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Review on β-lactams resistance among *Klebsiella pneumoniae* and *Escherichia Coli* clinical isolates

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ABSTRACT

Escherichia coli and *Klebsiella pneumoniae* are two important members of *Enterobacteriaceae* family. They are involved in sever community and hospital acquired infections such as urinary tract infections (UTIs), gastroenteritis, and pneumonia. Some of these diseases are associated with high mortality rates if not treated properly, so it is important to combat them with highly effective antibiotics. The most commonly used antimicrobial agents are beta-lactams such as penicillins, cephalosporins and other non-beta lactams such as aminoglycosides and quinolones. Extensive use of antimicrobials and disinfectants has promoted the rapid development of bacterial resistance. This bacterial resistance becomes a global health problem especially in developing countries. The increased rate of resistance towards different classes of antibiotics limits the treatment options for such infections. The antibacterial activity of some antimicrobial agents can be enhanced by the addition of new β-lactamase inhibitors. Further *in vivo* investigation is needed to confirm their therapeutic efficacy against local isolates.

Keywords: *Enterobacteriaceae, beta-lactamase inhibitors; antimicrobial activity, multi-drug resistance*

1. Family *Enterobacteriaceae*

Enterobacteriaceae is the most well-known Gram-negative family, that includes more than 30 genera and 100 species of the most familiar pathogens such as *Klebsiella, Escherichia coli, Proteus, Enterobacter and Citrobacter* **(Podschun and Ullmann, 1998)**.
Some of these pathogens are normal part of the gut flora, while others are naturally present in water and soil **(Williams *et* al., 2010)**. Most species are considered pathogenic to immunocompromised people as they are a common cause of urinary tract infections (UTIs), and diarrhea. In addition, These pathogens can spread to the bloodstream resulting in life-threatening complications **(Christensen, 2021)**.
Members of *Enterobacteriaceae* family are not spore-forming bacilli, typically measure 1–5 µm in length. They appear as medium to large-sized grey colonies on blood agar. Also, they are facultative anaerobes that could ferment sugars into lactic acid and other different end products. The majority of this family are most metabolically active and can grow well at 25-35°C. **(Perez and Van Duin, 2013)**.
Enterobacteriaceae members have peritrichous; type I fimbriae which is responsible for the adhesion of bacterial cells to their hosts. Some *enterobacteria* can produce endotoxins that reside in the cell wall and released when the cell dies. Moreover, other members can produce endotoxins when released into the bloodstream may cause a systemic inflammatory and vasodilatory response. The most severe form of this inflammatory response is known as endotoxic shock, which can be rapidly fatal **(Guh *et al*., 2015)**.

1.1 The Genus *Klebsiella*

Klebsiella is one of the most important members of the family *Enterobacteriaceae*; it was named after the German bacteriologist Edwin Klebs (1834-1913). *Klebsiella* has a prominent polysaccharide capsule

which encases the whole cell surface and accounts for the large appearance of organism on Gram stain; it also acts against many host defense mechanisms **(Dworkin *et.*, 2006).**

1.1.1 Taxonomic structure

The Genus *Klebsiella* was divided into seven species which are: *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella orithinolytica*, *klebsiella ozaenae*, *klebsiella terrigena*, *klebsiella rhinoscleromatis* and *Klebsiella planticola.* The most medically important species of this genus is *K. pneumoniae* **(Podschun and Ullmann, 1998).**

1.1.2. Epidemiology

Klebsiella probably have two common habitats, one being the environment where they are found in soil, sewage and on surface of plants, the other is the mucosal surface of mammals such as humans, horses and swine. *K. pneumoniae* is present as saprophyte in the nasopharynx and the intestinal tract. Carrier rates differ from study to study. The detection rate in stool samples ranges from 5 to 38%, while rates in the nasopharynx range from 1 to 6%. *Klebsiella spp*. is rarely found on the human skin because Gram-negative bacteria don't find good growth conditions there **(Brown and Seidler, 1973).**

