PalArch's Journal of Archaeology of Egypt / Egyptology

ESKAPE BACTERIA AND ANTIMICROBIAL RESISTANCE

Mohammad F. Al Marjani[1] ; Raad N. Hasan[2] ; Zahraa A. Khadam [1] ; Nadal A. Al-Saryi[1] and Aws H. Al Rahhal[3]

1 Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
2* Biotechnology and Environmental Center, University of Fallujah.
3Ministry of Higher Education and Scientific Research , Research and development Department
*raadalhasani@uofallujah.edu.iq

Mohammad F. Al Marjani; Raad N. Hasan; Zahraa A. Khadam; Nadal A. Al-Saryi and Aws H. Al Rahhal, ESKAPE Bacteria and Antimicrobial resistance-Palarch's Journal Of Archaeology OfEgypt/Egyptology 17(7),ISSN 1567-214x

Abstract

Infections caused by multidrug-resistant (MDR) bacteria continue to be a worldwide problem. Multidrug resistance is growing rapidly among Gram-positive and Gram-negative pathogens that cause infection in the nosocomial environment and the general community.

Several MDR pathogens especially, Klebsiella pneumoniae, Enterococcus faecium, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter spp. and Acinetobacter baumannii , known as ESKAPE pathogens cause the main infections within the nosocomial environment. Antibiotics resistance naturally occurs over time but it a slow process. However, the misuse or overuse of antibiotics has accelerated the number of resistant bacteria, which is difficult to eliminate. Despite, there are still possible treatment options available to eliminate these resistant bacteria in recent days, future attention should focus on avoiding further development of resistant pathogens. In this review, we will describe the ESKAPE, some of their antibiotics resistant mechanisms and bacterial species belonging to the ESKAPE group

Keywords:: *Enterococcus faecium, Acinetobacter baumannii*, nosocomial infections

Introduction

The acronym ESKAPE is used to describe six nosocomial pathogens that exhibit virulence and multidrug resistance, which includes : *Staphylococcus aureus, Klebsiella pneumoniae, Enterococcus faecium, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp*.(Yamaguchi *et al*., 2011). ESKAPE group causes most of nosocomial infections and can escape the biocidal action of antimicrobial agents (Van Melderen and DeBast,2009).

ESKAPE organisms are the common causes of life-threatening infections worldwide, especially in critically ill and immunocompromised patients (Wen et al.,2014). Further understanding of this group of organisms is important, as studies have shown that patients with ABR ESKAPE organisms are more likely to receive inappropriate antibiotic therapy, which leads to higher mortality rates.

Besides, spreading these organisms to other patients is a concern (Wang and Wood,2011).

1. Multi-drug resistance in ESKAPE

The resistance to an antimicrobial compound means non-susceptibility to a given antibiotic molecule. From practical aspects, one can distinguish between multi-resistant pathogens based on qualitative and quantitative profiles. A recent classification defines (i) multi-drug resistant (MDR) strains and isolates, which are not susceptible to (at least) one representative of each of three categories of antimicrobial compound families; (ii) extreme drug-resistant, (XDR),which are not susceptible to (at least) one representative of all but very few categories of antimicrobial compound families; and (iii) pan-drug resistant (PDR) ones, which are not susceptible to any of the tested representatives of all known antimicrobial compound families (Wozniak and Waldor, 2009).

1.1. *β*-Lactam antibiotics

These contain the four-member, nitrogen-containing, *beta*-lactam ring at the core of their structure (Forbes *et al*.,2007). *β*-lactam antibiotics are one of the most effective and commonly used agents in the treatment of infectious diseases (Livermore and Woodford, 2006). This drug class comprises the largest group of antibacterial agents such as penicillins, cephalosporins, cepha-mycins, monobactams and carbapenems. The popularity of these agents results from their bactericidal action and lack of toxicity to human (Forbes *et al*.,2007; Goering *et al*., 2012).The carbapenems are a subgroup of the *β*-lactam antibiotics and have the broadest spectrum of activity within this group. They are also structurally similar to the penicillins, but the sulphur atom in position 1 of the structure has been replaced with a methyl group (Marin and Gudiol,2003). Three major mechanisms have been proposed to generate resistance to *β*-lactam antibiotics: **(1)** Restricted access to drug targets either by preventing drug entry or enhanced drug efflux. **(2)** Alteration of drug targets. **(3)**The presence of drug-degrading enzymes (Fisher *et al*., 2005; Gao *et al*.,2017).

1.1.1 *β*-lactamase enzymes

The expression of β-Lactamase enzyme is a principal mechanism of Gram-negative resistance. There are four distinct classes of β-lactamases known: three classes of serine enzymes (A, C , and D) and one class of metal-dependent enzymes (B) (Fisher et al., 2005). β-Lactamases enzymes work by breaking the amide bond of the standard β-lactam ring until the antibiotic can enter the cell wall synthesis site, making antibiotics ineffective (Poole, 2004).

β-Lactamase enzyme expression is a fundamental resistance mechanism of Gram-negative. Four distinct*β*-lactamases classes are known: three serine-dependent enzyme classes (A, C and D) and one metal-dependent class (B) (Fisher *et al*.,2005). *β*-Lactamases enzymes work through disrupting the amide bond of the *β*-lactam ring, which renders antibiotics ineffective before reaching the site of cell wall synthesis, (Poole, 2004).

A; Ambler class A*β*-lactamase enzymes

Ambler class A *β*-lactamase enzymes group involves the main extended-spectrum *β*-lactamases (ES*β*Ls) such as SHV, CTX-M and TEM enzymes, which work through hydrolyze oxyimino *β*-lactams and monobactams but do not affect on the cephamycins and the carbapenems(Quale *et al*., 2002). While these are generally susceptible to *β*-lactamase inhibitors (Kim *et al*., 2008). A few class A enzymes, most noted the *K.pneumoniae* carbapenemase (KPC) are effective carbapenemases (Nordmann *et al*.,2009).

1.1.1.B: Ambler class B *β*-lactamase enzymes

They are also known as the metallo-*β*-lactamase(M*β*L), these enzymes contain in their active site one or two metal ions, usually zinc rather than a serine residue. Furthermore, these enzymes can hydrolyze all the *β*-lactam compounds except aztreonam, and are not inactivated by *β*-lactamase inhibitors (Walsh *et al*.,2005). Two major M*β*Ls have been reported worldwide, IMP and VIM family (Perez *et al*., 2007).

New Delhi metallo-*β*-lactamase (NDM-1) was first described in 2009 from New Delhi. This enzyme is widespread in *E.coli* and *K.pneumoniae* isolated from India and Pakistan. *ndm*-1 carrying bacteria have been introduced to other countries in the mid of 2010 (Walsh *et al*.,2011).

1.1.1.C: Ambler class C *β*-lactamase enzymes

They are more active on cephalosporins than benzylpenicillin and are usually resistant to inhibition by clavulanic acid and active on cephamycins. They have a high affinity for aztreonam (Bush,1988). Encodes the chromosomally *bla* AMP-Cwhich exhibits a typical cephalosporinase substrate profile but not cefepime or carbapenems and *bla* CMY (Cefipem resistance) (Perez *et al.*,2007).

1.1.1.D: Ambler class D *β*-lactamase enzymes

Class D (OXA) carbapenemases are another naturally occurring *β*-lactamase enzymes in *A.baumannii*, which leads to acquired resistance to carbapenem. Four group of class D carbapenemases CHDLs have been identified in *A.baumannii* including OXA- 23-like, OXA-24-like, OXA-51-like, OXA-58-like enzymes (Manchanda *et al*.,2010). Although, Class D enzymes are particularly prevalent in *A.baumannii*, they have been reported in many other clinically relevant organisms, such as *E. coli*, *Enterobacter spp*., *K.pneumoniae* and *P.aeruginosa*, among others (Evans and Amyes,2014).

