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MOLECULAR INVESTIGATION OF EXTREMELY DRUG RESISTANT ACINETOBACTER *BAUMANNII* OUTBREAK IN THE ICU OF A TERTIARY HEALTHCARE CENTER IN LEBANON

by
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ABSTRACT
OF THE THESIS OF

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Title: Molecular Investigation of Extremely Drug Resistant *Acinetobacter baumannii* Outbreak in the ICU of a Tertiary Healthcare Center in Lebanon.

Background: Antimicrobial resistance (AMR) represents one of the major global health issues today. Infections with extensively drug-resistant (XDR) and pan drug-resistant (PDR) bacteria are associated with prolonged treatment periods and high lethality, owing to the limited and sometimes absent therapeutic options. Due to the misuse of antibiotics by means of over-prescription and their abuse in agriculture, AMR is continuously spreading and causing dangerous outbreaks that prompt establishing persistent and accurate surveillance. Hospitals and other healthcare establishments are fertile ground for drug-resistant bacteria to flourish, especially biofilm-forming bacteria such as the WHO top priority pathogen *Acinetobacter baumannii*. In this study, we utilize next-generation sequencing (NGS) as a fast and effective diagnostic tool to gain insight into the genomic diversity and clonality of an *A. baumannii* outbreak at a tertiary care center in Beirut, Lebanon.

Methods: A total of 54 *A. baumannii* isolates from ICU patients and from the hospital environment collected from AUBMC Clinical Microbiology Laboratory were screened for their susceptibility using the Broth Micro-dilution assay (BMD) against 12 different antimicrobials from different antimicrobial classes. For the purpose of determining AMR genes, sequence type (ST) and international clones (IC), whole-genome sequencing (WGS) using Illumina sequencing was performed on all these isolates.

Results: According to CLSI guidelines, two *A. baumannii* isolates were found to be resistant to colistin. All isolates showed resistance to Bactrim. Resistance rates were 98% for imipenem, meropenem, tazocin, ciprofloxacin, levofloxacin, ceftazidime, and cefepime, while resistance to gentamicin and amikacin accounted for 93% and 94%, respectively. Furthermore, 96% of tested *A. baumannii* isolates can be categorized as XDR, 2% as PDR while 2% were susceptible to antibiotics. Additionally, many antimicrobial resistance genes were detected, including the carbapenem resistance gene OXA-23. All our isolates were of ST 2, with 93% of them belonging to IC 2.

Conclusion: The study focused on *A. baumannii* isolates and found that those belonging to ST-2 and IC-2 were the most prevalent. These isolates were clustered together and had similar antimicrobial resistance genes. WGS was crucial in identifying these differences and highlighting the importance of advanced techniques in tracking and identifying outbreaks accurately. The finding that most isolates detected belongs to IC-2 and ST-2 carrying *bla*OXA-23 suggests that the current outbreak strain might have originated from older isolates dating back to 2014-2020. This study emphasizes the importance of

utilizing NGS in monitoring the antibiotic-resistant pathogens as a way to prevent and control outbreaks.

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CHAPTER I

INTRODUCTION

Antimicrobial resistance (AMR) is a growing public health concern worldwide. The emergence of AMR poses a threat to the effectiveness of commonly used antimicrobial agents, making it difficult to treat infections and increasing the risk of mortality. AMR can lead to longer hospital stays, higher healthcare costs, and reduced productivity, which can negatively impact national economies. The primary cause of antimicrobial resistance on a global scale is the excessive or incorrect use of antimicrobials. This can be attributed to incorrect diagnoses, the irrational use of these agents, and inconsistent consumption, which may be due to incorrect prescriptions or non-compliance. According to the Centers for Disease Control and Prevention (CDC) in the United States, a minimum of two million Americans contract infections caused by bacteria resistant to antibiotics annually, and at least 23,000 individuals die each year due to these infections. The World Health Organization (WHO) has identified a list of priority pathogens that pose the greatest threat to public health due to their high levels of antimicrobial resistance, the critical priority group includes *Acinetobacter baumannii* (1, 2, 3).

Acinetobacter spp. are a group of gram-negative coccobacilli bacteria that do not ferment glucose. In recent years, they have become a significant cause of healthcare-associated infections, particularly in people with weakened immune systems or those who have undergone invasive medical procedures. Acinetobacter spp. are considered opportunistic pathogens, they can cause a range of infections, including pneumonia, urinary tract infections, bloodstream infections, and surgical site infections, among others. It is important to note that not all Acinetobacter species are pathogenic, and some

strains may be more virulent than others. Additionally, the emergence of antibiotic-resistant strains of Acinetobacter spp. has made them more difficult to treat, posing a significant challenge to healthcare providers. *A. baumannii* has been identified as one of the top antibiotic-resistant threats in healthcare settings by CDC. This bacterium can survive on surfaces and medical equipment for extended periods, making it easy for them to spread from person to person in healthcare facilities (4,5).

Next-generation sequencing (NGS) technology has emerged as a powerful tool for the detection and investigation of outbreaks of infectious diseases. NGS allows for rapid and high-throughput sequencing of microbial genomes, providing detailed information on the genetic makeup of microorganisms. This technology has been used successfully in the detection and investigation of outbreaks caused by a range of pathogens. By sequencing the genomes of pathogens, NGS can help identify the source of an outbreak, track the transmission of the pathogen, and determine the relatedness of different isolates (6,7). For that we aimed to dive deep into the genomic diversity and clonality of this *A. baumannii* using Whole Genomic Sequencing. We also aim to investigate and compare the current outbreak with previous outbreaks that occurred locally and internationally due to *A. baumannii.*

CHAPTER II

LITERATURE REVIEW

A. Antimicrobial Resistance

1. Causes of Antimicrobial Resistance

The emergence of new resistance mechanisms and the increasing prevalence of microorganisms that are frequently associated with nosocomial infections are placing a significant burden on healthcare systems and have a substantial impact on the global economy (2, 4). The development of antibiotic-resistant bacteria is proportional to the rate of antibiotic consumption, which has increased due to inappropriate prescribing and incorrect usage. This has led to the development of novel resistance mechanisms by bacteria and sustained the spread of existing resistant strains (1). Up to 50% of antibiotic prescriptions are either unnecessary or not the best option for the given condition (2). Another significant occurrence in recent years is the widespread use of antibiotics in agriculture, particularly as growth promoters for livestock. The indiscriminate use of antibiotics in this sector without prescription poses a severe threat to public health (1). Additionally, the production of new antibiotics by pharmaceutical industries has decreased due to the availability of low-cost antimicrobials. This decrease in the production of alternative antimicrobials is one of the main causes of antimicrobial resistance (AMR) (1). One of the most significant challenges in tackling antibiotic resistance is the lack of development of new antibiotics (4).

2. Consequences of Antimicrobial Resistance

Managing nosocomial infections caused by multidrug-resistant (MDR) bacteria has proven to be challenging over the past decades. AMR leads to prolonged hospital stays for patients with such infections and increased healthcare costs, as well as an increased risk of other healthcare-associated infections, particularly in susceptible patient populations (4). Moreover, the unresponsiveness of these bacteria due to their resistance to multiple classes of antibiotics, along with the limited efficacy of conventional therapies, makes treatment difficult (2). This puts a burden on healthcare systems to invest more in research and development to combat these infections. The development of new antibiotics is a critical need for healthcare, as antibiotic resistance continues to escalate worldwide, but developing new antibiotics is both time-consuming and expensive (7). Additionally, AMR causes an increase in both morbidity and mortality rates. The bacteria that account for the highest resistance rates are grouped under the name ESKAPE, which includes *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and Enterobacter species (2).

3. Mechanisms of resistance

Bacteria can be intrinsically resistant to antibiotics or can acquire resistance via spontaneous mutations in chromosomally located genes, plasmids, and bacteriophages, which is known as horizontal gene transfer (1). One of the most common ways bacteria evade antibiotics is by producing enzymes that can inactivate the drug. These enzymes can break down or chemically modify the active component of the antibiotic, rendering

it ineffective. This process is known as antibiotic hydrolysis and is frequently observed in β-lactamases, which are enzymes that can inactivate β-lactam antibiotics such as penicillin, cephalosporin, and carbapenems. In addition to inactivating antibiotics, bacteria can also pump out the drug molecules through efflux pumps, which are protein-based channels present on the bacterial cell. Furthermore, some bacteria can evade antibiotics by forming bacterial aggregates known as biofilms, making conventional antibiotics ineffective in these cases (2,10).

4. Definitions of Drug Resistant Bacteria

The emergence of drug-resistant isolates has made treatment extremely challenging. Various terminologies have been used to describe the extent of antimicrobial resistance, including 'multidrug-resistant (MDR),' 'extensively drug-resistant (XDR),' and 'pandrug-resistant (PDR)' (19). MDR is typically defined as strains that are resistant to at least one agent in three or more antimicrobial categories (18). XDR is defined as an isolate that is susceptible to one or two classes of antimicrobials but must also be resistant to carbapenems, an important class of antibiotics typically reserved for infections caused by MDR bacteria. PDR are those that are resistant to all antibiotics available, including last line treatment polymyxins like colistin (19,20).

B. *Acinetobacter baumannii*

1. Characteristics

Acinetobacter is a gram-negative bacterium that belongs to the Moraxellaceae family, which comprises 57 species, with *A. baumannii* being the most clinically important pathogen (27). *A. baumannii* is a non-fastidious, strictly aerobic organism that

appears coccobacilli-shaped under the microscope. It is immotile and derives its name from the Greek word "Akinetos," meaning not mobile. These microorganisms are considered non-fastidious as they do not ferment glucose, and the bacterium lacks both catalase and oxidase enzymes (1,4).

A. baumannii is an opportunistic pathogen that can be easily isolated from soil, hospital facilities, and water (8,9). Moreover, it can survive in dry conditions and persist on medical apparatus, curtains, and beds for days (8).

2. Infection

The WHO has identified Acinetobacter as a critical priority due to its ability to infect vulnerable hosts, particularly patients with weakened immune systems, such as those admitted to the intensive care unit (ICU) (4). This bacterium can colonize and cause infections in critically ill patients, particularly those with indwelling medical devices such as catheters and ventilators. Acinetobacter infections can lead to pneumonia, urinary tract infections (UTIs), bacteremia, endocarditis, meningitis, as well as complicated skin and soft tissue infections (3,8). Additionally, the bacterium can survive on surfaces for weeks, allowing for transmission not only between patients, but also through contaminated devices and inanimate objects (2).

3. Treatment

The use of broad-spectrum cephalosporins or extended-spectrum penicillin has been recommended in recent research as a treatment option for Acinetobacter infections. These antibiotics are often effective against many different types of bacteria, including Acinetobacter, and are commonly used as first-line treatments in hospitals for serious

infections. However, the choice of antibiotic therapy may vary depending on the specific type of Acinetobacter bacteria causing the infection and its susceptibility to different antibiotics, so it is important to consult with a healthcare provider for individualized treatment recommendations (23).

