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EVALUATION OF ANTIBIOTICS RESISTANCE PATTERNS OF SPUTUMS CULTURE EXAMINED IN MICROBIOLOGY LABORATORY OF NEAR EAST UNIVERSITY HOSPITAL: A RETROSPECTIVE STUDY

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OGHOGHO HAPPINESS AKPATA

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Supervisor:

Prof. Dr. KAYA SÜER

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APPROVAL

We certify that we have read the thesis submitted by **OGHOGHO HAPPINESS AKPATA** titled ``**EVALUATION OF ANTIBIOTICS RESISTANCE PATTERNS OF SPUTUMS CULTURE EXAMINED IN MICROBIOLOGY LABORATORY OF THE NEAR EAST UNIVERSITY HOAPITAL BETWEEN 2019 -2021, A RETROSPECTIVE STUDY**`` and that in our combined opinion it is fully adequate, in scope and quality as the thesis for the degree of Master of Educational Sciences.

Examining Committee	Name-Surname	Signature
Head of the Committee	Assist. Prof. Dr. Eşref Çelik	.....
Committee Member	Assist. Prof. Emrah Guler	.....
Supervisor	Prof. Dr. Kaya Süer	.....

Approved by Head of the Department

02/02/2023

Prof. Dr. Nedim Çakır

Head of Department

Yakın Doğu Üniversitesi Hastanesi
Prof.Dr. Nedim Çakır
Enfeksiyon Hastalıkları ve
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Approved by Institute of Graduate Studies

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This thesis has been approved by the above jury in accordance with the relevant articles of the Near East University Postgraduate Education. Teaching and Examination Directive and has been accepted by the decision of the Institute Board

...../...../20....

Prof. Dr. Kemal Hüsnü Can Başer

Head of the Institute

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DECLARATION

I hereby declare that all information, documents, analysis and results in this thesis have been

collected and presented according to the academic rules and ethical guidelines of Institute of

Graduate Studies, Near East University. I also declare that as required by these rules and

conduct, I have fully cited and referenced information and data that are not original to this study.

OGHOGHO HAPPINESS AKPATA

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ÖZET

Antibiyotik direnci artık dünya çapında en endişe verici konulardan biridir. 1900'lü yıllarda antibiyotik ilk kez kullanılmaya başlandığında, herkes mikroorganizmalara karşı savaşı kazandığımızı hissetti. Kısa bir süre sonra mikroorganizmaların kullanılan tüm ilaçlara karşı direnç geliştirebildiğini öğrendik. Görünüşe göre, patojenik mikroorganizmaların çoğu, en azından bazı antimikrobiyal maddelere karşı direnç geliştirme kabiliyetine sahiptir. Yakın Doğu Üniversitesi Hastanesinde 2408 örneğin toplandığı bu çalışma tasarlanmıştır. İzolatların tanımlanması, izolasyonu ve antimikrobiyal duyarlılık kontrolü standart mikrobiyolojik teknikler kullanılarak yapılmıştır. Örnekler 37 0 C'de 24 saat boyunca kanlı agar ve Eosin metilen mavisi agara ekildi. Her plakadaki temsili izolat üzerinde biyokimyasal testler yapıldı ve BD Phoenix Sistemi kullanılarak antibakteriyel duyarlılık paterni belirlendi. Örneklemlerinde çoğalma görülen hastaların bölümlerine göre değerlendirildiği çalışmada en fazla örneklerin Yoğun Bakım (%54,9, 732/1334), Kardiyoloji (%12,4, 166/1334) bölümlerinden gönderildiği belirlendi. ve Göğüs Hastalıkları ve Alerji (%11,8, 158/1334) hizmetleri. Laboratuvarımıza gönderilen aspirat/balgam örneklerinden en çok izole edilen mikroorganizmalar Acinetobacter baumannii/calcoaceticus kompleksi (%23,7, 316/1334), Pseudomonas aeruginosa (%17,5, 233/1334), Klebsiella pneumoniae ssp pneumoniae (%16,3, 218/1334) idi.), sırasıyla.

Aspirat/balgam örneklerinden en çok izole edilen enterik bakteriler Klebsiella pneumoniae ssp pneumonia, Escherichia coli ve Serratia marcescens'tir (sırasıyla %43, %21,7 ve %11,4). Enterik bakterilerde GSBL oranı %40 olarak belirlendi. Aspirat/balgam örneklerinden izole edilen gram pozitif bakteriler arasında en yaygın olanı Staphylococcus aureus (%65.9, 83/126) idi. MRSA oranı %42,2 idi.

Anahtar Kelimeler: Alt Solunum Yolu Enfeksiyonları, Metisilin Dirençli Staphylococcus Aureus, Methicillin Sensitive Staphylococcus Aureus, Antimikrobiyal Direnç, Antibiyotikler, Balgam.

ABSTRACT

Antibiotic resistance is a now one of the most concern issues worldwide. When antibiotic was first introduced in 1900s, everyone felt that we have won the war against microorganisms. After a short while, we found out that microorganisms could develop resistance to any of the drugs that were used. Apparently, most of the pathogenic microorganisms have the capability of developing resistance to at least some antimicrobial agents. This study was designed at Near East University Hospital in which 2408 samples were collected. Identification, isolati0on and antimicrobial susceptibility checking of isolates were done by using the standard microbiological techniques. The samples were plated on blood agar and Eosin methylene blue agar for 24 hours at 37^{0} C. Biochemical tests were carried out on the representative isolate on each plate, and antibacterial sensitivity pattern was determined using the BD Phoenix System. The study reveals that the patients whose samples were observed to grow were evaluated according to their departments and it was determined that the most common samples were sent from Intensive care (54.9%, 732/1334), Cardiology (12.4%, 166/1334) and Chest Diseases and Allergy (11.8%, 158/1334) services. The most isolated microorganisms from aspirate/sputum samples sent to our laboratory were Acinetobacter baumannii/calcoaceticus complex (23.7%, 316/1334), Pseudomonas aeruginosa (17.5%, 233/1334), Klebsiella pneumoniae ssp pneumoniae (16.3%, 218/1334), respectively. Enteric bacteria most isolated from aspirate/sputum samples were Klebsiella pneumoniae ssp pneumonia, Escherichia coli and Serratia marcescens (43%, 21.7% and 11.4%, respectively). The ESBL rate in enteric bacteria was determined as 40%. Of the gram-positive bacteria isolated from aspirate/sputum samples, Staphylococcus aureus (65.9%, 83/126) was the most common. The MRSA rate was 42.2%.

Key words: Lower Respiratory Tract Infections, Methicillin Resistant Staphylococcus Aureus, Methicillin Sensitive Staphylococcus Aureus, Antimicrobial Resistance, Antibiotics, Sputum.

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ABBREVIATIONS

EMB	Eosin Methylene Blue
MIC	Minimum Inhibitory Concentration
ICU	Intensive Care Unit
MIU	Motility Indole, Urease
TRNC	Turkish Republic Of Northern Cyprus
NCCL	National Committee for Clinical Laboratory Standards
WHO	World Health Organization
MRSA	Methicillin Resistant Staphylococcus Aureus
MSSA	Methicillin-Sensitive Staphylococcus Aureus
HA- MRSA	Hospital-Acquired Methicillin Resistant Staphylococcus Aureus
LRTIs	Lower Respiratory Tract Infections
AMR	Antimicrobial Resistance
CDC	Center for Disease Control
VRSA	Vancomycin Resistance S. Aureus
ARGs	Antibiotic Resistance Genes
MATE	Multidrug and Toxic Compound Extrusion
ATP	Adenosine Triphosphate
OMP	Outer Membrane Protein
DNA	Deoxyribonucleic Acid
RND	Resistance Modulation cell
PBPs	Penicillin Binding Proteins
DHPs	Dihydropteroate Synthase
AST	Antimicrobial Susceptibility Test
CLSI	Clinical and Laboratory Standard Institute
MIC	Minimum Inhibitory Concentration
SIR	Susceptible Intermediate Resistance
DHFR	Dihydrofolate Reductase

CHAPTER ONE

INTRODUCTION

According to (Russell, 2004) antibiotics also means antibacterial which is the opposite of life another word for it is death. Antibiotics are gotten from other microorganism to kill microorganisms too; antibiotics are met to fight against other microorganisms that can cause diseases in humans and animals. (Denyer et al.,2004).

Antibiotics serves different purposes in humans, some are met to kill microorganisms, while some are met to hinder the growth. The ones that kills are called bactericidal while the ones that does not allow the microorganisms to grow are called bacteriostatic (Walsh, 2003). As we generally know, antibiotics are known in different forms like, antibacterial (which inhibits bacterial), antifungal (inhibits or kill fungal), and antivirals (inhibits fungal). One of the first antibiotics that was produced was Penicillin, which was discovered by a Bacteriologist known as late Sir Alexander Fleming in September 1928, when he was doing his research fungus working on soil, this research was documented in 1929 (Aminov, 2010) they started using it on humans in 1940 for treatment, (Schlegel, 2003).

