An-Najah National University

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EVALUATION OF ANTIBIOTIC RESISTANCE AT A TERTIARY CARE HOSPITAL IN PALESTINE: A THREE- YEAR RETROSPECTIVE STUDY

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This thesis was defended successfully on 15/8/2022 and approved by

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Dedication

This thesis is dedicated …

To my beloved parents, for their spiritual, generous and emotional support and prayers, for all their unconditional love.

To my affectionate husband, for giving me a reason to keep going, for his patience and strength when I thought of giving up.

To my great brother, Mahmoud, for his invaluable advice and continuous support since childhood.

To my sweet little girl Nai, for her inspiring smile, for the time her mom took away from her to accomplish this work.

To Rania, Hend, Heba and the rest of my family and friends…for the support I have received from you all, I am forever grateful.

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My appreciation and thanks go to anyone who helped me directly or indirectly in the completion of this thesis.

Declaration

I, the undersigned, declare that I submitted the thesis entitled:

EVALUATION OF ANTIBIOTIC RESISTANCE AT A TERTIARY CARE HOSPITAL IN PALESTINE: A THREE –YEAR RETROSPECTIVE STUDY

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: Aya Ayed Abu-diak

Signature: 

Date: 15|8|2022

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EVALUATION OF ANTIBIOTIC RESISTANCE AT A TERTIARY CARE HOSPITAL IN PALESTINE: A THREE- YEAR RETROSPECTIVE STUDY

By
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Abstract

Background: Antimicrobial resistance is now one of the most critical medical threats worldwide. Historical facts and diverse data collected over the years have proved that something urgent should be done to control this growing phenomenon. The problem arises from the overuse and misuse of antibiotics. This has made —in some circumstances— the treatment of a simple infection a complicated process. Therefore, many countries around the world have found themselves compelled to devote efforts to control this antibiotic overuse and misuse in the hope of controlling it and its clinical, economic and societal consequences. Against this background, this study has sought to assess the frequency and antibiotic susceptibility patterns of the most common bacterial pathogens and candida isolated at An-Najah National University Hospital (NNUH) in Nablus, Palestine, between 2018–2020.

Methodology: A retrospective cross-sectional study was done to assess antimicrobial resistance patterns of the pathogens isolated from patients in different hospital wards during the study period (2018–2020), using sensitivity reports saved into the computerized database of NNUH microbiology lab. Data was then collected, coded and imported to Statistical Package for Social Sciences (SPSS) version 21 for further analysis. Descriptive statistics were run to generate frequency and percentages, tables and graphs. Approval was obtained from the university's Institutional Review Board.

Results: Out of 5, 585 clinical cultures, 46.9% were Gram-Negative Bacteria (GNB), 37.9% were Gram-Positive Bacteria (GPB) and 15.2% were candida. The most frequent Enterobacteriaceae was *ESBL E. coli* (15.6% of GNB) followed by *E. coli* (14%), *ESBL k. pneumoniae* (7.7%), *k. pneumoniae* (5.5%). *P. aeruginosa* and *A.*

baumannii were the most obtained Non-Fermenting Gram Negative Bacilli (NFGNB). For GBP, the most frequent isolate was *Coagulase-negative Staphylococci (CoNS)*, representing an isolation rate of 38.8% of GPB in total, followed by *E. faecalis* (18.6%), *S. aureus* (8.2%), and *VRE E. faecium* (7.5%). Clinical specimens were mostly collected from 27.7% of the urine samples. This was followed by wound specimens (19.6%) and blood specimens (19.2%). The total in-patients from all wards was 3, 895 cases (N%=69.7%). Of these, the surgical ward (SW) was the most prominent (14.6%) followed by the Intensive Care Unit (ICU): 10.7%. *A. baumannii* showed high resistance (over 85%) for ceftazidim, cefipim, gentamycin, ciprofloxacin, levofloxacin, piperacillin, and imipenem. *ESBL E. coli*. *ESBL k. pneumoniae* showed nearly complete resistance to ampicillin and cephalosporin agents. *MRSA S. aureus* was also nearly completely resistant to penicillin, oxacillin, amoxicillin\clavulinic acid, cefuroxime. *VRE E. faecium* showed full resistance to penicillin, amoxicillin\clavulinic acid, ampicillin, vancomycin, levofloxacin, ciprofloxacin, and erythromycin.

Conclusions: High rates of resistance were observed during the study period for most isolated bacteria species. Efforts need to be made on enhancing appropriate control and monitoring measures to stop the overuse and misuse of antibiotics and limit the prevalence of Multi-Drug Resistant Organisms (MDRO).

Keywords: Antibiotics; resistance; Palestine; MDRO.

Chapter One

Introduction and Literature Review

1.1 Overview

Antibiotics discovery in the mid-20th century was seen as a “medical miracle” that revolutionized modern medicine in many aspects. In fact, it has become one of the most significant medical interventions since it is believed to be the backbone of the success for many critical medical conditions, such as solid organ transplantation, surgical procedures, and sepsis (1). Moreover, it has been used extensively for cases at high risk for developing bacterial infections including patients with end-stage kidney disease, or patients receiving cancer therapy (chemotherapy), as well as procedures like intubation or catheterization (2).

They are likely the best medication class being used for enhancement of human wellbeing, which allowed for better life expectancies for patients with infectious diseases (3) after it was a leading cause of death (e.g., accounting for one-quarter of deaths in England up to the early 1900s), it is now treated with ease with this prodigious therapeutic agent (England’s mortality of infectious diseases retracted to less than 1% after antibiotics introduction) (4).

To assure the long-term availability of efficacious treatment for bacterial infections, appropriate consumption of existing antibiotics is a stage requirement (5). The excessive use of antibiotics at the societal level, lack of regulatory rules for the ideal use of antibiotics at the political level and diminished financial impetuses that inhibit new drug development are all direct causes of the antibiotic resistance crisis. Without rational antibiotic use, medicine will fail to treat even simple infections suggesting the payment of high costs in terms of mortality and morbidity (6).

Regrettably, the evolution of antibiotic resistance among the most important bacterial pathogens is considered as a main cause of concern that has a real impact on all parts of a human’s life. Efficacious prevention and treatment of an ever-expanding scope of diseases has been limited by antimicrobial resistance (AMR), which makes the treatment of patients a wearisome process and sometimes impossible (7). Indeed, the World Health Organization (WHO) has named antibiotic resistance as one of the three

most significant public health problems. Therefore, governments and individuals have to pay special attention to this global threat that may ruin the achievements of modern medicine. This leads many countries around the world to dedicate endeavors in the hope of controlling the problem and its clinical, economic, and societal impact (8).

There is a serious threat now about the emergence of bacteria that is resistant to many antibiotics, which increases the burden of infections and mortality rate, and poses a real challenge for physicians to manage critical cases (9). These multi-drug resistant (MDR) organisms referred to as ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.)* are now rendering classical generations of antibiotics inactive gradually, making the development of therapeutic alternatives a mandatory requirement (10).

Unfortunately, the rate in which antibiotic-resistant pathogens are rising exceeds new antibiotics development. The fields of molecular biology and genetics have not yet fully utilized genome revolution for new antibiotic's discovery and are assessing its resistance, even though it is known that the first living organism to have its entire genome sequenced was bacteria (11). However, if new drugs are discovered, without real changes in the way we prescribe and use antibiotics, it will still be an existing problem.

In Europe, most of the antibiotics dispensed for medical purposes were used by non-hospitalized patients, suggesting that the emergence of resistant organisms is not limited to a hospital environment but also identified in community settings, adopting the principle of "survival of the fittest". Luckily, resistant bacteria may be less fit than susceptible one. Therefore, reasonable use of antibiotics may help to overcome this crisis in the absence of new drug development by pharmaceutical industry (5).

1.2 Background and problem statement

Historical facts and diverse data collected over the years prove that something urgent should be done to control the increase in antibiotic resistance. This is a matter of global concern since the underlying causes are not fully comprehensive and there is a lack of strict strategies to control this potential catastrophe. Since the past decade, several

international organizations, such as WHO, the Centers for Disease Control and Prevention (CDC), and the World Economic Forum have listed antibiotic resistance as a high-priority issue and undertake drawing the community's attention through reports, conferences, and activities (12). In 2011, the WHO explained the problem of antibiotic resistance under the title "Antibiotic resistance: No action today, no cure tomorrow"(13).

1.2.1 Brief history of antibiotics

During the pre-antibiotics era, communicable diseases were the prime cause of human mortality and morbidity with a short lifespan of 47 years even in developed countries. The situation changed after the discovery of antibiotics. The leading cause of death became non-infectious diseases, and the average life expectancy improved remarkably (e.g., 78.8 years in the US) (14).

Antibiotics were first defined as a substance produced by a microbe that inhibits the growth of other microorganisms. The overwhelming majority of novel antibiotics with useful bioactivities were isolated from soil bacteria or other natural products (15). The genus *Streptomyces* alone was the source of more than 55% of the known natural products antibiotics classes discovered between 1945 and 1978 with the rest derived from other bacteria strains or fungi. As the chemical chemistry field developed, it became possible for researchers to modulate or synthesize natural antibiotics in the laboratory. However, the total synthesis of such antibiotics is a painstaking process since their molecule's structure is highly complex (16).

Old medical documents aged more than a millennium prove the use of substances with potential antibiosis activity to treat open wounds. Moldy bread and curative soil were used as anti-infecting agents (17). However, the first systematic screening approach for antibiotic's discovery was the arsenic-based drug salvarsan by Paul Ehrlich in 1909, which was the first drug against *Treponema pallidum*, the etiologic agent of syphilis (18). In 1937, Ehrlich's work was the inspiration for Domagk and his colleagues to discover the first effective broad-spectrum antibiotic in clinical use, which is sulfonamides. Its production was preceded by Fleming's discovery of penicillin in 1928 but its complete synthesis was done in 1949. In fact, 2022 is the 82nd anniversary of the first human use of penicillin (19).

The discovery and preparation of antibiotics increased dramatically between 1950s and 1970s in what is known as the golden age of antibiotics era. Regrettably, no new classes of antibiotics have been discovered since then; only modulations to existing groups (20).

1.2.2 Antibiotics classifications

Antibiotics can be grouped based on several different criteria including the mode of action, spectrum of activity, and molecular structure (21). Some antibiotics inhibit the bacterial action at its clinically used concentration called bacteriostatic. Whereas, the others are able to completely stop the bacterial growth and reproduction called bactericidal (22). Some drugs are known to have clinical activity against a wide range of gram-positive and negative bacteria regarded as broad-spectrum antibiotics (not differentiating pathogenic and beneficial bacteria). Whereas, the others are narrow-spectrum antibiotics that have a clinical effect against few types of organisms (23). The most commonly used classification is based on antibiotic chemical structure, which directly affects the mechanism of attacking the infecting bacteria (Table 1.1). To achieve bacterial inhibition, antibiotics have to disturb bacterial vital pathways or enzymes without exerting harmful effects to the host cells by targeting processes different or absent in eukaryotic cells, such as disturbing bacterial DNA transcription or replication, protein synthesis, folic acid metabolism, cell wall biosynthesis, and cell membrane integrity (24).

Table 1.1

Antibiotic classes and their mechanism of action (24)

	Antibiotic class	Examples	Spectrum	Mechanism(s) of action
1	Beta-lactams			
A	Penicillins	Amoxicillin, Ampicillin	varies depends on the sub-class	Disrupt the synthesis of bacterial cell wall peptidoglycan
B	Cephalosporins			
B.1	1st generation	Cefazolin,Cefalexine	Gram Positive	
B.2	2nd generation	Cefuroxime,Cefoxitine	Less Gram Positive more Gram Negative	
B.3	3rd generation	Cefotaxime,Ceftriaxone	Mainly Gram negative exept Pseudomonas	
B.4	4th generation	Cefepim	Covers Pseudomonas	
B.5	5th generation	Ceftroline,Ceftopiprole	For MRSA	
C	Carbabenems	Imipenim,Meropenim	Gram Positive and Negative	
D	Monobactams	Azetronam	Gram Negative	
2	Aminoglycosides	Amkacin,Gentamycin	Gram Negative	Disrupts bacterial protein synthesis by binding to 30s ribosomal sub-units
3	Glycopeptides	Vancomycin,Tecioplanin	Gram Positive	Inhibiting peptidoglycan synthesis
4	Macrolides	Erythromycin,Azithromycin	mostly Gram Positive	Disrupts bacterial protein synthesis by binding to 50s ribosomal sub-unit
5	Quinolons	Ciprofloxacin,Levofloxacin	Gram Positive and Negative	Inhibiting DNA replication and transcription
6	Tetracyclines	Tetracycline,Minocycline	Gram Positive and Negative	Disrupts bacterial protein synthesis by binding to 30s ribosomal sub-unit
7	Chloramphenicol	Chloramphenicol	Gram Positive and Negative	Disrupts bacterial protein synthesis by binding to 50s ribosomal sub-units
8	Lincosamides	Clindamycin	Gram Positive	Disrupts bacterial protein synthesis by binding to 50s ribosomal sub-unit
9	Streptogramins	Quinopristin\Dalfopristin	mostly Gram Positive	Disrupts bacterial protein synthesis by binding to 50s ribosomal sub-unit
10	Rifamycins	Rifampicin	Gram Positive and Negative	Inhibits RNA polymerase, the enzyme responsible for DNA transcription.
11	Polymyxin	Polymixin B,Colistin	Mostly Gram Negative	Disrupts bacterial cell membrane
12	Oxazolidinone	Linzolid	Gram Positive	Disrupts bacterial protein synthesis by binding to 30s,50s ribosomal sub-units

1.2.3 Overview of antibiotic resistance

As the natural products with antibiotic activity are ancient in nature, resistance is equally ancient and may even precede antibiotic's discovery, meaning that the evolution of resistance is an essential element of antibiotics and an inevitable result as it is one of the natural acclimatization strategies for bacterial existence (25).

Antibiotic resistance occurs when the antibiotic fails to stop the bacterial growth efficiently at its therapeutic level, which signifies a lack of sensitivity to those drugs. Bacteria can be said to be resistant when it continues to replicate even in the presence of suitable antibiotics designed to kill it (26).

The global collection of antibiotic resistance genes (ARGs), including those associated with pathogenic bacteria isolated in clinics or non-pathogenic environmental bacteria and all other resistance genes, is referred to as “antibiotic resistome” (27). In 1973, researchers found that environmental ARGs are genetically similar to clinical ARGs, which means that environment is a vast source of these genes, which is a main mediator for the resistance process. The term “antibiotic resistome” was proposed for the first time in 2006 after 30 years of being intensively studied (28).

1.2.4 Antibiotic resistance mechanisms

Indeed, antibiotic resistance is a trait that could be acquired and arises in previously susceptible bacteria in a process of horizontal gene transfer or it could be found intrinsically within the bacterial genome and disseminated vertically as a result of spontaneous mutations within chromosomal genes (29).

For antibiotics to have their desired effect, they should penetrate bacterial envelopes in a concentration high enough to cause the required action. In bacterial defense mechanisms, bacteria may even alter the antibiotic’s concentration or modify the target of the antibiotic within bacterial cell or alter the antibiotic itself (2). This could be achieved on a genetic basis or mechanistic basis. The genetic defensive strategy that bacteria adopts to repel the antibiotic attack depends on horizontal gene transfer by acquiring foreign DNA coding for resistance components through:

I. The conjugation process requires physical contact between a donor and recipient cells. When conjugation occurs between two plasmids (which is a genetic element replicated separately from chromosome), the transmission of ARGs that is carried within the plasmid may occur (30).
II. The transduction process occurs when a virus that infects a bacteria accidentally packages bacterial genetic material from the chromosome during phage assembly. Resulting in infecting a virus that may insert the bacterial DNA to a newly hosted bacteria (31).
III. Transformation process through which the bacterial cell picks and incorporates external naked DNA even in a natural way from the environmental cells or artificially to produce recombinant DNA in the host cell (32).

The second strategy used by the bacterial cell to overcome bacterial action is the mechanistic mechanisms. Based on the biochemical route used throughout the resistance process, we can categorize it as follows (1), (33):

1. Alterations to the drug itself even by chemical processes or by enzymatic destruction to the antibiotic molecule;
2. Reduction of the antibiotic's cellular concentration even by preventing the antibiotic molecule from entering the cell or by overexpressing the efflux system that extrudes the molecules outside the cell;
3. Modifications to the target site that the drug molecule binds to by protecting it from the binding process or mutating the target site.

1.2.5 Multi-drug resistance organisms

Since the first antibiotic introduced into commercial use until now, vast amounts of antibiotics are consumed globally, with manufacturing amount estimated to be 100000 tons each year. The overuse and misuse of antibiotics in humans, animals, and even fish have driven the rapid emergence of bacterial strains that are resistant to many classes of antibiotics, the phenomenon of multidrug resistance (34). It is considered one of the most serious threats in clinical practice, which poses a specific pressure on all life sectors. It is unquestionable that multi-drug resistance drives patients to life threatening conditions, increasing the morbidity and mortality rates. It causes a lack of confidence in conventional medicine because of the misgivings in diagnoses and the failures in treating many cases. It also affects the economic sector in particular since it increases the treatment cost, prolongs the patient's hospital stay, and reduces productivity in community (35). As example, the cost of prolonged hospitalizations due to MRSA infections in 31 countries that get involved in the European Antimicrobial Resistance Surveillance System was 44 million Euros in 2007 (36).

Antibiotics are widely used in animals husbandry since their production in 1940s. It is added to animal's food to control diseases or as a preventive. One of the antibiotics vital uses in veterinary is growth promotion. Antimicrobial growth promoters (AGPs) were used for the first time in the mid-1950s adding them to chicken and pig feed to improve their production (37). Inevitably, any time antibiotics are consumed extensively, whether by human or animal, it exacerbates the crisis (38). For example, ESBL

(extended spectrum beta lactamase) *K. pneumoniae* exhibiting resistance to sulfonamides and beta-lactams were found to be implicated in foodborne nosocomial outbreaks in Spain in 2008 (39). However, it is interesting to know that the antibiotic use in healthy food-producing animals exceeds their use for human diseases treatment (40).

1.2.6 Rate and economic burden of antibiotic resistance

Antibiotic resistance genes were isolated from cave systems and permafrost where it originated long before human interventions, adapting to extreme life conditions (19). However, bacteria remained susceptible to most natural antibiotics during the pre-antibiotics era as it lacked current selection processes (41). Resistance started to emerge after each new antibiotic discovery, but it was not marked as a real public health threat because of the presence of many alternatives for the resistant one. Additionally, each antibiotic that was rendering to be resistant was followed by the development of more potent antibiotic meant that it was always possible to find an effective therapy (42). It is very important to identify the parameters that affect the rate of antibiotic resistance development and dissemination to understand what makes specific antibiotics highly resistant, whereas other antibiotics less.

Vast antibiotic exposure is a major driving force leading to antibiotic resistance evolution and dissemination making it a global crisis that may lead us to a post-antibiotic era (41). In a study done to assess trends of antibiotics consumption in 76 countries around the world, they found that the global antibiotic consumption increased by 65% over 16 years (2000–2015) from 21.1 to 34.8 billion defined daily doses (DDDs) where the antibiotic consumption rate increased by 39% from 11.3 to 15.7 DDDs per 1,000 inhabitants per day over the same period. Supposing no policy changes, the predicted global antibiotic consumption by 2030 will be 200% higher than the number in 2015. The increased use of last-resort antibiotics, such as oxazolidinones and glycylcyclines is noteworthy (43). Another study done by (44) reported that the consumption of carbapenems increased by 45% and polymyxins use increased by 13% between 2000 and 2010, noting that both are last-resort compounds. Antibiotics are widely misused by community and over dispensed by health care providers for needless cases such as self-limited infections or less indicative cases such as viral infections (45). The relationship between antibiotic resistance and consumption is well documented by

many cross sectional studies, especially in high-income countries (46). A study done between 2005 and 2013 monitored the relationship between antibiotic consumption and antibiotic resistance in a tertiary care hospital, focusing on cephalosporines, especially on ceftriaxone since it is the most used one in this family. They found that the use of ceftriaxone increased from 3·6 DBD (2005) to 10·78 DBD (2013). The antibiotic resistance rate of E. coli and P. mirabilis to ceftriaxone changed significantly from 22% in 2005 to 47% in 2013, and from 31% in 2005 to 60% in 2013, respectively (47).

Another parameter is the bacteria itself. Spontaneous mutations occurs naturally within the bacterial population at a rate of 1 in 10^5 to 10^8 as a result of errors during the DNA replication process. This may create new functionalities for bacterial cells or disrupt the existing ones. However, bacteria may protect itself against this type of mutations but if the bacteria develops a high rate of mutations, mutational resistance may be the result. This mutation may transfer vertically or horizontally (48). Horizontal gene transfer makes it possible for resistant genes to spread within a large part of environmental bacteria, especially those sharing the same habitat and are phylogenetically closed. In contrast, resistance factors are much lower when transferred to environmental pathogens as they occupy different habitats and are not much phylogenetically close. When a resistant gene enters the human pathogen, it will spread more to another human pathogen, not an environmental one (49). Studies found that antibiotics even in its sub-minimum inhibitory concentration or sub-lethal concentration stimulates resistant gene production and transfer. The bacterial pathogens that acquired resistant genes will proliferate a number of resistant bacteria, which will accumulate in the environment and spread again through vertical gene transfer (50). Humans would be exposed to infectious bacteria via many routes, the main one is from other persons. It could be through direct contact, aerosols, or food prepared by an infected person. Another route is the environment. For example, transmission of resistant bacteria through dust, contaminated food, air-borne aerosols, plants, sewage, and water bodies. Poor hygienic routines in hospitals, clinics, and crowded places may lead to a resistant bacterial outbreak after the resistant bacteria being disseminated to many patients (49).