Aminoglycosides have also been used to treat infections with this bacterium (19,25). A retrospective review conducted in an ICU in France reported that amikacin, ceftazidime, or quinolones were the drugs used as first-line therapy for Acinetobacter (24). However, due to the increasing rate of resistance to these antibiotics, the carbapenem class of antibiotics is often used as a last resort in the treatment of serious infections (21,24). These drugs work by inhibiting the growth of bacteria, as they have outstanding bactericidal activity and stability toward a range of beta-lactamases (21). However, the overuse and misuse of carbapenems have led to the emergence of carbapenem-resistant bacteria (23). The emergence of these resistant bacteria is a major public health concern, as it limits the treatment options available for serious infections caused by MDR pathogens (22).

Most studies have shown that colistin and tigecycline are the most effective antibiotics used to treat multi-drug-resistant Acinetobacter (8,9). Tigecycline has been shown to be effective in the treatment of a variety of infections, including carbapenem-resistant *Acinetobacter baumannii* (22). Tigecycline belongs to the glycylcycline family and is a semisynthetic derivative of minocycline. It interrupts protein synthesis by binding to the 30S ribosomal subunit (21,22). This drug is a bacteriostatic agent, and it has proven its efficiency in vitro against various MDR *A. baumannii* infections (22). However, overuse and misuse of tigecycline can lead to the emergence of tigecycline-resistant bacteria, which can further limit treatment options (24).

Another potential treatment option for Carbapenem-resistant Acinetobacter baumannii (CRAB) infection is colistin. Colistin is an older antibiotic that fell out of favor due to its serious side effects, such as kidney damage and nerve damage (25). This polymyxin antibiotic has a bactericidal activity and leads to cell death after its interaction with the anionic lipopolysaccharide, mainly lipid A component. This also alters the cell permeability, in addition to displacing calcium and magnesium, thus causing cell leakage (26). With the emergence of PDR Acinetobacter that acquire colistin resistance, and the lack of newer antimicrobial compounds, combination therapies have been proposed to treat such infections. However, such regimens are not only more expensive, but the side effects and toxicity are also higher, and the efficacy is lower (19).

4. Resistance

A. baumannii is considered one of the most serious multidrug-resistant bacteria causing severe nosocomial infections. Due to its propensity for colonizing hospital environments and its rising susceptibility to commercially available antibiotics, MDR *A. baumannii* is distinguished by the CDC as a source of outbreaks on a worldwide scale (10). Resistance to almost all beta-lactam, quinolones, and aminoglycoside antibiotics is witnessed in an amplified manner over the past decade (8). Acinetobacter almost exhibits resistance to all current antimicrobial classes (3). These mechanisms are demonstrated in figure 1.

a. β-Lactamases

One of the primary mechanisms of beta-lactam antibiotic resistance is the changes in the structure or expression profile of penicillin binding proteins (PBPs), PBPs are

transglycosylases, transpeptidases, and carboxypeptidases that are found in the plasma membrane and work to create peptidoglycan, a crucial component of the bacterial cell wall (28).

As one of the most adaptable enzymatic families among -lactamases, carbapenemases belonging to class A of Ambler (1980). They can hydrolyze the majority of -lactam antibiotics, including carbapenems, penicillins, cephalosporins, and monobactams (28). These are also resistant to some commercial -lactamase inhibitors. A wide range of these extended-spectrum β-lactamases have been described of these TEM, SHV, CTX-M, GES, SCO, PER, and GES, most of them were reported in *A. baumannii* (29).

Class B of Ambler contains Metallo-β-lactamases, VIM-1 and NDM-1, these enzymes possess resistance to penicillins, cephalosporins, and carbapenems. But β-lactamase inhibitors have a significant role in inhibiting action of these enzymes, inhibitors such as clavulanic acid, sulbactam, and tazobactam (28).

Chromosome-based cephalosporinases (AmpC), which only slightly hydrolyze cephalosporins and penicillins, belong to class C of Ambler. The *bla*AmpC gene is overexpressed when the insertion element ISAba1 or ISAba125 is placed upstream of it, leading to resistance to extended-spectrum cephalosporins because upstream ISAba induces strong promoter sequence (28,29).

Class D of Ambler contains the *bla*OXA genes encode oxacillinases, the intrinsic class D oxacillinase proteins weakly hydrolyze second- and third-generation cephalosporins as well as carbapenems, and penicillins (28,29). However, the existence of an insertion sequence (IS) is thought to be a powerful promoter of increased oxacillin expression and distribution. Class D carbapenem-hydrolyzing enzymes (CHDLs) were

divided into six subgroups, including OXA-23, OXA-24, OXA-51, OXA-58, OXA-143, and OXA-23547. According to reports, oxacillinases have been isolated in clinical isolates of *A. baumannii* linked to hospital outbreaks (28). The most common enzymatic mode of carbapenem resistance is the production of oxacillinase, encoded by genes of the *bla*OXA-23, *bla*OXA-40, and *bla*OXA-58-like lineages. They may be plasmid or chromosomal localized, are not inhibited by clavulanic acid, and are found in most parts of the world (29).

b. Aminoglycosides:

Aminoglycosides (AG) a class of antibiotics that have used to treat a wide range of infections, resistance to this drug has been associated to at least nine different modifying enzymes present in different combinations in some strains (30). These genes are present in Acinetobacter in the form cassette genes within integrons. Three of these inactivating enzymes are known in Acinetobacter, aminoglycoside acetyltransferases (AAC), aminoglycoside nucleotidyltransferases (ANT) and aminoglycoside phosphotransferases (APH). Also, in *A. baumannii* strains, methylation of 16S rRNA by ArMA methylase has been reported, inactivating all aminoglycosides except streptomycin (31). Resistant genes can be located on plasmids such as aac(3)-Iva, ant(2”)-Ia, ant(3”)-Ia and armA, or they can be found on integrons like AAC(6’)-Ia family, or even found on chromosomal genomic island such as aph(6)-Id (32). However, AG main resistance mechanism is *i*through modifying enzymes, another mechanism to evade AG is by efflux pumps, AdeABC and AbeM pumps, gentamicin and netilmicin are successfully eliminated by these pumps thus rendering resistance to them (31,32).

c. Tetracycline s and Glycylcyclines:

Tetracycline antibiotics inhibit protein synthesis by binding to the 30S ribosomal subunit, thus interrupting the start of translation (32). Resistance to tetracycline and its derivatives in Acinetobacter isolates is achieved by two mechanisms: an efflux system or a ribosomal protection protein (RPP) (30,31). The genes responsible for resistance to this family of antibiotics are acquired on plasmids or transposons. In *A. baumannii*, the energy-dependent tetracycline efflux system is encoded by the tetA and tetB genes, while the tetM gene encodes ribosomal protection protein (RPP) (31).

The elimination of tetracycline is accomplished by RND pumps, predominantly AdeABC, which can cause increased minimum inhibitory concentrations for tigecycline, minocycline, and tetracycline (32). RPPs can cause resistance to tetracycline by modifying ribosomes through a non-covalent process, which subsequently nullifies the inhibitory impact of tetracycline on protein synthesis (32).

d. Fluoroquinolones:

Modification of DNA gyrase or topoisomerase IV by point mutations in the topoisomerase gyrA and parC genes are linked to resistance to fluoroquinolones. These genes are well described for *A. baumannii*, also these mutations interfere with target site binding. Quinolones like aminoglycosides are also substrates for multidrug efflux pumps, including the RND-type pump AdeABC and the MATE pump AdeM (35,27). To date, the qnr gene which is a plasmid-mediated quinolone resistance has been identified for *A. baumannii* (27).

e. *Colistin and polymyxin:*

Colistin and polymyxin are ancient antibiotics that are becoming a last resort against highly drug-resistant bacteria (33). Colistin act on modifying lipid A part of the lipopolysaccharide, however bacteria can avoid colistin by adding 4-amino-4-deoxy-l-arabinose (Ara4N) or/and phosphoethanolamine by that it acquires resistance to this drug. The attachment of these abolishes the negative charge of Lipid A and reduces the attraction of the positively charged colistin (34). Mutations in lipid A biosynthesis gene lpxA, lpxC or lpxD cause complete loss of LPS production in *A. baumannii*, this was tested and showed that these mutations are linked with colistin mutation (33,34). Studies has showed that the insertion sequence ISAba11 in either lpxA or lpxC revealed a reduced in the levels of colistin resistance (34). Although this phenotype is rare, colistin-resistant *A. baumannii* have been described (33).

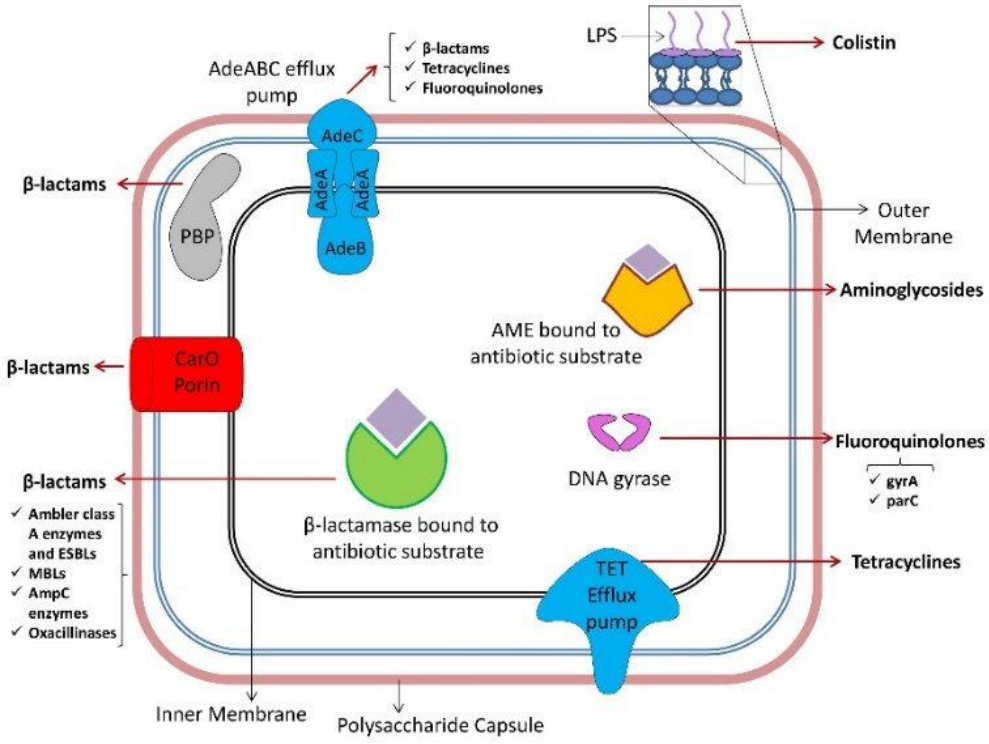

Figure1: Demonstrating the antimicrobial resistance mechanisms.

C. Epidemiology:

A. baumannii has a tendency to cause outbreaks in intensive care units worldwide and become endemic. These outbreaks occur frequently as proven by the detection of genetically related strains (36).