1.1.2: **Mechanisms of other antibiotics resistance**

Most pathogenic microorganisms can develop resistance mechanisms to at least some antibiotics. The main resistance mechanisms include: alteration of drugs target, drug inactivation, active efflux and reduced drug uptake. These mechanisms may be native or can be acquired from other microorganisms. Further understanding of resistance mechanisms should assist in finding better available options for the treatment of infective diseases and the development of

antimicrobial drugs that can withstand any microbial attempts to develop resistance (Martinez, 2014). Gram- negative bacteria are highly efficient at up-regulating or acquiring genes that code for antibiotic resistance mechanisms, especially in the present selection pressure of antibiotics (Boucher *et al*.,2009).

2. Biofilm as a virulence factor in ESKAPE

Some bacteria can attach to a specific area and produce exo-polymer, increased in adherent bacteria population leads to form a biofilm (Steinberg and Kolodkin-Gal,2015). Thus, biofilms are defined as an organized group of microorganisms living within a self-produced matrix of polymeric substances which gets attached to several surfaces (Hurlow *et al*.,2015). These microbial aggregations are found to be ubiquitous in almost every environment (Parsek and Singh, 2003).

Formation of biofilm in bacteria occurs as a response to different factors such as cellular recognition of attachment sites on the surface, nutritional cues, environmental stresses and exposure to sub-lethal concentrations of antibiotics (Hoffman *et al*., 2005).

2.1: Microbial biofilm composition

In biofilms, bacteria are generally embedded in the extra-polymeric matrix, which self-produced and consisted of polysaccharides, nucleic acids, lipids, and proteins . This extracellular matrix supports the stability of adhesiveness, cohesiveness, and three-dimensional architecture to the biofilm (Rice *et al*.,2016). Biofilm is mainly comprised of (80%–85%) exopolymer and (15%–20%) represent the microorganisms (Cowan, 2011).

2.1.1: Steps in Biofilm formation

Formation of biofilm in bacteria occurs as a response to different factors such as recognition of attachment sites on the surface, nutritional cues, environmental stresses and exposure to sub-lethal concentrations of antibiotics (Hoffman *et al*., 2005). Biofilm formation is a complicated process but some studied explained this process in a few steps: the first step is the initial contact/attachment to surfaces; then formation of microcolonies, maturation and then formation of the architecture of the biofilm; finally detachment/dispersion of the biofilm (Jamal *et al*.,2018)

In most cases, the base of the biofilm is a bed of dense, with thickness up 5 to 50 μm. This bed has consisted of substances produced by bacteria, which is a sticky mix of polysaccharides, other polymeric substances and water. Soaring 100 to 200 μm upwards are colonies of bacteria, shaped like mushrooms or cones. A mature biofilm may develop through several hours or may take several weeks, depending on the system. We will briefly describe the steps of biofilm formation:

A- Initial contact/attachment to the surface

The Initial step of the biofilm formation process begins when microbial cells attached to surfaces using their appendages like flagella and pilli . Also the

attachment can occur through other physical like forces electrostatic interactions and van der Waal's forces (Otto *et al*.,2013)

B-Microcolonies formation:

Following attachment of microorganisms into a biotic or an abiotic surface occurred and stabilized, microbial cells begin to multiplicate and divide, which initiated through particular chemical signaling within the EPS and as a result microcolonies formation completed.

C-Maturation and architecture

During this stage of biofilm formation, the commination between microbial cells occurs through auto-inducer signals (Papenfort and Bassler 2016)). These autoinducers activate quorum sensing, which plays a role in biofilm formation.

D-Detachment/dispersion of biofilm

In this phase, microbial cells within the biofilm perform quick multiplication and dispersion to convert from sessile into motile form. During the detachment process, microbial communities within biofilm produce different saccharolytic enzymes that help to release the surface of the microbes into a new area for colonization such as N-acetyl-heparosan lyase for the lysis of the EPS matrix and subsequent detachment (Arias *et al*.,2010). A detachment of microbial cells and transfer to a new site aid in the spreading of infections (AL-Marjani, 2013 ; Kadhim *et al*.,2018).

3. ESKAPE bacteria

3.1 *Enterococcus faeciu*

The two enterococcal species, *Enterococcus faecalis* and *Enterococcus faecium* are the most common species detected in clinical and food samples. *E. faecalis* was responsible for approximately 90% of the human infections and *E. faecium* for the remaining 10% in the first wave of nosocomial enterococcal infections, (Mateja *et al* .,2019).

Infections caused by multidrug-resistant Enterococci, especially multiple resistances to vancomycin, penicillin, and aminoglycoside (high-level resistance), are of major concern, making enterococcal infections a serious and life-threatening disease (Arias and Murray, 2012).

Enterococci resistance can be through intrinsic or acquired resistance to several antimicrobials, such as *b*-lactams, glycopeptides, and fluoroquinolones. They can exhibit a high level of resistance to aminoglycosides (streptomycin and gentamicin), leading to reduce therapeutic options for enterococci infections. Therefore, these bacteria are concerned as important pathogens with clinical relevance (Arias and Murray, 2012).

Vancomycin-resistant *E. faecalis* increased gradually in Baghdad hospitals and high dissemination of the *van*A gene, which encoded a high resistance level to vancomycin (Al Marjani , 2013).

3.2 *Staphylococcus aureus*

Staphylococcus aureus is found as a normal microflora in humans and animals. This bacterium is found on the skin or mucous membranes of healthy individuals. *S.aureus* can survive at different levels of oxygenation, and generally very hardy organisms. Also, this organism is an opportunistic pathogen often carried asymptomatically on the human body (Matthew , 2012).

3.2.1 Methicillin-Resistant *S. aureus* (MRSA)

Methicillin-Resistant *S. aureus* (MRSA) represents *S. aureus* strains that acquired a gene responsible for their methicillin resistance and essentially all other antibiotics belong to beta-lactam. MRSA strains first emerged in 1961, after methicillin was used to treat human infections with penicillin-resistant staphylococci (Lee *et al.,* 2003). Since then, these strains have known as a serious concern in human medicine. Although, these strains cause the same types of infections as other *S. aureus*, hospital-associated strains, they have acquired resistance to most known antibiotics. Thus, proper treatment can be a serious challenge (Lipsky *et al.,* 2010). In most patients, MRSA strains can be isolated and detected by swabbing the nostrils (Yan *et al.*, 2013). The spread of MRSA infections occurs through direct contact with pus from infected wounds; contact with objects such as clothing, sheets and towels; skin-to-skin contact, or athletic equipment used by an infected individual (Madigan and Martinko,2005).

3.2.1.1 Epidemiology of MRSA

The ecological niches of *S. aureus* strains are the anterior nares. In the case of nares are treated with antibiotics locally to eliminate nasal carriage as preventative strategies, the organisms also disappear from other sites of the body in most cases (Antony, 2011.) The percentage of *Staphylococcus aureus* infections caused by MRSA were increased steadily Between 1968-mid-1990s, In 1974, 2% of hospital-acquired *S. aureus* infections could be attributed to MRSA and this rate increased to 22% by 1995, later in 1997 and this rate reached 50% (Daum,2007). Genetic and epidemiologic evidence showed that CA-MRSA is caused by *S. aureus* strains that are different from those associated with HA- MRSA**.** CA-MRSA is differentiated from HA-MRSA based on an individual's exposure to healthcare settings (Nathwani*et al*., 2008).