It was estimated that antibiotic resistance resulted in about 35$ billion excess costs yearly in the US (51) , while the number is €1.1–1.5 billion yearly in the European Union and European Economic Area countries. Low-income regions suffer more (52).

In China, a study showed that MDR infection represented 15.7% of all bacterial infection. The economic burden of these resistant infections was estimated to be $77 billion during one year, which represents 0.37% of China's yearly gross domestic product (53). By 2030, a total of 24 million people would suffer from extreme poverty due to antimicrobial resistance (52).

1.2.7 Nosocomial bacterial infections

Nosocomial infections (NIs) are defined as hospital-acquired infections, developing within at least 48–72 hours of hospital admission. It could occur in different health care facilities, such as hospitals, clinics, and ambulatory settings. It could affect the health care providers or the patients before or even after discharge. The prognosis of NIs depend on the source of infection, which could be prosthetic devices, indwelling medical devices, and the etiologic bacteria (54).

The problem of NIs becomes wider each day. It affects all hospital departments, especially the ICU patients who usually suffer from underlying diseases and are immunocompromised. A study performed in 41 different hospitals in the Netherlands between 2007–2008 showed that the four major NIs sources are urinary tract infection that are usually associated with catheter use, primary bloodstream infections, surgical site infections and pneumonia usually associated with ventilator use. They accounted for about 70% of all types of NIs (55) and another study stated that the percentage is about 47.7% (56).

1.2.7.1 Surgical site infections

Surgical site infections (SSIs) are common complications for surgical procedures that lead to serious consequences, such as longer hospital stay, increased antibiotic use, increased economic burden, decreased patient's quality of life, and higher mortality rate. Many risk factors predispose for this surgical complication such as : surgery duration, classification of surgical wound, body mass index, and smoking (57). In fact, SSIs are reported as the third most frequent cause of all NIs causes. A study carried out in an Indian teaching hospital found that among 248 patients who underwent surgical interventions in the general surgery department, 45 of them had developed SSIs. They found that the infection rate was higher in urgent surgeries when compared with elective ones (58). However, the rate of developing SSI as well as the causative agents vary

widely from one setting to another based on the geographical location and the type of surgery. Another study performed in Oman reported that 58.1% of the checked surgical wound were positive for bacterial infection. The most predominant isolated pathogens were *S. aureus* (20.5%), followed by *P. aeruginosa* (13.7%), *K. pneumoniae* (9.6%), *and Methicillin-Resistant Staphylococcus aureus (MRSA)* (5.5%) (59). Another study carried out in Italy reported that the most frequent bacterial species that were isolated from surgical wounds are *S. aureus* (37%), followed by *P. aeruginosa* (17%), *P. mirabilis* (10%), *E. coli* (6%), and *Corynebacterium spp.* (5%) (60).

1.2.7.2 Ventilator associated pneumonia

The primary cause of infection in the ICU is the ventilator associated pneumonia (VAP). It is defined as pneumonia occurring within 48–72 hours after hospital admission. The prevalence rate ranges from 6–50 cases infected with VAP for each 100 ICU-admission and is estimated to occur in 9–27% of ventilated patients. There is sufficient evidence to suggest that the incidence of VAP increases with the length of mechanical ventilation, with the highest risk of developing the infection during the early days of hospitalization with an infection rate of 3% each day during the first 5 days in the ICU, the risk reduced after that to be 2% in days 5–10 of intubation. Early onset VAP that develops during the first 4 days of intubation occurs usually as a result of antibiotic-sensitive bacteria. Whereas, late onset VAP is usually attributed to MDR organism (61),(62). According to (63), the most common microorganisms attributed to cause VAP are: Gram-negative bacilli such as *E. coli, P. aerugenosa,* and *Acinetobacter spp.,* and Gram-positive cocci like *S. aureus*, especially the methicillin resistant one (MRSA).

1.2.7.3 Nosocomial urinary tract infections

Nosocomial urinary tract infection (NUTI) is considered the most frequent health care-associated infection (about 31.2% of NIs). The predominant risk factor for this complication is either having a permanent urethral catheterization which represents 80% of NUTIs or have had genitourinary or urological interventions (10–20%). The source of infection could be endogenous caused by the normal flora colonized in the rectum or vagina or exogenous caused by contaminated medical equipment or transmitted by contaminated medical-care providers hands (56). NUTI could be complicated (CNUTI)

or uncomplicated NUTI and the etiologic pathogens differ between these two categories. For uncomplicated NUTI, *E.coli* accounts for (75%–85%) of cases, followed by *K. pneumoniae* (about 6%), *S. saprophyticus* (about 6%), and the remaining agents are *Enterococcus spp*., *group B streptococcus (GBS)*, *Proteus spp*., and *P. aeruginosa*. In contrast, causative agents in CAUTI are *E.coli*, which accounts for about 24% of cases followed by *Candida spp.* (17.8%), *Enterococcus spp.* (13.8%), *P. aeruginosa* (10.3%), *Klebsiella spp.* (10.1%), *Proteus spp.* (4%), and others (64).

1.2.7.4 Primary bloodstream nosocomial infections

Primary bloodstream infections (BSIs) are considered a serious health care associated problem around the world. The highest frequency of this nosocomial infection found within the ICU department is about 49% according to the surveillance and control of pathogens of epidemiologic importance (SCOPE) data system in the US(65). A study was conducted over a duration of one year (2006), which had monitored all patients admitted to ICU to assess the development of BSIs. The results showed a rate of 75 per 1000 admission to ICU had developed BSI. The etiologic pathogen was mostly gram positive with a percentage of 69.1%. *MRSA* was the most frequent (18.9%), followed by *CoNS* (16%). Gram-negative bacilli were reported in 29.1%. *K. pneumoniae* was the most prevalent (10.3%) followed by *E. coli* (8.6%) and *Candida spp*., which was reported only in (1.7%) of isolated pathogens (66).

1.2.8 New drug development

The majority of the antibiotics available now in the clinical practice originated from discoveries made before 1970 (the golden age). In the last few decades, most pharmaceutical companies have ceased their investments in antibiotics research and development as a result of the limited positive clinical results and poor returns on investment in this research sector, rendering it to be less attractive for companies when compared with other therapeutic areas. In fact, 15 of the 18 largest pharmaceutical companies have stopped their research in this field in the last 30 years. Only few companies have taken on the challenge and continued their efforts in developing novel antibiotics (67), (68). Between 1980s and 2000, the number of new FDA approvals of antibiotics declined by 90%. Many reasons can be attributed to the failure of antibiotic developing programs. Conventional antibiotics that were derived from the natural

environment were large and complicated. Whereas, the newly developed ones are small and simple, thus, exposing it to rapid resistance selection. The other reasons are related to the inability to compete. Classical classes of antibiotics are able to target many binding sites allowing it to cover a variety of organisms involved in the infection, where newly developed drugs are genus, species or strain specific, suggesting the inability to cover all disease indications that could be caused by more than one bacterial strain. New antibiotics are also affected by marketing challenges since the cost of new drugs often is more expensive than generic antibiotics already available in clinical use (69). Between 1980 and 2009, 26 of the 61 antibiotics that entered the market were withdrawn mostly due to marketing failure (70).

Antibiotic development pipeline has to be reenergized to face the imminent global risk of multi-drug resistance. Today, many incentives are implemented nationally and internationally that are aimed at motivating the research and development (R&D) of antibiotics through special funding programs, such as Biomedical Advanced Research and Development Authority's (BARDA), Joint Programming Initiative on Antimicrobial Resistance (JPIAMR), and many others.

1.2.9 Candida infections

Candida is the most common cause of fungal infections. In fact, five candida species are responsible for about 90% of all fungal infections: *C. albicans, C. glabrata, C. tropicalis, C. krusei, and C. parapsilosis. C. albicans* is the most prevalent subtype, although its proportion relative to other candida species is decreasing with time. This is thought to be the result of the increasing incidence of *C. glabarta* as its sensitivity to Azole anti-fungals is going down (71).

Normally, candida may be found on the skin, mouth, vagina and gut without being clinically significant. However, it has the ability to provoke both systemic and superficial infections if it multiplies out of control or enters deep in the body. In fact, antifungal resistance is a growing issue in the whole world with geographical differences in the rate of infection. Although *C. albicans* is the most frequent strain, resistance among other strains is more common such as *C. glabarta*. Unfortunately, evidences suggest that patients with resistant candida are less likely to survive compered to patients having antifungal-sensitive candida infections (72).

1.3 Research question

- Does the problem of antibiotic resistance get worse with time in terms of higher frequency and percentage of resistant isolates between 2018–2020?
- Is there a relationship between multi-drug resistant organisms transmission and the hospital environment?
- Is there any hospital department affected by higher rates of drug resistance over others?
- Are there specific strains of bacteria that experience higher rates of resistance than others?
- What are the antibiotics that face the highest rate of resistance?

1.4 Hypothesis

Emergence of antibiotic resistance among the most important bacterial pathogens is considered as a serious challenge for the health care sector. What worsens the situation is the increasing incidence of multi-drug resistant isolates over years. Additionally, resistant organisms are disseminated in a high rate among hospitalized patients over out-patients, predicting that bacteria causing nosocomial infections are highly resistant. Moreover, we assume that the ICU department and surgical ward are affected by higher rates of resistant organisms over other hospital departments.

1.5 Main Objective

The general aim of thesis was to assess antimicrobial resistance patterns at NNUH in the period extending from 2018–2020.

1.6 Specific Objectives

1. To identify sample type (blood, urine, pus, etc.) and the percentage of bacterial isolate within each type;
2. To identify hospital departments and the percentage of bacterial isolate within each ward;
3. To record extraordinarily resistant bacteria and antibiotic resistance patterns;
4. To identify multi-drug resistant organisms;
5. To compare antibiotic resistance of the most common organisms in the above-mentioned three years.

1.7 Significance of the study

We carried out this study to assess antimicrobial resistance patterns of the most common clinical pathogens by analyzing data collected retrospectively in the period extending from 2018–2020 at NNUH in Palestine. This study provides us with sufficient information about the most prevalent bacteria in hospitals mainly and—with less extent—within out-patients, its resistance trends to the available antibiotics in the Palestinian market and the MDR bacteria disseminated in the highest frequency and the hospital wards showed its presence. To the best of our knowledge, the present study will be the first in Palestine performed over a period of three years, including all the hospital departments and includes both bacteria (gram positive and negative) and candida.

Results from this study will provide the best data for developing interventions and will help both policymakers and individuals to avoid this great threat to public health. Additionally, this may enhance the education programs within health care settings aimed at reducing the epidemiology of resistance and its economic burden.

1.8 Literature review

1.8.1 Overview

According to the Centers for Disease and Prevention's (CDC) report in 2021, at least 2.8 million people are infected with antibiotic resistant bacteria each year. Of them, more than 35,000 die as a result of the infection (73). Infections rang in their severity from simple, self-limited infections to serious life-threatening sepsis. Identifying emerging trends in antibiotic resistance is a critical need for health care workers, allowing the best antibiotic selection based on possible bacterial infections and known local susceptibilities (74).

1.8.2 Gram-negative bacteria

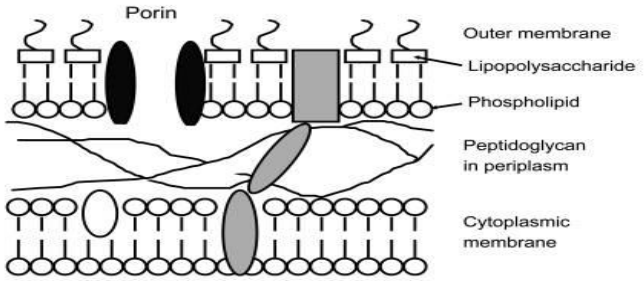
1.8.2.1 Appearance and groups

Gram-negative bacteria (GNB) are organisms of great clinical importance since they are highly resistant to antimicrobial agents, which complicates the treatment of serious medical conditions. Therefore, it leads to a higher risk of morbidity and mortality. GNB has two membranes: an outer membrane and an inner one. The outer membrane consists of a single layer of peptidoglycan containing lipopolysaccharide (LPS) in addition to

proteins and phospholipids. The LPS molecule is deemed as a virulence factor that could trigger a strong immune response when bacteria adhere to animal cells. This membrane is relatively permeable so it allows antibiotic diffusion through special channels called porins, excluding large and hydrophobic molecules. Sandwiched between the outer membrane and the inner membrane is a gel-like matrix found in the periplasmic space (the periplasm) that molecules have to invade after the outer membrane. It may also need to invade the inner membrane, often by energy-dependent uptake (75), (76). Figure 1.1 from (livemore,2012) shows GNB appearance.

Figure 1.1:

Appearance of gram negative bacteria (76)



Two main groups of GNB include most of clinically significant isolates: Enterobacteriaceae and the non-fermenters.

1.8.2.2 Studies related to Enterobacteriaceae resistance: Prevalence and epidemiology.

Enterobacteriaceae is a heterogeneous family widely diffused in nature, which includes numerous disease-causing pathogens. They represent about 80% of all gram-negative bacterial infections. The species that frequently cause human diseases are *Escherichia, Klebsiella, Proteus, Enterobacter, Citrobacter, Yersinia, Shigella, and Salmonella* (75).

A study was done in England (77) to find out trends in causative agents of bacteremia between 2004 and 2008, which reported that a percentage of 23% of bacteremia caused by *E.coli,* which represents the highest rate between all other microorganisms. According to (75), (76), *E.coli* is the most common cause of both nosocomial and community acquired urinary tract infection (UTI). It is also considered the major aerobic flora responsible for intra-abdominal infections. *Klebsiella spp. and Enterobacter spp.* are important isolates that contribute to cause pneumonia. Other

Enterobacteriaceae have been involved in diarrhea, meningitis, sepsis, endotoxic shock, peritonitis, cholangitis, and other infections..

In 2006, a study was conducted indicated that when third-generation cephalosporins as ceftriaxone, cefotaxime, and ceftazidime were first launched, it was almost universally active against Enterobacteriaceae. Within years of use, mutated strains of β-Lactamases that are resistant to β-lactams antibiotics started to emerge and spread. Within this family, resistance emerged first in *Enterobacter spp*., *Citrobacter freundii, and Serratia spp*. An example of resistant Enterobacteriaceae are the extended spectrum β-Lactamases (ESBL) producing organisms. They act by hydrolyzing penicillin, broad- and extended-spectrum cephalosporins and monobactams. In fact, genes that take the role of encoding ESBLs are found in the same plasmid that encode resistance to aminoglycosides and sulfonamides, this means that ESBL-producing Enterobacteriaceae are commonly multi-drug resistant organisms (78).

In 2016, a study (79) stated that a percentage of 60% or more of *E.coli* and *K. pneumoniae* are resistant to the most important β-lactams antibiotics in different parts of the world. The resistance of b-lactams and aminoglycoside among Enterobacteriaceae (especially *E.coli* and *K. pneumoniae*) have spread widely, making it mandatory to use other classes of antibiotics: carbapenems (such as meropenem) and fluoroquinolones (such as ciprofloxacin). However, co-resistance for these classes is well reported even in countries with strict polices in antibiotic use, such as Canada and Australia. For example, Ciprofloxacin resistance for *E.coli* isolates climbed from 5.4% in 2010 to 6.9% in 2012 in Australia, and from 21% in 2007 to 27% in 2011 in Canada.

A cross sectional study was conducted in West Bank-Palestine (80) to determine the prevalence and risk factors of ESBL-producing uropathogens. Out of 427 urine cultures checked for ESBL production, 163 were positive. The most frequently isolated uropathogen was *E. coli*, where the highest rate of ESBL production was in *K. pneumoniae*. After univariate and multivariate analysis were performed, risk factors for developing ESBL-uropathogen infections were identified. Recurrent UTIs represented the strongest risk factor (OR, 4.7:95% CI,2.68-8.27), followed by previous antibiotic use (OR, 3.07;95% CI,1.46-6.45). Other risk factors include hemodialysis, chronic kidney disease, diabetes mellitus, previous hospitalization, and catheterization. About

antibiotic susceptibility, ESBL-producing uropathogens were completely susceptible to fosfomycin and highly susceptible to piperacillin/tazobactam (94.5%), ertapenem (98.2%), meropenem (98.8%), amikacin and nitrofurantoin (93.9%).

In 2016, a review study was conducted (81) to assess antibiotic resistance in Enterobacteriaceae in Brazil, with special focus on β-lactams and polymyxins. With regard to β-lactams, the first published report about resistant Enterobacteriaceae to third generation cephalosporins was in 1994, in which cefepime resistance among ceftazidime-resistant Enterobacteriaceae was 52%. This was the first clue on the presence of ESBL-producing pathogens in Brazil. In 2000, ESBL production rate in *K. pneumoniae* reached 59.2% followed by *Enterobacter spp.* and *E.coli*. In 2005, after 20 years of being in clinical use, (82) reported the presence of CRE for the first time in Brazil. IMP-1 was the detected enzyme in a *K. pneumoniae* strain, which had been detected after that in a *P. rettgeri* isolate. However, KPC-2 is considered the most common carbapenem resistance-causing enzyme among Enterobacteriaceae. A publication was performed during 2011-2015 (83) proved the high jump of carbapenem resistance from 6.8% in 2011 to 35.5% in 2015. Unsurprisingly, 96.2% of the Carbapenem-resistant *K. pneumoniae* isolates were KPC-2 producers. With regard to polymyxin resistance in Brazil, a recent publication proved a growing problem. It described a jump in polymyxin b resistance from 0% in 2011 to 27.1% in 2015 among Carbapenem-resistant *K. pneumoniae* isolates.

A prospective study was performed in (2016) at An-Najah National University hospital in Nablus to assess the prevalence of ESBL production in isolates of *E. coli* and *E. cloacae.* Results showed high frequency of ESBL-producing *E. coli* in Nablus district. ESBL enzymes were detected in 73 out of 153 (47.7%) *E. coli* isolates and in 1 of 8 (12.5%) *E. cloacae* isolates. A randomized representative sample including 32 ESBL-producing *E. coli* was selected and subjected to polymerase chain reaction (PCR). Results proved the presence of 1 or 2 ESBL genes within each one of the 32 isolates. The most prominent gene was blaCTX-M, which was found within 30 isolates, whereas, blaTEM and blaSHV genes were detected in 2 and 1 isolates respectively (84).

A five-year retrospective study was performed to describe resistance trends of ESKAPE pathogens in South Africa. Among 64502 ESKAPE pathogens isolated between 2011–

2015, *K. pneumoniae* was the second most frequently isolated pathogen (22%) after *S. aureus* where *Enterobacter spp.* represented a percentage of 6.6%. Out of 14282 *K. pneumoniae* isolated pathogens, 54.5% were resistant to amoxicillin-clavulanate and 6.4% were resistant to meropenem. Increasing resistance rates among the years were clearly observed. Resistance to ceftriaxone increased from 54.6% in 2011 to 65.5% in 2015. Meropenem resistance increased from 5% or less between 2011–2014 to 16% in 2015. With regard to *Enterobacter spp.*, the average resistance rate was 5%, in which ciprofloxacin resistance was 16% for *E. cloacae* and 8% in *E. aerogenes* (85).

In 2020, a study was carried out in Gaza Strip to assess colistin resistance among Enterobacteriaceae isolates. Results emphasized the need for urgent action to control the growing issue of antibiotic resistance in general and colistin in particular. Out of 100 isolates tested for colistin susceptibility, 41 showed resistance. The highest percentage of colistin resistance among Enterobacteriaceae was seen within *Proteus spp.* (63.2%) followed by *Serratia* 57% where the lowest resistance was observed within *Klebsiella* isolates. As colistin is being used as a last-line agent for infections with CRE, the susceptibility of CRE to colistin was checked. They found that 39% of the meropenem-resistant Enterobacteriaceae and 45% of imipenem resistant Enterobacteriaceae were susceptible to colistin (86).

In 2020, a retrospective cohort study was done in Egypt about the Epidemiology of Carbapenem-resistant Enterobacteriaceae in Egyptian intensive care units between 2011–2017. The study found that about half of the isolated Enterobacteriaceae were carbapenem resistant, suggesting an incidence higher than other Arab, Asian, and African countries. The most isolated CRE producing pathogen was *K. pneumoniae* (85.1%) followed by *E.coli* and *Enterobacter spp*. CRE pathogens were most commonly isolated from blood samples (47%) compared to other sources. Contrary to what is known, the study did not find a significant relationship between carbapenem resistance in the ICUs and the use of medical devices, length of hospital stay, and undergoing surgical procedures (87).

