SELINUS UNIVERSITY
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Global rise of multidrug resistant organisms (MDRO), antibiotics research: MDRO in Bangladesh perspective and way forward

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Abstract

Multidrug resistance is a natural phenomenon and occurs when an organism is exposed to same antibiotic over and over or abuse or misuse of antibiotics. Microorganisms can produce various enzymes and become resistance to current antibiotics. Globally over 2 million people die each year due to bacterial infections. Approximately 25,000 patients die each year from MDR in the EU and about 1.5 billion Euro worth of extra healthcare costs and productivity losses due to infections by MDR organisms in the EU. More than 70% of the clinically significant or pathogenic bacteria possesses resistance to currently existing antibiotics. Methicillin resistance *Staphylococcus aureus* (MRSA), vancomycin resistance *Enterococcus* (VRE), Extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae, Carbapenemase resistance Enterobacteriaceae (CRE), Multidrug resistance *Pseudomonas* species are the key MDR mostly seen in the patients. According to The UK Government-commissioned O'Neill report 10 million people a year will die from MDR infections by 2050 if no urgent action is taken and recommended to boost the development of antibiotics. Bangladesh is an eighth most populous country in the world with a population of 161.03 million and with an area of 56,977 square miles which makes the country 94th largest by area. Food and waterborne diseases (bacterial and protozoal diarrhoea, hepatitis A and E, and typhoid fever) and vector-borne diseases (dengue, malaria) are highly prevalent. MDRO is a key problem in Bangladesh. Many factors contribute to the MDRO in Bangladesh such as over the counter sell antibiotics, uncontrol sells, lack of graduate pharmacist, lack of controlling and monitoring of antibiotics sells, patient's financial conditions, lack of medical and health access. Patient's financial incapacity of buying full antibiotics doses is among the most common cases of antibiotics misuses. Selling or prescribing inappropriate classes of antibiotics are another serious problem found in this study which also believed contributing to MDRO in Bangladesh. Immediate actions are needed in order to stop rising MDRO. Strong legislations, monitoring, training and education, professional and regulatory body, adequate medical professional and easy access to health and diagnosis to the diseases should be provided to overcome this crisis. Investing in antibiotics research should be encouraged and compensated by the government, charities and other NGOs are the way forward to this global MDRO crisis.

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Abbreviations

API = active pharmaceutical ingredients.
BA = Blood agar.
B.A. = Bachelor of arts.
B.Sc. = Bachelor of science.
BV = Bacterial vaginosis.
CNS = Central nervus system.
CNS = Coagulate negative *Staphylococcus*
CRE = Carbapenemase resistance Enterobacteriaceae.
ESBL = Extended spectrum beta lactamase.
EU = European union
EUCAST = European committee on antimicrobial susceptibility testing.
H.S.C = Higher secondary certificate
IV = Intravenous.
M.A. = Master of arts
MDRO = multidrug resistance organisms
M.Sc. = Master of science
MRSA = methicillin-resistant *S. aureus*.
MSTI = Mucosa and soft tissue infections.
NAATs = nucleic acid amplification tests.
OOP = out of pocket.
PCR = polymerase chain reaction.
S.S.C = Secondary school certificate
TB = Mycobacterium tuberculosis.
UTI = Urinary tract infections.
USA = United states of America.
UK = United Kingdom
USA = United State of America
UEL = University of East London
VRE = Vancomycin resistant *Enterococcus*

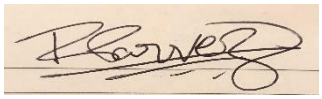
Acknowledgement

I am delighted to have an opportunity to thank everybody who has made this thesis possible. I am grateful to the Selinus university for giving the opportunity to do this research and believing in me for my capabilities. I would thanks to all participants both pharmacy personnel and customers for providing me with the necessary information needed for this study to understand the MDRO in Bangladesh and draw guidelines and conclusion to prevent the rise of MDRO in Bangladesh. I would firstly like to thank my Director of Studies, Professor Sally Cutler. I would express my deepest sense of gratitude, indebtedness, and sincere appreciation to Professor Cutler of UEL for teaching me scientific writings during my study at UEL.

I would like thank Dr Mukhlesur Rahman of UEL, my colleagues and mentors for providing me the necessary study materials for this research projects. Finally, I greatly appreciate for the support of my family, and I would like to dedicate my thesis to my beloved late father Abdus Samad.

Declaration

I do hereby declare that I am the sole author of this thesis and that its contents are only the result of the studies and research I have done. I also declare that I have not submitted this thesis to any other institution or university for any other degree.



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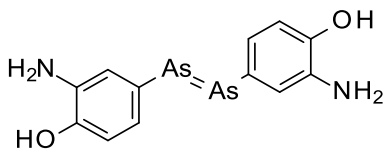
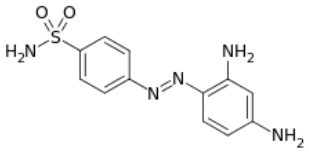
Chapter 1: Introduction

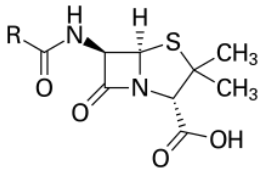
1.1 Pre-antibiotic era and discovery of first natural antibiotic

Microorganisms causes wide ranges infectious diseases from superficial infections such as boils, carbuncles, furuncles to a deep-seated or systemic infection such as necrotising fasciitis, meningitis and so on. Since the dawn of the human history microorganisms played a key role in health and infections. Mycobacterium tuberculosis (TB) was infection found in a mummified body around 3000 BC (Nerlich and Lösch, 2009). Another study showed that a 1000 years old Anglo-Saxon remedy can kill *Staphylococcus aureus* biofilms in soft tissue infection in an *in vitro* model and can kill methicillin-resistant *S. aureus* (MRSA) in a mouse chronic wound model (Harrison et al., 2015).

However, discovery of this tiny organism was possible for the invention of microscopy by Antony Von Leuwenhoek (Haden, 1939) and association of human infections and microorganisms were proposed by Robert Koch which popularly known as Koch's postulates (Breitschwerdt et al., 2013). Koch inoculated microorganism into experimental animal and reproduced the diseases. In order to cure infections, in the pre-antibiotic era, natural remedies such as herbs, honey, mouldy breads and animal faeces were used in different communities and countries (Gould, 2016). Paul Ehrlich who is regarded as the pioneer in antibiotic research showed scientific approach in screening antibiotics that led the development of Salvarsan, an arsenic based compound for the treatment of syphilis and trypanosomiasis (Aminov, 2010) in 1909 . Later a sulfa drug called prontosil (**2**) discovered by Gerhard Domagk (Gradmann, 2008) was a breakthrough to treat the infections until the availability of penicillin in early 1940s. The fortuitous discovery of penicillin (**3**) in 1928 (Fleming, 1929) is regarded as a new era of modern antibiotics from microbes that formed the foundation of current antibiotic researches (Gould, 2016).







1 **2** **3**

Figure 1.1: Structures of Salvarsan (**1**), prontosil (**2**) and penicillin (**3**)

1.2 Antibiotics challenges and rise of multi-drug resistance (MDR)

Soon after introduction of Penicillin 1941 and its wide uses, resistance to Penicillin had reported in *Staphylococcus aureus* in 1942 (Rammelkamp and Maxon, 1942) and now resistance to many other pathogens (Coates and Hu, 2007). Although antibiotic resistance is a current problem due to rise of multi drug resistance (MDR) and lack of discovery of new broad-spectrum antibiotics, but this phenomenon is an ancient by far several millenniums. Beringian permafrost sediments DNA from 30,000-year-old showed genes encoded resistance to beta-lactam, tetracycline, and glycopeptide antibiotics in metagenomic analyses. Study also confirmed VanA element of vancomycin resistance from this ancient DNA sample was similar to modern VanA variant by structure and functions (D'Costa et al., 2011a). Multidrug resistance is a natural phenomenon and occurs when an organism is exposed to same antibiotic over and over or abuse or misuse of antibiotics. Microorganisms can produce various enzymes and become resistance to current antibiotics. Globally over 2 million people die each year due to bacterial infections (Bérdy, 2012). Approximately 25,000 patients die each year from MDR in the EU and about 1.5 billion Euro worth of extra healthcare costs and productivity losses due to infections by MDR organisms in the EU. More than 70% of the clinically significant or pathogenic bacteria possesses resistance to currently existing antibiotics (Kitchel et al., 2009). Methicillin resistance *Staphylococcus aureus* (MRSA), vancomycin resistance *Enterococcus* (VRE), Extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae, Carbapenemase resistance Enterobacteriaceae (CRE), Multidrug resistance *Pseudomonas* species are the key MDR mostly seen in the patients (van Duin

and Paterson, 2016). According to The UK Government-commissioned O'Neill report 10 million people a year will die from MDR infections by 2050 if no urgent action is taken and recommended to boost the development of antibiotics (Antimicrobial resistance ,2019) . Hence this study was undertaken in search of novel antibiotic.

1.3 Approaches for antibiotic discovery

Varied approaches have been used in the past 40 years to discover and develop antibiotics from various sources. Technological advancement over the last few decades helped our understanding of the microbial resistance mechanisms. However, developing new antibiotics remains as a big challenge although various approaches have taken in antibiotic discovery.

1.3.1 Genomics Approaches

The whole genome sequence of *Haemophilus influenzae* in 1995 marked as genetic revolution and regarded as a potential new era for antibiotic discovery (Payne et al., 2007). Since then, there had been significant development in molecular microbiology and whole genome sequencing of 1000s of pathogenic and non-pathogenic microorganisms were done to understand their characteristics and find suitable drug target(s) (Livermore, 2011). On the other hand, metagenomic analysis of soil bacteria enabled to find secondary metabolites producing biosynthetic gene clusters (BGCs), such as nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) and these are the two largest classes of BGCs, encoded for most of the known antibiotics and antifungals metabolites (Sharrar et al., 2020). Whole genome sequences of *Streptomyces coelicolor* and *Streptomyces avermitilis* and their secondary metabolites analysis revealed more secondary metabolites initially scientists thought of (Bentley et al., 2002; Ikeda et al., 2003). AntiSMASH-3.0 tool can detect 44 different classes of BGCs present in a microbe (Weber et al., 2015). Most of the known antibiotics came from cultivable *Actinobacteria*, *Proteobacteria*, and *Firmicutes* member of bacterial family and yet the diverse uncultivable soil microbial communities are wealthy potential sources for secondary metabolites (Sharrar et al., 2020). Genomics revolution contributed significantly in diagnosis rather the antibiotic development.

1.3.2 Plant sources (phytochemical compounds)

Plant based remedies (bark, roots, leaf) had been in use for centuries and herbal medicines are still important in many communities and counties (Gould, 2016; Khameneh et al., 2019). Plant based compounds reported to be active against bacteria (Rahman et al., 2008a, 2008b; Wan et al., 1998), fungi (Hufford et al., 1993; Rana et al., 1997) including MDR strains (Dwivedi et al., 2019; Mun et al., 2014) and *Mycobacterium tuberculosis* (Hochfellner et al., 2015) and sweet wormwood (*Artemisia annua*) derived drug, Artemisinins, is currently in use for the treatment of malaria, caused by parasites, *Plasmodium* species, including drug-resistant strains (Krishna et al., 2008). Hence it remains as potential resources for antimicrobial compounds.

1.4 Main Source of Antibiotics

Most of the antibiotics currently in clinical uses come from soil microorganisms; for example, Streptomycin from *Streptomyces griseus,* Cephalosporins from *S. clavuligerus*, Bacitracin from *Bacillus licheniformis*, Polymyxin from *Bacillus polymyxa*, Chloramphenicol from *S. venezuelae*, Tetracycline from *S. aureofaciens*, Erythromycin *Saccharopolyspora erythraea*, Gentamicin from *Micromonospora purpurea*, Mupirocin from *Pseudomonas fluorescens* and more (de Lima Procópio et al., 2012). However, last antibiotics class Carbapenem was discovered in 1976 and came in clinical use in 1985 (Hutchings et al., 2019). There is a huge gap in the new class of antibiotic discovery, and it is an urgent need to find the new class of antibiotic.

Millions of microbial species exists in soil (Bollmann et al., 2007) but 1% can grow on laboratory media and rest are uncultivable (Ling et al., 2015). One-gram soil possibly can have millions of bacteria and fungi and a clear majority of these natural resources are not yet explored for their biodiversity and their bioactivity is not clearly known (Bérdy, 2012). Soil still offers a great potential for antibiotic discovery. Organisms in soil could produces multiple secondary metabolites with possible various functions, for example, to suppress the growth of competitors or as a predator, or even as signalling molecules to interact with eukaryotic hosts and this phenomenon is backed by the evidence of evolution of Streptomyces species and other filamentous actinomycetes and the plant species colonised earth circa 440 million years ago simultaneously (Hutchings et al., 2019).

1.4.1 Uncultivable Microorganisms

Metagenomic analysis of soil samples revealed that only 1% can grow on laboratory media and rest are uncultivable (Bollmann et al., 2007; Sharrar et al., 2020). The term uncultivable microorganisms mean, organisms those cannot grow in standard laboratory-based culture medium. Natural environments or simulated natural environments using a diffusion growth chamber can enable growth of some previously uncultivated microorganisms and this concept was proposed by Kaeberlein *et al*. in 2002 (Kaeberlein, 2002). Since then, several other techniques such as soil substrate membrane system (Ferrari et al., 2008), Hallow fibre membrane chamber (Aoi et al., 2009), iChip (Nichols et al., 2010), I-tip (Jung et al., 2014) were developed and employed to cultivate uncultivated microorganisms.

iChip is one of the simplest yet much efficient device that facilitate the growth of uncultivable environmental organisms in their natural habitats. The principle behind this approach is that the 100s of miniature diffusion chamber will take growth factors for the microbes from their natural environment and allow to grow *in situ* cultivation (Nichols et al., 2010). A novel broad spectrum antibiotic was discovered from previously uncultivated microorganism using iChip (Ling et al., 2015).

1.5 Aim of the study

Studying MDRO, use and abuse of antibiotics in Bangladesh perspective and way forward.

1.6 Objective of the study

1. Collecting data on antibiotics sales and practices in Bangladesh
2. Survey on Personnel involve in running pharmacies and antibiotics sales.
3. Uses and abuses of antibiotics
4. Providing guidelines for antibiotics sales, regulation and control uses.
5. Promoting social awareness of antibiotics uses and abuses.
6. Promoting antibiotics research using local resources.
7. Guidelines on antibiotic sales in Bangladesh

Chapter 2: Materials and methods

2.1 Introduction

Bangladesh is an eighth most populous country in the world with a population of 161.03 million and with an area of 56,977 square miles which makes the country 94th largest by area. 28.27% of the populations are below 14 years of age and 6.04% are above 65 years with a dependency ration 52.5%. The population growth rate is 1.05% (2016 est.) with a life expectancy at birth of 73.2 years. Yet food and waterborne diseases (bacterial and protozoal diarrhoea, hepatitis A and E, and typhoid fever) and vector-borne diseases (dengue, malaria) are highly prevalent. The economy has grown roughly 6% per year since1996 (Molla and Chi, 2017). About 55% of the world's population were living in urban areas in 2018 and expected to reach 60% at the end of 2030 according to latest agenda for sustainable development. It was estimated that more than 90% of future urban population growth would take place in low- and middle-income countries, including Bangladesh. Dhaka, the capital city of Bangladesh, will be the fourth most populous city after Delhi, Tokyo, and Shanghai. Dhaka is the largest city in Bangladesh, with around 21 million people. Dhaka is renowned as one of the fastest-growing megacities, and it is predicted to be one of the world's largest metropolises by 2025, along with Tokyo, Mexico City, Shanghai, Beijing, and New York City . It is the ninth largest and the sixth-most densely populated city globally. Dhaka is often recognised as one of the poorest megacities, grappling with many problems such as pollution, horrendous traffic jams, unregulated construction work, brick kilns and vehicles run on fuel containing higher levels of sulphur and other detrimental substances which pose grave threats to public health . Moreover, the largescale unplanned rural–urban migration and the continuous growth of Dhaka city have resulted in overloaded public services (Sarker et al., 2022).

Gazipur is another mega city in Bangladesh with a population of 4 million. Gazipur has the biggest industrial zone in Bangladesh including export processing zone (EPZ), Bangladesh small and cottage industries corporations (BSCIC), Many big and small garments industries. Gazipur is also a rapid growing city in the country located adjacent to Dhaka with an easy communication of Dhaka international airport. Job opportunities brings people to Gazipur from all over the country and international Garments buyers.

Bangladesh remains second position after China in garments export and Gazipur is the top producer of garments in Bangladesh.

2.2 Methodologies

In this study, MDR in Bangladesh will be assessed using the data from two of country's most important and megacities. Antibiotic sells policies, personnel involve in the sells, customers buying antibiotics and their socio-economic condition, training, education, and licensing policies for pharmacy business and other relevant factors will be assessed and studied to answer the key research questions and their findings. Literatures, books, magazine, prints and electronics, lectures, talks, presentations, articles on public health and MDR will be thoroughly studied, findings and guidance will be drawn as part of this Ph.D. research.

2.2.1 List of randomly selected pharmacies in Dhaka and Gazipur and personnel involve in the trades and sales

In this study, 100 pharmacies were selected randomly from various geographical areas in Dhaka and Gazipur. Areas in Dhaka were also selected based on the people's socio-economic conditions to understand how the socio-economical condition may contribute to the MDR and public health. Some area where richest or upper classes people live and some areas mixture of upper classes, upper middle classes, middle classes, poor and extreme poor people live. This will help the study to determine how pharmacy regulations are in Bangladesh and how it contributes to the MDR.

List of the randomly selected 100 pharmacies in Dhaka and Gazipur district of Bangladesh are given below in the table 2.1.

Table 2.1: List of the pharmacy personnel and their qualifications.

Pharmacies	Educations/certification level	Area and District
1	B.A. (Pass)	Savar, Dhaka
2	B.A. (Pass)	Savar, Dhaka
3	B.A. (Pass), M.A.	Savar, Dhaka
4	B.A. (Pass), Diploma in pharmacy	Savar, Dhaka
5	H.S.C.	Savar, Dhaka
6	B.A. (Honours), M.A.	Savar, Dhaka
7	B.Sc. (Honours).	Savar, Dhaka
8	B.Sc. (Honours), M.Sc.	Savar, Dhaka
9	H.S.C. (science), Diploma	Savar, Dhaka
10	B.Sc. (Honours).	Savar, Dhaka
11	B.A. (Pass), M.A.	Ashulia, Dhaka
12	B.Sc. (Honours).	Ashulia, Dhaka
13	B.Sc. (Honours). M.Sc.	Ashulia, Dhaka
14	B.A. (Honours), M.A.	Ashulia, Dhaka
15	B.Sc. (Honours). Diploma in Pharma	Ashulia, Dhaka
16	H.S.C., Diploma in Pharma	Ashulia, Dhaka
17	B.A. (Honours). M.A.	Ashulia, Dhaka
18	B.Sc. (Honours). M.Sc.	Ashulia, Dhaka
19	B.A. (Pass)	Ashulia, Dhaka
20	B.A. (Pass), M.A.	Ashulia, Dhaka
21	B.A. (Honours). M.A.	Uttara, Dhaka
22	B.A. (Honours). M.A.	Uttara, Dhaka
23	B.A. (Honours). M.A.	Uttara, Dhaka
24	B.Sc. (Honours). M.Sc.	Uttara, Dhaka
25	B.Sc. (Honours). M.Sc., Diploma	Uttara, Dhaka
26	B.Sc. (Honours). M.Sc.	Uttara, Dhaka
27	B.Sc. (Honours). M.Sc.	Uttara, Dhaka

28	B.Sc. (Honours). M.Sc.	Uttara, Dhaka
29	B.Pharm. (Honours)	Uttara, Dhaka
30	B.Sc. (Honours). M.Sc.	Uttara, Dhaka
31	B.Sc. (Pass).	Mugda, Dhaka
32	B.Sc. (Honours). M.Sc.	Mugda, Dhaka
33	B.A. (Pass)	Mugda, Dhaka
34	B.A. (Pass), MA	Mugda, Dhaka
35	B.A. (Pass), MA	Mugda, Dhaka
36	B.A. (Pass)	Mugda, Dhaka
37	B.A. (Honours), M.A.	Mugda, Dhaka
38	B.Sc. (Pass)	Mugda, Dhaka
39	B.Sc. (Honours), M.Sc.	Mugda, Dhaka
40	B.A. (Pass)	Mugda, Dhaka
41	B.A. (Pass), M.A., Diploma in pharmacy	Gulshan, Dhaka
42	B.A. (Honours), M.A.	Gulshan, Dhaka
43	B.Sc. (Honours)	Gulshan, Dhaka
44	B.Sc. (Honours), Diploma in pharmacy	Gulshan, Dhaka
45	B.Sc. (Honours), Diploma in pharmacy	Gulshan, Dhaka
46	B.Sc. (Honours), M.Sc.	Gulshan, Dhaka
47	B.Sc. (Honours)	Gulshan, Dhaka
48	B.A. (Honours), M.A.	Gulshan, Dhaka
49	B.Sc. (Honours), M.Sc.	Gulshan, Dhaka
50	B.Sc. (Honours), M.Sc. Dip. in pharmacy	Gulshan, Dhaka
51	B.A. (Honours), M.A.	Tongi bazar, Gazipur
52	B.A. (Honours), M.A.	Tongi bazar, Gazipur
53	B.Sc. (Pass)	Tongi bazar, Gazipur
54	B.Sc. (Honours), Dip. in pharmacy	Tongi bazar, Gazipur
55	B.Sc. (Honours), M.Sc.	Tongi bazar, Gazipur
56	B.A. (Honours)	Tongi bazar, Gazipur
57	B.Sc. (Honours), M.Sc.	Tongi bazar, Gazipur
58	B.A. (Honours)	Tongi bazar, Gazipur
59	B.Sc. (Honours), Dip. in pharmacy	Tongi bazar, Gazipur

60	B.A. (Pass)	Tongi bazar, Gazipur
61	B.A. (Honours), M.A.	College gate, Gazipur
62	B.A. (Pass), M.A.	College gate, Gazipur
63	B.A. (Pass), M.A.	College gate, Gazipur
64	B.A. (Honours), M.A.	College gate, Gazipur
65	B.Sc. (Honours)	College gate, Gazipur
66	B.Sc. (Honours), Dip. in pharmacy	College gate, Gazipur
67	B.Sc. (Honours), M.A.	College gate, Gazipur
68	B.Sc. (Honours), M.Sc., Dip. in pharmacy	College gate, Gazipur
69	B.A. (Honours), Dip. in pharmacy	College gate, Gazipur
70	B.A. (Pass), Dip. in pharmacy	College gate, Gazipur
71	B.A. (Pass), M.A.	Board bazar, Gazipur
72	B.A. (Pass), M.A.	Board bazar, Gazipur
73	B.A. (Honours)	Board bazar, Gazipur
74	H.S.C., Dip. in pharmacy	Board bazar, Gazipur
75	B.A. (Pass), Dip. in pharmacy	Board bazar, Gazipur
76	B.A. (Pass), Dip. in pharmacy	Board bazar, Gazipur
77	B.A. (Pass), Dip. in pharmacy	Board bazar, Gazipur
78	B.A. (Pass), M.A.	Board bazar, Gazipur
79	B.A. (Pass), M.A.	Board bazar, Gazipur
80	S.S.C.	Board bazar, Gazipur
81	B.A. (Pass)	Chowrasta, Gazipur
82	B.A. (Honours), M.A.	Chowrasta, Gazipur
83	B.A. (Pass), M.A.	Chowrasta, Gazipur
84	B.Sc. (Pass), M.A.	Chowrasta, Gazipur
85	B.Sc. (Pass), M.Sc.	Chowrasta, Gazipur
86	B.Sc. (Pass), Dip. in pharmacy	Chowrasta, Gazipur
87	H.S.C., Dip. in pharmacy	Chowrasta, Gazipur
88	B.A. (Pass), Dip. in pharmacy	Chowrasta, Gazipur
89	B.Sc. (Honours), M.Sc.	Chowrasta, Gazipur
90	B.Sc. (Pass), M.Sc.	Chowrasta, Gazipur
91	H.S.C.	Gazipur Sadar, Gazipur

92	B.Sc. (Pass), M.Sc. Dip. in pharmacy	Gazipur Sadar, Gazipur
93	B.Sc. (Honours), M.Sc.	Gazipur Sadar, Gazipur
94	B.Sc. (Honours), M.Sc.	Gazipur Sadar, Gazipur
95	B.Sc. (Honours), Dip. in pharmacy	Gazipur Sadar, Gazipur
96	B.A. (Honours), M.A.	Gazipur Sadar, Gazipur
97	B.A. (Pass), M.A.	Gazipur Sadar, Gazipur
98	B.A. (Pass), Dip. in pharmacy	Gazipur Sadar, Gazipur
99	B.Sc. (Honours), Dip. in pharmacy	Gazipur Sadar, Gazipur
100	B.Sc. (Honours), M.Sc., Dip. in pharmacy	Gazipur Sadar, Gazipur

Figure 2.1: Google map showing pharmacies in Ashulia area.

