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Contribution of Hospital Wastewater to the Spread of Antibiotic Resistance in Comparison to Non – Health Institution

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Department of medical technology, Faculty of science

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Declaration

"I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree of the university or other institute, except where due acknowledgement has been made in the text."

Author

Mai Marwan Mohsin

Signature:_____________________       date: ____________.
Abstract

A potential post-antibiotic era is threatening present and future medical advances. The current worldwide increase in resistant bacteria and, simultaneously, the downward trend in the development of new antibiotics have serious implications. This research conducted to study the resistance profile of bacterial isolates from wastewater samples effluent from Al-Shifa hospital in Gaza as a health institution and comparing their profile with bacteria isolated from wastewater samples effluent from a non-health institution. In this study, wastewater sample were collected from three different sewers receiving wastewater in Al-Shifa hospital, from three sewers receiving wastewater in Islamic university of Gaza (IUG), from inlet and outlet of Gaza wastewater treatment plant (WWTP) and from seawater. A total of 45 samples were collected and the total number of different bacterial species that was isolated was 154 different bacteria. From the isolated bacteria 30.5% E. coli, 33.1% Pseudomonas spp., 10.4% Klebsiella spp., 4.5% Proteus spp. and 21.4% Enterococcus spp. Isolates were subjected to antimicrobial susceptibility testing. The percent of resistance for Gram-negative bacteria to 15 antibiotics were as the following Cephalexin (52.1%), Co-Trimoxazole (41.3%), Tetracycline (41.3%), Chloramphenicol (39.7%), Nalidixic Acid (36.4%), Piperacillin (28.9%), Amoxycillin (35.5%), Ceftizoxime (14.0%), Azreomam (13.2%), Ciprofloxacin (12.4%), Tobramycin (11.6%), Gentamicin (10.7%), Ceftazidime and Amikacin (8.3%) and Imipenem (0.0%). The percent of resistance for Gram-positive bacteria (Enterococcus) to 5 antibiotics were as the following: Streptomycin (91.0%), Vancomycin (75.8%), Erythromycin (60.6%), Teicoplanin (9.1%) and Ampicillin (6.1%).

In conclusion we demonstrated that bacteria isolates from wastewater samples from Al-Shifa hospital and Laboratory building of IUG had higher number of antibiotic resistant bacteria than bacterial isolates from other sites.
مستخلص

إن الزيادة العامة لمشكلة مقاومة البكتيريا للمضادات الحيوية بالإضافة للانحدار في اتجاه تطوير مضادات حيوية جديدة له مضاعفات خطيرة. لقد صمم هذا البحث لدراسة صور مقاومة البكتيريا للمضادات الحيوية عن طريق عزل أنواع من البكتيريا من المياه العادية خارج من مستشفى الشفاء في قطاع غزة كمؤسسة صحية ومقارنة صور المقاومة هذه مع بكتيريا عزلت من مياه عامة خارجة من مؤسسات غير صحية. في هذه الدراسة جمعت عينات المياه العادة من ثلاث أماكن مختلفة من مستشفى الشفاء وثلاث أماكن مختلفة من الجامعة الإسلامية في قطاع غزة ومن مدخل ومخرج محطة غزة لمعالجة المياه العادة وعينة من مياه البحر بالقرب من مصب محطة المعالجة في البحر. مجموع العينات التي تم جمعها كان 45 عينة وقد كان مجموع البكتيريا المختلفة التي تم عزلها 154 بكتيريا من هذه العينات. وكانت نسبة الاليحبرية المعزولة كالآتي:

30.5% E. coli, 33.1% Pseudomonas spp., 10.4% Klebsiella spp., 4.5% Proteus spp. and 21.4% Enterococcus spp.

البكتيريا المعزولة اجري لها اختبار الحساسية للمضادات الحيوية، وكانت نسبة مقاومة البكتيريا السائبة الجرام

لخمسة مضادات حيوية على النحو التالي:

Cephalexin (52.1%), Co-Trimoxazole (41.3%), Tetracycline (41.3%), Chloramphenicol (39.7%), Nalidixic Acid (36.4%), Piperacillin (28.9%), Amoxycillin (35.5%), Ceftizoxime (14.0%), Azreomycin (13.2%), Ciprofloxacin (12.4%), Tobramycin (11.6%), Gentamicin (10.7%), Ceftazidime and Amikacin (8.3%) and Imipenem (0.0%).

وقد وجد أن البكتيريا المعزولة من عينات المياه العادة خارجة من مستشفى الشفاء ومن مبنى المختبرات في الجامعة الإسلامية تحتوي على عدد أكبر من البكتيريا مقاومة للمضادات الحيوية بالمقارنة مع الأماكن الأخرى.
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<td>API</td>
<td>Analytical Profile Index</td>
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<td>Ak</td>
<td>Amikacin</td>
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<td>Am</td>
<td>Amoxicillin</td>
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<td>Ao</td>
<td>Aztreonam</td>
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<td>A</td>
<td>Ampicillin</td>
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<tr>
<td>BHIB</td>
<td>Brain Heart Infusion Broth</td>
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<td>Ck</td>
<td>Ceftizoxime</td>
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<td>CN</td>
<td>Cephalexin</td>
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<td>Co</td>
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<td>Cf</td>
<td>Ciprofloxacin</td>
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<td>Ceftazidime</td>
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<td>DNA</td>
<td>Deoxy Ribonucleic Acid</td>
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<td>ETEC</td>
<td>Enterotoxigenic <em>E. coli</em></td>
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<td>ERE</td>
<td>Erythromycin Resistant Enterococci</td>
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<td>E</td>
<td>Erythromycin</td>
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<td>GWWTP</td>
<td>Gaza Wastewater Treatment Plant</td>
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<td>G</td>
<td>Gentamicin</td>
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<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
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<td>IUG</td>
<td>Islamic University of Gaza</td>
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<td>I</td>
<td>Imipenem</td>
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<tr>
<td>MRSA</td>
<td>Methicillin-Resistant <em>Staphylococcus aureus</em></td>
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<td>MDR</td>
<td>Multi Drug Resistant</td>
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<td>Na</td>
<td>Nalidixic Acid</td>
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<td>NETEC</td>
<td>Non-Enterotoxigenic <em>E. coli</em></td>
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<td>NCCLS</td>
<td>National Committee for Clinical Laboratory Standards</td>
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<td>PBP's</td>
<td>Penicillin Binding Proteins</td>
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<td>PNS</td>
<td>Penicillin Non-Susceptible</td>
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<td>Pc</td>
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<td>% R</td>
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<td>RTFs</td>
<td>Resistance Transfer Factors</td>
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<td>rRNA</td>
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<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
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<td>STPs</td>
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<td>tRNA</td>
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<td>UTI</td>
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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<td>Vancomycin Resistant Enterococci</td>
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<td>Va</td>
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To all workers at Gaza Waste Water Treatment Plant for all the help they present to me in the process of sample collection from the plant.

My family for nurturing my confidence, teaching me perseverance, and always encouraging me to pursue my goal.
Dedication

This thesis is dedicated to my father and my mother who have supported me all the way since the beginning of my studies.

Also, this thesis is dedicated to my husband and my son who have been a great source of motivation and inspiration.

Finally, this thesis is dedicated to all those who believe in the richness of learning.
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Chapter (I)
Introduction

1.1 Overview

In the past, bacteria were the most important cause of disease and mortality among humans. The introduction of antibiotics in human medicine has markedly reduced the impact of bacterial diseases on human mortality (1). In the past 50 years, antibiotics have been critical in the fight against many diseases and infections. Their discovery was one of the leading causes for the dramatic rise of average life expectancy in the 20th century and their significance to public health would be impossible to overstate. Antibiotics are defined as any compound which either kills or severely impedes the growth of bacteria (2).

Before the discovery of the first antibiotic, penicillin, in 1928, infections that are easily treated today killed millions of people around the world (3). These gains are now seriously jeopardized by another recent development: the emergence and spread of microbes that are resistant to cheap and effective first-choice or "first-line" drugs (4).

The first case of penicillin resistance in E. coli was reported in the 1950s (1). Since then, things have taken a turn for the worse. Today, antibiotic resistance represents an important problem in the therapy of various human pathogenic bacteria. Three bacterial species causing life-threatening infections (Pseudomonas aeruginosa, Mycobacterium tuberculosis and Enterococcus faecalis) can demonstrate resistance to any available antibiotic. Vancomycin is the only effective drug for the treatment of infections caused by methicillin-resistant Staphylococcus aureus (MRSA), but the occurrence of strains with reduced susceptibility to this antibiotic has already been reported. Problems may also occur in the therapy of hospital infections caused by Acinetobacter Baumannii, Haemophilus influenzae, Klebsiella pneumoniae, Neisseria meningitidis and Streptococcus pneumoniae (1).
The problem of antibiotic resistance is of particular concern for immunosuppressed patients, such as those affected by HIV, cancer or chronic diseases, as antibiotic therapy represents the only way to overcome bacterial infection for these people. Serious problems may also occur in developing countries where the use of new and expensive drugs is limited by their cost and availability. In addition to the risks for human health, this situation incurs a worldwide increase in the cost of hospital care, including the use of new expensive drugs, increased costs for bacteriological analysis and prolonged hospitalization (5).

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today (6). Confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem (6).

An important feature contributing to the dissemination of antibiotic resistance is the ability of the resistance genes to move into other bacteria by a variety of genetic means. The microbial environment has carried these various gene distribution systems over evolutionary periods, using them to defend itself against threats to its existence, such as those posed by antibiotics (6).

Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. However, higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats, indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment (7, 8).

The emergence and spread of antimicrobial resistance are complex problems driven by numerous interconnected factors (4). The widespread and often
inappropriate administration of antibiotics in livestock, pets, and humans has been shown to result in the development of antibiotic-resistant bacteria and is generally accepted to be the primary pathway for proliferation of antibiotic-resistant bacteria in the environment (9).

Possible mechanisms by which humans enhance the spread of antibiotic resistance among environmental bacteria include the deliberate or accidental introduction of antibiotics, resistant bacteria and resistance genes into the environment. Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance (9, 10).

The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogen (9, 10). There are several routes of entry of antimicrobial agents into the environment. Studies have shown that introduction by these routes has changed the antibiotic susceptibility of the microbes in those environments (11).

One of these route is the sewage, the antibiotics that we take in are not all processed by our bodies. Some of them are expelled as waste and wind up in our wastewater treatment plants. Of bacteria isolated from sludge remaining after wastewater treatment at one plant, 46.4% were resistant to multiple antibiotics. Sewage from hospitals and pharmaceutical plants has been shown to contribute to antibiotic resistance in treatment plants (11).

The volume of antibiotics used in hospitals and private households and released into effluent and municipal sewage indicates a selection pressure on bacteria (12). Waste effluent from hospitals contains high numbers of resistant bacteria and antibiotic residues at concentrations able to inhibit the growth of susceptible
bacteria (13,14,15). Accordingly, hospital waste effluent could increase the numbers of resistant bacteria in the recipient sewers by both mechanisms of introduction and selection for resistant bacteria (16).

1.2 Aim of the study

Bacteria in waste effluents from hospital are exposed to high levels of various chemicals including antimicrobials that are disposed into wastewater systems. Antimicrobials may exert selective pressure on bacteria leading to the elimination of sensitive strains and allowing resistant ones. Resistant bacteria can transfer their resistance characteristics to other bacteria in the surrounding environments through several mechanisms. Al-Shifa hospital is one of the largest hospitals in the Gaza Strip. It discharges sewage into the sewage system connected to the Gaza Wastewater Treatment Plant. Along the way to the GWWTP, it combines with sewage from local community. The efficiency of the GWWTP is believed by many to be low and in several documented occasions discharged untreated wastewater directly to the sea. This poses a serious public health threat to the locals because of the fact that sewage contains pathogenic microorganisms and the threat becomes more and more real if these pathogens are originating from hospital or has the opportunity to acquire resistance through contact with resistant hospital pathogens.