1.1.3. Pathogenesis

Klebsiella is an opportunistic pathogen and can cause severe diseases such as urinary tract infections (UTI), pneumonia and septicemia. Typically, *Klebsiella* infections are nosocomial. The hospitalized immunocompromised patient is the main target of these bacteria. *Klebsiella* spp ranks second (behind *Escherichia coli*) as a cause of nosocomial Gram-negative bacteremia **(Podschun and Ullmann, 1998).**
Klebsiella have the ability to spread extensively among patients, leading to nosocomial outbreaks, especially in neonatal units and intensive care units (ICUs). *K. pneumoniae* can spread among different hospitals and even across country borders through the transfer of infected patients. The mortality rates can exceed 50% in vulnerable patients even when treated with appropriate antibacterial drugs **(Munoz-Price *et al.*, 2013).**
The infections with *Klebsiella* are often linked to some host related factors such as the use of catheters, indicating that bacterial adhesion and biofilm formation are important for the establishment of infection. Other host features predisposing to *Klebsiella* nosocomial infection include extremes of age, diabetes mellitus, chronic alcoholism, chronic pulmonary diseases, cardiac diseases, and renal diseases **(Munoz-Price *et al.*, 2013).**

1.2. The Genus *Escherichia*

Escherichia coli is one of the most important model organisms, discovered in 1885 by Theodor Escherich, a German bacteriologist. Also, it is one of the first organisms to have its genome sequenced. The complete genome sequence of *E. coli K12* was published in 1997 **(Dabke *et al.*, 2014)**.
On one hand, there are the commensal *E. coli* strains that live in the human gut in symbiosis with their host, helping to prevent colonization of pathogenic bacteria through contaminated food or water and can benefit their hosts by producing vitamin K2. On the other hand, there are the pathogenic *E. coli* strains that can cause severe diseases like food poisoning, bloody diarrhea, meningitis, and UTIs **(Koyama *et al.*, 2010)**.

1.2.1. Taxonomic Structure:

E. coli strains can be divided into three different pathotypes which are, the harmless commensal strains, the intestinal pathogenic strains including enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrageic *E. coli* (EHEC) causing diarrhea, and finally the extraintestinal pathogenic strains including sepsis associated *E. coli* (SEPEC), meningitis *E. coli* causing newborn meningitis, and uropathogenic *E. coli* (UPEC) causing UTI and pyelonephritis **(Kaper *et al.*, 2004)**.
E. coli can also be classified into hundreds of strains on the basis of different serotypes. *E. coli* O157:H7, for example, is a well-studied strain, the "O" in the name refers to the cell wall antigen (somatic) and the "H" refers to the flagella antigen **(Dabke *et al.*, 2014)**.

1.2.2. Epidemiology:

E. coli infection can be transmitted to humans through feco-oral route, or consumption of contaminated food, such as raw or undercooked ground meat products like undercooked hamburgers, dried cured salami, also raw milk and dairy products for example yogurt, and unpasteurized cheese. Cross-contamination during food preparation like beef and other meat products, contaminated surfaces and kitchen utensils, will also lead to infection **(Sargeant *et al.*, 2004)**.
Contamination may also be due to contact with domestic or wild animals feces, as a consequences people living in rural areas may be at greater risk of infection due to greater exposure to livestock **(Heaton and Jones, 2008)**.
Waterborne transmission has also been reported from both contaminated drinking-water, and from unhygienic food preparation due to irrigation of crops with contaminated water or direct consumption of

sewage-contaminated water. In addition, person-to-person contact is an important mode of *E. coli* infection transmission, where an asymptomatic carrier with no clinical signs of disease is capable of infecting others **(Denny *et al*., 2008)**.
Therefore, the prevention of infection requires control measures at all stages of the food chain, from agricultural production to processing, manufacturing and preparation of foods in both commercial establishments and household kitchens **(Denny *et al*., 2008)**.