1. Worldwide:

Annual surveillance reported that carbapenem-resistant *A. baumannii* is now a concern throughout Asia and the Americas, except for Japan and Canada (37). The International clone II was the major clone identified in 34 countries in Europe, Asia, Africa, Australia, USA, and South America, where *bla*$_{\text{OXA-23}}$ was the major carbapenem-resistant gene identified (39). Carbapenem resistance in this bacterium is achieved through horizontal transfer of OXA genes mainly OXA-23, -40, -58 belonging to class D and NDM-1 genes belonging to class B β-lactamases (42). The most prevalent clinical carbapenem-resistant gene in *A. baumannii* in the European regions is the *bla*$_{\text{OXA-58}}$ gene, this gene is associated with the reduction in imipenem MICs (41). As Miltgen et al. stated in their work, that the first NDM-1- and OXA-23-coproducing Acinetobacter spp., was detected in April and December 2015 in Mayotte Island and Reunion Island, respectively (43). The co-existence of both genes was reported in India and China back in 2010 (42), whereas the first outbreak of carbapenem and colistin-resistant *A. baumannii* was described in Spain in 2009. In Italy, Venditti et al. showed that the majority of the isolates in their study belonged to sequence type ST2. There the group recorded the existence of *bla*$_{\text{OXA-66}}$ encoding the OXA enzyme belonging to the intrinsic OXA-51-like enzymes, *bla*$_{\text{OXA-23}}$ carbapenemase and the cephalosporinase-encoding *bla*$_{\text{ADC-25}}$ gene, in addition to the ISAba1 insertion sequence (43). A study done in the western United States stated

the presence of rare OXAs, such as OXA-235 and OXA-237, in an outbreak that occurred in Oregon between 2012 and 2014. Moreover, CRAB harboring OXA-72 have been identified in countries in eastern Asia, Latin America, and southern Europe (44). A recent study done in an ICU in Mexico occupied by COVID-19-infected patients aimed to recognize and characterize ESKAPE bacteria during this pandemic, to detect their possible clonal spread on medical devices, inert surfaces, medical personnel, and patients. They showed that the most frequent among the MDR pathogens found was *A. baumannii* at 52% (53). Another study conducted in a hospital in New Jersey during the COVID-19 pandemic reported an *A. baumannii* outbreak harboring OXA-23 (53).

2. MENA region:

Multidrug-resistant (MDR) *A. baumannii* strains are now widely disseminated worldwide, with the mediterranean region reporting the highest rates of carbapenem resistance at over 90%, which places a significant burden on healthcare systems (37). $bla_{\text{OXA-58}}$ gene was the most identified carbapenem-resistance gene in *A. baumannii* between 1999 to 2009 in many mediterranean countries such as Lebanon, Italy, Greece, and Turkey. However, after that period, OXA-23-harboring isolates were predominant over OXA-58-harboring ones. This newer gene belonging to the international clonal I and II lineages has been observed globally (45). *bla*OXA-58- and bla_{PER}-like genes from Iraqi *A. baumannii* strains were discovered through sequencing as early as 2006. Six years later, researchers found the carbapenemase genes $bla_{\text{OXA-40}}$, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-58}}$, and ISAbal on plasmids and/or chromosomes, upstream of the intrinsic $bla_{\text{OXA-51}}$. Additionally, genes similar to $bla_{\text{OXA-40}}$ were found in Acinetobacter strains found in the Iraqi Kurdistan region, while $bla_{\text{NDM-1}}$ was identified in carbapenem-resistant *A.*

baumannii strains from Syria. *bla*$_{\text{OXA-23}}$ was found to be the most frequently occurring beta-lactam resistance gene in all evaluated geographical areas. Furthermore, fluoroquinolones resistance genes reported in Syrian strains were the gyrA and parC genes. Meanwhile, strains detected in Libyan patients harbor the carbapenemase-encoding genes *bla*$_{\text{NDM-1}}$, *bla*$_{\text{OXA-23-like}}$, and *bla*$_{\text{OXA-40like}}$ (38). In 2017, reports showed that *bla*$_{\text{TEM}}$ was the most prevalent ESBL gene isolated from *A. baumannii* in Egypt, Saudi Arabia and Turkey (45). During the COVID-19 pandemic, a Turkish group studied the link between COVID-19 and *A. baumannii* co-infections, the study was done during pre-pandemic period (January 2018–March 2020) and the pandemic period (April 2020–June 2022) and showed that *A. baumannii* outbreaks increased during the pandemic period (53).

3. Lebanon:

The first study revealing epidemiological data on *A. baumannii* infections was in 1992, this was done by Matar et al. in the American University Hospital of Beirut, in Lebanon (90). In Beirut-Lebanon between 2004 and 2007, the only reported gene responsible for carbapenem resistance was *bla*$_{\text{OXA-58}}$ carried by a plasmid called pABIR, this is because of inadequate reports investigating outbreaks at that time. These bacteria producing such gene belong to the three international clones (I, II and III) (39,50). Moreover, isolates belonging to ST2 carrying *bla*$_{\text{OXA-66}}$ were found to be predominant in North Lebanon and Beirut likely indicating a spread among hospitals (39). It is worth mentioning that Rafei et al. in their study reported the first *bla*$_{\text{OXA-23}}$ and *bla*$_{\text{OXA-24}}$ detections in Lebanon. The percentage of Acinetobacter resistant to imipenem increased significantly from 57.6% in 2011 to 84.5% in 2013, according to a retrospective study

conducted across the country by the Lebanese Society of Infectious Diseases (40). In 2012 the incidence of *A. baumannii* resistant to imipenem accounted for 88% according to the study done by Hammoudi et al. Moreover, they have proven the presence of $bla_{\text{OXA-23}}$ and $bla_{\text{GES-11}}$ in the majority of the isolates tested (46). Prevalence of *A. baumannii* isolates harboring the $bla_{\text{OXA-23}}$-like gene accounted for 91.3% in a tertiary healthcare center during 2013-2014 (47). The $bla_{\text{OXA-66}}$ gene variant followed by *bla*-$_{\text{OXA69}}$ gene variant were the most detected genes from different hospitals in Tripoli, these genes were associated with ST2 and ST1 respectively in addition to the NDM-1 gene detection that occurred in one of the Tripoli hospitals (48). The NDM-1 gene was detected in Lebanon in isolates collected from Syrian patients during the Syrian war (46). In 2015, a study revealed that the majority of *A. baumannii* carried the $bla_{\text{OXA-23}}$ and $bla_{\text{GES-11}}$ genes. Moreover, another study revealed that $bla_{\text{OXA-23}}$-harboring isolates belong to the international clone II lineage (45). Regarding colistin resistance, Lebanon accounted for the highest percentage (17.5%), among 41 different countries (49). In their study, Osman et al. responded to two different outbreaks that occurred between January–September 2016 and April– July 2017 in 3 different hospitals in Tripoli. Their results showed that the majority of the isolates belonged to ST2 and IC2 and carried the $bla_{\text{OXA-23}}$. It is worth noting that the chief source of carbapenem resistance was recognized by the Lebanese government as the OXA-23 gene which was detected in ST2 isolates that accounted for 60 to 90% of studied isolates (52).

D. **Whole genome sequencing:**

Whole genome sequencing (WGS) is becoming more widely recognized as a potent tool for epidemiologic investigations. This is achieved by the utilization of core

regions or core single nucleotide polymorphism (SNP) phylogenies present in each pathogen, this technique is effective in most cases (57). WGS delivers high resolution in distinguishing outbreaks in real time and in a retrospective manner. Moreover, it is used to track the spread of pathogens in an occurring outbreak, by identifying resistance genes and mutations occurring in these genes. Studies done to test WGS investigation compared to conventional methods are limited, Kanamori et al. proved in their work that two distinct epidemiologically outbreaks were genetically connected (54). Sequencing technique is becoming a standard method to identify and track bacterial outbreaks due to the detailed information that can be generated through. This is getting much easier especially after the arrival of bench-top sequencers, these technique delivers a simple and quicker ways for bacterial identification (55). *Acinetobacter baumannii* an opportunistic pathogen capable of causing series hospital-acquired infections (HAI), particularly patients in the ICU. This MDR bacteria can thrive hospital environment for long time and is potentially transmissible. WGS with its discriminatory power has the advantage to identify this bacterium and differentiate between closely related isolates. Thus, WGS is better suited for typing and determining transmission between patients compared with traditional typing methods, especially when the same pandemic ST isolates are found locally (56). MLST schemes such as Oxford and Pasteur can also provide useful information for tracking and identifying the spread of *A. baumannii* (58). WGS can help understand the spread and phylogeography of many lineages of *A. baumannii* in several countries by generating genomic variation within the species (57).

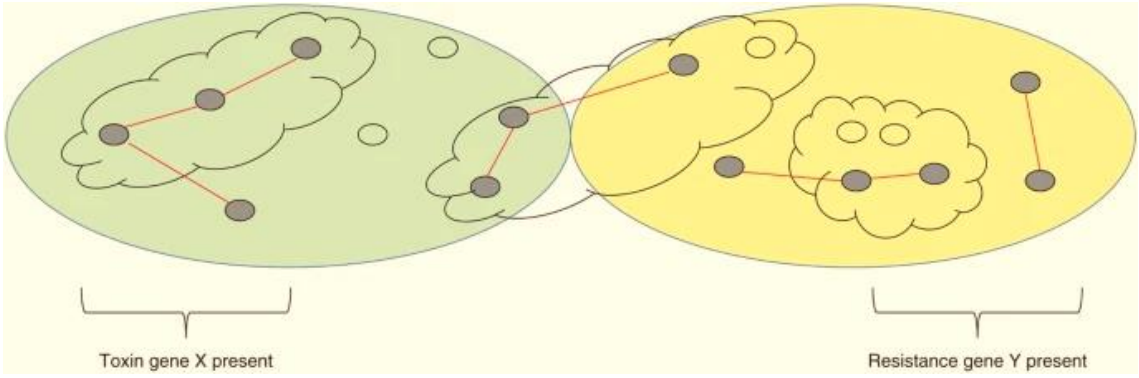

Figure 2: Demonstrations of the High resolution of WGS

As Robinson et al. illustrated in this figure. Besides shedding light on virulence factors (such as toxin gene X and resistance gene Y in figure (2)) and other aspects of pathogen biology, WGS offers a superior precision compared to conventional methods. The precision of whole-genome sequencing allows for higher resolution typing, distinguishing and linking of different isolates compared to conventional laboratory techniques. Genotypically grouped isolates obtained pre-WGS can be represented in figure (2) by small ovals. Clusters generated by epidemiological data are illustrated as clouds. WGS delivers data, in this case links between same-genotype sequenced isolates and across genotypes (filled small ovals), that would be otherwise missed by older laboratory typing techniques, as represented by the red lines in figure (2). (55).

CHAPTER III

MATERIALS AND METHODS

A. Sample collection

A total of 52 *Acinetobacter baumannii* isolates were recovered from clinical samples obtained from patients admitted to the ICU of the American University of Beirut Medical Center (Table 1). Environmental swabs from the ICU were also received (Table 2). All samples were received during the period between August 2022 and January 2023. Samples were processed once received or stored at 4°C and analyzed within 24 hours (h).