3.2.1.2 Pathogenicity

MRSA presents a major concern in hospitals, nursing homes and prisons, where patients with open wounds, weakened immune systems and invasive devices are at greater risk of infections than healthy individuals (Vainio, 2012). *Staphylococcus*

can cause mastitis or abscess of breasts in breastfeeding women. Staphylococcal breast abscesses can cause the spread of this bacteria into the mother's milk (Iwase,2010).

MRSA strains may progress substantially within 24-48 hours of initial topical symptoms. MRSA can take hold in human tissues and eventually become resistant to treatment after 72 hours (Lipsky *et al*., 2010). The initial symptom of MRSA is small red bumps that resemble pimples, spider bites, or boils; fever and occasionally rashes may be accompanied. Bumps become larger and more painful within a few days. These bumps eventually open into deep, pus-filled boils (Raygada and Levine,2009).

3.2.2 Methicillin-Resistant *S.aureus* andantimicrobialssusceptibility

The resistance of methicillin occurs via the mecoperon, part of the staphylococcal cassette chromosome mec (SCCmec) (Berglund et al., 2009). The resistance of MRSA strains is conferred by the expression *mecA* gene , this the gene encodes for PBP2a protein, which is a protein with a low affinity to β-lactam antibiotics(cephalosporins, penicillins and carbapenems). This group of antibiotics is used to treat complicated skin and skin structure infections and bacterial pneumonia caused by *S. aureus* (Kanafani and Corey, 2009).

Quinolones are a family of broad-spectrum antibioticswith bactericidal activity by inhibiting nucleic acids synthesis. First-generation drugs (*e.g*., Nalidixic acid) achieved minimal serum levels and required a high inhibitory concentration. While second-generation quinolones (*e.g*., ciprofloxacin) developed to improve the spectrum of activity. Third-generation drugs (*e.g*., levofloxacin) developed to expand their activity against gram-positive bacteria and atypical pathogens (Ivanov and Budanov, 2006**)**. Levofloxacin is used to treat chronic bacterial prostatitis caused by both methicillin-susceptible and resistant*Staphylococcus*, cellulitis, , impetigo, pyoderma, abscesses, furuncles and wound infections due to MSSA**(**Wright *et al*.,2002).The fourth-generation of quinolone drugs (currently only trovafloxacin) added a significant efficiency against anaerobic organisms**.** Aminoglycosides are a group of antibiotics, their mechanism of action by inhibition of bacterial protein synthesis through interaction with A-site on the 16S ribosomal RNA of the 30S ribosome.

Until now, three main resistance mechanisms that are known associated with aminoglycoside include:, ribosomal mutations, aminoglycoside modifying enzymes and efflux-mediated resistance. The usage of these antibiotics is limited due to the potential side effects, which can cause kidney and ear damage (Ramirez and Tolmasky,2010; Kareem *et al*.,2020). Vancomycin is recommended by The Infectious Disease Society of America as the first line of treatment for complicated skin infections, endocarditis, bloodstream infections, joint and bond infections and meningitis infections caused by MRSA **(** Liu *et al*., 2008). Macrolides are a class of antibiotics that contain macrolide ring as part of their

chemical structure. This ring structure is responsible for the chemical activity of drugs (MacDougall and Chambers ,2011).

3.4 *Acinetobacter baumannii*

*Acinetobacter baumannii*lipopolysaccharides (LPS) are potent stimulators of white blood cells to release pro-inflammatory substances (Erridge *et al*., 2007). LPS are toxic to neutrophils and inhibit their migration as well as phagocytosis (Kurcik-Trajkovska, 2009). The biofilms formation an important virulence factor related to bacterial survival and antibiotic resistance for *A.baumannii* (Luo *et al*., 2015).

3.4.1 Hospital-acquired *Acinetobacter* pneumonia

The majority of *A.baumannii* strains were isolated from the respiratory tracts of patients at hospitals. However, *A.baumannii* is the second most common etiologic agent among all the Gram-ve bacteria (Luna and Aruj, 2007). Nosocomial pneumonia occurs in intensive care units with a frequency of 3–5% and with crude death rates of 30–75% being reported (Doughari *et al*., 2011).

3.4.2 *Acinetobacter* andcommunity-acquired pneumonia

Acinetobacter can easily inhabit tracheotomy sites leading to and tracheobronchitis and community acquired bronchiolitis in immuno-compromised adults and healthy children. However, it rarely causes sepsis and community-acquired pneumonia (Whitman *et al*., 2008).

3.4.3 Epidemiology of *A.baumannii*

Acinetobacter baumannii is primarily hospitals related to the pathogen. It has reported that it is a cause of outbreaks and nosocomial infections (Vashist *et al*., 2011). Multi-drug resistant *Acinetobacter* only has a minimal threat to patients' family members or healthcare workers because it rarely causes any serious infection in healthy people.

3.4.3.1 Global epidemiology of *A.baumannii*

*A. baumannii*is found in water and soil besides health care environments, causing human colonizers in the hospital (Kanafani and Kanj, 2014, Villegas and Hartstein,2003). Several studies have shown the incidence of MDR *A.baumannii* infections in different countries and often associated with nosocomial infections (Doughari *et al*., 2011). An escalation in the number of highly resistant isolates of *A.baumannii calcoaceticus* complex was detected between The United Kingdom and US militaries. These isolates infected individuals among the army, who were wounded during deployment to Afghanistan and Iraq (Peleg *et al*., 2008).

3.4.3.2 Middle East epidemiology of *A.baumannii*

In the middle East, many cases associated with MDR *A.baumannii* have been shown from hospitals in Saudi Arabia, Bahrain, Palestine, the United Arab Emirates and Lebanon (Mugnier *et al*.,2009). In Riyadh Military Hospital, Saudi Arabia, a retrospective study to show a prevalence of MDR bacteria at the

Intensive care units (ICUs), indicated that the most common bacterial type isolated from ICUs patients belong to *A.baumannii*, which represents 40.9% percent of collected samples (Saeed *et al*., 2010).

There are specific resistant clones, which are the predominant cause of the outbreak and three European clones (designated as I, II and III) have geographically disseminated in separate areas. The majority of *A.baumannii* isolates usually belong to a single clone (Zowawi *et al*., 2015).

3.5. *Klebsiella pneumoniae*

The pathogenicity of *K.pneumoniae* isolates is mediated by several virulence factors that allow it to evade host innate immune responses. They are typically enveloped by a polysaccharide capsule, which protects the bacterium from phagocytosis and prevents killing by bactericidal serum factors (Podschun *et al*., 2000). Adhesions or colonization factors: Pili or fimbrial adherence factor required for epithelial cell attachment and host colonization such as type 1 and type 3 (Struve *et al.,* 2008).