1.2.3. Pathogenesis

Some *E. coli* strains are capable of infecting humans in addition to animals like dogs, goats, horses and sheep. *E. coli* O157:H7 is an important infective strain that can cause food poisoning, belongs to enterohemorrhagic strains which produce shiga-like toxin the main virulence factor of *E. coli* **(Pallett and Hand, 2010)**.
Shiga-like toxin can target an organ such as kidneys, binds to receptors on the cell membrane, then enters the cells and prevents them from producing their essential proteins by inactivating 60S ribosomal subunits, blocking mRNA translation, as a result cells lose the ability to maintain their functions and die through either apoptosis or necrosis **(Chase-Topping *et al*., 2008).**
About 80% to 90% of urinary tract infections are caused by *E. coli* which often gains access to the urinary tract via stool. UTIs can cause a wide range of symptoms, including bladder fullness, burning urination, pelvic pain and foul-smelling, cloudy urine. Unfortunately, infections that spread all the way up to the kidneys can be particularly serious. Such infections can be found in both adult males and females, and some infants can be infected as well **(Mohawk and O'Brien, 2011)**.
Escherichia coli represents the most common cultivable, gram-negative bacteria. *E. coli* are divided into four phylogenic groups (A, B1, B2, and D) according to the acquisition of virulence factors. Phylogroups A and B1 are normally not pathogenic, whereas phylogroups B2 and D are involved in intestinal and extra-intestinal diseases. Interestingly, some *E. coli* strains from phylogroup B2 are associated with Crohn's disease; a chronic inflammatory bowel disease known to be a risk factor for colorectal cancer **(Collins *et al*., 2011)**.
The most important symptom of *E. coli* infection is acute and severe diarrhea either bloody or not. Other symptoms include abdominal pain, vomiting, loss of appetite, fever, and kidney failure. Symptoms usually disappear on their own in one to three days with no treatment required **(Pallett and Hand, 2010)**.

2. Antimicrobial therapy for infections caused by *K. pneumoniae* and *E. coli*

Antimicrobial agents that are generally used for treating *E. coli* infections are β-lactam antibiotics, quinolones, and aminoglycosides. They are used as monotherapy or even as a combination. Patients who suffer from severe illness, a combination treatment course of aminoglycoside in addition to β-lactams including extended-spectrum cephalosporin is recommended **(Martinez-Medina and Garcia-Gil, 2014)**.
In most cases of *E. coli* infections, patients will recover within a couple of days without need of antibiotics. However, the aim of treatment is to avoid dehydration resulting from diarrhea as electrolyte balance and fluid levels are essential to prevent the development of hemolytic-uremic syndrome **(Kotra *et al*., 2002)**.
Some antimicrobials are often used to treat hospital and community infections due to E. coli by effectively inhibiting its growth. Examples for these agents are fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, and β-lactams **(Pitout, 2012).**

2.1. β-lactam antimicrobial agents:

β-lactam drugs are the most popular antibiotic class for treating bacterial infections. The first discovered β-lactam drug was benzyl-penicillin in 1928 and its first clinical use was in 1940 **(Papp-Wallace, 2019)**. The success of penicillin led to the development of other different β-lactam antibiotics include penicillin derivatives (penams), cephalosporins (cephems), carbapenems and monobactams **(Mortazavi-Tabatabaei *et al*., 2019).**
β-lactam drugs contain a β-lactam ring in their chemical structure which is essential for their activity. The β-lactam groups differ from each other by additional rings other than the β-lactam ring for instance; Double ring structure for carbapenem, thiazolidine ring for penicillin, cephem nucleus for cephalosporin, none for monobactam **(Lima *et* al., 2020).**
Earlier β-lactam antibiotics were active mainly against Gram-positive bacteria, while the discovery of broad spectrum β-lactam antibiotics which are active against Gram-negative organisms has increased their usefulness **(Thompson *et al*., 2010)**.

2.2. Mode of action of β-lactams

Bacterial cell wall consists of peptidoglycan layer that provides mechanical stability and responsible for cell wall structural integrity and rigidity. This layer is composed of glycan chains made of N-acetylglucosamine and N-acetylmuramic acid subunits **(Drawz and Bonomo, 2010)**.
β-lactams are bactericidal agents, they act by inhibiting the final step in peptidoglycan synthesis by inhibiting the transpeptidase involved in cross-linking peptides to form peptidoglycan. The targets for their action are

transpeptides known as penicillin-binding proteins (PBPs), the structure similarity of β-lactam antibiotics which are analogues of D-alanyl-D-alanine, the terminal amino acid of N-acetylmuramic acid/N-acetylglucosamine (NAM/NAG) facilitates their binding to the active site of penicillin-binding proteins. This binding, in turn, interrupts the terminal transpeptidation process and induces loss of viability and lysis of bacterial cell **(Livermore, 1996 and Darmanin, 2006)**.