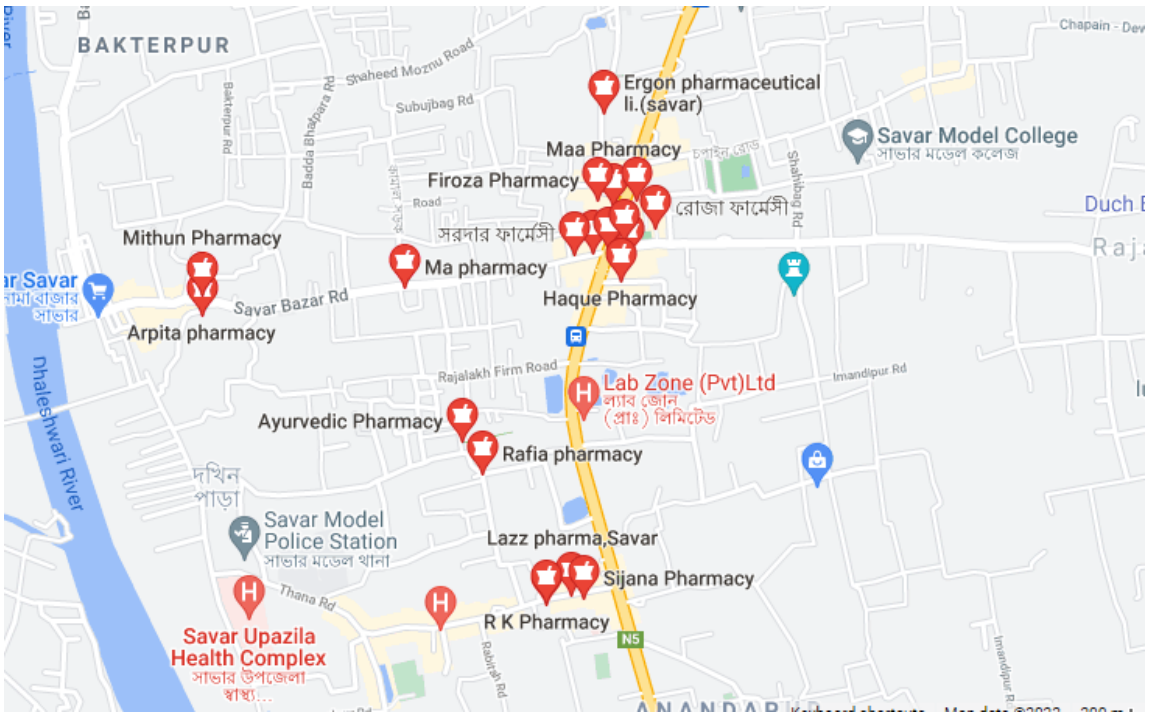


Figure 2.2: Google map showing pharmacies in Savar area, Dhaka.

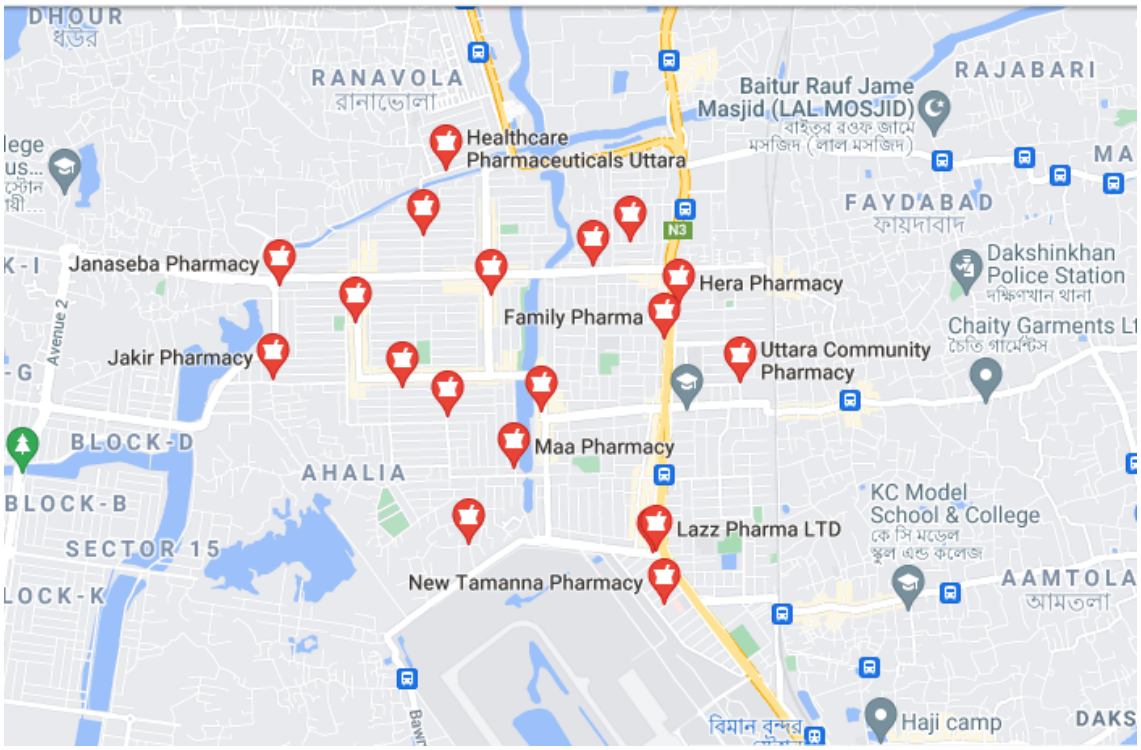


Figure 2.3: Google map showing pharmacies in Uttara area, Dhaka.

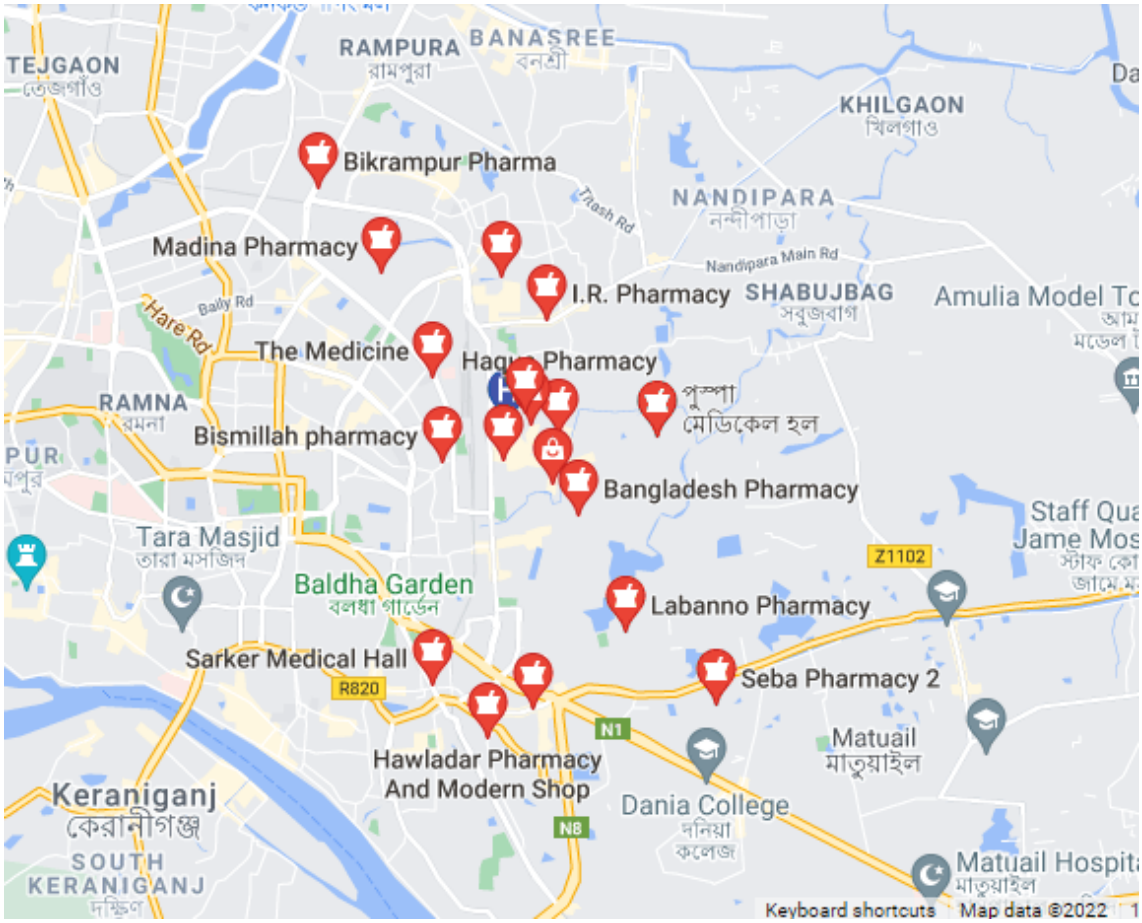


Figure 2.4: Google map showing pharmacies in Mugda area, Dhaka.

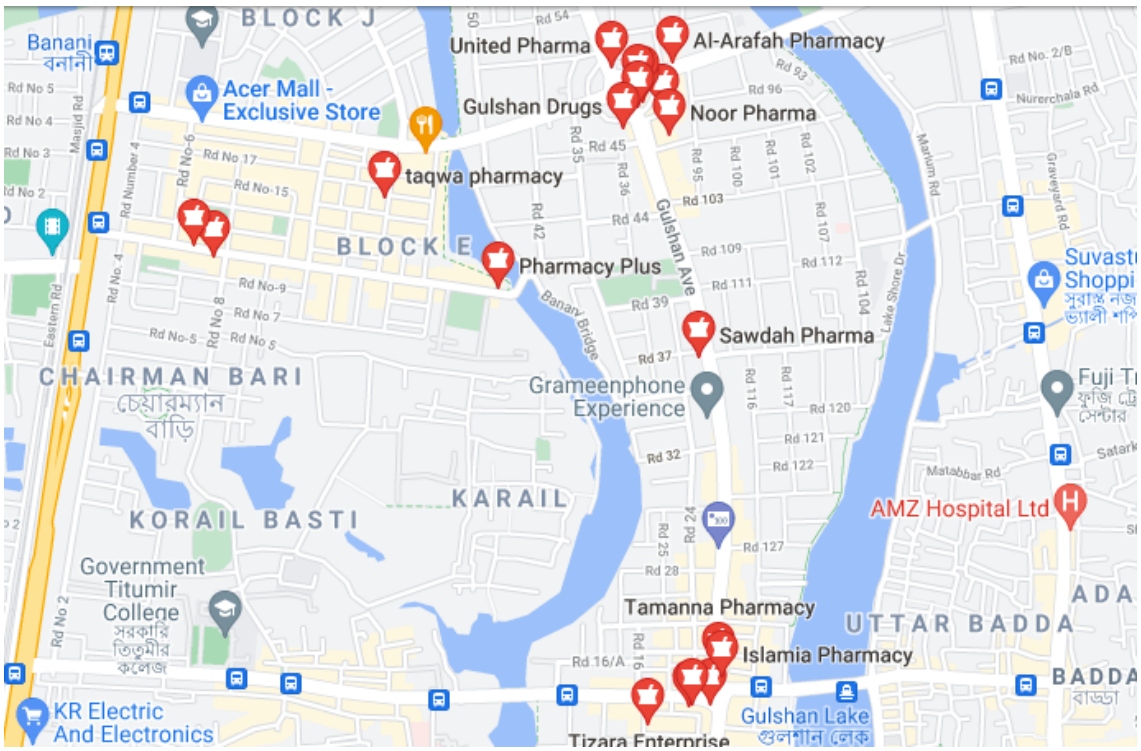


Figure 2.5: Google map showing pharmacies in Gulshan area, Dhaka.

2.2.2 Customer survey in relation to antibiotic purchases

Bangladesh remains one of the poorest healthcare systems in the world. Only 3% of the GDP is spent in healthcare in Bangladesh which about US$37 per capita per annum. Healthcare expenditure is out of pocket (OOP) payments based which bring financials catastrophes to many families and leading to poverty. According to a study 5% of the non-poor family became poor in 2011 due to OOP in healthcare cost (Rahman et al., 2020). In this study, a questionnaire was set for the customer who bought antibiotics from the pharmacy to analyse the uses and abuses of the antibiotics and contribution to the MDR. A set of questionnaires was also developed for pharmacy personnel how they sell antibiotics or control abuse of antibiotics and what were the common antibiotics given for some of the common community infections and why.

2.2.2a Questionnaire for pharmacy personnel

In this set of questionnaires, key questions were set to understand the pharmacy personnel's professional qualifications, trainings and competencies, professional affiliations, professional regulations, and reliabilities. How these people are qualified and capable enough to prescribe or sell antibiotics and how it contributes to the MDR.

2.2.2b Questionnaire for customers buying antibiotics

In this set of questionnaires, key questions were set to understand the customer's antibiotic uses and abuses. How customers buy antibiotics i.e., do they see doctors and get prescriptions for the antibiotics? Are they capable of seeing doctor using OOP expenses? Do they use antibiotics as they are supposed to? Are they capable of buying full course of antibiotics? Socio-economical condition of the customer and their level of educations, living conditions, employment status, genders, age and family incomes and so on. These questions are essential to understand the use and abuse of the antibiotics and contribution the MDR. It will also help this study to conclude and draw a guide for antibiotic control to fight the MDR and tackle the problem that we are facing in this era.

Chapter 3: Infections and microorganisms

3.1 Introduction

Infections are caused by various types of microorganisms such bacteria, virus, fungus, and parasites. These microorganisms can cause mild infection such as common cold to life threating infections such as meningitis, septicaemia and so on. This research mainly focuses on infections caused by bacterial pathogens and uses of antibiotics to treat them.

3.2 Urinary tract infections (UTIs)

Urinary tract infections (UTI) are caused due to the presence and multiplication of microorganisms in one or more anatomic area of the urinary tract system with capability of tissue invasion (Drekonja and Johnson, 2008). Wide variety of clinical syndromes can be manifested in UTIs which typically include acute and chronic pyelonephritis (kidney and renal pelvis), cystitis (bladder), urethritis (urethra), epididymitis (epididymis) and prostatitis (prostate gland). Infection occasionally spread to surrounding tissues (e.g., perinephric abscess) or to the bloodstream and cause septicaemia.

3.2.1 UTIs Incidences

UTIs incidences can be influenced by various factors such as age, sex or by other predisposing factors e.g., impaired immune defence mechanisms 2 . One of the common bacterial infections incur in children is UTI. However, symptom manifestations are often non-specific, and diagnosis can be difficult (Svanborg, 2013). UTIs in children is often associated with renal tract abnormalities and mostly seen in male child in their first three months of life and common infection in preschool boys too, due to congenital abnormalities. Rapid diagnosis of this condition is essential as it might leave a renal scarring and eventually renal function loss. On the other hand, UTIs are more commonly seen in older female children. The vesicoureteric reflux, a condition in which urine may passage from bladder to urethra or up to kidney, might be predisposing children to UTI or may also be results of UTIs (Malcolm G. Coulthard et al., 2014; Malcolm G Coulthard et al., 2014) and can affect 25-40% children according to a study published (Tullus, 2015). Laboratory diagnosis is very important in children UTIs and often difficult in children due the quality of the sample collected, which is often contaminated with faecal organisms. Two subsequent samples yielding same microbial growth increase the

probability of diagnosis of UTIs ("SMI-Investigation of urine," n.d.) . Growth of a single species of $\geq 10^3$ cfu/mL in voided urine may be diagnostic of UTIs. Generally, a pure growth of 10^4 -10^5 cfu/mL in carefully take midstream urine sample is diagnostic of UTI. Negative cultures or growth of $<10^4$/mL from bag urine may be diagnostically useful. Counts of $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) should be confirmed by culture of a more reliable specimen, either a single urethral catheter specimen or, preferably, an SPA. Bacteriuria usually exceeds $\geq 10^8$ cfu/L ($\geq 10^5$ cfu/mL) in SPAs from children with acute UTI, although any growth is potentially significant.

Among adults, UTIs cases are highest in young women and approximately 10–20% of women will experience a symptomatic UTI at some points. Women with acute symptomatic UTI may have as low as 10^2 cfu/mL counts of a single microbial species in voided urine (Kunin et al., 1993). Culture results must be interpreted with care and consider the factors such as age and storage of specimen, level of contamination is determined by presence of number of squamous epithelial cells, and the sensitivity of the method. However, UTIs in adult men are complicated and related to abnormalities of the urinary tract, although a low incidence occurs spontaneously in otherwise healthy young men.

Incidence of UTIs increases with age for both sexes and is one of the most common infections associated with the older age group 33-35. Asymptomatic bacteriuria is common over the age of 80 and the incidence rates are estimated 10% in males and 20% in females (Nicolle, 2009). Underlying health condition greatly contribute to UTIs with the rises of resistance strains of the pathogens and diagnosis can be difficult.

Four percent of pregnant women have shown asymptomatic bacteriuria (persistent colonisation of the urinary tract without urinary symptoms) according to a Pregnancy Studies in the UK (Law and Fiadjoe, 2012) . Delayed diagnosis of this condition in pregnant women may increase the risk of premature birth and pyelonephritis affecting maternal and foetal outcome. especially at the time of delivery, about 30% of patients may suffer from acute pyelonephritis (Krieger, 1986; Pedler and Bint, 1987) and 20–40% of pregnant women would develop pyelonephritis if the condition left untreated (Pedler and Bint, 1987). A routine and sensitive urinary screening programmes are essential for

the early diagnosis bacteriuria in pregnancy. In asymptomatic pregnant women if infection is suspected, then it should be ruled out by repeat culture (MacLean, 2001).
A higher incidence of asymptomatic bacteriuria is seen in women with diabetes than those without (Geerlings, 2008; Zhanel et al., 1991). However in the case of man, there is no difference in the prevalence of bacteriuria with or without diabetes (Zhanel et al., 1991). It is argued as to whether conditions such as glycosuria, age or instrumentation are contributory factors in the high prevalence of UTI, but bladder dysfunction due to diabetic neuropathy may be the major contributory factor (Geerlings, 2008). It remains unclear whether diabetes play any significance role in incidences of symptomatic infection, but, when they do occur, patients with diabetes seems to have more severe UTIs (Aswani et al., 2014; Schneeberger et al., 2014).

Patients with impaired bladder innervation in neuromuscular disorders due to congenital or acquired disorders such as spina bifida, spinal cord injury have higher UTIs risk and rate of mortality is significantly high in this patient's group (Litza and Brill, 2010). This may be because of impaired bladder function that unable a complete emptying of the bladder or require instrumentation of the urinary tract for a complete void of urine (Gołębiewska et al., 2011; Kawecki et al., 2011). In renal transplant patient, UTIs are mostly seen soon after transplantation, mainly due to catheterisation, ureteric drainage tube, or the case of previous UTIs whilst on dialysis. Less frequently, infections are introduced via the donor kidney.