In 2018, a cross sectional study was carried out in Gaza strip hospitals to assess the carbapenem resistance among Gram-negative isolates. They reported that carbapenem resistance within Enterobacteriaceae isolates was (30/226) 13.2% and all the them were

100% MDR. Of them, *Klebsiella spp*. was the most resistant to carbapenems 13/90 (14.4%), followed by *E. coli* 9/91 (9.8%), while in other Enterobacteriaceae resistance to carbapenem was 8/45 (17.7%). The ICUs showed the highest resistance rate to carbapenems followed by the surgical ward (SW)—(52.9%) and (37.5%), respectively. The outpatient clinics exhibited a rate of (6.1%) (88).

A review article was conducted in 2019 that includes articles published in peer reviewed journals between 2013–2018 to assess available data about carbapenem- resistant Enterobacteriaceae (CRE) in Saudi Arabia. The article stated that a disconcerting dissemination of CRE producers is an issue with significant concern to the ministry of health and healthcare providers in Saudi Arabia. A recent study in Riyadh detected carbapenemase genes more frequently in *K. pneumoniae* (63%) isolates, followed by *E.coli* (55%). Another two studies from Makah found that 48.4% and 38%, respectively, of the checked *K. pneumoniae* were positive for carbapenemase production. However, a recent study from Madinah has been done to assess antibiotic susceptibility of *E. coli* isolated from different clinical sources and reported that all the isolates have good susceptibility to imipenem (89).

1.8.2.3 Studies related to non-fermenting GNB resistance: Prevalence and epidemiology.

ting gram negative bacilli (NFGNB) is the second most clinically important sub-group of gram-negative bacteria. It includes a variety of bacterial pathogens that are aerobic, non-sporing, and do not use glucose as its own source of energy or use it oxidatively. They are mostly saprophytic organisms that were considered as contaminants isolated from water, soil, plants, insects, and many other sources due to their ability to use diversity of substrates as energy sources, and to develop within extreme environments providing only few nutrients (90). However, nowadays they are considered as the main health care associated pathogens responsible for many clinically significant infections, such as UTI, ventilator pneumonia, meningitis, surgical site infections (SSIs), and bacteremia. The most common species of NFGNB with clinical significance are *P. aeruginosa, A. baumannii,* and *S. maltophilia*. NFGNB stands for about 15% of all clinical bacterial isolates in most microbiological laboratories, whereas *P. aeruginosa* and *A. baumannii* both represent about 10% of the nosocomial infections in American

ICUs. In fact, most NFGNB are MDR pathogens intrinsically, leaving only few options of antibiotics with therapeutic effectiveness (91) (92).

A study was conducted in India to identify the clinical significance and resistance patterns of the non-fermenters pathogens. Out of 4248 clinical specimens, 189 specimens showed the presence of 193 NFGNB. Regarding the source of the clinical sample, NFGNB were isolated from 117 pus samples, 24 sputum samples, 23 urine samples, 22 blood cultures, and 3 fluid samples. Of them, *P. aeruginosa* was the most frequent isolate (53.8%), followed by *A. baumannii* (22.2%), *P. fluorescence* (10.8%). Most of the isolated *P. aeruginosa* were susceptible to imipenem (94.2%), cefoperazone (70.5%), and amikacin (69%). They found that *P. aeruginosa* was more sensitive to all of antibiotics tested compared to *P. fluorescence. A. baumannii* was 100% susceptible to imipenem and 70% to piperacillin (92).

A retrospective study was conducted in 2010 to evaluate clinical characteristics and risk factors for the fatality of bloodstream NFGNB. 215 patients proved to have 221 NFGNB bacteremia episodes. Out of them, the most frequent isolate was *P. aeruginosa* (55.6%), followed by *A. baumannii* (17.5%), and *S. maltophilia* (9%). However, no significant difference in the fatality was found between the isolated organisms. When hospital-acquired NFGNB bacteremia episodes developed, 42.5% of the patients were in the ICU department and 57.5% had a central venous catheter (CVC) placement. The three most common sources of infection within patients included in the study were lung, urinary tract, and biliary tract. Regarding mortalities, 80 of the included patients died, 57 of the deaths were related directly to the bacteremic episodes, where 23 of them had not direct relationship with bacteremia. Compared to patients who survived, patients who died from NFGNB bacteremia had a higher proportion of liver cirrhosis, steroid use, NFGNB bacteremia secondary to pneumonia, ICU-acquired bacteremia, and septic shock (93).

A study was performed between 2006 and 2014 to characterize carbapenem-resistant *A. baumannii* which were collected from 69 hospitalized patients from five Palestinian districts. Susceptibility testing revealed that all the isolates were MDR with complete resistance to β-lactams including carbapenems. They were also highly resistant to aminoglycosides, cotrimoxazle, and macrolides. On the other hand, all of the 69 isolates

were susceptible to colistin sulfate. They stated that as *A. baumannii* is recognized by the CDC as a source of global outbreaks because of its clonal fashion of dissemination within health care facilities and even between countries, it is critical to implement certain measures, starting with following the CDC's infection control guidelines and recommendations regarding sterilization, hand hygiene, and isolation precautions. Additionally, adequate cleaning for surfaces and equipment that may act as reservoir for *A. baumannii* is important, such as catheters, ventilators, and humidifiers. Finally, they recommend to implement antibiotics stewardship programs to control the spread of this easily disseminated organism (94).

A retrospective study was conducted to identify NFGNB contributed to respiratory tract infections rather than *P. aeruginosa* and *Acinetobacter spp.*. Results showed that NFGNB accounted for 16.4% of the respiratory specimens that showed significant growth. *P. aeruginosa* and *Acinetobacter spp.* represented 96% of the isolated NFGNB. Other than that, they found 4% (N=33) of the isolated NFGNB included organisms like *S. maltophilia, B. cepacia, S. paucimobilis*, etc. A high rate of pathogenicity was observed among these isolates such as community-acquired pneumonia, which was attributed to them in 8 patients (out of 33). (95).

A retrospective study was performed in Turkey in 2014 to assess the changes in the prevalence of resistant-Acinetobacter infections among ICU patients based on carbapenem consumption by dividing the study period to carbapenem non-restricted period (CNRP) and carbapenem restricted period (CRP). During CNRP, a total of 10.82 (DDD/100 ICU days) of anti-pseudomonal carbapenem were used. This number was decreased to 6.95 during CRP. MDR *A. baumannii* was isolated from 42 patients in CNRP and from 14 patients in CRP, meaning that the rate of infection was 2.24 folds higher without implementing restrictions on carbapenems use during CNRP. They found that nosocomial infections density in general was lowered during CRP. (96).

A retrospective study was performed in 2017 in Morocco to identify the prevalence and resistance pattern of *A. baumannii* isolated from blood cultures of ICU-patients, and to detect the most prevalent resistance genes among the isolates. *A. baumannii* was the second most frequent organism after *CoNS* with a percentage of 9.2%. Results showed an increase in the prevalence and resistance rate of *A. baumannii* among years of the

study (2010–2014). A high resistance rate to ceftazidime, cefotaxime, ciprofloxacin, and gentamicin was confirmed, with special attention to the increasing resistance to imipenem from 50% in 2010 to 75% in 2014. However, *A. baumannii* showed high sensitivity to colistin (99%). Since oxacillinases are the main enzymes responsible for the breakdown of carbapenems, their presence was detected. All of the imipenem resistant *A. baumannii* expressed blaOXA-51 genes where a high percentage of them were positive for blaOXA-23. (97)

1.8.3 Gram-positive bacteria

1.8.3.1 Appearance and groups

Gram-positive bacteria are organisms with a wide range of variety in development and resistance patterns. They are characterized by their thick wall of peptidoglycans surrounding the plasma membrane and gives the bacterial cell its strength and rigidity (98). It is subdivided to cocci, bacilli, and branching filaments. For cocci, *Streptococcus* bacteria includes *S. pneumonia, S. pyogenes, S. agalactiae, enterococci, and S. viridans*, it also includes *Staphylococcus* bacteria, which belongs to *Micrococcaceae* family and classified to either coagulase positive, such as *S. aureus* or coagulase negative as *S. epidermidis* (99). Gram-positive bacilli vary in their ability to form spores and divided as a result of spore-forming (*Bacillus* and *Clostridia*) and non-spore forming (*Listeria* and *Corynebacterium*) (100). Also, the branching filament rods includes *Nocardia* and *Actinomyces* (101).

1.8.3.2 Studies related to gram-positive bacteria resistance: Prevalence and epidemiology.

According to (102)), the introduction of new antibiotics to counter the spread of S aureus was unfortunately always followed by the emergence of resistant strains. All *S. aureus* were sensitive to penicillin G when first launched into markets in 1940s. Few years later, resistance strains started to emerge. However, all strains of *S. aureus* are resistant nowadays to penicillin G, aminopenicillins and antipseudomonal penicillins. As the main mechanism of resistance depends on penicillinase production, new penicillinase-resistant penicillin such as methicillin were developed. Unfortunately, this success did not last long because another *methicillin resistant S. aureus (MRSA)* strain had appeared and was disseminated initially in a hospital setting only and in a

community setting later. Fluoroquinolones (Ciprofloxacin in particular) was the first recommended oral antibiotic to be used in MRSA infections. However, only after one year of use, many hospitals had reported the presence of remarkable increase in the resistance to ciprofloxacin. Vancomycin was the ideal agent to treat *MRSA* for many years until the observable increase in the resistance as a result of the high consumption of vancomycin especially in patients on dialysis. Since the past decade, new agents with anti-MRSA activity have been developed and used (linezolid, tigecycline, daptomycin, quinupristin-dalfopristin).

A review study was conducted in 2006 to explain the development of antibiotic resistance in *S. aureus*, focusing on methicillin and vancomycin resistance. *S. aureus* has the ability to cause various life-threatening infections, including bloodstream, soft tissues, lower respiratory, and many other infections. Apart from that, its significance stems from the extraordinary possibility of causing antimicrobial resistance. Shortly after methicillin was welcomed as the first semi-synthetic penicillinase-resistant penicillin, chromosomal changes occurred within the penicillin-binding protein caused the production of a new one (PBP2a) with lower affinity and resistance as a result to methicillin and all β-lactams including cephalosporins. With time, clonal dissemination has occurred for the resistant strains (*MRSA*) around the world with remarkable regional variation in the prevalence (103). For example, a study showed many folds variation in the prevalence of *MRSA* from less than 1% in northern Europe to more than 40% in Greece, the UK, and Italy. This may be explained by the control measures followed within the health care facilities (104).

In 2017, a study was performed in Iran to assess linezolid activity against gram positive cocci within three teaching hospitals between 2013–2015. Considering the definition of MDR organisms as any isolate showing resistance to three or more antibiotics, susceptibility patterns of the isolated organisms showed that all were MDR. The collected isolates were 31 *MRSA*, 32 *VRE*, 15 *penicillin non-susceptible pneumococci* (PNSP) and 28 *Group B streptococci (GBS)*. The results proved the excellent effectivity of linezolid against the isolated gram-positive cocci since resistance was not observed. The MIC50 value for *MRSA* and *VRE* was 2 mg/ml and it was 0.5 m g/ml for *PNSP* and *GBS* strains (105).

A retrospective study was performed in 2019 to evaluate the clinical efficacy of fosfomycin against a variety of gram-positive cocci. The study includes all patients with severe gram-positive infections who received fosfomycin within their infection's treatment regimens between 2011–2017. The results found that fosfomycin as a part of the antimicrobials approach for gram-positive cocci is an effective alternative to the non-susceptible antibiotics. Out of 75 patients treated with fosfomycin combinations, 81% of them were successfully treated. The combination of fosfomycin and daptomycin was the most successful among the others with a 93% success rate. Where fosfomycin and vancomycin combinations showing poor efficacy with only 47% eradication rate (106).

In 2016, a study was performed in China to assess the prevalence of quinupristin/ dalfopristin (Q/D) resistance among *E. facium* clinical isolates. (Q/D) is a combination antibiotic that consists of streptogramin A and B, expressing a bactericidal activity against the majority of multi-drug resistant gram-positive cocci. Although *E. faecalis* is intrinsically resistant to Q/D, it has been showing high effectiveness in the treatment of *Vancomycin-resistant E. faecium (VREf*) infections. However, resistance to Q/D started to disseminate in many countries around the world. Out of 911 *E. faecium* clinical isolates during the study period (2012–2015), 9 of them were resistant to Q/D. All Q/D-resistant isolates were susceptible to vancomycin, teicoplanin, and tigecycline but resistant to penicillin, ampicillin, and erythromycin. What is worth mentioning that Q/D is not available in the Chinese market, which suggests that resistance has been acquired by routes other than consumption of Q/D. They reported that the cross resistance of Q/D may occur as a result of animals being extensively subjected to in-feed use of virginiamycin. In this study, although the resistant-Q/D *E. faecium* frequency was low, it was higher for the intermediate resistance among the isolated organisms (107).

A study performed to analyze national trends in antibiotic resistance of *Coagulase-negative staphylococci (CoNS)* with special attention to *S. epidermidis* isolates as the most common *CoNS* pathogen, using data from the Surveillance Network (TSN) of the US over a period between1999–2012. Over the study period, 540000 *CoNS* blood isolates were submitted to the surveillance system and of them 80000 were *S. epidermidis*. The results showed a significant increase in the prevalence of resistant *CoNS* isolates including MDR pathogens during 1999–2005. However, this was

followed by another significant decrease in MDR trends thought to be the result of the decrease in levofloxacin resistance patterns within the same period. Wilcoxon-Mann-Whitney rank sum test was performed and proved that the difference between the two periods was statistically significant ($P< 0.01$). They found that *S. epidermidis* resistance to levofloxacin was positively and significantly correlated to levofloxacin prescription rate. Results showed the same resistance pattern if we describe all the *S. epidermidis* isolates or only the bloodstream *S. epidermidis* isolates (108).

A study was conducted in 2009 to evaluate susceptibility pattern of *L. monocytogenes* strains isolated from food environments to the most common antibiotics. Certain antibiotics are used in animal feeding to control infectious diseases that may affect animals or as growth promoters. This can create new antimicrobial-resistant zoonotic foodborne bacterial pathogens that may easily disseminate to humans as food contaminants or to other animals. *L. monocytogenes* is an ideal foodborne pathogen that is usually susceptible to most antibiotics. However, the first MDR *L. monocytogenes* was isolated in 1988 in France. 120 L. monocytogenes were tested for 19 antibiotics used widely in veterinary and human therapy, in which results showed the high sensitivity of *L. monocytogenes* to theses' agents. All strains were sensitive to ampicillin/sulbactam, benzylpenicillin, imipenem, gentamicin, and teicoplanin. Resistance to 12 and intermediate resistance to 6 within the antibiotics used in the study was observed. Clindamycin was the most non-susceptible antibiotic with 4 isolates showing complete resistance and 6 isolates showing an intermediate susceptibility to this antibiotic. Linezolid was the next one of the least susceptible followed by ciprofloxacin, ampicillin, rifampicin, TMP–SMX, and finally, vancomycin and tetracycline (109).

A study was performed in 2011 to evaluate resistance within *S. pneumoniae* pathogens collected from two Lebanese hospitals from 2005–2009. A total of 121 isolates of *S. pneumoniae* were associated with many pneumococcal diseases were subjected to more studies. Results showed that about half of the strains were isolated in winter, and 73.5% were isolated from respiratory tract specimens. The MICs90 seem to fluctuate within the same range within the study period, with the exceptions of clarithromycin and azithromycin that were high during the four years. All isolated strains showed complete sensitivity to amoxicillin/clavulanic acid and cefpodoxime, where 58 were susceptible

to penicillin, 61 were intermediate, and 2 were completely resistant to this antibiotic (110).

1.8.4 Studies related to candida resistance: Epidemiology and prevalence.

According to CDC (2020), about 7% of blood specimens tested for fungal infections by CDC were resistant to fluconazole. Unfortunately, *C. glabarta* has shown high level of resistance to another class of antifungals (echinocandins), which are considered as the drug of choice for fluconazole-resistant *C. glabarta* infections. Patients with resistant infection to these both classes have very few remaining treatment options such as amphotericin B, which can be harmful to some patients. Evidences suggest that patients with resistant candida are less likely to survive compared to patients having antifungal-sensitive candida infection.

According to (112) candida species distribution and antifungal resistance patterns to echinocandins and azole antifungal agents were compared in 779 patients with ICU-associated BSIs due to *Candida spp*. and 973 infected non ICU-patients collected from 79 medical center between 2008–2009. Of the 1752 Candida isolates, the most common isolate was *C. albicans* (48.7%), where (17.8%) were *C. glabrata*, (17.2%) *were C. parapsilosis*, (10.0%) were *C. tropicalis*, and (2.1%) were *C. krusei*. Minor differences were observed according to the distribution of the isolates between ICU and non-ICU patients. About isolates susceptibility to antifungals, resistance to the echinocandins was rare within the five main species of candida causing BSIs both in ICU and non-ICU patients, where fluconazole resistance was found in 5.0% of ICU isolates and 4.4% of non-ICU isolates. *C. glabrata* was the main isolate showing resistance to azoles and echinocandins within both settings.

A retrospective study was conducted in 2015 to find out candida profiles and antifungal resistance development over a decade in Lebanon. Results showed that among the 1,300–1,500 Candida isolates isolated per year, *C. albicans* rates reduced from 86% in 2005 to about 60% in 2014. On the other hand, the non-albicans infection rates increased from 14% in 2005 to about 40% in 2014, the most frequent species of which were *C. tropicalis*, *C. glabrata*, and *C. parapsilosis*. All these were 100% susceptible to amphotericin B but showed high resistance (35%–79%) against itraconazole. Fluconazole remained highly effective (94%–100%) for *C. albicans*, though a trend of

higher MIC values and lower susceptibility was observed during the period 2012–2014 compared to previous periods. *C. glabrata* showed higher resistance than other species. However, the latest trend showed better susceptibility. Few numbers of *C. parapsilosis* were tested and showed high susceptibility (86%–100%) to fluconazole, and the trend showed a decrease in susceptibility over time (113).

Chapter Two

Methodology

Antibiotic resistance is one of the most disconcerting issues in our world today. Even though there is real lack of local researches as well as antimicrobials resistance monitoring system. This has pushed us to study the burden of antimicrobial resistance patterns in Palestine, taking An-Najah National University Hospital (NNUH) in Nablus as an example.

2.1 Study design and setting

This study was conducted in Palestine between 2018–2020, designed as a retrospective cross-sectional study. We chose NNUH as the study site due to its complete and comprehensive data system, allowing a reliable data collection. NNUH was established in 2013 as the only teaching hospital in Palestine and is considered one of the most important referral institutions in the health care field. With 120 beds in an area of 17,000 square meters, the hospital contains an emergency room, medical laboratory department, dialysis department, completely equipped intensive care unit, and many other departments. Moreover, various modern services can be provided, such as cardiac care, eye surgery, bone marrow transplantation, liver surgery, general and orthopedic surgery, and many services introduced for children in particular, such as advanced spinal surgery and pediatric cancer. Since Nablus is the second largest Palestinian city in the West Bank and positioned in the center of Northern towns and cities, it is considered as a main health care center providing medical services for more than a million people.

2.2 Study sample and population

The study sample was the whole population, which consists of all the clinical samples that were sent for culturing to microbiology lab of NNUH during the study period of 2018–2020 collected retrospectively. The hospital's medical laboratory department receives culture specimens from all hospital departments. Additionally, it receives clinical samples from patients who refer to the outpatient clinics and out-patients visiting the emergency department without the need for hospital admission. Thus, our study includes both in- and out-patients.

2.3 Data collection

As this is a retrospective survey, sample selection for specimen collection was not possible for us, and the sampling criteria was recorded as requested by the physician. Permission for the study to be done within NNUH, and for the microbiology lab database access was obtained from the hospital's medical director.

Samples from different sources (e.g., blood, wound, urine, tissue, stool, pus, cerebrospinal fluid (CSF), sputum, etc.) were cultured using appropriate culture media (e.g., Blood Agar, Chocolate Agar, and MacConkey Agar). Pure isolates of bacterial culture were taken and inserted to Vitek 2 Compact by the microbiology laboratory staff.

After tests were performed and the results of resistance and susceptibility were extracted, data were entered to Microsoft Excel on a routine basis. For this study, we have collected the required data that belongs to the study period from the electronic medical records for patients who had bacterial or fungal infections confirmed by any clinical sample. We only included the first collected sample for the same subject having the same isolate to avoid duplication, but if the patient is re-admitted after recovery or visiting the clinics again with new infection, we considered that as multiple cases.

Data was extracted and entered directly to Microsoft Excel 2016 for good organization. An excel sheet was prepared and named for each bacterial species (such as E. coli) for most WHO priority pathogens list (WHO PPL) (Table 2). It depends on the emergence of the pathogen within the institute's environment, with pathogens rarely cultured being gathered together under the title of (others) once for gram-positive and the second for gram-negative pathogens followed by the removal of duplicated culture reports. Patient information was entered including their name, source of specimen, date of collection, department, resistance and susceptibility pattern for each antibiotic, with the (intermediate) sensitivity being merged within resistance ones. Those with incomplete data, meaning any information that was not recorded for either the sample source, department, isolated organism or antibiotics susceptibility were excluded.