2.3. Classical β-lactam antibiotics

2.3.1. Penicillins

Penicillins are the oldest and one of the most used groups of antibiotics at present. All penicillins have the same basic structure, they are considered as N-acyl substitution of 6-amino penicillanic acid (6APA), which is a thiazolidine ring attached to β-lactam ring **(Livermore, 1996)**.

They can be divided into two groups, namely natural and semisynthetic penicillins. Natural penicillins are produced from the fermentation of the *Penicillium chrysogenum* fungi. While the semisynthetic penicillins are prepared from (+)-6-aminopenicillanic acid **(Dowell *et al*., 1998)**.

The antibacterial activity spectrum varies with each class of penicillin family. The naturally occurring penicillins are generally effective against Gram-positive bacteria; the aminopenicillins are effective against Gram-positive and some Gram-negative bacteria; the carboxy and uriedopenicillins against Gram-positive and Gram-negative organisms, including pseudomonas; and the antistaphylococcal penicillins against staphylococcus and streptococcus **(Drawz and Bonomo, 2010)**.

Penicillins are used to treat many infections like skin infections, upper and lower respiratory tract infections, urinary tract infections and endocarditis **(Livermore, 1996)**.

2.3.2. Cephalosporins

The first agent was cephalothin that was discovered in 1964, the cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA) was derived from cephalosporin C, which is an analogue to the penicillin nucleus and consists of a β-lactam ring fused to a dihydrothiazine ring **(Malik and Figueras, 2019).**

Modification of the 7-ACA side chains provides different generations of antibiotics that are classified into five groups according to their sequence of discoveries and their spectrum of coverage against Gram-positive and Gram-negative bacteria. Cephalosporins have low toxicity and are generally safe. The most common side effects of cephalosporins are nausea, vomiting, abdominal pain, and lack of appetite **(Loder *et al*., 1961)**.

First generation cephalosporins include cefazolin, cefadroxil and cephalexin. They are moderate spectrum agents active against *E. coli, K. pneumoniae* and *Proteus mirabilis* but have no activity against *Pseudomonas* and methicillin-resistant *Staphylococci* **(Loder *et al*., 1961)**.

Second generation cephalosporins for example cefuroxime and cefaclor have greater activity against Gram-negative organisms and show more resistance to β-lactamase **(Ozbek and Otuk, 2010)**.

Third generation cephalosporins are active mainly against *Enterobacteriaceae* family, *Neisseria spp.,* and *H. influenza.* They have less coverage against Gram-positive bacteria. Members of this generation include ceftriaxone, cefotaxime, and ceftazidime. These antibiotics are more stable to β-lactamases than second generation cephalosporins and capable of passing through the outer cell membrane of Gram-negative bacteria **(Ferech *et al*., 2006)**.

Fourth generation cephalosporins for instance, cefepime and cefpirome are extended spectrum antibiotics having greater resistance to β-lactamases. They possess similar coverage as third generation cephalosporins but with additional coverage against Gram-negative bacteria with antimicrobial resistance. They are also active against *Pseudomonas aeruginosa* infections **(Ozbek and Otuk, 2010)**.

Fifth generation cephalosporins include ceftobiprole and ceftaroline. Ceftobiprole has an extended activity against Methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant *pneumococci,* while ceftaroline has broad spectrum activity against many of the commonly community acquired Gram-positive and Gram-negative pathogens **(Steed and Rybak, 2010)**.

2.4. Non classical β-lactam antibiotics

2.4.1. Carbapenems

Imipenem and meropenem for example, are considered antibiotics of last resort. Physicians prescribe carbapenems when infected patients become very ill or are suspected of harboring resistant bacteria because they have the broadest spectrum of activity, the greatest resistance to β-lactamases and the greatest potency against Gram-positive and Gram-negative bacteria **(Bradley *et al*., 1999)**.

2.4.2. Monobactams

The chemical structure of monobactams consists of a single β-lactam ring with no other ring attached, for example, aztreonam and nocardicin A that have a wide range of activity to aerobic Gram-negative bacteria **(Bush *et al*., 1995)**.

