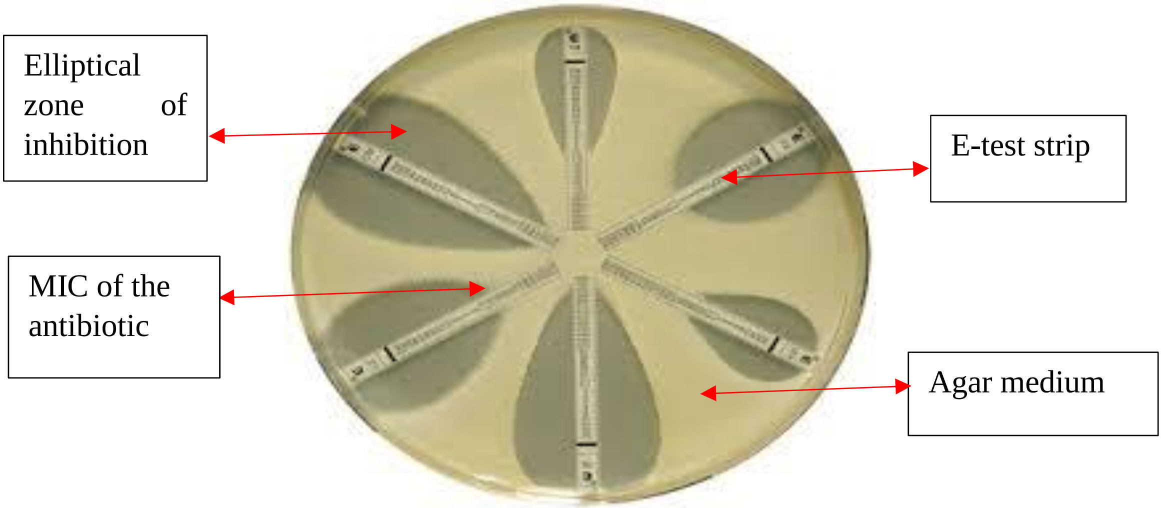


Fig 2.6: An agar plate with E-test strip showing elliptical zones of inhibition (Tortora et al., 2013)

2.10 *Escherichia coli*

Escherichia coli are a gram-negative lactose fermenting anaerobic and non-spore forming bacilli found mainly in the gut of humans and other warm-blooded mammals. After birth, these microorganisms can be found in the peri-urethral region of infants for a few hours (Linshaw, 1996). *E. coli* exist mainly as a gut commensal which co-habit with the animal host (Scott et al., 1990). Most *E. coli* strains are harmless and hence do not cause disease unless when found outside the normal gut flora such as in the case of a breach in the gastrointestinal tract resulting in peritonitis or when found in immunocompromised patients such as HIV/AIDS and diabetes patients (Epoke et al., 2000). The harmless strains are also involved in the synthesis of vitamin K_2 for the host as well as preventing the invasion of pathogenic microorganisms within the intestines (Dippold & Vogt, 2005). Transmission of the pathogenic strains is mainly faeco-oral. It is a facultative anaerobe that colonizes the mucosal layer of the mammalian colon and are the most abundant facultative anaerobes among the human microflora (Kaper et al., 2004). Several strains of *E. coli* exist and these strains are grouped into two main classes namely; enteric/diarrhoea causing and the extra-intestinal disease-causing type (Marrs et al., 2005).

2.10.1 Extra-intestinal *E. coli*

These strains are mainly the causative microorganisms in urinary tract infections as well as meningitis. However, the strains that cause urinary tract infections are collectively referred to as uropathogenic *E. coli*.

2.10.1.1 Uropathogenic *E. coli*

These refers to both pathogenic and non-pathogenic *E. coli* strains that move from the normal gut flora, particularly from the colon of the host and colonize the urinary tract system where they persist and multiply (Sweeney et al., 1996). These microorganisms may undergo mutations or transformations into DNA sequences which confer potential virulence factors thus resulting in pathogenicity. This, however, enables the microorganisms to adapt and thrive in the urine-abundant environment.

2.10.2 Enteric/diarrhoea *E. coli*

These stains are known to cause diarrhoea in the host and six different pathotypes have been identified namely the enterotoxigenic (ETEC), enteropathogenic (EPEC), enterohaemorrhagic (EHEC), enteroaggregative (EAEC), diffused adherence (DAEC) and enteroinvasive (EIEC) *E. coli*.

2.11 Chelation

Some antibiotics have the tendency of reacting with other compounds leading to the formation of complexes with reduced rates of absorption and increased rates of elimination. A classical example is the interaction between Ciprofloxacin and multivitamins, calcium and milk products (Uivarosi, 2013). Most patients are prescribed multivitamins and antacids, usually salts and bases of calcium, aluminium, magnesium and sodium, together with Ciprofloxacin because of the untoward side effects of the antibiotic which includes anaemia and gastric irritation. However, concurrent administration of multivitamin formulations, especially with mineral contents and Ciprofloxacin reduces the bioavailability of the antibiotic. Metallic contents bind and form complexes with the antibiotic which reduces the rate at which the drug is absorbed into the blood stream whiles making it more water soluble to be excreted from the body via the renal system. Calcium found in antacids and milk products also bind and form complexes with the Ciprofloxacin moiety which reduces the rate of absorption of the drug with subsequent increase in the rate of elimination. Milk also inhibits the absorption of Ciprofloxacin and other antibiotics through adsorption at the surface of milk protein molecules which is usually casein (Dey et al., 2015). Most pharmaceutical industries employ these principles, as safety mechanisms to reduce the exposure of workers to the raw antibiotic powder. Absorption of the raw antibiotic powder they are exposed to is inhibited while promoting the elimination of the drug from the body. This, however, decreases sensitization of microbes to the antibiotic leading subsequently to decrease in the development of resistant strains.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Methodology

3.1.1 Study design

A cross-sectional study, employing in vitro experimental procedures to analyse samples. It was carried out among pharmaceutical industry workers selected from various pharmaceutical industries located in the Greater-Accra Region. The pharmaceutical industries selected for the research included those that were involved in the production of antibiotics such as Amoxicillin, Cefuroxime and Ciprofloxacin. The study was carried out within a 1-month period where urine samples, as well as vital information from the study participants were collected for analyses. The analyses of the various samples, which included isolation, susceptibility testing and minimum inhibitory concentration determinations, were carried out at the laboratory department of the North Legon Hosptial, Legon-Accra, Ghana.

3.1.2 Study area

The study area comprised of three pharmaceutical industries. Two of these industries were located in Spintex in the Ledzokuku-Krowor Municipal Assembly in the Greater-Accra region of Ghana. The assembly is bounded to the west by the Accra Metropolitan Assembly, to the east by the Tema Metropolitan Assembly and to the south by the Gulf of Guinea, thus making fishing the major occupation in the municipality even though there exist other occupations due to the dense industries located in the municipality. The other was located at the Tema heavy industrial area in the Tema Metropolitan Assembly also in the Greater-Accra Region of Ghana. The metropolitan assembly is no different from that of LEKMA in terms of occupation as inhabitants engaged mostly in fishing activities as well as industrial work. The selected pharmaceutical industries have among their product portfolios, antibiotics used in the treatment of infections such as urinary tract infections.