This research aims at studying the resistance profile of bacterial isolates from Al-Shifa hospital as a health institution and comparing their profile to a non-health institution. The following specific objectives were achieved:

- The pattern of antibiotic resistance of five bacterial strains (E. coli, Pseudomonas spp., Proteus spp., Klebsiella spp., and Enterococcus spp.) isolated from the sewage of Al-Shifa hospital in comparison to the same isolates from the sewage of Islamic University of Gaza.
• The contribution of resistant strains by the different wards in the hospital.

• Study the pattern of resistance of the same five bacterial strains isolated from Gaza Wastewater Treatment Plant (GWWTP) and the effect of wastewater effluent from GWWTP on the seawater.

1.3 Significance

Antimicrobial resistance is driving up health care costs, increasing the severity of disease, and increasing the death rates from certain infection. Human activities other than indiscriminate use of antibiotics in human medicine, animal husbandry and agriculture, and inappropriate wastewater treatment and disposal may disrupt the microbial balance in favor of resistant bacteria. This study would highlight and demonstrate the extent of hospital effluents contribution to the resistance phenomena in Gaza. Thus providing data for policy makers and local authorities, this may assist in future planning in an attempt to reduce antibiotic resistance and associated burdens.

There are numerous studies that are concerned with applying antibiotic profiles in tracing source of bacterial contamination. This trend was tested during the course of this work.
Chapter (II)
Literature Review

2.1 Antibiotics

2.1.1 Definition of an antibiotic

Antibiotics are substances produced by living organisms, which are able to kill or inhibit the growth of other microorganisms (17). Antibiotics are product of the earth, more specifically of soil; they are byproducts of cellular metabolisms; antibiotics are "all natural" (18). According to the literal sense of the word, substances produced synthetically (e.g. sulfonamides or quinolones) should not be termed antibiotics, and the use of broader term (i.e. Antimicrobial agents) would be more appropriate to indicate the complex of all substances having a harmful effect on microorganisms (17). However, the term antibiotic is used throughout the present thesis as a synonym of antimicrobial agents.

2.1.2 History of antibiotic development

Interest in antimicrobial chemotherapy was kindled as soon as microorganisms were understood to be agents of infectious disease. In earlier times, plant products were sometimes used successfully in the treatment of disease, but neither doctors nor patients know the basis for the action of these therapeutic agents. Many early medicines were used to cure protozoan diseases, rather than bacterial diseases. As early as 1619, it was known that malaria could be treated with the extract of cinchona bark (quinine) and that amoebic dysentery could be treated with ipecacuanha root (emetine) (19,20). Only a few antibacterials, such as mercury, which was used to treat syphilis, were in use when the era of true chemotherapy began.

It was in the early 1900's when Paul Ehrlich first hypothesized that dyes could be used as antimicrobial drugs, based on their differential affinities for various tissues. In 1904, Ehrlich and Shiga discovered that a red dye called trypanrot was effective against trypanosomes (21). It was around this time that
arsenicals drew Ehrlich's interest. Ehrlich, along with Sahachiro Hata in 1909, found that arsphenamine (named Salvarsan) was active against spirochetes and, therefore, was an effective cure for syphilis (19).

The seminal work of Joseph Lister, Louis Pasteur, Robert Koch, and others-identifying microbes as agents of disease and devising means for avoiding infections by the use of disinfectants and antiseptics-made possible rational approaches to the treatment of infectious diseases (22).

True antimicrobial therapy became available only in the 1930s with the discovery of the sulfonamides by Gerhardt Domagk (22). Gerhard Domagk, a German doctor, announced the discovery of a synthetic molecule with antibacterial properties (2). In 1932, two scientists at the Bayer company, Mietzsch and Klarer, synthesized Prontosil red, a red dye bound to a sulfanamide group. Domagk (23) showed, in 1935, that infections in mice caused by hemolytic streptococci were cured by Prontosil red (19, 20).

Unfortunately for Bayer, Prontosil red was shown to have no antibacterial activity in vitro. This lack of activity was explained by Trefouel et al. (24) when they showed that Prontosil red is split in vivo into its component dye and sulfanilamide, the active antibacterial agent and a previously described molecule that was already in the public domain. From that point, sulfanilamide was manufactured by a number of companies and work was begun to modify the molecule to enhance performance, leading to decreased side effects and a broader spectrum of action (20).

Although penicillin was the first natural antibiotic to be discovered, the idea of using microorganisms therapeutically was not new. Fungi had been used in poultices for many years, and by 1899, a product called pyocyanase, which was an extract from Pseudomonas aeruginosa, was used in the treatment of wounds (20).
In 1928, Alexander Fleming was searching for potential antibacterial compounds. He noticed that a patch of the mould *Penicillium notatum* had grown on a plate containing the bacteria Staphylococcus and that around the mould there was a zone where no Staphylococcus could grow. After more research, he was able to show that culture broth of the mold prevented growth of the Staphylococcus even when diluted up to 800 times. He named the active substance penicillin but was unable to stably isolate it. Several years later, in 1939, Ernst Chain and Howard Florey developed a way to isolate penicillin and used it to treat bacterial infections during the Second World War (2). By 1941, Ernst Chain, Howard Florey, and Norman Heatley had shown the therapeutic value of penicillin (25), but they were also unable to produce enough penicillin for commercial use. Collaboration with Andrew Moyer and Robert Coghill (26) at the USDA's Northern Regional Research Laboratory in Illinois led to much higher production yields of penicillin by 1943. After a worldwide search for *Penicillium* strains that could produce more penicillin, Raper and Fennel (27) found a strain of *Penicillium chrysogenum* on a moldy cantaloupe at a local market that was capable of even higher yields of penicillin (28). The new drug came into clinical circulation in 1944 and made a huge impact on public health. For these discoveries Fleming, Chain and Florey were awarded the Nobel Prize for Medicine in 1945. Their discovery and development revolutionized modern medicine and paved the way for the development of many more natural antibiotics (2).

The discovery of penicillin stimulated search for antibiotics in many parts of the world and numerous substances were discovered, products of various kinds of moulds, bacteria, or plants which would kill other bacteria. But the great majority of these were unsuitable for medical use. They were either too poisonous or too difficult to purify or liable to degenerate on keeping; or, for some reason or other, they failed to meet the special requirements needed for a chemotherapeutic drug. Researchers began to feel that perhaps penicillin was unique in the field of antibiotics. It certainly is a remarkable coincidence that the first antibiotic to be tested at all thoroughly proved such a wonderful drug, whereas hundreds which were subsequently tested proved duds (29).
In 1940, Selman Waksman began searching for antibiotic compounds produced by soil microorganisms (19). In 1943, one of Waksman's students discovered streptomycin (30), leading to a flood of researchers combing the world for new drugs. It was in this same period that Rene Dubos (31) discovered gramicidin, the first antibiotic active against gram-positive bacteria. Chlortetracycline, chloramphenicol, and others were discovered shortly thereafter (20). Many discoveries were of drugs that were too toxic for human use, or that had already been discovered. Nevertheless, this work did lead to many new drugs and within only 10 years, drugs comprising the major classes of antibiotics were found (19). In addition to soil, many of these drugs were discovered by isolating the producing microorganisms from interesting and unusual sources. For example, some antibiotic-producing bacteria were isolated from a wound infection and others from sewage, a chicken's throat, and a wet patch of wall in Paris (20).

In 1962, one of the later discoveries was a synthetic drug, nalidixic acid, the first of the quinolones to be described, and although not therapeutically important by itself, modification of nalidixic acid led to the production of the highly effective fluoroquinolones. Members of this class, such as ciprofloxacin, norfloxacin, enrofloxacin, and ofloxacin, have become very important in the treatment of diseases in both humans and animals (21). Since the 1960's, there have been few discoveries of new antibiotic drugs. The drugs developed since have mostly been chemical modifications of existing drugs. These modifications have been very useful in treating infectious diseases, leading to enhanced killing of pathogens, increased spectrum of action, reduced toxicity, and reduced side effects. Unfortunately, since the 1970's, only one new class of antibiotics has been introduced (32) and a recent trend in antibiotic therapy has been to employ combinations of drugs with different mechanisms of action, in order to increase their effectiveness and to overcome the problem of drug resistance.
2.1.3 Classification
Antibiotics are classified based on their chemical structure. Each class of antibiotics is characterized by a typical core structure and the various members of the class are differentiated by the addition or subtraction of secondary chemical structures from the core structure. The main classes of antibiotics currently used in clinical practice include penicillins, cephalosporins, tetracyclines, aminoglycosides, fluoroquinolones, potentiated sulfonamides, macrolides and glycopeptides (16). Antibiotics can also be classified as broad, intermediate or narrow spectrum, depending on the range of bacterial species against which they are active (16).

2.1.4 Mechanisms of action
Antibiotics constitute quite a heterogeneous group of chemicals. Depending on the chemical structure, antibiotics exert an effect on different structures or functions of the bacterial cell. The major mechanisms of action are inhibition of the cell wall synthesis, damage of the cell membrane function, inhibition of protein synthesis, inhibition of the nucleic acid synthesis, and metabolic antagonism (16).

2.1.4.1 Inhibition of cell wall synthesis
There are two major groups of cell wall synthesis inhibitors, the β-lactams and the glycopeptides. As bacterial cell walls are wholly unlike the membranes of eukaryotes, they are an obvious target for selectively toxic antibiotics. The β-lactams include the penicillins, cephalosporins, and the carbapenems. These agents bind to the penicillin binding proteins (PBP's) that cross-link strands of peptidoglycan in the cell wall. In gram negative cells, this leads to the formation of fragile spheroplasts that are easily ruptured. In gram positive cells, autolysis is triggered by the release of lipoteichoic acid (19).

The mechanism of β-lactam resistance is via the action of the β-lactamases. These enzymes catalyze hydrolysis of the β-lactam ring and, thereby, inactivate these antibiotics. Many bacteria contain chromosomally encoded β-lactamases
necessary for cell wall production and it is only through over-production of these enzymes that resistance occurs (19). β-lactamases encoded on plasmids or other transmissible elements can lead to such overproduction and, therefore, to resistance (33). There are also some bacteria that possess altered PBP's that result in reduced penicillin binding (19).

Since the discovery of penicillin and resistant bacteria, various new versions of the β-lactams have been used that have different spectrums of activity and different susceptibility to β-lactamases. Since the 1970s, several compounds, such as clavulanic acid, have been discovered that have the ability to bind irreversibly to β-lactamases and, thereby, inhibit their action. Combinations of these compounds with β-lactam drugs have been very successful in treatment of disease (34).

The glycopeptides are a group of antibiotics that include vancomycin, avoparcin, and others that bind to acyl-D-alanyl-D-alanine. Binding of this compound prevents the addition of new subunits to the growing peptidoglycan cell wall. These drugs are large molecules that are excluded from gram negative cells by the outer membrane, thus limiting their action to gram positive organisms.

Glycopeptide resistance was long thought to be rare, but has recently been shown to be quite common (34). Resistance in enterococci has developed through newly discovered enzymes that use d-alanyl-d-lactate in place of acyl-D-alanyl-D-alanine, allowing cell wall synthesis to continue. Other mechanisms of resistance involve the over-production of peptidoglycan precursors which overwhelm the drug (19).

2.1.4.2 Inhibitors of protein synthesis

There are many types of antibiotics that inhibit bacterial protein synthesis. These drugs take advantage of structural differences between bacterial ribosomes and eukaryotic ribosomes.
The aminoglycoside antibiotics are a group whose mechanism of action is not completely understood. The three major groups of aminoglycosides are the streptomycins, neomycins, and kanamycins. These drugs enter bacterial cells by an active transport that involves quinones that are absent in anaerobes and streptococci, thus excluding these organisms from the spectrum of action. Streptomycins act by binding to the 30S ribosomal subunit. Kanamycins and neomycins bind to both the 50S subunit and to a site on the 30S subunit different from that of streptomycin (19). Activity involving initiation complexes and cell membrane proteins that contribute to cell death plays a role in the action of these antibiotics, but this is poorly understood (19, 34).

There are three mechanisms of aminoglycoside resistance that have been identified to date. The first involves only streptomycin. Since streptomycin binds to one particular protein on the ribosome, alteration of this protein, even by a single amino acid in its structure, confers high-level resistance to the drug (34). The other mechanisms involve decreased uptake of the antibiotic and in one of these the cell membrane is altered, preventing active transport of the drug. In the other, one of many enzymes alters the antibiotic as it enters the cell, causing a block in further active transport (34).