Penicillin was the first antibiotics discovered in the year 1920, and it was introduced into the medical system in 1940, it has been of great help on the medical field ever since, according to White and Cox, 2013, it has helped fight against bacterial infections. Antibiotics do not, however, operate in a selective manner against bacteria. When fighting against bacterial that causes diseases, what they do is that they fight both the bacteria that help us to fight against diseases and the ones that we don't need, most especial those useful bacterial presents in our gastrointestinal tract (Walsh, 2003). The way antibiotics has been prescribe and taken is of great concern to its effectiveness in or body. That is why it's very important to know how it works in our body, before introducing it into our medical system.

We have just been able to define antibiotics, how the name was found and who discovered it. Let's now discuss the different classification of antibiotics.

1.1 BACKGROUND OF STUDY

1.1.1 What are antibiotics?

An antibiotic is a sort of drugs or antibacterial used to kill other bacteria. They serve as agent used to fight other disease causing bacterial, antibiotics are being used to inhibits or kill other microorganisms which can cause infections in man. One of the major use of antibiotics is to destroy or fight against the growth of bacteria. Examples of some antibacterial are metronidazole, cefoperazone, furazolidone, daptomycin, tetracycline, erythromycin.

1.2 CLASSIFICATION OF ANTIBIOTICS

We are going to be classifying antibiotics but based on the atomic composition, the way they react and scope of activities (Calderon and Sabundayo, 2007). The other ways we should put into considerations are the ways they are been administered either through oral taking of the drugs, through the veins which is also known as injections and topical means. Antibiotics that are on the same systemic group have a way of commonly showing same way of efficacy, virulent and sensitive which are likely to cause some side effects. Most antibiotics are grouped according to their molecular and chemical structure, those group of antibiotics are Oxazolidinones, Sulponamides, tetracycline, Beta-lactam, Aminoglycosides, Quinolones, Macrolides and Glycopeptides, 2012, Frank and Tacconelli, 2011,Van Hoek et al.; 2012; and Adzitey,2015).

1.2.1 Beta-lactams

These group of antimicrobial have 1 hydrogen and 3 carbons, they are very active. What they do is to interfere with proteins which are used to breakdown bacterial cell walls, in a way by killing or causing hinderances in their growth. In a nutshell, some bacterial enzymes known as Penicillin Binding Proteins (PBP) are the ones that are liable for peptide unit cross-linking when peptidoglycan is broken down. these PBP enzymes can be attached to each other by beta-lactam antibiotics, and when that activity is going on, they obstruct the synthesis of peptidoglycan which can then lead to breaking down and cell death (Heeseman 1993). The class of beta-lactams` most significant examples are known to be Penicillins, Cephalosporins, Monobactams Penicillin, Carbapenems and Cephalosporins.

1.2.2 Penicillin

Penicillin was the first antibiotics found; it was discovered in the year 1929 by Alexander Fleming. (McGeer et al., 2001). They were several compounds combined to penicillin, all of them ended with the appendix -cillin. These Beta-lactam compounds has 6-aminopenicillanic acid ring (nucleus) and some other chains found on the ring side. Bacterial are like humans they protect themselves from attack from some antibiotics, what they do is to break down every stimulant in humans so that they do kill them. Because of this, antibiotics like ampicillin, carbenicillin and amoxicillin create in themselves an artificial side chain. What this side chains do is to give antibiotics the power of penetrate and kill some of the enzymes that the bacteria produce and to speed up the motility of antimicrobial everywhere around the outer membrane of the bacterial cell walls. Augmentin is an example of penicillin that was produced by combining the non-antibiotic compound that can kill the action of bacterial penillinase enzyme. Augmentin is made up of amoxicillin which is clavulanic acid and antibiotics which is a non-antibiotic compound. (Poirel et al., 2005)

1.2.3 Tetracyclines

Benjamin Duggar was the one who discovered Tetracycline in the year 1945, it was discovered in bacterium found in the soil which is from the genus of Streptomyces, Sanchez and others, 2004. Chlortetracycline served as the class`s fundamental chemical (Aureomycin). Tetracycline has four hydrocarbon rings, which were also known by their name with the appendix -cycline.

The Tetracycline was first gotten from the combination of compounds. Tetracycline, Chlortetracycline, Oxytetracycline and Demeclocycline were also gotten by fusion. Tigecycline is one of the antibiotics gotten from total synthesis unlike the others, so it was considered the Third generation (Fuoco, 2012). Medical News Today of 2015 stated that Tetracycline always targets the ribosome of a bacterial, they are damaging the amino acid to polypeptide chains that allows the protein synthesis inside a bacterial organelle (2015) Medical News Today. Tetracycline should be taken by patients two to three before meals so that that it can fully absorb into their systems. And also, patients below 8 years are not advice to take it because it can affect their teeth by changing the color of the teeth, even pregnant women are also to abstain from it because of the effects on the fetus. This is the reason why patients are informed to take

tetracyclines at minimum of two or more hours before they eat or the same time after eating their meals for better assimilation. (Sanchez et al., 2004).

1.2.4 Quinolones

Scientists looking for antimalarial medicines made the initial discovery of the antibiotic, nalidixic acid. The early 1960s saw the discovery of this Nalidixic acid as an impurity during the quinine development process. They can interfere with bacterial DNA replication and transcription. From the basic molecules he developed two main groups of compounds, which are quinolones and naphthyridines which also includes ofloxacin, norfloxacin, quinolones, ciproxacin, temafloxacin, etc. They have a structure that specifically have two rings but with new discoveries of quinolones, it shows that they have an additional ring added to their structure, which aids in extending their antibacterial effectiveness against some bacteria, particularly those that were previously resistant to quinolones and are unable to survive in the presence of oxygen.

1.2.5 Aminoglycosides

According to Mahajan and Balachandran, 2012, another member we need to discuss or talk about is Streptomycin, it was the first drug that was discovered in the group of Aminoglycosides, it was found in the year 1943. Streptomycin has been of great important in the treatment of Mycobacterium tuberculosis, which causes tuberculosis in humans.

Compound known as aminoglycosides are made of 3-amino sugars connected by glycosidic sugars connected by glycosidic linkages. They are soil derived Actinomycetes. Aminoglycosides are effective against a variety of microorganisms. They become active against aerobic Gram-negatives bacilli and certain Gram-positive bacteria after binding to a ribosomal subunit and inhibiting bacteria protein synthesis (Peterson, 2008). According to Talaro and Chess (2008), tuberculosis, tularemia, and the bubonic plague have all been treated with streptomycin, the earliest aminoglycoside, as we`ve already mentioned. It has mostly been used for treating infections, streptomycin is also highly toxic. And because of the toxicity, research was needed to find new classes of aminoglycosides which are still active against bacteria and doesn’t cause much harm to humans. The research yielded results, new antibiotics like Neomycin, Tobramycin Neomycin and Amikacin was discovered. According to

Gilbert, 2000, Gentamicin was discovered to be less toxic and now it is generally used for treating diseases that are been a result of Gram-negative rods like (Pseudomonas, Salmonella, Shigella and Escherichia). Tobramycin is used specifically to treat Pseudomonas infections in cystic fibrosis patients following tularemia, tuberculosis.

1.2.6 Sulphonamides

,Sulphonamides typically combat both Gram-positive and Gram-negative bacteria, including Chlamydia trachomatis, Nocardia, Shigella, E.coli, Salmonella, and Enterobacter, as well as a few species of protozoa. They are frequently used in the treatment of a number of illnesses, including meningococcal meningitis, septicemia, tonsillitis, bacillary dysentery, and few UTI infections (Eyssen and others., 1971). Xu et al., 2014 , Stawinski et al., 2013; states that Sulphonamides can also hinder the effective eness of agents causing cancer.

1.2.7 Glycopeptides

Originally derived as natural compounds, glycopeptide antibiotics, or GPAs for short, are now available as semi-synthetic derivatives with improved action and pharmacokinetic characteristics (Kahne et al.,2005, Van Bambeke et al., 2004,). Glycopeptide are naturally created from cyclic peptide of 7 amino acids, to which 2 sugars are attached, thus the name (Kang and Park, 2015). Yim and Associates do a good job of presenting the structures of different types of glycopeptides (2014). The antibiotic binds to its target by forming 5-hydrogen bonds with the peptide support the medication. The backbone of a medicine can occasionally has an extra chlorine atom or sugar bonded to it during synthesis, as is the case with inoritavancin. Drugs having such extra attachments are known to bind to the target location n more effectively (Allen and Nikas, 2003).