The hospital antibiogram in general covered the following antimicrobials: penicillin, oxacillin, amoxicillin/ clavulanic acid, cefuroxime, ceftriaxone, cefotaxime, ceftazidime, cefeipime, clindamycin, erythromycin, gentamycin, ciprofloxacin, levofloxacin,

moxifloxacin, amikacin, vancomycin, tigecycline, rifampin, tetracycline, quinupristin/dalfopristin, linezolid, co-trimoxazole, ampicillin, piperacillin, piperacillin/tazobactam, ticarcillin, ticarcillin/clavulanic acid, imipenem, meropenem, ertapenem, norfloxacin, nitrofurantoin, colistin, aztreonam, minocycline, tobramycin, fluconazole, voriconazloe, caspofungin, micafungin, flucytosine, and amphotericin.

Table 2.1

WHO global priority pathogens list (114)

Priority 1: CRITICAL	Priority 2: HIGH	Priority 3: MEDIUM
Acinetobacter baumannii, carbapenem-resistant	Enterococcus faecium, vancomycin-resistant	Streptococcus pneumoniae, penicillin-non-susceptible
Pseudomonas aeruginosa, carbapenem-resistant	Staphylococcus aureus, methicillin-resistant, vancomycin intermediate and resistant	Haemophilus influenzae, ampicillin-resistant
Enterobacteriaceae*, carbapenem-resistant, 3 rd generation cephalosporin-resistan	Helicobacter pylori, clarithromycin-resistant	Shigella spp., fluoroquinolone-resistant
	Campylobacter, fluoroquinolone-resistant	
	Salmonella spp., fluoroquinolone-resistant	
	Neisseria gonorrhoeae, 3 rd generation cephalosporin-resistant, fluoroquinolone-resistant	

**Enterobacteriaceae include: K. pneumonia, E. coli, Enterobacter spp., Serratia spp., Proteus spp., and Providencia spp., Morganella spp.*

2.4 Data analysis

Data collected within Microsoft Excel was coded and imported to Statistical package for social sciences (SPSS) version 21 for further analysis. Descriptive statistics were run generating frequency and percentages tables and graphs. Many custom tables and crosstabs were generated as descriptive analysis, starting with the sources of infection, frequencies, and percentages. Frequency and percentage of isolated microorganisms, distribution of sample type and bacterial isolate, rates of antibiotics resistance of the most common isolates, MDRO frequency and percentage, frequency and percentage of in\out patients during months, frequency and percentage of in\out patients of the most common isolates. Each of them was done for the three years of the study 2018–2020.

2.5 Ethical consideration

Ethical authorization was gained from institutional review board (IRB) of An-Najah National University. Additionally, approvals were obtained from the hospital administration and the clinical research unit of NNUH. This research did not involve human subjects directly; thus, no consent process was needed. All patient names have been anonymized prior to analysis. The study did not involve any risk of psychological

or informational harms. All data collected in the course of the study were kept confidential and used only for this study.

Chapter Three

Results

3.1 Isolates total frequency

In total, N=5585 patients having complete data reserved within computerized database in the microbiology lab. N=1572 patient's samples were collected in 2018, N= 2039 in 2019 and N=1974 in 2020. GNB represented the majority of isolates with 46.9%, whereas GPB was isolated in lower percentage 37.9%, and candida 15.2% within the three years (Table 3.1).

Table 3.1

Frequency of gram positive, gram negative and candida during study period.

		2018	2019	2020	Total
Gram negative	N	767	972	880	2619
	%	48.8	47.6	44.6	46.90
Gram positive	N	609	749	758	2116
	%	38.7	36.7	38.4	37.9
Candida	N	196	318	336	850
	%	12.5	15.6	17	15.2
Total	N	1572	2039	1974	5585
	%	100	100	100	100

3.2 Samples sources

Samples were collected from many sources, including blood, wound, urine, tissue, respiratory origins, fluids and (others), which include sources were mentioned in a few numbers, such as ear swab, vaginal swab, stool, catheter tip, etc. Of them, urine specimens were the most collected with a total percentage of 27.7% among the three years. Followed by wound specimens (19.6%) and blood specimens (19.2%). The least numbers of samples were collected from tissue sources with a total of 178 sample (3.2%) and (other) sources 238 samples (4.2%) (Table 3.2).

Table 3.2

Frequency of common infection sources during the study period.

		2018	2019	2020	Total
Urine	N	466	542	537	1545
	%	29.6	26.6	27.2	27.7
Wound	N	318	443	331	1092
	%	20.2	21.7	16.9	19.6
Blood	N	340	350	383	1073
	%	21.6	17.2	19.4	19.2
Respiratory origin	N	261	396	368	1025
	%	16.6	19.4	18.6	18.3
Fluids	N	86	139	209	434
	%	5.4	6.8	10.6	7.8
Tissue	N	52	78	48	178
	%	3.3	3.8	8.4	3.2
Others	N	49	91	98	238
	%	3.1	4.4	5.1	4.2
Total	N	1572	2039	1974	5585
	%	100	100	100	100

3.3 location of patients

With regard to hospital departments, the highest frequency of bacterial and fungal cultures isolated from in-patients were from the SW with a percentage of 14.6% of all the hospital departments during the study years, followed by ICU with a percentage of 10.7% isolated cultures. The third one was the medical ward (MW) (N=518, N%=9.3%). The total inpatients from all the wards were 3895 (N%=69.7%). For outpatients, most bacterial and fungal specimens were isolated from clinics with a frequency of 756 cases, followed by emergency room outpatients (N=597) (Table3.3). Note: Itihad and Ateel's clinics are departments outside NNUH but belong to the hospital.

Table 3.3

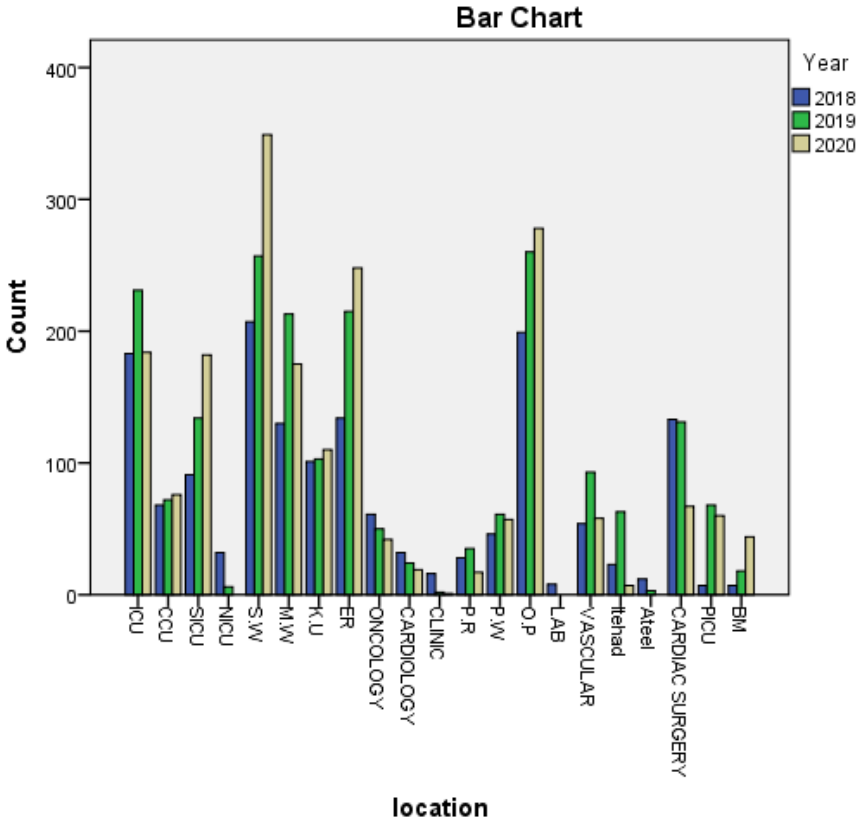
Frequency of hospital departments from which clinical specimens were obtained during the study period.

	Department	Frequency	Percent
In-patients	Surgical ward (SW)	813	14.6
	Intensive care unit (ICU)	598	10.7
	Medical ward (MW)	518	9.3
	Surgical ICU (SICU)	407	7.3
	Cardiac surgery	331	5.9
	Cardiac care unit (CCU)	216	3.9
	Vascular ward	205	3.7
	Pediatric ward (PW)	164	2.9
	Oncology ward	153	2.7
	Pediatric ICU (PICU)	135	2.4
	Itehad hospital	93	1.7
	Private room	80	1.4
	Cardiology ward	75	1.3
	Bone marrow transplant ward	69	1.2
	Neonatal ICU (NICU)	38	0.7
	Total	3895	69.7
Out-patients	Clinics	756	13.5
	Emergency room (ER)	597	10.7
	Kidney unit (KU)	314	5.6
	Ateel clinics	15	0.3
	Hospital's Lab	8	0.1
	Total	1690	30.2

Chart 3.1 shows the distribution of hospital departments from which the bacterial and fungal cultures were isolated over the years of study (2018–2020).

Chart 3.1

Frequency of hospital departments from which clinical specimens were obtained during the study period.



3.4 Frequencies of common GNB, GPB and candida.

When we observe the total frequencies of GNB over the three years, we found that *E. coli, P. aeruginosa, A. baumannii,* and *K. pneumoniae* were the most prominent isolates. However, many GNB were isolated in small numbers over the years. We collected them under the name (other gram-negative bacteria), which all together represented 3.2% of the total isolates over the three years (see appendix). Some of them are: *A. hydrophila, R. radiobacter, B. bronchiseptica, M. catarrhalis, Pantoea spp*., etc.) (Table 3.4).

Table 3.4

Frequency of common gram-negative bacteria during the study period.

		2018	2019	2020	Total
Acinetobacter spp.					
A. baumannii	Count	94	72	57	223
	% within YEAR	6.0	3.5	2.9	4.0
Other Acinetobacter spp.	Count	14	23	8	45
	% within YEAR	0.90%	1.1	0.4	0.8
Pseudomonas spp.					
P. aeruginosa	Count	107	141	109	357
	% within YEAR	6.80%	6.9	5.5	6.4
Other Pseudomonas spp.	Count	13	19	17	49
	% within YEAR	0.80%	0.9	0.9	0.9
E. coli					
CRE E. coli	Count	1	4	11	16
	% within YEAR	0.10%	0.2	0.6	0.3
ESBL E. coli	Count	115	146	148	409
	% within YEAR	7.30%	7.2	7.5	7.3
E. coli	Count	104	132	133	369
	% within YEAR	6.60%	6.5	6.7	6.6
K. pneumoniae					
ESBL k. pneumoniae	Count	56	74	72	202
	% within YEAR	3.6	3.6	3.6	3.6
CRE k. pneumoniae	Count	10	20	33	63
	% within YEAR	0.6	1.0	1.7	1.1
k. pneumoniae	Count	38	54	51	143
	% within YEAR	2.4	2.6	2.6	2.6
Enterobacter spp.					
CRE E. cloacae	Count	1	2	2	5
	% within YEAR	0.1	0.1	0.1	0.1
ESBL E. cloacae	Count	2	0	0	2
	% within YEAR	0.1	0.0	0.0	0.0
E. cloacae	Count	35	40	31	106
	% within YEAR	2.2	2.0	1.6	1.9
Other Enterobacter spp.	Count	5	6	7	18
	% within YEAR	0.3	0.3	0.4	0.3
Proteus spp.					
CRE. Proteus	Count	2	0	1	3
	% within YEAR	0.1	0.0	0.1	0.1
ESBL. Proteus	Count	4	4	3	11
	% within YEAR	0.3	0.2	0.2	0.2
Proteus spp.	Count	27	47	22	96
	% within YEAR	1.7	2.3	1.1	1.7

For gram-positive bacteria, the most frequent isolate was *Coagulase-negative Staphylococci (CoNS)* (most of *CoNS* were *S. epidermidis*, *S. haemolyticus*, and in small numbers found *S. hominis*, *S. sciuri*, *S. cohnii*, *S. warneri*, *M. luteus*, *S. saprophyticus*, *S. capitis* and *S. xylosus*). *CoNS* represented 14.7% in total for the three years, followed by *E. faecalis* (7%). Small numbers of gram positive isolates collected under the name (other gram positive), such as *Actinomyces spp.*, *Lactobacillus*, *L. monocytogenes*, *Clostridium spp.*, etc. (see appendix B) (Table 3.5).

Table 3.5

Frequency of gram-positive bacteria during the study period.

		2018	2019	2020	Total
S. aureus					
MRSA S. aureus	Count	42	50	44	136
	% within YEAR	2.7	2.5	2.2	2.4
S. aureus	Count	40	66	68	174
	% within YEAR	2.5	3.2	3.4	3.1
CoNS	Count	234	277	310	821
	% within YEAR	14.9	13.6	15.7	14.7
E. faecalis					
VRE E. faecalis	Count	0	0	1	1
	% within YEAR	0.0	0.0	0.1	0.0
E. faecalis	Count	111	154	128	393
	% within YEAR	7.1	7.6	6.5	7.0
E. faecium					
VRE E. faecium	Count	44	52	62	158
	% within YEAR	2.8	2.6	3.1	2.8
E. faecium	Count	32	38	39	109
	% within YEAR	2.0	1.9	2.0	2.0
Other Enterococcus spp.		2			
Other VRE Enterococcus spp.	Count	0	5	12	17
	% within YEAR	0.0	0.2	0.6	0.3
Other Enterococcus	Count	14	9	6	29
	% within YEAR	0.9	0.4	0.3	0.5
Streptococcus spp.					
S. pneumoniae	Count	4	5	0	9
	% within YEAR	0.3	0.2	0.0	0.2
S. agalactiae	Count	6	25	25	56
	% within YEAR	0.4	1.2	1.3	1.0
Other Streptococcus spp.	Count	68	36	27	131
	% within YEAR	4.3	1.8	1.4	2.3

For candida species, the most frequent species was *C. albicans* with 317 isolate (5.7%) in total, the second was *C. glabrata* (3.6%), and *C. tropicalis* (3.2%). Other uncommon candida species were collected under (other candida) and represented only 2.7% among the three years, such as *C. lusitaniae, C. parapsilosis, C. krusei* (Table 3.6 in appendix B).

3.5 Distribution of sample type and microbiological isolates.

Table 3.7 describes the overall distribution of sample type and microbiological isolates. For GNB, *A. baumannii and P. aeruginosa* were isolated in the highest percentage from wound samples followed by respiratory samples, while *E. coli* and *K. pneumoniae* isolates (including ESBL and CRE producers) were found mostly within urine samples.

*S. aureus (*including *MRSA S. aureus)* was isolated mainly from wound samples while *CoNS* from blood samples. *E. faecalis and E. faecium* were obtained in the highest frequency from urine samples. Candida species were isolated mainly from respiratory-origin samples and urine samples. (Distribution of sample type and bacterial isolate in 2018, 2019, 2020 are within tables in appendix B).

Table 3.7

The overall distribution of sample type and microbiological isolates.

	Blood	Wound	Urine	Tissue	Respiratory origin	Fluids	Others
	%	%	%	%	%	%	%
Gram negative							
Acinetobacter							
A. baumannii	10.7	36.3	11.2	2.2	30.9	5.3	3.1
Other Acinetobacter spp.	24.4	24.4	13.3	2.2	26.6	8.8	0.0
Pseudomonas							
P. aeruginosa	15.1	35.8	17.6	3.3	17.6	5.3	5.0
Other Pseudomonas spp.	30.6	18.3	6.1	4.0	16.3	20.4	4.0
E. coli							
CRE E. coli	12.5	31.2	31.2	6.2	12.5	6.2	0.0
ESBL E. coli	8.3	18.0	58.4	2.4	3.1	9.0	0.4
E. coli	9.2	17.3	55.8	1.3	4.6	9.2	2.4
K. pneumoniae							
ESBL k. pneumoniae	7.4	21.7	49.5	0.9	13.3	5.4	1.4
CRE k. pneumoniae	14.2	20.6	30.1	3.1	14.2	3.1	14.2
k. pneumoniae	16.7	13.9	30.7	2.1	27.2	7.6	1.4
Enterobacter							
CRE E. cloacae	25.0	25.0	25.0	0.0	0.0	25.0	0.0
E. cloacae	17.9	26.4	15.0	7.5	20.7	10.3	1.8
Other Enterobacter spp.	5.5	22.2	16.6	5.5	33.3	11.1	5.5
Proteus							
ESBL Proteus	0.0	63.6	36.3	0.0	0.0	0.0	0.0
Proteus	2.0	50.0	35.4	3.1	4.1	2.0	3.1
Gram positive							
S. aureus							
MRSA S. aureus	27.2	43.3	2.2	5.1	11.0	1.4	9.5
S. aureus	29.3	33.3	3.4	5.1	17.2	6.9	4.6
CoNS	57.3	4.9	4.3	6.3	7.3	11.9	7.6
E. faecalis	7.6	26.7	41.7	2.5	11.9	6.3	3.0
E. faecium							
VRE E. faecium	5.0	24.0	40.5	3.1	22.1	4.4	0.6
E. faecium	5.5	26.6	37.6	3.6	11.0	11.0	4.5
Other Enterococcus	20.6	24.1	13.7	6.9	17.2	17.2	0.0
Streptococcus spp.							
S. agalactiae	1.7	8.9	71.4	0.0	10.7	1.7	5.3
Other Streptococcus spp.	19.8	12.9	21.3	3.0	10.6	28.2	3.8
Candida							
C. tropicalis	7.9	6.7	34.4	0.5	42.9	3.9	3.3
C. albicans	5.0	7.8	34.3	1.5	44.4	3.7	2.8
C. glabrata	12.4	6.9	52.7	1.0	19.9	5.9	1.0

Table 3.8 in appendix B describes the frequency of in- and out- patients of the most common isolates within study period. In 2018, the highest organism isolated from in-patients (N=1102) was *CoNS* with a percentage of 14.2% (N=156), followed by *A. baumannii* with a percentage of 7.2%(N=79). In 2019, *CoNS* had reported the highest frequency again with N=188 cases (12.9%), followed by *P. aeruginosa* N=107 (7.3%). In 2020, *CoNS* had reported 196 cases (14.6%), followed by *ESBL E. coli* with a rate of 7% (N=93) (see Table 3.8 in appendix B).

3.6 Frequency of common MDRO.

In 2018, the most frequently isolated MDRO was *ESBL E. coli* with a percentage of 7.3% of the whole organisms isolated in that year, the second was *A. baumannii* that represented a percentage of 5.2% (N=82), followed by *ESBL K. pneumoniae* (3.6%). In 2019, it was *ESBL E. coli*, still the top frequent organism, with a rate of 7.2% (N=146). The next was *ESBL K. pneumoniae* (N=74), and the third most frequent one was *A. baumannii* with a percentage of 2.84% (N=58). In 2020, the most two frequent MDRO were the same as 2019, *ESBL E. coli* (7.5%), and *ESBL K. pneumoniae* (3.6%). The third organism was *VRE E. faecium* with a percentage of 3.1%.

Table 3.9

MDRO frequency during the study period (sorted by most prevalent).

MDRO		YEAR			
		2018	2019	2020	Total
ESBL E. coli	Count	115	146	148	409
	% within YEAR	7.3	7.2	7.5	7.3
ESBL K. pneumoniae	Count	56	74	72	202
	% within YEAR	3.6	3.6	3.6	3.6
A. baumannii	Count	82	58	53	193
	% within YEAR	5.2	2.8	2.6	3.4
VRE E. Facium	Count	44	52	62	158
	% within YEAR	2.8	2.6	3.1	2.8
P. aeruginosa	Count	46	63	34	143
	% within YEAR	2.9	3.0	1.7	2.5
MRSA S. aureus	Count	42	50	44	136
	% within YEAR	2.7	2.5	2.2	2.4
CRE K. pneumoniae	Count	10	20	33	63
	% within YEAR	0.6	1.0	1.7	1.1
VRE Enterococcus spp.	Count	0	5	12	17
	% within YEAR	0.0	0.2	0.6	0.3
CRE E. coli	Count	1	4	11	16
	% within YEAR	0.1	0.2	0.6	0.2
ESBL Proteus	Count	4	4	3	11
	% within YEAR	0.3	0.2	0.2	0.1
CRE E. cloacae	Count	1	2	2	5
	% within YEAR	0.1	0.1	0.1	0.0
CRE Proteus	Count	2	0	1	3
	% within YEAR	0.1	0.0	0.1	0.0
ESBL E. cloacae	Count	2	0	0	2
	% within YEAR	0.1	0.0	0.0	0.0
ESBL K. oxytoca	Count	0	1	0	1
	% within YEAR	0.0	0.0	0.0	0
CRE K. oxytoca	Count	0	0	1	1
	% within YEAR	0.0	0.0	0.1	0
VRE E. Fecalis	Count	0	0	1	1
	% within YEAR	0.0	0.0	0.1	0

3.7 Frequency of common isolates with regard to hospital departments

With regard to the distribution of the isolated organisms based on the departments of the hospital, we found that *A. baumannii* was isolated in the highest number from the ICU (N=52) and SW (N=41). *P. aeruginosa* was isolated from 62 out-patients visiting the hospital's clinics and 46 cases from the SW. For *ESBL E. coli* and *E. coli* that were isolated in the highest frequency were referred to the clinics. *K. pneumoniae* and *ESBL*

K. pneumoniae were isolated mainly from the SW (N=22, 42), respectively. (Table 3.10)

MRSA S. aureus and *S. aureus* were isolated in the highest number from clinics (N=17, 22), respectively. *CoNS* were isolated mostly from the KU in a frequency of 112 cases, followed by 90 specimens from clinics. *E. faecalis* was found in 61 specimens from the SW and 56 specimens from clinics. *VRE E. faecium* was isolated from 27 cases within the ICU and 25 cases from the SW, where *E. faecium* was found in the highest number within the SW specimens (N=26). *Streptococcus spp.* were isolated in the highest frequency from clinics (N=4, 28), respectively. (Table 3.10)

C. tropicalis was isolated from 33 specimens within the ICU, and 32 specimens from the MW. *C. albicans* was isolated from 68 specimens from the ICU department, and 49 specimens from the MW. *C. glabarta* was found in 40 specimens from the ICU and 29 specimens from the SW. (Table 3.10)

Table 3.10

The overall frequency of common isolates with regard to hospital departments.