Chloramphenicol is a broad-spectrum antibiotic that, although naturally occurring, is produced by chemical synthesis. Chloramphenicol inhibits peptide bond formation on 70S ribosomes (34). This drug is especially useful in that it can penetrate eukaryotic cells and cerebrospinal fluid, making it a drug of choice for treatment of meningitis and intracellular bacterial infections such as those caused by Chlamydia. It is not in widespread use, however, because of potentially fatal side-effects, namely, aplastic anemia (19).

Resistance to Chloramphenicol is conferred by the enzyme Chloramphenicol acetyl-transferase. A number of these enzymes have been discovered, each altering the Chloramphenicol molecule to prevent binding to the bacterial ribosome. Chloramphenicol resistance in gram negative cells can also arise
from alteration in outer membrane permeability that prevents the drug from entering the cell (34).

The tetracyclines are another group of broad-spectrum antibiotics that inhibit bacterial protein synthesis. They are brought into the cell by active transport and, once there, bind to the 30S subunit to prevent binding of aminoacyl tRNA (35).

Resistance to the tetracyclines occurs via three mechanisms. First, production of a membrane efflux pump which remove the drug as rapidly as it enters and there are several genes encoding these pumps. Second, several ribosome protection proteins act to prevent tetracycline from binding to the ribosome, thus conferring resistance. Third, a protein found only in Bacteroides spp., enzymatically inactivates tetracycline (35). Interestingly, efflux pump inhibitors have recently been discovered that may allow combinations of these inhibitors and tetracyclines to be used against previously resistant strains (36).

The macrolides are a group of antibiotics commonly used to treat gram positive and intracellular bacterial pathogens. Erythromycin was the first of these, and several other important macrolides have been discovered since, including clarithromycin and azithromycin. Azithromycin has a longer plasma half-life which allows treatment with a single dose for some pathogens or one daily dose for others. Clarithromycin has enhanced absorption and causes less gastrointestinal discomfort (37). It was originally believed that erythromycin inhibited protein synthesis by competing with amino acids for ribosomal binding sites, but newer research shows several mechanisms are involved (20). The macrolides are now believed to promote dissociation of tRNA from the ribosome, inhibit peptide bond formation, inhibit ribosome assembly, and prevent amino acid chain elongation (37).

There are two major mechanisms of macrolides resistance. First, an efflux pump has been found that removes the drug from the cell. Second, modification
of the ribosome can confer resistance. Mutations at several sites of the ribosome can allosterically prevent macrolides binding and a common alteration is dimethylation of one nucleotide on the 23S rRNA. This dimethylation not only prevents macrolides binding, but also confers resistance to lincosamide and streptogramin antibiotics (37).

The streptogramins are another class of antibiotic that inhibits bacterial protein synthesis, mostly in gram positive organisms (due to decreased permeability of the gram negative outer membrane). These antibiotics are actually combinations of structurally different drugs, types A and B, that act synergistically. These compounds bind to separate sites on the 50S subunit. Type A drugs block attachment of substrates at two sites on the 50S subunit, whereas type B drugs cause release of incomplete protein chains. The synergistic effect arises from a conformational change induced by the binding of type A drug which significantly increases affinity of type B drugs (38). Streptogramins currently in use include virginiamycin, pristinamycin, and quinupristin/dalfopristin.

Resistance to streptogramin antibiotics can be found in several forms. Efflux pumps for both type A and B streptogramins have been identified. Type A streptogramins can be inactivated by one of the virginiamycin acetyltransferases, and several enzymes have been identified that can inactivate type B streptogramins. Alteration of bacterial ribosomal proteins or RNA can also confer resistance. A common mutation is the dimethylation of one nucleotide on the 23S rRNA, mentioned previously, that gives rise to resistance to type B drugs, as well as macrolides and lincosamides (38).

2.1.4.3 Inhibitors of nucleic acid synthesis

The sulfonamides and the diaminopyrimidines should be discussed together, in that both only indirectly inhibit nucleic acid synthesis by inhibiting folate synthesis. Folate is a coenzyme necessary for the synthesis of purines and pyrimidines. Although both types of drugs are useful on their own, they exhibit a
synergistic effect when combined. Sulfonamides are currently not used commonly in medicine, but the combination drug trimethoprim-sulfamethoxazole is sometimes used in the treatment of urinary tract infections. Sulfonamides serve as an analog of $p$-aminobenzoic acid. Therefore, they competitively inhibit an early step in folate synthesis. Diaminopyrimidines, of which trimethoprim is the most common, inhibit dihydrofolate reductase, the enzyme that catalyzes the final step in folate synthesis (19).

There are several resistance mechanisms that microorganisms employ against each of the anti-folate drugs. For example, sulfonamides are rendered ineffective by over-production of $p$-aminobenzoic acid or production of an altered dihydropteroate synthetase. The substrate for dihydropteroate synthetase is $p$-aminobenzoic acid, and the altered form has a much lower affinity for sulfonamides than for $p$-aminobenzoic acid (39). Trimethoprim resistance can also result from several mechanisms, e.g., over-production of dihydrofolate reductase or production of an altered, drug-resistant form can lead to resistance (34). In addition, both drugs can be enzymatically inactivated, resulting in resistance (39).

The quinolones are a chemically varied class of broad-spectrum antibiotics widely used to treat many diseases, including gonorrhea and anthrax. Drugs in this class include nalidixic acid, norfloxacin, and ciprofloxacin. These drugs are commonly used and, worldwide, more ciprofloxacin is consumed than any other antibacterial agent (40). Quinolones inhibit bacterial growth by acting on DNA gyrase and topoisomerase IV, which are necessary for correct functioning of supercoiled DNA (19). Although quinolones target both enzymes, in gram negative organisms the primary target is DNA gyrase and, in gram positive organisms, the primary target is topoisomerase IV (41).

There are three main mechanisms of resistance to quinolones. Resistance to some quinolones occurs with decreased expression of membrane porins. Cross-resistance to other drugs requiring these porins for activity also results
from these changes. A second mechanism of resistance is expression of efflux pumps in both gram negative and gram positive organisms (42) and the third is alteration of the target enzymes. Several mutations have been described in both quinolone target proteins that result in reduced binding affinities (41). It is believed that high-level quinolone resistance is brought about by a series of successive mutations in the target genes, rather than a single mutation (42).

2.1.5 Antibiotics used in this research

**Amoxicillin:** is broad spectrum semisynthetic penicillin with antibacterial activity against certain gram negative and gram positive organisms. It is in activated by penicillinases including those produced by *Staphylococcus aureus, E. coli, Pseudomonas, Klebsiella, and Enterobacter (43).*

Amoxicillin is considered the drug of choice for the treatment of acute sinusitis, acute otitis media, and of acute exacerbations of chronic bronchitis. In these cases it is superior to ordinary penicillin. It is also suitable for the treatment of streptococcal pharyngitis (e.g. in children) (44).

**Amikacin:** an aminoglycoside antibiotic a semisynthetic derivative of Kanamycin. Amikacin has the same spectrum of activity as gentamicin and tobramycin, but it is less susceptible to enzymatic inactivation. This makes amikacin valuable in managing infections caused by gram-negative bacilli resistant to gentamicin and tobramycin. Amikacin’s use can include coverage against some aerobic gram negative bacteria, which include *E. coli, Klebsiella, Proteus, Pseudomonas, Salmonella, Enterobacter, Serratia and Mycoplasma.* Used in serious gram-negative bacillary infections, In Bacteremia or Septicemia, or bone and joint infections

**Aztreonam:** is the only clinically available member of a unique class of beta-lactam antibiotics called 'monobactams'. As such, it is structurally related to the other beta-lactams: penicillins, cephalosporins, and carbapenems (e.g.
imipenem, meropenem). Its spectrum of activity is specific to gram negative organisms, including *Haemophilus influenzae* and *Pseudomonas aeruginosa*. It has no anti-gram positive activity and therefore has no role in the treatment of *Staphylococcus aureus* (including MRSA) or *Streptococcus pneumoniae* (45).

**Ceftazidime** is a semisynthetic, broad-spectrum, beta-lactam antibiotic, Cephalosporin, Third Generation. Effective against a broad range of gram-positive and gram-negative bacteria. It is effective against bacteria resistant to ampicillin and other cephalosporins. Ceftazidime may be indicated in an extremely wide variety of gram-positive and gram-negative infections of the respiratory tract, the skin, urinary and genital tracts, septicemia, the abdominal cavity, and the central nervous system. Ceftazidime inhibits one of the enzymes involved in the synthesis of the bacterial cell walls.

**Ceftizoxime**: is Cephalosporin, Third Generation. Use to treat infections due to sensitive gram-positive and gram-negative bacteria. All third generation cephalosporins are more active against certain gram-negative bacteria than second generation but are less active against gram-positive bacteria, notably *Staphylococcus aureus*.

**Cephoxitin**: is Cephalosporin, Second Generation. Broad spectrum antibiotic. Cephoxitin may be indicated in cases of lower respiratory tract infections, urinary tract infections, gynecological infections, intra-abdominal infections, skin, bone and joint infections and septicemia.

**Chloramphenicol**: is a protein synthesis inhibitor, has a broad spectrum of activity but it exerts a bacteriostatic effect. Chloramphenicol was originally discovered and purified from the fermentation of a *Streptomyces*, but currently it is produced entirely by chemical synthesis. Chloramphenicol inhibits the bacterial enzyme peptidyl transferase, thereby preventing the growth of the polypeptide chain during protein synthesis (46).
Ciprofloxacin: is the generic international name for the synthetic antibiotic belonging to a group called fluoroquinolones. Ciprofloxacin is bactericidal and its mode of action depends on blocking of bacterial DNA replication by binding itself to an enzyme called DNA gyrase, which allows the untwisting required to replicate one DNA double helix into two. Notably the drug has 100 times higher affinity for bacterial DNA gyrase than for mammalian. Ciprofloxacin is a broad-spectrum antibiotic that is active against both gram-positive and gram-negative bacteria (47).

Co-Trimoxazole: is a combination of two drugs that act together: Trimethoprim and Sulfamethoxazole (48). Cotrimoxazole is active against a broad spectrum of gram-negative and gram-positive bacteria, as well as Nocardia and Toxoplasma. This combination is used to treat gram-negative urinary tract infections, and is the agent of choice for Nocardia infections. Cotrimoxazole is also used to treat and prevent *Pneumocystis carinii* and toxoplasma infection.

Gentamicin: is an aminoglycoside antibiotic, exert its activity by binding bacterial ribosomes and preventing the initiation of protein synthesis. Gentamicin is broad spectrum antibiotic, effective against a broad range of gram-positive and gram-negative bacteria (46).

Imipenem: is the first of a new class of beta-lactam antibiotics called carbapenems, related to the penicillin/cephalosporin family of antibiotics. The antibacterial spectrum of imipenem exceeds any antibiotic investigated to date and includes gram-positive, gram-negative, and anaerobic organisms. It has the ability to kill a wide variety of bacteria. It works by interfering with their ability to form cell walls, and therefore the bacteria break up and die. Not destroyed by most beta-lactamases including those that mediate resistance to cefuroxime, cefotaxime and ceftazidime.

Nalidixic Acid: is a synthetic chemotherapeutic agent which has activity mainly against gram-negative bacteria. Nalidixic acid belongs to a group of compounds called quinolones. Nalidixic acid is a bactericidal agent that binds to the DNA
gyrase enzyme which is essential for DNA replication. Binding of the drug inhibits DNA gyrase activity. The main use of nalidixic acid is in treatment of lower urinary tract infections (UTI). The compound is unusual in that it is effective against several types of Gram-negative bacteria such as *E. coli*, *Enterobacter aerogenes*, *K.pneumonia* and proteus species which are common causes of UTI (46).

**Piperacillin:** is a semisynthetic broad-spectrum penicillin. It works by interfering with their ability to form cell walls and therefore the bacteria break up and die. Piperacillin is active against certain groups of bacteria especially *Pseudomonas aeruginosa*.

**Tetracycline:** is broad-spectrum antibiotic with a wide range of activity against both gram-positive and gram-negative of bacteria.