B. Isolation and purification of *A. baumannii*

Clinical samples were received in the form of disc diffusion petri plates, subcultured on both MacConkey (MAC) agar No.3 and Luria-Bertani (LB) agars (Neogen, Lansing, MI), isolated and purified. However, environmental swabs were processed first by plating on meropenem-based MacConkey and CHROMagar™ Acinetobacter Base (CHROM agar). The agar plate was left in the incubator for 24 hrs. If growth was not detected on the plate, the isolate was recorded as meropenem-susceptible and Acinetobacter-Free. If growth was observed, isolates were subcultured on separate MAC agar plates. Two to three bacterial colonies are then added to 3 ml of Mueller-Hinton cation-adjusted broth (MH cab) (Sigma Aldrich, St. Louis, MO) in polystyrene tubes and left in the incubator for 24 h. After 24 h, if turbidity was detected, 600 μL of bacterial mixture were transferred to 50% glycerol (Sigma Aldrich, St. Louis, MO) and stored at -80°C.

C. Antimicrobial susceptibility testing

1. Broth-Microdilution Methods

Antimicrobial susceptibility of *A. baumannii* isolates was tested by broth microdilution against 12 different antimicrobials from different antimicrobial classes. Meropenem (AstraZeneca, Cambridge, United Kingdom), Imipenem (Merck, Rahway, NJ), amikacin, gentamicin, tigecycline, ceftazidime hydrate, colistin sulfate, ciprofloxacin, levofloxacin (Sigma Aldrich, St. Louis, MO), cefepime (Julphar, Ras-Al-Khaimah, UAE), Bactrim (trimethoprim-sulfamethoxazole) (Somerset therapeutics LLC, Karnataka, India), and tazocin (piperacillin-tazobactam) (Wyeth, Philadelphia, PA).

The selected bacterial isolates were streaked on suitable agar plates using sterile loops and incubated overnight in an incubator at 37 °C. 180 µL of MH Cab was added to the first column, 90 µl was added from columns 2 to 11 and rows A, B, C, and D in column 12, and 100 µL was added to rows E, F, G, and H in column 12. MHCAB was poured into a reagent reservoir and added to each plate using a 300 µL Multichannel pipette. To column 1, 20.5 µL of the 5 mg/mL antibiotic suspension was added to have a total volume of 180 µL, then serial dilution was done by transferring 90 µl from column 1 into column 2, 2 to 3, and further on till column 11 where 90 µL are discarded after mixing. Antibiotics were added using the micropipettes (based on the volume required) and serial dilution was done using the 300 µL multichannel pipette. After that, columns 1 to 11 should contain 90 µl of antibiotics and broth, rows (A, B, C, and D) and rows (E, F, G, and H) in column 12 should contain 90 and 100 µl of broth only and served as positive and negative controls respectively. To prepare a bacterial suspension of 5 $x10^6$ CFU/mL, a bacterial suspension of 0.5 McFarland (equivalent to 10^8 CFU/mL) was

prepared as follows: In a 15 mL falcon tube, 3 mL of MH cab broth is added, OD (optical density) is measured using a densitometer and the value is recorded. Then, 1-2 colonies of the overnight bacterial culture are added and the suspension is mixed well by vortexing. OD is then remeasured to have a bacterial suspension of 0.5 McFarland. To reach a bacterial suspension with a concentration of 5 x10^6 CFU/mL, 500 µl of the 0.5 McFarland bacterial suspension is added to 9.5 mL of MH Cab. From the diluted bacterial suspension, 10 µL is added to columns 1 to 11 and rows A, B, C, and D in column 12 to reach a final volume of 100 µL in all the wells. The plate is placed on a shaker (at 160 rpm) in the incubator (37 °C) for 18-24 hours. Once the incubation period is done, the plate is removed from the incubator and the results are recorded. The negative control is checked to ensure the absence of contamination. The positive control for bacterial growth is also checked to ensure that the bacterial suspension was properly prepared. Wells 1-11 are checked for bacterial growth, the well preceding the first well with bacterial growth, is referred to as the well containing the MIC. All the experiments were run in duplicates. Control strain *Escherichia coli* ATCC® and *A. baumannii* DSM 30008 were used in parallel to monitor the MIC results. Compare the MIC values with the Clinical and Laboratory Standards Institute (CLSI) guidelines to interpret the results as susceptible or resistant. Tigecycline breakpoint was obtained from the British Society for Antimicrobial Chemotherapy.

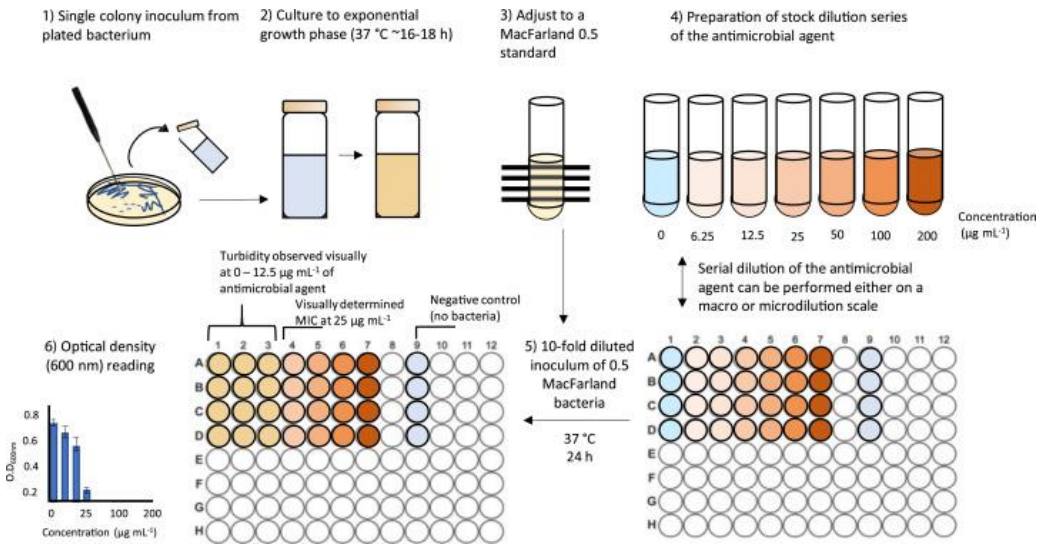

Figure 3: Broth microdilution procedure

D. DNA Extraction

Bacterial strains were cultured on MacConkey in order to perform DNA extraction using the ZymoResearch Quick-DNA™ Fungal/Bacterial Miniprep Kit (D6005. According to the manufacture's protocol, a loop full of bacterial cells is suspended in up to 200 µL of nuclease free water and added to ZR Bashing Bead™ Lysis Tube (0.1 mm & 0.5 mm). 750 µL BashingBead™ Buffer is added a to the tube. Then, the tube is secured in a bead beater fitted with a 2 ml tube holder assembly and process at maximum speed for 30 minutes using Disruptor Genie ™. The ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) is centrifuged in a micro-centrifuge at 10,000 x g for 1 minute. Supernatant is transferred to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged at 8,000 x g for 1 minute. 1,200 µL of Genomic Lysis Buffer is added to the filtrate in the Collection Tube. 800 µL of the mixture is added to a Zymo-Spin™ IICR Column® in a Collection Tube and centrifuged at 10,000 x g for 1 minute. The flow through from the Collection Tube is discarded and the previous step is repeated. 200 µL DNA Pre-Wash Buffer is then added to the Zymo-Spin™ IICR Column in a new

Collection Tube and centrifuged at 10,000 x g for 1 minute. Followed by the addition of 500 µL g-DNA Wash Buffer to the Zymo-Spin™ IICR Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin™ IICR Column is transferred to a clean 1.5 ml microcentrifuge tube and 35 ul minimum DNA Elution Buffer is added directly to the column matrix and centrifuged at 10,000 x g for 30 seconds to elute the DNA.

E. DNA Clean and Concentrate

This is done using the ZymoResearch Genomic DNA Clean & Concentrator™ (D4010, D4011) kit and according to the manufacture's protocol. In a 1.5 ml microcentrifuge tube,70 ul of DNA Binding Buffer is added to each volume of DNA sample and mixed briefly by vortexing. The mixture is transferred to a provided Zymo-Spin ™ Column in a Collection Tube and centrifuged for 30 seconds at 13,000 x g. The flow-through is discarded.200 ul DNA Wash Buffer is added to the column and centrifuge for 30 seconds at 13,000 x g. This wash step is repeated. 22 ul DNA Elution Buffer is added directly to the column matrix and incubated at room temperature for one minute. The column is transfer to a 1.5 ml microcentrifuge tube and centrifuged for 30 seconds to elute the DNA. Ultra-pure DNA is now ready for use. Recovered DNA was quantified using Nanodrop technology and stored at -20°C.

F. Library preparation and sequencing

For sequencing using the Illumina MiSeq sequencer, libraries were prepared using the Illumina DNA prep kit (Illumina GmbH, Munich, Germany). For sequencing on Oxford Nanopore MinION, libraries were prepared using the Rapid Barcoding Kit 96 (SQK-RBK110.96) and sequenced using R9.4.1 flow cells (FLO-MIN106).

1. Illumina library preparation:

Tagmentation of the genomic DNA was done using Bead-Linked Transposomes (BLT), which is a process that fragments and tags the DNA with adapter sequences. First, 2–30 µl DNA was added to each well of a 96-well PCR plate so that the total input amount is 100–500 ng. To prepare the tagmentation master mix, equal volumes of BLT and TB1 (Tagment Buffer 1) (10 µl of each per sample) were added. The mix was vortexed and 20 µl was transferred to each well of the plate containing a sample and mixed well. Then, the plate was placed on the preprogrammed thermal cycler and the TAG program was run.

Post Tagmentation Cleanup is then performed by washing the adapter-tagged DNA on the BLT before PCR amplification. Thus, 10 µl TSB (Tagment Stop Buffer) was added to the plate and slowly each well was pipetted 10 times to resuspend the beads, and then sealed. The plate was placed on the preprogrammed thermal cycler and the PTC program was run. When the program ends, the plate was placed on the magnetic stand until liquid is clear. Using a multichannel pipette, supernatant was removed and discarded. Then double washes were done by removing the sample plate from the magnetic stand and adding 100 µl TWB (Tagment Wash Buffer) directly onto the beads to fully resuspend them. The plate was then placed on magnetic stand to remove and discard supernatant. After that, TWB was added and the plate was kept on the magnetic stand until the next step.

Amplification of the Tagmented DNA is done through a limited-cycle PCR program. The PCR step adds Index 1 adapters, Index 2 adapters, and sequences required for sequencing cluster generation. To prepare the PCR Master Mix, equal volumes of

EPM (Enhanced PCR Mix) and Nuclease-free water (10 µl of each per sample) was added. With the plate still on the magnetic stand, supernatant was removed and discarded. After removing the plate from the magnet, 40 µl PCR Master Mix was added immediately and directly onto the beads in each sample well. Pipetting is done to mix until the beads are fully resuspended and then appropriate index adapters are added to each sample. Then the plate was sealed and placed on the preprogrammed thermal cycler and the BLT PCR program was run.