3.1.3 Study variables

The study variables included age, sex, educational level, current station at the industry, duration at current station, availability of PPE, frequency of use of PPE, availability of chelating agents, frequency of use of chelating agents and antibiotic susceptibility patterns.

Independent Variables – Age, sex, educational level, current station, duration of stay at the current station, availability of PPE, frequency of use of PPE, availability of chelating agents and frequency of use of chelating agents. These factors influence the dependent variable which is susceptibility patterns of microorganisms to the antibiotics produced.

The prevalence of the *E. coli* strains among the collected urine samples from the pharmaceutical industrial workers was calculated as the ratio of the *E. coli* positive plates to the total samples expressed as a percentage. The urine samples were collected into sterile plastic 20 mL urine containers with air-tight screw caps. The containers were distributed among the participants and early morning mid-stream urine samples of volume between 10 – 20 mL were collected. Each urine container was labelled with a reference code of the participant. The collected urine samples were transported on ice in ice chests to the laboratory and tested within 2 hours of collection.

All the collected urine samples were inoculated into UriSelect agar and MacConkey agar and incubated at 37^{o}C for 24 hours and for another 24 hours in case no growth was observed after the first 24-hour incubation period. The appearance of pink colonies following the incubation was indicative of *E. coli* strains and such plates were recorded as positive for *E. coli* strains whiles plates that showed no growth or different coloured colonies other than pink were recorded as negative for *E. coli*.

The agar disk diffusion method was used for susceptibility testing of the isolated strains of *E. coli* against the selected antibiotics. Antibiotic disks were placed on the inoculated set agar and incubated at 37^{o}C for 24 hours.

The activity of the various antibiotics against the isolated strains of *E. coli* was measured as a function of the zones of inhibition where plates with zones of inhibition of less than 14 mm for both Amoxicillin and Cefuroxime and less than 15 mm for Ciprofloxacin were recorded as resistant for the respective antibiotics. Zones of inhibition of (15 – 16) mm for Amoxicillin, (15 – 17) mm for Cefuroxime and (16 – 20) mm for Ciprofloxacin were recorded as intermediate whiles zones of inhibition of greater than 18 mm for both Amoxicillin and Cefuroxime and greater than 21 mm for Ciprofloxacin were recorded as sensitive. MIC determinations were carried out on both the sensitive and intermediate strains to determine the concentrations that just inhibited the growth of the *E. coli* strains. These concentrations were recorded as the minimum inhibitory concentrations for the various antibiotics.

3.1.4 Study population

The population for the study were pharmaceutical industry workers stationed at various units of the production process such as on the production floor, warehouses (raw materials and finished goods), blistering department, packaging unit., quality assurance department and quality control laboratory. Quality assurance department is concerned with documentations as well as quality checks along the various stages of the product formulation whiles quality control laboratory is concerned with laboratory testing to ensure that the quality of the product is not compromised.

3.1.5 Sample size

The study population was selected from three antibiotic producing pharmaceutical industries in the Greater-Accra region of Ghana. One hundred and one (101) pharmaceutical industry workers were selected from various units of the selected pharmaceutical industries for the study. The sample size estimate for the study was calculated based on an assumed prevalence of 10%, using a confidence interval of 95% and 5% margin of error. Being an exploratory study with limited resources including funding and time, the size of 101 samples was considered adequate.

3.1.5 Sampling method

The simple random sampling technique was used to select the study participants. Google random number generator was used to generate random numbers from the total number of pharmaceutical industry workers present on the production plant for each of the three pharmaceutical industries. An average of thirty-four pharmaceutical industry workers (34) was selected from each of the three pharmaceutical industries.

3.1.6 Inclusion and Exclusion criteria

3.1.6.1 Inclusion criteria

- The study participants were selected to include personnel directly on the production plants as well as personnel stationed at different units of the production process such as secondary packaging, quality assurance department and quality control laboratories.

3.1.6.2 Exclusion criteria

- The study excluded personnel whose operations did not involve any of the production processes such as the personnel involved in administrative duties. Such personnel were excluded from the study because the selected pharmaceutical industries had their manufacturing plants separated from their administrative buildings.

3.1.7 Data collection

A structured questionnaire was used to collect vital information of the study participants. The vital information collected included variables such as age, sex, level of education, current station and duration at current station, availability and frequency of use of chelating agents and the availability and frequency of use of personal protective equipment.

Urine samples were collected from the study participants and analysed experimentally.

3.1.8 Data analysis

The association between the current station of the personnel and the development of resistant strains was obtained by cross tabulating current station against the susceptibility patterns of the various antibiotics. Associations between the current station and the dependent variable were determined using the chi – square test. The results were presented in tables, graphs.

3.1.9 Data processing

Data obtained from the questionnaires as well as the *in vitro* laboratory analysis were processed using Stata IC version 14.

3.1.10 Quality control

Samples were aseptically collected and stored in sample collection containers and transported on ice in ice chests to the laboratory. The collected samples were stored in the refrigerator between the temperature range of 4–8°C. Aseptic procedures were employed during processes such as isolation, susceptibility testing to the various antibiotics and minimum inhibitory concentration determinations. The various procedures were repeated to ensure reproducibility of the test results.

3.1.11 Ethical clearance

Ethical approval was sought from the Ghana Health Service ethical review committee. Antibiotic resistance as an issue of global public health concern required approval from the ethical review committee concerned with the health of the population of the country.

3.1.12 Ethical considerations

Approvals were sought from the Human resource managers and production managers of the selected pharmaceutical industries. The selected pharmaceutical industries were Entrance Pharmaceuticals and Research Centre, Spintex-Accra, Danadams Pharmceutical Industry Limited, Spintex-Accra and Ernest Chemists Limited, Tema-Accra. Approval was also sought from the Administrator of North Legon Hospital.

3.1.12.1 Potential risks/benefits

Handling of the collected samples for analysis was identified as the major area that posed a greater risk to the execution of the research. There was a greater possibility of contamination of the collected samples which would have resulted in false results when analysed experimentally. This, was however reduced through handling of fewer samples at a time and also implementation of strict aseptic procedures during transport and experimental procedures.

The study also sought to determine an association between industrial exposure to antibiotics and susceptibility patterns of microorganisms to the antibiotics produced.

3.1.12.2 Consenting process

Informed consent of the study participant was sought for prior to participation and sample collection.

3.1.12.3 Privacy/Confidentiality issues

Due to competition among the local pharmaceutical manufacturing companies, confidentiality of information obtained from any of them was very key o as to prevent leakage of vital information to competitors. The samples collected will be assigned arbitrary numbers to ensure confidentiality. The samples collected were also used for academic purposes only.

3.1.12.4 Data storage, security and usage

Data collected from the study were stored on a password protected external hard-disk drive and put under lock and key in a cabinet which was only be accessible to the principal investigator, co-principal investigators and the supervisors of the study. The collected and electronic data were destroyed after three months following submission of the final dissertation. The questionnaires were burned and the data from the experiments appropriately discarded. The data obtained from the study were used for academic purposes only.