Overall UTIs incidences are not higher within the immunocompromised patients group compared with those who are not except diabetic and renal transplant patients (Korzeniowski, 1991). Some studies have suggested that acquired immunodeficiency syndrome (AIDS) patients may be at higher risk of bacteriuria, and symptomatic UTIs often leading to severe septicaemia and eventually death (De Pinho et al., 1994). Moreover, MDR are the main contributor in UTIs in these patients group due to long-term use of antibiotics for other infections.

Catheterisation is one of the most cause of UTIs . It is difficult to determine true bladder pathogen from the urine culture results from patients with indwelling catheters due to the presence of several microbial species. Hence the culture results should be interpreted

cautiously along with other factors. There are no set established criteria for differentiating asymptomatic the urinary tract colonisation to a symptomatic infection (Stamm, 1991).

3.2.3 Organisms associated with UTIs and types of UTIs

Organisms those implicated in UTIs both complicated and uncomplicated are given below:

3.2.3a Organisms Common in Acute, Uncomplicated UTI's

Acute or uncomplicated UTIs are mainly caused by a single bacterial species.

Escherichia coli is prevalent in faecal flora that remains as the common isolated in uncomplicated UTIs which accounts for 77% of all isolates. Certain virulence factors favour the development of pyelonephritis whilst others favour cystitis or asymptomatic bacteriuria (Kahlmeter and ECO.SENS, 2003). The more virulence factors a strain possesses, the more severe infection it can cause. Virulence factors includes increased adherence to vaginal and uroepithelial cells, resistance to bactericidal activity, haemolysin production and a greater quantity of K antigen (Johnson, 1991).

Proteus mirabilis is common in young boys and males and is associated with renal tract abnormalities (calculi). *Proteus* species may cause chronic infections.

Coagulase negative *Staphylococci* is often considered contaminants, as they are part of the perineal skin flora. However, they may cause complicated infections in patients of either sex.

S. saprophyticus can adheres to uroepithelial cells better than *S. aureus* or other coagulase negative staphylococci which may be seen in uncomplicated UTIs.

Streptococci rarely cause uncomplicated UTI'S, except Lancefield Group B and enterococci.

3.2.3b Organisms Common in Complicated UTIs:

Complicated UTIs which occur in the abnormal or catheterised urinary tract are caused by a variety of organisms, many of them with increased antimicrobial resistance because of the prolonged use of antibiotics.

E. coli remains the most common isolate. Other frequent isolates include *Klebsiella*,

Enterobacter and *Proteus* species, *Enterococcus* species (usually associated with instrumentation and catheterisation) and *Pseudomonas aeruginosa* (associated with structural abnormality or permanent urethral catheterisation). *S. aureus* rarely causes infection and is associated with renal abnormality or as a secondary infection to bacteraemia, surgery, or catheterisation. *Mycobacterium tuberculosis* and other *Mycobacterium* species may infect the urinary tract.

Less common organisms causing infection include *Haemophilus influenzae*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Corynebacterium urealyticum* and anaerobes.

3.2.4 Antibiotics in treatment of UTIs

Antibiotics are chosen to treat the UTIs based on the organisms associated with the infection. Antibiotics susceptibility testing are done with a combination of different classes of antibiotics (details of the antibiotic classes are discussed in the latter chapter). In the UK, antibiotic susceptibilities are performed based European committee on antimicrobial susceptibility testing (EUCAST) guidelines. The common antibiotics susceptibility testing performed in the laboratory for the treatment of UTIs are summarised in the table below

Table 3.2: Common antibiotics tested in the lab for UTIs

Organisms	Antibiotics
Staphylococci species	Ampicillin (only *S. saprophyticus*, inferred from penicillin), gentamycin, penicillin (*S. aureus* and *S. lugdunensis*),trimethoprim, ciprofloxacin, cefoxitin, mupirocin, nitrofurantoin (only *S. saprophyticus*).
Beta haemolytic *Streptococci*	Penicillin, cefuroxime, cefalexin, co-amoxiclav, nitrofurantoin (*S. agalactiae* only), levofloxacin.

Enterococci species	Ampicillin, co-amoxiclav, ciprofloxacin, nitrofurantoin *E. faecalis* only), linezolid, trimethoprim (always reported as resistance).
Coliforms	Ampicillin, co-amoxiclav, cefalexin, ciprofloxacin, nitrofurantoin (*E.* coli only), trimethoprim, amikacin, gentamycin, ceftazidime, piperacillin/tazobactam, meropenem.
Pseudomonas species	amikacin, gentamycin, ceftazidime, piperacillin/tazobactam, meropenem.

3.3 Genital Infections both Sextually Transmitted Infections (STIs) and Non-STIs.

There are several types of vaginal infections other then STIs which typically includes Bacterial Vaginosis, Vaginal thrush, vaginitis and vulvovaginitis. Vaginal infections are diagnosed in this lab by means of microscopy and cultures.

3.3.1 Non-STIs

3.3.1a Bacterial vaginosis (BV)

Bacterial vaginosis (BV) is vaginal condition that can produce vaginal discharge as a result of an overgrowth of predominantly anaerobic organisms (*Gardnerella vaginalis*, *Mobiluncus* species and vaginal anaerobes) in the vagina, leading to replacement of *lactobacilli* and an increase in pH from less than 4.5 to as high as 7.0. BV is not believed to be sexually transmitted. In the past, the condition was called *Gardnerella* vaginitis, after the bacteria that were thought to cause the condition and was originally described by Gardner and Dukes in 1955.

The symptoms of bacterial vaginosis are vaginal discharge and “Fishy” odour. Usually, there are no other symptoms. The amount of vaginal discharge that is considered normal

varies from woman to woman. Therefore, any degree of vaginal discharge that is abnormal for a particular woman should be evaluated.

Presence of mix organisms especially Gram-negative rods are one of the indications of BV although anaerobic organisms are Gram variable. Gram negative organisms that are seen in the BV are curved rod/bacilli. Mixed organisms attached to the epithelial cells are the clue cells for BV.

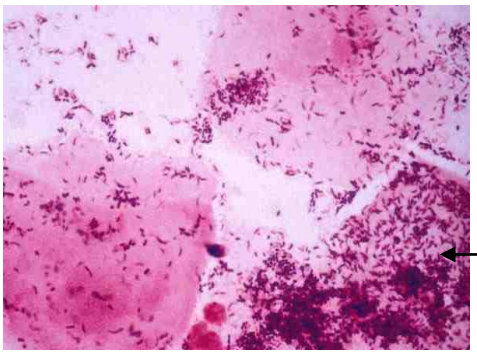


Figure-3.1: Film suggest of Bacterial vaginosis

3.3.1b Vaginitis and vulvovaginitis

Vaginitis is an inflammation of vagina where vulvovaginitis is referred to an inflammation of both vagina and vulva. Vaginitis and vulvovaginitis can be caused by various microorganisms such as *Candida* species, beta haemolytic Streptococci, S. aureus and H. influenza.

Table 3.2: Difference between vaginitis and vulvovaginitis

Vaginitis	Vulvovaginitis
Vaginitis can be caused by *Candida* species and *T. vaginalis*.	Vulvovaginitis can be caused by *Streptococcus, Staphylococcus aureus, C.*

	albicans, Haemophilus influenzae, N. Gonorrhoeae.
Vaginitis can occur at any stage of life. Particularly, in children, infections caused by β-haemolytic streptococci and *S. aureus*.	Vulvovaginitis is mainly seen in children, especially, prepubertal females, but may affect women of any age.
In woman, reduced endogenous oestrogen causes the epithelium to thin, contributing to a reduction in lactic acid production and an increase in vaginal pH. This change causes overgrowth with mixed flora and the disappearance of lactobacilli and lead to vaginitis.	It may be associated with poor hygiene, skin irritation due to soaps, or with streptococcal throat carriage.
Main symptom of vaginitis is vaginal discharge with polymorphonuclear leucocytes and small round basal epithelial cells.	Symptoms include irritation, soreness and discharge.
Atrophic vaginitis is a rare condition usually associated with the elderly.	Other unusual organisms may cause vulvovaginitis including *Salmonella* and *Shigella* species.

3.3.2 STIs

STIs can be caused by bacteria, viruses, and parasites. However, only STIs caused by bacterial pathogens are included in this study.

3.3.2a Gonorrhoea

Neisseria gonorrhoea is the causative organism for gonorrhoea. It is a Gram-negative diplococcus. It is an intracellular and fastidious organism that does not survive for a long outside the host cell. It is highly susceptible to adverse environmental conditions such as drying and extreme temperatures and can be killed during transportation from general physician practices, STIs clinics or hospital to the diagnostic lab. Hence, molecular

diagnosis such as polymerase chain reaction (PCR) or nucleic acid amplification tests (NAATs) is recommended for the definite diagnosis of the infections. When organisms grow on the culture medium, following antibiotics susceptibility is performed in accordance with EUCAST guideline

Table 3.3: Gonorrhoea antibiotics test panel.

Organism	Antibiotics
Neisseria gonorrhoea	Ceftriaxone, Azithromycin, Ciprofloxacin, and tetracycline.

3.3.2b Chancroid

Chancroid is an STI that cause genital ulceration, lymphadenitis with formation of bobu. It is mostly seen in the tropics such as Africa, South America, and Asia, however the incidence of Chancroid was increased dramatically in North America during the late 1980s. It is caused by *Haemophilus ducreyi* which enters in the epithelium via broken skin. Chancroid ulcers are vascular, painful and the granulomatous base bleeds easily.
Ulcer lesions occur mainly on and around the genital area (Lewis and Ison, 2006; Morse, 1989). Asymptomatic carriage appears to be rare. Infection rarely presents as urethritis alone without any genital ulcers 21. The chancroid infection rate is reportedly getting high in many areas, although laboratory diagnosis is poor due to difficulties growing the organisms in the culture medium for its fastidious growth nature and often diagnosis is made on clinical grounds alone and may thus be inaccurate. Chancroid, in common with other sexually transmitted diseases, is assumed to be an important contributory factor in HIV transmission in the tropics (Trees and Morse, 1995). Diagnostic lab routinely performs Gram stain on the materials obtain from genital ulcers. However Gram stain has very less diagnostic value due to the poor sensitivity and specificity (Morse, 1989). Application of immunofluorescence and molecular techniques are of great diagnostic value, but further evaluation is need (Lewis and Ison, 2006).

3.3.2c Genital Chlamydia

Chlamydia trachomatis is an intracellular bacterium that causes genital chlamydia infections among young and other sexually active persons. Multiple sex partners and unprotected sexual intercourses contribute to the infection processes. The infection rate has done up since 2000s. Salpingitis, ectopic pregnancy, infertility and, to a lesser extent, epididymitis may arise from untreated *C. trachomatis* infections. *C. trachomatis* doesn't grow on culture medium and diagnosis is performed by NAATs or PCR as they are reliable and non-invasive procedures are applied for specimen collection such genital swab or urine (Bébéar and de Barbeyrac, 2009).

3.3.2d Syphilis

Syphilis is a sexually transmitted infections caused by *Treponema pallidum* and the clinical manifestations are often subtle and varied. Based on clinical manifestation, syphilis can be divided into different stages such as primary, secondary, latent, and tertiary stages. A vertical transmission of syphilis can occur from mother to the foetus resulting in congenital syphilis, and occasional transmission of the disease by blood transfusion as a non-sexual contact. Diagnosis is mainly by dark field microscopy in early syphilis and by serological tests (LaFond and Lukehart, 2006).

3.4 Gastrointestinal Infections

Gastro-intestinal infections often refer to diarrhoea, which is characterised by frequent and watery bowel movement. A wide range of pathogens e.g., bacteria, viruses and parasites can cause infections of the gastrointestinal tract. Some of these pathogens are found in both humans and animals, while others are strictly human pathogens. Main source of infection is contaminated food, drinks and hands and the transmission is faecal oral route.

Many bacteria can cause GI tract infection. Different bacteria have a different mode of action that contributes to GI tract infections. Following are the important bacterial pathogens that cause GI tract infections.

3.4.1 *Salmonella* species

There are more than 2500 strains have been identified on the basis of differences in cell wall (O) and flagellar (H) antigens. There are two main categories of *Salmonella* infections:

Non-typhoid *Salmonella* is one of the common bacteria that cause gastrointestinal infection and the commonest forms of food poisoning worldwide. Although there are over 2,500 different types of salmonella but they all produce a similar clinical picture to other forms of infective gastroenteritis. There are numerous serotypes of salmonella exist and serogroups A to E are the ones that usually cause disease in humans. Serogroups B, C, and D are responsible for most infections. *S. enteritidis* is serogroup D and is the most common cause of salmonella gastroenteritis. Other important species is S. typhimurium. Salmonella species are commonly found in many animals of domestic, agriculture and wild origin. Contamination occurs from animal faeces and human infection usually associated with consumption of food of animal origin, drinking contaminated water or person to person contact. Usually, the peaks of incidence of Salmonellosis are in summer and autumn and may also be associated with foreign travel. Symptoms include watery diarrhoea, stomach cramps and sometimes vomiting and fever. It is particularly likely to cause severe illness in the very young and very old.

3.4.2 *Shigella* species

Shigella species are highly infectious organisms that cause a gastrointestinal infection known as shigellosis or bacillary dysentery. Only a small amount is required to cause infection and infective dose of Shigellosis is as low as 10-100 Shigella organisms. Shigella species present with range of symptoms and the disease is characterized by a short period of watery diarrhoea with intestinal cramps and general malaise, soon followed by permanent emission of bloody, mucoid, often mucopurulent stools. Outbreaks of dysentery due to S. dysenteriae type 1 are frequent in poor populations living in crowded settings where hygiene is poor and sanitation non-existent. Acute complications may occur that include peritonitis and septicaemia, especially in malnourished children, and the severe Haemolytic Uremic Syndrome (HUS) with renal failure due to the production of Shiga toxin, which is similar to the verotoxin, produced by *E.coli* 0157.

Mode of transmission is primarily person to person by the faecal-oral route. There are four *Shigella* species: *S. sonnei, S. dysenteriae, S. flexneri* and *S. boydii*. All four species can cause dysentery but three major species of Shigella are responsible for bacillary dysentery: *S. sonnei, S. flexneri and S. dysenteriae*. A fourth species, *S boydii*, is responsible for scattered disease foci. These species are further subdivided into subtypes on the basis of the antigen specificity of the O-polysaccharide portion of their LPS. *S. flexneri* is the most common isolate worldwide (60%) whereas *S. sonnei* is predominant is developed country accounts for 77% of cases compared with developing country that accounts 15% of cases. Enterotoxin is produced by *Shigella*, but the role is unclear as toxin-negative mutants also cause disease.

3.4.3 *Campylobacter species*

Campylobacter species is the most common cause of GI infection in the UK which is characterised by severe diarrhoea and abdominal pain. Mainly two species Campylobacter accounts for the majority of infections: *C. jejuni* and *C. coli* with a seasonal peak from May to September. C. jejuni is the most common type responsible for 90% of infection, although we do not distinguish between species in the laboratory.

Campylobacter is a microaerophilic and able to grow on an anaerobic condition. It is a slow growing organism requires up to 48 hours growing onto a culture medium. Presumptive colonies are confirmed by testing their reaction with oxidase, which should be positive, and by performing a gram stain as they have a specific small Gram-negative S-shaped appearance.

3.4.4 *E. coli*

Most strains of *E. coli* form part of the normal intestinal microbial flora in humans and warm-blooded animals. However, some strains have the ability to cause disease in humans through the presence of specific virulence factors. Currently, there are four recognized classes of enterovirulent *E. coli.* Verotoxin-producing *Escherichia coli* (*E.coli* 0157) is a mutant form which lives in the intestines of some cattle, sheep and goats but is not naturally found in the intestines of human. It can be passed from eating infected food and drink. E. coli symptoms can range from mild diarrhoea to abdominal cramps and

blood in the stools. Some patients also suffer from a complication called haemolytic uremic syndrome (HUS), which kills red blood cells and can cause kidney failure. Currently this laboratory routinely looks for E. coli 0157 strain only.

3.4.5 *Vibrio cholerae*

Cholera is life threatening diarrheal disease characterised by a severe watery stool caused by toxigenic *Vibrio cholerae* that colonises the small intestine and produces an enterotoxin called cholera toxin (CT). Cholera is endemic in the tropical and sub-tropical countries and mostly in southern Asia and parts of Africa and Latin America. Seasonal outbreaks are widely seen, and poverty and poor sanitation are the key contributory factors in the outbreaks. Two important properties of *V. cholerae* are taken into consideration to assess the public health significance which includes the production of CT, which is responsible for the severe diarrhoea, and the possession of the O1 or O139 antigen, which acts as a marker of epidemic potential. However, molecular analysis has revealed that in addition to genes encoding CT, all strains capable of causing cholera invariably carry genes for a colonisation factor known as toxin-coregulated pilus (TCP) and a regulatory protein, ToxR, which coregulates the expression of CT and TCP (Faruque et al., 1998).

3.4.6 Other bacterial diarrhoea

Aeromonas species and *Yersinia* species are bacterial pathogens less commonly seen in the UK. Selective agar medium is used for the isolation of these bacteria if clinically suspected due to exposure of the environment where bacteria are prevalence.

3.4.7 Bacterial toxin associated diarrhoea

GI tract infection can cause by wide range of bacterial, but this laboratory does not check all of them routinely. Because these bacteria not commonly associated with GI infection and main associated with particular situation or in an outbreak. If clinical information suggests any out breaks or a situation which might be associated with GI tract infection then we will look for those organisms.

3.4.7a *Bacillus cereus*

B. cereus is a large Gram-positive spore forming bacillus which causes food poisoning by the ingestion of toxin rather than actual bacillus spores. Spores are found on many

foods, especially rice, pulses and vegetables. Two clinical syndromes may be associated with food poisoning.

- ✓ The symptoms of B. cereus diarrhoeal type food poisoning can mimic those of C. perfringens food poisoning. The onset of watery diarrhoea, abdominal cramps and pain occurs 6-15 hours after consumption of contaminated food due to heat labile enterotoxin.
- ✓ The emetic type of food poisoning is characterised by nausea and vomiting within 0.5 to 6 hours after consumption of contaminated foods which mimic those of S. Aureus food poisoning. Duration of the symptom is generally less than 24 hours.

3.4.7b *Staphylococcus aureus*

S. aureus is Gram positive cocci which form the part of human normal flora. Some strains are capable of producing highly heat stable toxin that causes human infection including the food poisoning. Staphylococcal food poisoning is usually rapid (0.5 to 6 hours) and in many cases acute, depending on individual susceptibility to the toxin, the amount of toxin being eaten, or the amount of contaminated food being consumed. The most common symptoms are nausea, vomiting, abdominal cramp and prostration. Some individuals may not be presented with all the symptoms associated with the illness. As *S. aureus* forms part of normal gut flora, to conclude infection, large numbers must be seen in the stool culture. Isolated *S. aureus* should be tested for enterotoxin production and epidemiologic studies performed by a reference laboratory.

3.4.7c *Clostridium perfringens*

Some strains of C. perfringens (typically type A2) are associated with a mild food poisoning. The main symptoms are watery diarrhoea with severe abdominal pain and the incubation period is 8-24 hours. In order to confirm C. perfringens food poisoning, the same serotype must be isolated from the faeces of the infected person and from the food or enterotoxin must be detected in the faeces of the infected individual.

3.4.7d *Clostridium botulinum*

Clostridium botulinum produces a deadly neurotoxin known as Botulinum toxin (abbreviated either as BTX or BoNT) and causes a clinical condition called botulism. The

clinical syndrome of botulism can occur following ingestion of contaminated food, from colonization of the infant gastrointestinal tract, or from a wound infection.

BoNT is broken into 7 neurotoxins (labelled as types A, B, C [C1, C2], D, E, F, and G), which are antigenically and serologically distinct but structurally similar. Human botulism is caused mainly by types A, B, E, and (rarely) F. Types C and D cause toxicity only in animals.

3.4.8 Antibiotic associated diarrhoea

Clostridium difficile is the leading cause of infectious diarrhoea in hospital worldwide, because of its virulence, spore forming ability and persistence. *C. difficile* associated diseases are induced by antibiotic treatment or disruption of normal gastrointestinal flora. Recently morbidity and mortality resulting from C. diff associated disease have increased significantly due to change in virulence of causative strain and antibiotic use pattern. It is the causative agent of pseudomembranous colitis. Although a small percentage of healthy adults are colonised with *C. difficile* many patients acquire this organism through nosocomial infection (Abt et al., 2016).