3. Beta lactamase enzymes

Beta lactamases are bacterial enzymes that inactivate β-lactam antibiotics by hydrolysis, resulting in ineffective compounds. Production of these enzymes is the most important mechanism of resistance among the clinical isolates of *Enterobacteriaceae* to penicillins, cephalosporins and monobactams and hence protects the enzyme producers against the lethal effects of these antibiotics **(Mortazavi *et al.*, 2019).**

Gram-positive bacteria release the enzyme into the surroundings medium which is called group protection, while Gram-negative bacteria release the enzyme into the periplasmic space, so it is called individual protection **(Samaha-Kfoury and Araj, 2003).**

Resistance to β-lactamases has become a particular problem in last few decades as β-lactamase producing bacteria are increasing in number and causing more severe infections. Originally, genes encoding these enzymes were identified on the bacterial chromosome. In 1965, the first report of plasmid encoded β-lactamase in Gram negative bacteria appeared from Greece **(Timofte *et al.*, 2014).**

3.1. Extended Spectrum Beta Lactamases (ESβLs)

ESβLs are known as extended spectrum because they are capable of hydrolyzing broader spectrum β-lactams more than the simple parent β-lactamase from which they are derived. They render the bacteria resistant to extended spectrum cephalosporins (first, second, third and fourth generations) containing oxyimino-group (e.g., ceftriaxone, cefotaxime, cefuroxime, ceftazidime, and cefepime) and oxyimino -monobactam, e.g. aztreonam **(Peirano and Pitout, 2010).** Generally, they are inhibited by β-lactamses inhibitors, such as clavulanic acid, sulbactam and tazobactam **(CLSI, 2014)**. In 1983, the first ESβL was identified during a hospital outbreak of *Klebsiella pneumoniae* infections in Germany **(Knothe *et al*., 1983).**

It is recommended that any organism which is confirmed for ESβL production should be reported as resistant to all broad spectrum β-lactams and, for these strains, the carbapenems are the main remaining treatment option **(Uchil *et al.*, 2014).**

3.2. Classification of β-Lactamases.

A. Classification based on molecular structure

Beta lactamases are grouped into four classes designated A to D Classes based on amino acid similarity. Beta lactamases of class A, C and D possess serine amino acid at their active site, while class B has zinc binding thiol-group at their active site **(Bush *et al.*, 1995).**

B. Classification based on function

This classification began when cephalosporinases, β-lactamases with high hydrolysis rates for cephalosporines were differentiated from penicillinases, which are β-lactamases with high hydrolysis rates for penicillins **(Bush *et al.*, 1995).** Functional classification schemes that have been accepted included:

1. The classification of penicillinases and cephalosporinases by using the response to antisera as an additional discriminator **(Sawai *et al.*, 1968).**

2. Richmond and Sykes (1973), scheme that included all of the β-lactamases from Gram-negative bacteria described at that time. They are classified on the basis of substrate profile into five major groups.

3. The extension of the Richmond and Sykes (1973) scheme by Sykes and Matthew (1976), which emphasizes on the plasmid-mediated β-lactamases that could be differentiated by isoelectric focusing.

C. Classification based on function and molecular structure:

The scheme proposed by **Bush *et al.* (1995)** described the molecular classes, the substrate and inhibitory specificities of β-lactamases. It was the first one to correlate these three elements. The scheme classified the β-lactamases as follow:

Functional Classification which includes Group 1 cephalosporinases, Group 2 serine β-lactamases, Group 3 Metallo β-lactamases.

Molecular Classification which includes: OXA, CTX-M, SHV, and other ESBLS.

Some point mutations in genes that encodes for ESBL enzymes which are mainly derived from SHV and TEM β-Lactamases can cause expansion in the enzyme active site and increase its susceptibility to β-Lactamase inhibitors through enhancing its ability to hydrolyze extended-spectrum cephalosporins. The first to be discovered was TEM-3 now more than 90 TEM β-Lactamases have been approved to have ESBL phenotype **(Paterson and Bonomo, 2005).**

Pseudomonas aeuroginosae exhibit mainly OXA-type ESBL which is responsible for its high resistance rate compared to low resistance rate when cloned in *E. coli* as it hydrolyzes cloxacillin, oxacillin and third generation cephalosporins rapidly **(Bradford, 2001).**
CTX-M also characterized by its high ability to hydrolyze cefotaxime and is inhibited better by tazobactam than sulbactam and clavulanic acid β-Lactamase inhibitors **(Cantón *et al.*, 2012).**
SHV β-Lactamases are mostly found in *Klebsiella pneumoniae* but unfortunately have few derivatives exhibiting ESBL phenotype **(Paterson and Bonomo, 2005).**