3.1.12.5 Compensation

The study participants were refreshed appropriately after collection of samples to serve as a means of appreciation for their participation in the study.

3.1.12.6 Conflict of interest

The principal investigator had no conflict of interest in this study.

3.2 Laboratory Analysis

3.2.1 Materials

- Pure Amoxicillin impregnated disc
- Pure Ciprofloxacin impregnated disc
- Pure Cefuroxime impregnated disc

- Petri dishes
- Urine sample collection containers
- MacConkey agar
- UriSelect agar
- Nutrient agar and broth
- 96 – well micro-titre plate
- Micro-titre pipette
- Gloves
- Incubator
- Reciprocal water bath
- Autoclave
- Incubator
- Filter paper
- Hot air oven
- Measuring cylinders
- Beakers
- Test tubes
- Syringes and needle
- 3-4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT)

3.2.2 Isolation of *Escherichia coli*

0.1mL of the urine samples was seeded into freshly prepared and stabilized UriSelect agar and MacConkey agar and rolled in the palms to allow for uniform distribution of the sample throughout the liquid agar medium. It was then poured into a sterile petri dish, allowed to stand for about 20 minutes for the agar to solidify and incubated at 37^{o}C for 18-24 hours and for 48 hours in case no growth was observed after 24 hours.

After incubation, pink – red colonies with bile precipitates was indicative of *Escherichia coli strains*.

3.2.3 Susceptibility testing of *Escherichia coli* isolates to Amoxicillin, Cefuroxime and Ciprofloxacin using the agar disk diffusion method

Test tubes of 20mL UriSelect agar and MacConkey agar were seeded aseptically with 0.1mL of the urine samples and aseptically poured into sterile petri dishes and allowed to stand for about 20 minutes for the agar to solidify. Antibiotic discs containing different concentrations were put on the set agar and allowed to stand for about 30-45 minutes to allow for efficient diffusion of the antibiotics into the agar. The petri dishes were then incubated at 37^{o}C for 18-24 hours after which zones of inhibition around each disk was measured and recorded.

3.2.4 Minimum Inhibitory Concentration determinations (MIC) using the broth micro-dilution method

The minimum inhibitory concentration (MIC) of the antibiotic samples was determined using the broth micro-dilution technique. Each of the 96-well micro-titre plate was filled with 50 uL double strength MacConkey broth and 10 uL of the urine samples. Stocks of 40 µg/mL of the antibiotics were prepared. Appropriate volumes of sterile water and the stocks were added to each well to make up to 100 uL. The plate was covered and incubated ay 37^{o}C for 24 hours. Following incubation, 5 uL of 1.25 mg/mL of 3-4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT) was added to the content of each well. The observation of blue to purple colour was indicative of microbial growth whiles the persistence of the yellow colour of the MTT dye was indicative of the absence of microbial growth. The MIC of the drug solution for the particular organism was recorded as the lowest concentration of the drug solution where no growth was observed.

CHAPTER 4

RESULTS

4.1 Prevalence of *E. coli* urine isolates

A total of 101 urine samples were collected and analysed using microbial assays to isolate and identify urine microbes. A total of 47 bacterial species were isolated from the 101 urine samples which included *Klebsiella spp., Enterobacter spp., Non-lactose fermenters and Escherichia coli.* 20 out of the 47 bacterial urine isolates were identified to be *E. coli* strains. The prevalence of *E. coli* urine isolates among the urine samples collected from the pharmaceutical industry workers was calculated as a percentage fraction of the isolated *E. coli* strains to the total urine samples collected.

Mathematically, Prevalence of *E. coli* = $\frac{20}{101} \times 100 = 19.80\%$

Thus, the prevalence of *E. coli* strains among the recruited pharmaceutical industry workers in Accra was 19.80%.

4.2 Demography of the study participants

A total of 101 pharmaceutical industrial workers consisting of 73 males and 28 females volunteered to participate in the study. The study participants included personnel from both the production floor and non-production floor. The production floor personnel included workers from the various production departments such as dispensing, granulation, compression, coating, blistering, sachet filling and encapsulation. The non-production floor personnel included workers from departments such as raw materials and finished goods warehouses, secondary packaging, quality assurance department as well as quality control laboratory. The production floor personnel were those who by virtue of their working conditions and tasks performed are greatly exposed to the raw pharmaceutical ingredients whiles the non-production floor personnel were those who were less exposed to the raw pharmaceutical ingredients.

Out of the total sample size of 101, 72.28% of the study participants were males and 27.72% were females. Majority (51.48%) of the participants were within the age range of 31 – 40 whiles 26.73% and 21.78% of the participants were within the age ranges of 21 – 30 and 41 – 50 respectively. All the study participants had had some form of formal education with the highest proportion achieving secondary level of education and lowest proportion achieving primary level of education. Majority (63.37%) of the participants were stationed on the production floor where they carried out their normal dispensation of duties whiles 36.63% were stationed away from the production floor, hence their normal dispensation of duties did not involve the production floor. Out of the total study participants, 85.15% had spent more than 6 months at their current station whiles 2.97% and 11.88% had spent (1 – 3) months and (3-6) months respectively at their current station. All the study participants had access to daily PPE but did not have access to chelating agents. Out of the total 101 samples collected; urine microbes were present in 46.53% and absent in 53.47%. Out of the identified urine microbes, 19.8% were identified to be *E. coli* strains whiles 80.20% were identified to be strains other than *E. coli*.

Table 4.1: Demographic characteristics of the study participants

Variable	Frequency	Percentage
Sex		
Male	73	72.28
Female	28	27.72
Age		
21-30	27	26.73
31-40	52	51.48
41-50	22	21.78
Educational level		
Primary	2	1.98
JHS	16	15.84

SHS	66	65.35
Tertiary	17	16.83
Current station		
Production floor worker	64	63.37
Non-Production floor worker	37	36.63
Duration of stay at current station		
1-3 months	3	2.97
3-6 months	12	11.88
> 6 months	86	85.15
Availability of PPE		
Yes	101	100
Frequency of use of PPE		
Daily	101	100
Availability of chelating agents		
No	80	79.21
Not applicable	21	20.79
Urine microbes		
Present	47	46.53
Absent	54	53.47
***E. coli* isolates**		
Present	20	19.80
Absent	81	80.20

Out of the 20 participants with urine *E. coli* isolates, 55% were males and 45% were females. Majority (50%) of them were within the age range of 31 – 40, whiles 40% and 10% were within age ranges of 21 – 30 and 41 – 50 respectively. Majority (85%) of the participants had attained secondary level of education whiles 15% had attained tertiary level of education. Out of the 20 participants, 45% were stationed on the production floor where daily dispensation of duties had to do with the production floor whiles 55% were stationed away from the production floor. Majority of the participants had spent more than six months at their current station whiles 25% of the participants had spent 3 – 6 months at their current station. All the study participants had access to daily PPE but did not have access to chelating agents.