	SW	**ICU**	**Clinics**	**ER**	**MW**	**SICU**	**Cardiac surgery**	**KU**	**CCU**	**Vascular**
Gram negative										
Acinetobacter										
A. baumannii	41	52	22	8	12	36	9	4	6	10
Other Acinetobacter spp.	8	3	3	2	7	2	5	3	3	4
Pseudomonas										
P. aeruginosa	46	33	62	37	27	26	28	9	7	25
Other Pseudomonas spp.	8	6	4	5	6	2	0	8	1	1
E. coli										
CRE E. coli	3	0	2	1	4	4	0	0	0	0
ESBL E. coli	76	17	82	65	32	20	17	11	10	19
E. coli	53	21	106	58	25	10	13	12	12	9
K. pneumoniae										
ESBL K. pneumoniae	42	12	22	29	17	14	17	14	5	5
CRE K. pneumoniae	17	7	2	4	4	15	3	0	5	1
K. pneumoniae	22	9	18	14	16	6	16	6	5	5
Enterobacter										
CRE E. cloacae	1	0	1	0	0	1	0	0	0	1
ESBL E. cloacae	0	0	0	0	0	0	0	0	0	1
E. cloacae	15	5	9	14	9	3	5	10	6	3
Other Enterobacter spp.	2	1	2	1	3	2	2	0	0	2
Proteus										
CRE Proteus	0	0	0	1	1	0	0	0	0	0
ESBL Proteus	0	0	1	1	0	0	0	0	0	3
Proteus	9	7	15	8	5	1	5	4	1	6
Total	**343**	**166**	**351**	**248**	**168**	**168**	**120**	**81**	**61**	**95**
Gram positive										
S aureus										
MRSA S. aureus	9	4	17	7	8	0	5	12	3	7
S. aureus	7	6	22	18	12	0	4	21	5	3
CoNS	87	90	80	86	64	55	38	112	35	20
E. faecalis										
VRE E. faecalis	0	0	0	0	0	1	0	0	0	0
E. faecalis	61	45	56	52	46	24	22	8	6	24
E. faecium										
VRE E. faecium	25	27	10	11	22	15	9	1	10	7
E. faecium	26	10	5	15	12	14	5	0	3	3
Other Enterococcus spp.										
Other VRE Enterococcus spp.	6	1	1	1	2	0	2	0	1	0
Other Enterococcus	10	0	1	1	0	4	1	4	1	2
Streptococcus spp.										
S. pneumoniae	0	0	4	0	1	0	1	0	1	0
S. agalactiae	2	2	28	10	2	1	1	3	1	0
other Streptococcus spp.	19	6	31	10	4	6	9	10	5	1
Total	252	191	255	211	173	120	97	171	71	67
Candida										
C. tropicalis	22	33	7	10	32	24	11	0	10	4
C. albicans	46	68	17	25	49	28	23	0	22	6
C. glabrata	29	40	9	21	27	28	15	2	11	6

3.8 Antibiotics resistance pattern of common GNB in 2018–2020

We found that *E. coli* was resistant in a high rate for ciprofloxacin, norfloxacin, ampicillin, co-trimoxazole, and cephalosporin agents. *K. pneumoniae* was highly resistant to amoxicillin\ clavulanic acid, cephalosporins, ampicillin, ciprofloxacin, levofloxacin, norfloxacin, amikacin, gentamycin and co-trimoxazole with remarkable resistance to carbapenems. *A. Baumannii* was highly resistant (over 85%) for Ceftazidime, cefipim, ciprofloxacin, piperacillin, piperacillin\tazobactam, ticarcillin, ticarcillin\ Clavulanic acid, imipenem, meropenem (see table 3.11) (see figures 3.2, 3.3, 3.4, 3.5). (Rates of antibiotics resistance of more gram-negative bacteria species in 2018, 2019, and 2020 are within tables in appendix B).

3.9 Antibiotics resistance pattern of common GPB in 2018–2020

Concerning antibiotic resistance, we notice that *S. aureus* was highly resistant to penicillin during the study period and less resistant to erythromycin and clindamycin. For *CoNS*, it was extra-resistant to penicillin, oxacillin, amoxicillin\ clavulanic acid, cefuroxime, erythromycin, ciprofloxacin and less resistant to clindamycin, gentamycin, moxifloxacin, tetracycline, and co-trimoxazole. *Enterococcus spp.* was resistant in a high rate for tetracycline, quinupristin/dalfopristin, erythromycin, levofloxacin, and ciprofloxacin. (See Table 3.12) (see figures 3.6, 3.7, 3.8, 3.9). (Rates of antibiotic resistance of more gram-positive bacteria species in 2018, 2019, and 2020 are within tables in appendix B).

3.10 Anti-fungal drugs resistance pattern of most common candida species in 2018–2020

In general, candida have a good sensitivity for anti-fungal drugs. In 2018, N=1 *C. albicans* was resistant for amphotericin,N=2 *C. glabarta* were resistant for fluconazole and voriconazole, and N=5 were resistant for caspofungin. In 2019, N=4 *C. tropicalis* were resistant for fluconazole, N=3 *C. albicans* were resistant for voriconazole and caspofungin. In 2020, N=1 *C. albicans* was resistant for flucytosine and N=1 *C. glabarta* was resistant for voricoanzle (see Table 3.13 in appendix B).

Figure 3.2

Trends of resistance of A. baumannii during the study period.

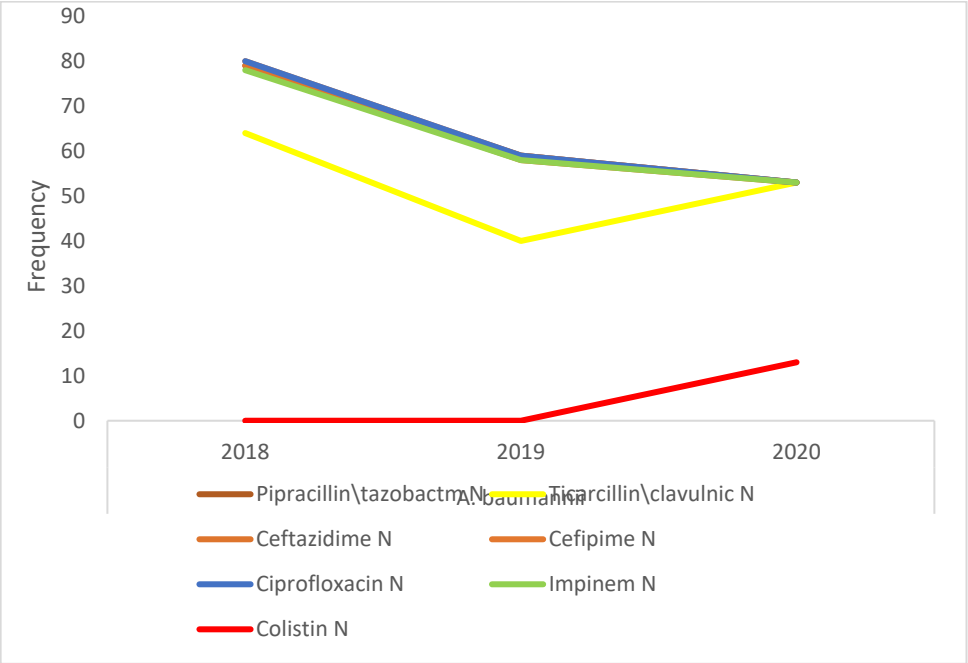


Figure 3.3

Trends of resistance of P. aeruginosa during the study period

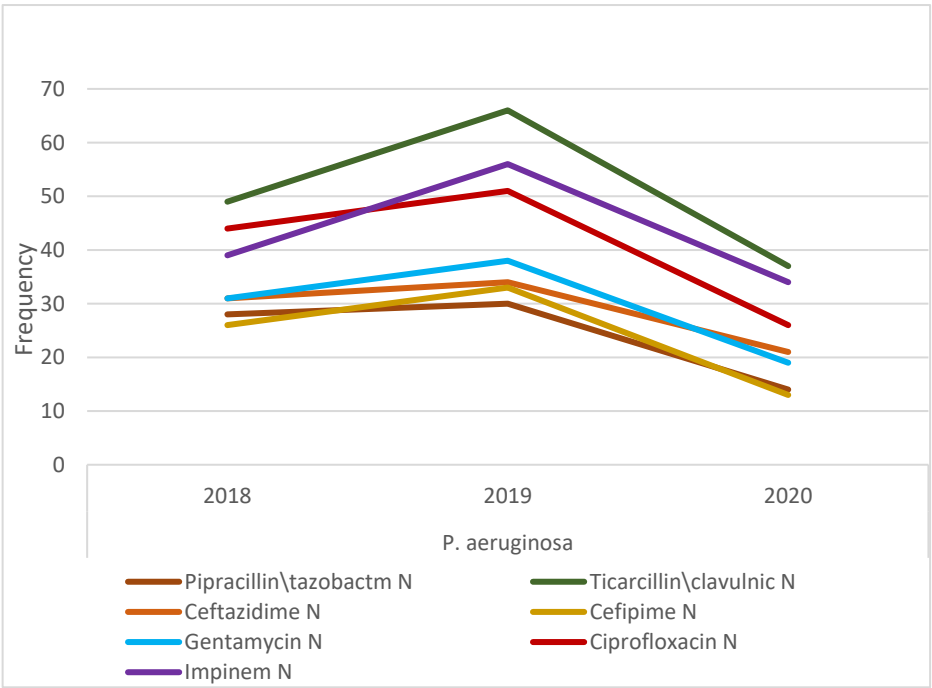


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Figure 3.4

Trends of resistance of E. coli during the study period.

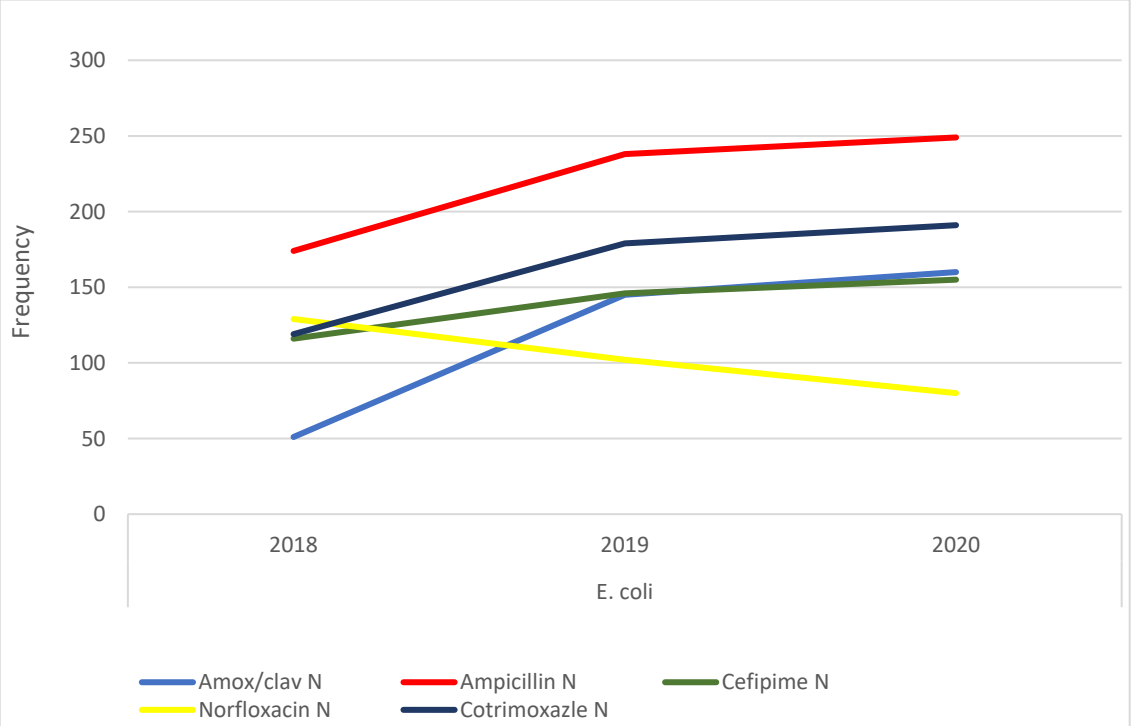


Figure 3.5

Trends of resistance of K. pneumoniae during the study period.

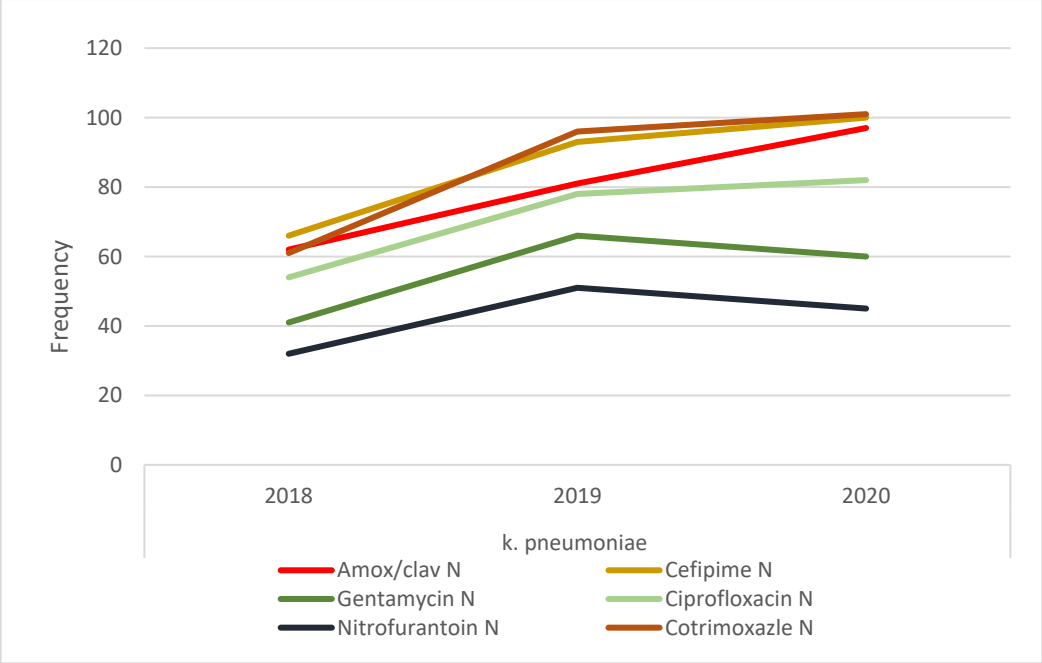


Figure 3.6

Trends of resistance of CoNS during the study period.

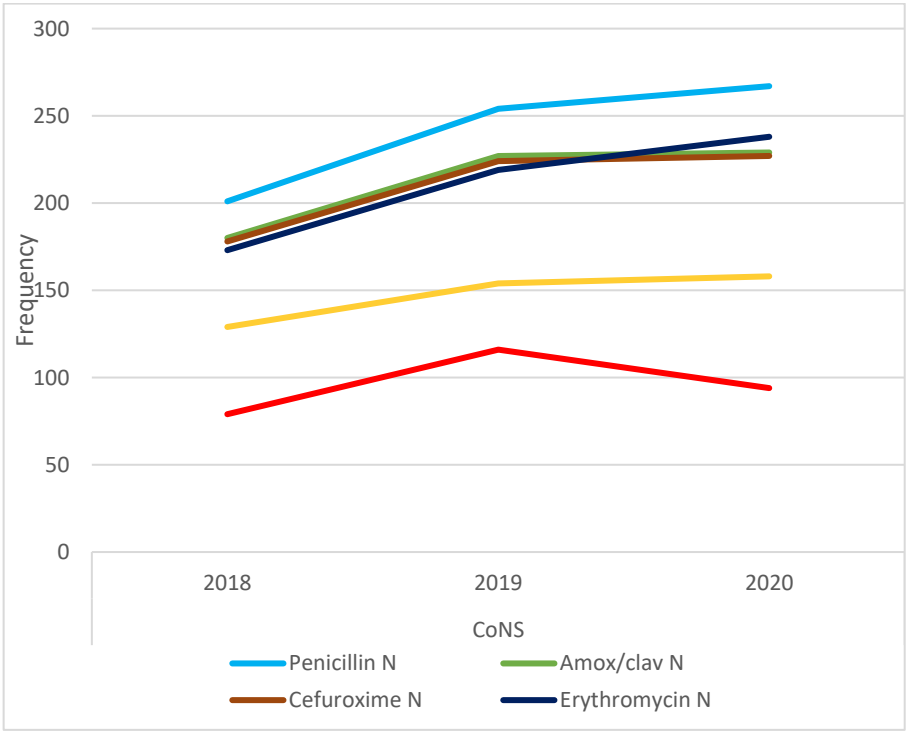


Figure 3.7

Trends of antibiotic resistance of S. aureus during the study period.

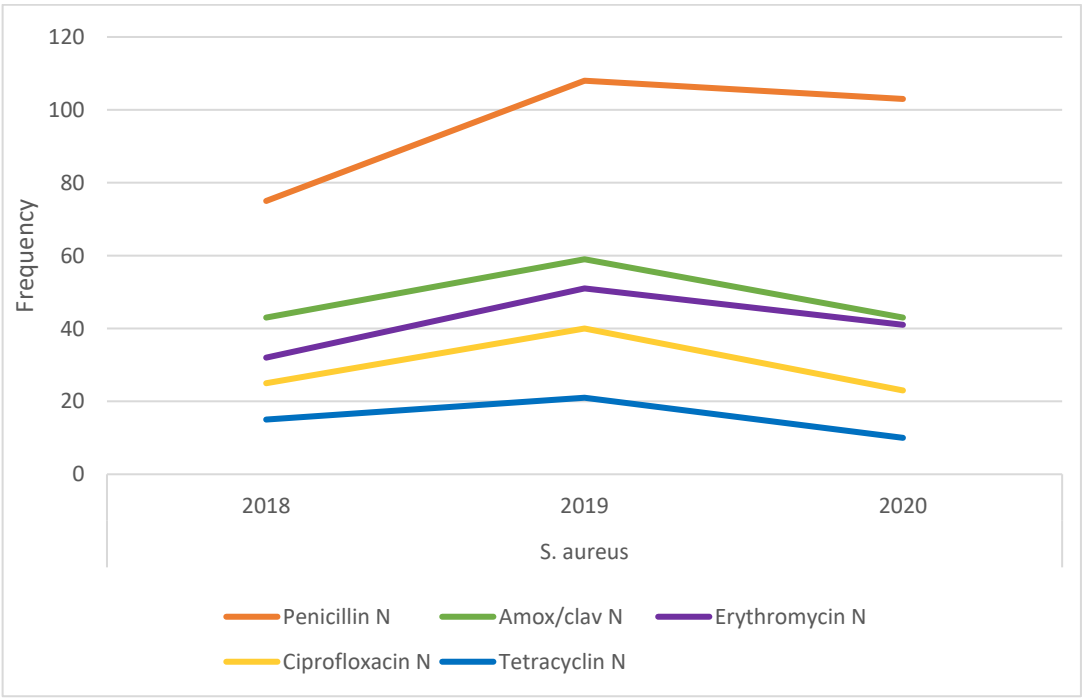


Figure 3.8

Trends of antibiotic resistance of Enterococcus spp. during the study period.