3.5.1 **Infections with *K.pneumoniae***

3.5.1.1 Hospital-acquired cases of pneumonias (HAPs).

Hospital-acquired cases of pneumonias with *K.pneumoniae* are far more prevalent than Community-acquired pneumonia. *K.pneumoniae* is the underlying cause of ~11.8 % of HAPs (Magill *et al*., 2014). This bacterium is responsible for destructive changes to human lungs inflammation and hemorrhage with cell death, necrosis that sometimes produces thick, bloody, and mucoid sputum in a patient with chronic pulmonary disease, and in immune-compromised patients (Jagessar and Alleyne, 2011)

3.5.1.2 Community-acquiredcases of pneumonia (CAPs):

Community-acquired pneumonia cases are common infections. These infections are serious and progress rapidly leading to intensive care unit (ICU) and hospitalization stays, besides high rates of mortality and morbidity (Restrepo *et al*., 2013)

3.5.2 Epidemiology

It is an important emerging pathogen in community-acquired liver abscess worldwide with the prevalence rate is as high as 78% in Taiwan and 41% in the USA (Rahimian *et al*., 2004). Frequency as an etiological agent of pneumonia is rarely reached to (3–5 %) in North America, Europe, and Australia. However, *K. pneumoniae* is a more common etiological agent of CAPs in Asia and Africa by (15%) following *Streptococcus pneumoniae* as the underlying agent (Magill *et al*.,2014).These findings suggest that environmental factors, such as diet, may play a role in the epidemiologic differences observed concerning this pathogen(Chung *et al*.,2012).

The most prevalent type of nosocomial infections caused by *K.pneumoniae* is urinary tract infections, which are commonly caused by biofilm-forming isolates on catheters (Lundberg *et al*., 2013). *K.pneumoniae* was the most Gram-negative bacteria isolated from Baghdad hospitals and its prevalence rate was 90% among hospitalized patients (Al Jailawi *et al.*, 2014).

3.6 Pseudomonas aeruginosa

P.aeruginosa is an opportunistic human pathogen as well, was known for its ability to produce pigments, which were toxic to numerous bacteria, fungi and damages mammalian cells. (Kerr *et al*., 1999; Ran *et al*., 2003).

P.aeruginosa was considered by many as a facultative anaerobic, as it was admirably adapted to proliferate in conditions or partial or total oxygen depletion (Hassett, 1996).

3.6.1 Pathogenicity and epidemiology of *P.aeruginosa*.

P.aeruginosa is an opportunistic bacterium , which can invade areas lack of normal host defense mechanisms , such as damaged tissue due to burn wounds, which lead to mucous membranes and skin disruption (Harvey *et al.*, 2013). *P.aeruginosa* pathogenesis has been extensively studied and proven to be a multifactorial process, mediated by quorum sensing. *P.aeruginosa* possesses two quorum-sensing two quorum sensing systems, *las* and *rhl* that facilitate cell – cell communication by signaling molecules production termed autoinducers to target specific receptors for activation (O'Loughlin *et al*.,2013; Streeter & Katouli, 2016).Localized infections**:** These may occur in the eye, most commonly after injury or surgical procedures (causing keratitis and endophthalmitis following trauma). Otitis media is the most common type of ear infection while the bacterial cause of ear infections is the most important in humans. *P.aeruginosa* is the most common pathogen causing ear infection in pediatrics, the bacterium is often found in mild otitis externa in swimmers, it may cause invasive and necrotizing (malignant) otitis externa, particularly in older adult diabetic or trauma patients (Wei *et al*., 2014; Ayub *et al*., 2015). Urinary tract infection (UTI); Urinary tract infections by *P.aeruginosa* are associated with high mortality in hospitalized patients, whichincreases significantly in hospitalized patients who have been subjectedto catheterization, instrumentation, irrigating solutions, surgery, renaltransplantation or those with severe concomitant diseases such as, chronicrenal failure, advanced liver disease or diabetes mellitus (Elkhatib &Noreddin, 2014; Lamas Ferreiro *et al*., 2017).

Relatively, gastrointestinal tract infections ranging from mild to severe children diarrheal illness, infants necrotizing enterocolitis and neutropenic cancer patients. *P.aeruginosa* causing meningitis and brain abscesses, when introduced by lumbar puncture, trauma, neurosurgical procedures, or tumors of the head or neck (Brooks *et al.*,2013).

3.6.1.1 Quorum Sensing systems (QS)

Quorum sensing first discovered by Dr. peter Greenberg in 1994 in the bioluminescent bacterium *Vibrio fischeri* which consisted of two regulatory proteins, LuxI / LuxR. Lux1 protein works as an autoinducer synthase enzyme and

the second protein as a promoter binding protein (Lee & Zhang, 2015). All activities depend on the QS system, which depends on the production, secretion, and detection of the small diffusible signaling molecules, named Autoinducers (AIs) (Kalia &Purohit, 2011).

In this bacterium, the autoinducer is encoded by the *lasI* genes. Las enzyme catalyzes the transfer of an acyl group bound to acyl carrier protein (ACP) from fatty acid biosynthesis to S-adenosyl–L-methionine (SAM) (Rutherford and Bassler, 2012), whereas RhlI catalyzes sequential ordered mechanism with SAM by forming an amide bond between the amino group of homocysteine and the Acyl group of ACP before binding the acyl–ACP , then autoinducer is produced by the release of Methylthioadenosine .The acyl transfer reaction yields an acyl–SAM intermediate, which then undergoes lactonization to form the N-acyl–homoserine lactone (Li Z, 2012; Dong*et al*., 2017).

3.6.2 Multidrug Resistance of *P.aeruginosa* (MDR).

Traditionally, serious *P.aeruginosa* infections have not been eradicated by using single-drug therapy because successful treatment rate is low using such therapy. Moreover, the bacteria can develop a rapid resistance when single drugs used (Brooks *et al*.,2013).

Epidemiological studies have indicated that infections caused by MDR *P.aeruginosa* are associated with increases in morbidity, mortality, length of hospital stay and chronic care, need for surgical intervention, and overall cost of treating the infection. Even more problematic is the development of resistance during therapy, a complication that has been shown to double the length of hospitalization and the over all cost of patient care (Akingbade *et al*., 2012). *P.aeruginosa* can develop antibiotics resistance either through mutational processes or by the resistance genes acquisition on mobile genetic elements (*i.e*., plasmids). Both strategies of drug resistance can severely limit available options to treat serious infections (Streeter and Katouli, 2016).

Fluoroquinolones resistance.

The resistance of *P.aeruginosa* tofluoroquinolone has been linked only mutational changes in chromosomal genes characterize the fluoroquinolone targets (*gyrA* and *gyrB* and/or *parC* and *parE*)) and/or overexpression of multidrug efflux pumps (Mishra *et al*.,2016). Although plasmid-encoded DNA gyrase protection protein quinolones resistance (Qnr) and fluoroquinolone-modifying enzyme *AAC(6')Ib-cr* can develop a resistance to fluoroquinolone group of antibiotics among isolates of *Enterobacteriaceae*, these two mechanisms have not been identified between *P.aeruginosa* isolates yet (Yang *et al*., 2015).

***β*-Lactam groups resistance.**

The most common imported *β*-lactamases in *P.aeruginosa* isolates are penicillinases belonging to the molecular class A serine *β*- lactamases (TEM PSE and CARB).The most prevalent enzymes in this group belong to the PSE family (Zafer *et al*., 2014). The therapeutic impact of these penicillinases is relatively limited since they do not impact the clinical efficacy of extended-spectrum cephalosporins, carbapenems or monobactams (Tankhiwale, 2016).

Class A extended-spectrum *β*-lactamases have been found in isolates of *P.aeruginosa* and involve enzymes from the TEM, , CTX-M , VEB , SHV, PER, GES, and IBC families (Zafer *et al*., 2014). The D, OXA-type enzymes of extended-spectrum *β*-lactamases have also been described within *P.aeruginosa* isolates (Zafer *et al*., 2014).