Table 4.2: Demographic characteristics of participants with *E. coli* urine isolates

Variable	Frequency	Percentage
Sex		
Male	11	55.00
Female	9	45.00
Age		
21-30	8	40.00
31-40	10	50.00
41-50	2	10.00
Educational level		
SHS	17	85.00
Tertiary	3	15.00
Current station		
Production floor worker	9	45.00
Non-Production floor worker	11	55.00
Duration of stay at current station		
3-6 months	5	25.00
> 6 months	15	75.00
Availability of PPEs		
Yes	20	100
Frequency of use of PPEs		
Daily	20	100
Availability of chelating agents		
No	14	70.00
Not applicable	6	30.00

4.3: Susceptibility patterns of *E. coli* isolates to the selected antibiotics

Out of the 20 isolated strains of *E. coli*, none was sensitive to amoxicillin as all strains showed resistant patterns to it. Some of the isolates (2 of 20) showed sensitivity patterns to cefuroxime whiles 3 of the 20 isolates showed intermediate resistant patterns to it. Most of the isolates (15 of 20) showed resistant patterns to cefuroxime. For ciprofloxacin, 8 of the 20 isolates showed sensitivity patterns whiles 3 of the 20 isolates showed intermediate resistant patterns to it. Most of the isolates (9 of 20) showed resistant patterns to it.

Table 4.3: Antibiotic susceptibility patterns of *E. coli* isolates

	***E. coli* Isolates (n=20)**			
Antibiotic	**Sensitive**	**Intermediate**	**Resistant**	**Resistance rate (%)**
Amoxicillin	0	0	20	100
Cefuroxime	2	3	15	75
Ciprofloxacin	8	3	9	45

4.4: Test of association between industrial exposure to antibiotics and development of resistant strains of microorganism

A cross tabulation was used to test the association between industrial exposure to antibiotics and the development of resistant strains of microorganism. Workers were grouped into production floor and non-production floor workers based on their current station in the industry. Production floor workers included personnel stationed in units such as dispensing, granulation, compression and raw materials warehouses whiles non-production floor workers included personnel stationed in units such as quality assurance department and quality control department. The production floor workers had daily direct contacts with raw active pharmaceutical ingredients whiles the non-production floor workers hardly came into contact with the raw active pharmaceutical ingredients. For the duration at current station, workers were grouped into those who had spent 3–6 months and those who had spent more than 6 months.

Table 4.4: Test of association between current station and development of resistant strains of microorganism

	Amoxicillin Susceptibility Pattern					
Current Station	**S**	**I**	**R**	**Total**	**x^2**	**p-value**
Production floor worker	0	0	9	9	-	-
Non-Production floor worker	0	0	11	11		
Total	0	0	20	20		
	Cefuroxime Susceptibility Pattern					
Current Station	**S**	**I**	**R**	**Total**	**x^2**	**p-value**
Production floor worker	0	1	8	9	5.3222	**0.033***
Non-Production floor worker	2	2	7	11		
Total	2	3	9	20		
	Ciprofloxacin Susceptibility Pattern					
Current Station	**S**	**I**	**R**	**Total**	**x^2**	**p-value**
Production floor worker	1	1	7	9	7.4860	**0.024***
Non-Production floor worker	7	2	2	11		
Total	8	3	9	20		

Table 4.5: Test of association between duration at current station and development of resistant strains of microorganism

	Amoxicillin Susceptibility Pattern					
Duration at current station	**S**	**I**	**R**	**Total**	x^2	**p-value**
3 – 6 months	0	0	5	5	-	-
> 6 months	0	0	15	15		
Total	0	0	20	20		
	Cefuroxime Susceptibility Pattern					
Duration at current station	**S**	**I**	**R**	**Total**	x^2	**p-value**
3 – 6 months	1	0	4	5	4.6889	0.049
> 6 months	1	3	11	15		
Total	2	3	15	20		
	Ciprofloxacin Susceptibility Pattern					
Duration at current station	**S**	**I**	**R**	**Total**	x^2	**p-value**
3 – 6 months	1	0	4	5	5.4815	0.045
> 6 months	7	3	5	15		
Total	8	3	9	20		

Fig 4.1: ***E. coli*** **isolates and their susceptibility patterns to the antibiotics using the current station of the workers**