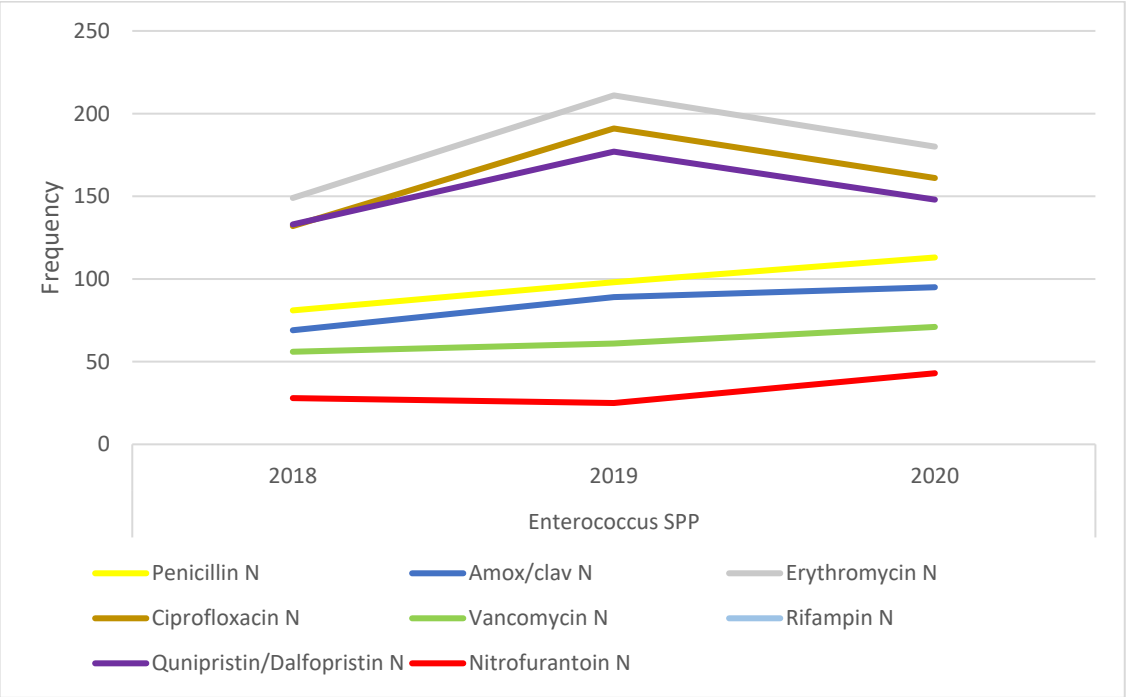
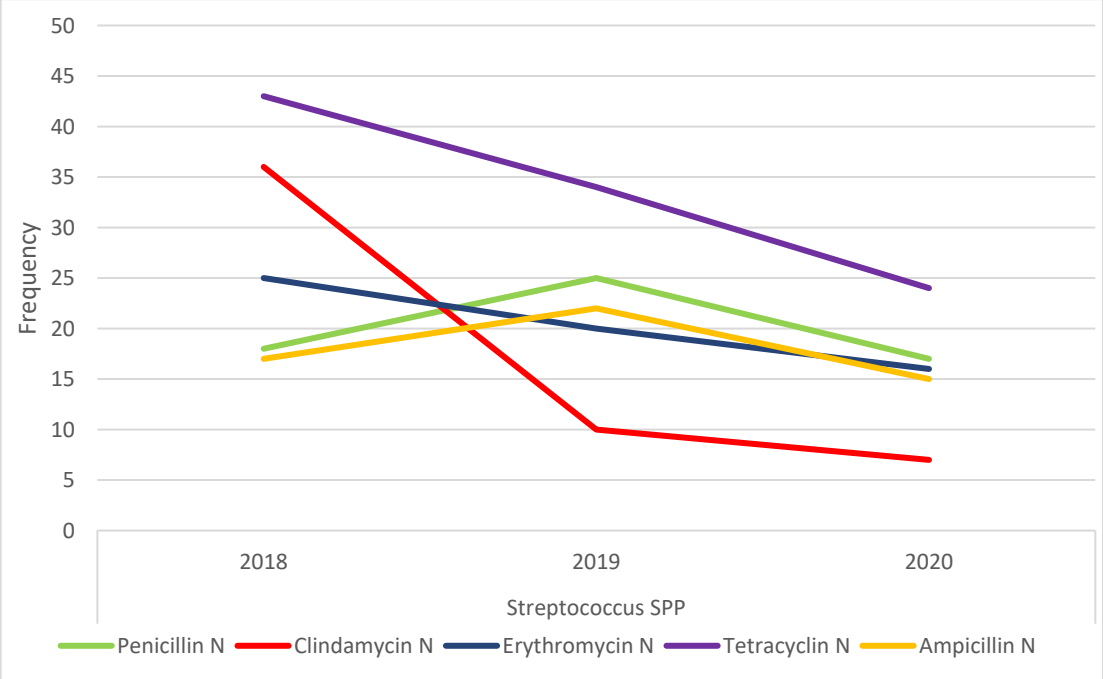


Figure 3.9

Trends of antibiotic resistance of Streptococcus spp. During the study period.



Chapter Four

Discussion

4.1 Overview

The emergence of antibiotic resistance among the most clinically significant bacterial pathogens is considered as a major public health threat that affects human life. Efficacious prevention and treatment have been limited as a result, and the treatment of some resistant infections becomes a complicated process and sometimes impossible. It is critical now to identify local susceptibility patterns in order to select the most suitable empiric antimicrobial agents with the least cost and time of treatment. Our study was done with the aim of assessing antimicrobial resistance patterns in a three year period (2018–2020) at a tertiary care hospital in Palestine. To the best of our knowledge, the present study will be the first in Palestine that performed over three years, including all the hospital departments and all types of isolated microbes (gram- positive and negative bacteria and candida). The available studies in Palestine where in one department level (115), shorter study period (116), evaluating single infection source (117), including one causative agent or family (94) (88), or studying resistance to single antimicrobial agent among specific bacterial family (86).

The present study includes 5585 samples. The majority of infections were caused by GNB (46.9%), while 37.9% of the positive samples represented GPB growth, and 15.2% showed candida presence. Similar findings were reported by another study in Palestine (118) in which they found 45.8% were GNB, 39.6% were GPB, and 14.6% were fungal isolates. Another study (119) reported a much higher percentage of GNB isolation rate (72.8%) than GPB isolation rate (27.2%). However, another study in Palestine (127) showed a higher isolation rate of GPB 46.4% than GNB (42.3%).

Regarding the sources of the collected samples, we found that urine specimens were the most common source with a total percentage of 27.7% among the three years, followed by wound specimens (19.6%), and blood specimens (19.2%). Close results were reported by a study from Kuwait (120), while another study performed in India (121) found that clinical samples were obtained most predominantly from stool (19.2%), urine cultures (10.8%), and blood cultures (12.1%). Another inconsistent study was

performed in 2019 (122) reported that blood specimens were the predominant infection source (88.7%), followed by cerebrospinal fluid (CSF) (5.3%) and pleural fluid (4%).

Various bacterial and candida samples were obtained from different hospital wards. From in-patients (69.7%), SW was the department from which the most number of clinical samples were obtained (14.6%), followed by the ICU (10.7%). While for out-patients (30.2%), they were represented mostly by clinic patients (13.5%). However, another study in India reported that most clinical samples were received from ICU (123), while another study stated that nosocomial infection are most common among patients in the medical ward than surgical ward. The highest incidence of both GNB and GPB in our findings was found within clinics followed by surgical wards with slight difference in the frequency between them, while the highest incidence was reported in the ICU and the least in SW in another study performed in Palestine (88). Additionally, a study was performed in the neonatal intensive care unit (NICU) in León, Nicaragua (124) reported much higher rates of GNB isolation (74%) than GPB (26%). The isolation rate of *A. baumanii* was highest among ICU-patients while (119) reported that it was obtained mostly from patients in the MW. High frequency of *CoNS* was found within the ICU and SW. This is considered similar to (125) findings.

With regard to frequencies and percentages of GNB, the most frequent Enterobacteriaceae isolates in the present study was *ESBL E. coli* (15.6% of GNB), followed by *E. coli* (14%), *ESBL K. pneumoniae* (7.7%), *K. pneumoniae* (5.5%), and *E. cloacae* (4%). Many studies reported *E. coli* to be the most prominent Enterobacteriaceae. A study in Palestine (86) mentioned *E. coli* (including *ESBL E. coli*) as the most obtained Enterobacteriaceae with an occurrence rate of 51% followed by *Klebsiella spp.* (19%). Other studies that reported similar findings are(127),(80) and (128). Similar to what is known about non-fermenting gram negative bacilli (NFGNB), *P. aeruginosa* and *A. baumannii* were the most obtained NFGNB in the present study with a percentage of 13.6% and 8.5% of GNB, respectively. According to (97) and (128), the prevalence of *P. aeruginosa* and *A. baumannii* in blood cultures was too close while (127) reported higher incidence of *A. baumannii* (20%) than *P. aeruginosa* (12%).

For GBP, the most frequent isolate was *Coagulase-negative Staphylococci (CoNS)* representing isolation rate of 14.7% of all isolated organisms and a percentage of 38.8%

of GPB for the three years, followed by *E. faecalis* (18.6% of GPB), *S. aureus* (8.2%), and *VRE E. faecium* (7.5%). Close results were obtained in another study in Palestine (128). The isolation rate was different in another study (123). They reported that out of 314 gram positive cocci, about 80% were *Coagulase Positive Cocci* followed by *Enterococci.* Another study (102) reported that *CoNS* is responsible for 31.9% of mono-microbial nosocomial bloodstream gram positive infections followed by *S. aureus* (15.7%) and *Enterococci (*11.1%).

For candida, the most frequent species was *C. albicans* with a percentage of 5.7% in total, the second was *C. glabrata* (3.6%) and *C. tropicalis* (3.2%). (113) reported similar findings according to the most frequent candida species. In contrast to our findings, a study from Saudi Arabia reported *C. parapsilosis* to be more frequent than *C. glabrata* (129).

What is critical more than the frequency of samples isolated from different wards is the rate of resistant organisms and the types of most frequent bacteria within each department. A study was performed in Taiwan to compare the colonized bacteria between files from the surgical ICU and the SW reported that 90% of files in the surgical ICU and 72.2% in the SW were contaminated with pathogenic or potentially pathogenic organisms. It is noteworthy that many of the isolated bacteria were MDR, such as *A. baumannii* and *K. pneumoniae* (130). Another study was performed in the US that stated that the resistance rates were the highest within the ICU and the least within the out-patients group (131). Another study in Uganda agreed with the previous studies indicating the presence of high level of resistance in the SW (132). This is considered similar to the hypothesis adopted by our study; resistant organisms are disseminated in a high rate among hospitalized patients over out-patients, predicting that bacteria causing nosocomial infections are highly resistant. Moreover, we assume that the ICU department and SW are affected by higher rates of resistant organisms over other hospital departments). In our study, *A. baumannii* and *VRE E. facium* were mostly isolated from the ICU while these organisms were the second-most frequently isolated organisms from the ICU after *CoNS* over five years in a study performed in Morocco (97). Additionally, *ESBL K. pneumoniae*, *CRE K. pneumoniae* and *VRE Enterococcus spp.* were isolated mostly from the SW. However, the hypothesis has not been proven correct for some other MDR organisms, such as *ESBL E. coli*, *P. aeruginosa*, and *MRSA*

S. aureus that were found most frequently within the out-patients group (specifically the clinics).

As mentioned, the isolates investigated in this study were obtained from different samples group. In general, *A. baumannii, P. aeruginosa, E. cloacae, Proteus spp.,* and *MRSA S. aureus* were isolated in the highest rate from wound samples, taking into consideration that wound samples group in our study also included pus and bed sore samples. These findings were in-line with (92) regarding *A. baumannii* and *P. aeruginosa,* while it was disagreed with (85), in which these two organisms were mainly isolated from respiratory samples. Additionally, (133) reported that *E. cloacae* and *Proteus spp.* were isolated in the highest rate from urine samples in contrast to our findings.

Urine samples in the present study have yielded many organisms in marked numbers, such as *ESBL E. coli, E. coli, ESBL K. pneumoniae, E. faecalis,* and *VRE E. faecium*. This was completely agreed with in (133) , (134) and (86). However, different findings were reported by (85) regarding *E. faecium*.

4.2 Antimicrobial resistance patterns among GNB

Investigating antimicrobial resistance patterns is a stage requirement for both physicians and policy makers to stop the growing issue of resistance and implement the appropriate empirical therapy. In general, we notice that remarkable resistance to most of antibiotics is more common among GNB compared to GPB, in-line with many other studies, such as (133) and (135). As we mentioned, *ESBL E.coli* was the prominent GNB and the most frequent MDRO within specimens in the present study. Another less occurred ESBL producing agent is *K. pneumoniae* although studies from Palestine showed low ESBL rates 20 years ago (35.3%) (136). Among different antibiotics that were tested against ESBL producing organisms, they showed very high resistance to cephalosporins, fluoroquinolones and ampicillin, while effective choices were limited to carbapenem, amikacin and nitrofurantoin. This resistance pattern was agreed with another study in Palestine (80) but not consistent with a study in Saudi Arabia that reported amikacin to be the less resistant agent and 20% resistance rate to carbapenems (137). However, carbapenems are considered now as the best choice for ESBL producing organisms as more than 98% of these organisms are still susceptible to this

group (138) (80). This is considered the same to our findings in the three years. Some studies mentioned fosfomycin and piperacillin\tazobactam among the efficient treatment options for ESBL- producing organisms (74), (139). However, these two agents were not checked for their susceptibility to ESBL in the present study.

The susceptibility patterns of Enterobacteriaceae and non-fermenter clinical isolates showed remarkable resistance to commonly used antibiotics. Starting with *E.coli,* which demonstrated high rate of resistance to ampicillin, co-trimoxazole, and fluoroquinolones, especially ciprofloxacin in the present study. Similar resistance pattern among Ethiopian *E. coli* clinical isolates was noted (133). On the other hand, no resistance was reported for amikacin; this finding is similar to the study in Saudi Arabia (140). About *K. pneumoniae,* the data suggested high resistance rate for ampicillin among the three years in line with (140) and moderate resistance to nitrofurantoin in 2018 and 2020 while it was decreased in 2019 to 7% only similar to the Indian study, which reported mild sensitivity to nitrofurantoin (48%) (141). However, other studies showed much more complexed resistance pattern for *K. pneumoniae* (142). *E. cloacae* in our study showed the highest resistance for amoxicillin\clavulanic acid in line with (133) and cefuroxime. For *Proteus,* remarkable resistance was noted for ampicillin, nitrofurantoin, co-trimoxazole and imipenem, which ranges between 14-43% among the three years similar to (143) in which imipenem resistance was 31%. *ESBL Proteus* was highly resistant to cephalosporins, fluoroquinolones, ampicillin, and co-trimoxazole while it show effectivity to meropenem the same as *ESBL E. cloacae* (N=2).

Carbapenems have been used as the “last-resort” treatment options for infections caused by resistant Enterobacteriaceae, including ESBL producers. However, carbapenem-resistant Enterobacteriaceae (CRE) are now one of the greatest public health threats. The most clinically relevant carbapenemase producers are: *E. coli, K. pneumoniae, E. cloacae,* and *Proteus* while the most common pathogen for CRE cases was *K. pneumoniae* followed by *E. coli*. This is in agreement with the Egyptian study (87) and other reports from the Middle East (144). The overall percentage of CR among GNB was (88\2619) 3.4%. This is lower than Jordan 5.6% (145) and Colombia 8.8% (146). Regarding CRE resistance pattern in the present study, it showed high resistance to most ß-lactam antibiotics including penicillins, cephalosporins, carbapenems, especially imipenem. Additionally, resistance to structurally unrelated classes such as

aminoglycosides and fluoroquinolones was remarkably noted. The optimal treatment choices for CRE infections are largely unknown depending mainly on the susceptibility report for each case and using few antibiotic classes that remains effective for CRE such as polymyxins, fosfomycine, tigecycline, and aminoglycosides (147) (148). In fact, these antibiotics were not checked for their susceptibility in the current study, except aminoglycosides that showed moderate to mild susceptibility among the three years.

Non-fermenting gram negative bacilli (NFGNB) are important health care associated organisms that exhibit remarkable resistance to many clinically used antibiotics. In the present study, the most common non-fermenters are *P. aeruginosa* (N=375) followed by *A. baumanii* (N=223). *A. baumanii* have a much more complicated resistance pattern compared with *P. aeruginosa* since *A. baumanii* have extremely drug resistance profiles to most antibiotics tested, similar resistance profiles was described by another study in Palestine (94) and other regions in the world (149). Resistance to carbapenems, which is considered as treatment choice for *A .baumanii* was above 80% among the three years. This percentage varies largely between studies, 48% in Algeria (150), 60–90% in Tunisia (151), 50–75% in Morocco (97). On the other hand, more than 90% of the isolated *A. baumanii* are carbapenem susceptible in a study from the US (152) and 100% susceptible to imipenem in India (92). However, *A. baumanii* showed good sensitivity to colistin in our study. This is in agreement with another study in Palestine (94). *P. aeruginosa* showed low to moderate resistance to third generation cephalosporins, carbapenems, aminoglycoside and fluoroquinolones. Close resistance profile was reported in a study from Saudi Arabia (153) while lower rate of resistance to carbapenems and higher to aminoglycoside and fluoroquinolones was noted in the Indian study (154).

4.3 Antimicrobial resistance patterns among GPB

GPB are important nosocomial pathogens having wide variety of resistance patterns with particular concern to *MRSA* and *VRE*. Starting with *S. aureus*, it was ranked within the highest frequent isolates (N=310). Of them, 43.8% were *MRSA* (N=136). This rate varies widely among studies. Another study in Palestine reported a rate of 80% (155), 31% Jordan (156), 61% Iraq (157), 82% in Egypt (158). Regarding resistance pattern, nearly complete resistance was reported among penicillin, oxacillin, amoxicillin/clavulanic acid and cefuroxime while no resistance was noted for

vancomycin, tigecycline, linezolid, and Quinupristin/dalfopristin among MRSA isolates. This is in agreement with a study from South Africa that reported no resistance for vancomycin and linezolid (85). Similar results are observed from Jordan (156) and Afghanistan (159).

Another common GBP infections are the *Enterococci*, which stands for the first letter in ESKAPE organisms. Since it was highlighted by the WHO as a growing cause of antibiotic resistance infections in the few last decades, its local susceptibility profile should be clearly set. According to the results, *E. faecalis* was isolated in a rate higher than *E. faecium*. However, *Vancomycin-resistant enterococci (VRE)* was more common among *E. faecium* in line to what is known in the literature (120), (160). *VRE E. faecium* showed nearly 100% resistance to many important antibiotics: vancomycin, amoxicillin\clavulanic acid, penicillin, ampicillin, erythromycin, ciprofloxacin, and levofloxacin. While high resistance level was reported for streptomycin HLA and gentamycin HLA. Another Italian study reported full sensitivity to vancomycin among *Enterococcus spp.* (161). On the other hand, our results showed good susceptibility to linezolid and tigecycline in accordance to many studies. (160) reported that linezolid is the only FDA-approved antibiotic against *VRE* infections and tigecycline considered the last salvage choice for *VRE*. Other studies reported the absence of resistance to linezolid among *Enterococcus spp.* in Bangladesh (162). However, (163) reported 2% resistance to linezolid and only 14% to vancomycin among *Enterococcus spp.* .

Coagulase negative staphylococci (CoNS) were considered as contaminant organisms in the past. Now, they are a well-known source of nosocomial bloodstream infections. In the present study, results showed extremely high resistance for penicillin, oxacillin, amoxicillin/clavulanic acid, cefuroxime, and erythromycin. Close resistance rate was reported for penicillin (96%) and erythromycin (70%) by (164) and (165). Nearly 100% sensitivity was found among *CoNS* isolates for vancomycin, tigecycline and quinupristin/dalfopristin in accordance with (165) for vancomycin and tigecycline. Another Turkish study reported high sensitivity for vancomycin and teicoplanin (125). Although, new studies reported increased vancomycin resistance among *CoNS* isolates that may be illustrated by the increased prevalence of *S. warneri* (166) or *S. capitis* (167).

4.4 Antifungal drugs resistance patterns among candida species

The evolution of antifungal drug resistance in Candida species has been considered as the main cause of increased morbidity and mortality among immunocompromised patients. Understanding species prevalence and antifungal drug resistance patterns is a critical step for new drug development and fungal infections control. In the present study, all candida species showed good sensitivity to anti-fungal drugs. Only few cases of resistance was noted, especially for caspofungin and voriconazole among *C. glabarta* isolates. An inconsistent study showed that all candida species including *C. glabarta* were 100% susceptible to voriconazole and caspofungin (168). While another study reported nearly full sensitivity to amphotericin-B in line with our findings (169). In agreement to our findings, many studies in the literature showed that resistance to anti-fungal drugs is much higher in *C. glabarta* than other common candida species and specially to echinocandins, which have replaced the azoles as first-line therapy (170), (171).

4.5 Strengths and limitations

Strengths:

1. The objectives are clearly stated.
2. There is no conflict of interest.
3. This is the first study in Palestine to address the antibiotic resistance pattern within three years including this large number of samples (5585 samples).

Limitations:

1. The study discussed the antibiotic resistance pattern without associating it with antibiotic consumption.
2. Due to the short duration (3 Ys), we were not able assess the trends of antibiotics resistance.
3. The study does not implement the sexes and ages of the patients.
4. The study was performed in one healthcare setting that made it less representative.