The *P.aeruginosa* porin OprD is a substrate-specific porin that has been shown to facilitate the basic amino acid diffusion, small peptides with these basic amino acids, and carbapenems inside the cell (Poonsuk & Chuanchuen, 2014). Lack or reduced expression of OprD assists in resistance development of *P.aeruginosa* isolates to carbapenems (Ocampo-Sosa *etal*., 2012; Shaaban *et al*.,2017).

P.aeruginosa, a common cause of nosocomial infections is infamous for its resistance multiple drugs that are mainly attributed to the synergy between low outer membrane permeability and multidrug efflux systems expression, particularly the Resistance-Nodulation-Cell Division (RND) family mainly MexAB-OprM and MexXY-OprM that represent a principal resistance mechanism in *P.aeruginosa.* (Lau *et al*., 2014)

RND family (as well as the remaining superfamilies) is secondary active transporters (symporters, antiporters, and uniporters). They derive the required energy for compound extrusion by proton motive force. The primary active transporters of the ABC superfamily, utilize ATP hydrolysis for energy, (Poonsuk & Chuanchuen, 2014).

Aminoglycosides group resistance.

The resistance mechanism to aminoglycosides occurs through enzymatic inactivation of the antibiotic molecule by chemical modification. Many studies have uncovered many aspects related to molecular characterization, Prevalence, and clinical significance of aminoglycoside inactivating enzymes in *P.aeruginosa* (Streeter and Katouli, 2016). These enzymes are categorized into the following three families, depend on the chemical modification they mediate: **(i)** aminoglycoside phosphoryl-transferase enzymes phosphorylate the drug molecule by (*aph(3')-V*), **(ii)**aminoglycoside acetyl-transferase (AAC) enzymes acetylate the drug molecule by (*aac(6')-I, aac(6')-II*) and **(iii)** aminoglycoside nucleotidyltransferase (ANT) enzymes adenylate the drug molecule by (*ant(2'')-I*) (Teixeira *et al*.,2016).

Polymixins resistance.

Polymyxins have been reintroduced for antimicrobial therapy after many reports indicated an increase in resistance among G-ve bacteria. Some bacteria, such as *P.aeruginosa*, *Klebsiella pneumoniae,*and *A.baumannii*, developed their resistance mechanism to polymyxins through the acquired resistance (Hashemi *et al*., 2017). However, other bacterial species(such as *Proteus* spp., *Serratia* spp., and *Burkholderia* spp.) are naturally carried resistance to these drugs. Recently, many studies have reported cases of polymyxin resistance between clinical isolates carrying both intrinsic and acquired resistance.

This increased in resistance has been concerned an important issue due to the low number of currently available treatment choices (Hashemi *etal*., 2017).

MacNair *et al*.(2018) indicated that plasmid-borne colistin resistance mediated by *mcr-1* may contribute to the dissemination of pan-resistant G-ve bacteria, otherwise combination of colistin and clarithromycin showed an efficient therapy against *mcr-1*-positive *K. pneumoniae* in murine thigh and bacteremia infection models. These data suggested that the combination between colistin and other antibiotics can be effective against G+ve bacteria and presents a viable alternative therapy for highly drug-resistant G-ve pathogens expressing *mcr-1*.

3.7 Enterobacter spp

Enterobacter belongs to ESKAPE bacterial group, which includes most resistant bacterial isolates. Until now, there are 22 species belong to this genus. These species are found in the environment and many studies reported them as opportunistic pathogens caused by the plant, human and animal infections . (Davin-Regli *et al*.,2019). Also, their antimicrobial resistance has been extensively explored and these bacteria showed different resistance mechanisms via various local and global regulator genes and the modulation of different proteins expression including membrane transporters such as porins and efflux pumps or enzymes (β-lactamases, *etc*.),.

Many *Enterobacter* strains have carbapenemases and ESBLs, including VIM, Metallo-β-lactamase-1, OXA, and KPC (Castanheira *et al.*,2008) . Also, stable derepression of the AmpC β-lactamases can be expressed at high levels by a mutation in this bacterial group. These MDR strains shows resistant to almost all antimicrobial drugs, except tigecycline and colistin (Boucher *et al*., 2009).
The *Enterobacter aerogenes* and *E. cloacae* complex show a multidrug-resistant phenotype, which emerges a concern about a cascade regulation role in these clones (Davin-Regli *et al.*,2019).

References

Akingbade, O. A.;Balogun, S. A.;Ojo, D. A.;Afolabi, R. O.;Motayo, B. O.;Okerentugba, P. O. and Okonko, I. O. (2012). Plasmid profile analysis of multidrug-resistant Pseudomonas aeruginosa isolated from wound infections in South West, Nigeria. World ApplSci J, 20(6), 766-775.

Al-Jailawi, M.H.; Zedan,T.H. and Jassim,K.A. (2014). Multiplex-PCR assay for identification of Klebsiellapneumoniae. Int. J. Pharm. Sci. Rev. Res. 26(1): 112-117.

AL-Marjani, M. F. (2013). vanA in vancomycin-resistant *Enterococcus faecalis* isolated in Baghdad. African Journal of Microbiology Research, 7(2), 115-119.

Antony, A. (2011). Study of Biofilm forming capacity of pathogens involved in Chronic Rhinosinusitis. Master Thesis. Auckland University of Technology. Auckland.

Arias, C. A. and Murray, B. E. (2012). The rise of the *Enterococcus*: beyond vancomycin resistance. Nature Reviews Microbiology, 10(4), 266.

Arias, C. A.; Contreras, G. A. and Murray, B. E. (2010). Management of multidrug-resistant enterococcal infections. Clin microbiol infec , 16(6), 555-562.

Ayub, M.; Islam, A.;Moiz, A. and Fahad, M. (2015). Acute otitis media: identification of causative pathogens with antimicrobial comparative efficacy. J App Pharm, *7*(205), 2.

Berglund, C. T.; Ito, T.; Ma,X.X .;Ikeda,M.; Watanabe,S.; Soderquist,B. and Hiramatsu,K. (2009). Genetic diversity of methicillin-resistant *Staphylococcus aureus* carrying type IV SCCmec in Orebro County and the western region of Sweden. J. Antimicrob. Chemother. 63:32-41.

Boucher,H.W; Talbot,G.H.; Bradley,J.S.; Edwards,J.E.; Jr.; Gilbert,D.; Rice,L. B.; Scheld,M.; Spellberg,B. and Bartlett,J.(2009). Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America. Clin. Infect. Dis. 48:1 –12.

Brooks, G.F; Carroll, K.C; Butel, J.S.; Mores, S.A. &Mietzner,T.A.(2013). Jawetiz, Melnick&Adelberg's Medical Microbiology. 26thedition. McGraw-Hill. U.S.A. Pp:245-248.

Bush,K. (1988). Beta-Lactamase inhibitors from laboratory to clinic. Clin. Microbiol. Rev. 1(1):109–123.

Castanheira, M., Sader, H. S., Deshpande, L. M., Fritsche, T. R., and Jones, R. N. (2008). Antimicrobial activities of tigecycline and other broad-spectrum antimicrobials tested against serine carbapenemase- and metallo-beta-lactamase-producing Enterobacteriaceae: report from the SENTRY antimicrobial surveillance program. Antimicrob. Agents Chemother. 52, 570–573.

Chung, D. R.; Lee, H.; Park, M. H.; Jung, S. I.; Chang, H. H.; Kim, Y. S.; Ko, K.S.; Kang, C.I.; Peck, K.R. ; Song,J.H. & Shin, S. Y. (2012). Fecal carriage of serotype K1 *Klebsiellapneumoniae* ST23 strains closely related to liver abscess isolates in Koreans living in Korea. Eur Eur. J. Clin. Microbiol. Infect. Dis., *31*(4), 481-486.