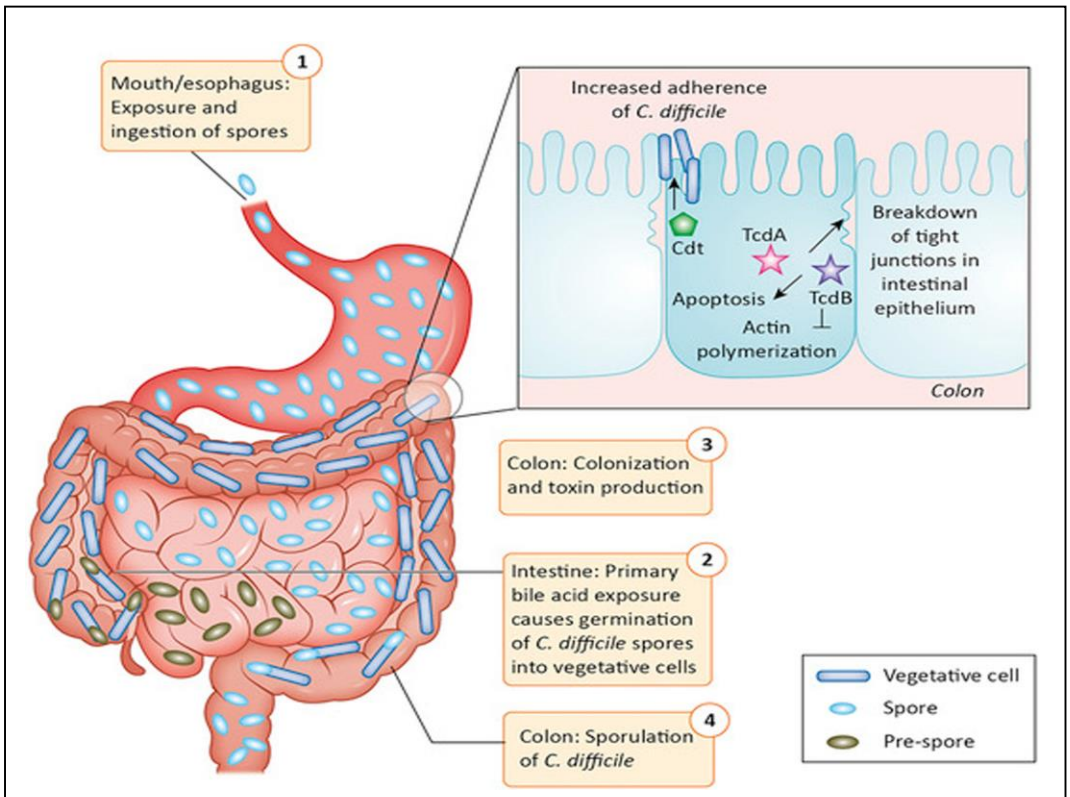


Figure 3.2: Figure shows mechanisms of *C. difficile* toxin related diarrhoea.

The production of two toxins A (enterotoxin) and B (cytotoxin) cause characteristic mucosal damage. The spectrum of disease ranges from a self-limiting mild diarrhoea to severe characteristic pseudomembranous colitis as shown in the figure above (Sandhu and McBride, 2018).

Culture for *C. difficile* is not a reliable method of diagnosis of *C. difficile* associated disease (CDAD) or diarrhoea, as some strains may be non-toxigenic. Non-toxigenic strains are generally assumed to be avirulent. Demonstration of toxins of *C. difficile* in diarrhoeal stools is generally regarded as suggestive of CDAD in the absence of any other cause of the gastrointestinal upset.

Enzyme Immunoassay (EIA) kit is used for the detection of C. difficile toxins A and B. Breakaway microwells are coated with toxin-specific monoclonal and polyclonal antibodies. The toxins bind to the antibodies in the wells and with the addition of a substrate is recognized as positive by a colour change reaction.

3.4.9 Antibiotic susceptibility testing for GI tract infections

The UK standard microbiology investigation (SMI) recommends following antibiotics testing.

Table 3.4: Antibiotics tested in the lab for GI tract infections.

Organisms	First line of antibiotics	Second line of antibiotics
Shigella species	Ciprofloxacin Azithromycin Amoxicillin Co-trimoxazole	Ceftriaxone Ceftazidime Meropenem Pefloxacin
Salmonella species	Ciprofloxacin Azithromycin Amoxicillin Co-trimoxazole	Ceftriaxone Ceftazidime Meropenem Pefloxacin

Campylobacter species	Ciprofloxacin Erythromycin	Tetracycline Gentamicin

3.5 Mucosa and soft tissue infections

Mucosa and soft tissue infections (MSTI) can involve any anatomical part of the body. Depending on the anatomical sites and infection types, laboratory investigation and uses of antibiotics can be varied. Examples some common MSTIs (Dryden, 2010) are given below in the table

Table 3.5: Mucosa and soft tissue infections list

Infection	Description	Pathogens
Cellulitis	Spreading infection of the skin involving deep layers and subcutaneous tissues	*S. pyogenes* *S. aureus*
Erysipelas	Spreading superficial infection of the skin involving the upper dermis and superficial lymphatic system	*S. pyogenes* *S. aureus*
Impetigo	Superficial infection producing erythematous lesions	βHS: A, C, G *S. aureus*
Paronychia	Superficial infection of the nail fold	*S. pyogenes* *S. aureus* Yeasts Anaerobes
Folliculitis	Infection and inflammation of a hair follicle	*S. aureus* *P. aeruginosa* Candida
Carbuncles & furuncles	Deep and extensive abscesses involving hair follicles and sebaceous glands.	*S. aureus*

Table 3.6: Necrotising skin and soft tissue infections.

Infection	Description	Pathogens
Meleney's gangrene	Barrowing lesion or chronic gangrene usually following abdominal operations	*S. aureus*, *Streptococci*, *enterococci*, Enterobacteriales, anaerobic GNB
Gas gangrene	Toxaemia and gas present in the tissues, usually after traumatic accident.	*C. perfringens* and *Clostridium* species.
Fournier's gangrene	Applies to non-sporing anaerobes. Important cause of infection in pelvic and scrotal areas. Also common in diabetic limbs.	Enterobacteriales, *Streptococci* and *Clostridium* species
Spontaneous gangrene	No apparent trauma or mild or non-penetrating trauma, commonly seen in carcinoma, leukaemia, or neutropenia.	*C. perfringens* and *Clostridium* species.
Actinomycosis	Suppurative infections characterised by abscess formation and sulphur granules production.	*Actinomyces* species
Necrotising fasciitis	Serious infection affecting subcutaneous fat, superficial fascia of muscles and overlaying soft tissues	*S. pyogenes*.

Table 3.7: Other skin infections and expected pathogens.

Infection	Pathogens
Abscess in IVDU	Oral Streptococci, *S. anginosus* group, *S. aureus*, Anaerobes, *B. anthracis*
Animal bites	*P. multocida*, *P. canis*, *S. aureus*, AHS, *S. anginosus* group. Rare causes: Anaerobes, *Capnocytophaga*, *Ekenella corrodens*, *Haemophilus*, CNS, *Streptobacillus moniliformis*, *S. intermedius.*
Burns	*S. aureus*, BHS, *Pseudomonas*, *Acinetobacter*, *Bacillus*, Enterobacteriales, Filamentous fungi, Candida, CNS
Contact with animal	Fisherman, butcher, and abattoir workers: *Erysipelothrix rhusiopathoae.* Water related wounds: *Aeromonas species*, *Vibrio species*, *Edwardsiella tarda*. Agricultural workers: *B. anthracis*.

Table 3.8: Eye infections

Infection	Description	Pathogens	
		Any age	Additional for neonate:
Conjunctivitis	Acute or chronic infection of the conjunctiva causing red and sticky eyes.	*S. aureus* *S. pneumoniae* *H. influenzae*	*N. gonorrhoeae*, *H. parainfluenzae*, BHS group B&D, Enterobacteriales, *P. aeruginosa*.
Keratitis	Infection of the cornea may be caused by bacteria, fungi or parasites.	Staphylococci Streptococci Pseudomonas Enterobacteriaceae Corynebacterium	*Serratia* *Haemophilus* *N. gonorrhoeae* *Aspergillus* Candida & Moulds

		Moraxella	*Propionibacterium*
Endophthalmitis	Infection of intraocular fluids and tissue	*S. aureus* Streptococci CNS	*P. acnes* Yeast & Moulds *P. aeruginosa*
Orbital cellulitis	Infection of the orbital tissue caused by trauma, surgery, or paranasal sinus infections.	*S. aureus* Streptococci Anaerobes	*H. influenzae* *P. aeruginosa*
Blepharitis	Blepharitis	*S. aureus* *S. epidermidis* *Streptococcus sp.*	*Moraxella sp* *Corynebacterium* *P. acnes*

Table 3.9: Ear infections

Infection	Description	Pathogens
Otitis externa	Infection of the external auditory canal. Can be subdivided in to acute localised, acute diffuse, chronic and invasive ('malignant'). 'Swimmer's ear'.	*S. aureus* *S. pyogenes* *P. aeruginosa* Anaerobes Enterobacteriaceae Fungi
Malignant otitis externa	Severe necrotising infection that spreads from the epithelium of the canal in to surrounding tissues, cartilage and bone.	*P. aeruginosa*
Acute otitis media	Defined by the co-existence of fluid in the middle ear and signs and symptoms of acute illness.	*S. pneumoniae* *H. influenzae* *M. catarrhalis*

		Less common causes: *S, pyogenes* *S. aureus* Gram negative bacilli
Chronic suppurative otitis media	Very destructive infections which can result in hearing loss	*Pseudomonas* species. MRSA Anaerobes

Table 3.10: Sinusitis

Infection	Description	Pathogens
Community sinusitis	Community acquired sinusitis may be bacterial, viral, mixed.	*S. pneumoniae* *H. influenzae* *S. anginosus group* *M. catarrhalis* *S, pyogenes* *S. aureus* Anaerobes
Nosocomial sinusitis	Often a complication of endotracheal intubation and mechanical ventilation.	*S. aureus* *P. aeruginosa* Coliforms
Chronic sinusitis	May be pre- or post-surgical	*S. pneumoniae* *H. influenzae* *S. anginosus group* *M. catarrhalis* *Pseudomonas* *S, pyogenes* *S. aureus* Anaerobes
Fungal sinusitis	Usually due to filamentous fungi, may be locally invasive in immunocompromised patients.	*Aspergillus* species *Rhizopus* species *Mucor* species

3.5.1 Wounds/abscesses

Wounds and abscesses can be formed any part of the body. Depending on the wounds and abscesses and site of the body swabs are taken from are inoculated onto different kinds of agar medium which support the growth various types of microorganisms. Depending on the composition of the agar medium it may support the growth of one group of organisms and suppress the growth of others. These agar compositions sometimes contain specific nutrient type or antibiotic to support the growth of wanted microorganisms and suppress the unwanted one. Blood agar (BA), MacConkey agar for coliforms, CNA agar for Staphylococci and Streptococci isolation, ARIA, or FHB for the isolation of anaerobic microorganisms. Followings are the most common pathogens isolated on routine investigation from the wounds and abscesses swabs-

3.5.1a *Staphylococcus aureus*

Staphylococcus aureus is an aerobic, facultatively anaerobic, Gram-positive spherical organism, 0.7 to 1.2 μm in diameter and pairs or grape like clusters formed as a result of incomplete separation of daughter cells after the cell division in three perpendicular planes. It is the most common organism isolated from the wound swabs. It is a non-fastidious organism can grow with minimum requirement. It is commonly colonised human skin and mucosa without causing any harm. It can also cause disease, particularly if there is an opportunity for the bacteria to enter the body, for example through broken skin or a medical procedure and can cause mild to a life-threatening problem.

Panton-Valentine Leukocidin (PVL) is a toxin that destroys white blood cells and is a virulence factor in some strains of *Staphylococcus aureus*. PVL has been associated with virulent, transmissible strains of *S. aureus* including MRSA and predominantly causes skin and soft tissue infections.

On CNA agar or chocolate agar *Staphylococcus aureus* colonies are circular, 2 - 3 mm in diameter with a smooth, shiny surface, and are frequently pigmented such as golden-yellow, fawn or cream. *Staphylococcus aureus* are coagulase positive, catalase positive and DNASE positive. Differentiation of *Staphylococcus* species is based initially on coagulase test.

3.5.1b Beta haemolytic Streptococcus – *Streptococcus pyogenes*

Streptococci are a large and diverse group of gram-positive organisms that grows in pairs or chains. Three types of haemolysis reaction are seen after growth of streptococci on sheep blood agar (alpha, beta and gamma). Alpha refers to partial haemolysis with a green coloration (from production of an unidentified product of haemoglobin) seen around the colonies; beta refers to complete clearing and gamma means there is no lysis. It was not until 1930 the role of *Streptococcus pyogenes* was clearly understood its association with infection. Lancefield in the 1930s showed that most pathogenic streptococci possess specific carbohydrate antigens which can be used to classify streptococci into groups.
Streptococcus pyogenes is one of the common isolates from soft tissue and mucosal infection. *Streptococcus pyogenes* is commonly known as Group-A *Streptococcus*. Group-A streptococcal organisms are referred to as β-haemolytic. Following a 24-hour incubation period, these facultative aerobic organisms, when grown in blood enriched media (especially in anaerobic conditions), tend to show large zones of clear β-haemolysis around colonies. They are approximately 0.5 mm in diameter, translucent and domed. It's then tested using a streptococcal grouping kit to identify which Lancefield group the organism belongs to.

Figure 3.3: Commercial grouping kits for Streptococcal Lancefield groupings.

3.5.1c *Streptococcus milleri*

Streptococcus milleri group are part of the normal flora of human mucous membrane of the oral cavity, oral pharynx, gastrointestinal tract, and genitourinary tract. Although they are commensal organisms, they can become pathogenic and lead to an infection to the surrounding or distant sites after mucosal disruption caused by trauma. Streptococcus milleri group is formed of three distinct species of streptococci namely *Streptococcus intermedius, Streptococcus constellatus,* and *Streptococcus anginosus*. S. milleri group organisms are associated with localised abscess formation most notably in the liver and brain.

3.5.1d Anaerobes

Anaerobes are very often responsible for deep wound infections and abscesses. They are often difficult to grow on culture medium as they can be slow growing and need very specific requirements or prolonged incubations. So, when clinical details or wounds type suggest the possible anaerobic infection, anaerobic culture should be prolonged. Robertson's cook meat and brain heart infusion broth can be used for enrichment and subcultured from the enrichment after 2days of incubation onto standard agar plate as per SOPs. Robertson's cook meat broth is very useful as it contains unsaturated fatty acids which take up oxygen and reaction is catalysed by haematin in meat and thus helps to enrich the growth of anaerobes. Two most common anaerobes that isolated on the routine cultures are C. Perfringens and Bacteroides species.

3.5.2 Tissue infections

The most common pathogens those are associated with tissue infections are Staphylococcus aureus, Beta haemolytic Streptococci and anaerobes. Depending on the infection type various types of pathogenic microorganism may be associated with the tissue infections e.g., *Clostridium perfringens* in gas gangrene. Other organisms may also be associated in tissue infections are such as Enterobacteriales, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Haemophilus* species, *Actinomycosis, Mycobacterium* species, *Nocardia* species, *Streptomyces* species and various fungi and so on.

3.5.3 Orthopaedic infections

To isolate the microorganisms those are associated with tissue infections require a special preparation for culture. Tissue sample is taken into the safety cabinet for the preparation where tissue sample is cut into a require piece and grind it using the Griffiths tube with an addition of approximately 0.5 ml. nutrient broth. This will homogenise the tissue sample. Orthopaedic samples are submitted for microbiological investigation in the case of osteomyelitis i.e., inflammation bone due to an infection. Orthopaedic samples are treated differently to most other tissue samples due to several reasons. This is a progressive infective process involving the various components of bone and may be acute or chronic condition. Osteomyelitis can be haematogenous origin or by direct inoculation from a contiguous site or device associated. Organisms those involve in orthopaedic samples vary due to age and mode of infection. Classically haematogenous osteomyelitis is seen in childhood, but it can occur at any age especially when there are risk factors such as a recent intravascular device, haemodialysis, intravenous drug usage or recurrent infections elsewhere (such as urinary tract infections). Beta haemolytic group B Streptococci, *Staphylococcus aureus* and *Escherichia coli* are mainly involved in neonatal haematogenous osteomyelitis whereas between the ages of one and sixteen, *S. aureus*, and *Haemophilus influenzae* type B predominate. Diagnosis can be made by culturing an infective bone biopsy in appropriate culture media and laboratory techniques. Blood culture may also aid to the diagnosis.

3.5.4 Antibiotics tested in laboratory for MSTI

Any significant organisms those isolated in the lab will be tested for their susceptibility to the antibiotics and resistance pattern will be determined. In the UK most of laboratory uses EUCAST guidelines for their susceptibility, however, CLSI guidelines are also used for certain rare microorganisms and are mostly used in the reference laboratory services. Some common organisms and their susceptibility testing are given in the table below

Table 3.11: Organisms vs antibiotics

Organisms	Antibiotics
Staphylococcus	Penicillin, gentamicin, co-trimoxazole, erythromycin, fusidic acid, tetracycline, linezolid, rifampicin, cefoxitin (screening for MRSA), mupirocin, trimethoprim, ciprofloxacin.
Enterobacteriales	Ampicillin, co-amoxiclav, cefuroxime, cephalexin, ciprofloxacin, co-trimoxazole, piperacillin/tazobactam, gentamicin, amikacin, meropenem, ceftazidime, cefpodoxime,
Pseudomonas species	piperacillin/tazobactam, gentamicin, amikacin, meropenem, ceftazidime, gentamicin, ciprofloxacin, ceftolozane-tazobactam.
Beta haemolytic Streptococcus species	Penicillin, vancomycin, tetracycline, erythromycin, linezolid, levofloxacin, clindamycin.
Anaerobes	Penicillin, metronidazole, clindamycin, vancomycin

3.6 Respiratory tract infections

Respiratory tract infections are mainly divided as upper respiratory tract infections e.g., pharyngitis, tonsilitis and lower respiratory tract infections e.g., pneumonia.

Table 3.12: Upper respiratory tract infections and their causative bacterial and fungal agents

Infection	Description	Pathogens
Oral mucositis	A painful complication of chemotherapy or head and neck radiotherapy.	Yeasts Oral bacteria

Erythematous and pseudomembranous candidiasis	The most frequent presentations or oral fungal infection	C. albicans Other Candida species.
Angular chelitis	Infections of the angles of the mouth and lips.	S. aureus Candida species S. pyogenes
Pharyngitis	Inflammation of pharynx (sore throat)	S. pyogenes BHS group C/G Acronobacteria haemolytic
Diphtheria	Acute infectious disease of URT and occasionally the skin	Toxigenic C diphtheriae, C. ulcerans C. pseudotuberculosis
Vincent's angina	Characterised by ulceration of the pharynx and gums and occurs in adults with poor mouth hygiene or systemic disease.	Borrelia vincentii Fusobacterium species
Epiglottitis	Inflammation of the epiglottis, commonly affecting children.	H. influenza
Tonsillitis	Inflammation of tonsil, can be due to viral or bacterial infection	S. *pyogenes* S. *dysgalatiae* *Fusobacterium* sp. Anaerobes
Laryngitis	Inflammation of the larynx usually caused by viruses.	*C. diphtheriae,* *S. pyogenes* *S. pneumoniae* *H. influenzae*

Table 3.13: Lower respiratory tract infections

Infections	Description	Pathogens
Tuberculosis	Infection of lungs usually caused by *M. tuberculosis*.	*M. tuberculosis*
Pneumonia	Inflammation of air sacs in one or both lungs.	*K. pneumoniae*
Bronchiectasis	A chronic lung condition, where lungs airways become widen and causes mucus build up and make prone to infections.	*H. influenzae* *P. aeruginosa* *M. catarrhalis* *S. aureus*

Table 3.14: Common microbes in respiratory infections and antibiotic test profiles

Organisms	Antibiotics
Streptococcus pneumoniae	Levofloxacin, vancomycin, tetracycline, erythromycin, oxacillin.
Haemophilus species	Penicillin, ampicillin, co-amoxiclav, tetracycline, ceftriaxone.
Moraxella catarrhalis	ampicillin, co-amoxiclav, tetracycline, cefuroxime, erythromycin (reported as clarithromycin), ciprofloxacin

4 Different Antibiotic classes in clinical use and their mode of actions.

4.1 Introduction

Antibiotics are the chemical derivatives or substances that inhibits the growth of bacteria or kill the bacteria. These chemical compounds are produces by different microorganism or can be synthesised in the laboratory. Different classes of antibiotics are current in use and classed them based on their chemical compositions. Although different classes of antibiotic might have different chemical structure, their mode of actions can have similar mode of actions as others or completely different as shown in table 4.1.

Table 4.1: Mode of action vs antibiotic classes.

Mode of action	Antibiotic classes
Cell Wall Synthesis	Penicillins, Cephalosporins, Vancomycin, Beta-lactamase inhibitors, Carbapenems, Aztreonam, Polymyxin, Bacitracin
Protein Synthesis Inhibitors	Inhibit 30s Subunit:- Aminoglycosides (gentamicin). Inhibit 50s Subunit:- Macrolides, Chloramphenicol, Clindamycin, Linezolid, Streptogramins.
DNA Synthesis Inhibitors	Fluoroquinolones, Metronidazole
RNA synthesis Inhibitors	Rifampicin
Mycolic Acid synthesis inhibitors	Isoniazid
Folic Acid synthesis inhibitors	Sulphonamides, Trimethoprim

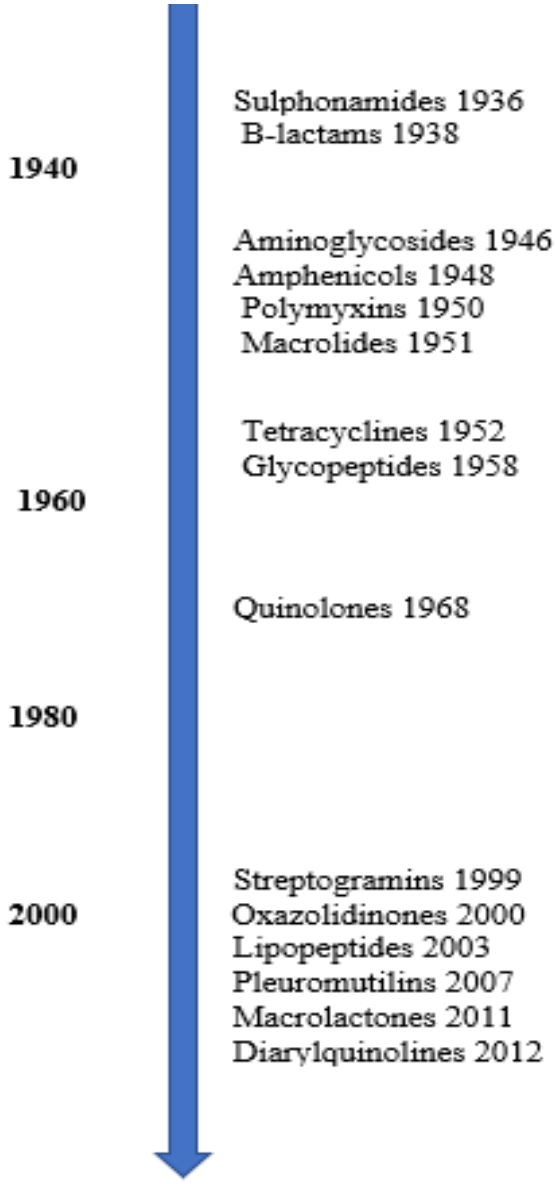


Figure 4.1: Timeline of the antibiotic's classes for their first clinical approval.