4. β-lactamase inhibitors (BLIs)
Beta-lactamases are enzymes secreted by pathogenic bacteria as a defense mechanism against β-lactam antibiotics. They act through the hydrolysis of β-lactam ring in β-lactam antibiotics, and divided into four classes based on the primary sequence homology and the differences in hydrolytic mechanisms: A, B, C, and D **(Bush *et* al., 1995)**. Classes A, C, and D β-lactamases are serine enzymes, which can hydrolyze the β-lactam ring via a serin-bound acyl intermediate in the active site, while class B β-lactamases (known as metallo-β-lactamases) present one or two zinc ions in the active site, which are necessary for their activity **(Tooke *et* al., 2019)**.
However, the inappropriate and excessive use of β-lactams, has led to the spread of resistance to extended-spectrum cephalosporins (e.g., cefotaxime, ceftriaxone, and ceftazidime), and more recently to carbapenems (imipenem, meropenem, and doripenem). As a consequence, a worldwide health public problem has raised, which is the proliferation of carbapenem-resistant bacteria due to carbapenem-hydrolyzing β-lactamases, such as *Klebsiella pneumoniae* carbapenemase (KPC) belonging to class A, New Delhi metallo-β-lactamase (NDM) belonging to class B, and oxacillinase (OXA-48) belonging to class D **(Khanna and Gerriets, 2021)**. The only solution to overcome β-lactam resistance is the development of broad-spectrum β-lactamase inhibitors which mimic the β-lactam core, thus blocking β-lactamases, including cephalosporinases and serine-based carbapenemases **(Tehrani and Martin, 2018)**.
β-lactamase inhibitors (BLIs) have poor antimicrobial activity. They are co-administrated with β-lactam antibiotics to prevent their inactivation by binding to the β-lactamases, which is the only purpose of use of such BLIs. β-lactamase inhibitors include calavams, for example, the naturally occurring member clavulanic acid. They also include penicillanic acid sulfones as sulbactam, tazobactam which inhibit many class A β-lactamases, avibactam which is a non-β-lactam inhibitor, and enmetazobactam**,** the novel ESBL inhibitor structurally similar to tazobactam **(Livermore, 1996)**.

4.1. Clavulanic acid
Clavulanic acid is an antibiotic isolated from *Streptomyces clavuligerus*, having a β-lactam ring, and differs from penicillin G and penicillin V in its second ring, which is an oxazolidine instead of a thiazolidine ring. It binds powerfully to β-lactamase near its active site, that results in blocking its enzymatic activity and improving the antibacterial effects **(Tooke *et* al., 2019)**.
Clavulanic acid was among the first BLIs to be approved for use. However, these BLIs profiles are largely limited to class A serine penicillinases (e.g., TEM-1, SHV-1) and ESBLs (e.g., CTX-M-15) in addition to some class C and D β-lactamases (e.g., AmpC and OXA-1) **(Kazmierczak *et al.*, 1990)**. In the clinical setting, clavulanic acid is currently combined with β-lactams including amoxicillin and ticarcillin. Amoxicillin/ clavulanic acid combination has antimicrobial activity against many Gram-positive bacteria such as methicillin-sensitive *Staphylococcus aureus* (MSSA), *Staphylococcus epidermidis, Enterococcus faecalis, Streptococcus pyogenes, and Streptococcus pneumoniae.* In addition, it shows significant antimicrobial activity against many non-*Enterobacteriaceae* Gram-negative species, such as *H. influenzae* **(Khanna and Gerriets, 2021)**. Despite over 20 years of clinical use, Aminopenicillin/β-lactamase inhibitor combinations (e.g., amoxicillin/clavulanic acid) are well established in the therapy of a wide range of infections because of their broad-spectrum activity and good tolerance **(Huttner *et* al., 2020; Uto and Gerriets, 2022).**
These agents are particularly suitable for the prophylaxis and treatment of polymicrobial infections. Moreover, many studies have verified their efficacy in treatment of diabetic foot infections, intra-abdominal infections, pulmonary infections, brain abscesses and pelvic inflammatory disease. Also, they have been effective in the prophylaxis of after surgery infections involving abdominal, pelvic, head and neck surgeries. The increase in consumption of amoxicillin/clavulanic acid combination resulted in organisms resistant to both drugs **(Huttner *et* al., 2020).**