1.2.8 Carbapenems

It was necessity that led to the discovery of this class of antibiotics in 1976. Before that, in the latter half of the 1960s, the emergence of his Beta-lactamase in bacteria seriously threatened the effectiveness of penicillin. According to Papp-Wallace et al., 2011, bacterial beta-lactamase grants defense to bacteria when they are protecting themselves against Penicillin. Apparently, this shocking scheme made researchers to undertake great research on how to fight against this beta-lactamase. In 1976, according to Butterworth et al., 1979 and Brown et al., 1976, Olivanic acid was discovered, it is produced by Gram positive bacteria, which is call Streptomyces

clavuligerus, this bacterium was able to fight against the beta-lactamase. But chemically this acid was not strong enough to easily pierce through the bacterial cell. Reading and Farmer, 1984, it was these difficulties that now caused the delay in the olivanic acid, not long after the delay, two greater or superior beta-lactamase inhibitor were uncovered. The first one is clavulanic acid which was also gotten from S. clavuligerus, while the second is thienamycin which was gotten from Streptomyces cattleya ((Brown et al., 1976), and Kropp et al., 1976). According to Papp-Wallace et al., 2011, it was examined that Thienamycin is believably the first Carbapenem and supply as model for every other carbapenem. Since then, numerous more carbapenem species have been identified (Cassidy and others, 1981). Research has shown that Carbapenems played a very great part in the fight against diseases caused by bacterial. It can do this because they have the ability to withstand the hydrolytic operation of beta-lactamase enzyme. Carbapenems has proven to possess the widest scope of activities and the greatest potency against both gram negative bacteria even with its LPS and gram-positive bacteria that has no cell wall when compared to all the well-known beta-lactams. Torres et al., 2007, they are typically used on patients who have developed a resistance to other antibiotics and have resisted healing over their course of treatment. Carbapenems have different types which are,

Imipenem –it has the widest scope of efficacy, which it uses to inhibit both aerobic and anaerobic bacteria, and they are normally taking orally, and they function at low concentrations with low amount of allergic.

Meropenem – they also have a wide scope of efficiency just that this only limited to non-fermentative Gram-negative bacteria most especially against those diseases that are contacted.

Ertapenem – Brink et al., 2004, they narrowed their activities against non-fermentative Gram-negative bacilli. According to Patel and Bonomo, 2011, the most concerned issue to scientist now is the fact that the increase of pathogenic bacteria resistance against Carbapenems is on the high side worldwide, which is growing so wide internationally (Papp-Wallace et al., 2011).

1.3. Let's now discuss the way antibiotics carry out their activities

The functions of antibiotics?

All antibiotics have their different target they act on, their effectiveness also depends on the classes of antimicrobial agents they belong to, every bacterial have their individual characteristic formation.

The process by which antibiotics carry out their different activities.

- Obstruction of cell wall composition – this process whereby the drugs hinder both the growth and multiplication of the bacterial, example of such drug is Penicillin.
- The disruption of cell membrane structure – The cell membrane`s primary function is to stand as a barricade by keeping the components of the cell in and unwanted substance out, it also allows the transport of important nutrients into the cells and flushing out of the waste products.
- Restriction of formation and role of nucleic acids- the main antibiotics that interfere with the structure and function of nucleic acid is quinolones.
- Hindrances of protein fusion- they stop or slow down the movement of bacterial proteins. Example is aminoglycosides.
- Hindrances of key metabolic pathway- the main cause of this, is when an enzyme goes missing or loss its power, the pathway is blocked, and this can lead to deficiency in products which can cause diseases.

Wright (2010), Madigan and Martinko (2006), Talaro and Chess (2008).

1.4 Antibiotics resistances and their causes.

When we talk about antibiotic resistance this can happen when bacteria are treated with an antibiotic. What the drug is supposed to do is to kill the germs causing diseases. But a few numbers of these organisms survive. This resistance has been of great concern to the world at large, because it has led to the increase in morbidity and mortality rate. Both gram-positive and gram-negative bacteria have evolved a strong capacity to resist be killed by antibiotics because of this it is now very difficult to treat infections cause by them. Since the infectious organisms are known and their antimicrobial resistance in patients are on the open, it is very necessary to put all measures in to actions to make sure the drugs been introduced can fight against the pathogenic bacteria and making sure that there is no irrelevant use of drugs. As a result of the quick recognition of pathogenic micro-organisms and the drugs been used to

inhibit their growth or killed them (antimicrobial) vulnerability structures in humans that have bacteremia and some other serious diseases is needed in many healthcare centres , extensive broad antibiotics are copiously and mostly unnecessarily used. There has been a great increase resulting in resistance occurrences and with addition to poor health care services, pathogenic bacteria are contagious, which means that they can easily be contacted from patients to patients in the same hospital or health care facilities. (M. Akova, 2016) The increase in antibiotic resistance is of great worries to health practitioners all over the world. There has been a great challenge in the fighting these pathogenic bacterial with their diseases, but because of the lack and shortage of efficient drugs, very few antibiotics are available to combat these diseases which has make the increase in resistance to go on a high level. (S. Mühlen, P. Dersch, 2016).
The emanation and the increase of bacterial resistance in those pathogenic bacterial is creating big problem in the humans. It was discovered that it not just antibiotic resistance genes (ARGs) that we come across are causing health diseases are of concern, even the commensal that we come across in our environment can also cause diseases, they have the ability to change their genetic forms and viruses or bacterial replicating in another bacteria cell, they form like a tank of ARGs whereby the infectious bacteria can get their resistance through an horizontal gene transfer (HGT). It has been found that HGT has triggered antibiotics resistance to increase from been a mutual and normal species found in our environment to a disease causing bacterial, which causes the antimicrobial resistance hike. While transformation and transduction are considered not to be really necessary, researchers are letting know that this might not be the case. Finding out the level of the resistome and the way it gathers pathogenic bacteria can be very helpful in controlling the spread of the resistance genes. (C.J. Von Wintersdorff, et al, 2016*)*

1.5 Bacteria resistance are caused by:

Antimicrobial resistance can be better understood by studying the different ways the drugs are be been produced and used by patients, the following also need to be put in consideration; manufacturing, dissemination, prescription, dispensing, and most especially the way the drugs are been used by the patients [Quick J, Bremer K; 1997]. Therefore, any careless practice can also cause resistance to the antibiotics been used.

1.5.1 Drug dispensers and drug quality

When there is no proper control of the sales of antibiotics, it can lead to the misuse of the drugs, which eventually leads to resistance of the drugs. Most of the developing countries can buy antibiotics without the prescription which are been sold by the street vendors who are not professionals. These drug vendors do not care about the effects of these drugs to the users all they care about is the profits they make from the sales. There are also some pharmacies that operates without license, they also don't ask the public for prescription when they come to buy drug so they are easily accessible to the general public, because they don't charge for consultation fees and are ready to settle with the treatment option the patients are ready to pay because of their financial standard. Most of these pharmacies are those small pharmacies that sell in small quantities and are mostly found in developing countries like Africa, (Kwena Z, et al., 2008). When the drugs are not kept at the appropriate places it can also lead to the deterioration of the drugs, which can make the drugs to loss its potency. Another thing we should also consider is when the drug does not meet the quality standard, it wouldn't be active because the substance use for the production is low. (Cockburn R, et al., 2005).

1.5.2 Health professionals

The health care professionals have a great role to play when it comes to treating and preventing diseases, if they are careless about this can cause a great effect on the patients they are treating. When it comes to the instructions of taking antimicrobial drug, it varies among different health care workers and the countries they are. Sometimes the way the antimicrobial is inappropriate given or prescribe can cause resistance to the drugs. Another reason can be a shortage of physicians in most developing countries, most doctors are devasted because of the workload on them, so they don't really have time for their patients or might not even have time to listen to them to know the adequate drugs or prescription to give to them, what is most necessary when treating a patient is the ability to communicate with them and guide them on how to take the drugs that are been prescribed to them.

One of the main reason for resistance is lack of diagnosis before treatment, which can result to you treating the wrong diseases. (Usluer G, Ozgunes I, Leblebicioglu H, 2005). Physicians in rural areas that does not have the ability to carry out AMR which

makes it difficult to know which antibiotics that can work better for a particular disease. (Neu HC., 1992). Because some physicians are scared of making mistakes by not treating the patients adequately they resort to using multiple prescription for antimicrobial treatments. (Reynolds L, McKee M., 2009).

1.5.3 Patients

Another very important thing that can cause AMR are the patients (Malfertheiner P., 1993). Some patients miss their dosage when placed on medication some because they forgot others because they do it intentionally. Despite the fact that the majority of patients are aware of the harmful effects of alcohol use, some patients that are placed-on antibiotics have to skip the intake because they want party and think about drinking alcohol. These practices can give way for the microorganism that are not yet inhibited by the antibiotics to increases the chances of developing resistance (Calva J, Bojalil R., 1996). While in some developing countries like some countries in Africa some people when they are sick first go for herbal treatment, some is because of poverty while others is because of what they believe. Some of them even end up combing antibiotics with herbal treatment from the traditional healers. Some of these things are done by these patients either because of poverty or because of lack of knowledge. The mixture of both traditional and antibiotics can cause resistance to the infection been treated. Which can lead to giving more strength to pathogenic bacteria.