4.2 Penicillins

Penicillins are one of the primary B-lactam antibiotics which has diverse chemical structures but unique to one specific Penicillins antibiotic containing B-lactam ring (shown in the figure below). Important members of this antibiotics are Penicillin G, Penicillin V, ampicillin, ampicillin-sulbactam, amoxicillin, amoxicillin and clavulanic acid, oxacillin, piperacillin, methicillin, flucloxacillin, mecillinam (pivmecillinam). First member of this class was penicillin G which was discovered by Fleming in 1928 and came into clinical use in 1938 (Fair and Tor, 2014).

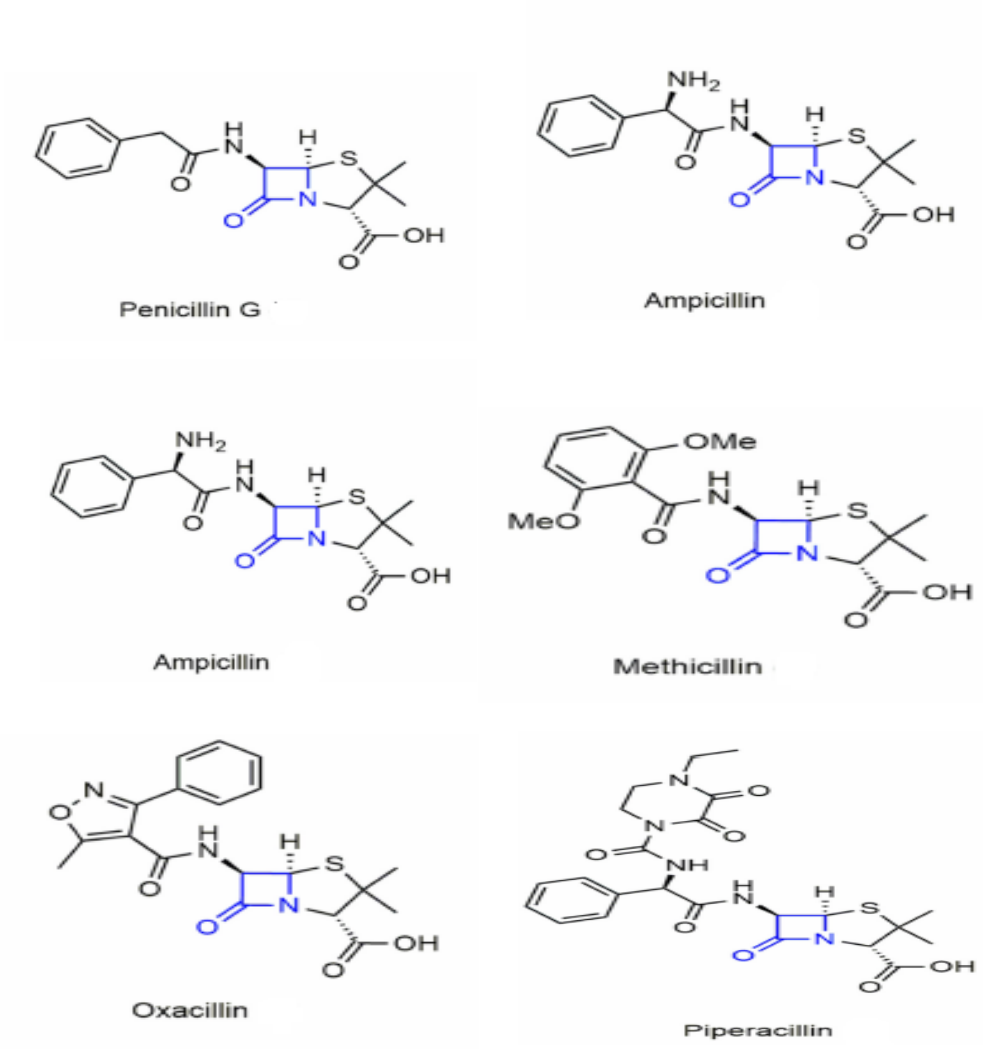


Figure 4.2: Different Penicillins antibiotics and their chemical structures.

4.3 Cephalosporins

Cephalosporins are a greatly significant classes of B-lactam antibiotic discovered from a filamentous fungus *Acremonium chrysogenum*, Cephalosporins have clear advantages compared to first-discovered B-lactam antibiotic, penicillin, as they are more resistant to penicillinase enzyme and most effective against several penicillin-resistant strains. Moreover, reports are less on incidence of antagonistic effects of cephalosporins compared to penicillin antibiotics and other anti-bacterial agents. Therefore, they exist as the most widely used anti-bacterial drugs in clinical practice (Das et al., 2019). Cephalosporin C (CPC) metabolite of *A. chrysogenum* is the major resource for 7-amino cephalosporanic acid (7-ACA) production which is considered as an important precursor/intermediate that can be used in the industrial production of many first line cephalosporins antibiotics and was discovered in 1948.

First and second generations of cephalosporins mainly sought-after improving pharmacokinetics of the antibiotics and targeting broad spectrum activities against gram-negative pathogens mostly focusing the increased cellular penetration. Third and later generations have focused mainly on combating β-lactam resistance and fifth generation cephalosporin have shown excellent safety profiles and increased spectrum of activity which made cephalosporins as the most demanding and therapeutic choice of first line antibiotics (Fair and Tor, 2014).

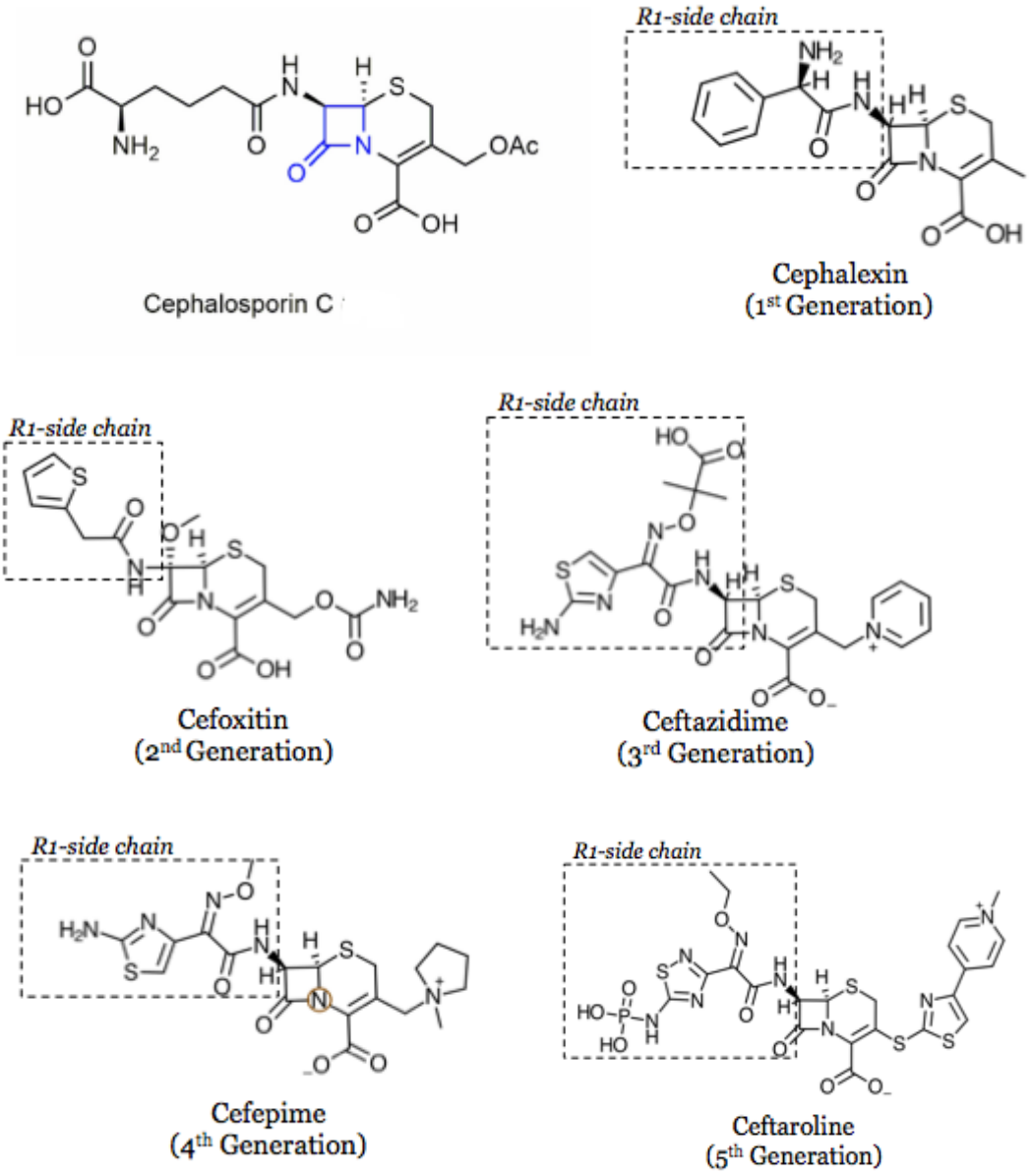


Figure 4.3: Primary structures of Cephalosporin C and example different generation of cephalosporins antibiotic's structures.

4.4 Carbapenems

Carbapenems are another class of beta lactam antibiotics. The first naturally occurring β-lactam antibiotic containing the carbapenem ring system was reported in 1976. Thienamycin was the first generation of carbapenems antibiotic that was produced by *Streptomyces cattleya* and other *Streptomycetes* as well as by unicellular bacteria such as *Erwinia* and *Serratia*. Thienamycins, epithienamycins, and olivanic acid derivatives are various naturally occurring products those forms part of the carbapenem group.

Carbapenems are the most prescribed antibiotics in the treatment of MDR and nosocomial infections. Carbapenems have showed the efficacy against various Gram-positive and Gram-negative bacteria, such as K. pneumoniae, *Haemophilus* spp., *P. aeruginosa*, *Staphylococcus aureus* and so on.

Imipenem was the first of a new class the carbapenems antibiotic released for clinical use. Subsequently, ertapenem and meropenem were developed and licensed for the clinical uses. Doripenem is the latest carbapenem produced and marketed.

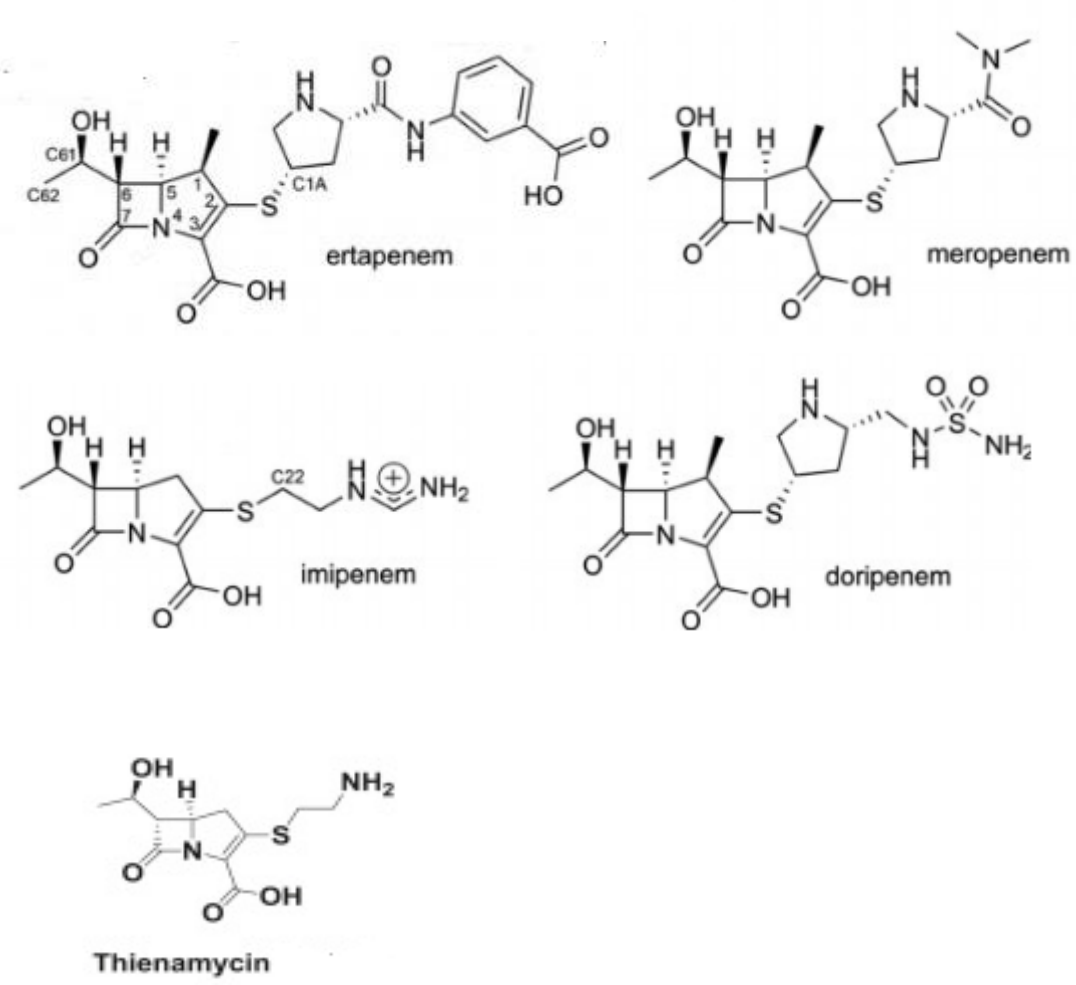


Figure 4.4: Different carbapenems structure with core b-lactam and carbapenems side chain.

4.5 Fluoroquinolones

Bicyclic ring is the basis of chemical structure of the potent antimicrobial agents' fluoroquinolones. Position C-6 containing fluorine atom and various functional groups on the basic quinolone structure made the fluoroquinolones antibiotics group. Nalidixic acid, cinoxacin and oxolinic acid were the first quinolone agents and each had basic bicyclic quinolone ring. These quinolones agent showed narrow-spectrum activity which limited their use in clinical practice. Addition of functional groups on certain part of quinolone ring increased potency of agents namely, cyclopropyl or difluorophenyl in position C1, a fluorine in position C6 and a halogen, metoxy or fused third ring in position C8. Having a piperazin in position C7 in quinolones made the agent more effective on Gram-negatives and made the capable of targeting topoizomerase IV. Agents targeting both gyrase and topoisomerase IV result have broad-spectrum effect (Kocsis et al., 2016).

The addition of fluorine and other functional groups on the basic quinolone structure made the fluoroquinolones such as ofloxacin, ciprofloxacin, norfloxacin, pefloxacin, levofloxacin, moxifloxacin, and several other agents. Structural changes in the fluoroquinolone's agents enhanced their tissue penetration and help them to achieve therapeutic concentrations in kidney, lung and intestine. These structural changes improved pharmacokinetic parameters and helped to achieve broad spectrum activities and showed bactericidal effect against numerous pathogens including Gram-positives, Gram-negatives, aerobes and anaerobes and their antibacterial effect is considered to be concentration-dependent.

Ciprofloxacin is the most widely used fluoroquinolone agent with a potency against Gram-negatives. Levofloxacin (stereoisomer of ofloxacin) has bactericidal effect against Gram-negative and Gram-positive pathogens. Moxifloxacin is characterized by antibacterial effect mainly against Gram-positives including anaerobes, although they lack potency against Gram-negative anaerobes (e.g.: *Bacteroides* sp.) (Kocsis et al., 2016).

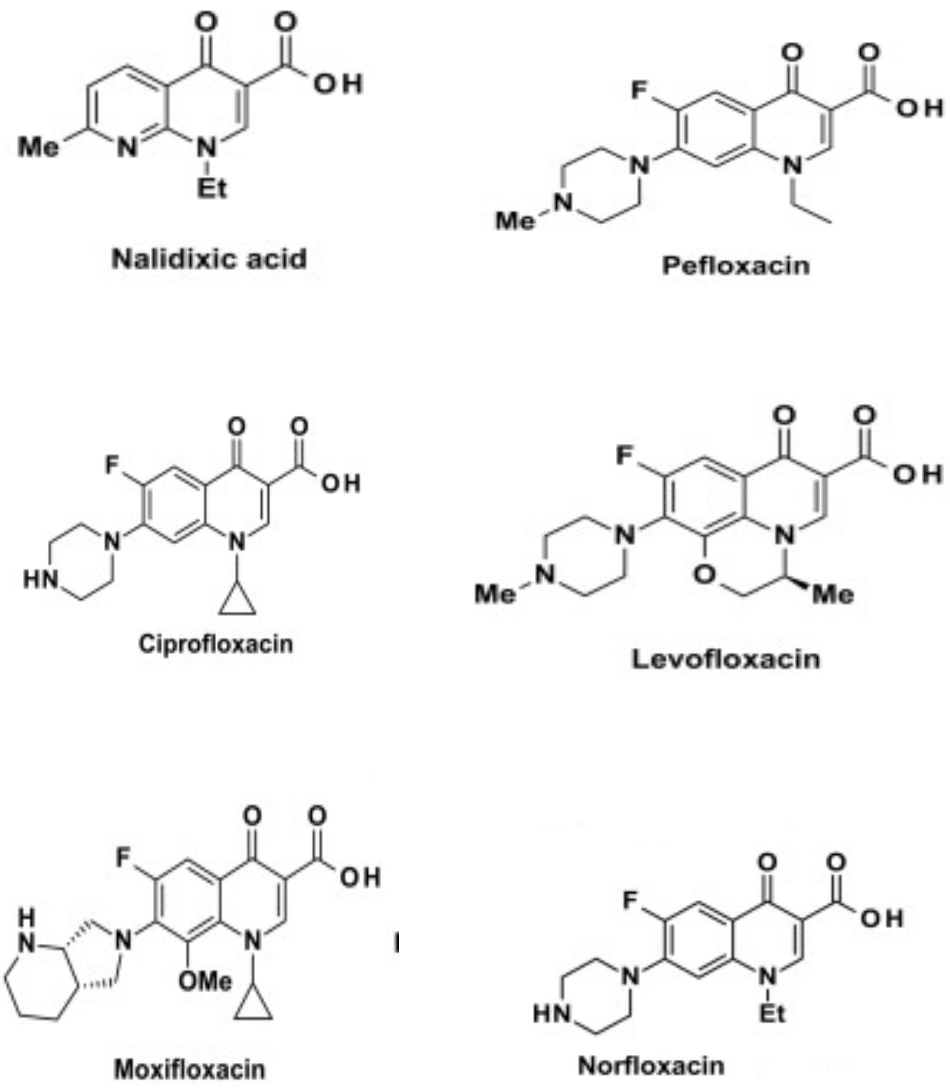


Figure 4.5: Chemical structure of different fluoroquinolones antibiotics

4.6 Aminoglycosides

Aminoglycosides structures a basis of a hexose ring via glycosidic linkages various amino sugars are attached to them. based on the aminocylitol nucleus aminoglycosides can be classified into two main structural classes streptidine (streptomycin) and deoxystrepatamine (gentamicin, tobramycin, amikacin, kanamycin, neomycin, and plazomicin). All aminoglycosides exhibit concentration-dependent bactericidal activity through inhibition of protein synthesis despite of their structural differences. The structural difference seems to play an important role in escaping the bacterial resistance mechanisms, especially by offering structural robustness against metabolizing enzymes, such as Aminoglycoside Modifying Enzymes (AMEs), and target-modifying 16S rRNA methyl transferases (16S-RMTases), produced by the bacteria. Amikacin and plazomicin have been shown to have increased stability against AMEs compared to gentamicin. All currently marketed aminoglycosides are affected by 16S-RMTases, rendering them inactive against the organisms producing the enzyme (Childs-Kean et al., 2019).

Antimicrobial activities of aminoglycoside were first discovered in the 1940s and originally isolated from *Actinomycetes* metabolites. Streptomycin was the first aminoglycoside isolated from *Streptomyces griseus* and introduced into clinical uses for the tuberculosis treatment. Selman Waksman won the Nobel Prize for his discovery of streptomycin along with Albert Schatz in 1952 and Selman Waksman was recognised as the one for the first to coin the term “antibiotic”. Several aminoglycosides had been discovered as metabolites from the *Streptomyces* group (“mycin” aminoglycosides, e.g., neomycin, kanamycin, tobramycin) or *Micromonospora* group (“micin” aminoglycosides, e.g., gentamicin, sisomicin) species, or developed through chemical modifications using existing aminoglycoside scaffolds (e.g., amikacin, netilmicin, arbekacin, plazomicin). Plazomicin is an aminoglycoside that was engineered to overcome aminoglycoside-modifying enzymes (AMEs), the most common aminoglycoside resistance mechanism in *Enterobacteriaceae*, and is the first aminoglycoside to be approved by the FDA (June 2018) since the approval of amikacin in 1981, marking the beginning of a class rejuvenation (Serio et al., 2018).