Clean Up Libraries is done using a double-sided bead purification procedure to purify the amplified libraries. First, the plate was centrifuged and placed on the magnetic stand. Then, 45 µl supernatant was transferred from each well of the PCR plate to the corresponding well of a new plate. Next, illumina purification master Mix was prepared by mixing 45 µl IPB and 40 µl nuclease-free water per sample. The plate was placed on the magnetic stand and then 125 µl supernatant was transferred from each well of the first plate to a new plate and 15 µl of undiluted IPB (Illumina Purification Beads) was added to each well. The plate was incubated at room temperature for 5 minutes then placed on the magnetic stand until the liquid is clear. Without disturbing the beads, supernatant was removed and discarded. After that, double washes were done while the plate was on the magnetic stand with the addition of 200 µl fresh 80% EtOH without mixing, and then incubation took place for 30 seconds. Supernatant was removed and discarded. Air-drying on the magnetic stand was done next for 5 minutes. The plate was removed from the magnetic stand and 32 µl RSB (Resuspension Buffer) was added to the beads and resuspended by pipetting. Incubation is done at room temperature for 2 minutes then the plate was placed on the magnetic stand until the liquid is clear. Finally, 30 µl supernatant

was transferred to a clean 1.5 ml microcentrifuge tube that can be stored at -25℃ to -15°C for up to 30 days.

When the DNA input is 100-500 ng, quantifying and normalizing individual libraries generated in the same experiment is not necessary. However, the final yield of libraries generated in separate experiments can vary slightly. To achieve optimal cluster density, equal library volumes was pooled and quantified before sequencing. 5 µl of each library was combined in a 1.7 ml microcentrifuge tube. Vortexing is done to mix, and then centrifuged. The library pool was quantified using Qubit fluorometer (Invitrogen, Waltham, MA).

2. MinION library preparation:

The extracted DNA was prepared in nuclease-free water by transferring 50 ng of genomic DNA per sample into a 1.5 ml Eppendorf and adjusting the volume to 9 µl with nuclease-free water. Second, in 0.2 ml thin-walled PCR tubes, 9 µl of template DNA and 1 µl of Rapid Barcodes (RB01-96, one for each sample) were mixed thoroughly by pipetting. The tubes were incubated at 30°C for 2 minutes and then at 80°C for 2 minutes. Then, they were put briefly on ice to cool. All barcoded samples were pooled noting the total volume. After resuspending the AMPure XP Beads (AXP, or SPRI) by vortexing, an equal volume to the entire pooled barcoded sample of it was added and mixed by flicking the tube. Then, it was incubated on a Hula mixer (rotator mixer) for 5 minutes at room temperature. The sample and pellet were spinned down on a magnet and the supernatant was pipetted off. While the tube was kept on the magnet, the beads were washed with 1.5 ml of freshly prepared 80% ethanol in nuclease free water without

disturbing the pellet. The ethanol was removed using a pipette and the previous step was repeated. Next, the tube was briefly spinned down and placed back on the magnet. Any residual ethanol was pipetted off. The pellet was allowed to dry for 30 seconds, but not to the point of cracking. After that, the tube was removed from the magnetic rack and the pellet was resuspended in 15 µl Elution Buffer (EB) and incubated for 10 minutes at room temperature. The beads were pelleted on a magnet until the eluate was clear. 15 µl of eluate which contains the DNA library was removed and retained in a clean 1.5 ml Eppendorf DNA LoBind tube and the pelleted beads were disposed. 11 µl of the sample was transferred into a clean 1.5 ml Eppendorf DNA LoBind tube, and 1 µl of Rapid Adapter F (RAP F) was added to 11 µl of barcoded DNA. The tube was gently mixed by flicking and spinned down. Finally, the reaction was incubated for 5 minutes at room temperature and the prepared library was used for loading into the flow cell.

G. Bioinformatics Analysis

Reads quality control and trimming was done using Trimmomatic (v.1.2.14) after which assembly of the genome was performed using Unicycler on the Galaxy platform (https://usegalaxy.org/). Antimicrobial resistance genes were acquired through CARD (https://card.mcmaster.ca/). Sequence types were identified using MLST on Galaxy.

Long reads generated by the minION sequencing technique were analyzed using EPI2ME platform (https://epi2me.nanoporetech.com/).

CHAPTER IV

RESULTS

A. Isolation and purification of ACN bacteria:

Fifty-four *A. baumannii* clinical isolates were obtained from the Clinical Microbiology Laboratory at AUBMC, 52 clinical isolates were received in the form of disc diffusion purified and stored for further testing. Out of 42 environmental swabs only 1 swab tested positive on both Mero-based MacConkey and Acinetobacter chromogenic agar. These bacteria were purified and stored as separate isolates labeled according to the media stored from.

B. Antimicrobial susceptibility testing

To determine the antimicrobial susceptibility profile of each isolate, broth microdilution (BMD) testing was performed. All isolates were tested for susceptibility to colistin as well as several other antimicrobial agents, including imipenem (MIC ≥ 8), meropenem (MIC ≥ 8), tazocin (MIC ≥ 128/4), ciprofloxacin (MIC ≥ 4), levofloxacin (MIC ≥ 8), ceftazidime (MIC ≥ 32), cefepime (MIC ≥ 32), gentamicin (MIC ≥ 16), and amikacin (MIC ≥ 64). According to CLSI guidelines, two isolates (ACN_202209-357 and ACN_202208-348) were found to be resistant to colistin with MICs of 512 µg/mL. All isolates showed resistance to Bactrim. Resistance rates were 98% for imipenem, meropenem, tazocin, ciprofloxacin, levofloxacin, ceftazidime, and cefepime, while resistance to gentamicin and amikacin accounted for 93% and 94%, respectively. Table 2 shows the MIC values the tested antimicrobial agents.

XDR *A. baumannii* in our study reached 89%, 7% of the isolates full under the MDR description. Adding to that, only 1 isolate had the characteristic of PDR where it was resistant to all classes of antibiotics tested in this study.

C. Sequencing Data:

For the purpose of determining AMR genes, sequence type (ST) and international clones (IC), whole-genome sequencing (WGS) using Illumina sequencing was performed on all these isolates, which were of ST 2, with 93% of them belonging to IC 2. In terms of AMR genes, 91% of the isolates contained the 4 tetracycline and fluoroquinolone genes (adeG, adeL, adeH, and adeF). The macrolide, aminocoumarin genes (adeS) and peptide antibiotic gene LpsB were present in all the isolates tested. However, 89% of the isolates had the macrolide, gene (msrE), while the other macrolide gene was present in 91% of the isolates. The three genes adeK, adeJ, and adeI, belonging to macrolide, fluoroquinolone, lincosamide, carbapenem, cephalosporin, and tetracycline, had a percentage of 94%. The glycylcycline and tetracycline genes adeC, adeR, and adeA were found in 91% of the isolates. ADC-73, responsible for resistance to cephalosporin, was present in 91% of the isolates. However, ADC-163 and ADC-186 were found in 2 different strains. The carbapenem-resistant genes OXA-23 and OXA-66 were found in 93% of the isolates, while 1 bacterium harbored the OXA-121 gene. The three fluoroquinolone genes AbaQ, gyrA, and parC were found in 94% of the isolates. Aminoglycoside genes were the most diverse; one bacterium had 6 genes, while 56% of isolates contained APH (6)-Id, APH (3")-lb, ANT (3")-LIC, and armA genes. The other 23% contained APH (6)-Id, APH (3")-lb, ANT (3")-LIC, armA, and APH (3')-Via, while

21% contained these genes in different combinations. Tetracycline genes tetB and tetR were found together in 89% of the isolates. The results are present in table 4.

The monobactam gene TEM-1 was found only in 11% of the isolates; however, the sulfonamide gene sulf-2 was found only in 4%. Finally, 1 isolate contained NDM-1 and BRP(MBL) genes, responsible for carbapenem and glycopeptide antibiotic resistance, respectively. The results are present in table 5.

Phylogenic tree:

All the isolates belonging to IC-2 and ST-2 clustered well with old isolates from AUBMC dating back to 2014-2020. Some of the isolates have a different sequence type than the main set, even though they have the same susceptibility, indicating that they are not part of the same outbreak cluster. This information can only be determined using NGS.

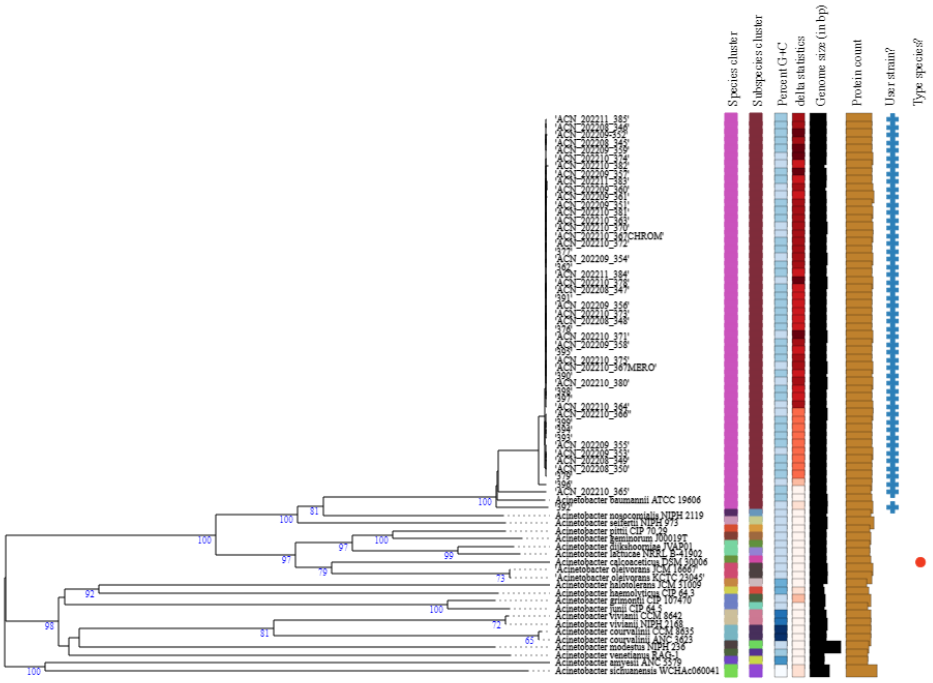

Figure 4: Phylogenic tree

CHAPTER V

DISCUSSION

Antimicrobial resistance (AMR) became the leading public health threats of the 21st century as stated by the WHO (59). It is estimated that the Mediterranean and eastern regions have the highest AMR rates due to the massive utilization of antimicrobial agents in many life sectors and the absence of the infection prevention and control practices that limit and plan the consumption of such agents (59,60). *A. baumannii* the member of ESKAPE pathogens has been recognized recently by the WHO as a major threat to global health. The availability of NGS for the detection of resistant genes has replaced conventional laboratory techniques in the investigation of outbreaks, especially ones occurring because of MDR organisms (61).