1.5.4 Lacking observation and inadequate laboratory antimicrobial sensitivity testing. Accessibility of standard antimicrobial sensitivity testing can deliver data on resistance developments, which means involving rising resistance which plays a great role for regular clinical activity, and which can also contribute to the evolution of efficient procedures against antimicrobial resistance. Antibiotic sensitivity experiment is not always done in many rural labs because of the shortage of equipment needed. When is deficiency is related to a particular patient been resistant to antibiotics sensitivity testing, or a particular region been resistant antimicrobial in order to treat illnesses with particular sensitive antimicrobials in particular villages or regions, observational data may be crucial. Given that resistance rates might change at any time across the nation`s regions, this kind of testing must be carried out frequently and continually.

1.5.5 Antimicrobial resistance management policies

AMR challenges has worsened of by the statistic that the majority of world pharmaceutical companies believe that researching for new antimicrobials has no much profit research and some are also considering the fact that even when they produce new antimicrobial, more bacteria will still with time come out to be resistance to those that are been produced. Therefore, they would rather put their money into the advancement of medications for prolonged infections (diabetes and hypertension) so also other drugs that are used to advance style of living (e.g., Cialis, Viagra, etc.) Brandenburg K, Schürholz T., 2015. Thus, the long-term resolution has to be centered on approaches to keep away the occurrence of resistance and also the increase of resistant organisms between humans.

1.5.6 Sanitation and cleanliness

Apart from the ridiculous use of antibiotics, the way we use our environment is also of great concern, some very important conditions can also cause illness or infections spreading, over population and low maintenance of the environment can facilitate the transmission and the spread of bacterial resistance. human to human contact with already contaminated water, food, and vectors spreads up the spread of infections that are resistant of treatment. Cleaning and taking care of our immediate environment has a great role to play in the reduction of diseases. Developing infection avoidance and control in hospitals will bring down a great deal the spread of that is nosocomial from spreading from one patient to another, or microorganisms that are be contacted from someone that is acquired resistance like as *Staphylococcus aureus* as well as others.

1.6 Aim and Objective

This research study is mostly concern about how to know the cause of antibiotics resistance, how it can cause harm in humans, and what we need to do to reduce that rate of the resistance. Because of the rise in mortality rate due to the increase in antibiotics resistance all over the world, it has become very necessary to look into the cause and solutions to the problem, so that mortality rate can drop.

CHAPTER TWO

LITERATURE REVIEW

When bacteria have the tendency to resist a particular drug from killing it or hindering its growth completely, it is known as antibiotic resistance. On this level bacteria become resistant which makes them to continue to increase even with the presence of antibiotics that were supposed to kill them. (2017). Accessed: June 19)

Normally antibiotics are commonly active against microorganisms, when the microorganisms are now less sensitive or resistant, it means that what is needed is a higher concentration than the normal ones been taken to kill the bacteria. When new antimicrobial compounds are been introduced you start observing antimicrobial resistance which shows the effects of antimicrobial compounds. Chadwick DJ, Goode J. John, Chichester; 2007.

There can be a resistance to antibiotics if the natural selection empowers the bacteria with some level of lower resistance. Levy SB; Springer, 1992. 1–12.

According to a study, 1998, Hoge CW, et al;, it explains how ampicillin, tetracycline sulfamethoxazole and trimethoprim (TMP-SMZ) were used in the pasted years for treating bacterial infections but of recent scientist found out that it is not used anymore in places like Thailand for treating non-cholera diseases.

While another study shows that this same drug that is not effective in another country is effective in Bangladesh and shows how effective it has been treating the same diseases or infections that it couldn't treat in Thailand. (Rahman AE, et al, 2017).

Research shows that resistance has been on even before we started the use of antibiotics to combat infections. (Nature. 1940, 146:837). By not using antibiotics in the appropriate ways can cause microorganisms to be resistance to the drugs. It was shown that since sulfonamides was found in 1937, it was produced because of a specific mechanism of resistance, it was used to fight against the resistance. In the 1930s, sulfonamide resistance was discovered, it shows the same composition of resistance, which is still on till today, which is more than more than 80 years later (Int J Pharm. 2002).

2.1 Bacteria is a one of the common causes of LTRIs and the way they resist antibiotics.

Lower respiratory tract infections are known to be between the most mutual infectious diseases which can be very dangerous to humans. The most frequently described infection in humans is the respiratory tract infection, which is highly prevalent. Upper and lower respiratory tract infections serve as a general division of these infections (LRTIs). Meanwhile they are not harsh, temporary and sometimes they are self-limiting, because of this most people that are infected does not really count it important to treat. (Ndip RN. et al., 2008). Report shows that out of almost 4 million death every year all over the world, about 34.6 percent of them from the South-East region do die of respiratory tract infection. It is even worst in countries like Africa, and they are not easy to control because of the complications that follows the analysis process and the way the treatment is being administered.

Lower respiratory tract infection is not just one infection, nonetheless it is a collection of diseases with different genes, etiologies, clinical presentations, and results. The examination and signs of lung infections depends on their gender, season, age, the type of population that are in danger, with other conditions. This is according to: Dawadi S, Rao BS, Khan GM., 2005. The diagnosis factors of LRTIs are not decided clinically and it is different from region to region. *Streptococcus pneumonia and Staphylococcus aureus,* etc are gram positive so likewise *Pseudomonas*, *Acinetobacter*, *Haemophilus influenza*e, and *Klebsiella* species which are gram negative bacteria are recovered from LRTIs, Ozyilmaz E. et al., 2005.

Observing the antimicrobial resistance methods of the analysis are required for more than just helping the physicians during the treatment of the diseases but also needed when managing antibiotic medication, it also helps to direct these infections. According to, Keith T, et al., 2010, bacteria are known to cause serious illness or terrible infections, and in the majority of cases, they need to be treated with lead. Cases of respiratory tract infections respond to antibiotic therapy however, using antibiotics inappropriately can result in respiratory tract infections, which are frequent, especially in developing nations like Africa and this can might lead to resistance. Sherchan JB, et al, 2012 make it clear that antibiotic resistance methods that are analyzed for respiratory tract bacteria vary between different countries, all of them are different

depending on the bacterium and the treatment that is been explore. Although we don't know much of the microbial agents that can cause LRTIs in North Cyprus. We have to consider Age of the patients, their gender, and time must be designated to disturb the occurrence of LRTIs. This little we know about the occurrence of LRTIs is important for normal observations on the modification in the design of antibiogram for these organisms.

2.2 Drug efflux

Bacteria are known to have chromosomally concealed genetic factors for efflux pumps. Others are triggered or overexpressed whereas some are sent primarily, this happens mostly through mutation, these are conducted under specific environmental conditions, if the stimulus or suitable substrate is present the important thing is these drains, pumping is done to rid the bacterial cells of harmful materials. In essence, certain of these pumps move the majority of chemicals (Multidrug drainage pump, MDR). The resistance efficiency of most of these pumps can be determined by the amount of available carbon sources. Cox G, Wright GD; 2013.

The majority of bacteria possess a variety of these efflux pumps, which are categorized into five kinds in bacteria, and they are grouped based on the ATP-binding cassette (ABC) family, the multidrug hazardous compound extrusion (MATE) family, the ATP-dependent protein kinase (ATPase) family, and MFS known as the main implementer superfamily and SMR, short for small multidrug resistance family, and RND, short for resistance nodulation cell division family, respectively. The main thing we should understand the majority of these efflux pump types function as single-component pumps that transfer substrates through the cytoplasmic membrane. These multi-component pumps from the RND family, which are primarily found in gram-negative bacteria, collaborate to efflux as an underlayer surrounding the entire cell envelope with periplasmic membrane fusion protein (OMP-porin). Of other circumstances, other members of the efflux group in gram negative bacteria move with other cellular components as multicomponent pumps. Blair JM, Richmond GE, Piddock LJ; 2014.