4.6 Conclusions

In general, we found that the antibiotic resistance rate was relatively high at NNUH during the study period to most commonly used agents. High prevalence of MDRO was

detected especially among ESKAPE pathogens. High level of resistance for wide-spectrum antibiotics was clearly noted, such as third-generation cephalosporins, aminoglycosides, and carbapenems.

The current findings showed that GNB represented the majority of the isolated pathogens. Of GNB, The most frequent Enterobacteriaceae was *E. coli* while *P. aeruginosa* and *A. baumannii* were the most obtained NFGNB. For GBP, the most frequent isolate was *CoNS*. Clinical specimens were collected in the highest frequency from urine samples and mostly from in-patients. The SW was the most prominent department followed by the ICU.

Commonly prescribed antibiotics such as penicllins, cephalosporins, and carbapenems have become poorly efficacious for many pathogens such as: *A. baumannii* which showed high resistance (over 85%) for ceftazidim, cefipim, gentamycin, ciprofloxacin, levofloxacin, piperacillin, and imipenem. *ESBL E. coli* and *ESBL k. pneumoniae* showed nearly complete resistance to ampicillin and cephalosporin agents. *MRSA S. aureus* was nearly completely resistant to penicillin, oxacillin, amoxicillin\clavulinic acid, cefuroxime. *VRE E. faecium* showed full resistance to penicillin, amoxicillin\clavulinic acid, ampicillin, vancomycin, levofloxacin, ciprofloxacin, and erythromycin.

Regarding trends of antibiotic resistance during study period, we were not able to notice obvious changes in the trends of resistance since we think that a period of three years was not enough to definitively detect the susceptibility changes of the bacteria towards each antibiotic.

4.7 Recommendations

This study demonstrated the urgent need for conducting surveillance studies detecting local antimicrobial patterns and resistance rate of the most common organisms to control the emergence and dissemination of resistant strains and to encourage the best usage of antimicrobials in the treatment of infectious diseases. Additionally, there is a real need for educational programs discussing infection control and prevention parameters. Policies regarding antibiotics use and prevention measures should be a priority for strategic health plans as part of the global antibiotic resistance reduction

target. Physicians should reduce the empirical use of antibiotics in non- complicated infections and depend more on sensitivity tests for specific treatment.

We strongly advocate the implement of Antibiotic Stewardship Programs in NNUH that can improve and measure the appropriate use of antimicrobial agents. There is a strong evidence of the significance role that the clinical pharmacist can play in such programs suggesting the need of hiring more clinical pharmacists in the different health care settings.

List of Abbreviations

Abbreviations	Meaning
AMR	Antimicrobial Resistance
ARG	Antibiotic Resistance Genes
AGP	Antimicrobial Growth Promoters
BARDA	Biomedical Advanced Research and Development Authority's
BSI	Primary Bloodstream Infections
CCU	Cardiac Care Unit
CDC	Centers for Disease Control and Prevention
CNRP	Carbapenem Non-Restricted Period
CNUTI	Complicated Nosocomial Urinary Tract Infection
CoNS	Coagulase negative Staphylococci
CRE	Carbapenem-Resistant Enterobacteriaceae
CRP	Carbapenem Restricted Period
CSF	Cerebrospinal Fluid
CVC	Central Venous Catheter
DDD	Defined Daily Doses
ER	Emergency Room
ESBL	Extended Spectrum B-lactamases
FDA	Food and Drug Administration
GBS	Group B Streptococcus
GNB	Gram-Negative Bacteria
GPB	Gram-Positive Bacteria
ICU	Intensive Care Unit
IRB	Institutional Review Board
JPIAMR	Joint Programming Initiative on Antimicrobial Resistance
LPS	Lipopolysaccharide
MDR	Multi-Drug Resistant
MW	Medical Ward

MRSA	Methicillin-Resistant Staphylococcus Aureus
NFGNB	Non-Fermenting Gram Negative Bacilli
NI	Nosocomial Infection
NNUH	An-Najah National University Hospital
NUTI	Nosocomial Urinary Tract Infection
PBP2a	Penicillin-Binding Protein
PCR	Polymerase Chain Reaction
PICU	Pediatric Intensive Care Unit
PW	Pediatric Ward
PNSP	Penicillin Non-Susceptible Pneumococci
Q/D	Quinupristin/Dalfopristin
SCOPE	Surveillance and Control Of Pathogens of Epidemiologic importance
SPSS	Statistical Package for Social Sciences
SSI	Surgical Site Infections
SICU	Surgical Intensive Care Unit
SW	Surgical Ward
TSN	The Surveillance Network
TMP–SMX	Trimethoprim-sulfamethoxazole
UK	United Kingdom
UTI	Urinary Tract Infection
VAP	Ventilator Associated Pneumonia
VISA	Vancomycin-Intermediate Staphylococcus aureus
VRE	Vancomycin-Resistant Enterococcus
VREf	Vancomycin-Resistant Enterococcus faecium
VRSA	Vancomycin-Resistant Staphylococcus aureus
WHO	World Health Organization
WHO PPL	World Health Organization Priority Pathogens list

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Appendices

Appendix A

Institutional Review Board (IRB) approval letter.

An-Najah
National University
Health Faculty of medicine&
Sciences
IRB

جامعة النجاح
الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref: Mas. August /2020/6

IRB Approval Letter

Study Title:
"*Evaluation of antibiotic resistance at a tertiary care hospital in Palestine: a three year's retrospective study.*"

Submitted by:
Aya Abu Diak

Supervisor:
Adham Abu Taha

Date Approved:
13th August 2020

Your Study Title "***Evaluation of antibiotic resistance at a tertiary care hospital in Palestine: a three year's retrospective study.***". was reviewed by An-Najah National University IRB committee and was approved on 13th August 2020.

Hasan Fitian, MD
IRB Committee Chairman
An-Najah National University

IRB

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Appendix B

More Detailed Tables of the Results

Table 3.6

Frequency of common candida species during the study period.

		2018	2019	2020	Total
C. tropicalis	Count	37	78	62	177
	% within YEAR	2.40	3.80	3.10	3.20
C. albicans	Count	86	112	119	317
	% within YEAR	5.50	5.50	6.00	5.70
C. glabrata	Count	42	84	75	201
	% within YEAR	2.70	4.10	3.80	3.60

Table 3.8

Frequency of in- and out- patients of the most common isolates within the study period

	2018				2019				2020			
	In pt.		Out pt.		In pt.		Out pt.		In pt.		Out pt.	
Gram negative	N	%	N	%	N	%	N	%	N	%	N	%
Acinetobacter												
A. baumannii	81	86	13	14	65	90	7	10	47	83	10	18
Other Acinetobacter SPP	14	100	0	0	19	83	4	17	7	88	1	13
Pseudomonas												
P. aeruginosa	80	75	27	25	108	77	33	23	70	64	39	36
Other Pseudomonas spp.	13	100	0	0	14	74	5	26	13	77	4	24
E. coli												
CRE E. coli	1	100	0	0	4	100	0	0	8	73	3	27
ESBL E. coli	79	69	36	31	90	62	56	38	96	65	52	35
E. coli	59	57	45	43	72	55	60	46	71	53	62	47
k. pneumoniae												
ESBL k. pneumoniae	37	66	19	34	53	72	21	28	61	85	11	15
CRE k. pneumoniae	9	90	1	10	19	95	1	5	29	88	4	12
k. pneumoniae	29	76	9	24	40	74	14	26	42	82	9	18
Enterobacter												
CRE E. cloacae	0	0	1	100	2	100	0	0	2	100	0	0
ESBL E. cloacae	2	100	0	0	0	0	0	0	0	0	0	0
E. cloacae	28	80	7	20	34	85	6	15	21	68	10	32
Other Enterobacter spp.	4	80	1	20	4	67	2	33	7	100	0	0
Proteus												
CRE Proteus	1	50	1	50	0	0	0	0	1	100	0	0
ESBL Proteus	3	75	1	25	3	75	1	25	2	67	1	33

Proteus	17	63	10	37	34	72	13	28	9	41	13	59
Gram positive												
S. aureus												
MRSA S. aureus	31	74	11	26	37	74	13	26	31	71	13	30
S. aureus	23	58	17	43	43	65	23	35	42	62	26	38
CoNS	199	85	35	15	225	81	52	19	230	74	80	26
E. faecalis												
VRE E. faecalis	0	0	0	0	0	0	0	0	1	100	0	0
E. faecalis	87	78	24	22	112	73	42	27	86	67	42	33
E. faecium												
VRE E. faecium	38	86	6	14	46	89	6	12	53	86	9	15
E. faecium	27	84	5	16	32	84	6	16	30	77	9	23
Other Enterococcus spp.												
Other VRE Enterococcus spp.	0	0	0	0	5	100	0	0	10	83	2	17
Other Enterococcus	13	93	1	7	9	100	0	0	5	83	1	17
Streptococcus spp.												
S. pneumoniae	2	50	2	50	3	60	2	40	0	0	0	0
S. agalactiae	3	50	3	50	9	36	16	64	6	24	19	76
Other Streptococcus spp.	46	68	22	32	23	64	13	36	21	78	6	22
Candida												
C. tropicalis	32	87	5	14	70	90	8	10	58	94	4	7
C. albicans	79	92	7	8	99	88	13	12	97	82	22	19
C. glabrata	35	83	7	17	72	86	12	14	64	85	11	15

Table 3.11

Overall rates of antibiotics resistance of the most common gram-negative bacteria during the study period.

		A. baumannii	***P. aeruginosa***	***E. coli***	***K. pneumoniae***
Total number of isolates		223	357	794	408
Amoxicillin/ clavulanic acid	N			356	240
	R%			52	59.36
Ampicillin	N			661	403
	R%			84.2	100.0
Piperacilli	N	189	126		
	R%	85.9	37.0		
Pipracillin\tazobactam	N	191	72	89	157
	R%	86.0	21.1	17.7	38.6
Ticarcillin	N	176	161		
	R%	79.2	47.0		
Ticarcillin\ clavulanic acid	N	157	152		
	R%	90.0	46.6		
Cefuroxime	N				179
	R%				66.7
Ceftriaxone	N			420	264
	R%			53.5	65.0
Cefotaxime	N			421	264
	R%			53.5	65.0
Ceftazidime	N	190	86	428	268
	R%	85.2	24.2	53.9	65.5
Cefepime	N	192	72	417	259

	%	86.0	20.1	52.8	63.4
Gentamycin	N	170	88	245	167
	R%	76.9	24.7	34.6	40.9
Amikacin	N			52	38
	R%			6.5	15.0
Tobramycin	N	164	85		
	R%	74.5	24.6		
Ciprofloxacin	N	192	121	466	214
	R%	86.0	33.8	58.8	52.6
Levofloxacin	N				92
	R%				39.3
Norfloxacin	N			311	93
	R%			71.1	50.3
Imipenem	N	189	129	20	64
	R%	85.5	36.1	2.5	15.0
Meropenem	N	191	96	20	65
	R%	85.6	26.8	2.5	15.1
Ertapenem	N			19	63
	R%			2.4	14.6
Nitrofurantoin	N			40	128
	R%			9.1	84.5
Co-trimoxazole	N	125		489	258
	R%	60.0		61	63.1
Minocycline	N	41	11		
	R%	18.1	100.0		
Colistin	N	13	2		
	R%	11.30	1.6		

Table 3.12

Overall rates of antibiotics resistance of the most common gram-positive bacteria during the study period.

		CoNS	*S. aureus*	*Enterococcus spp.*	*Streptococcus spp.*
Total number of isolates		821	310	707	196
Penicillin	N	722	286	292	60
	R%	94.50	92.9	41.5	34.3
Ampicillin	N			281	54.0
	R%			39.6	29.5
Oxacillin	N	644	143		
	R%	83.4	48.0		
Amoxicillin/clavulanic acid	N	636	145	253	7
	R%	80.4	48,1	44.3	5.8
Cefuroxime	N	629	141		
	R%	83.9	46.9		
Erythromycin	N	630	124	540	61
	R%	78.3	40.3	92.5	65.7
Gentamycin	N	254	23		
	R%	33.0	7.6		
StreptoHLA	N			407	
	R%			58.7	
GentaHLA	N			427	
	R%			61.0	
Ciprofloxacin	N	441	88	484	
	R%	54.4	28.6	69.4	
Levofloxacin	N	355	87	476	19
	R%	44.5	28.1	74.8	10.7
Moxifloxacin	N	153	39		15

	R%	20.6	14.4		38.4
Tetracycline	N	237	46	491	101
	R%	30.9	15.1	69.8	53.0
Clindamycin	N	387	82		53
	R%	48.1	26.5		38.2
Vancomycin	N	2	1	188	1
	R%	0.2	0.3	26.8	40.0
Linezolid	N	0	0	33	0
	R%	0.0	0.0	4.8	0.0
Tigecycline	N	0	0	3	0
	R%	0.0	0.0	0.4	0.0
Rifampin	N	54	6		
	R%	7.0	1.8		
Quinupristin/dalfopristin	N	3	0	458	0.0
	R%	43.0	0.0	77.7	0.0
Co-trimoxazole	N	289	24		7.0
	R%	36.2	7.9		77.5
Nitrofurantoin	N			96	
	R%			39.0	

Table 3.13

Overall rates of anti-fungal drugs resistance of the most common candida species during the study period.

		2018		**2019**		**2020**	
		C. albicans	**other candida**	***C. albicans***	**other candida**	***C. albicans***	**other candida**
Fluconazole	N	0	15	3	19	0	23
	R%	0.0	13.8	2.7	16.2	0.0	16.4
Voriconazole	N	0	7	3	6	0	3
	R%	0.0	6.4	2.7	3.0	0.0	1.4
Caspofungin	N	0	0	0	14	0	0
	R%	0.0	0.0	0.0	7.0	0.0	0.0
Micafungin	N	0	0	0	1	0	1
	R%	0.0	0.0	0.0	0.5	0.0	0.5
Flucytosine	N	1	9	1	17	1	17
	R%	1.1	8.2	0.9	8.3	0.8	8.3
Amphotericin	N	1	2	0	0	0	0
	R%	1.1	1.9	0.0	0.0	0.0	0.0

Table 3.14

Distribution of sample type and bacterial isolate in 2018.

	Blood	Wound	Urine	Tissue	Respiratory origin	Fluids	Others
	N	N	N	N	N	N	N
Gram negative							
Acinetobacter							
A. baumannii	11	31	10	1	30	7	4
Other Acinetobacter spp.	2	3	3	0	4	2	0
Pseudomonas							
P. aeruginosa	13	55	21	2	9	6	1
Other Pseudomonas spp.	7	4	1	0	0	1	0
E. coli							
CRE E. coli	0	1	0	0	0	0	0
ESBL E. coli	13	19	73	3	2	5	0
E. coli	5	15	69	0	6	7	2
K. pneumoniae							
ESBL k. pneumoniae	4	14	28	1	7	1	1
CRE k. pneumoniae	2	3	3	0	0	0	2
k. pneumoniae	5	4	11	2	15	1	0
Enterobacter							
CRE E. cloacae	0	0	1	0	0	0	0
ESBL E. cloacae	1	1	0	0	0	0	0
E. cloacae	7	9	6	4	6	2	1
Other Enterobacter spp.	1	1	0	0	3	0	0
Proteus							
CRE Proteus	0	0	2	0	0	0	0
ESBL Proteus	0	3	1	0	0	0	0
Proteus	1	13	10	1	1	0	1
Gram positive							
S. aureus							
MRSA S. aureus	15	15	2	1	5	2	2
S. aureus	14	12	0	3	7	2	2
CoNS	162	7	9	21	11	20	4
E. faecalis	10	35	46	1	11	5	3
E. faecium							
VRE E. faecium	6	10	19	2	3	3	1
E. faecium	2	12	11	1	4	2	0
Other Enterococcus	5	4	2	1	2	0	0
Streptococcus spp.							
Streptococcus spp.	10	9	25	3	5	12	4
S. pneumoniae	0	0	0	0	1	0	3
S. agalactiae	0	2	3	0	1	0	0
Candida							
C. tropicalis	1	2	17	0	16	0	1

C. albicans	6	6	30	1	36	3	4
C. glabrata	9	1	27	0	2	3	0

Table 3.15

Distribution of sample type and bacterial isolate in 2019.

	Blood	**Wound**	**Urine**	**Tissue**	**Respiratory origin**	**Fluids**	**Others**
	N	N	N	N	N	N	N
Gram negative							
Acinetobacter							
A. baumannii	6	28	7	2	26	1	2
Other Acinetobacter spp.	7	6	2	1	5	2	0
Pseudomonas							
P. aeruginosa	23	49	23	7	25	4	10
Other Pseudomonas spp.	2	2	0	2	7	5	1
E.coli							
CRE E. coli	1	1	2	0	0	0	0
ESBL E. coli	5	32	88	4	5	11	1
E. coli	11	33	66	3	6	7	6
K. pneumoniae							
ESBL k. pneumoniae	3	18	41	0	5	6	1
CRE k. pneumoniae	5	5	5	1	3	0	1
k. pneumoniae	10	9	17	1	11	4	2
Enterobacter							
CRE E. cloacae	1	1	0	0	0	0	0
E. cloacae	5	10	5	3	14	3	0
Other Enterobacter spp.	0	2	2	0	1	1	0
Proteus							
ESBL Proteus	0	3	1	0	0	0	0
Proteus	1	21	19	2	1	1	2
Gram positive							
S. aureus							
MRSA S. aureus	15	24	0	5	4	0	2
S. aureus	16	26	2	5	9	5	3
CoNS	149	15	12	18	25	30	28
E. faecalis	8	41	64	7	19	9	6
E. faecium							
VRE E. faecium	0	17	17	2	16	0	0
E. faecium	3	8	15	1	4	4	3
Other Enterococcus spp.							
Other VRE Enterococcus spp.	1	1	1	1	0	0	1
Other Enterococcus	0	3	1	1	3	1	0
Streptococcus spp.							

S. pneumoniae	0	0	0	0	3	0	2
S. agalactiae	0	1	17	0	4	1	2
Other Streptococcus spp.	9	4	0	0	5	17	1
Candida							
C. tropicalis	7	4	29	0	33	3	2
C. albicans	5	9	37	0	53	4	4
C. glabrata	9	8	40	2	22	2	1

Table 3.16

Distribution of sample type and bacterial isolate in 2020.

	Blood	**Wound**	**Urine**	**Tissue**	**Respiratory Origin**	**Fluids**	**Others**
	N	N	N	N	N	N	N
Gram negative							
Acinetobacter							
A. baumannii	7	22	8	2	13	4	1
Other Acinetobacter spp.	2	2	1	0	3	0	0
Pseudomonas							
P. aeruginosa	18	24	19	3	29	9	7
Other Pseudomonas spp.	6	3	2	0	1	4	1
E. coli							
CRE E. coli	1	3	3	1	2	1	0
ESBL E. coli	16	23	78	3	6	21	1
E. coli	18	16	71	2	5	20	1
k. pneumoniae							
ESBL k. pneumoniae	8	12	31	1	15	4	1
CRE k. pneumoniae	2	5	11	1	6	2	6
k. pneumoniae	9	7	16	0	13	6	0
Enterobacter							
CRE E. cloacae	1	1	1	0	0	2	0
E. cloacae	7	9	5	1	2	6	1
Other Enterobacter spp.	0	1	1	1	2	1	1
			Proteus				
ESBL Proteus	0	1	2	0	0	0	0
Proteus	0	14	5	0	2	1	0
Gram positive							
S. aureus							
MRSA S. aureus	7	20	1	1	6	0	9
S. aureus	21	20	4	1	14	5	3
CoNS	160	19	15	13	24	48	31
E. faecalis	12	29	54	2	17	11	3
E. faecium							
VRE E. faecium	2	11	28	1	16	4	0
E. faecium	1	9	15	2	4	6	2

Other Enterococcus spp.													
Other VRE Enterococcus spp.	1	5	2	0	0	4	0						
Other Enterococcus	1	0	1	0	0	4	0						
Streptococcus spp.													
S. agalactiae	1	2	20	0	1	0	1						
Other Streptococcus spp.	7	4	3	1	4	8	0						
Candida													
C. tropicalis	6	6	15	1	27	4	3						
C. albicans	5	10	42	4	52	5	1						
C. glabrata	7	5	39	0	16	7	1						

Table 3.17

Rates of antibiotics resistance of the most common gram-negative bacteria in 2018.