Acinetobacter baumannii became the significant driver of hospital-acquired infections caused by MDR and XDR globally (61), since 1980s this bacterium was the major pathogen causing nosocomial infections with higher morbidity and mortalities, exclusively in the (ICUs) (62). A Research from Iran revealed that MDR *A. baumannii* isolated from clinical samples has a high incidence (65). Due to the high prevalence of *A. baumannii* and its recurrent outbreaks in a tertiary healthcare center in Lebanon, we aimed to investigate it between the period of 2022-2023 by utilizing NGS. After the investigation, some isolates occurred previously were further compared. So, for that clinical samples were obtained from the AUBMC processed and stored. 52 known Acinetobacter isolates were received from various clinical samples swabs, urine, sputum, deep tracheal aspiration (DTA), etc.… These were collected from patients in the ICU. Furthermore, because of the increased occurrence of this bacterium in the ICU and the

fact that this bacterium persists in the environment and live on inanimate objects especially hospital environments (63), screening the environment for the presence of Acinetobacter spp. was performed. Two different isolates were isolated from the same site in this unit which was RCU nursing station (Table 1). The cross-contamination of ICU environment by bacterial isolates by patients and healthcare workers' hands has been reported in the literature, and this is proved in the work of Ababneh et al. where they had isolated to MDR *A. baumannii* from handle and surface of the same sink (84).

To assess the antimicrobial susceptibility of these isolates, broth microdilution was performed. *Acinetobacter baumannii* is known by its extraordinary capacity for the acquisition of resistance to most antibiotics available (61). In the current outbreak, Imipenem, Meropenem, Tazocin, Ciprofloxacin, Levofloxacin, Ceftazidime, Cefepime had the highest resistance rate observed which accounted for 98% followed by amikacin 94% and Gentamicin 93%. However, resistance to colistin was observed only in two isolates. A study done in Romania stated the highest resistance rates were found against ceftazidime and imipenem antibiotics (65). Few isolates were obtained from previous outbreaks occurring in the same hospital. The isolates chosen showed a 100% rate of resistance to all antibiotic tested except 2 isolates (ACN2015_276 2273 ACN (3), ACN_201810_142Tony 31) were susceptible to gentamicin. Interestingly, these 15 isolates were all resistant to colistin. The worldwide emergence of colistin resistance is achieved when this drug has been reestablished in clinical practice as a last resort against XDR pathogens in addition to its consumption in the agriculture sector (64).

The resistance levels in *A. baumannii* to the most antibiotics especially carbapenems has risen and reached alarming values globally. The incidence of MDR-*A. baumannii* and XDR-*A. baumannii* accounted to 84% and 48% respectively in 2018 (80).

Recently XDR *A. baumannii* in our study reached 89%, 7% of the isolates full under the MDR description. Adding to that, only 1 isolate had the characteristic of PDR where it was resistant to all classes of antibiotics tested in this study. A study done in the period of 2012-2013 in an ICU of a tertiary healthcare center, showed that the rate of XDR-AB acquisition is 15.6% (81). Another one done in Iran revealed that XDR *A. baumannii* prevalence was high and accounted to 92.4% these were resistant to almost all tested antibiotics (79).

Furthermore, to confirm the resistance in these isolates WGS was performed to detect AMR genes. WGS provides information at the molecular level, which can also detect the relatedness between isolates (66). Another application of this high-throughput technique is detecting the source of outbreaks and tracking the way of spread between patients (67). In the recent outbreak, higher levels of AMR genes were detected. Carbapenem resistance was mediated by the 2 OXA genes OXA-23 and OXA-66. A study done in Lebanon in 2012 showed that most *A. baumannii* isolates harbored OXA-23 resistant gene in addition to OXA-51 gene which was conserved in these isolates and helped in the confirmation of ACN isolates (68). These genes were also present in the previous outbreak. It is worth noting that the chief source of carbapenem resistance was recognized by the Lebanese government as the OXA-23 gene which was detected in ST2 isolates that accounted for 60 to 90% of studied isolates (52).

The prevalence of carbapenem resistance has increased globally, this is linked by the carbapenemases belonging to class D β-lactamases. Three important genes were detected in *A. baumannii bla*OXA-23-like, *bla*OXA-40-like, and *bla*OXA-58-like genes. The OXA-23 gene was detected first in Scotland and then reported worldwide (69). OXA-

51 like enzymes these are chromosomally encoded and are considered a marker for the detection of *A. baumannii* (70).

One isolate harbored NDM-1 gene, it is known that the NDM-1 gene was detected in Lebanon in isolates collected from Syrian patients during the Syrian war (46). NDM gene, also referred to as New Delhi metallo-beta-lactamase 1 belongs to Metallo-beta-lactamase. It was initially discovered in *Klebsiella pneumoniae* and *Escherichia coli* isolated from a Swedish patient in an Indian hospital. Bacteria harboring this gene appear to be resistant to all antimicrobial agents except colistin and sometimes tigecycline (73). NDM-1 is known to transfer horizontally by conjugation, it was recognized as a chimeric gene formed after the fusion of the aminoglycoside-resistance gene aphA6 with a mannose-binding lectin gene. The emergence of this gene was detected at higher rates in *A. baumannii* compared to Enterobacteriaceae. NDM-1 harboring bacteria has been detected in many countries including Germany, Spain, Egypt, Switzerland, Libya, India, Pakistan, and Nepal as Joshi et al. stated in their article (72). Isolate ACN-396 of ST-570 carrying NDM-1 in addition to OXA-23, OXA-66.

Narrow spectrum beta-lactamases TEM-1 were observed ubiquitously in Acinetobacter isolates (78). This gene was present in 11% of the isolates of the recent outbreak, also TEM-1 was one of the genes harbored by the previous isolates. Dagher et al. in their work done in the Saint-George Hospital in Beirut in 2016 showed that all isolated *A. baumannii* harbored the ß-lactamase TEM-1 gene (45).

Another beta-lactamase is cephalosporinases belonging to class C, these are intrinsically available called Acinetobacter-derived Cephalosporinases (ADC), responsible for resistance to extended spectrum of antibiotics belonging to this family such as ceftazidime (70). A study done in a hospital in Nepal between 2014-2015 showed

a high prevalence of cephalosporin resistance mediated by ADC genes in 90.1% of the isolates tested (72). ADC-73 was the main contributor to cephalosporin resistance in the current outbreak in 91 % of the isolates. However, two other ADC genes were identified, ADC-163 and ADC-186 were found in 2 different strains. Compared to the older isolates this gene wasn't available, the ADC-25 was the mediator of cephalosporin resistance. ADC-25 is naturally available in *A. baumannii* mediates resistances to cephalosporin, it was reported in 2 isolates resistant to carbapenem isolated from patients in a hospital in Tunisia in 2012 (71).

Coming to aminoglycosides resistance, Acinetobacter produces enzymes that modify aminoglycosides thus rendering resistance to this drug. ArmA gene mediate resistance to aminoglycosides in a different mechanism, by methylating the 16S rRNA, this gene is transmitted by plasmids between bacteria (74). The same genes were present in the two outbreaks but in different variations. In the recent one, APH (6)-Id; APH (3")-lb; ANT (3")-LIC; armA these four genes were present together in 56 % of the isolates. However, these 4 genes in addition to APH (3')-Via were present in 23% of these isolates. The remaining isolates harbored different combinations of aminoglycoside genes. A recent study in Lebanon showed that resistance to aminoglycosides is being reported at higher levels (50). A study done in China between 2006 and 2009 revealed the increased rates of resistance to aminoglycoside in *A. baumannii*, moreover, they showed that the coexistence of these genes was demonstrated in almost all strains (75). Moreover, in Europe, 84% of *A. baumannii* are harboring minimum of 2 aminoglycoside-modifying enzymes (50).

Trimethoprim/sulfamethoxazole (SXT) is a folate antagonist belonging to the sulfonamide family, recently resistance to this drug has been recorded (77). Sulfonamide

resistance differs between bacterial species and the source of isolation of each bacterium (76). Sulfonamide gene sul-2 was present in 4% of the isolates in the recent period and this gene was reported in the previous isolates obtained. A study done in 5 hospitals in Algiers between the period 2008-2014 showed SXT resistance in 97.87% of the isolates. Both sul1 and sul2 were highly detected in the Acinetobacter strains obtained from this study (78). Another study done in southwest Iran between 2015-2016 showed that the percentage sul2 positive isolates were 67.4%, and that sul2 was of plasmid origin (79).

CRAB isolated from patients in a hospital in Thailand in 2019 showed that macrolide resistance was carried by mphE and msrE which were present in 79.64% of the isolates. These 2 genes are usually detected in MDR in *A. baumannii* isolates. Each has a different mode of resistance against macrolides, the mphE gene inactivates the macrolides however, msrE gene is responsible for the inactivation of the target site (82). These two genes were detected in our isolates both the current and previous ones, msrE containing isolates were 89% however that with mphE was 91% reported from the current isolates. A study done at the Lebanese American University in 2016 showed that macrolide resistance in a Lebanese hospital was 31.7% for mph(E) and 44% for msr(E) harboring isolates (83).

The Ade ABC pump confers resistance to carbapenems, cephalosporins, aminoglycosides, and tetracycline agents. Mutations in the regulator protein of this pump AdeS has been reported in *A. baumannii* (85). And this Ade's gene was reported in all our isolates collected during the last period. The tetracycline-specific efflux pumps are the most mechanism to resist tetracycline, tetA, and tetB efflux pumps with varying affinities to tetracycline derivatives. TetB act on tetracycline, doxycycline, and minocycline. This later when present with AdeIJK acquire extended resistance to tigecycline and

minocycline (85). Tet B was the efflux pump detected in most of our isolates, and the ade IJK was present in 94% of the isolates. The recent isolates harbored extra tetracycline gene which was tet -R, the availability of both was in 89% of the isolates. As reported in an Iranian study tetB harboring isolates was 67.8% between 2016-2018 (86).

Moreover, WGS is used as a tool to strain typing, it differentiates bacterial strains at the levels of nucleotide (87). By the use of MLST we can detect what sequence type and international clones our isolates belong to. MLST stands for multi-locus sequence typing, it is a method used in molecular biology to differentiate bacterial strains by the utilization of the housekeeping genes in each bacterium. The change in these genes is monitored and recorded (88). Of our isolates, 50 isolates (93%) belonged to sequence type 2 in addition to 10 of the older samples. In addition, all these isolates belonged to the international clone 2 (IC2). ST 2 is the most disseminated type in *A. baumannii* worldwide, and it is known that OXA-23 is the most detected beta-lactamase gene isolated from carbapenem-resistant *A. baumannii* (89). In their study, Osman et al. responded to two different outbreaks that occurred between January–September 2016 and April– July 2017 in 3 different hospitals in Tripoli. Their results showed that the majority of the isolates belonged to ST2 and IC2 and carried the *bla*OXA-23 (52).