**Tobramycin:** is an “aminoglycoside” antibiotic used to treat infections caused by many different bacteria. It acts by binding to the ribosomal 30S subunit and to prevent it from joining to the 50S subunit during protein synthesis. It may has a bactericidal effect because this leads to cytoplasmic accumulation of dissociated 30S subunits, which is apparently lethal to the cell.

**Teicoplanin:** is a narrow spectrum antibiotic, belongs to a group of antibiotics called glycopeptides. Bacteria have an external cell wall that is reinforced by molecules called peptidoglycans. The cell wall is vital for protection against the normal environment of the body in which the bacteria live. Teicoplanin works by blocking the formation of these peptidoglycans. By doing this the walls of the bacteria become weak and this results in the death of the bacteria. Teicoplanin is used to treat serious infections caused by gram-positive bacteria in heart and blood (49).

**Vancomycin:** is a narrow spectrum antibiotic, belongs to a group of antibiotics called glycopeptides. It works by inhibiting a step in cell wall synthesis.
Vancomycin is not effective against gram-negative bacteria because it cannot penetrate their outer membrane. However, it has become important in clinical usage for treatment of infections by strains of *Staphylococcus aureus* that are resistant to virtually all other antibiotics (46).

**Ampicillin:** is broad spectrum semisynthetic penicillin with antibacterial activity against certain gram-negative and gram-positive organisms. It works by inhibiting a step in cell wall synthesis (46).

**Erythromycin:** it belongs to a group of antibiotics called macrolides, characterized by structure that contain large lactone rings linked through glycoside bonds with amino sugars. It acts by inhibiting bacterial protein synthesis. Erythromycin is active against most gram-positive bacteria, Neisseria, Legionella and Haemophilus (46).

2.2 Antibiotic resistance

2.2.1 Definition

Antibiotic resistance is a relative term. A bacterial strain can be defined resistant if it survives in the presence of higher antibiotic concentrations in comparison with phylogenetically related strains (50). Thus, antibiotic resistance is not a bacterial property that can be determined by studying a single strain, but only by comparison under identical conditions of two or more strains belonging to the same genus or species.

The above-mentioned definition of antibiotic resistance refers to *in vitro* conditions. Under *in vivo* conditions, antibiotic resistance is a context dependent term as it depends on the location of the bacterium and the bioavailability of the drug. For example, bacteria are less susceptible to antibiotics when assembled in biofilms (complex communities of microorganisms embedded in a matrix of extracellular material) compared with the same organisms living separately (51). In aquatic environments, binding of the antibiotic molecule with ions or
substances present in sediment strongly reduces both the activity of the drug and its absorption in the fish intestine (52).

2.2.2 History of antibiotic resistance

There is evidence that although resistant microorganisms existed in nature before the use of antibiotics, such microorganisms were mostly absent from human flora (53). However, in the intervening years, antibiotic resistant microorganisms have become frighteningly common. Almost as soon as antibiotics were discovered, researchers began to find microorganisms resistant to the new drugs. Even by 1909, when Ehrlich first began to study dyes and arsenicals, he found drug resistant trypanosomes (19). Resistant strains of Staphylococcus aureus in hospitals grew from less than 1% incidence, when penicillin first came into use, to 14% in 1946, to 38% in 1947, to more than 90% today (19). Worldwide, ampicillin and penicillin resistance can be found together in more than 80% of S. aureus strains (53). After World War II, sulfonamides were widely used to treat Shigella infections in Japan, but by 1952, only 20% of isolates were susceptible. As the Japanese began to switch to tetracycline, chloramphenicol, and streptomycin, Shigella strains that were multiply-resistant quickly began to appear (54). Within 30 years of their discovery, sulfonamides ceased to be an effective treatment for meningococcal disease (53). In the years since, reports of resistance have grown increasingly common, and pathogens that are resistant to almost all antibiotics have been found. It has become painfully obvious that antibiotic resistance is reaching a crisis stage and some clinicians have even forecasted that we are facing a return to the devastating diseases of the pre-antibiotic era (32, 53, 55).

2.2.3 Molecular mechanisms of antibiotic resistant

Bacterial resistance to antibiotics can be caused by different molecular mechanisms. The most common mechanisms include: reduced drug uptake (e.g. membrane impermeability to cephalosporins); active drug efflux (e.g. tetracycline efflux pumps); drug deactivation (e.g. hydrolysis of penicillins by beta-lactamases), modification of the drug target (e.g. mutations of the DNA
gyrase leading to quinolones resistance); increased concentration of the drug target (e.g. increased folic acid production that counteracts the inhibition of such production by sulfonamides), or alternative pathways to elude the drug effect (e.g. synthesis of folic acid using an enzyme which is not affected by sulfonamides) (56).

2.2.4 Natural and acquired resistance
An important distinction should be made between natural and acquired resistance. Bacteria are termed naturally, intrinsically or constitutively resistant when resistance is due to characteristic features typical of the species. For example, *Pseudomonas aeruginosa* is naturally resistant to penicillins, due partly to the inability of the drug to diffuse through the outer membrane (57) and partly to the deactivation of the drug by chromosomally encoded enzymes (i.e. Beta lactamases) (58).

In contrast, acquired resistance emerges in a bacterial population that was previously susceptible, because of modifications of the bacterial DNA caused by either chromosomal mutation and selection (sometimes referred to as vertical evolution) (18) or horizontal gene transfer. Natural resistance results from a long process of genetic evolution, whereas, acquired resistance can arise within a short time (one or few generations) (56).

**Vertical evolution:** A spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population. In the selective environment of the antibiotic, the wild types (non mutants) are killed and the resistant mutant is allowed to grow and flourish. The mutation rate for most bacterial genes is approximately $10^{-8}$. This means that if a bacterial population doubles from $10^8$ cells to $2 \times 10^8$ cells, there is likely to be a mutant present for any given gene. Since bacteria grow to reach population densities far in excess of $10^9$ cells, such a mutant could develop from a single generation during 15 minutes of growth.
**Horizontal evolution** is the acquisition of genes for resistance from another organism. For example, a streptomycete has a gene for resistance to streptomycin (its own antibiotic), but somehow that gene escapes and gets into *E. coli* or Shigella. Or, more likely, some bacterium develops genetic resistance through the process of mutation and selection and then donates these genes to some other bacterium through one of several processes for genetic exchange that exist in bacteria (18).

Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation. **Conjugation** involves cell-to-cell contact as DNA crosses a sex pilus from donor to recipient. During **transduction**, a virus transfers the genes between mating bacteria. In **transformation**, DNA is acquired directly from the environment, having been released from another cell. Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant). It is common for DNA to be transferred as plasmids between mating bacteria. Since bacteria usually develop their genes for drug resistance on plasmids (called **resistance transfer factors** or RTFs), they are able to spread drug resistance to other strains and species during genetic exchange processes (18).

The combined effects of fast growth rates, high concentrations of cells, genetic processes of mutation and selection, and the ability to exchange genes, account for the extraordinary rates of adaptation and evolution that can be observed in the bacteria. For these reasons bacterial adaptation (resistance) to the antibiotic environment seems to take place very rapidly in evolutionary time: bacteria evolve fast (18).

**2.2.5 Impact of antibiotic resistance**

In the last decades, bacterial resistance to antibiotics has assumed an increasing importance with regard to its impact on both public health and ecology. Obviously, the primary problem is represented by the emergence of antibiotic resistance among bacteria pathogenic to humans and animals, which
makes difficult the treatment of some life-threatening infections. However, independent from the risks for human health, is the spread of antibiotic resistance and the problems raised in ecological nature. In fact, the introduction and selection of resistant bacteria in the environment can lead to structural changes in the composition of microbial communities, with possible deleterious effects on the balance of natural ecosystems (17).

To combat the occurrence of resistant bacteria, pharmaceutical companies must constantly research, develop and test new antimicrobials in order to maintain a pool of effective drugs on the market. Five years ago, there were approximately 150 antibiotics available to the public with new drugs appearing every 8-10 years. This appears to be a substantial amount. However, these numbers are misleading as many of these drug targets are similar. Since the drug development process is very costly, pharmaceutical companies often concentrate on finding antimicrobials similar to the ones already found to reduce the risk of producing an unmarketable drug. This means that it is easy for microorganism to develop resistance to a similar drug to which it already has resistance. Past and current strategies to combat resistance are not effective (18).

Today, antibiotic resistance represents an important problem in the therapy of various human pathogenic bacteria. Three bacterial species causing life-threatening infections (Pseudomonas aeruginosa, Mycobacterium tuberculosis and Enterococcus faecalis) can demonstrate resistance to any available antibiotic (1). Vancomycin is the only effective drug for treatment of infections caused by methicillin-resistant Staphylococcus aureus (MRSA), but the occurrence of strains with reduced susceptibility to this antibiotic has already been reported (59). Problems may also occur in the therapy of hospital infections caused by Acinetobacter baumannii, Haemophilus influenzae, Klebsiella pneumoniae, Neisseria meningitidis and Streptococcus pneumoniae (1).

The problem of antibiotic resistance is of particular concern for immunosuppressed patients, such as those affected by HIV, cancer or chronic
diseases, as antibiotic therapy represents the only way to overcome bacterial infections for these people. Serious problems may also occur in developing countries where the use of new and expensive drugs is limited by their cost and availability. In addition to the risks for human health, this situation incurs a worldwide increase in the cost of hospital care, including the use of new expensive drugs, increased costs for bacteriological analysis and prolonged hospitalization (5).

2.3 Sources of resistance in the environment

Concern over resistance was originally confined to acquisition of resistance by microorganisms which cause epidemic disease and was an issue only with respect to clinically isolated strains. However, in recent years, antibiotic resistant bacteria have been isolated from virtually every environment on earth. Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats (60). This came as a surprise to many clinicians, because resistance was found in regions never exposed to human impacts. Even as awareness of environmental resistance has increased, many investigators have continued to restrict their concern to only those pathogens that survive in the environment. It was believed that they posed a danger to humans only if the disease they caused involved resistance to antibiotics.

The occurrence of resistant bacteria in nature may have originated from antibiotic producing organisms, as suggested by the evidence that in some cases the mechanisms and genes protecting these organisms from the antibiotics they produce are similar to those responsible for resistance in clinical isolates (60). However, higher numbers of resistant bacteria occur in polluted habitats compared with unpolluted habitats (7, 8), indicating that humans have contributed substantially to the increased proportion of resistant bacteria occurring in the environment.

The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The ability of resistant bacteria
and resistance genes to move from one ecosystem to another is documented by the various cases in which transmission of resistant bacteria has been demonstrated between animals and humans (9, 10).

Non-pathogenic organisms serve as a source from which pathogens can acquire genes conferring resistance, and in turn, they can become resistant by acquiring genes from pathogens discharged into the environment, e.g. via sewage or agricultural runoff. Thus, dissemination of resistant bacteria is not only a problem of the resistant pathogens themselves, but also availability of resistance genes to pathogens via gene transfer.

Although resistant organisms can be found naturally in the environment, most resistance is associated with man-made impacts of some type, either agricultural or direct human impact (61). Possible mechanisms by which humans enhance the spread of antibiotic resistance among environmental bacteria include the deliberate or accidental introduction of antibiotics, resistant bacteria and resistance genes into the environment. Antibiotics exert a selection in favor of resistant bacteria by killing or inhibiting growth of susceptible bacteria; resistant bacteria can adapt to environmental conditions and serve as vectors for the spread of antibiotic resistance; resistance genes can be taken up by indigenous bacteria and spread by mechanisms of genetic transfer (9, 10).

Antibiotic use in humans can lead to resistance in the environment via discharge of domestic sewage, hospital wastewater, and/or industrial pollution. In addition to use in humans, antibiotics are added to animal feed to treat infections, as prophylactics, and in sub-therapeutic doses as growth promoters (61).
2.4 Human impacts

Humans can have significant impact on the occurrence of antibiotic resistance in the environment. Antibiotic resistant organisms from the human gastrointestinal tract, as well as unabsorbed antibiotics, can enter the environment via sewage. Domestic wastewater, however, often has less effect than hospital wastewater, since, in the latter, heavy antibiotic concentrations increase the impact.