4.2. Sulbactam
Sulbactam is a semi-synthetic β-lactamase inhibitor. Its β-lactam ring binds irreversibly to a β-lactamase, thus blocks the β-lactamase enzyme activity and prevents degradation of β-lactam antibiotics. Sulbactam is generally combined with ampicillin and cefoperazone. This combination provides a wide range of activity against both Gram-positive and Gram-negative pathogens, particularly in the ratio of 2:1.

Ampicillin/sulbactam can be used in ventilator-associated pneumonia and in lower respiratory tract, and intra-abdominal infections. It's also effective in acute diabetic foot infections, and skin and soft tissue infections **(Rafailidis *et* al., 2007; Betrosian and Douzinas, 2009)** .
On the other hand, the combination is not active against *P. aeruginosa*, while it is considered particularly active against *A. baumannii* infections. The drug is indicated as empirical treatment for a wide range of community-acquired diseases in adults as well as children, and it is effective in both parenteral (ampicillin-sulbactam) and oral (as a reciprocal sultamycin prodrug) forms **(Betrosian and Douzinas, 2009)**.
Moreover, sulbactam has been combined with cefoperazone, either at a fixed level of 8 mg/L sulbactam or at a fixed cefoperazone: sulbactam ratio (2:1). Many studies proved that cefoperazone/ sulbactam has better antimicrobial activity against *Enterobacteriaceae, P. aeruginosa,* and *A. baumannii,* compared to treatment with cefoperazone alone. Also, cefoperazone/sulbactam, in a ratio of 1:1 or 1:2, presents higher *in vitro* activity against MDR organisms, extended-spectrum β-lactamase-producing, and AmpC-producing *Enterobacteriaceae* **(Ku and Yu, 2021)**.
Previously approved β-lactamase inhibitors such as clavulanic acid and sulbactam have no activity against important classes of β-lactamases, like *Klebsiella pneumoniae* carbapenemases (KPCs), New Delhi metallo-beta-lactamase (NDM-1), and AmpC-type Beta-lactamase. Because of their limited spectrum and the spread of antimicrobial resistance especially in Gram-negative pathogens, novel β-lactamases inhibitors with expanded profiles are required **(Ku and Yu, 2021)**.

4.3. Avibactam

In the last three decades, avibactam was the first non-β-lactam β-lactamase inhibitor -it does not contain a β-lactam core-, to be approved in the USA for clinical use. Avibactam belongs to the class of azabicycloalkanes in which the amino hydrogen is replaced by a sulfooxy group at position 6 **(Tooke *et* al., 2019)**.
It presents an unusual mechanism of inhibition as it acts via the opening of the avibactam ring, but the reaction is reversible, because the deacylation leads to the regeneration of the compound and not to hydrolyse and turnover. This mechanism highlights that avibactam is highly effective in providing protection to β-lactam antibiotics against hydrolysis caused by chromosomal and plasmid β-lactamases **(Khanna and Gerriets, 2021)**.
It exhibits an excellent spectrum, covering essentially all class A (KPCs, CTX-M, TEM, SHV), and class C β-lactamases and some class D serine β-lactamases such as OXA-23 and OXA-48 **(Wang *et al*., 2016)**.
It is used in the form of sodium salt in combination with ceftazidime and meropenem. Ceftazidime–avibactam shows *in vitro* activity against several Gram-negative bacteria, including many extended-spectrum β-lactamase-, AmpC-, *K. pneumoniae* carbapenemase-, and OXA-48-producing *Enterobacteriaceae* and MDR *P. aeruginosa* isolates **(Shirley, 2018).** In Europe, ceftazidime–avibactam (available in market as Zavicefta®) is administered for the treatment of hospital-acquired pneumonia, including ventilator-associated pneumonia, complicated urinary tract infections, intra-abdominal infections, and other infections caused by aerobic Gram-negative pathogens, it represents a suitable choice for severe and difficult-to-treat infections **(Shirley, 2018).**