Cowan,T. (2011). Biofilms and their management: From concept to clinical reality. J. Wound Care. 20(5): 222–226.

Daum,R.S. (2007).Skin and soft -tissue infections by methicillin –resistant*Staphylococcus aureus* . New England Journal of Medicine 357 (4): 380-390 .

Davin-Regli A, Lavigne J-P, Pagès J-M. (2019). *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin Microbiol Rev 32:e00002-19.

Dong, S. H.;Frane, N. D.; Christensen, Q. H.; Greenberg, E. P.;Nagarajan, R. and Nair, S. K. (2017). Molecular basis for the substrate specificity of quorum signal synthases. Proceedings of the National Academy of Sciences, 114(34), 9092-9097.

Doughari,H.J.; Ndakidemi,P.A.; Human,I.S. and Benade,S. (2011). The ecology, biology and pathogenesis of *Acinetobacter* spp.: an overview. Microbes Environ. 26(2): 101 –112.

Elkhatib, W. and Noreddin, A. (2014). *In vitro* antibiofilm efficacies of different antibiotic combinations with zinc sulfate against *Pseudomonas aeruginosa* recovered from hospitalized patients with urinary tract infection. Antibiotics, *3*(1), 64-84.

Erridge, C.;Moncayo-Nieto, O. L.; Morgan, R.; Young, M. and Poxton, I. R. (2007). *Acinetobacter baumannii* lipopolysaccharides are potent stimulators of human monocyte activation via Toll-like receptor 4 signalling. Journal of medical microbiology, 56(2), 165-171.

Evans, B.A. and Amyes, S.G. (2014). OXA β-lactamases. Clin. Microbiol. Rev. 27(2):241–263.

Fisher, J. F.;Meroueh, S. O. and Mobashery, S. (2005). Bacterial resistance to β-lactam antibiotics: compelling opportunism, compelling opportunity. *Chemical reviews*, *105*(2), 395-424.

Forbes, B. A.;Sahm, D. F. and Weissfeld, A. S. (2007). *Study guide for Bailey & Scott's diagnostic microbiology*. USA: Mosby.

Gao, M.; Glenn, A. E.;Blacutt, A. A. and Gold, S. E. (2017). Fungal lactamases: their occurrence and function. Front Microbiol 8: 1775.

Goering, R.;Dockrell, H.; Zuckerman, M.;Roitt, I. andChiodini, P. L. (2012). *Mims' medical microbiology*. Elsevier Health Sciences.

Harvey, R.A.; Cornelissen,C.N. & Fisher, B.D. (2013). Lippincott'sIllustrated Reviews: Microbiology.3rd edition.Lippincott Williams &Wilkins. Wolters Kluwer Company, Philadelphia, USA. Pp:137-139.

Hashemi, M. M.;Rovig, J.;Weber, S.; Hilton, B.;Forouzan, M. M. and Savage, P. B. (2017). Susceptibility of colistin-resistant, Gram-negative bacteria to antimicrobial peptides and ceragenins. Antimicrobial agents and chemotherapy, 61(8), e00292-17.

Hassett, D. J. (1996). Anaerobic production of alginate *by Pseudomonas aeruginosa*: alginate restricts diffusion of oxygen. Journal of bacteriology, *178*(24), 7322-7325.

Hoffman,L.R.; D'Argenio,D.A.; MacCoss,M.J.; Zhang,Z.; Jones,R.A. and Miller,S.I.(2005). Aminoglycoside antibiotics induce bacterial biofilm formation. Nature. 436(7054): 1171 –1175.

Hurlow,J.; Couch,K.; Laforet, K.; Bolton, L.; Metcalf, D. and Bowler, P. (2015). Clinical biofilms: a challenging frontier in wound care. Adv.Wound Care. 4(5):295–301.

Ivanov, D.V.andBudanov, S.V. (2006). Ciprofloxacin and antibacterial therapy of respiratory tract infections .Antibiot. Khimioter. (in Russian) 51 (5): 298–37.

Iwase, T.; Uehara,Y.; Hitomi,S.; Akiko,T.; Hiromi,S.; Koji,T.; Agata,T. and Yoshimitsu,M. (2010). "*Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization". Nature 465 (7296): 346–9.

Jamal, M., Ahmad, W., Andleeb, S., Jalil, F., Imran, M., Nawaz, M.A., Hussain, T., Ali, M., Rafiq, M. and Kamil, M.A. (2018) Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81(1), pp.7-11.

Jagessar, R.C. and Alleyne,R. (2011). Antimicrobial potency of the aqueous extract of leaves of Terminaliacatappa. Acad. Res. Int. 1(3):362-371.

Rafiq, M.and Kamil, M. A. (2018). Bacterial biofilm and associated infections. Journal of theChinese Medical Association, *81*(1), 7-11.

Kadhim,A.A. , Salman ,J.A.S. , Haider,A.J. (2018). Antibacterial and Anti virulence Factors activity of ZnO nanoparticles Biosynthesized byLactococcuslactis ssp. Lactis. Indian Journal of Public Health Research and Development., 9(12):1228-1234.

Kalia, V. C. and Purohit, H. J. (2011). Quenching the quorum sensing system: potential antibacterial drug targets. *Critical reviews in microbiology*, *37*(2), 121-140.

Kareem, SM; Aljubori, SS and Ali, MR (2020). Novel determination of spa gene diversity and its molecular typing among Staphylococcus aureus Iraqi isolates obtained from different clinical samples. New Microbes and New Infections, 34: 100653

Kamolvit,W.; Sidjabat, H.E. and Paterson, D.L. (2015). Molecular epidemiology and mechanisms of carbapenem resistance of Acinetobacter spp. in Asia and Oceania. Microb. Drug Resist. 21(4):424–434.

Kanafani, A.Z. and Kanj, S.S.(2014). Ministry of Health, Kingdome of Saudi Arabia.

Kanafani, Z.A. and Corey GR (2009). "Ceftaroline: a cephalosporin with expanded Gram-positive activity". Future Microbiology 4: 25–33.

Kerr, J. R.; Taylor, G. W.;Rutman, A.;Høiby, N.; Cole, P. J. and Wilson, R. (1999). *Pseudomonas aeruginosa* pyocyanin and 1-hydroxyphenazine inhibit fungal growth. Journal of clinical pathology, 52(5), 385-387.

Kim,J.W.; Heo, S.T.; Jin, J.S.; Choi,C.H.; Lee,Y. C.; Jeong, Y.G., Kim, S.J. and Lee, J.C. (2008). Characterization of *Acinetobacter baumannii* carrying blaOXA-23, blaPER-1 and armA in a Korean hospital. Clin. Microbiol. Infect. 14(7): 716-718.

Kurcik-Trajkovska, B. (2009). *Acinetobacter* spp.-A serious enemy threatening hospitals worldwide. Macedonian Journal of Medical Sciences, *2*(2), 157-162.

Lamas Ferreiro, J. L., Álvarez Otero, J., González González, L., Novoa Lamazares, L., Arca Blanco, A., Bermúdez Sanjurjo, J. R., Rodríguez Conde, I., Fernández Soneira, M., and de la Fuente Aguado, J. (2017). *Pseudomonas aeruginosa* urinary tract infections in hospitalized patients: Mortality and prognostic factors. PloS one, 12(5): e0178178.