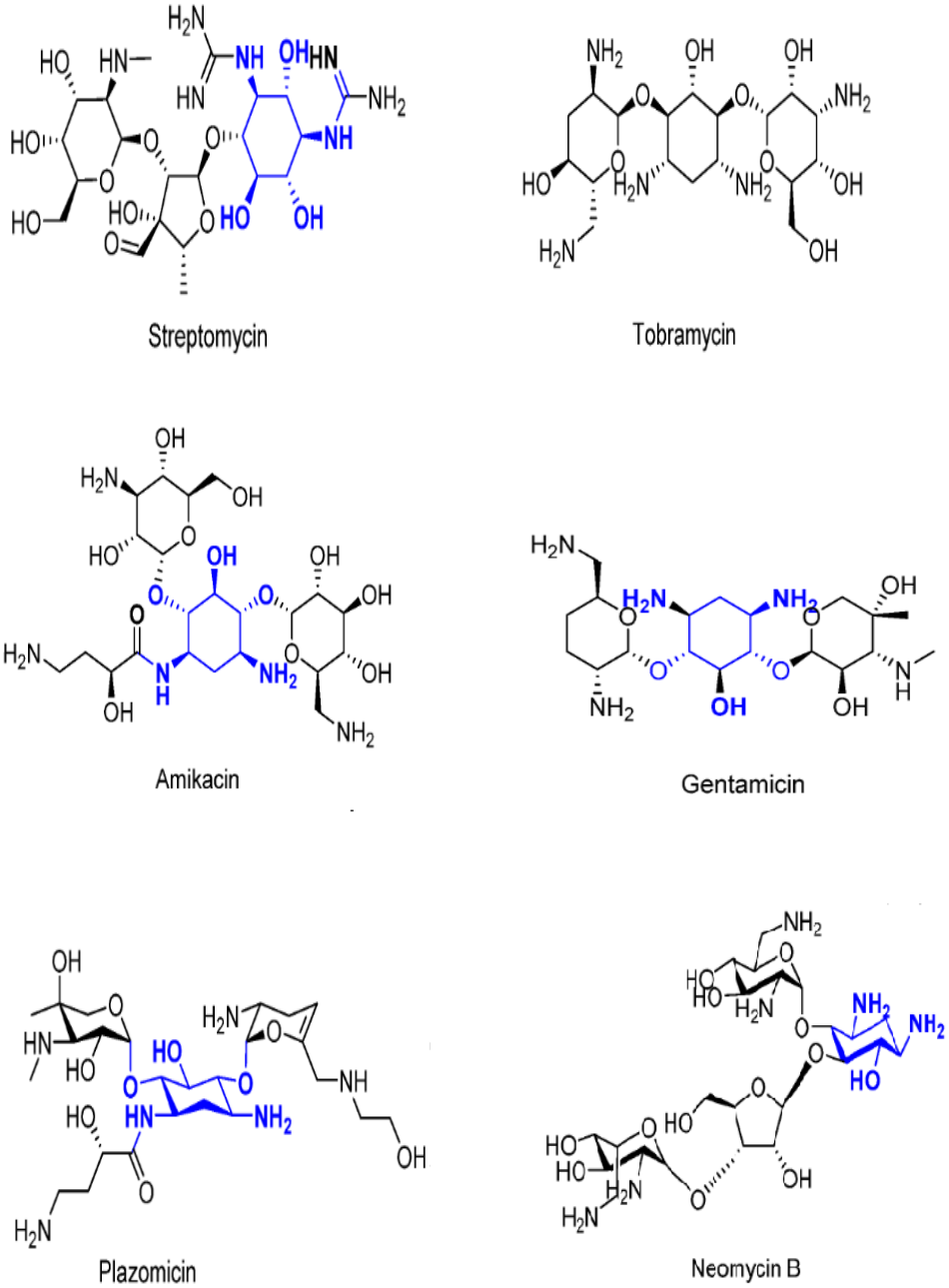


Figure 4.6: Different aminoglycosides structures and their functional groups.

4.7 Glycopeptides and lipoglycopeptides

Glycopeptide antibiotics are actinomycete-derived antibiotics with unique tricyclic or tetracyclic heptapeptide cores that are usually glycosylated and sometimes have additional lipophilic fatty acid side chains (Figure 4.7). Vancomycin was the first glycopeptide class member of the of antibiotics discovered in the 1953 by Eli Lilly & Co (Indianapolis, USA) from an actinomycete, *Streptomyces orientalis* (now called *Amycolatopsis orientalis*) was isolated from the soil sample sent by missionary in Borneo, Indonesia. Two more *Streptomyces orientalis* strains from soils samples from India were identified over next few years that also produced vancomycin in optimised fermentation and isolation conditions. It has a vital role in the treatment of infections caused by Gram-positive pathogens, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and the gut anaerobe *Clostridium difficile* till to date (Butler et al., 2014).

According to the substituents and the type of residues at positions 1 and 3 of the heptapeptide, Lancini in 1989 had divided glycopeptide antibiotics into four distinct structural subclasses (I–IV) and latter, it was summarised by Nicolaou *et al.* in 1999 (Nicolaou et al., 1999). glycopeptide containing valine-1 and asparagine-3/glutamine-3 residues in their structures are classified as Type I, for example vancomycin, whereas type II is an example of β-avoparcin glycopeptide that have aromatic residues at positions 1 and 3 that are not linked. Type III glycopeptides have the position 1 and 3 aromatic residues linked via an aryl ether, whereas Type IV glycopeptides such as teicoplanin A_2-2 (**2**) are a subclass of Type III with an additional fatty acid component attached to an amino sugar (Butler et al., 2014). Type V class of glycopeptide aglycones such as complestatin containing a tryptophan in place of a phenyl group in the heptapeptide core had been designated by Nicolaou *et al.* and analogues with an oxindole-alanine in place of the tryptophan such as neoprotectin A and B have also been reported (Butler et al., 2014).

Teicoplanin was first reported in 1978 by Lepitit Research Centre from *Actinoplanes teichomyceticus*, which was derived from an Indian soil sample, and it is a ristocetin-type lipoglycopeptide complex exemplified by its major component teicoplanin A_2-2. NMR and other mass spectrometry had elucidated the complex components of teicoplanin

structure in 1984 and revealed that the teicoplanin A_2 complex differed from vancomycin by additional glycosylation, an acyl chain and ether linked 4-hydroxyphenylglycine and 3,5-dihydroxyphenylglycine groups. Initially Teicoplanin (Targocid) was first approved in Europe for its clinical uses in 1988 and were available in many other countries in the world (Butler et al., 2014).

Dalbavancin is considered as a second-generation of glycopeptide antibiotic along with other newer members, oritavancin (formerly LY-333328) and telavancin (formerly TD-6424) of the glycopeptide class. Like first generation of glycopeptides, for example, vancomycin or teicoplanin, dalbavancin inhibits bacterial cell wall biosynthesis by formation of a complex with the C-terminal D-alanyl-D-alanine of growing peptidoglycan chains. Moreover, dalbavancin has the unique capability to dimerise and anchor its lipophilic side chain in the bacterial membranes and therefore increase the potentiality of dalbavancin for its target and achieve better antimicrobial activities. However, *in vitro* lab results showed more potent bactericidal activity of dalbavancin than vancomycin or teicoplanin against many resistant Gram-positive organisms including MRSA.

Dalbavancin was developed by Vicuron Pharmaceuticals Inc., (Fremont, CA, USA) dalbavancin was chemically developed from a naturally occurring teicoplanin-like glycopeptide produced by the actinomycete *Nonomuria* spp. from parent compound A-40926, Modifications of the parent compound included derivatization of functional groups such as the C-terminus and N-terminus of the peptide, removal of sugars and the addition of acyl moieties (Chen et al., 2007).

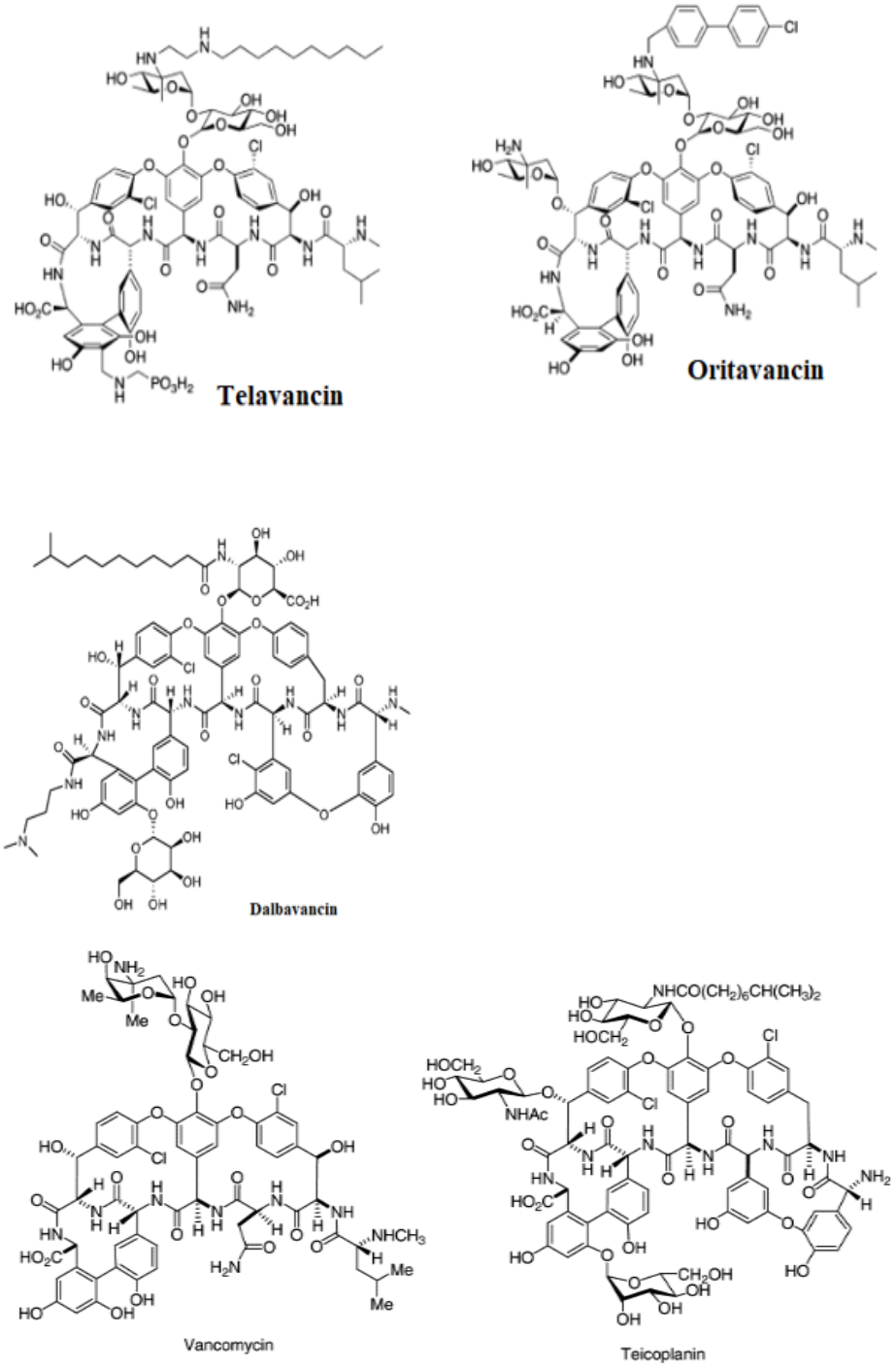


Figure 4.7: Structures of glycopeptide (vancomycin) and lipoglycopeptide (Teicoplanin) and other newer members of this classes.

4.8 Macrolides, lincosamides and streptogramins

As part of the Eli Lilly and Co's an intense screening program to find new antibiotics, erythromycin was discovered in 1949 from the bacteria *Saccharopolyspora erythreus* was from a soil sample collected from the Philippines. Macrocyclic lactone is core of the macrolide antibiotics that contains a large ring to which one or more deoxysugar can be attached to form different member of this class. Macrolide antibiotics has a characteristic 14-membered, 15-membered or 16 membered lactone rings. Macrolide rings viability in acid enrolment is unstable and at gastric pH erythromycin convert into anhydro-erythromycin and thus lose antibacterial activity and can cause nausea and vomiting. To overcome the acid instability, semi-synthetic second generation of macrolide antibiotics such as roxithromycin, clarithromycin and azithromycin were developed and were acid stable. Although erythromycin is known as first macrolide antibiotic, in fact pikromycin, a 14-membered macrolide without a cladinose sugar, was the first macrolide antibiotic identified but due to low activity, it was not developed, and erythromycin became the core for new macrolide development (Fernandes et al., 2017).

Erythromycin and other macrolides are widely used as therapeutic agents to treat respiratory, genital and skin infections caused by Staphylococcus aureus, Beta-haemolytic Streptococci and other Gram positive and Gram-negative organisms including STI such as gonorrhoea. The macrolide class properties have several advantages for example, oral bioavailability, high concentrations in tissues and fluids typically in the lung and pulmonary epithelial lining fluid, intracellular concentration with antimicrobial capabilities, limited antimicrobial spectrum that does not disrupt gut anaerobic Gram-negative microflora that plays a significant role gut health and mostly safe and well-tolerated. The macrolides possess strong anti-inflammatory properties that help inflammatory pain relief due to cytokines released at the infection site (Fernandes et al., 2017).

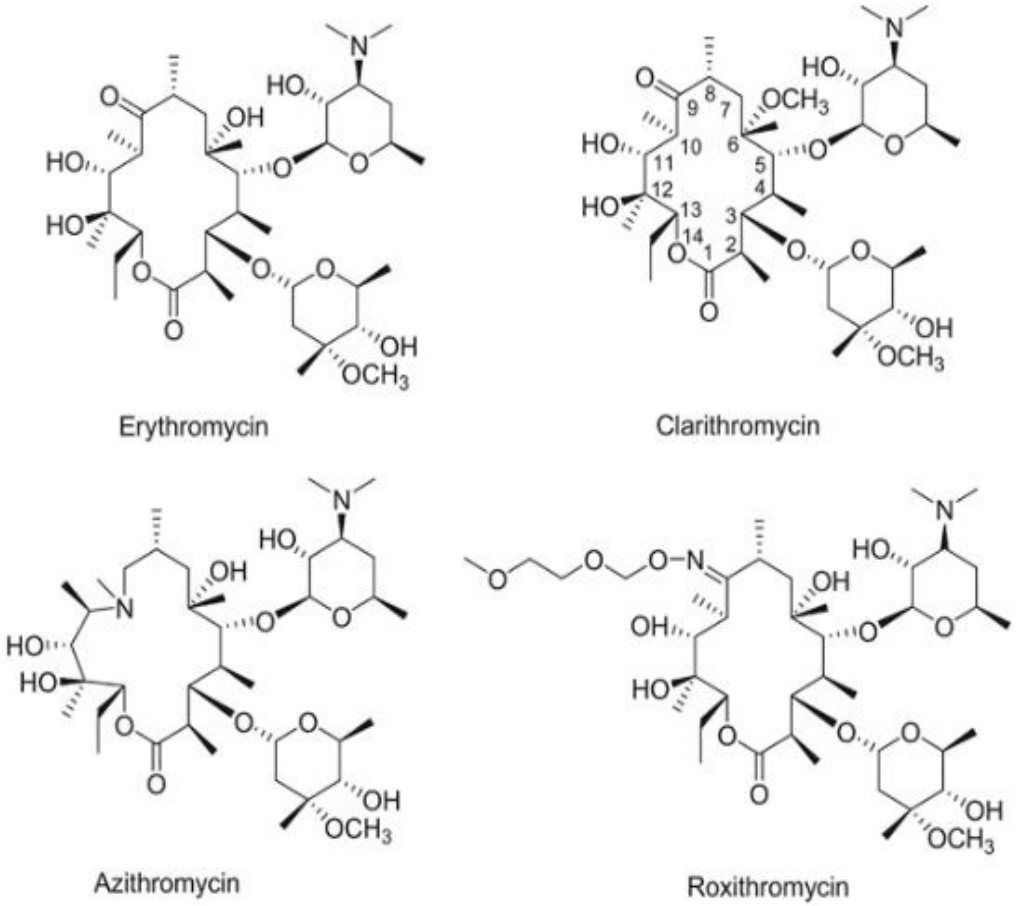


Figure 4.8: Structure macrolide antibiotics.

Lincomycin is a secondary metabolite from produce by soil saprophytic bacteria, *Streptomyces lincolnensis*. Clindamycin is chemical modified form of lincomycin by synthetic methods containing a chlorine atom at the C-7 position with 7(*S*)-configuration. Clindamycin showed better antimicrobial activities than the mother compound lincomycin. However, like lincomycin, clindamycin is also not effective against resistant pathogens those have *erm* gene present in them.

Both lincomycin and clindamycin inhibit protein synthesis in bacteria similar to the macrolide antibiotics. X-ray crystallographic can reveal that lincomycin and macrolides

has closely located binding site and numbers of major interactions by hydrogen bonding in 23S rRNA. Owing to that, lincomycin are effective against pathogens containing *mef* gene in clinical isolate. In conclusion, clindamycin showed several positive characters such as, it is available for both p.o. and i.v. administrations and possible to change therapeutic route, it has good tissue and cells distribution, it can suppress toxin production in Streptococcal strains, and it is reasonably cost effective. Hence, lincomycin analogues will be more clinically desirable compared to macrolide antibiotics, in the case of their effectiveness against pathogens with *erm* gene (Wakiyama et al., 2017).

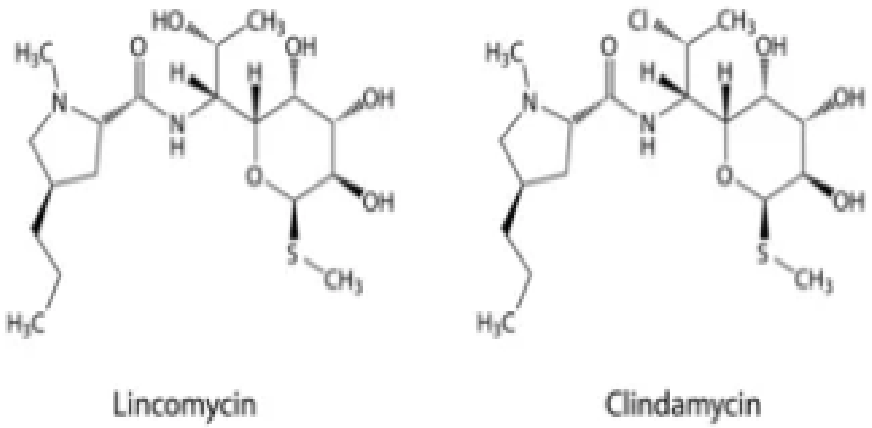


Figure 4.9: Chemical structure of Lincosamides antibiotics

Streptogramins are cyclic peptide antibiotic with a similar antimicrobial effect as macrolides and lincosamides. Synercid® (quinupristin and dalfopristin) is the first member of this antibiotic class. Streptogramins was isolated as the secondary metabolites from *Streptomyces* species mainly Streptomyces . Dalfopristin and quinupristin are type of streptogramins A and B respectively and both has a biostatic mechanism by binding to the 50s subunit of bacterial chromosome and inhibit the protein synthesis. However, when Dalfopristin and quinupristin are combined, they can be bactericidal, and the highest

bactericidal effects are seen in a ratio 70:30 of dalfopristin and quinupristin. Chemically they are cyclic peptide antibiotics where group A streptogramin is a peptide that has 23 membered unsaturated rings with peptide and lactone bond and group B streptogramin is a depsipeptides. This antibiotic is mainly used to treat resistant Gram-positive bacteria such as MRSA, VRE and resistant *Streptococcus pneumoniae* ("Streptogramin - an overview | ScienceDirect Topics," n.d.)

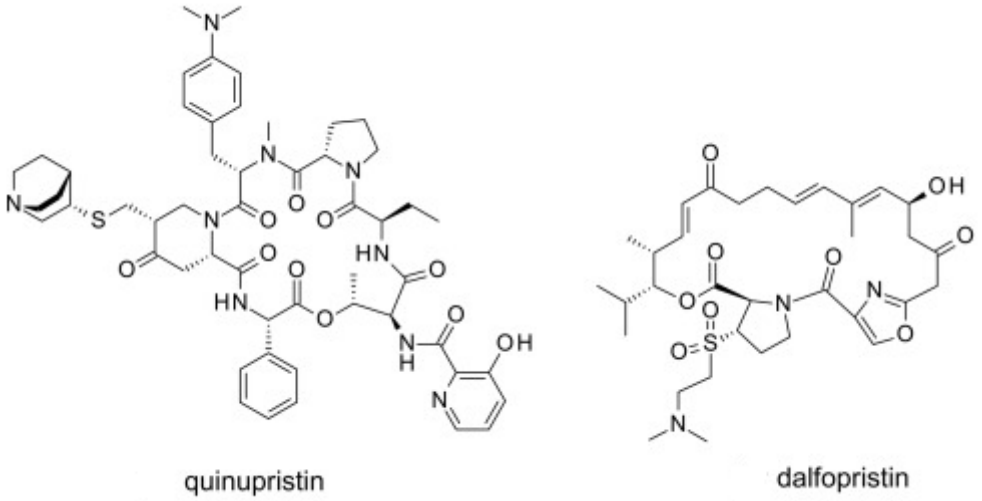


Figure 4.10: Chemical structures of Streptogramins antibiotics.

4.9 Tetracyclines

Tetracyclines were first broad-spectrum antimicrobial agents which showed activities against wide range of pathogens and diseases such as cholera (*Vibrio cholerae*), typhoid fever (*Salmonella enterica* subsp. *enterica* ser. Typhi), syphilis (*Treponema pallidum*), Legionnaire's disease (*Legionella pneumophila*), and anthrax (*Bacillus anthracis*). Some members of this drug class are also used in the treatment of malaria (*Plasmodium* parasites), Lyme disease (*Borrelia burgdorferi, Borrelia afzelii, Borrelia garinii*), tuberculosis (*Mycobacterium tuberculosis*), query fever (*Coxiella burnetii*), Rocky Mountain spotted fever (*Rickettsia rickettsii*), and leprosy (*Mycobacterium leprae*) (Ramachanderan and Schaefer, 2021). Use of the tetracyclines were discovered from thousand years old mummy's bones from Nubia which is believed that early people consumed tetracyclines accidently or on purpose to treat different diseases as these antibiotics can easily be formed in bread dough or beer fermentation (Ramachanderan and Schaefer, 2021).