2.3 Effect of antimicrobial resistance for individual bacteria

It will be of great use for us to know and understand how many of these resistance mechanisms each bacterium has in stock, one other best example to use is the MRSA. This increased cost is subject to additional charges longer hospital stays, more tests required, providing medical and rehabilitation, which mean that the amount of time you stay in the hospital can also affect the cost, which can increase the number of tests you have to do and also can have effect on the medical and recovery service you get. Another important element we should put into consideration is the morbidity and mortality that are been caused by MRSA, which includes the noticeable increases in diseases aggravation. Filice GA., et al; 2010

Is the same as the necessary number of virulence factors between Methicillin Susceptible Staphylococcus Aureus (MSSA) and MRSA strains which includes secreted chemicals and surface molecules that promote growth which grant the intrusion and causes harm to host cells. Bacteria can use these factors to cause multiple types of diseases. MRSA is known to cause infections of the skin and other related tissues, these infections can easily spread from one person to another, which can cause nosocomial. Research have shown that MRSA mortality rate is 2-3 times higher than that of MSSA strains. Which means that MRSA strains are more resistant to antibiotic which can reduce the impact of antimicrobial treatment. Hübner C. et al; 2014.

2.4 Antibiotic resistance mechanism,

There are four means that antimicrobial resistance mechanisms fall into: (1) limitation of drugs taken; (2) Altering the drug target area; (3) suspending the use of a medication; (4) emanation of active drug. Inherit resistance may cause limitation of drugs taking, suspension of the use of medication, and even emanation of active drugs; obtained resistance mechanisms can also change drug destination, suspending the use of a particular drug, and emanation of active drug, which are caused by difference in system, etc., compared to gram positive bacteria, gram negative bacteria use different sorts of processes that are subject to mutation. According to, Chancey ST, Zähner D, Stephens DS., 2012 the method gram negative bacteria employ is different from that used by Gram negative bacteria. Gram-positive bacteria Gram-negative bacteria use four different mechanisms Gram-positive bacteria consume less of it, but usually only in limited amounts drug intake. Gram-positive bacteria cannot use other bacteria

mechanism at the emission of active substances stated by 2014. Mahon CR, Lehman DC, and Manuselis G.

2.4.1 Limitation of drugs taken

According to, Richmond GE, Blair JM, and Piddock LJ., 2014, Gram negative bacteria can be resistance to antibiotic because of the design and activities of LPS, it is this structure that helps to stand as a hindrance to some particles. Which gives bacteria a resistance common to some class of huge antimicrobial substances. Mycobacteria have an extremely lipid-rich outer membrane, and it makes it very difficult for drugs to dissolve, drugs like rifampicin and the fluoroquinolones can easily works on the cell because they dissolve easily, but hydrophilic drugs which does not dissolve easily, do have lower access (Kumar A, Schweizer HP., 2005, Lambert PA., 2002).

Some bacteria such as Mycoplasma and Bacteria that lack a cell wall, such as *Mycoplasma* and similar species, are mostly resistant to all drugs that target the cell wall including β-lactams and glycopeptides (Bébéar CM, Pereyre S., 2005). But because of lack of LPS in gram positive bacteria it is not found to be resistant to most antibiotic drugs. When it comes to enterococci the polar molecules have problem entering the cell wall gives inherent resistant to aminoglycosides. Gram-positive bacteria such as *Staphylococcus aureus*, have been discovered to be resistant to vancomycin. The way S. aureus has been able to fight against vancomycin is that the bacteria yield a broad cell wall, this massive cell wall makes it difficult for drug to enter the cell, and provides an central resistance to vancomycin. These strains are delegated as VISA strains (Lambert PA., 2002, Miller WR., et al., 2014).

According to Blair JM.et al, 2014, for porins channels, which are often used by gram-negative bacteria and give access to hydrophilic bacterium molecules, are the common entry points for substances into the cells of bacteria with a thick outer membrane. A reduction in the quantity of porins accessible is one way that porin changes limit drug consumption, secondly, mutation porin channel selectivity. (Kumar A, Schweizer HP., 2005). The *Enterobacteriaceae* is another group of bacteria which cannot be inhibit or killed by antibiotics, they resist antibiotics by reducing the number of porins (Cornaglia G., et al.,1996). The transformation that can cause changes in the porin channel have been discovered to be *E. aerogenes.* (Gill MJ., et al.1996, Thiolas A., et al., 2004).

The formation of biofilm is another way bacteria used for migrating. Biofilms sometimes contain dominate organisms, (such as by *Pseudomonas aeruginosa* in the lung), it can also have a broad range of organisms, these are normal flora of the gut found in biofilm community. The pathogenic bacteria are being protected from the attack of the host immune system because of the formation of biofilm, it also gives them protection from antimicrobial agent. Biofilm contains polysaccharides, it is thick and sticky, it contains proteins and DNA from the resident bacteria, all these are the things that makes it tough for antibacterial agents to spread through the bacteria. For more efficiency, the drugs needed must be high in concentration. Biofilm specializes in transferring genes horizontally which promotes the closeness of bacterial cells. It means that sharing of antimicrobial resistance genes is possibly easier for these bacterial communities. (Mah TF.,2012; Van Acker H, Van Dijck P, Coenye T., 2014).

2.4.2. Altering the drug target area

Bacterial cell has so many elements that are been targeted by antimicrobial agent, so likewise bacteria have many ways to change it forms to enable it resistant to those drugs. For the B-lactam drugs, all gram positive needs to do is to change their structure or numbers of PBPs (Penicillin Binding Proteins). These PBPs are transpeptidase, their job is to manufacture peptidoglycan in the cell wall. When there is any change in PBPs it also has a great impact in the number of drugs that are been used. And also when there is change in the structure, it may also affect the drug been used or even hinders the drugs required. (Reygaert WC., 2009; Beceiro A, Tomás M, Bou G. 2013).

Another one we need to consider is glycopeptides (example is: vancomycin) what is does is to inhibit lipopeptides, such as daptomycin, which depolarizes the cell membrane, and cell wall formation. However, gram-negative bacteria are highly resistant to these medications, Randall CP., et al., 2013). The ability for gram negative bacteria to remain resistance toward vancomycin is of great concern in the enterococci and in *Staphylococcus aureus*. (Cox G, and Beceiro A, 2013).

Kumar S, Mukherjee MM, Varela MF., 2013; and Roberts MC., 2004 states that antibiotics resistance to drugs that target the ribosomal subunits can happen through ribosomal alteration in (aminoglycosides, oxazolidinones), this ribosomal subunit methylation which are, (aminoglycosides, macrolides—gram positive bacteria, oxazolidinones, streptogramins) are most usually connecting *erm (erythromycin*

ribosome methylase) genes, or ribosomal protection (tetracyclines). What this does, is to inhibit the drug from attaching itself to the ribosome. This is not always the same at all the levels.

Some drugs the metabolic pathways, in this process, it normally happens through bacteria mutation in their enzymes (DHPS—dihydropteroate synthase, DHFR—which is also known as dihydrofolate reductase) this is involved in the folate biosynthesis pathway and/or overproduction of resistant DHPS and DHFR enzymes (sulfonamides—DHPS, trimethoprim—DHFR). Sulfonamides and trimethoprim are known to bind to their various enzymes because of their structural comparisons of the natural substrates (sulfonamides—*p*-amino-benzoic acid, trimethoprim—dihydrofolate). The action of these drugs is through competitive inhibition by binding in the active site of the enzymes. Mutations in these enzymes are habitually localized at the active site, which can lead to fundamental changes in the responsible enzyme which is also capable of inhibiting drug binding while allowing essential substrates to tie. (Huovinen P, et al., 1995; Guay GG, Austria NE, Vedantam G, , et al., 1998).

2.4.3. Suspending the use of a medication

According to 2013, Kumar S.,et al.; states that, bacteria can suspend the uses of drugs in two ways: by physically deteriorating the drug, and also by transferring of a chemical group to the drug. β-lactamases are very enormous category of drug hydrolyzing enzymes. Tetracycline is another drug that can be inactivated by hydrolyzation, via the *tetX* gene (Blair JM, Webber MA, Baylay AJ, et al., 2015).

The most often used transfers of acetyl, phosphoryl, and adenyl groups are used in drug inactivation through transmission of a chemical set to the drug. There have been so many transferases discovered. One of the devices that are frequently used during drug deactivation is Acetylation, and it is also known to work against the aminoglycosides not just that, also chloramphenicol, the fluoroquinolones and the streptogramins. Additionally, it has been observed that adenylation and phosphorylation are frequently used against aminoglycosides (2015 Blair JM, et al., Schwarz S, et al., 2004)

2.4.4 β-lactamases

β-lactam drugs are commonly used as an antimicrobial (antibiotics). B-lactam has a four-sided ring and share a distinct core structure. According to, Pfeifer Y, Cullik A, Witte W., 2010; resistance to the β-lactam drugs can happen in three ways: (1) it prevent the relation between the target PBP and the drug, by changing the capacity of the drug to attach itself to the PBP; (2) efflux (discharge)pumps that can dismiss β-lactam drugs when it is present; (3) another way is the disintegration of those drugs by β-lactamase enzymes (Bush K, Bradford PA., 2016).