Lau, C. H. F.; Hughes, D. and Poole, K. (2014). MexY-promoted aminoglycoside resistance in *Pseudomonas aeruginosa*: involvement of a putative proximal binding pocket in aminoglycoside recognition. MBio, *5*(2), e01068-14.

Lee, J,H.(2003). Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains.Appl Environ Microbio. 69(11):6489-94.

Lee, J. and Zhang, L. (2015). The hierarchy quorum sensing network in Pseudomonas aeruginosa. Protein & cell, *6*(1), 26-41.

Lipsky, B.A.; Tabak, Y.P.; Johannes, R.S.; Vo L, Hyde, L and Weigelt JA, (2010). "Skin and soft tissue infections in hospitalized patients with diabetes: culture isolates and risk factors associated. with mortality, length of stay and cost. US National Library of Medicine National Institutes of Health. 53(5):914-23.

Livermore, D. M. and Woodford, N. (2006). The β-lactamase threat in *Enterobacteriaceae, Pseudomonas* and *Acinetobacter*. Trends in microbiology, *14*(9), 413-420.

LiZ, N.S.K. (2012). Quorum Sensing: how bacteria can coordinateactivity and synchronize their response to external signal? J. Protien Sci. 21:1403-1417.

Luna,C.M. and Aruj,P.K. (2007). Nosocomial *Acinetobacter pneumonia*. Respirology. 12(6): 787–791.

Lundberg, U.; Senn, B. M.; Schüler, W.; Meinke, A. and Hanner, M. (2013). Identification and characterization of antigens as vaccine candidates against *Klebsiella pneumoniae*. Hum. Vaccin. Immunother. 9(3), 497–505.

Luo,L-m.; Wu,L-j.; Xiao,Y-l.; Zhao,D.; Chen,Z-x.; Kang,M.; Zhang,Q. and Xie, Y. (2015). Enhancing pili assembly and biofilm formation in *Acinetobacter baumannii* ATCC19606 using non-native acyl-homoserine lactones. BMC Microbiol. 15:62.

MacDougall C. and Chambers H.F. (2011): Protein Synthesis Inhibitors and Miscellaneous Antibacterial Agents. Chapter 55. In: *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, Twelfth Edition. Brunton LL, Chabner BA, Knollmann BC (Editors). McGraw-Hill.

MacNair, C. R.; Stokes, J. M.;Carfrae, L. A.;Fiebig-Comyn, A. A.;Coombes, B. K.;Mulvey, M. R. and Brown, E. D. (2018). Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. Nature communications, 9(1), 458.

Madigan, M. and Martinko, J. (2005). Brock Biology of Microorganisms (11 ed.). Prentice-Hall. ISBN 0-13-144329-1.

Magill,S.S.; Edwards,J.R.; Bamberg,W.; Beldavs,Z.G.; Dumyati,G.; Kainer, M. A.; Lynfield,R.; Maloney,M.; McAllister-Hollod,L.; Nadle,J.; Ray,S.M.; Thompson,D.L.; Wilson,L.E. and Fridkin,S.K. (2014). Multistate point-prevalence survey of healthcare-associated infections. N. Engl. J. Med. 370(13):1198-1208.

Marin, M. and Gudiol, F.(2003). Antibioticosbetalactamicos. Enferm. Infecc. Microbiol. Clin. 21: 42-55.

Martinez, J. L. (2014). General principles of antibiotic resistance in bacteria. Drug Discovery Today: Technologies, 11, 33-39.

Mateja, M.; Pate, M.;Kušar, D.;Dermota, U.;Avberšek, J.;Papić, B. and Zdovc, I. (2019). Antimicrobial Resistance and Virulence Genes in *Enterococcus faecium* and *Enterococcus faecalis* from Humans and Retail Red Meat. BioMed research international. (11):1-12.

Matthew, J.T.(2012). Synthesis of the accessory gene regulator autoinducing peptide in *Staphylococcus aureus*. University of Iowa.

Mishra, S.; Kumar, M.; Vyas, A. &Dalal, A.S. (2016)). Antibiogram ofClinical Isolates of *Pseudomonas aeruginosa* against SelectedQuinolones. *Int. J. Curr. Microbiol. App. Sci*. 5(6):771-777.

Mugnier, P.D.; Bindayna, K.M.; Poirel, L. and Nordmann, P. (2009). Diversity of plasmid-mediated carbapenem-hydrolysingoxacillinases among carbapenemresistant *Acinetobacter baumannii* isolates from Kingdom of Bahrain. J. Antimicrob. Chemother. 63:1071 –1073.

Nathwani, D.; Morgan, M.; Masterton, R.G.; Dryden, M.; Cookson, B.D.; French, G.; and Lewis, D.(2008). Guidelines for UK practice for the diagnosis and management of methicillin-resistant *Staphylococcus aureus*(MRSA) infections presenting in the community. J. Antimicrob. Chemother. 61:976–994.

Nordmann, P.; Cuzon, G. and Naas, T. (2009). "The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria". Lancet Infect. Dis. 9 (4): 228–236.

O'Loughlin, C.T.; Miller, L.C.; Drescher, K.; Semmelhack, M.F. &Bassler, B.L. (2013). A quorum-sensing inhibitor block *Pseudomonas aeruginosa* virulence and biofilm formation. Proc. Natl. Acad. Sci. USA. 29;110(44):17981-6

Ocampo-Sosa, A. A.; Cabot, G.; Rodríguez, C.; Roman, E.;Tubau, F.;Macia, M. D.and Domínguez, M. A. (2012). Alterations of OprD in carbapenem-intermediate and-susceptible strains of *Pseudomonas aeruginosa* isolated from patients with bacteremia in a Spanish multicenter study. Antimicrobial agents and chemotherapy, *56*(4), 1703-1713.

Otto, M. (2013). Staphylococcal infections: mechanisms of biofilm maturation and detachment as critical determinants of pathogenicity.Annual review of medicine, 64, 175-188.

Parsek, M.R. and Singh, P.K. (2003). Bacterial biofilms: an emerging link to disease pathogenesis. Annu. Rev. Microbiol. 57:677-701.

Papenfort, K. and Bassler, B. L. (2016). Quorum sensing signal-response systems in Gram-negative bacteria. Nature reviews. Microbiology, 14(9), 576–588.

Peleg, A.Y.; Seifert, H. and Paterson, D.L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. Clin. Microbiol. Rev. 21(3): 538–582.

Perez, F.; Hujer, A.M.; Hujer, K.M.; Decker, B.K.; Rather, P.N. and Bonomo, R.A. (2007). Global challenge of multidrug-resistant *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 51(10): 3471 –3484.

Podschun, R.; Fisher, A. and Ullmann, U.(2000). Characterization of *Klebsiella terrigena* strains from humans: Hemagglutinins, serum resistance, siderophore synthesis and serotypes. Epidemiol. Infect., 125:71 -78.

Poole, K. (2004). Resistance to β-lactam antibiotics. *Cellular and Molecular Life Sciences CMLS*, *61*(17), 2200-2223.

Poonsuk, K. and Chuanchuen, R. (2014). Detection of the mex efflux pumps in *Pseudomonas aeruginosa* by using a combined resistance-phenotypic markers and multiplex RT-PCR. Open Journal of Medical Microbiology, 4(3), 153.pp. 1–12, 2009.

Quale, JM.; Landman, D.; Bradford, PA.; Visalli, M.; Ravishankar, J.; Flores, C.; Mayorga, D.; Vangala, K. and Adedeji, A. (2002). Molecular epidemiology of a citywide outbreak of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* infections. Clin. Infect. Dis. 35(7): 834-841.