Antibiotics consumed by humans can act to select for resistant organisms in the human gut. Both the resistant microorganisms and antibiotic residues are excreted, entering the sewage system. Although most people consider the environment to be generally safe from contamination with untreated sewage, breaches occur frequently where leakage or overflow into groundwater or natural waters occurs (62). Raw domestic sewage contains high numbers of bacteria, often including antibiotic resistant bacteria. One report showed that, in healthy people, 80.5% of fecal samples contained resistant organisms (63). Although VRE in the U.S. is associated with hospitals, in Europe, VRE is widespread in the community. See tables 2.1 and 2.2.

**Table (2.1):** Low-level Vancomycin Resistant Enterococci from Domestic Wastewater (64, 65, 66)

<table>
<thead>
<tr>
<th>Country</th>
<th>Percentage of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sweden</td>
<td>60&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>U.K.</td>
<td>52</td>
</tr>
<tr>
<td>Germany</td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> strains were also resistant to erythromycin (100%)
<sup>b</sup> strains were also resistant to erythromycin (26%)
<sup>c</sup> strains were also resistant to ampicillin (62%) and ciprofloxacin (2.5%)
Table (2.2): High-level Vancomycin Resistant Enterococci from Hospital Wastewater (62, 67)

<table>
<thead>
<tr>
<th>Country</th>
<th>Percentage of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>0.4</td>
</tr>
<tr>
<td>U.S.A</td>
<td>6.25</td>
</tr>
<tr>
<td>U.S.A</td>
<td>5.21</td>
</tr>
</tbody>
</table>

In Czechoslovakia, a study comparing resistance in municipal wastewater to that of clinical isolates showed higher resistance rates in wastewater within the same species. Gentamicin resistance in *E. coli* was 13.9% and 30% for *Klebsiella* and *Enterobacter* strains (68).

Although sewage treatment processes reduce the numbers of bacteria in wastewater, the effluent will still generally contain large numbers of both resistant and susceptible bacteria. Schwartz et al. (66) showed a decrease in VRE from 16% in untreated wastewater to 12.5% at the outlet. High numbers of resistant coliforms have also been found in treatment plant effluents (63) and rivers that receiving effluent from treatment plants have higher numbers of resistant organisms. Even as early as 1983, Bayne, Blankson and Thirkell (69) found a significant increase in resistance to tetracycline, erythromycin, streptomycin, ampicillin, and penicillin in enterococci, when comparing isolates downstream from a treatment plant to those upstream. Coliforms isolated from sites downstream of a treatment plant in the Tama river in Japan showed significant increases in resistance to ampicillin and tetracycline (70).

In Spain, resistance in Aeromonas isolates increased from 50% upstream to 90% downstream of a treatment plant effluent, and resistance in enteric bacteria increased from 30% to 50% (71). In Sweden in 2003, erythromycin resistant enterococci were isolated from 63% of received water samples and VRE were isolated from 3% (64).

Due to heavy antibiotic use, hospital wastewater contains larger numbers of resistant organisms than domestic wastewater. In Florida, vancomycin resistant *E. faecium* were isolated, without enrichment, from hospital wastewater (62).
VRE were found in 35% of the hospital sewage samples in two Swedish studies (64, 65). Twenty-five percent of enterococci were vancomycin resistant in a German study of biofilms from hospital wastewater, and of these, many were multiply resistant (66). Reinthaler et al. (63) showed significantly higher percentages of *E. coli* in the inlet water of a treatment plant receiving hospital waste than two other treatment plants. A study of *Acinetobacter* showed that an increase in the prevalence of oxytetracycline resistance was correlated with hospital wastewater (72).

Industrial pollution can also influence the incidence of antibiotic resistance, with pharmaceutical plants yielding a particularly strong effect. Until the 1970s, it was common for pharmaceutical plant waste to be disposed of in regular landfills, but drug residues leaching from these landfills were detected in nearby groundwater systems (73). Guardabassi et al. (72) found high levels of multiply resistant *Acinetobacter* in pharmaceutical plant effluents. Higher levels of antibiotic resistance have also been associated with heavy metals from industrial pollution. McArthur and Tuckfield (74) were able to show that antibiotic resistance was correlated with the heavy metal content of sediments downstream of a nuclear reactor complex.

2.5 Spread of resistance

Historically, researchers have assumed that the danger posed by resistant organisms in the environment would be minimal, since bacteria (especially pathogens) introduced to an environment were generally believed to survive only for a relatively short time. In the last 20 years, however, work by many investigators (75, 76, 77, 78) has shown that many bacterial species can survive far longer than once thought, and that organisms such as *Vibrio cholerae*, long considered to have a reservoir only in humans, not only survive, but are actually autochthonous to aquatic environments (79, 80). This drastically increases the probability that humans will come into contact with resistant pathogens from runoff or sewage. Additionally, even resistant non-pathogens can have a large
2.5.1 Spread of antibiotic resistance in sewage
Sewage is waste matter resulting from the discharge into the sewers of human excreta and wastewater originating from the community and its industries. Sewage contains a high content of both organic and inorganic matter, as well as high densities of living organisms, including pathogenic, commensal and environmental bacteria. This characteristic composition makes sewage a particularly suitable ecological niche for the growth and spread of antibiotic resistance.

2.5.2 Antibiotic selective pressure
The acquisition of antibiotic resistance genes is generally independent of the presence of antibiotics. However, the exposure of bacteria to antibiotics confers an ecological advantage to resistant strains on susceptible strains, allowing them to become predominant in the bacterial population. This situation is commonly termed as antibiotic selective pressure and can occur in either the host in vivo (e.g., human or animal body) as a consequence of chemotherapy or in the environment, for example when antibiotic residues are introduced in sewage (81).

Residues of antibiotics administered to humans and animals reach the sewage systems in urine or feces, in the form of either parent compound or degraded metabolites depending on the pharmacology of the specific antibiotic. Furthermore, an unknown amount of antibiotics enter the sewers by waste derived from antibiotic production and disposal of a surplus of drugs. Indeed, various antibiotics have been found in municipal sewage, including fluoroquinolones, sulfonamides and erythromycin metabolites (81, 82). The antibiotic concentrations found in sewage vary between 1 and 100 µg per liter. Such concentrations are 100- to 1000 fold lower compared with those necessary to inhibit resistant bacteria, but are sufficient to affect susceptible
bacteria (16, 83). Therefore, the occurrence of such antibiotic concentrations in sewage has the potential to select for antibiotic resistance.

The fate of antibiotics in sewage depends on their chemical properties. Lipophilic and non-readily degradable substances are likely to be retained in the sludge, whereas, hydrophilic substances may be able to pass through treatment plants and end up in the natural recipients receiving treated sewage (84). It also appears that the solubility in water of drug metabolites is generally higher compared with the parent compounds (84). Thus, it is likely that a large proportion of the antibiotic residues introduced into the sewage system can reach surface waters through municipal sewage effluents.

2.5.3 Non-antibiotic selective pressure
Among the multitude of substances occurring in sewage, there are some that have the potential to select for antibiotic resistance, even though they are not antibiotics. Heavy metals and biocides are two important groups of non-antibiotic substances showing this property. Heavy metals are widespread in sewage as a consequence of industrial pollution. Biocides are introduced into sewage by hospitals, farms, slaughterhouses and food-processing establishments; where these agents are used for the disinfection of environments and utensils, or by the community, due to the presence of these agents in house-hold products, such as soaps and dish washing detergents (85).

There are two possible ways by which heavy metals and biocides can select for antibiotic resistance. The genes encoding resistance to heavy metals and biocides can be located together with antibiotic resistance genes on either the same genetic structure (e.g. plasmid), or different genetic structures within the same bacterial strain. Alternatively, bacteria can have unspecific mechanisms of resistance to different substances, including heavy metals, biocides and antibiotics. In both cases, exposure to one substance results in the selection of bacterial strains also able to resist the other substance (co-selection) (85).
Genes encoding resistance due to heavy metals and antibiotics often co-exist on plasmids (85). In addition, unspecific mechanisms conferring resistance to both heavy metals and antibiotics are known to exist in some bacterial species (e.g. active pump-efflux system encoded by the marA gene in E. coli). The co-selective property of heavy metals is confirmed by the indirect evidence that bacteria isolated from heavy metal-polluted marine sediment are significantly more resistant to antibiotics compared with bacteria isolated from unpolluted sites (86).

Although genes encoding resistance to biocides have been found on plasmids and integrons (87), these substances are more likely to select for antibiotic resistance by induction of unspecific mechanisms of multiple resistance. Laboratory experiments have shown that biocides such as triclosan and pine oil can select for resistance to different antibiotics when bacteria are exposed to low concentrations of biocide (88, 89). Accordingly, the co-selective effect of biocides for antibiotic resistance could be particularly marked when these substances are dispersed in the environment, because of dilution and formation of concentration gradients.

2.5.4 Optimal conditions for horizontal gene transfer

Sewage is a suitable habitat for the transfer of resistance genes across different groups of bacteria. In this habitat, environmental bacteria meet resistant bacteria selected by use of antibiotics in human and veterinary medicine. Consequently, resistance genes occurring in bacteria of human and animal origin can be transferred to environmental bacteria, contributing to the formation of an environmental pool of resistant bacteria and resistance genes.

The high concentrations of bacteria, nutrients and suspended solids in sewage are all factors enhancing horizontal gene transfer (90, 91). High bacterial concentrations increase the chance that donor and recipient cells come in contact. Nutrients are more likely to have an indirect influence on the occurrence of gene transfer by increasing the concentration and the metabolic
activity of bacteria. Suspended solids provide ideal surfaces on which the various components contributing to the process of horizontal gene transfer (bacteria, free DNA and bacteriophages) are concentrated.

Plasmids and transposons harboring antibiotic resistance genes are widespread in the bacterial flora of sewage (92, 93). Multiple-resistant bacteria isolated from sewage can transfer plasmid-mediated antibiotic resistance at high frequencies in the laboratory (94, 95). Experiments performed using membrane chambers immersed in sewage have shown that high frequencies of transfer may also occur under real conditions (96, 97).

2.6 Antibiotic resistance in Gaza Strip

The rate of antibiotic resistance is high in Gaza strip, this high rate of resistance is likely due, in part, to the selective pressure resulting from the uncontrolled, unwise and frequent administration of those drugs and by antimicrobial agent policy that permits an easy access of the Palestinian health centers to those agents. This is also associated with the relatively low cost of these antimicrobial agents (98).

One study in Gaza strip showed decreased susceptibility to many antimicrobial drugs used for empiric treatment of infections, especially amoxicillin, cotrimoxazole, doxycycline and penicillin. Antimicrobial resistance among gram-negative bacilli in this study was notable. E. coli resistance to amoxicillin was 80.1%, 58.5% to cotrimoxazole, 9.1% to ciprofloxacin and 3.0% to the amikacin. The high resistance rates of E. coli may be due to the mechanisms that involve alternations in the outer membrane protein and in the antibiotic efflux system in the cell membrane. Concerning the antimicrobial susceptibility patterns among gram-positive isolates, the results show that vancomycin resistance of S. aureus and Enterococcus faecalis was 1.8% and 3.2%, respectively (98).

Second study conducted on the southern area of the Gaza Strip to identify the microorganisms that cause "community-acquired" urinary tract infections, found high proportion of the isolates resistant to amoxicillin (73.6%), doxycycline
(68.6%) and trimethoprim-sulfamethoxazole (66.1%). A high percentage of multiple-drug resistance was also observed for the majority of the isolates (99).

According to third study investigated urinary tract bacterial isolates in Gaza strip. High rates of resistance were found to amoxicillin (82.5%), followed by cotrimoxazole (64.4%) and doxycycline (63.1%) while the lowest resistance was to amikacin and ceftazidime (10.0%). The resistance rate to ciprofloxacin was 15.0% (100). Whereas, in a previous study (2000) carried out in Gaza Strip, lower resistance to ciprofloxacin (4.1%) was reported (99). The widespread and more often the misuse of antimicrobial drugs in Gaza Strip have led to a general rise in the emergence of resistant bacteria, particularly to ciprofloxacin (100).