Bush K, Jacoby GA., 2010 states that what β-lactamases does is to deactivate β-lactam drugs by determining in a specific site in the β-lactam ring structure, causing the ring to open. The open-ring drugs are not able to bind to their target PBP proteins. The known β-lactamases are wide-spread, and the group contains enzymes that can inactivate any of the current β-lactam drugs. One of the most common resistance structures in the formulation of β-lactamases which is used by gram negative bacteria against β-lactam drugs, and the most important resistance mechanism against penicillin and cephalosporin drugs

Antibiotic resistance is of great concern worldwide, because of its effect on the world. World Health Organization, 2014; requires all antibiotics use to be reserved or used only when local interventions are insufficient to control infections.

CHAPTER THREE

Materials and Procedures

3.1 Study Areas

Northern Cyprus, known as TRNC is its constituent state, it is the northeastern region of Cyprus, an island in the eastern Mediterranean that has been bordered by Turkey and Greece since the late 20th century. There are thought to be 382,000 people living in TRNC (2018). Between January 2019 and December 2021, the current investigation was carried out at North Cyprus` Near East University Hospital.

3.2 Sampling

2408 samples were collected for this study samples considered from the period of 2019 January 1st to 2021 December 31st and were gathered from Near East University Hospital in North Cyprus. The seventeen different samples were collected from different departments. The demographic information which includes their age, sex and was gotten from the medical records of patients. The samples were labeled according to their departments and where they were collected and were subjected for screening in MCB Lab at Near East University Hospital, Lefkosia North Cyprus.

3.3 Sputum Culture

A sputum culture is a test that is use to know the amount of bacteria or other form of organism that can cause infection in the lungs of a patients or that can disrupt the airways leading to the lungs. Another name for sputum is phlegm, it is a thick type of mucus that is formed in the lungs of the patients. These samples are gotten through the deep cough material from the bronchi. The Upper respiratory bacteria usually infect sputum or bronchoscopic specimens.

The test use for determining sputum culture is known as Gram stain. It is a test that are used for determining bacteria at the site of a suspicious infection or in the fluid produced by the body such as the blood or urine. It is also used to help identify the particular type of infection the person may have.

3.4 Isolation of Pure Culture

The entire material was initially purified to obtain a clean culture using Blood agar and EMB agar.

3.4.1 Eosin Methylene blue (EMB) medium

A very little amount of eosin methylene blue agar from OXIDE was employed. The medium was autoclaved at 121oC for 20 minutes after being organized in accordance with the company`s instructions. After autoclaving, 25ml of the media is transferred under sterile conditions into sterile petri plates (99 mm in diameter). Samples are inoculated using a clean inoculating loop under aseptic circumstances once the medium has solidified. The plates are incubated at 37oC overnight following inoculation.

3.4.2 Blood Agar

Blood agar is made out of a base with a protein source (tryptone, for example), soy protein digestion, sodium chloride (Nacl), and agar containing 5% sheep blood. The blood agar base is arranged according to the manufacturer. The media is been sterilized at the autoclave at 121 ^{o}c for 15 minutes after which it been transferred to the prepared blood culture medium base which is then put inside bowl of water to cool to 50^{0}C. then, the culture medium base has been put in cool water to get to 50^{0}C, then you add a sterilized blood neatly and mix well carefully so that air bubbles not to form. Then pour into a clean petri dish.

3.4.3 Gram Staining

A smear of each isolate was created, dried, and heat-fixed on glass slides using sterile procedures. Crystal violet was applied to the smear and left on for a minute. After being drenched with Gram`s iodine for a minute, it was then rinsed with distilled water. Following a decolorizing step with 95% ethyl alcohol, it was once more rinsed with distilled water. It was then cleaned with distilled water and counterstained for 45 seconds with safranin. Oil emulsion was used to magnify the slide 100 times while it was dried and analyzed.

3.5 BIOCHEMICAL TESTS

3.5.1 Preparation of cell suspension

For the purpose of conducting biochemical assays, cell suspension was organized. The McFarland turbidity standard solution was compared to the cell suspension that had been prepared in saline water (0.85% Nacl), see Gomes et al., 2001.

3.5.2 Catalase Test

Catalase test is been used to detect catalase enzyme. To perform this test, 23ml of hydrogen peroxide are used. Then, using glass or wood sticks, you remove a colony of bacterial culture from the nutrient agar plate and add hydrogen peroxide to it. The presence of foams indicates the validity of the test.

3.5.3 Oxidase Test

The oxidase reagents are applied to a piece of filter paper to conduct this test. Use a disposable loop to remove some recent growth from the culture plate, then rub the growth on the filter paper. After which you wait for a blue color to show up within 10 seconds for positive test.

3.5.4 Indole production test

By using a sterilized technique, new organism is been introduced into its properly labeled deep tube containing motility indole urea (MIU) media by using a cable loop. At 37oC, the tubes were incubated for 24 hours. After which you can then add Kovac's reagent and wait to see a red color within 10 min.

3.5.5 Citrate utilization test

Using sterile methods, organisms has been inoculated into Simmons citrate agar in citrate utilization test you use streak inoculation. Then the culture has been cultivated for 24 hours at 37 ^{0}C. then detect the transformation in the color of media changing from green to blue color.

3.5.6 Motility Test

You insert tubes containing semisolid nutritional agar with a clean culture by drilling a hole deeper than halfway down the center of the medium column. Incubation of the tubes takes place in an anaerobic environment for 24-48 hours at 35oC.

3.5.7 Preparation of the inoculums

The 0.85% Nacl solution in 5ml of normal saline is being used to prepare the bacterial suspension. This is the reason, a fresh culture of 24 hours old is being used. After that, a platinum wire loop is used to collect two to three carefully isolated colonies. The bacterial suspension was compared to the 0.5% Mc Farland standard after you must have shaken it.

3.6. Antimicrobial susceptibility testing methods.

Bacteria are subjected to AST by which they were isolated into pure culture derived from the sample provided,

In order to identify the relevant microorganisms consistently and correctly to the genus and /or species level, standard reference procedures were applied.

The most significant of the isolated bacteria, together with a sample of others, are being preserved for later analysis (either through lyophilization or cryogenic preservation at -70oC -80oC).

AST are being influenced by the following factors which are been considered, enhanced and recorded in a thorough principle for functioning measure:

Following the bacterium`s isolation in pure culture, the ideal inoculant concentration was established to provide precise susceptibility data. AST testing employed bacteria or other organisms from a fresh culture,

How the agar and broth media were made and prepared to be used (e.g. pH, they cations, thymidine, they make use of the additional media) The way the media has been performed and sterilized are also identified, documented, and put into practice processes. Antibiotics used in microtiter plates, disks, strips, and tablets vary in size depending on the carrier, Creating substance and solvents to generate antibacterial standard solution. Circumstances for growth and maturation (time, temperature, atmospheric factors like CO2) Culture medium intensity, The quantity of dosages evaluated per dilution of broth and agar, The reference organisms, along with the test controls, were employed, The following explicative measures cut-off values in epidemiology, clinical breaking points.

Due to these factors, emphasis is being placed on following correct processes and permitted, well-documented methodologies. Through the application of such approach, an acceptable level pf reproducibility was achieved.

When we use these three methods and follow them accurately it will give us the consistent and memorable outcomes, CLSI, 2008; and 2007, Walker: The three methods are as follows,

3.6.1. Disk diffusion method

In this method, the solid culture medium is implanted with a desired inoculum that has been isolated in pure culture and the antimicrobial agent is circulated in a precise concentration using dis, strips or tablets. We can evaluate how susceptible the microbes are to the antibiotics in the disk-thanks to-disk diffusion.

Anytime you spread antibiotics into the culture media, the outcome of the antibiotics are being seen around the antimicrobial angle. Then after the antimicrobial concentration has diluted, it loses its potency and no longer cripple the growth that are supposed to occur in the bacteria, the area where the disk has dissolved on will be separated from the other part. The diameter area of the barrier within the antimicrobial disk serves as the minimum inhibitory concentration (MIC) for that bacterium and antimicrobial combination, the area of restraint is in opposition to test bacterium's MIC.

3.6.2 Broth concentration method

Broth concentration is used to measure the amount of bacterium of a proposed choice, or the desired concentration and this concentration is tested against another which is made up of antimicrobial agent and this is done on a liquid medium which has been prepared for that purpose, documented interpretation. The two ways to perform broth dilution methods are, first by using tubes that contain a minimum volume of 2ml this is also known as microdilution, the second method is using a smaller volume by making use of microtitration plates (microdilution). The quantity of irregularities that could occur during the production and dilution of antimicrobials from different laboratories might be decreased when we utilize the same microdilution plates. The use of these plates and a written test methodology with the specification of relevant

reference organisms would be beneficial for comparing the results obtained from different laboratories.