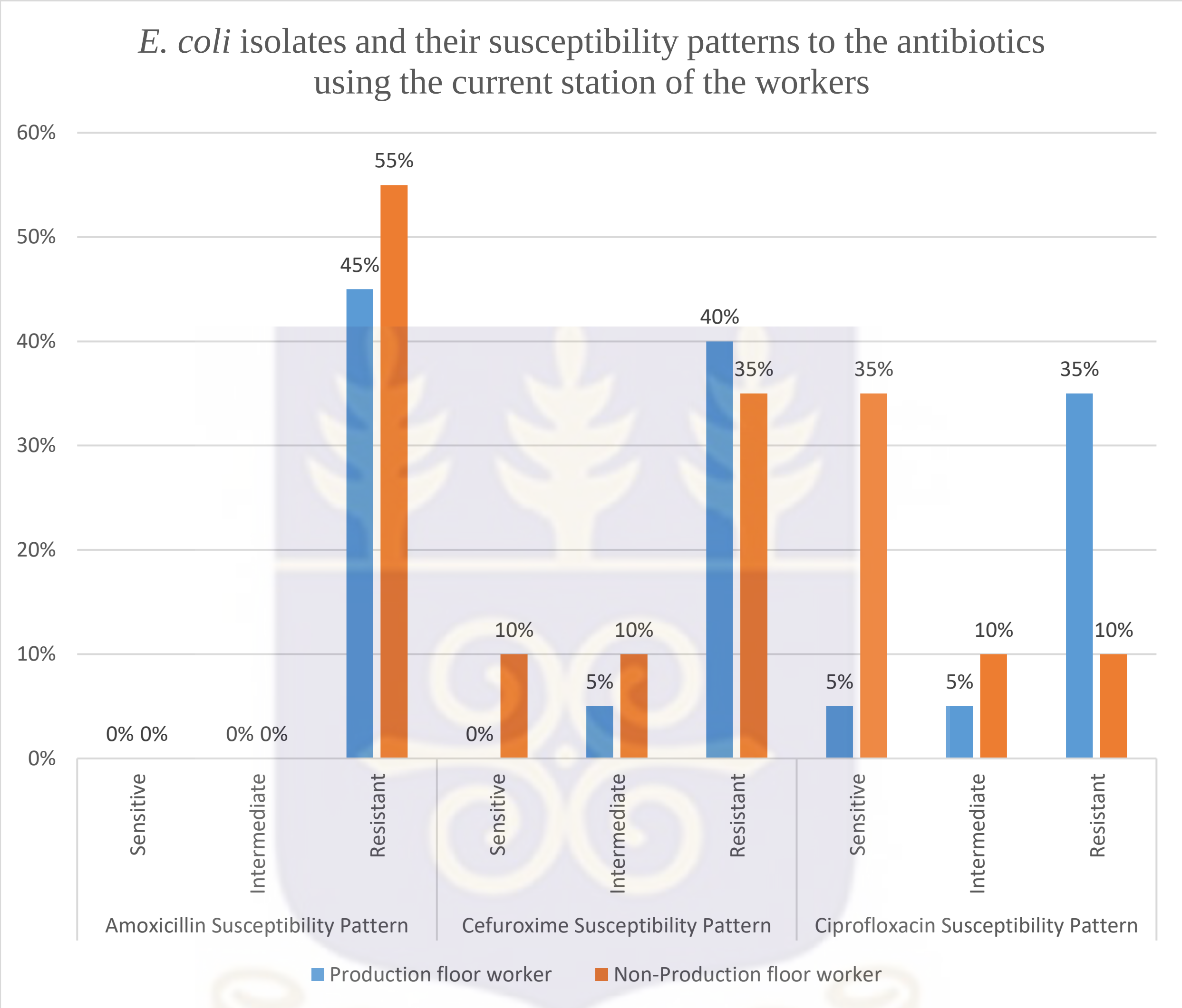
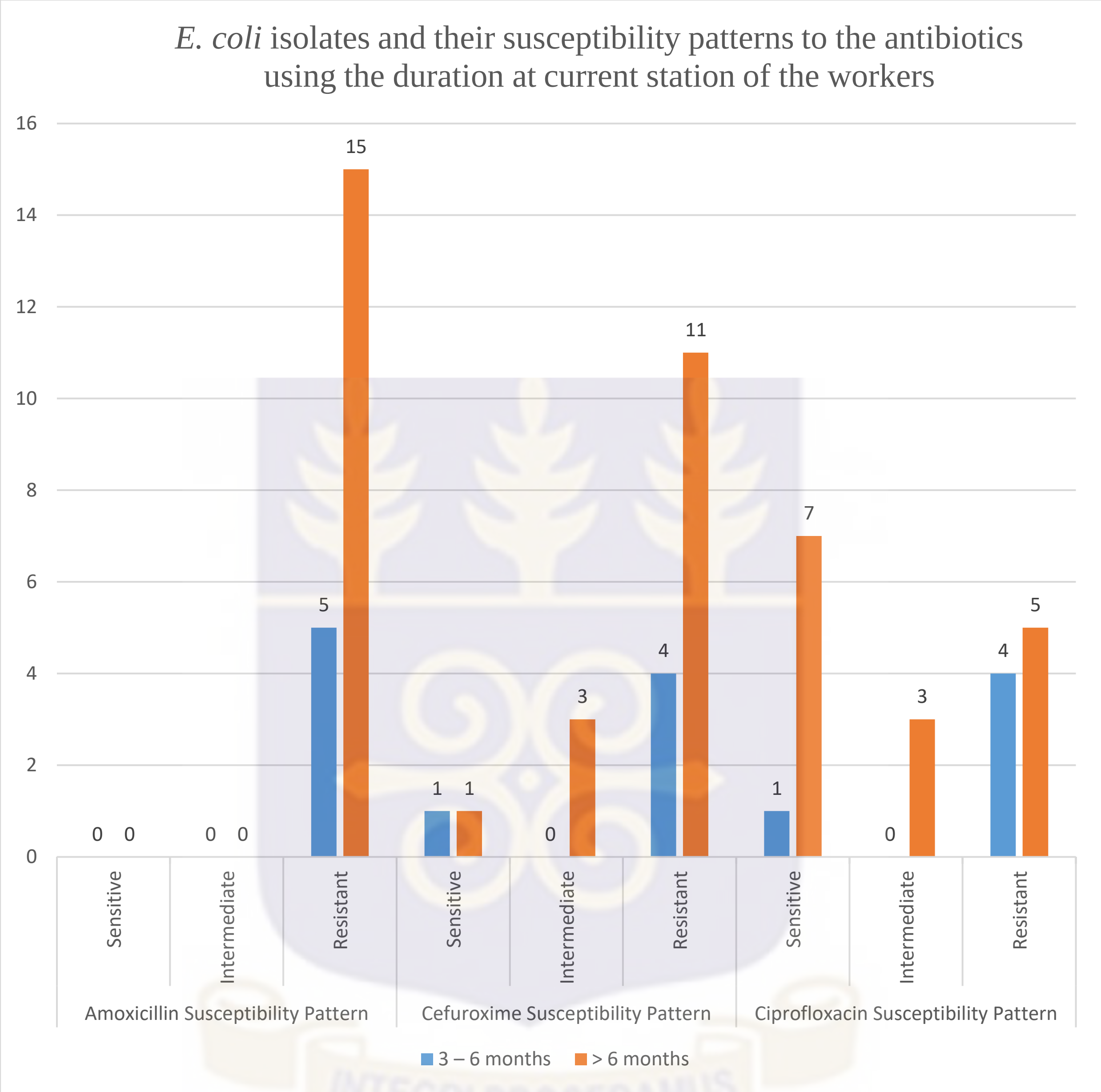


Fig 4.2: *E. coli* isolates and their susceptibility patterns to the antibiotics using the duration at current station of the workers



4.5: Minimum inhibitory concentrations of antibiotics against the *E. coli* isolates

The minimum inhibitory concentrations were determined for determined for the antibiotics to which the isolated microorganism showed sensitivity and intermediate resistant patterns. None of the isolated microorganisms showed sensitivity or intermediate resistance patterns to Amoxicillin. Cefuroxime had five of the isolated strains showing sensitivity and intermediate resistance patterns to it whiles Ciprofloxacin had eleven of the isolated strains showing sensitivity and intermediate resistance to it. The MIC for Cefuroxime ranged from 20-30 µg/mL whiles that of Ciprofloxacin ranged from 3-5 µg/mL.

Table 4.6: Minimum inhibitory concentrations of antibiotics against the *E. coli* isolates

	MIC (µg/mL)		
***E. coli* Isolates**	**Amoxicillin**	**Cefuroxime**	**Ciprofloxacin**
001	-	-	5
002	-	-	3
003	-	-	5
004	-	-	5
005	-	30	3
006	-	-	4
007	-	20	3
008	-	25	4
009	-	30	3
010	-	30	4
011	-	-	4

CHAPTER 5

DISCUSSION

5.1 Discussion

Antibiotic resistance continues to be an issue of global public concern because of the increasing resistant strains of microorganisms to the current antibiotics on the market. These agents were however very effective in the treatment or control of the infections caused by these strains but development of resistance to these agents have limited their use in current treatment regimens for most infections. There is however, limited synthesis of newer antibiotics as most pharmaceutical giants finds the antibiotic market as not profitable compared to the market of chronic infections. Antibiotic regimens are usually short-lived lasting usually between 7-14 days after which treatment is terminated unlike in the case of chronic diseases where treatment usually lasts for a lifetime. It is therefore imperative to prudently and effectively manage the already existing antibiotics so as to reduce the incidence of increasing resistance whilst remaining effective for our generation and the generations yet to come. Antibiotic resistance can arise from various factors such as exposure to sub-therapeutic doses of the chemicals or drugs, the use of antibiotics for indications which do not require antibiotic therapies, the use of antibiotics in animal feed among others (Kelesidis et al., 2007). The study thus aimed at assessing the activity of Amoxicillin, Cefuroxime and Ciprofloxacin against *Escherichia coli* among pharmaceutical industrial workers in Accra. Pharmaceutical industrial workers are a vulnerable group of individuals who by virtue of their operations are repeatedly exposed to sub-therapeutic amounts of the antibiotics they produce through inhalation and skin contacts (Uivarosi, 2013).