The finding that all isolates belonging to IC-2 and ST-2 cluster well with old isolates from AUBMC dating back to 2014-2020 suggests that the current outbreak strain might have originated from these older isolates. This could indicate that the outbreak has been ongoing for a longer period of time than initially suspected. However, it's worth noting that some of the isolates have a different sequence type than the main set, despite having the same susceptibility. This suggests that these isolates are not part of the same outbreak cluster as the main set. This information can only be determined using NGS,

highlighting the importance of using advanced techniques to accurately track and identify outbreaks. By using NGS, it is possible to gain a more comprehensive understanding of the genetic relatedness of isolates, which is crucial for effective outbreak management and prevention of further spread. The International clone II was the major clone identified in 34 countries in Europe, Asia, Africa, Australia, USA, and South America, where *bla*$_{\text{OXA-23}}$ was the major carbapenem-resistant gene identified (39). It is worth noting that the chief source of carbapenem resistance was recognized by the Lebanese government as the OXA-23 gene which was detected in ST2 isolates that accounted for 60 to 90% of studied isolates (52).

In this study, Acinetobacter isolates belonging to sequence type 2 (ST-2) and IC-2 were found to be the most prevalent. These isolates were shown to cluster together and were found to have approximately the same antimicrobial resistance genes. Interestingly, some isolates had a different sequence type than the main set, despite having the same susceptibility, indicating that they were not part of the same outbreak cluster. The use of NGS was critical in identifying these differences and highlighting the importance of advanced techniques in accurately tracking and identifying outbreaks. The finding that all isolates belonging to IC-2 and ST-2 clustered well with old isolates from AUBMC dating back to 2014-2020 suggests that the current outbreak strain might have originated from these older isolates. Overall, the study underscores the importance of vigilant monitoring and surveillance of antibiotic-resistant pathogens to prevent and control outbreaks.

APPENDIX

Number	Site
1	PN 1 Neuro-ICU phone
2	CNC Neuro-ICU
3	RN 2 ICU
4	ICU DR 1 phone Medical
5	RN 1 RCU Phone
6	ICU 8 Environment
7	ICU 7 Environment
8	Phone Inhalation Therapist ICU
9	DR 2 ICU Steto (Nephro)
10	PN 2 RCU Phone
11	RCU nursing station
12	RN 1 Neuro-ICU Phone
13	PN-RCU 1 phone
14	Keyboard 1 ICU
15	ICU Dialysis Machine SN 02796
16	PN 1 ICU
17	Neuro-ICU Nursing station
18	Emergency carriage Neuro-ICU
19	DR-Neuro-ICU medical phone
20	RN 2 RCU Phone
21	RN 1 RCU
22	DR 2 ICU Surgical Phone
23	Vent. 840-32
24	DR 1- Stet Medical (ICU)
25	X-RAY ICU
26	Ventilator V60 #6
27	V60 #7 outlet/surface
28	V60 #6 outlet
29	GS5 otlet
30	GSI #3 I surface/outlet
31	HFNG #5 outlet
32	HFNG #5 surface
33	GS5 I Surface
34	Vent V60-6 surface
35	V60 #6 Surface
36	V60-6 Vent. Outlet
37	X-Ray Machine New-ED (WET)
38	X-Ray Machine New-ED (DRY)
39	X-Ray Machine ICU (WET)

40	X-Ray Machine ICU (DRY)
41	X-Ray Machine 90412 (WET)
42	X-Ray Machine 90412 (DRY)

Table 1: Sites of Environmental swabs in the ICU.

Internal ID	XDR /PAN	AST											
		Amikacin (≥ 64)	Bactrim (R≥ 4/76)	Ciprofloxacin (R≥ 4)	Levofloxacin (R≥ 8)	Ceftazidime (≥ 32)	Cefepime (≥ 32)	Colistin (≥ 4)	Gentamicin (≥ 16)	Imipenem (≥ 8)	Meropenem (≥ 8)	Tazocin (≥ 128/4)	Tigecycline (>2)
ACN_202208-345	XDR	>512 R	16 R	512 R	64 R	512 R	256 R	1 S	>512 R	256 R	128 R	512 R	1 S
ACN_202208-346	XDR	>512 R	32 R	>512 R	64 R	>512 R	256 R	2 S	>512 R	128 R	128 R	512 R	2 S
ACN_202208-347	XDR	>512 R	32 R	128 R	16 R	512 R	256 R	<0.5 S	>512 R	128 R	128 R	512 R	1 S
ACN_202208-348	PAN	>512 R	32 R	>512 R	64 R	256 R	64 R	512 R	>512 R	128 R	64 R	256 R	2 S
ACN_202208-349	XDR	>512 R	32 R	256 R	64 R	512 R	128 R	1 S	>512 R	64 R	64 R	256 R	4 R
ACN_202208-350	XDR	>512 R	64 R	256 R	64 R	256 R	128 R	1 S	>512 R	64 R	64 R	256 R	2 S
ACN_202209-351	XDR	>512 R	32 R	>512 R	128 R	512 R	256 R	1 S	>512 R	128 R	128 R	512 R	2 S

ACN_202209-352	XDR	>512 R	32 R	>512 R	128 R	512 R	256 R	1 S	>512 R	128R	128 R	512 R	2 S
ACN_202209-353	XDR	>512 R	32 R	256 R	64 R	256 R	64 R	1 S	>512 R	32 R	32 R	128 R	2 S
ACN_202209-354	XDR	>512 R	16 R	512 R	64 R	512 R	256 R	1 S	>512 R	128 R	32 R	512 R	2 S
ACN_202209-355	XDR	>512 R	32 R	256 R	64 R	512 R	128 R	< 0.5 S	>512 R	64 R	64 R	256 R	2 S
ACN_202209-356	XDR	>512 R	16 R	512 R	64 R	512 R	256 R	1 S	>512 R	128 R	128 R	512R	2 S
ACN_202209-357	XDR	>512 R	16 R	512 R	64 R	256 R	64 R	512 R	>512 R	128 R	128 R	256 R	2 S
ACN_202209-358	XDR	>512 R	16 R	512 R	64 R	512 R	256 R	1 S	>512 R	128 R	128 R	512R	2 S
ACN_202209-359	MDR	>512 R	32 R	512 R	64 R	512 R	256 R	1 S	2 S	128 R	128 R	512 R	2S
ACN_202210-360	XDR	>512 R	32 R	256 R	64 R	512	256 R	1 S	>512 R	64 R	64 R	512 R	1

ACN_202210-361	XDR	>512 R	32 R	512 R	64R	512	256 R	1 S	>512 R	128 R	128 R	512 R	2
ACN_202210-362	XDR	>512 R	128 R	512 R	64 R	>512	256 R	<0.5 S	>512 R	64 R	64 R	512 R	2
ACN_202210-363	XDR	>512 R	32 R	512 R	64 R	512	128 R	1 S	>512 R	64 R	64 R	512 R	1
ACN_202210-364	XDR	>512 R	32 R	>512 R	128 R	>512	256 R	<0.5 S	>512 R	64 R	64 R	512 R	1
ACN_202210-365	Susceptible	2 S	4 R	<0.5 S	<0.5 S	4 S	8 S	<0.5 S	<0.5 S	<0.5 S	<0.5 S	<0.5 S	16 R
ACN_202210-366	XDR	512 R	>512 R	128 R	16 R	512 R	256 R	1 S	>512 R	128 R	128 R	>512 R	2 S
ACN_202210-367ch	XDR	> 512 R	32 R	256 R	16 R	512 R	256 R	<0.5 S	>512 R	128 R	128 R	> 512 R	4 R
ACN_202210-367M	MDR	256 R	32 R	256 R	16 R	512 R	256 R	< 0.5 S	8 S	128 R	128 R	> 512 R	2 S
ACN_202210-370	XDR	>512 R	32 R	512 R	64 R	>512 R	>512 R	<0.5 S	>512 R	64 R	64 R	512 R	2 S

ACN_202210-371	XDR	>512 R	32 R	512 R	64 R	512 R	256 R	1 S	>512 R	128 R	128 R	512 R	1 S
ACN_202210-372	XDR	>512 R	32 R	512 R	64 R	>512 R	256 R	1 S	>512 R	128 R	128 R	512 R	2 S
ACN_202210-373	XDR	>512 R	32 R	512 R	64 R	512 R	256 R	1 S	>512 R	128 R	64 R	512 R	1 S
ACN_202210-374	XDR	>512 R	32 R	512 R	64 R	512 R	256 R	<0.5 S	>512 R	128 R	128 R	512 R	2 S
ACN_202210-375	XDR	>512 R	32 R	512 R	64 R	>512 R	256 R	1 S	>512 R	128 R	64 R	512 R	2 S
ACN_202210-376	XDR	>512 R	32 R	256 R	64 R	>512 R	256 R	<0.5 S	>512 R	64 R	64 R	512 R	1 S
ACN_202210-377	XDR	>512 R	32 R	512 R	64 R	512 R	128 R	<0.5 S	>512 R	128 R	64 R	512 R	2 S
ACN_202210-378	XDR	>512 R	32 R	128 R	16 R	512 R	256 R	<0.5 S	>512 R	128 R	128 R	512 R	2 S
ACN_202210-379	XDR	>512 R	32 R	256 R	64 R	512 R	256 R	<0.5 S	>512 R	128 R	128 R	512 R	2 S

ACN_202210-380	XDR	>512 R	32 R	512 R	64 R	512 R	256 R	<0.5 S	>512 R	128 R	128 R	512 R	2S
ACN_202210-381	XDR	> 512 R	32 R	512 R	64 R	512 R	256 R	1 S	>512 R	256 R	128 R	> 512 R	1 S
ACN_202210-382	XDR	> 512 R	32 R	512 R	64 R	512 R	256 R	<0.5 S	> 512 R	128 R	128 R	>512 R	2 S
ACN_202211-383	XDR	> 512 R	32 R	256 R	32 R	512 R	512 R	<0.5 S	> 512 R	64 R	128 R	512 R	4 R
ACN_202211-384	XDR	> 512 R	32 R	256 R	64 R	512 R	256 R	2 S	>512 R	256 R	128 R	512 R	1 S
ACN_202211-385	XDR	>512 R	32 R	512 R	64 R	512 R	512 R	1 S	>512 R	256 R	128 R	512 R	4 R
ACN_202211-386	XDR	>512 R	32 R	512 R	128 R	512 R	256 R	1 S	>512 R	256 R	128 R	512 R	4 R
ACN_202212-387	XDR	>512 R	8 R	128 R	16 R	256 R	64 R	< 0.5 S	>512 R	64 R	32 R	256 R	2S
ACN_202212-388	XDR	>512 R	32 R	128 R	16 R	512 R	512 R	< 0.5 S	>512 R	256 R	256 R	> 512 R	2S

ACN_20 2212-389	XDR	>512 R	64 R	> 512 R	128 R	256 R	64 R	< 0.5 S	>512 R	64 R	64 R	256 R	2 S
ACN_20 2212-390	XDR	>512 R	65 R	128 R	16 R	512 R	256 R	< 0.5 S	>512 R	64 R	64 R	512 R	1 S
ACN_20 2212-391	XDR	>512 R	256 R	512 R	64 R	512 R	256 R	< 0.5 S	>512 R	128 R	64 R	256 R	1 S
ACN_20 2301-392	XDR	>512 R	512 R	> 512 R	256 R	256 R	64 R	< 0.5 S	> 512 R	32 R	32 R	256 R	4 R
ACN_20 2301-393	XDR	>512 R	32 R	> 512 R	64 R	512 R	512 R	1 S	> 512 R	64 R	64 R	512 R	4R
ACN_20 2301-394	MDR	2 S	32 R	512 R	128 R	> 512 R	256 R	1 S	8 S	128 R	64 R	512 R	2 S
ACN_20 2301-395	XDR	>512 R	16 R	512 R	128 R	> 512 R	256 R	1 S	>512 R	64 R	128 R	512 R	2 S
ACN_20 2301-396	MDR	8 S	8 R	256 R	32 R	> 512 R	512 R	< 0.5 S	16 R	256 R	512 R	512 R	2 S
ACN_20 2301-397	XDR	> 512 R	32 R	512 R	32 R	> 512 R	256 R	2 S	> 512 R	128 R	64 R	256 R	2 S

ACN_20 2301- 398	XDR	> 512 R	64 R	256 R	32 R	> 512 R	256 R	< 0.5 S	> 512 R	128 R	64 R	512 R	2 S
ACN_20 2301- 399	XDR	> 512 R	64 R	512 R	128 R	512 R	128 R	1 S	> 512 R	64 R	64 R	512 R	2 S

Table 2: Minimum Inhibitory Concentrations of Antibiotics for Isolates of Current Outbreak.