3.6.3. Agar concentration method

This process involves incorporating different antimicrobial concentrations substance put all of them into an agar medium, by using the standard number of cells you are supposed to use on the surface of the agar plate. You can read the plates by checking properly the least of the antimicrobial concentration that you see kill the bacterial growth. The results are always treated as a very good one, the test bacterium/antimicrobial combination`s MIC is then determined using this information.

3.7. The BD Phoenix System.

The disposable plates, broths used for ID and AST, AST indicator, an appliance, software, and broths are all components of the BD Phoenix System. Improved conventional fluorogenic, and chromogenic substrates are used in this ID method. The AST technique is a mini dilution test based on broth that enhances the detection of organism growth by using a redox indicator. The NMIC/ID-26 panels were therefore also utilized in this study. In the ID broth, the test organism underwent a 0.5 McFarland deferment. After confirming the suspension`s density using a crystal Spec Nephelometer, 25gL of the suspension was added to the AST broth. The AST soup has previously received one drop of the AST indicators. After confirming the suspension`s density using a crystal Spec Nephelometer, 25 gL of the suspension has already had single drop of the AST indication added to the AST broth. The panel`s ID wells were then cultured using the suspension in the ID broth, while the AST wells received an inoculation using the suspension in the AST broth. The panels were loaded into the apparatus, and it read the panels at 20-minute intervals. Group measurements, IDs and Minimum Inhibitory Concentrations (MICs) are created. The evaluation of every antibacterial agent`s MIC values resulting in Susceptible, Intermediate, or Resistant (SIR) results of every antibacterial agent`s categories uses organism identification. Even though the majority of IDs took 2-3 hours to complete, and MICs took 6-8 hours, the final findings for ID and AST are available in 2-12 hours and 4-16 hours, respectively. The BD Expert system software is another component of the Phoenix system. It assesses ID AST outcomes against pre-established benchmarks and

notifies the user of anomalous outcomes and patient circumstances that may necessitate further intervention. Nadarajah R and others (2004).

3.8 Statistical Analysis:

Following the effective data collection, statistical consultations using SPSS version 22 were used to analyze the data, and the outcome will be evaluated against existing literature.

CHAPTER FOUR

4.1 RESULT

In this retrospective study, between 01.01.2019 and 31.12.2021, Near East University (NEU) Hospital, Aspirate and sputum samples of 2408 patients sent to the Microbiology Laboratory evaluated. Of the patients, 1488 (61.8%) are male, 920 (38.2%) are female, and their average age are 68.64±17.17 (between 0-98 years old). Growth was observed in 1334 (55.4%) of the samples sent, while non-growth was observed in 1074.There was no reproduction (44.6%). Of the patients with reproduction, 846 (63.4%) were male, 488 (36.6%) were female and their mean age was 70.89±15.60 (between 0-98 years). Statistically significant between the average age of patients with and without growth in their samples a difference was observed. Accordingly, average age of patients with reproduction was compared to those without reproduction was found to be high (p=<0.0001). Thus, high age is a risk factor for bacterial pneumonias. We can say it is when gender and reproductive status were compared, male patients It was determined that the growth in aspirate/sputum cultures was higher than in women (p=0.037). When the aspirate/sputum cultures over the years are evaluated, 54.5% (360/660) in 2019, There was a reproduction rate of 53.1% (436/821) in 2020 and 58.0% (538/927) in 2021 Although there was an increase in the year compared to other years, no statistical difference was detected not (p=0.103). In addition, the reproductive rate in inpatients compared to outpatients was found to be significantly higher (p=0.001). Table 1 shows gender, years, and inpatient/outpatient Reproductive status according to the parameters are shown.

Table 1

The Growth, Number of growth p value

	Growth	**No of growth**	**p value**
Male	836 (%63.4)	642 (%59.8)	0.037
Female	488 (%36.6)	432 (%40.2)	
2019	360 (%27.0)	300 (%27.9)	0.103
2020	436 (%32.7)	385 (%35.8)	
2021	538 (%40.3)	389 (%36.2)	
In-patient	1283 (%96.2)	1002 (%93.3)	0.001
Out-patient	51 (%3.8)	72 (%6.7)	

The growth versus the value in general for male and female, we have 0.037. For the three years which is between 2019-2021, the prevalence is 0.103. For the in-patients and the out-patients, the p value is 0.001, which shows that the in-patients dominated the out-patients.

Table 2:

Patients with growth observed in their samples according to their department

Department

		Frequency	Percent
Valid	Emergency	6	.4
	Cardiology	166	12.4
	Ear Nose Throat	2	.1
	Neurology	17	1.3
	Oncology	62	4.6
	Orthopedics and Traumatology	13	1.0
	Urology	3	.2
	Intensive care	732	54.9
	Infectious Diseases	61	4.6
	Brain surgery	28	2.1
	Child Health and Diseases	11	.8
	Internal medicine	25	1.9
	General surgery	17	1.3
	Geriatrics	31	2.3
	Chest Diseases and Allergy	158	11.8
	Gynecology and Obstetrics	2	.1
	Total	1334	100.0

The patients whose samples were observed to grow were evaluated according to their departments and it was determined that the most common samples were sent from Intensive care (54.9%, 732/1334), Cardiology (12.4%, 166/1334) and Chest Diseases and Allergy (11.8%, 158/1334) services.

Table 3:

Distribution of growing microorganisms

Microorganism

		Frequency	Percent
Valid	Streptococcus pneumoniae	4	.3
	Citrobacter koseri	6	.4
	Escherichia coli	110	8.2
	Enterococcus faecium	22	1.6
	Enterococcus faecalis	17	1.3
	Enterobacter cloacae	37	2.8
	Klebsiella oxytoca	18	1.3
	Enterobacter aerogenes	13	1.0
	Citrobacter freundii	2	.1
	Candida species	95	7.1
	Burkholderia cepacian	3	.2
	Stenotrophomonas maltophilia	54	4.0
	Acinetobacter baumannii/calcoaceticus complex	316	23.7
	Staphylococcus aureus	83	6.2
	Serratia marcescens	58	4.3
	Pseudomonas aeruginosa	233	17.5
	Providencia species	5	.4
	Proteus mirabilis	38	2.8
	Morganella morganii	2	.1
	Klebsiella pneumoniae ssp pneumoniae	218	16.3
	Total	1334	100.0

The most isolated microorganisms from aspirate/sputum samples sent to our laboratory were *Acinetobacter baumannii/calcoaceticus* complex (23.7%, 316/1334), *Pseudomonas aeruginosa* (17.5%, 233/1334*), Klebsiella pneumoniae ssp pneumoniae* (16.3%, 218/1334), respectively. *Acinetobacter baumannii/calcoaceticus* complex grew significantly higher in aspirate/sputum samples sent from the intensive care unit (ICU) at the hospital compared to other services (p=<0.0001)

Table 4

			department2				
			Intensive care	Cardiology	Chest Diseases and Allergy	Others	Total
MO2	Acineto	Count	215	35	29	37	316
		Expected Count	173.4	39.3	37.4	65.9	316.0
		% within MO2	68.0%	11.1%	9.2%	11.7%	100.0%
		% within department2	29.4%	21.1%	18.4%	13.3%	23.7%
	Other	Count	517	131	129	241	1018
		Expected Count	558.6	126.7	120.6	212.1	1018.0
		% within MO2	50.8%	12.9%	12.7%	23.7%	100.0%
		% within department2	70.6%	78.9%	81.6%	86.7%	76.3%
Total		Count	732	166	158	278	1334
		Expected Count	732.0	166.0	158.0	278.0	1334.0
		% within MO2	54.9%	12.4%	11.8%	20.8%	100.0%
		% within department2	100.0%	100.0%	100.0%	100.0%	100.0%

Enteric bacteria most commonly isolated from aspirate/sputum samples were *Klebsiella pneumoniae ssp pneumonia*, followed by *Escherichia coli and Serratia marcescens*