The agar disc diffusion method was used for the susceptibility testing whiles the broth micro-dilution method was used for the minimum inhibitory concentration determinations (MIC) of the selected antibiotics against the isolated strains of *E. coli*. Zones of inhibition around the antibiotic discs following the 24-hour incubation of the microbial plates were measured, recorded and appropriate inferences of susceptibility patterns were made. Microbial plates that gave zones of inhibition of less than 14 mm for both Amoxicillin and Cefuroxime were recorded as resistant to the antibiotics whiles plates that gave zones of inhibition of less than 15 mm for Ciprofloxacin was recorded as resistant to the antibiotic. Microbial plates that gave zones of inhibition of (15-17) mm for both Amoxicillin and Cefuroxime were recorded as intermediately resistant to the antibiotics whiles plates that gave zones of inhibition of (16-20) mm for Ciprofloxacin were recorded as intermediately resistant to the antibiotic. Microbial plates that gave zones of inhibition of greater that 18 mm for both Amoxicillin and Cefuroxime were recorded as sensitive to the antibiotics whiles plates that gave zones of inhibition of greater than 21 mm for Ciprofloxacin were recorded as sensitive to the antibiotic.

The prevalence of urine *E. coli* strains among the recruited pharmaceutical industrial workers in Accra was 19.80%. These are however, healthy asymptomatic people who showed no signs and symptoms of urinary tract infection. *E. coli* is the indicative microorganism in most urinary tract infections (Schaechter 2009). It is a normal flora of the gut but become pathogenic once it leaves the normal gut flora into the urinary tract (Tortora et al., 2013). All the strains of *E. coli* isolated from the study participants were resistant to Amoxicillin at the test concentrations. Amoxicillin is however an antibiotic employed in the treatment of urinary tract infections as it is renally excreted hence concentrates in urine (BNF 2017). Amoxicillin is a broad-spectrum antibiotic with marked activities against genitourinary tract isolates such as *E. coli*, *P. mirabilis* and *E. faecalis*. The observed resistance of the isolated strains to the antibiotic could be due to the industrial exposure of sub-therapeutic amounts as they carry out their normal daily operations at the industry. Amoxicillin, being one of the most widely abused antibiotic on the

Ghanaian market could also be a contributory factor to the observed resistance of both the production floor personnel isolates and the non-production floor personnel isolates.

15 out of the 20 isolated *E. coli* strains were resistant to Cefuroxime whiles 3 out of the 20 isolated strains showed intermediate resistance to Cefuroxime. Only 2 of the isolated strains were sensitive to Cefuroxime. 53.3% (8 of 15) of the resistant strains were isolated from urine samples of production floor personnel whiles 46.7% (7 of 15) were isolated from urine samples of non-production floor personnel. None of the sensitive strains were isolated from urine samples of production floor personnel whiles 100% (2 of 2) were isolated from urine samples of non-production floor personnel. 33.3% (1 of 3) of the intermediate resistant strains were isolated from urine samples of production floor personnel whiles 66.7% (2 of 3) were isolated from urine samples of non-production floor personnel. The high prevalence of resistance among the production floor personnel urine isolates may be due to the exposure to sub-therapeutic amounts of the raw Cefuroxime powder as they carry out the various production processes to give out finished products. Cross resistance between penicillin (Amoxicillin) and cephalosporin (Cefuroxime) is very common as these two drugs are similar in structure and exhibit the same mechanisms of action (Grimm, 1984). This may however be the reason for the resistance among the non-production personnel urine *E. coli* isolates. However, the strains that exhibited intermediate resistance patterns to the antibiotic may be in the process of transition from sensitive to resistance and hence infections caused by such strains of microorganisms usually will require treatment regimens higher than the normal therapeutic doses (Ryan & Ray 2014). Cefuroxime is a cephalosporin with a broad spectrum of activity affecting both gram-negative and gram-positive strains. Like Amoxicillin, Cefuroxime is renally excreted and hence concentrates in the urine making it effective for the treatment of urinary tract infections BNF 2017).

Ciprofloxacin is a fluoroquinolone with broad spectrum antibiotic with marked gram-negative activity. It is employed for the treatment of most urinary tract infections as it concentrates in the urine due to renal excretion (BNF 2017). 9 out of the 20 *E. coli* urine isolates were resistant whiles 3 out of the 20 strains showed intermediate resistance to Ciprofloxacin. 8 of the 20 isolated strains were sensitive to Ciprofloxacin. 77.8% (7 of 9) of the resistant strains were isolated from urine samples of production floor personnel whiles 22.2% (2 of 9) of the resistant strains were isolated from urine samples of non-production floor personnel. 12.5% (1 of 8) of the sensitive strains were isolated from urine samples of production floor personnel whiles 87.5% (7 of 8) of the sensitive strains were isolated from urine samples of non-production floor personnel. 33.3% (1 of 3) of the intermediate resistant strains were isolated from urine samples of production floor personnel whiles 66.7% (2 of 3) were isolated from urine samples of non-production floor personnel. The high prevalence of resistance among the production floor personnel urine isolates may be due to industrial exposure to sub-therapeutic amounts of the antibiotic. These personnel, through inhalation and skin contact, are exposed to sub-therapeutic amounts of the antibiotic which sensitizes the microorganisms to develop resistant mechanisms towards it. The high prevalence of the sensitive strains among the non-production floor personnel urine isolates may be due to limited exposure to the antibiotics as the daily activities of these personnel do not require them to come into direct contact with the antibiotics. Thus, there was a significant association between current station of the workers and development of resistant strains to antibiotics produced (p-value of 0.033 for Cefuroxime and 0.024 for Ciprofloxacin).

From the results, it can also be observed that most of the production floor personnel urine isolates which were resistant to both Amoxicillin and Cefuroxime were however sensitive to Ciprofloxacin. This may however be due to the fact that cross-resistance between fluoroquinolones (Ciprofloxacin) and β-lactam antibiotics (Amoxicillin and Cefuroxime) is rare. Ciprofloxacin exhibits an entirely different structure and mechanism of action from the β-lactam antibiotics by inhibiting the DNA gyrase enzyme responsible for unwinding the DNA double helix structure during cell division thus promoting breakage of the double-stranded DNA (BNF 2017, Tortora et al., 2013 & Schaechter 2009).

The duration of current station also showed a similar trend as personnel who had spent more than six months at their current station showed increased resistant patterns to the antibiotics (p-value of 0.049 for Cefuroxime and 0.045 for Ciprofloxacin) as compared to personnel who had spent less than six months at their current station. This, however, can attest to the fact that extended exposure to the sub-therapeutic amounts of the antibiotics can significantly lead to the development of resistant strains of microorganisms (Grimm, 1984 & Uivarosi, 2013).