R: Resistant S: Susceptible

Secondary ID	Colistin (R ≥ 4)	Amikacin (R ≥ 64)	Bactrim (R ≥ 4/76)	Levofloxacin (R≥ 8 µg/mL)	Tigecycline (R ≥ 0.5)	Meropenem (≥ 8)	Imipenem (≥ 8)	Gentamicin (≥ 16)	Cefepime ((≥32)	Tazocin (≥ 128/4)	Ceftazidime (≥ 32)	Ciproflox acin (R≥ 4)
ACN_201810_145Tony 36	512 R	>4096 R	8 R	32 R	16 R	16 R	16 R	>1024 R	128 R	256 R	512 R	128 R
ACN_201810_142Tony 31	512 R	128 R	16 R	8 R	16 R	8 R	16 R	8 S	64 R	128 R	128 R	64 R
ACN_201810_124Tony 19	512 R	128 R	32 R	16 R	64 R	32 R	128 R	>1024 R	256 R	256 R	256 R	256 R
ACN2014_278 R1314 ACN(5)	512 R	>4096 R	64 R	16 R	64 R	64 R	128 R	>1024 R	256 R	512 R	512 R	64 R
ACN2015_276 2273 ACN(3)	512 R	>4096 R	32 R	32 R	64 R	8 R	16 R	2 S	128 R	256 R	256 R	128 R
ACN2015_274 2090 ACN(1)_new	512 R	>4096 R	32 R	16 R	32 R	64 R	256 R	>1024 R	256 R	512 R	512 R	64 R
ACN2015_275 2258 ACN(2)	64 R	>4096 R	64 R	64 R	32 R	128 R	128 R	>1024 R	128 R	512 R	256 R	128 R
ACN_201812_189Tony 40	8 R	>4096 R	64 R	32 R	32 R	128 R	64 R	>1024 R	265 R	512 R	512 R	128 R
ACN_201810_149Tony 34	8 R	>4096 R	64 R	32 R	64 R	128 R	128 R	>1024 R	256 R	512 R	512 R	64 R
ACN_201811_157Tony 39	4 R	>4096 R	64 R	32 R	32 R	256 R	128 R	>1024 R	512 R	1024 R	512 R	64 R
ACN_201812_194Tony 48	4 R	>4096 R	64 R	32 R	64 R	128 R	64 R	>2048 R	128 R	512 R	256 R	64 R

ACN_201812_193Tony 47	4 R	>4096 R	64 R	32 R	64 R	128 R	128 R	>2048 R	512 R	256 R	512 R	64 R
ACN_201810_150Tony 38	4 R	>4096 R	128 R	32 R	64 R	128 R	128 R	1024 R	256 R	1024 R	512 R	64 R
ACN_201810_125Tony 20	4 R	>4096 R	128 R	64 R	64 R	32 R	64 R	>1024 R	512 R	512 R	512 R	128 R
ACN_201810_116Tony 11	4 R	>4096 R	64 R	16 R	64 R	128 R	256 R	>1024 R	256 R	512 R	128 R	64 R

Table 3: Minimum Inhibitory Concentrations of Antibiotics for the previous Isolates

R: Resistant S: Susceptible

Internal ID	**ST**	**IC**	**fluoroquinolone, tetracycline**	**Macrolide**			**macrolide, fluoroquinolone, lincosamide, carbapenem, cephalosporin, tetracycline**	**glycylcycline, tetracycline**	**cephalosporin**	**carbapenem, cephalosporin, penam**	**fluoroquinolone**	**peptide antibiotic**	**aminoglycoside**	**tetracycline**
ACN_202208-345	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202208-346	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202208-347	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202208-348	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR

ACN_20 2208-349	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2208-350	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-351	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-352	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	AdeR	ADC-74	OXA-23; OXA-67	gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-353	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2209-354	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2209-355	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-356	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-357	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-358	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2209-359	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR

ACN_20 2210-360	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	-	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC	tetR
ACN_20 2210-361	abaum annii	2	adeG; adeL; adeH;adeF	ade S	-	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-362	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC, armA	tet(B); tetR
ACN_20 2210-363	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-67	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC, armA	tet(B); tetR
ACN_20 2210-364	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-365	abaum annii_1 50	2	adeL; adeH;adeF	ade S	-	-	adeK; adeJ; adeI;adeN	adeR	ADC-163	OXA-121	AbaQ; parC	LpsB	ANT(3")-LIC	-
ACN_20 2210-366	abaum annii_2	2	adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via,APH(3')-Ia	tet(B); tetR
ACN_20 2210-367ch	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	-	-	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; APH(3')-Vla	tet(B); tetR
ACN_20 2210-367M	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-370	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-371	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-372	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR

						hE								
ACN_20 2210-373	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2210-374	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-375	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2210-376	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2210-377	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2210-378	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-379	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-380	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_20 2210-381	abaum annii_2	2	adeG; adeL;adeF	ade S	msrE	mphE	adeK; adeJ; adeI	adeC;adeR	-	OXA-66	AbaQ; gyrA; parC	LpsB	APH(3")-lb; ANT(3")-LIC; armA	tetR
ACN_20 2210-382	abaum annii_2	2	adeF; adeL	ade S	msrE	mphE	adeK; adeI	adeA	-	OXA-23	gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC	tet(B); tetR
ACN_20 2211-383	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	msrE	mp	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR

						hE								
ACN_202211-384	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202211-385	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202211-386	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202212-387	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202212-388	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202212-389	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Via	tet(B); tetR
ACN_202212-390	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202212-391	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	msrE	mphE	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_202301-392	abaumannii_717	2	adeG; adeL;adeF	adeS	msrE	mphE	adeN; adeJ; adeI	AdeC	ADC-186	OXA-23; OXA-69	AbaQ; gyrA; parC	LpsB	ANT(3")-LIC;APH(3')-Via;ABTI(2")-Ia	-
ACN_202301-393	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	-	-	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA; APH(3')-Ia	tet(B); tetR
ACN_202301-394	abaumannii_2	2	adeG; adeL; adeH;adeF	adeS	-	-	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; APH(3')-Ia	tet(B); tetR

ACN_20 2301-395	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2301-396	abaum annii_5 70	2	adeG; adeL; adeH;adeF	ade S	-	-	adeK; adeJ; adeI;adeN	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	ANT(3")-LIC; APH(3')-Ia; APH(3')-Via	-
ACN_20 2301-397	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2301-398	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA	tet(B); tetR
ACN_20 2301-399	abaum annii_2	2	adeG; adeL; adeH;adeF	ade S	m sr E	m p h E	adeK; adeJ; adeI	adeC;adeR; adeA	ADC-73	OXA-23; OXA-66	AbaQ; gyrA; parC	LpsB	APH(6)-Id; APH(3")-lb; ANT(3")-LIC; armA;APH(3')-Ia	tet(B); tetR

Table 4: AMR genes of current Outbreak

Internal ID	AMR Genes			
	monobactam	sulfonamide	carbapenem	glycopeptide
ACN_202210-366	TEM-1	sul2	-	-
ACN_202212-388	TEM-1	-	-	-
ACN_202301-392	-	sul2	-	-
ACN_202301-393	TEM-1	-	-	-
ACN_202301-394	TEM-1	-	-	-
ACN_202301-395	-	-	-	-
ACN_202301-396	TEM-1	-	NDM-1	BRP(MBL)
ACN_202301-399	TEM-1	-	-	-

Table 5: AMR genes of Current Outbreak (continued)

secondary_id	ST_p	ST_scheme_p	aminoglycoside_variants	Sulphonamide variants	Tetracycline variants	macrolide variants	beta-lactam variants	OXA_type
ACN_201810_145 Tony 36	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	-	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201810_142 Tony 31	2	abaumannii_2	armA|||aph(3')-Ia	-	-	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201810_124 Tony 19	187	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	-	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN2014_278 R1314 ACN (5)	187	abaumannii_2	armA|||aph(3')-Ia|||aph(3'')-Ib|||aph(6)-Id|||aph(3')-Via	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN2015_276 2273 ACN (3)	187	abaumannii_2	armA|||aph(3')-Ia|||aph(3'')-Ib|||aph(6)-Id|||aph(3')-Via	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN2015_274 2090 ACN (1) new	187	abaumannii_2	armA|||aph(3')-Ia|||aph(3'')-Ib|||aph(6)-Id|||aph(3')-Via	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN2015_275 2258 ACN (2)	187	abaumannii_2	armA|||aph(3')-Ia|||aph(3'')-Ib|||aph(6)-Id|||aph(3')-Via	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201812_189 Tony 40	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201810_149 Tony 34	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	-	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201811_157 Tony 39	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||aph(3')-Via	sul2	tet(B)	-	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25	OXA_23_like_OXA_51_like
ACN_201812_194 Tony 48	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201812_193 Tony 47	2	abaumannii_2	armA|||aph(3')-Ia|||aph(3'')-Ib|||aph(6)-Id|||aph(3')-Via	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-225|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201810_150 Tony 38	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	-	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like
ACN_201810_125 Tony 20	2	abaumannii_2	aph(3'')-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	-	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like

ACN_201810_116 Tony 11	2	abaumannii_2	aph(3")-Ib|||aph(6)-Id|||armA|||aph(3')-Ia	sul2	tet(B)	mph(E)|||msr(E)	*bla*OXA-66|||*bla*OXA-23|||*bla*ADC-25|||*bla*TEM-1D	OXA_23_like_OXA_51_like

Table 6: AMR genes of old isolates.

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