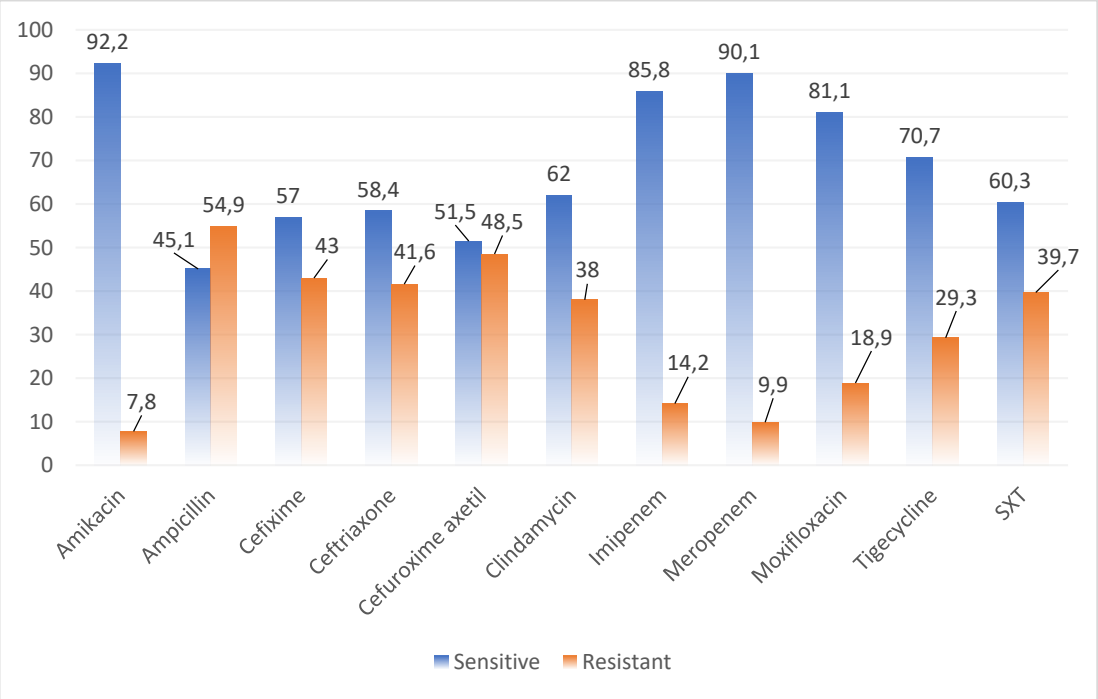
which has the following percentage (43%, 21.7% and 11.4%, respectively). The resistance rates of enteric bacteria to antibiotics are given in Graph 1. The ESBL rate in enteric bacteria was determined as 40%.

Table 5

Enteric bacteria

		Frequency	Percent
Valid	Klebsiella oxytoca	18	3.6
	Escherichia coli	110	21.7
	Enterobacter cloacae	37	7.3
	Enterobacter aerogenes	13	2.6
	Citrobacter koseri	6	1.2
	Citrobacter freundii	2	.4
	Serratia marcescens	58	11.4
	Providencia species	5	1.0
	Proteus mirabilis	38	7.5
	Morganella morganii	2	.4
	Klebsiella pneumoniae ssp pneumoniae	218	43.0
	Total	507	100.0

Figure 1: Resistance rates of enteric bacteria to antibiotics



Of the gram-positive bacteria isolated from aspirate/sputum samples, *Staphylococcus aureus* (65.9%, 83/126) was the most common. The MRSA rate was 42.2%.

Table 6

Gram positive bacteria

		Frequency	Percent
Valid	Streptococcus pneumonia	4	3.2
	Enterococcus faecium	22	17.5
	Enterococcus faecalis	17	13.5
	Staphylococcus aureus	83	65.9
	Total	126	100.0

4.2 DISCUSSION

This study`s primary objective is to determine the percentage of the microorganisms infecting Near East Hospital and how susceptible they are to medications. 2408 aspirate/sputum samples were collected between January 2019 and December 2021, of these, 1334 (55.4%) yielded significant bacterial growth on culture media, with a heightened propensity in aspirate samples linked to sputum. A comparable percentage was reported in Duan, Nu, J., Huang, C., and 2020`s articles when compared to ours. Males were more likely than females to have LRTIs (61.8% vs. 38.2%). According to Singh and Sharma (2020), smoking, drinking, and COPD are some linked risk factors for respiratory tract infections that may account for the increased prevalence of LTRIs in man. The incidence of LRTIs grows quickly with age, peaking between 60 and 79 years old, according to the age range distribution. Similar investigations have found that samples from individuals with lower respiratory tract infections primarily contain types of gram-negative bacteria. In current analysis, single species was discovered in 74.7% of the cases, but polymicrobial (single species) whereas polymicrobial growth was only found in 80% of the instances, growth was seen in 20% of the cases (S. Khan, Priti, and Ankit, 2015). *Acinetobacter baumanni*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, were the primary pathogens responsible for LTRI. This research is related to earlier investigations, such as those by Christopher Aye Egbe and colleagues, in which Klebsiella spp. and Pseudomonas spp. were the most common causes of mixed infections (Rouze, A., Boddaert, P., et al., 2020 and Shah, M.U. and others.)

Colistin was the most efficient antibiotic almost 100% sensitivity, whereas the lest efficient antimicrobial were Amoxicillin/Clauvulanic acid, Cefotaxime, Oxacillin, Ciprofloxacin, Ertapenem, Gentamicin, Imipenem, and Trimethoprim/Sulfamethoxazole (98-100% resistance) when tested against *Acinetobacter baumannii* isolates. The factors might be connected to the increased susceptibility of this soecies for acquiring resistance genes easily as well as its capacity of endure and thrive in a medical setting. The high incidence of first-line antibiotic resistance in A. baumannii isolates medications underscore the need to discover additional potent compounds to migrate this danger (2018) Xie, R., et al. Pseudomonas aeruginosa is the most prevalent species of isolated Gram-negative bacteria. Unlike Ciprofloxacin and piperacillin/Tazobactam, which are less effective than the others

(40-50% resistance), the three antibiotics that were most effective against P. aeruginosa were Colistin, Gentamycin and Amikacin (less than 20% resistance).

Staphylococcus aureus is one of the most isolated Gram-positive bacteria, are often resistant to Penicillin G and in some way to Oxacillin, you can also include Macrolides because some of them are resistant to it also, Lincosamides and Fluoroquinolones also belong to that group. One antibiotic that effectively combats S. aureus is Tigecycline, Vancomycin, Gentamicin, Tetracycline, Teicoplanin, and Linezolid are other examples of antibiotics. These medications all have a rate of resistance less than 10%. Very low resistance rates are also displayed by Oxacillin, Vancomycin, and Teicoplanin, nonetheless, investigations have revealed that resistance rates are rising. This information leads me to conclude that the present surge in resistance is due to a spike in MRSA strains of S. aureus that are resistant to Oxacillin and are thus treated with vancomycin and Teicoplanin (Petrillo, F. and others, 2020). *P. aeruginosa* is one of Gram-negative bacteria another on is *Klebsiella pneumoniae*, they are most resistant to Fluoroquinolones, Cephalosporins and Penicillin. The most powerful antibiotic is Colistin, which has resistance rates higher than 10%. Tetracyclines, carbapenems, and aminoglycosides also have higher resistance rates, at about 45%. With the exception of Piperacillin/Tazobactam, which has less resistant than what is shown in our investigation, with a stated resistance rate of 18.2%, K, pneumoniae exhibits the same resistance rates in Ahmed et al. According to Prestinaci et al (2015), this variation may result from a different actual antimicrobial treatment and a distinct geographical area. According to recent study, gram negative bacteria are a major contributor to LRTIs Regha, I., Sulekha, B., 2018) and have been linked to K. pneumoniae`s increased resistance to carbapenems and fluoroquinolones. In a different way, S. aureus is the most commonly isolated and reported gram-positive bacterium, and it has a high resistance to the antibiotic's vancomycin and linezolid. In underdeveloped nations, LTRI tops the list of infectious disorders, according to recent WHO assessment on their epidemiology. This study shows the prevalence of the main LTRI causing bacteria and how easily the most popular antibiotics can treat them in healthcare facilities. Our research demonstrates the isolated bacteria`s strong resilience. More quickly than ever before, fluoroquinolone and current cephalosporin resistance is emerging. This can be caused by different things like abusing of drugs and change in the structure or form of infectious organisms. [Manyi-Loh, C.; Mamphweli, S.; et al., 2018].

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

Bacterial that causes diseases are different from one region to another, and population of a particular community also matters a lot. Since this retrospective study, it focused mostly on LRTIs, it is very important that we increase our focus on how to control these bacterial to limit the cause of these diseases. Our great concern right now is to be able to easily identify these organisms that are causing infections and how they are resistance to antibiotics, so that we will be able to know the right antibiotics to use, but for us to be able to do that, we must carry out a susceptibility test using the available antibiotics to be able to take the most points and effective antibiotic therapy. However, our research has certain limitation because it only focuses on three (3) years data of patients at Near East University Teaching Hospital.

Between 1st of January 2019 to 31st of December 2021, according to the retrospective research carry out at the Near East University teaching Hospital, microbiology laboratory, there was an increase in microorganisms which were resistance to antibiotics, according to our reports some of those organisms are (*Acinetobacter baumannii/ calcoaceticus* complex with 23.7%, followed by *Pseudomonas aeruginosa* with 17.5%). Antibiotic resistance is not just a problem found in Cyprus it is a global issue and more ambiguous in developing countries where data are difficult to assessed, carrying out examination programs to determine the ubiquity of different resistant pathogens will play a great role in helping to manage patients care in the hospitals and health care centers.

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