In general, it was observed that being a production floor personnel (dispensing, granulation, compression, in-process quality control, coating, blistering, sachet filling, bottle filling and encapsulation) puts one at risk of developing resistant strains of bacteria to the antibiotics they produce than being a non-production floor personnel (secondary packaging, quality assurance department and quality control laboratory). The calculated risk for the production floor personnel developing resistant strains of bacteria to Amoxicillin was no different for that of non-production personnel. The calculated risk for the production floor personnel developing resistant strains of bacteria to Cefuroxime was 1.2 compared to that for non-production floor personnel. The calculated risk for the production floor personnel developing resistant strains of bacteria to Ciprofloxacin was 2.4 compared to that for non-production floor personnel However, exposure of the production personnel to the active pharmaceutical ingredients such as the raw antibiotic powder can be minimised through the use of personal protective equipment which would protect the workers from exposure through inhalation and dermal contacts as well as the products from contaminations. They can as well be given chelating agents which will complex with the antibiotics, making them more water soluble to reduce absorption whiles promoting excretion. In these ways, exposure to the antibiotics will be reduced thus minimizing or possibly preventing sensitization of the microorganisms to the antibiotics. From the findings, it can be inferred that the use of personal protective equipment alone as means of reducing or preventing exposure is not enough. This is because even though they wore personal protective equipment whiles performing their daily operations, they still developed resistant strains of microorganisms to the antibiotics produced (Uivarosi, 2013).

The personal protective equipment, even though were provided on daily basis to these workers could not offer complete protection against the effects of the raw pharmaceutical ingredients as most of these PPE were incomplete and lacked some components. Chelation on the other hand was not practised among any of the selected pharmaceutical industries, thus left personnel at risk of the effects of exposure to the raw pharmaceutical ingredients which included development of resistant strains of microorganisms.

The MIC of Cefuroxime against *E. coli* fell in the range of 20–30 µg/mL whiles that of Ciprofloxacin fell in the range of 3–5 µg/mL. This, however, affirms the fact that Ciprofloxacin exhibits more gram-negative activity than Cefuroxime as the MIC range for Ciprofloxacin were lower than that of Cefuroxime.

However, comparing the results obtained from this study to a similar study carried out among children of school going age in Nigeria; it was observed that the resistance patterns of the urinary isolates of *E. coli* were not different in both studies as most of the isolates that were resistant to penicillin (Amoxicillin) showed cross resistant patterns to cephalosporins (Cefuroxime). This sensitivity patterns were however different among fluoroquinolones (Ciprofloxacin) as most of the strains showed sensitivity patterns to it with only a few showing resistance patterns to it (Aiyegoro et al., 2007).

Findings from the study also indicated that, the recruited pharmaceutical industries did not practice chelation as means of reducing exposures to the raw pharmaceutical ingredients. For this reason, a policy can be formulated to enforce the use of chelating agents so as to ensure the health and safety of the workers through reduction in exposures. Antibiotic resistance as an issue of global health concern can be reduced significantly if exposure to antibiotics is markedly reduced which includes industrial exposure. Also, a policy can be formulated to ensure that the PPE supplied are complete thus reducing exposures to the barest minimum. The recruited industries supplied PPE in pieces where some components such as googles and glove sleeves were not available. The policies governing health and safety at the industries should be enforced thereby making the industrial environment safe to work in. Health and safety officers should be made mandatory for all industries where they will ensure that the working environment is safe for people to operate in.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of *E. coli* urine isolates among pharmaceutical industrial workers in Accra was 19.80%. Some of the isolated strains of *E. coli* were sensitive to both Cefuroxime and Ciprofloxacin whiles all the isolated strains were resistant to Amoxicillin. The MIC ranges for Cefuroxime and Ciprofloxacin were 20–30 µg/mL and 3–5 µg/mL respectively. There was significant association between working in the pharmaceutical industry as a production floor personnel and development of resistant strains of microorganisms to the antibiotics produced. The use of personal protective equipment alone to prevent industrial exposure to raw pharmaceutical ingredients is not enough as both production and non-production floor workers had resistant strains of *E. coli.*

6.2 Recommendations

Out of the 101 urine samples collected for analyses, only 20 of the cultured samples gave growth that were identified to be isolates were identified to be *E. coli* strains. The study thus, should be repeated using a much larger sample size to confirm the findings of this study since the sample size was small.

Since none of the selected pharmaceutical industries practiced chelation as a means of preventing exposure, further studies should be conducted to find out industries that practise chelation and subsequently ascertain the role of chelation in the prevention of industrial exposure.

A more sophisticated study such as a case-control study should be conducted comparing antibiotic producing pharmaceutical industry workers to non-antibiotic producing pharmaceutical industry workers to confirm the role of industrial exposure to the development of resistant strains.

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APPENDIX 1A: MATERIALS, APPARATUS, GLASSWARE AND EQUIPMENT

Table 1A.1: Consumables

Consumables	Manufacturer/Source
Nutrient agar	Oxoid Ltd, Basingstoke, United Kingdom
Nutrient broth	Oxoid Ltd, Basingstoke, United Kingdom
UriSelect agar	Oxoid Ltd, Basingstoke, United Kingdom
MacConkey agar	Oxoid Ltd, Basingstoke, United Kingdom
MacConkey broth	Oxoid Ltd, Basingstoke, United Kingdom
Citrate	Biomark Laboratories, Pune, India
Peptone water	Biomark Laboratories, Pune, India
Triple sugar iron (TSI) agar	Biomark Laboratories, Pune, India
96-well micro-titre plate	Thermo Fisher Scientific, United Kingdom
Micro-titre pipette tips	Fisher Scientific GMBH, Schwerte, Germany
Gloves	Kama Health Services, Ghana
Antibiotic disk	Axiom Laboratories, New delhi, Delhi, India

Table 1A.2: Equipment, Apparatus and Glassware

Equipment, Apparatus and Glassware	Manufacturer/Source
Petri dishes	Fisher Scientific GMBH, Schwerte, Germany
Electronic weighing balance	Ohaus Corporation, Pine Brook, NJ, USA
Autoclave	Basildon Ltd, UK
Reciprocal water shaker bath	New Brunswick Scientific, Edison NJ, USA
Refrigerator	Sharp Corporation, UK
Incubator	Weiss Technik, UK
Laminar flow cabinet	Gelaire BSB6, Germany

APPENDIX 1B: PREPARATION OF MACCONKEY AGAR

MacConkey agar is a selective and differential media used for the isolation of gram–negative enteric bacteria and differentiation of lactose fermenting from non – lactose fermenting gram negative bacteria.

49.53 grams of dehydrated medium was suspended in 1000 mL of purified or distilled water. The mixture was heated to boiling to completely dissolve the medium. The media was then sterilized by autoclaving at 121°C for 15 minutes. The media was well mixed and poured into sterile petri dishes for inoculation.