First member of the tetracyclines antibiotics discovered were chlortetracycline and oxytetracycline which were isolated from *Streptomyces aureofaciens* and *S. rimosus* respectively in 1940s. Latter members of this group of antibiotics were discovered from other natural sources or developed semi synthetically in the lab. Minocycline, doxycycline and tigecycline are the other important member of the tetracycline group of antibiotics. Although early tetracyclines were effective enough, yet scientists sought to improve them for better water solubility and pharmacokinetics. the vinylogous acid at A ring and a keto–enol system at BC rings plays an important structural role in the pharmacology and bioavailability of the tetracyclines antibiotics (Chopra and Roberts, 2001).

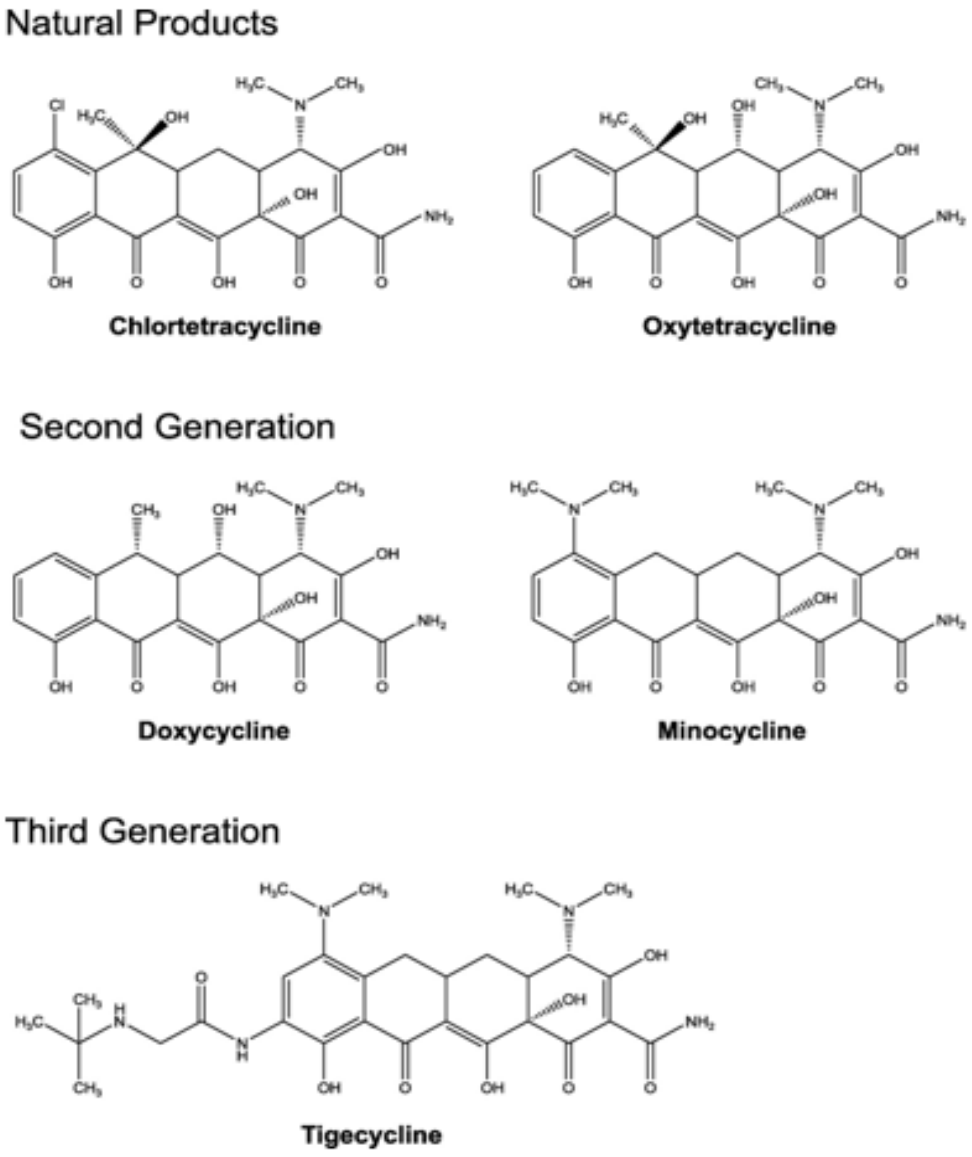


Figure 4.11: Chemical structures of important members of tetracyclines antibiotics.

4.10 Oxazolidinones

Oxazolidinones are the newest class of synthetic antibiotics with an unique chemical structures and linezolid is the first antibiotic member of this class approved for the clinical uses against MDR Gram positive organisms such as MRSA, VRE, and penicillin resistant Streptococcus pneumoniae. Linezolid was first approved in USA by FDA in the year 2000, chemically it is 3-(fluorophenil)-2-oxazolidine which possesses a morphin-lyl group substitution in its core structures (Marchese and Schito, 2001). Although macrolides, lincosamides and chloramphenicol also bind to 50S, linezolid showed unique binding mechanisms in 50S when compared with them.

Linezolid can be taken orally or intravenously, and bioavailability is almost 100% with very few side effects. Linezolid inhibits the early steps of protein synthesis by binding to 50S ribosomal subunits and prevent translation of protein by distorting site for formyl methionyl RNA ($tRNA^{fMet}$) and inhibiting ternary initiation complex formation. Linezolid penetrates well in lung, skin, and other tissue types and work perfectly even in the case of hepatic or renal dysfunctions without any dose's adjustments. Serum half-life of linezolid antibiotic is around 5 hours, hence administration of antibiotic twice a day is sufficient.

Tedizolid is the second generation of novel oxazolidine antibiotic approved by FDA, USA in 2014 for its clinical use. Main difference of tedizolid from linezolid is by its modified side chain at position 5 of the oxazolidine nucleus which confers antagonistic effect on certain pathogens those are linezolid resistance. Tedizolid also has an optimised C and D ring system that enhances interaction of tedizolid at its binding site (Zhanel et al., 2015). Tedizolid has a serum half-life of 12 hours, hence administration of single daily dose is enough to achieve the bactericidal effects.

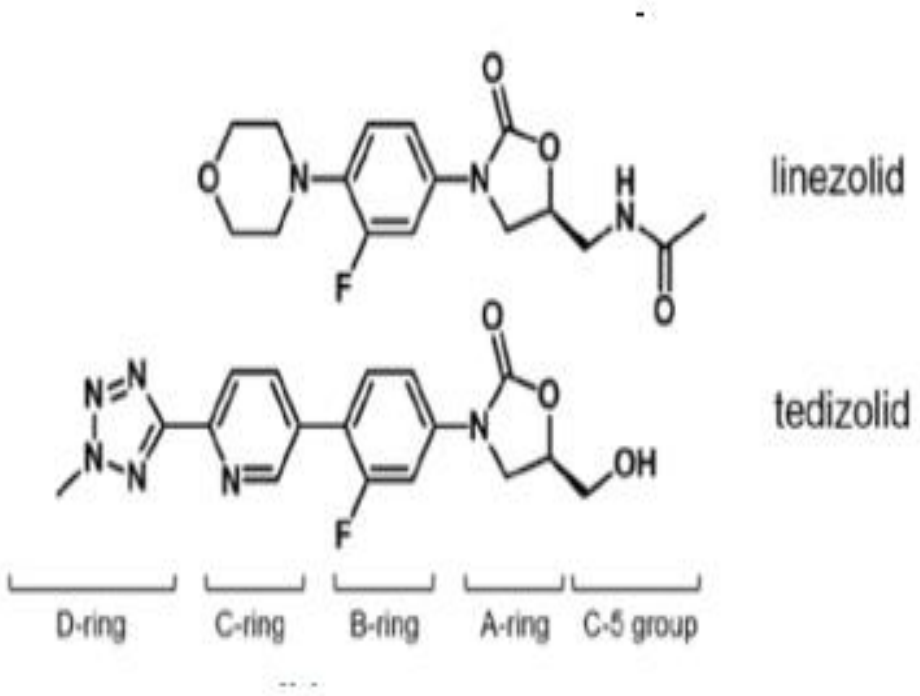


Figure 4.12: Chemical structures of oxazolidines antibiotic.

4.11 Folate pathway antagonists

To understand the basis of antifolates antagonists, it is essential to focus on the core folic acid molecular structure and their metabolic pathways. Folic acid or folate is vitamin B family metabolite which are small water-soluble molecules act as enzymatic cofactors to carry out various metabolic functions. The tetrahydrofolate molecule (THF), which is made up of a pteridine portion, a *p*-aminobenzoic acid (PABA) and a L-glutamate residue are mainly referred as folate in terms of folate antagonists (Figure: 4.11a) and over 150 molecules under this term (Fernández-Villa et al., 2019).

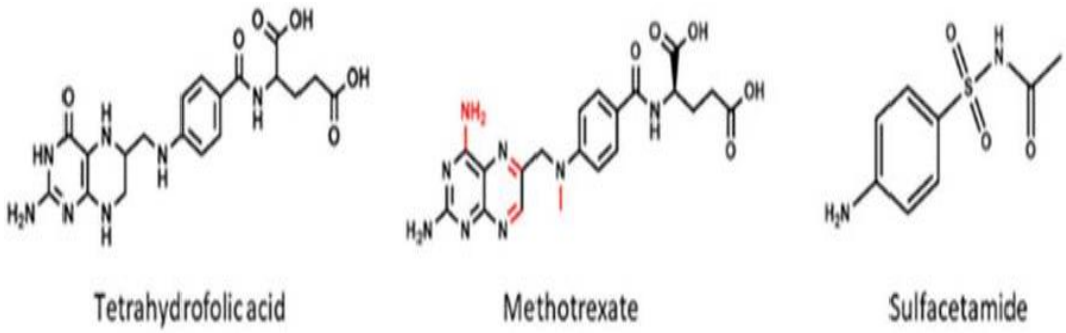


Figure: 4.13 "Chemical structures of tetrahydrofolic acid and two antifolates: methotrexate (classical antifolate) and sulfacetamide (non-classical antifolate), commonly used as folic acid antagonists. Differences between tetrahydrofolate molecule (THF) and methotrexate are pointed out in red" (Fernández-Villa et al., 2019).

Folate is essential for all living cell, and it participate in different pathways of biosynthesis depending on the organisms for their appropriate protein synthesis. Folate also participates in some amino acids synthesis reactions for example, methionine, serine, glycine, histidine, or glutamate and in the formylation of transference RNAs which are essential steps for protein synthesis. Blocking any of these pathways in microorganisms will ensure an antagonistic effect.

Trimethoprim and trimethoprim-sulfamethoxazole are the commonest antibacterial agent widely used in clinical practice. They are fully synthetic chemical compounds developed in 1960s and are commonly used for UTIs, skins and soft tissue infections. Folate antibiotics are active against both Gram positive and Gram-negative organisms.

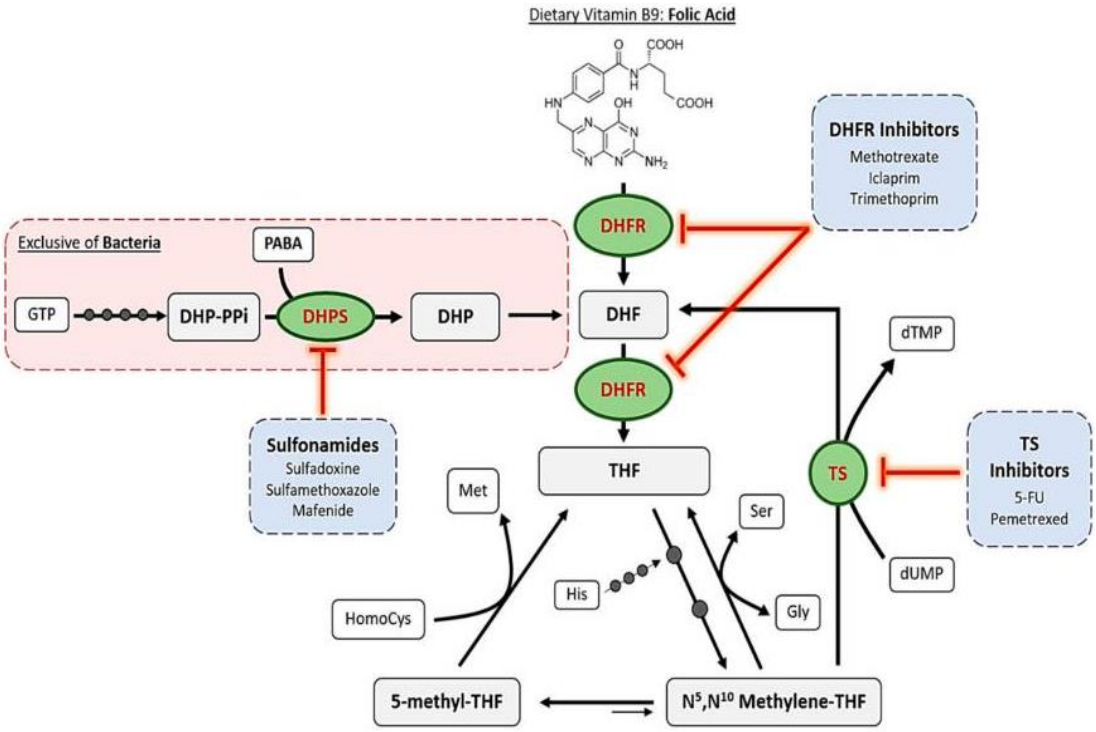


Figure: 4.14: "Summarized pathway of folic acid metabolism, including bacterial de novo synthesis, reduction and TS-mediated feedback loop. Principal enzymes targeted by antifolates are highlighted in green circles. Examples of the different inhibitors are listed in the blue boxes. Small dark grey circles over the arrows indicate an enzymatic reaction. Abbreviations (Abbs.): DHP = dihydropteroate, DHP-PPi = dihydropteroate pyrophosphate, DHPS = dihydropteroate synthase, DHF = dihydrofolate, DHFR = dihydrofolate reductase, Gly = glycine, GTP = guanosine triphosphate, His = histidine, HomoCys = homocysteine, Met = methionine, PABA = p-aminobenzoic acid, Ser = serine, THF = tetrahydrofolate, and TS = thymidylate synthase" (Fernández-Villa et al., 2019).

4.12 Phenicols

First phenicols antibiotic is chloromycetin, which was isolated from soil bacteria *Streptomyces venezuelae* in 1940s by Parke-Davis team. Chemical structure of this phenicol antibiotic was revealed in 1949 by Parke-Davis team and named chloramphenicol. It was the first totally synthetic antibiotic. Chloramphenicol is a broad-spectrum antibiotic used treat various infections such conjunctivitis, meningitis, cholera, typhoid fever. However, chloramphenicol can cause various side effects which includes aplastic anaemia, neurotoxic, and some form of cancer. WHO classified chloramphenicol as possible human carcinogen in 2007. Chloramphenicol is used rarely now a days as a systemic antibiotic and mostly used as topical agent to fight conjunctivitis. It inhibits the

bacterial multiplications my means of inhibition of protein synthesis. Chloramphenicol can also be used for brain abscesses and meningitis and for MDR *Salmonella typhi*. Chloramphenicol distribution in the body is greater compared to many other antibiotics and is easily absorbed by tissues and CNS.

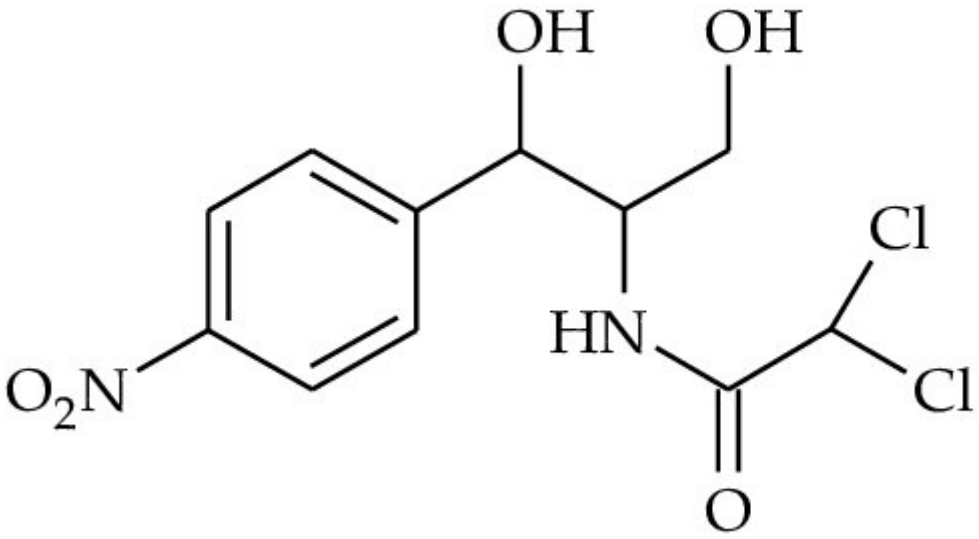


Figure: 4.15: Chemical structure of Chloramphenicol

4.13 Nitrofurantoin

Nitrofurantoin is a broad-spectrum antibiotic was approved for the use UTIs in 1953 by FDA, USA. It's a synthetic antibiotic developed in 1940s, but mechanisms of actions are still poorly understood. However, nitrofurantoin uses various mechanisms, such as DNA, RNA and protein synthesis and production of other enzymes and thus inhibits the bacterial growth. Nitrofurantoin remains active against ESBLs, VRE, MRSA clinical isolates and doesn't interfere with those MDR mechanisms (Squadrito and del Portal, 2022). Nitrofurantoin resistance mechanism is unique to on its own. Certain organisms process intrinsic resistance to nitrofurantoin such as *Proteus* species, *Providencia* species, *Morganella* species.

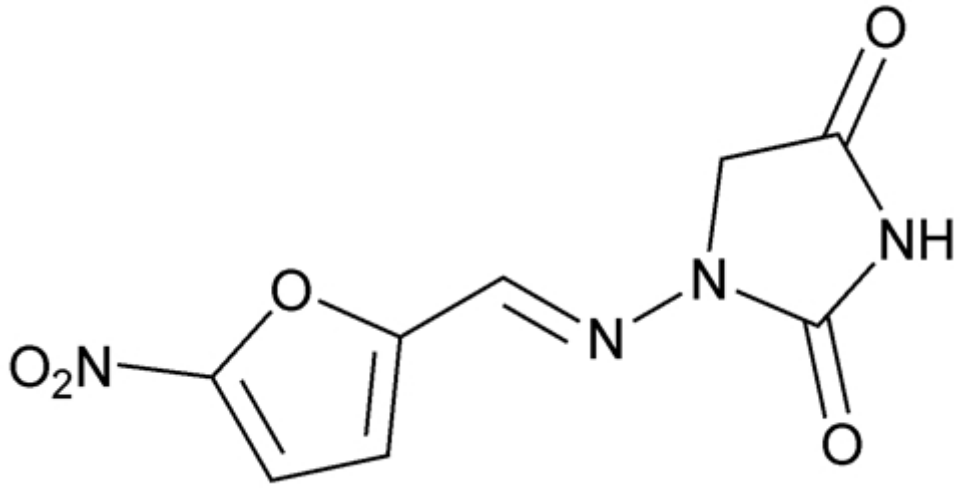


Figure: 4.16: Nitrofurantoin chemical structure.

4.14 Fosfomycin

Fosfomycin was developed in 1969 in Spain from the fermentation broth of *Streptomyces fradiae* and it's a broad-spectrum antibiotic with unique mechanisms and with a low molecular weight. It's available as both IV and oral doses. A single dose of Fosfomycin is uses in the treatment of UTIs caused by E. coli and Enterococcus species. It can be active against MRD due its different mechanisms of action from other antibiotics. Fosfomycin inhibits the cell wall synthesis on both Gram-positive and Gram-negative organisms by inhibiting the initial step of phosphoenolpyruvate synthetase.

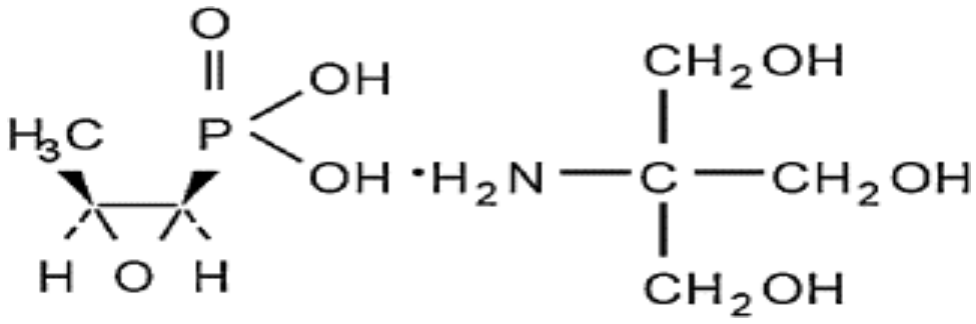


Figure: 4.17: Chemical structure of Fosfomycin.

Chapter 5: MDRO, mechanisms and clinical isolate types

5.1 Introduction

Discovery of the antibiotics are the blessings for us. Antibiotics are not just used to fight infections but also plays important roles in patient management in the cases of surgery to prevent post-surgical infections, in transplant patients, cancer patients and patient with other complications (Munita and Arias, 2016). Since their discovery, antibiotic have saved the life of millions from deadly infectious diseases. Moreover, antibiotics are also used in dairy, poultry industries and other livestock. However, emergence of MDRO are the main problem in current time due to overuse of antibiotics and literary every antibiotics in clinical uses has resistance strains of bacteria. Over 20,000 potential resistance genes of 400 different types have been enlisted in the data base so far from bacterial genome sequencings (Davies and Davies, 2010). Luckily resistance marker genes or genotypes in clinical isolates are much smaller than overall resistance genes found from whole genome sequence.