Fourth study conducted in the Khan Younis hospital laboratory to evaluate susceptibility patterns in *Pseudomonas aeruginosa* causing nosocomial infections. The results demonstrated that most common resistance was to ampicillin, followed by cephalexin. The most effective antimicrobial agents were meropenem and amikacin, respectively. The highest resistance to ciprofloxacin was found among ICU and surgery sections (101).

### 2.7 Antibiotic resistance in neighboring countries

Not only Gaza that suffer from this increasing problem but also in other countries, "Israel" for instance as shown in a study conducted to compare susceptibility patterns of 8338 community urinary isolates collected during 1995 with 6692 isolates from 1999. This study demonstrated that ampicillin, first generation cephalosporins and sulphamethoxazole/trimethoprim could no longer be considered first line drugs for empirical treatment of clinically evident urinary tract infection because of very high resistant rates. Ampicillin remained a good choice for urinary infections caused by enterococci, 98% of the strains being susceptible. It was found that 1.25% of the gram-negative uropathogens isolated during 1999 were extended spectrum beta-lactamase producers (102).

Another study was executed in Israel on Methicillin-Resistant *Staphylococcus aureus* showed that hospital-acquired MRSA isolates were persistently highly
resistant to chloramphenicol (69% in 1988 and 100% in 1997), gentamicin (89% in 1988 to 94% in 1997), and ciprofloxacin (87% in 1988 to 96% in 1997). The resistance to clindamycin (62% in 1988 to 92% in 1997), fusidic acid (6% in 1988 to 14% in 1997), and rifampicin (21% in 1988 to 76% in 1997) increased significantly. All isolates were sensitive to vancomycin (103).

A third study also in Israel on Streptococcus pneumoniae showed that of the 437 isolates, 276 (63.4%) were antibiotic resistant and 156 (35%) were penicillin nonsusceptible (PNS). The isolates were mostly nonsusceptible to SXT (51%), penicillin (35%), and erythromycin (10%). Resistance to tetracycline, clindamycin, and chloramphenicol was found in 11, 5, and 2% of the isolates, respectively. Of the 154 isolates that were nonsusceptible to penicillin, 97 of 154 (63%) were intermediately susceptible and 37 of 154 (37%) were highly penicillin resistant. These two groups comprised 22 and 13% of all S. pneumoniae isolates, respectively (104).

In Jordan a study on antibiotic sensitivity of Enterobacteriaceae isolated from patients with community acquired urinary tract infections demonstrated that high rates of resistance were found against ampicillin (95%), tetracycline (86%), carbenicillin (84%), trimethoprim/sulphamethoxazole (48%), and amoxicillin/clavulanic acid (45%). For the antibiotics tobramycin, aztreonam, ceftriaxone and gentamicin 7% of the isolates were resistant, while resistance varied from 9-18% for amikacin, ciprofloxacin, norfloxacin, nalidixic acid and cefuroxime (105).

In Egypt a study on antimicrobial susceptibility of E. coli and Shigella spp. isolated from rural Egyptian pediatric populations with diarrhea between 1995 and 2000, showed that the cumulative rates of resistance for E. coli and Shigella spp. were high (68.2% and 54.8% for ampicillin, 24.2% and 23.5% for ampicillin-sulbactam, 57.2% and 42.5% for trimethoprim-sulphamethoxazole, and 50.9% and 75.4% for tetracycline respectively). Non-enterotoxigenic E. coli
(NETEC) isolates had a consistently higher level of antimicrobial resistance than did enterotoxigenic *E. coli* (ETEC) isolates (106).

Another study in Egypt which reviewed the antimicrobial susceptibility patterns of bloodstream isolates of Gram-positive cocci and Gram-negative bacilli in five hospitals in Cairo, Egypt, from 1999 to 2000. In addition, susceptibilities of non-bloodstream isolates of *Streptococcus pneumoniae* and *Enterococcus* spp. were analyzed. High rates of resistance were found in most of the bacteria studied. Staphylococci were highly resistant to erythromycin, co-trimoxazole, clindamycin and doxycycline; all isolates were susceptible to vancomycin. Susceptibility of *S. pneumoniae* isolates to penicillin, ceftriaxone and fluoroquinolones was 63%, 84% and 82%, respectively. Vancomycin susceptibility of the enterococci was 96%; susceptibility to high-level gentamicin and streptomycin was 54% and 48%, respectively. Resistance to most relevant antimicrobials was commonplace among the Gram-negative bacilli; however, most remained susceptible to imipenem. The percentage of bloodstream isolates of *Escherichia coli* susceptible to common antimicrobial agents was as follows: ampicillin (6%), ampicillin–sulbactam (38%), co-trimoxazole (38%) and aminoglycosides (52%). The susceptibility of isolates of *E. coli*, *Klebsiella* and *Enterobacter* spp. to ceftazidime was 62%, 40% and 46%, respectively (107).

### 2.8 Global problem of antibiotic resistance

From the above studies we can easily conclude that antimicrobial resistance among bacterial pathogens is a global problem. No country on its own can isolate itself from resistant bacteria. Antibiotic resistance is a growing international problem affecting both current and future generations. Resistance that develops in one area of a country may easily spread nationwide. Globalization, with increased migration, trade and travel, has widened the range for infectious diseases. A resistant strain of *Streptococcus pneumoniae*, first identified in Spain, was soon afterwards found in Argentina, Brazil, Chile, Taiwan, Malaysia, the USA, Mexico, the Philippines, the Republic of Korea, South Africa and Uruguay (108). Such examples underline the fact that no single country can protect itself from the threat of resistant bacteria as
pathogens are spreading across international, cultural and ethnic boundaries. Although the effects of antibiotic resistance are more documented in industrialized countries, there is a greater potential for harm in the developing world, where many of the second and third line therapies for drug-resistant infections are unavailable and unaffordable (109).
Chapter (III)
Materials and Methods

3.1 Materials

3.1.1 Media and reagents
Blood Agar Base, MacConkey Agar, Bile Esulin Azide Agar, M-Enterococcus Agar Base Modified, Pseudomonas Agar Base, HiCrome UTI Agar modified, Muller Hinton Agar, Brain Heart Infusion Broth, Triple Sugar Iron Agar (HiMedia). Oxidase reagent, antimicrobial susceptibility test HiDiscs Cartridges (HiMedia) and API 20 E (biomeroux). All media and reagents were prepared as recommended by the manufacturers.

3.1.2 Equipment
- Autoclave (Tutanuer)
- Incubator 37°C (Memmert)
- Balance
- Binocular microscope (OLYMPUS)
- Sewage collection tool (Home made)
- Bacteriological loop

3.2 Methods

3.2.1 Sampling sites selection and identification
As part of the study, samples of sewage were collected from sewers and sewage treatment plants. Four different sites were selected for the study and from each site samples were collected from different locations. Table (3.1) and figure (3.1) illustrate the sampling sites and locations. Figure (3.2) illustrate the path that the wastewater take through its way to GWWTP on Gaza map.
Table (3.1): Sampling sites, numbers and locations

<table>
<thead>
<tr>
<th>Sites</th>
<th>Number of sample</th>
<th>Location of sample collection at each site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al-Shifa-Hospital</td>
<td>15</td>
<td>Intensive care unit sewer, Burn unit sewer and Laboratories sewer</td>
</tr>
<tr>
<td>IUG</td>
<td>15</td>
<td>L building sewer, M building sewer and Laboratories building sewer</td>
</tr>
<tr>
<td>GWWTP</td>
<td>10</td>
<td>From inlet and outlet</td>
</tr>
<tr>
<td>Seawater</td>
<td>5</td>
<td>About 20 meters from the point of sewage discharge in seawater</td>
</tr>
</tbody>
</table>

Figure (3.1): A schematic presentation of the sampling locations

3.2.1.1 Sampling sewers

Samples of sewage were collected from three separate sewers receiving waste effluent from three different units in Al-Shifa hospital, and samples of sewage were collected from three separate sewers receiving waste effluent from three different buildings in Islamic University-Gaza.

The selection of the sampling sites was restricted by the access to the sewer system for the collection of samples. Differences in the occurrence of resistant
bacteria between these sites were used to evaluate the impact associated with the discharge of waste effluent.

3.2.1.2 Sampling Gaza Wastewater treatment plant (GWWTP)
Samples of raw sewage and treated sewage were collected from inlet and outlet of the treatment plant as shown in figure (3.3 & 3.4).

3.2.1.3 Sampling seawater
Samples of seawater about 20 meters from the point of sewage discharge from GWWTP in the sea as shown in figure (3.5 & 3.6) were collected, to see the impact of sewage discharge in the sea on the bacteria present in the sea. Samples were collected below the surface at the shoreline, using sterile bottles, and transported to the laboratory in an insulated cooler.
3.2.2.1 Sampling Frequency and Duration

From each site the sample were collected five times. Samples collection lasted from Jul. 2005 to Sep. 2005.

3.2.2.2 Sample Containers

Five hundreds ml plastic bottles were used. The sample bottles were sterilized by autoclaving at 121 °C for 15 minutes.

3.2.2.3 Preservation and Storage of Samples

Samples were collected in sterile containers and stored on ice until analyzed as recommended by American Public Health Association Standard Methods for the Examination of Water and Wastewater (110).

3.3 Sample Processing

Loop full of each specimen was inoculated on blood agar, MacConkey agar, M-Enterococcus Agar, Pseudomonas Agar, HiCrome UTI Agar plates and Brain heart infusion broth tubes using bacteriological loop and incubated aerobically at 37°C for 24–48 hours. Growing colonies were identified biochemically in a systematic way according to standard methods (111).

All Gram-negative rods were identified by using API 20E strips. The initial characterization of enterococci was based on catalase reaction, hemolysis, and colony morphology. Further identification of enterococci was accomplished by the use of bile esculin test.

It is important to note that some bacterial strains were isolated from the same sample more than once; the sensitivity test was done for this isolate, if the sensitivity patterns was the same for the two isolates one of them was neglected.
Each specimen was inoculated on the following media and incubated aerobically at 37°C for 24–48 hours:

- MacConkey plate
- Blood Agar plate
- BHIB
- M-enterococcus Agar plate
- Pseudomonas Agar plate
- HiCrome UTI Agar plate

After 24 hr subcultures were made from BHIB on the same media:

Subculture

- MacConkey plate
- Blood Agar plate
- M-enterococcus Agar plate
- Pseudomonas Agar plate
- HiCrome UTI Agar plate

Yellow colonies considered as presumptive:

- Small red colonies

Enterococcus formed dark brown or black colonies:

- Oxidase
- API 20E

Confirmed by biochemical tests:

- E. coli pink to red
- Proteus mirabilis light brown
- Pseudomonas aeruginosa colorless
- Klebsiella pneumoniae blue to purple
- Enterococcus fecalis blue small

**Figure (3.7):** Flow diagram of the isolation and identification procedures
3.4 Antimicrobial susceptibility testing

Bacterial susceptibility testing was done by the disk diffusion method according to Kirby-Bauer method (112) following the NCCLS assessment criteria (113). Bacterial inocula were prepared by suspending the freshly grown bacteria in 4-5 ml sterile BHIB and the turbidity was adjusted to that of a 0.5 McFarland standard. The inoculum suspension was spread in three directions on a Mueller Hinton agar plate surface with a sterile swab Filter paper disks containing designated amounts of the antimicrobial drugs obtained from commercial supply firms (HiMedia).

The antimicrobial disks tested for all isolates were: Amikacin 30µg, Amoxycillin 30µg, Aztreonam 30µg, Ceftazidime 30µg, Ceftizoxime 30µg, Cephoxitin 30µg, Chloramphenicol 30µg, Ciprofloxacin 30µg, Co-Trimoxazole 23.75µg, Gentamicin 10µg, Imipenem 10µg , Nalidixic Acid 30µg , Piperacillin 100µg, Tetracycline 30µg, Tobramycin 10µg, were tested against Gram negative bacteria. On the other hands, Streptomycin 10µg , Tetracycline 30µg, Teicoplanin 30µg,Vancomycin 30µg, Ampicillin 10µg, Erythromycin 15µg, were tested against Gram positive bacteria. The plates were incubated aerobically at 37°C for 18-24 hours. Zone of inhibition around antibiotic disks were recorded and using the chart provided by the antimicrobials manufacturer, results were interpreted as sensitive, intermediate or resistant.