		A. baumannii	***P. aeruginosa***	***ESBL E. coli***	***E .coli***	***ESBL k. pneumoniae***	***CRE k. pneumoniae***	***k .pneumoniae***	***ESBL E. cloacae***	***E .cloacae***	***CRE Proteus***	***ESBL Proteus***	***Proteus***
Amox/clav	N	75	85	34	17	11	9	9	2	35	2	0	0
	R %	98	98	30	16	20	100	24	100	100	100	0	0
Cefuroxime	N	75	74	77	7	43	8	2	1	30	2	4	3
	R %	98	96	100	9	97	100	9	50	90	100	100	15
Ceftriaxone	N	77	74	109	2	55	9	1	2	12	2	4	1
	R %	100	96	98	2	100	100	2	100	34	100	100	3
Cefotaxime	N	1		109	2	55	9	1	2	14	2	4	1
	R %	50		98	2	100	100	2	100	40	100	100	4
Ceftazidime	N	79	31	113	3	56	10	1	2	12	2	4	1
	R %	84	29	98	2	100	100	2	100	34	100	100	3
Cefepime	N	80	26	113	2	55	10	1	1	10	2	4	1
	%	85	24	98	1	98	100	2	50	28	100	100	3
Gentamycin	N	75	31	43	18	32	4	2	1	6	0	3	7
	R %	81	29	37	17	57	40	5	100	17	0	75	25
Ciprofloxacin	N	80	44	84	45	39	6	7	0	7	1	3	6
	R %	85	41	73	43	69	60	18	0	20	50	75	22
Levofloxacin	N	58	36	71	37	30	3	5	0	6	1	3	6
	R	85	42	78	44	73	50	16	0	18	50	75	24

	%												
Amikacin	N	0	30	1	0	1	6	0	0	1	0	0	0
	R	0	28	0.9	0	1	60	0	0	2	0	0	0
	%												
Rifampin	N	3											
	R	18.80%											
	%												
Cotrimoxazole	N	52		80	38	49	8	4		7	2	4	15
	R	55		69	36	87	80	10		25	100	100	55
	%												
Ampicillin	N			112	59	55	9	36			2	4	11
	R			100	57	100	100	100			100	100	45
	%												
Piperacillin	N	79	53								2	0	2
	R	85	52								100	0	7
	%												
Pipracillin\tazobactm	N	79	28	14	6	10	10	4	2	7			
	R	84	28	12	5	17	100	10	100	20			
	%												
Ticarcillin	N	64	52										
	R	83	51										
	%												
Ticarcillin\clav	N	64	49										
	R	84	57										
	%												
Imipenem	N	78	39	0	0	0	9	1	0	2	2	1	5
	R	83	37	0	0	0	100	2	0	5	100	33	18
	%												
Meropenem	N	79	33	0	0	0	8	1	0	2		0	0
	R	84	30	0	0	0	80	2	0	5		0	0
	%												
Ertapenem	N			0	0	0	6	1	0	1	0	0	0
	R			0	0	0	85	2	0	3	0	0	0
	%												
Norfloxacin	N			68	61	21	0				1	2	8
	R			93	89	87	0				100	100	88
	%												
Nitrofurantoin	N			1	1	8	0	8		1	1	2	9
	R			1	1	34	0	57		20	100	100	100
	%												
Colistin	N	0	0										
	R	0	0										
	%												
Minocycline	N	19	11										
	R	21	100										
	%												
Tobramycin	N	74	31										
	R	80	29										
	%												

Table 3.18

Rates of antibiotics resistance of the most common gram-positive bacteria in 2018

		MRSA S. aureus	*S. aureus*	*CoNS*	*E. faecalis*	*VRE E. faecium*	*E. faecium*	*Other Enterococcus*	*S. agalactiae*	*Other Streptococcus*
Penicillin	N	42	33	201	10	44	23	4	0	11
	R%	100	86	95	9	100	74	28	0	17
Oxacillin	N	42	1	182						
	R%	100	2	86						
Amox/clav	N	42	1	180	2	44	22	1	0	
	R%	100	2	81	1	100	71	16	0	
Cefuroxime	N	40	1	178	66	27	25	7		0
	R%	97	2	86	95	100	100	100		0
Ceftriaxone	N									12
	R%									41
Cefotaxime	N									11
	R%									37
Ceftazidime	N									0
	R%									0
Cefepime	N									0
	R%									0
Clindamycin	N	16	4	106					1	31
	R%	38	10	46					100	57
Erythromycin	N	27	5	173	79	28	27	11		21
	R%	64	12	75	91	100	100	91		70
Gentamycin	N	7	0	98						0
	R%	16	0	43						0
Ciprofloxacin	N	21	2	129	58	43	20	4	0	1
	R%	50	5	55	52	97	64	30	0	16
Levofloxacin	N	18	2	121	55	43	20	4	2	6
	R%	42	5	52	50	97	64	28	33	9
Moxifloxacin	N	10	2	85	8			1	2	4
	R%	23	5	37	44			33	33	12
Vancomycin	N	0	0	0	1	44	2	9	1	0
	R%	0	0	0	0.9	100	6	64	16	0
Tigecycline	N	0	0	0	0	1	0	0	0	0
	R%	0	0	0	0	2	0	0	0	0
Rifampin	N	1	0	20						
	R%	2	0	9						
Tetracycline	N	13	2	95	101	11	20	8	4	36

	R%	31	5	42	91	25	64	57	66	56
Quinupristin/dalfopristin	N	0	0	1	108	4	6	11	0	0
	R%	0	0	0.5	99	9	19	78	0	0
Linezolid	N	0	0	0	1	1	0	1	0	0
	R%	0	0	0	1	2	0	7	0	0
Cotrimoxazole	N	7	0	79						
	R%	16	0	35						
Ampicillin	N				2	44	22	2	0	11
	R%				1	100	71	14	0	16
Nitrofurantoin	N				4	11	5			
	R%				9	64	45			
StreptoHLA	N				63	34	17			
	R%				57	77	56			
GentaHLA	N				69	35	13	7		
	R%				62	81	41	50		

Table 3.19

Rates of antibiotics resistance of the most common gram-negative bacteria in 2019

		A. baumannii	***P. aeruginosa***	***ESBL E. coli***	***E. coli***	***ESBL K.. pneumoniae***	***CRE k. pneumoniae***	***k. pneumoniae***	***CRE E. cloacae***	***E. cloacae***	***ESBL Proteus***	***Proteus***
Amox/clav	N			47	21	28	20	4	2	34	1	4
	R%			32	15	37	100	7	100	100	25	9
Cefuroxime	N					24	10	0	2	12	0	7
	R%					100	100	0	100	63	0	28
Ceftriaxone	N			141	1	74	20	0	2	8	0	7
	R%			97	1	100	100	0	100	20	0	15
Cefotaxime	N			141	1	74	20	0	2	9	0	7
	R%			97	1	100	100	0	100	23	0	15
Ceftazidime	N	58	34	144	1	74	20	0	2	6	0	7
	R%	80	24	98	0.8	100	100	0	100	15	0	14
Cefepime	N	59	33	142	0	73	20	0	2	3	0	7
	R%	81	23	97	0	98	100	0	100	7	0	14
Gentamycin	N	46	38	64	29	45	17	4	0	4	2	15
	R%	63	27	43	22	60	85	7	0	10	50	31
Ciprofloxacin	N	59	51	104	60	46	18	4	0	5	2	18
	R%	1	2	70	80	12	5	0	50	2	0	6

Levofloxacin	N	36	15			15	10	2	0	0	1	8
	R%	97	46			68	100	10	0	0	100	50
Amikacin	N		36	4	0	6	16	0	0	3	0	0
	R%		25	2	0	8	80	0	0	7	0	0
Cotrimoxazole	N	35		102	73	66	19	11	1	5	2	29
	R%	56		69	55	90	95	20	50	13	50	63
Ampicillin	N			145	89	74	20	53			2	30
	R%			100	67	100	100	100			50	66
Piperacillin	N	57	51									
	R%	80	37									
Pipracillin/tazobactam	N	59	30	18	9	13	20	1	2	7	0	1
	R%	81	22	12	6	17	100	1	100	17	0	2
Ticarcillin	N	58	71									
	R%	81	52									
Ticarcillin/calv	N	40	66									
	R%	95	48									
Imipenem	N	58	56	1	0	1	20	0	2	0	1	20
	R%	80	40	0.7	0	1	100	0	100	0	25	42
Meropenem	N	58	42	1	0	1	20	0	2	0	0	2
	R%	80	30	0.7	0	1	100	0	100	0	0	4
Ertapenem	N			1	0	2	20	0	2	0	0	0
	R%			0.7	0	2	100	0	100	0	0	0
Norfloxacin	N			65	34	25	4	4			0	8
	R%			78	53	64	100	26			0	50
Nitrofurantoin	N			1	0	13	4	1			1	16
	R%			1	0	32	100	6			50	100
Colistin	N	0	2									
	R%	0	11									
Minocycline	N	14										
	R%	19										
Tobramycin	N	45	36									
	R%	63	26									

Table 3.20

Rates of antibiotics resistance of the most common gram-positive bacteria in 2019

		MRSA S. aureus	***S. aureus***	***CoNS***	***VRE E. faecalis***	***E .faecalis***	***VRE E. .faecium***	***E. faecium***	***Other Enterococcus***	***S. agalactiae***	***Other Streptococcus***
Penicillin	N	50	58	254	0	13	52	32	1	0	9
	R%	100	87	95	0	8	100	84	12	0	27
Oxacillin	N	50	5	228							
	R%	100	8	85							
Amox/clav	N	50	9	227		4	52	32	1	0	3
	R%	100	14	84		2	100	84	12	0	20
Cefuroxime	N	47	10	224						0	
	R%	94	15	86						0	
Ceftriaxone	N										8
	R%										25
Cefotaxime	N										9
	R%										28
Clindamycin	N	15	15	127							8
	R%	30	23	46							22
Erythromycin	N	32	18	219		97	50	30	4		16
	R%	64	28	80		77	100	87	66		53
Gentamycin	N	7	2	82							
	R%	14	3	30							
Ciprofloxacin	N	24	16	154		96	52	27	4		1
	R%	0	0	56		62	100	73	44		0
Levofloxacin	N	7	9	126		95	52	27	2		4
	R%	14	13	45		61	100	71	25		11
Moxifloxacin	N	3	3	50						0	1
	R%	6	4	18						0	5
Amikacin	N			0							
	R%			0							
Vancomycin	N	0	1	2		0	52	1	3	0	0
	R%	0	1	0.7		0	100	2	33	0	0
Tigecycline	N	0	0	0		0	1	0	0	0	0
	R%	0	0	0		0	1	0	0	0	0
Rifampin	N	1	2	20							
	R%	2	3	7							
Tetracycline	N	10	11	76		137	11	28	7	21	10
	R%	20	16	28		89	21	73	77	84	28

Quinupristin/dalfopristin	N	0	0	1	150	10	5	3	0	
	R%	0	0	0.4	98	19	13	42	0	
Linezolid	N	0	0	0	6	0	1	1	0	0
	R%	0	0	0	4	0	2	11	0	0
Cotrimoxazole	N	8	3	116						
	R%	16	4	42						
Ampicillin	N			0	5	52	32	1	0	9
	R%			0	3	100	84	12	0	25
Nitrofurantoin	N				0	10	1	0	1	
	R%				0	83	6	0	20	
Streptomycin HLA	N				88	34	25	2		
	R%				57	65	65	28		
Gentamycin HLA	N				95	39	24	2		
	R%				62	75	63	33		

Table 3.21

Rates of antibiotics resistance of the most common gram-negative bacteria in 2020.

		A . baumannii	***P. aeruginosa***	***CRE E. coli***	***ESBL E. coli***	***E. coli***	***ESBL k .pneumoniae***	***CRE k .pneumoniae***	***k. pneumoniae***	***CRE E. cloacae***	***E. cloacae***	***Other Enterobacter spp.***	***CRE Proteus***	***ESBL Proteus***	***Proteus***
Amox/clav	N				57	18	28	33	4	2	31	7	1	1	0
	R%				39	14	39	100	8	100	100	100	100	100	0
Cefuroxime	N						61	31	0				1	1	0
	R%						100	100	0				100	100	0
Ceftriaxone	N			11	146	3	72	33	0	2	4	3	1	3	0
	R%			100	99	2	100	100	0	100	13	50	100	100	0
Cefotaxime	N			11	147	3	72	33	0	2	4	3	1	3	0
	R%			100	99	2	100	100	0	100	13	50	100	100	0
Ceftazidime	N	53	21	10	146	3	72	33	0	2	5	4	1	3	0
	R%	93	19	91	99	2	100	100	0	100	16	67	100	100	0
Cefepime	N	5	1	10	14	3	68	32	0	1	0	0	1	3	0

		3	3		1										
	R	9	1	91	95	2	94	97	0	50	0	0	100	100	0
	%	3	2												
Gentamycin	N	49	19	5	50	25	31	21	6	0	1	1	1	2	2
	R	86	17	46	34	19	43	64	12	0	3	14	100	67	9
	%														
Ciprofloxacin	N	53	26	11	106	41	27	30	9	1	1	0	1	2	3
	R	93	24	100	72	31	39	91	18	50	3	0	100	67	14
	%														
Amikacin	N			0	3	0	7	9	9	0	0	0	0	0	0
	R			0	2	0	10	27	18	0	0	0	0	0	0
	%														
Vancomycin	N														
	R														
	%														
Cotrimoxazole	N	38		9	119	63	60	27	14	2	3	2	1	3	12
	R	72		82	81	47	86	82	28	100	10	29	100	100	55
	%														
Ampicillin	N			11	146	88	72	33	51				1	3	10
	R			100	100	66	100	100	100				100	100	46
	%														
Piperacillin	N	53	22	11	22	13									
	R	93	21	100	15	10									
	%														
Pipracillin/tazobactam	N	53	14				10	33	5	2	4	3	0	0	0
	R	93	13				14	100	10	100	13	50	0	0	0
	%														
Ticarcillin	N	54	38												
	R	95	37												
	%														
Ticarcillin/clavulanic acid	N	53	37												
	R	95	36												
	%														
Imipenem	N	53	34	11	0	3	0	32	0	2	0	0	1	0	3
	R	93	31	100	0	2	0	97	0	100	0	0	100	0	14
	%														
Meropenem	N	54	21	10	0	3	0	32	0	2	0	0	1	0	0
	R	95	19	91	0	2	0	97	0	100	0	0	100	0	0
	%														
Ertapenem	N				0	3	0	33	0	2	0	0	1	0	0
	R				0	2	0	100	0	100	0	0	100	0	0
	%														
Norfloxacin	N				49	2	9	9	1		1	0		1	0

	R%				68	231	32	90	7		20	0		50	0
Nitrofurantoin	N			0	3	2	9	4	5		0			2	6
	R%			0	4	3	35	31	33		0			100	100
Aztronam	N	13	0												
	R%	23	0												
Minocycline	N	8	0												
	R%	14	0												
Tobramycin	N	45	18												
	R%	79	17												

Table 3.22

Rates of antibiotics resistance of the most common gram-positive bacteria in 2020

		MRSA S .aureus	***S .aureus***	***CoNS***	***VRE E. faecalis***	***E. faecalis***	***VRE E. faecium***	***E. faecium***	***Other VRE Enterococcus spp.***	***Other Enterococcus***	***S. agalactiae***	***Other Streptococcus***
Penicillin	N	44	59	267	1	12	62	32	6	0	0	4
	R%	100	86	92	100	9	100	82	50	0	0	16
Oxacillin	N	43	2	234								
	R%	97	2	78								
Amox/clav	N	41	2	229	1	0	62	32			0	
	R%	97	2	75	100	0	100	82			0	
Cefuroxime	N	41	2	227								
	R%	97	2	79								
Ceftriaxone	N											5
	R%											20
Cefotaxime	N											5
	R%											20
Clindamycin	N	15	17	154								6
	R%	34	25	51								24
Erythromycin	N	24	15	238	1	58	53	27	7	2		16
	R%	55	22	79	100	53	100	79	58	40		66
Gentamycin	N	3	0	74								
	R%	6	0	24								

Ciprofloxacin	N	17	6	158	1	68	62	27	3	0		
	R%	38	9	51	100	53	100	69	25	0		
Levofloxacin	N	5	2	108	1	66	62	27	3	0	2	5
	R%	11	3	35	100	51	100	69	25	0	8	19
Moxifloxacin	N	0	0	18							2	3
	R%	0	0	6							8	13
Vancomycin	N	0	0	0	1	0	62	1	3	4	0	0
	R%	0	0	0	100	0	100	2	25	66	0	0
Tigecycline	N	0	0	0	0	0	0	1	0	0	0	0
	R%	0	0	0	0	0	0	2	0	0	0	0
Rifampin	N	0	0	14								
	R%	0	0	4								
Tetracycline	N	8	2	66	1	109	16	24	11	2	16	8
	R%	18	3	21	100	86	25	63	91	33	64	32
Quinupristin/dalfopristin	N	0	0	1	1	127	5	4	1	4	0	
	R%	0	0	0.4	100	99	8	10	9	66	0	
Linezolid	N	0	0	0	0	3	0	0	0	0	0	0
	R%	0	0	0	0	2	0	0	0	0	0	0
Cotrimoxazole	N	5	1	94								
	R%	11	1	30								
Ampicillin	N				1	0	62	32	5	0	0	4
	R%				100	0	100	82	41	0	0	14
Nitrofurantoin	N			0		1	19	6				
	R%			0		1	76	37				
Streptomycin HLA	N				1	69	35	25	5	1		
	R%				100	54	58	64	50	16		
Gentamycin HLA	N				1	74	44	22	3	1		
	R%				100	58	72	56	27	16		

جـامعـــة النجاح الوطنية

كليـــة الدراســـات العليـــا

تقييم مقاومة المضادات الحيوية في مستشفى إحالة في فلسطين: دراسة بأثر رجعي لمدة ثلاث سنوات

إعداد

آية عايد أبو دياك

إشراف

د. أدهم أبو طه

قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الصيدلة السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس– فلسطين.

2022

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الملخص

الخلفية: مقاومة مضادات الميكروبات هي الآن واحدة من أكثر الأمور الطبية خطورة وتعقيدا في جميع أنحاء العالم. تثبت الحقائق التاريخية والبيانات المتنوعة التي تم جمعها على مر السنين أنه ينبغي القيام بشيء عاجل للسيطرة على هذه القضية المتنامية، ما دفع بالعديد من دول العالم إلى تكريس محاولات السيطرة على هذه القضية وتبعاتها الطبية والاقتصادية والمجتمعية. هدفت هذه الدراسة إلى تقييم تواتر وأنماط استجابة مضادات الميكروبات لمسببات الأمراض البكتيرية والفطرية الأكثرشيوعًا في مستشفى جامعة النجاح الوطني (NNUH) في مدينة نابلس في فلسطين خلال 2018–2020.

المنهجية: تم إجراء دراسة وصفية مقطعية بأثر رجعي لتقييم أنماط مقاومة مضادات الميكروبات لمسببات الأمراض المهمة سريريًا التي تم جمعها من المرضى المتواجدين في أجنحة المستشفى المختلفة خلال فترة الدراسة (2018–2020) ، باستخدام تقارير الاستجابة التي تم حفظها في قاعدة البيانات المحوسبة لمختبر الأحياء الدقيقة في مستفى جامعة النجاح الوطنية، تم جمع هذه البيانات ضمن برنامج Microsoft Excel، وتم ترميزها وإرسالها إلى برنامج (SPSS) الإصدار 21 من أجل تحليل البيانات. تم عمل إحصاء وصفي لإنشاء جداول ورسوم بيانية للتكرار والنسب المئوية وتم الحصول على الموافقة من (IRB).

النتائج: من بين 5585 عينة تم زراعتها، 46.9٪ من البكتيريا كانت سالبة الجرام (GNB)، 37.9٪ من البكتيريا موجبة الجرام (GPB). أكثر أنواع البكتيريا السالبة شيوعًا كانت ESBL E. coli (15.6٪ من GNB) تليها E. coli (14٪). بينما كان P. aeruginosa و A. Baumannii أكثر (NFGNB) شيوعا. بالنسبة ل GPB: كانت البكتيريا الأكثر

شيوعًا هي (CoNS) بنسبة 38.8٪ من إجمالي GPB، تليها E. faecalis و S. aureus. جمعت العينات السريرية في الغالب من عينات البول بنسبة 27.7٪. تليها عينات الجروح (19.6٪)، وعينات الدم (19.2٪). كان إجمالي المرضى المنومين من جميع الأجنحة 3895 حالة، ومن بين هؤلاء كان قسم الجراحة هو الأبرز ويليه وحدة العناية المركزة (ICU). أظهر A. Baumannii مقاومة عالية (أكثر من 85٪) لسيفتازيديم، سيفيبيم، جنتامايسين، سيبروفلوكساسين، بيبيراسيلين وإيميبينيم. E.coli أظهر مقاومة لكل من السيفالوسبورين، سيبروفلوكساسين، نورفلوكساسين، أمبيسلين. كانت S. aureus مقاومة للبنسلين واقل مقاومة للايرثرومايسين والكلينداماسين. أظهرت Enterococcus SPP مقاومة للتتراسيكلين، ايرثرومايسين، ليفوفلوكساسين، سيبروفلوكساسين.

الاستنتاجات: لاحظنا ارتفاع معدلات المقاومة خلال فترة الدراسة لمعظم أنواع البكتيريا التي تم جمعها. يجب التركيز على تعزيز أساليب الرقابة على صرف واستخدام المضادات لوقف إساءة استخدامها والحد من انتشار الميكروبات المقاومة للأدوية بالذات ال (MDRO).

الكلمات المفتاحية: مضاد حيوي ، مقاومة ، فلسطين ، البكتيريا متعددة المقاومة للمضادات الحيوية.