Rafiq, M.and Kamil, M. A. (2018). Bacterial biofilm and associated infections. Journal of the Chinese Medical Association, 81(1), 7-11

Rahimian, J.; Wilson, T.; Oram, V. and Holzman, R.S.(2004). Pyogenic liver abscess: recent trends in etiology and mortality. Clin. Infect. Dis. 39:1654-1659.

Ramirez, M. S. and Tolmasky, M. E. (2010) Aminoglycoside modifying enzymes. Drug Resistance Update 13, 151-171.

Ran, H.;Hassett, D. J. and Lau, G. W. (2003). Human targets of *Pseudomonas aeruginosa* pyocyanin. Proceedings of the National Academy of Sciences, 100(24), 14315-14320.

Raygada, J.L .and Levine, D.P. (2009). "Managing CA-MRSA Infections: Current and Emerging Options". Infections in Medicine 26 (2).

Restrepo,M.I.; Peterson, J.; Fernandez, J.F.; Qin, Z.; Fisher, A.C. and Nicholson, S.C. (2013). Comparison of the Bacterial Etiology of Early-Onset and Late-Onset Ventilator-Associated Pneumonia in Subjects Enrolled in 2 Large Clinical Studies. Respir. Care. 58(7):1220–1225.

Rice,S.A.; Wuertz,S. and Kjelleberg,S.(2016). Next-generation studies of microbial biofilm communities. Microb. Biotechnol. 9(5): 677–680.

Rutherford, S. T. and Bassler, B. L. (2012). Bacterial quorum sensing: its role in virulence and possibilities for its control. Cold Spring Harbor perspectives in medicine, *2*(11), a012427.

Saeed, N.K.; Kambal, A.M. and El-Khizzi, N.A. (2010). Antimicrobial-resistant bacteria in a general intensive care unit in Saudi Arabia. Saudi Med. J. 31(12): 1341 -1349.

Steinberg N. and Kolodkin-Gal I. (2015) The matrix reloaded: Probing the extracellular matrix synchronizes bacterial communities. J. Bacteriol. 197:2092–2103.

Shaaban, M.; Al-Qahtani, A.; Al-Ahdal, M. and Barwa, R. (2017). Molecular characterization of resistance mechanisms in *Pseudomonas aeruginosa* isolates resistant to carbapenems. The Journal of Infection in Developing Countries, *11*(12), 935-943.

Streeter, K. and Katouli, M. (2016). *Pseudomonas aeruginosa*: A reviewof their Pathogenesis and Prevalence in Clinical Settings and theEnvironment. J. Infect. Epidemiol. Med. 2(1): 25-32.

Struve, C.; Bojer, M. and Krogfelt, K. A. (2008). Characterization of *Klebsiella pneumoniae* type 1 fimbriae by detection of phase variation during colonization and infection and impact on virulence. Infect. Immun. 79(9):4055-4065.

Tankhiwale, S. (2016). Beta-lactamases in P. aeruginosa: a threat to clinical therapeutics. Current Pediatric Research. 20: 253-257

Van Melderen, L. and DeBast, M. S. (2009). Bacterial toxin–antitoxin systems: more than selfish entities?. PLoS genetics, *5*(3), e1000437.

Vashist, J.; Tiwari, V.; Das, R.; Kapil, A. and Rajeswari, M.R. (2011). Analysis of penicillin-binding proteins (PBPs) in carbapenem-resistant *Acinetobacter baumannii*. Indian J. Med. Res. 133(3): 332- 338.

Vasudevan, R. (2014). Biofilms: microbial cities of scientific significance. J Microbiol Exp, 1(3), 00014.

Villegas, M.V. and Hartstein, A.I. (2003). *Acinetobacter* outbreaks, 1977–2000. Infect. Control Hosp. Epidemiol. 24(4):284-295.

Walsh,T.R.; Toleman, M. A.; Poirel, L. and Nordmann, P. (2005). Metallo-beta-lactamases: the quiet before the storm?.Clin. Microbiol. Rev. 18(2): 306-325.

Walsh,T.R.; Weeks,J.; Livermore,D.M. and Toleman,M.A. (2011). "Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study". Lancet Infect. Dis. 11 (5): 355–362.

Wang, X. and Wood, T. K. (2011). Toxin-antitoxin systems influence biofilm and persister cell formation and the general stress response. Appl. Environ. Microbiol., *77*(16), 5577-5583.

Wei, C.; Zhu, M.; Petroll, W.M. & Robertson, D.M. (2014).*Pseudomonas aeruginosa* infectious keratitis in a high oxygen transmit-ssible rigid contact lens rabbit model. J. Invest. Ophthalmol. Vis. Sci.55:5890–5899.

Wen, Y.;Behiels, E. and Devreese, B. (2014). Toxin–Antitoxin systems: their role in persistence, biofilm formation, and pathogenicity. Pathogens and disease, *70*(3), 240-249.

Whitman, T.J.; Qasba, S.S.; Timpone, J.G.; Babel, B.S.; Kasper, M.R.; English, J.F.; Sanders, J.W.; Hujer, K.M.; Hujer, A.M. and Endimiani, A. (2008). Occupational transmission of *Acinetobacter baumannii* from a United States

serviceman wounded in Iraq to a health care worker. Clin. Infect. Dis. 47: 439–443.

Wozniak, R. A. and Waldor, M. K. (2009). A toxin–antitoxin system promotes the maintenance of an integrative conjugative element. PLoS genetics, *5*(3), e1000439.

Wright, D. H.; Gunderson, B. W.; Hovde, L. B.; Ross, G. H.; Ibrahim, K. H. and Rotschafer, J. C. (2002). Comparative pharmacodynamics of three newer fluoroquinolones versus six strains of staphylococci in an *in vitro* model under aerobic and anaerobic conditions. Antimicrobial agents and chemotherapy, 46(5), 1561-1563.

Yamaguchi, Y.; Park, J. H.and Inouye, M. (2011). Toxin-antitoxin systems in bacteria and archaea. Annual review of genetics, *45*, 61-79.

Yan, Miling.; Pamp.; Sünje J.; Fukuyama, Julia; Hwang, Peter H.; Cho, Do-Yeon.; Holmes, Susan.; Relman. and David, A. (2013). "Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage". Cell Host & Microbe 14 (6): 631–640.

Yang, X., Xing, B., Liang, C., Ye, Z., & Zhang, Y. (2015). Prevalence and fluoroquinolone resistance of *Pseudomonas aeruginosa* in a hospital of South China. International Journal of Clinical and Experimental Medicine, 8(1), 1386.

Zafer, M. M.; Al-Agamy, M. H.; El-Mahallawy, H. A.; Amin, M. A.;and Ashour, M. S. E. D. (2014). Antimicrobial resistance pattern and their beta-lactamase encoding genes among *Pseudomonas aeruginosa* strains isolated from cancer patients. BioMed research international, Volume 2014, Article ID 101635, 8 pages.

Zowawi, H.M.; Sartor,A.L.; Sidjabat,H.E.; Balkhy,H.H.; Walsh, T.R.; Al Johani, S.M.; Aljindan, R.Y.; Alfaresi, M.; Ibrahim, E.; Al-Jardani, A.; Al Salman, J.; Dashti, A.A.; Johani, K. and Paterson, D.L. (2015). Molecular Epidemiology of Carbapenem-Resistant *Acinetobacterbaumannii* Isolates in the Gulf Cooperation Council States. Dominance of OXA-23-type producers. J. Clin. Microbiol. 53(3): 896–903.