3.5 Analysis of data

Data generated from the study was tabulated as excell files and uploaded to Statistical Package for Social Sciences (SPSS version 11). Crosstabulation of variables were generated.
Chapter (IV)
Results

This study was conducted during the period from June to September, 2005, and attempted to isolate *E. coli*, *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp., and *Enterococcus* spp. from wastewater sample for the purpose of studying the possible contribution of Al-Shifa hospital to the increasing problem of antibiotic resistance. Standard antimicrobial susceptibility test was performed for all isolates.

A total of 45 wastewater samples were collected from 9 different sampling points. Each point was sampled 5 times with 2 weeks intervals. Three sampling points at Al-Shifa hospital (burn unit sewer, ICU unit sewer and laboratory sewer), another three from Islamic University-Gaza (L building sewer, M building sewer and laboratory building sewer), two from the Gaza Wastewater Treatment Plant (Inlet and outlet of the plant) and one from the seawater near the GWWTP discharge point. From the 45 wastewater samples, 154 bacterial strains were isolated. The highest number of bacteria isolated from Al-Shifa hospital and accounted for 32.5% (50 isolates) of the isolated bacteria followed by sites from the Islamic University-Gaza, 29.9% (46), GWWTP, 26.6% (41) and seawater 11.0% (17), as illustrated in details in Table 4.1 and in Figure 4.1.

<table>
<thead>
<tr>
<th>Site</th>
<th><em>E. coli</em></th>
<th><em>Pseudomonas</em></th>
<th><em>Klebsiella</em></th>
<th><em>Proteus</em></th>
<th><em>Enterococcus</em></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Burn</td>
<td>6</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>2. ICU</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>16</td>
</tr>
<tr>
<td>3. Laboratory</td>
<td>3</td>
<td>7</td>
<td>2</td>
<td>-</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>4. L Building</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td>5. M building</td>
<td>5</td>
<td>6</td>
<td>2</td>
<td>-</td>
<td>5</td>
<td>18</td>
</tr>
<tr>
<td>6. Lab. U</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>-</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>7. Inlet</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>3</td>
<td>7</td>
<td>21</td>
</tr>
<tr>
<td>8. Outlet</td>
<td>7</td>
<td>7</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>9. Sea</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>51</td>
<td>16</td>
<td>7</td>
<td>33</td>
<td>154</td>
</tr>
</tbody>
</table>
Figure (4.1): Distribution of bacterial isolates by sampling site.

The most frequently identified bacterium was *Pseudomonas* spp. (33.1%) followed by *E. coli* (30.5%), *Enterococcus* spp. (21.4%), *Klebsiella* spp. (10.4%) and *Proteus* spp. (4.5%) as illustrated in Table 4.1.

Figure (4.2): Frequency of different bacterial isolate.

The gram-negative isolates showed wide variation in their response to the tested antimicrobial drugs as shown in Table 4.2. High resistance rate to amoxicillin (43.1%), ceftizoxime (33.3%), nalidixic acid (72.5%), cephalexin
(90.2%), aztreonam (19.6%), and ceftazidime (13.7%) was observed among Pseudomonas spp. The highest resistance rate was for tetracycline (100.0%), amikacin (100.0%), chloramphenicol and co-Trimoxazole (71.4%) was exhibited by Proteus spp. For imipenem, there was no resistance at all.

The resistance pattern for each bacterium varied according to the site from which the bacteria were isolated. For E. coli the highest resistance rate to tetracycline, amoxicillin, ciprofloxacin and chloramphenicol was for those isolated from the inlet of the GWWTP and were (66.7%), (66.7%), (66.7%) and (33.3%) respectively. For tobramycin, nalidixic acid, cephalexin, Co-Trimoxazole, piperacillin, gentamicin and Ceftazidime the highest rate of resistance for E. coli which was isolated from the laboratory building of IUG and account for (40.0%), (40.0%), (80.0%), (80.0%), (80.0%), (40.0%) and (40.0%) respectively as shown in Table 4.3.

With regard to Pseudomonas spp., the resistance rate is shown to be high for most antibiotics and reached 100.0% for nalidixic acid, cephalexin, Co-Trimoxazole and chloramphenicol. The Pseudomonas spp. strains isolated from Al-Shifa hospital and sea were more resistant to antibiotics than Pseudomonas spp. isolated from other sites as shown in Table 4.4.

The most resistant Klebsiella spp. isolate was that isolated from the laboratory building of the IUG. The highest resistance rate of Klebsiella was observed against piperacillin (100.0%) as shown in Table 4.5.

Proteus spp. constituted the lowest number of the isolated bacteria and showed high rate of resistance to tetracycline, Co-Trimoxazole, piperacillin ciprofloxacin and chloramphenicol which account for (100.0%) as shown in Table 4.6.

The only Gram-positive isolate was Enterococcus spp. and showed the highest rate of resistance to streptomycin followed by vancomycin and erythromycin.
This high resistance rate observed for all *Enterococcus* spp. regardless of the isolation site as shown in Table 4.7.

**Table (4.7):** Percentage resistance of *Enterococcus* spp. isolated from different sites to antibiotics

<table>
<thead>
<tr>
<th>Site</th>
<th>No. of isolates</th>
<th>A</th>
<th>Te</th>
<th>S</th>
<th>E</th>
<th>Va</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burn</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>60.0</td>
<td>80.0</td>
<td>60.0</td>
</tr>
<tr>
<td>ICU</td>
<td>3</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>50.0</td>
<td>100.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Laboratory</td>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>28.2</td>
<td>85.7</td>
<td>100.0</td>
<td>85.7</td>
</tr>
<tr>
<td>L building</td>
<td>1</td>
<td>33.3</td>
<td>0.0</td>
<td>100.0</td>
<td>33.3</td>
<td>100.0</td>
<td>33.3</td>
</tr>
<tr>
<td>M building</td>
<td>5</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>100.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Lab. B</td>
<td>3</td>
<td>25.0</td>
<td>0.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>50.0</td>
</tr>
<tr>
<td>Inlet</td>
<td>7</td>
<td>0.0</td>
<td>0.0</td>
<td>66.7</td>
<td>33.3</td>
<td>33.3</td>
<td>33.3</td>
</tr>
<tr>
<td>Outlet</td>
<td>2</td>
<td>0.0</td>
<td>0.0</td>
<td>100.0</td>
<td>20.0</td>
<td>60.0</td>
<td>40.0</td>
</tr>
<tr>
<td>Sea</td>
<td>3</td>
<td>0.0</td>
<td>33.3</td>
<td>66.7</td>
<td>33.3</td>
<td>66.7</td>
<td>0.0</td>
</tr>
</tbody>
</table>

* A: Ampicillin, Te: Tecoplanin, S: Streptomycin, E: Erythromycin, Va: Vancomycin and T: Tetracycline

In Vitro activities of 6 different antibiotics against the *Enterococcus* spp. is illustrated in Table 4.8 and Figure 4.3. The highest resistance rate was to streptomycin (91.0%). Also it was, 75.8%, 60.6%, 39.3%, 9.1% and 6.1%, to vancomycin, erythromycin, tetracycline, teicoplanin and ampicillin, respectively. The lowest resistance was to ampicillin (6.1%).

**Table (4.8):** Activities of 6 different antibiotics against *Enterococcus* spp. isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>% Resistance</th>
<th>% Intermediate</th>
<th>% Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>6.1</td>
<td>51.5</td>
<td>42.4</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>60.6</td>
<td>27.3</td>
<td>12.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>91.0</td>
<td>9.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>9.1</td>
<td>12.1</td>
<td>78.8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>39.3</td>
<td>21.2</td>
<td>39.3</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>75.8</td>
<td>0.0</td>
<td>24.2</td>
</tr>
</tbody>
</table>
Figure (4.3): Bars represent the activities of 6 different antibiotics against the Enterococcus isolates

In Vitro activities of 15 different antibiotics against the gram-negative bacterial isolates is illustrated in Table 4.9. A high resistance rate among gram-negative bacteria was observed against cephalexin (52.1%) followed by Co-Trimoxazole and tetracycline (41.3%), chloramphenicol (39.7%), nalidixic acid (36.4%) and amoxicillin (35.5%). The lowest resistance was to amikacin and ceftazidime (8.3%).
Table (4.9): Antibiogram of 15 different antibiotics against the gram-negative isolates

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Susceptibility Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistance</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>35.5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8.3</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>13.2</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>52.1</td>
</tr>
<tr>
<td>Co-Trimoxazole</td>
<td>41.3</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>39.7</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>12.4</td>
</tr>
<tr>
<td>Ceftazidine</td>
<td>8.3</td>
</tr>
<tr>
<td>Ceftizoxime</td>
<td>14.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10.7</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.0</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>36.4</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>28.9</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>41.3</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>11.6</td>
</tr>
</tbody>
</table>

A high proportion of the isolated strains showed resistance to more than two drugs. The percent of bacteria that are not resistant to any antibiotics is low compared with these with multiple resistant pattern. The multiple drug resistance of the isolates is illustrated in Table 4.10. Pseudomonas showed superiority to other isolates in terms of resistance to more than four antibiotic.
Table (4.10): Multiple resistance patterns of the isolated strains.

<table>
<thead>
<tr>
<th>Resistance</th>
<th>E. coli N=47</th>
<th>Pseudomonas spp. N=51</th>
<th>Enterococcus spp. N=33</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Not resistant</td>
<td>11   23.4</td>
<td>2   3.9</td>
<td>1   3.00</td>
</tr>
<tr>
<td>To one antibiotic</td>
<td>10   21.3</td>
<td>2   3.9</td>
<td>3   9.1</td>
</tr>
<tr>
<td>To two antibiotics</td>
<td>5    10.6</td>
<td>4   7.8</td>
<td>11   33.3</td>
</tr>
<tr>
<td>To three antibiotics</td>
<td>6    12.8</td>
<td>8   15.7</td>
<td>9    27.3</td>
</tr>
<tr>
<td>To four Antibiotics</td>
<td>6    12.8</td>
<td>8   15.7</td>
<td>8    24.2</td>
</tr>
<tr>
<td>More than 4</td>
<td>9    19.1</td>
<td>27   52.9</td>
<td>1    3.0</td>
</tr>
</tbody>
</table>
Chapter (V)
Discussion

There is growing concern about bacterial resistance to antimicrobials. Almost since the beginning of the antibiotic era, bacterial resistance has been seen as the major obstacle to successful treatment (113).

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today (6). Confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem. Selection of resistant forms can occur during or after antimicrobial treatment; antibiotic residues can be found in the environment for long periods of time after treatment. Besides antibiotics, there is the mounting use of other agents aimed at destroying bacteria, namely the surface antibacterial agents available now in many household products. These too enter the environment. The stage is thus set for an altered microbial ecology, not only in terms of resistant versus susceptible bacteria, but also in terms of the kinds of microorganisms surviving in the treated environment. The world currently face multiresistant infectious disease organisms that are difficult and, sometimes, impossible to treat successfully (6).

Antibiotic-resistant bacteria (115,116,117,118) and antibiotics (119) are discharged in various amounts in the environment as a result of the increasing and often indiscriminate use of antibiotics in medical, veterinary, and agricultural practices.

Resistant bacteria may be selected by antibiotic substances in hospital effluent, municipal sewage, aeration tanks, the anaerobic digestion process of STPs or in soil. Furthermore, resistant bacteria are excreted and discharged into sewage or soil and other environmental compartments. Resistant and even multi-resistant pathogenic bacteria have been detected in wastewater and STPs, as well as in
other environmental compartments (72,120,121) Furthermore, in arid regions, wastewater containing resistant bacteria and antibiotics is used for irrigation, and sewage sludge serves as a fertilizer. This allows resistant bacteria to enter the food chain directly. Concentrations below therapeutic levels may play a role in the selection of resistance and its genetic transfer in certain bacteria. Exposure of bacteria to sub-therapeutic antimicrobial concentrations is thought to increase the speed at which resistant strains of bacteria are selected. Resistance can be transferred to other bacteria living in other environments such as ground water or drinking water (122).