Scientists were always puzzled where these resistances come from? Some scientists proposed, it might come from the antibiotic producer organisms as the antibiotic producers are not affected by their secondary metabolite, rather they suppress the growth of other organisms in the environment to ensure the availability of nutrition and secure their further growth. They might transfer their genes to other organisms or other organisms might hack their genes by horizontal genes transfer and thus they become resistance. It was seen that aminoglycoside modifications enzymes found in Actinomycetes species had similar biochemical activities as clinical isolate (Peterson and Kaur, 2018). Although pathogens and producers have similar biochemical mechanisms in terms of resistance, however their G+C contents in genes are different and hence it's not certain that pathogen may have their genes from producer organisms. Another proposal is that resistance genes those present in the environmental organisms which are non-pathogenic and non-antibiotic producer might play and important role in evolution of resistance. Resistance genes are much higher than originally it was thought. Moreover, resistances are ancient, a DNA sample from dental plaque of ancient human revealed the resistance genes encoded for β-lactams, aminoglycosides, macrolides, tetracycline, and bacitracin similar to those in clinical isolates (Warinner et al., 2014). 30,000-year-old

permafrost DNA sample revealed the existence of *tet*M, *van*X, and *bla* genes in metagenomic analysis by one of the studies (D'Costa et al., 2011b).

5.2 Intrinsic resistance

Intrinsic resistance is defined as when some organisms naturally resistance to certain antibiotics as their evolutionary tactic to survive along with those organisms produces antibiotics. Generally chromosomal genetic materials are encoded for the intrinsic mechanisms, and it allow them to thrive. Different genus of the bacteria or species may possess intrinsic resistance mechanism to one or more antibiotics, and it varies between organisms. Various intrinsic mechanisms might involve deactivating the antibiotics for example, drug targets affinity, inaccessibility of the drug into the bacterial cells, efflux pump, and possessing genes encoded for antibiotic degrading enzymes. Table below enlisting some clinical isolates those are intrinsically resistance to different antibiotics.

Table 5.1: List of some common clinical isolates those possesses intrinsic resistance.

Microorganisms	Intrinsically resistance antibiotic agent or class
All Gram-positive bacteria	Aztreonam
All Gram-negative bacteria	Glycopeptides and Lipopeptides
E. coli	Macrolides
Proteus, Morganella, Providencia species	Nitrofurantoin
K. pneumoniae	Ampicillin
Pseudomonas	Ampicillin, 1st and 2nd generation of cephalosporins, chloramphenicol, tetracycline
Enterococcus species	Cephalosporins, aminoglycosides
Serratia marcescens	Macrolides, cephalosporin
Enterobacter cloacae	Macrolides, cephalosporin
Stenotrophomonas maltophilia	Aminoglycosides, beta-lactams, carbapenems, quinolones.
Acinetobacter species	Ampicillin and glycopeptides
Listeria monocytogenes	Cephalosporins

Bacteroides species	Aminoglycosides, many beta-lactams, quinolones.

5.3 Acquired resistance

Bacteria can acquire genetic materials from other closely related microorganism when present in the same environment. It is usually done by means of transformations, transpositions and conjugations which most often referred as horizontal gene transfer (HGT). These changes in bacteria might be temporary or permanent. Often HGT is responsible for resistance in bacteria (Munita and Arias, 2016). transformation occurs through the incorporation of naked DNA between two organisms whereas transduction is a phage mediated process, and conjugation is referred as bacterial "sex". Transformation is perhaps the simplest most HGT perhaps is transformation, however, only minority of clinically significant bacterial species are capable to incorporate naked DNA to develop resistance naturally. On the other hand, resistance in the hospital environment frequently involves conjugation which is a very efficient way of gene transfer that involves cell-to-cell contact and is possibly occur in the human gastrointestinal tract while under antibiotic treatment (Munita and Arias, 2016).

β-lactamase enzymes are perhaps the most distributed globally and extended spectrum β-lactamases is in the rise too. The β-lactamase genes are millennium old and scattered in the wide environment. They can be easily transferred from genus to genus or species for example extended-spectrum β-lactamase (CTX-M) was first found in environmental Kluyvera strains which later seen among the clinical isolates in the 1990s and it was the first enzyme known to hydrolyse cephalosporins at a clinically significant level. The CTX-M genes and its subsequent variants can transmit very successfully in worldwide and pose a significant threat (Davies and Davies, 2010). Such efficient HGT of r-gene epidemic is extremely difficult to control (Figure: 5.3).

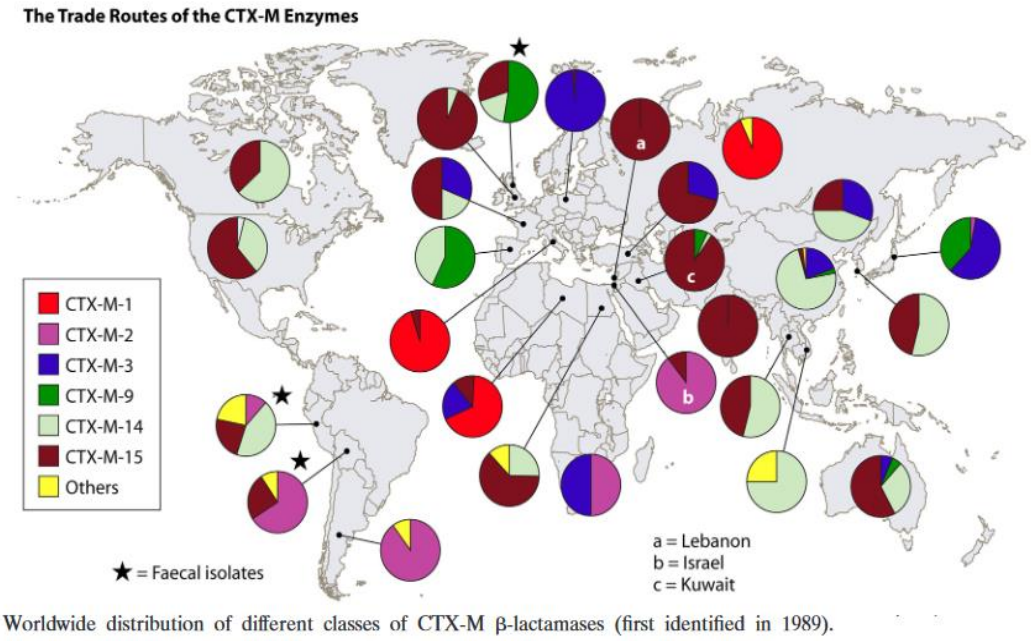


Figure: 5.1: Global distribution of CTX-M enzymes (Davies and Davies, 2010).

5.4 Efflux mechanisms

Bacterial efflux pump is another important resistance mechanisms possesses by many clinically important bacteria. It is mainly seen in Gram-negative bacteria for example efflux pump in E. coli is denoted as AcrAB-TolC where AcrA act as a periplasmic linker protein, AcrB as an inner membrane efflux transporter and TolC as an outer membrane channel (Wen et al., 2018). Gram-negative bacteria have advantages of possessing the outer membrane, which in terns, forms a permeability barrier and offers an intrinsic resistance mechanism for protection against hydrophilic antibiotics and other antimicrobial agents including various toxic substances out from their body (Peterson and Kaur, 2018). Genetic information of efflux pumps can be encoded in the bacterial chromosome or plasmid. Efflux mechanisms might also contribute to the bacterial biofilm formation and thus difficulties in patient with urinary catheter or other devices where it can be easy to form bacterial biofilm. Different bacteria might have different or specific efflux protein for example MexXY-OprM multidrug efflux system of *P. aeruginosa* whereas OtrC found in oxytetracycline producer *Streptomyces rimosus* as a self-resistance efflux system that shows multidrug specificity, and this organism also possesses OtrB protein.

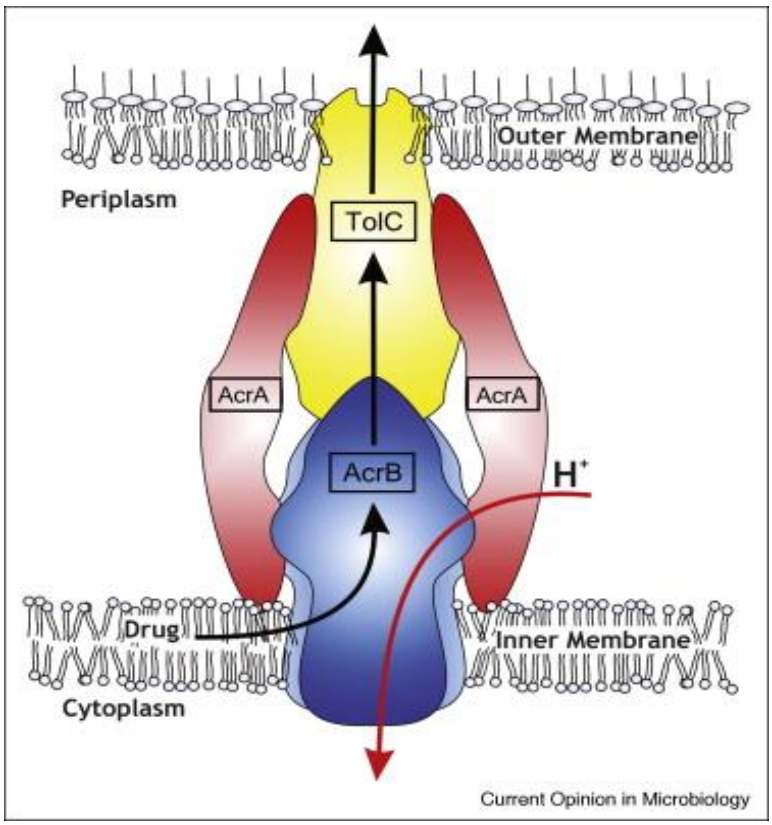


Figure 5.2: Example of an efflux pump system in Gram-negative bacteria (Blair and Piddock, 2009).

Genes encoding for efflux pumps mechanisms can be either intrinsic or acquired. *Acr*AB & *Tol*C in *E. coli*, *nor*A in *S. aureus*, and *lmr*A in *Lactococcus lactis* are some examples of efflux protein which can be acquired from other bacteria and form resistance to single or multiple antibiotics. Among these efflux proteins, tripartite RND pump AcrAB/TolC is the most studied and well understood system which carries out efflux of a very broad spectrum of compounds (Peterson and Kaur, 2018).

5.5 Target modification

Target modifications are seen as protection mechanisms in many clinical isolates. The classical example of one of such organisms is MRSA strains of Staphylococcus aureus where β-lactams resistance is conferred by penicillin binding protein (PBP) mainly PBP2a. whose transpeptidase domain is insensitive to the action of several different β-lactams. PBP2a is encoded by the *mec*A gene, which is located on a large MGE called SCC*mec* (Staphylococcal chromosomal cassette) in *S. aureus*. Many different types of SCC*mec* cassettes have been described, which contain varying numbers of accompanying

resistance elements (Fishovitz et al., 2014; Liu et al., 2016). Another example of target modification is vancomycin resistance, which results from acquisition of the *van* gene cluster and is commonly a problem in enterococci (Miller et al., 2014). Of the many known types of *van* clusters, *van*A and *van*B, in particular, are a problem in clinical strains as they occur on MGEs. The similarities in the sequence and arrangement of *van* genes in producer and clinical strains suggest that they are evolutionarily linked.

Chapter 6: Results and finding of the study

6.1 Introduction

Pharmacies are the first calling point for over-the-counter (OTC) medicine or non-prescription drugs. In Bangladesh, many people use pharmacies as their sole source of health care and advice as they cannot afford doctor's fees. It is very common to buy prescription drugs from the pharmacies without prescription which are given by the pharmacy salespersons or dispensers. Many of these salesmen has little or no appropriate training to sell prescription drugs such as antibiotics or they are licensed to sell prescription drugs. Many pharmacies also sell counterfeit drugs or low-quality drugs those might contain far less active pharmaceutical ingredients (API) as they claimed for. For example, antibiotic that should contain 200 milligrams of API has only 120 to 160 mg API per capsule or tablet.

6.2 Overall results and findings of the study

In this study, main focus was given on how antibiotics are sold in Bangladesh and how it contributes to the MDR. Study also considered other parameters such socio-economical condition of the consumers, key regulations in selling antibiotics, people in involved in sell and dispensing.

6.2.1 Qualifications and training of the people involved in selling and dispensing antibiotics

Studies were conducted in Dhaka (capital of Bangladesh) and Gazipur city corporation of Gazipur district, Bangladesh. To understand the educations and training levels of the people involved in pharmacies sells and dispensing, 100 pharmacies were selected randomly in various geographical areas of Dhaka and Gazipur. Highest level of education and professional training and qualifications were checked and recorded for this study.

Table 6.1: Educational qualification of pharmacy personnel

Pharmacy graduate	1
Postgraduate qualifications (non-pharmacy)	55
Graduate (non-pharmacy)	24
Diploma	27
Job based training	72
Higher secondary certificate	6
Secondary school certificate	1

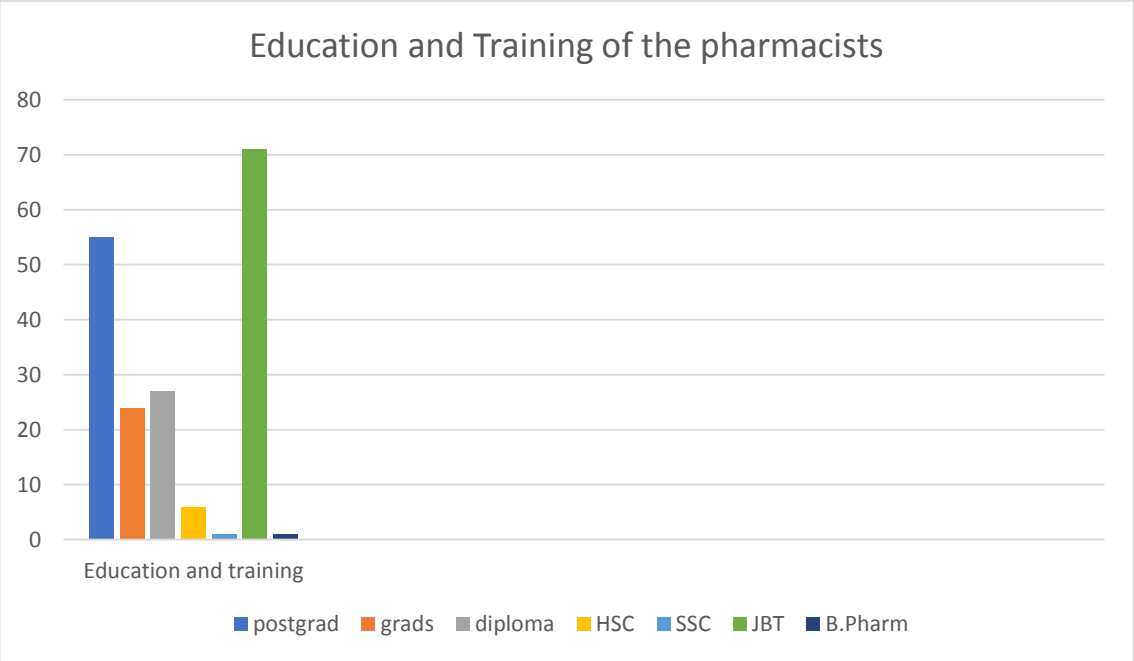
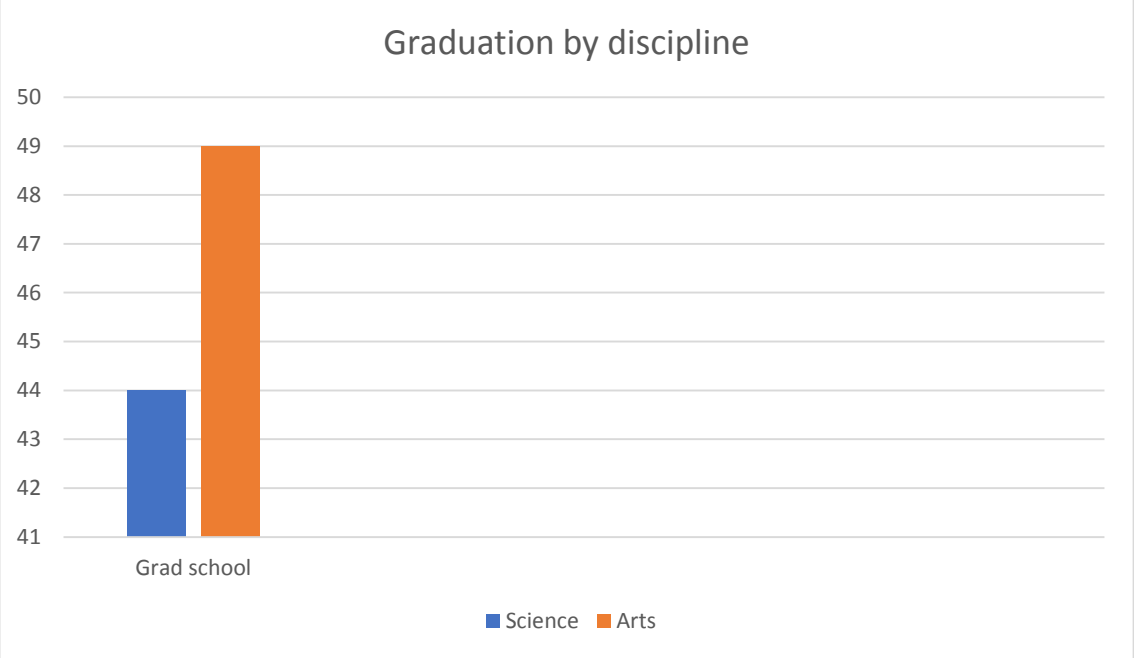


Table 6.2: Educational qualification by discipline

Graduate discipline	Science	44
	Arts	49



More than half of the people engaged in the trade never studied science. Those personnel graduated from science discipline also found to have not studied chemical science or biological science. When asked about MDRO, they found to have no knowledge or vague idea about. 27 percent only had 6 months diploma in pharmacy course certificate whereas 72 percent people had job-based training. Only 1 graduate had pharmacy degree.

6.2.2 Consumers and their socioeconomical conditions

In randomly selected 100 pharmacies, ten thousand consumers i.e., 100 consumers from each pharmacy were interviewed and data were collected for their socioeconomical conditions, frequencies they visited to pharmacies to buy antibiotics, visit to primary healthcare, hospitals, or private clinics for consulting doctors (data not included in this thesis) which are summarised in the table 6.1.2 below

Table 6.3: Household income ranges of the consumers

Household Income (in BDT)	Number of people	Prescription/doctor
<10,000	109	no
10,000-15,000	3784	no
15,001-25,000	3821	no
25,001-35,000	1455	Partly
35,001-50,000	703	Partly
50,001-100K	107	Yes
100K+	21	Yes

Chapter 7: Discussion and conclusion

7.1 Discussion of overall findings of the study

MDR is one of the biggest threats to human health and economy. MDR is in rise in many countries and left undetected, under reported or unmonitored. People are dying from a simple wounds or post operative infections due to MDR. Every year millions of people dying from infectious diseases and many due to right course of antibiotics, Overuses, abuses or misuses are the key contributors to the rise of MDR. Emergence of MDROs is the serious public health threat and must take urgent action to prevent the rise of MDROs.

Bangladesh is a lower middle-income country with 8th largest population in the world despite of being 92nd largest country by landmass. Minimum monthly salary in Bangladesh is BDT 8,000 or USD 84.15 and average salary is BDT 9690 or USD 114.11. In this study, randomly selected one thousand people were interviewed who visited pharmacies mainly for buying antibiotics. People were asked what type of infection they have, whether they consulted my medical professional, whether they have prescription and their household incomes. People with a monthly income up to BDT 25,000 (USD 263.11) has never consulted doctor or produced any prescription. Part of the People with a monthly income up to BDT 50,000 (USD 526.33) visited doctor and had prescription for their antibiotics whereas all people over BDT 50,000 (USD 526.33) monthly income had prescription and consulted doctor. Moreover, people with income below BDT 10,000 were unable to buy the complete antibiotics dose.

Personnel involved in antibiotics sells and dispensing were subjected in this study. It was vital to know whether pharmacy personnel have enough training and appropriately qualified to sell and dispense antibiotics. Out of hundred people only 27 people had diploma in pharmacy and 1 had pharmacy degree (B. Pham) which are significantly low in number. Also, vast majority of the people had no science degree whatsoever and trained in the pharmacy shops or learnt about the product from the pharmacy sales representative. Many of the were not sure about different classes of antibiotics and different generations of those antibiotics, their mode of actions, side effects. Moreover, vast majority were completely unaware about MDRO or MDR which severely impact on public health as

contributing to the MDR. Pharmacy shops regulation in Bangladesh is weak and there are over 100,000 unlicensed pharmacies in Bangladesh.

Selecting appropriate antibiotics and their doses for suspected infection is very important tackling the diseases and MDR. Antibiotic should be selected carefully depending on microorganism involved in the infections and the type of the infection. Susceptibility of the organisms to the antibiotic is key to control the infection. Specific susceptibility can only be possible after appropriate susceptibility testing in the lab. Without lab diagnosis using any broad-spectrum antibiotic is rather will harm the patients as it can also kill beneficial gut microorganism. For example, using ciprofloxacin for long-term can eradicate healthy can flora and contribute to the antibiotic associated diarrhoea. Another example is using penicillin would be ideal for treating simple bacterial sore throat rather than using cephalosporin which is a broad-spectrum antibiotic. As pharmacist in many pharmacy shops are not well qualified, trained and have appropriate level of knowledge, they very often use wrong classes of antibiotics and wrong doses and hence contribute to the MDR.

7.2 Guideline and further works

1. Increasing the patients and doctor's ratio and make easy medical access to the lower, lower middle and middle incomed people.
2. Pharmacy profession should be regulated as like UK, USA, and other developed country and only pharmacy graduate should handle certain classes of drugs especially antibiotics.
3. Antibiotics should not be sold without prescription.
4. Creating awareness on antibiotic uses and abuses.
5. Antibiotic resistance screening and monitoring.
6. Control on antibiotics uses in poultry, dairy, and other industries.
7. Investing on research in noble antibiotic discoveries.

7.3 Conclusion

MRDO is great threat to public health which should be tackle by working together, educating people, making appropriate guidelines for drugs antibiotics sells and control.

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