Composition

Ingredients	**Gram/Litre**
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin	17.000
Lactose monohydrate	10.000
Bile salts	1.500
Sodium chloride	5.000
Crystal violet	0.001
Neutral red	0.030
Agar	13.500
pH after sterilization at 25°C	7.1±0.2

APPENDIX 1C: PREPARATION OF URISELECT AGAR

UriSelect agar is a non–selective chromogenic agar medium used for the isolation, differentiation and enumeration of urinary tract pathogens.

56.80 grams of the dehydrated medium was suspended in 1000 mL of distilled water and continuously stirred to obtain a homogenous suspension. The suspension was boiled while stirring frequently until optimal dissolution of the agar and subsequently adjusted to a pH of 7.3. The suspension was then sterilized by autoclaving at 121oC for 15 minutes and poured into sterile petri dishes.

Composition

Ingredients	**Grams/Litre**
Peptone mix	21
Silica	20
Chromogenic mix	<1
Tryptophan	1
Agar	16
Final pH	7.2±0.2

APPENDIX 1D: PREPARATION OF DOUBLE STRENGTH MACCONKEY BROTH

The double strength MacConkey broth was be used for the minimum inhibitory concentration determination where it will be diluted with antibiotics, microorganisms and sterile water to give a final single strength broth.

80.15 grams of the MacConkey broth is suspended in 1000mL of sterile distilled water. The mixture is heated to dissolve the mixture completely. The resultant mixture is distributed into test tubes with inverted Durham tubes and sterilized by autoclaving at 121^{o}C for 15 minutes. Cool the tubes before inoculation.

Composition

Ingredients	**Gram/Litre**
Peptic digest of animal tissue	40.000
Lactose	20.000
Bile salts	10.000
Sodium chloride	10.000
Neutral red	0.150
Final pH (at 25^{o}C)	7.4±0.2

APPENDIX 1E: RELATIVE RISK CALCULATIONS

For Amoxicillin, using non-production floor workers as the reference,

Risk ratio= $\frac{9/9}{11/11} = 1.00$

There was no difference between production floor workers and non-production floor workers.

For Cefuroxime, using non-production floor workers as the reference,

Risk ratio= $\frac{9/9}{9/11} = 1.20$

Production floor workers were more likely to develop resistant strains than non-production floor workers.

For Ciprofloxacin, using non-production floor workers as the reference,

Risk ratio= $\frac{8/9}{4/11} = 2.44$

Production floor workers were more likely to develop resistant strains than non-production floor workers

APPENDIX 2A: PARTICIPANT CONSENT FORM – PHARMACEUTICAL INDUSTRY WORKER

School of Public Health

College of Health Sciences

University of Ghana

Project Topic

Assessment of the activity of selected antibiotics against *Escherichia coli* among selected pharmaceutical industry workers in Accra.

Background

Dear Participant,

I wish to invite you to participate in an academic research involving pharmaceutical industry workers in Accra. My name is Joseph Poku Asumadu, a student of the School of Public Health, University of Ghana. I am undertaking a study on the topic: Assessment of the activity of selected antibiotics against *Escherichia coli* among selected pharmaceutical industry workers in Accra. The objective of the study is to determine how industrial exposure to raw active pharmaceutical ingredients influences the sensitivity patterns of the isolated microorganisms to the selected antibiotics which are employed in the treatment of majority of the infections caused by these microorganisms. This will thus make the workers aware of the harmful effects of the exposure on their health. and ways which can be employed to protect themselves from exposure to these active ingredients. This will also inform health professionals about the treatment regimens to opt for during treatment of infections among these workers. This research would form part of my work for the award of a Masters' degree in Public Health.

Procedures

The study seeks to analyse urine and sputum samples collected from pharmaceutical industry workers from which isolated strains of *Escherichia coli* will be tested against the selected antibiotics. A structured questionnaire will be used to collect vital information from the workers where appropriate associations between the data from the questionnaire and the *in vitro* experiment will be calculated for and the appropriate inferences made.

Risks and Benefit

The data obtained from both the questionnaires and *in vitro* experiment will give information about the relationship between industrial exposure and antibiotic resistance. This will thus help reduce the spread of resistant strains of bacteria and also help protect the workers from the harmful effects of the raw active pharmaceutical ingredients. Your participation in this study would only take 20 minutes of your time. However, be assured that the samples to collected and the information you will provide would be used purely for academic purposes and also treated with the uttermost confidentiality and anonymity.

Right to refuse

Participation in this study is voluntary and you can choose not to partake. You are at liberty to withdraw from the study at any time. However, I will encourage your full participation since your participation is important.

Pharmaceutical Industry Worker's Consent

I, ………………………………………………… declare that the purpose, procedures as well as risks and benefits of the study have been thoroughly explained to me and I have understood them. I hereby agree to take part in this study.

Signature of participant/ thumbprint: ……………………………………

Date: ……………… / ………………….. / …………………………

Interviewer's Statement

I, the undersigned, have explained this consent form to the subject in simple language that he/she understands, clarified the purpose of the study, procedures to be followed as well as the risks and benefits involved. The subject has freely agreed to participate in the study.

Signature of interviewer: ………………………………..

Date: ……………….. / …………………… /………………..

Address

Joseph Poku Asumadu

Post Office Box 435, Konongo

In case of any concern you can contact the Ethics administrator, Ms Hannah Frimpong, GHS/ERC on: 0243 235225 / 0507 041223

APPENDIX 2B: STRUCTURED QUESTIONNAIRE FOR COLLECTION OF BASELINE INFORMATION

A RESEACH TO STUDY THE ACTIVITY OF AMOXICILLIN, CEFUROXIME AND CIPROFLOXACIN AGAINST ISOLATES OF *Escherichia coli* AMONG PHARMACEUTICAL INDUSTRY WORKERS IN ACCRA

Respondent Number: ………………………………….

1. Sex

a. Male b. Female

2. Age

a. Below 20	b. 21 – 25	c. 26 – 30	d 31 – 35
e. 36 – 40	f. 41 – 45	g. 46 – 50	h. Above 50 years

3. Highest educational level attained

a. None

b. Primary

c. Junior High School (JHS)

d. Senior High School (SHS)

e. Tertiary Education

4 Current Station at the industry

a. Warehouse – (i) Raw materials warehouse

(ii) Finish goods warehouse

b. Production floor – (i) Dispensing

(ii) Granulation

(iii) Compression

(iv) Coating

(v) In-process quality control laboratory

c. Packaging unit – (i) Primary packaging – (i) Blistering

(ii) Sachet filling

(iii) Bottle filling

(iv) Encapsulation

(v) Automation

(ii) Secondary Packaging

d. Other - Specify

5. Length of stay at current station

a. Less than a week

b. 1 – 4 weeks

c. 1 month – 3 months

d. 3 months – 6 months

e. Greater than 6 months

6. Availability of personal protective equipment

a. Yes

b. No

If yes, frequency of use of personal protective equipment

a. Daily

b. Weekly

c. Every one month

d. Every three months

e. After every three months

7. Availability of chelating agents

a. Yes

b. No

If yes, frequency of use of chelating agents

a. Daily

b. Weekly

c. Every one month

d. Every three months

e. After every three months