This study was conducted to examine the possible contribution of wastewater effluent from different parts of Al-Shifa hospital on the prevalence of resistant bacteria in the recipient sewers in comparison with the contribution of wastewater effluent from non-health institution (Islamic University-Gaza) on the prevalence of resistant bacteria, and to see the impact of wastewater effluent from GWWTP in the seawater.

In this study, different aspects concerning the occurrence and fate of antibiotic resistant bacteria in sewage were investigated:

- The rate of resistant bacteria in the recipient sewers at Al-Shifa Hospital.
- The susceptibility pattern of bacteria isolated from wastewater effluent from IUG and GWWTP.
- The impact of wastewater effluent from GWWTP on the prevalence of resistant bacteria in the recipient seawater.

In the present study, 45 wastewater samples were collected from 9 different sampling points. Out of those 45 wastewater samples, 154 bacterial strains were isolated (Refer to table 4.1 and figure 4.1). The highest number of bacteria isolated from Al-Shifa hospital and accounted for 32.5% (50 isolates) of the isolated bacteria followed by sites from the Islamic University-Gaza, 29.9% (46), GWWTP, 26.6% (41) and seawater 11.0% (17). The most frequently identified
bacterium was *Pseudomonas* spp. (33.1%) followed by *E. coli* (30.5%), *Enterococcus* spp. (21.4%), *Klebsiella* spp. (10.4%) and *Proteus* spp.

In the present study, table 4.2 showed that a high percentage of antibiotic resistance among gram-negative isolates. For this isolates, high resistance rate to amoxicillin, ceftizoxime, nalidixic acid, cephalexin, aztreonam, and ceftazidim (43.1%), (33.3%), (72.5%), (90.2%), (19.6%) and (13.7%) respectively was observed among *Pseudomonas* spp. The highest resistance rate for tetracycline, amikacin, chloramphenicol and co-Trimoxazole (100%), (100%) and (71.4%) respectively was exhibited by *Proteus* spp. For imipenem antibiotic there was no resistance at all, it is important to indicate that the use of imipenem antibiotic in our hospitals is very restricted for life threatening infections, and for this reason there is no resistance at all to this antibiotic.

For gram-positive isolate (*Enterococcus* spp.), the highest resistance rate was to streptomycin (91.0%). Also it was, 75.8%, 60.6%, 39.3%, 9.1% and 6.1%, to vancomycin, erythromycin, tetracycline, teicoplanin and ampicillin, respectively. The lowest resistance was to ampicillin (6.1%), referred to Table 4.8.

From this study we observed that the resistance rate against most antibiotics was high, particularly those isolated from wastewater samples which were collected from Al-Shifa hospitals, GWWTP, the laboratory building of IUG and from seawater. The resistance rate for tetracycline was high for most of the isolated bacteria, tetracycline resistance rates similar or higher than those found in this study have been reported (72, 115,116,118).

Also the resistance rate for nalidixic acid (Quinolones) was high, previous studies have demonstrated that quinolone resistance was less than 25% among environmental isolates (72,115,123). The high resistance rate for nalidixic acid may be due to the fact quinolones antibiotics are excreted mostly as unchanged substances, and they are among the most persistent antibiotics in the environment (119).
In this study the resistance rate was high for chloramphenicol antibiotic for most of the isolates. In contrast, chloramphenicol resistance are rare in most studies (116,117,118), possibly as the result of the restricted use of this drug.

In comparison with the levels of antibiotic resistance reported in the literature for clinical isolates, gram-negative isolates particularly *E. coli* and *Pseudomonas* spp. that isolated from sewage were generally more susceptible to antimicrobial agents. This result confirms the data, reported previously by other authors (124,125). *Acinetobacter* spp. from sewage were also more susceptible to antimicrobial agents (72).

The resistance pattern for each bacterium varied according to the site from which the bacteria were isolated. For *E. coli* the highest resistance rate to tetracycline, amoxicillin, ciprofloxacin and chloramphenicol was for those isolated from the inlet of the GWWTP, the same result was reported in previous study and indicated that the highest resistance rates were found in *E. coli* strains of a sewage treatment plant which treats not only municipal sewage but also sewage from hospitals (63) and another study indicated that the percentages of *E. coli* resistance in raw sewage were significantly high (70). For tobramycin, nalidixic acid, cephalaxin, Co-Trimoxazole, piperacillin, gentamicin and ceftazidime the highest rate of resistance was for *E. coli* which was isolated from the laboratory building of IUG.

With regard to *Pseudomonas* spp., the resistance rate is shown to be high for most antibiotics particularly for nalidixic acid, cephalaxin, Co-Trimoxazole and chloramphenicol, high resistant rate for *Pseudomonas* spp. Isolated from clinical sources against the same antibiotics was also demonstrated in another study conducted in Gaza Strip hospitals (98). The *Pseudomonas* spp. isolated from Al-Shifa hospital and sea were more resistant to antibiotics than *Pseudomonas* spp. isolated from other sites as shown in table 4.4. Another author found a higher incidence of resistance in the bacteria isolated from remote uplant tars
than those isolated from a polluted lake or sewage, with the highest value being observed for pseudomonas, which are naturally multiresistant organisms (126).

The most resistance *Klebsiella* spp. isolates were those isolated from the laboratory building of the IUG. The highest resistance rate of *Klebsiella* was observed against piperacillin (100%).

The high resistance rate found in samples collected from the laboratory building of IUG is likely due to heavy metals, biocides and various chemicals that are discharged in sewage of this building and these substances have the potential to select for antibiotic resistance (127).

*Enterococcus* spp. showed the highest rate of resistance to streptomycin followed by vancomycin and erythromycine. This high resistance rate observed for all *Enterococcus* spp. regardless of the isolation site, results from other study indicate that the majority of the vancomycin resistance enterococci (VRE) were resistant to at least two of the tested antimicrobial agents besides vancomycin (65), another study indicated that VRE and ERE isolates were detected in most of the wastewater (128).

Concerning the antibiogram of all bacteria that were isolated from the seawater, the results of this study indicated that, the percentage of resistance to antibiotics is generally high. Similarities in antimicrobial resistance rates and pattern between seawater isolates and AL-Shifa as well as GWWTP isolates were found. This high resistant rate for bacteria that were isolated from seawater may be due to the fact that only few compounds were partially biodegraded in under test conditions in aquatic systems (16) and most were persistent. And this result is consistent with those reported in another study and reported that, the river, which is contaminated by treated wastewater with many kinds of pollutants, is also contaminated with antibiotic resistant coliform group bacteria and *E. coli* (70). Similarly, another author have reported that, An increase of resistances was also observed in strains isolated from rivers
receiving urban discharge (117) or hospital and pharmaceutical plant wastewaters (72).

As shown by the results of this research, the resistant rate for bacteria that were isolated from the inlet of GWWTP was generally higher than the resistant rate for those that isolated from the outlet of the plant, but for some antibiotics the resistant rate was the same for both isolates, for some the resistance rate was higher in the outlet isolates, and this result is slightly different from what have been reported in another study which demonstrated that, the percentages of resistance in raw sewage were significantly higher than those in the river water and in the treated water and thus the percentages of resistant bacteria in the wastewater treatment plant were decreasing during the treatment process (70). But it is important to note that the GWWTP was overloaded during the period of our study and the plant was not properly working during this period.

This study indicates a high percentage of multiple drug resistance for the majority of the isolated strains. And this result is consistent with that reported in another study done in Gaza Strip but the isolated bacteria were isolated from patient's samples and indicated a high percentage of multiple drug resistance (99) and in this study the author correlated this finding to many factors that may contribute to this alarming situation, including the use of antimicrobial agents as prophylactics in surgery, antibiotic use in animal feeds, under-dosing of antibiotics, the widespread use of broad spectrum antibiotics and the sale of antibiotics over the counter and self-treatment with antibiotics. In this study we can attribute this to the fact that, sewage contains a high content of both organic and inorganic matter, as well as high densities of living organisms, including pathogenic, commensal and environmental bacteria. This characteristic composition makes sewage particularly suitable ecological niche for the growth and spread of antibiotic resistance (126).

In general, we demonstrated that bacterial isolates from wastewater samples, Al-Shifa hospital and Laboratory building of IUG contained higher number of
antibiotic resistant bacteria than bacteria that were isolated from other sites. Accordingly, previous studies have shown that waste effluent from hospitals contain higher levels of antibiotic-resistant enteric bacteria than waste effluent from other sources. Hospital waste effluent could increase the numbers of resistant bacteria in the recipient sewer by both mechanisms of introduction and selection for resistant bacteria. (13,14, 15).

This finding is likely due, in part, to the selective pressure resulting from the exposure of bacteria that present in sewage to antibiotics and non-antibiotics substances that have the potential to select for antibiotic resistance. The waste effluent from Al-Shifa hospital contain residues of antibiotics administrated to patients reach the sewage systems in urine or feces, in the form of either parent compound or degraded metabolites depending on the pharmacology of the specific antibiotic. Furthermore, unknown amount of antibiotics enter the sewers by waste derived from disposal of a surplus of drugs. This result is quite similar to that reported by another author, who has stated that, indeed, various antibiotics have been found in municipal sewage, including flouroquinolones, sulfonamides and erythromycin metabolites (81, 82). The antibiotic concentrations found in sewage vary between 1 and 100 μg per liter. Such concentrations are 100- to 1000 fold lower compared with those necessary to inhibit resistant bacteria, but are sufficient to affect susceptible bacteria (16, 83). Therefore, the occurrence of such antibiotic concentrations in sewage has the potential to select for antibiotic resistance.
Chapter (VI)
Conclusions and Recommendations

Environmental pollution with bacteria of public health significance and the likelihood of this bacteria gaining access to the food chain are the most critical areas of concern. To our knowledge, this was the first study done in Gaza Strip demonstrating the impact of waste effluent from hospital on the occurrence of resistant bacteria in sewage. From the present investigation, we can conclude that the release of wastewater from the hospital under study was associated with an increase in the prevalence of antibiotic resistance.

6.1 Conclusions

1. A high percentage of antibiotic resistance was demonstrated among gram-negative and gram-positive isolates.

2. The resistance pattern for each bacterium varied according to the site from which the bacteria were isolated.

3. The resistant rate was high for most bacteria that isolated from the laboratory building of IUG.

4. *Enterococcus* spp. showed highest rate of resistance to streptomycin followed by vancomycin and erythromycin. This high resistance rate observed for all *Enterococcus* spp. regardless of the isolation site.

5. Antibiogram of all bacteria that were isolated from seawater was similar to bacterial isolates from other sites particularly from Al-Shifa hospital and GWWTP.

6. Resistant rate for bacteria that isolated from the inlet of GWWTP is generally higher than the resistant rate for those that isolated from the outlet of the plant with some exceptions.

7. This study indicated a high percentage of multiple drug resistance for the majority of the isolated strains.

8. In general, we demonstrated that bacteria isolates from wastewater samples from Al-Shifa hospital and Laboratory building of IUG had higher number of antibiotic resistant bacteria than bacterial isolates from other sites.
9. These results indicate that waste effluents from hospitals are an important source for the occurrence and selection of resistant bacteria in sewage

6.2 Recommendations

One major public health concern is the spread of resistant bacteria into the environment and community which would complicate management and increase the mortality rate of infectious diseases. Considering the result of this study, the followings are recommended:

1. Hospitals should adapt strict infection control measures to prevent nosocomial infections.

2. Governmental actions to regulate the process of prescribing antimicrobial should be carefully planned and implemented.

3. It is important to educate health professional on proper disposal of antimicrobials left over in order to minimize their presence in the environment.

4. It is also recommended that hospitals should have a small scale treatment plant for their wastewater before discharged into the public sewers.

5. It is also important to educate the public about the proper use of antimicrobials through seminars, publications and media.

6. Protocols on antibiotic usage should be regularly reviewed by experts based on local antimicrobial profiles data collected from various hospitals
7. This study suggests the necessity for continued monitoring of multidrug resistance in hospital and the environments.

8. There is a new trend of monitoring antimicrobial resistance in a particular area that utilizes fecal indicators as makers for the overall resistance situation. The existing monitoring programs depend totally on data obtained from clinical isolates. This new trend could be a good research area to be explored.

9. As this study was conducted on a single hospital, further investigation is needed to assess the role of waste effluent from hospitals on selection and introduction of resistant bacteria in sewage.
References


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