4.4. Enmetazobactam

Enmetazobactam (formerly known as AAI101) is a novel ESBL inhibitor structurally similar to tazobactam. It possess potent inhibitory activity towards a wide range of class A β-lactamases, including ESBLs (e.g., CTX-M, TEM, SHV, and other class A β-lactamases, as well as some class C and class D β-lactamases **(Isler *et al*., 2021)**.Enmetazobactam is being developed in combination with cefepime, which is stable against hydrolysis by OXA-48 and AmpC β-lactamases. Together, this combination represents a novel β-lactam–β-lactamase inhibitor combination with broad-spectrum antimicrobial activity against MDR *Enterobacteriaceae* **(Johnson *et* al., 2020).**The efficacy of cefepime-enmetazobactam is currently being compared with that of piperacillin-tazobactam in a phase III clinical trial of patients suffer from complicated urinary tract infection and/or acute pyelonephritis. In this phase III trial, cefepime-enmetazobactam demonstrated superiority over piperacillin-tazobactam at the primary efficacy endpoint. On the other hand, the potential utility of cefepime-enmetazobactam for a range of other serious hospital infections remains to be determined **(Das *et* al., 2020).**

5. β-Lactam/ β-Lactamase inhibitor combinations.

The most frequently used antimicrobial agents for *Enterobacteriaceae* infections are β-lactam antibiotics including penicillins, cephalosporins, monobactams and carbapenems **(Pitout, 2012)**. Extensive use of these antimicrobials has promoted the rapid development of bacterial resistance **(Kadry *et al*., 2017)**. β-lactamase enzymes are major contributors of cephalosporins resistance, some β-lactamases have activity even against 3rd and 4th generation cephalosporins and monobactams, known as the extended spectrum β-lactamases (ESBLs). Therefore, third generation cephalosporins-resistant *Enterobacteriaceae* have been categorized as "critical

priority" pathogens and such resistance becomes a global health problem especially in developing countries. So, multiple approaches to develop antimicrobial agents that act actively against ESBLs have been pursued, and different β-lactamase inhibitors (BLIs) have been developed and joined with β-lactam antibiotics to overcome such resistance against the first combinations of β-lactam/β-lactam inhibitors that have been used over a long period are amoxicillin/clavulanate, ampicillin/sulbactam and piperacillin/tazobactam combinations **(Mogasale *et al*., 2021)**.

Despite the fact that, the strategy of combining β-lactams along with classical β-lactamase inhibitors has already yielded effective combinations, the problem of misuse and overuse of these antimicrobials resulted in the emergence of MDR species with high resistance rates towards these combinations. As a result, new approaches to develop and expand the number and effectiveness of β-lactamase inhibitors are in progress **(Huemer *et al*., 2020)**.

Recently, different (β-lactam/ β-lactamase inhibitor) combinations have been approved to exhibit *in vitro* synergistic activities against multidrug-resistant (MDR) organisms, like cefoperazone/sulbactam, ceftazidime/avibactam, and cefepime/enmetazobactam **(Yahav *et al*., 2020).**

Regarding cefoperazone/sulbactam combination, cefoperazone is a third generation cephalosphorin that inhibits the final stage of bacterial cell wall synthesis of actively dividing cells by binding to specific penicillin-binding proteins (PBPs). Unfortunately, it is susceptible to degradation by β-lactamases, so it is combined with sulbactam, a penicillanic acid sulfone that inhibits β-lactamase activity, resulting in preventing cefoperazone inactivation and enhancing the cefoperazone spectrum of activity. It does not exert clinically significant antibacterial effect alone, except against *Neisseriaceae* and *Actinobacter* **(Alfei and Schito, 2022)**.

Ceftazidime/avibactam combination branded as (Avycaz) and developed by AstraZeneca was approved by the FDA in February 2015 for the treatment of complicated urinary tract infections and complicated intra-abdominal infections (cIAI) caused by multi-drug resistant Gram-negative bacterial pathogens. It is highly potent against *Enterobacteriaceae* carrying bla_{KPC} and bla_{OXA-48}. The spectrum of activity of ceftazidime/avibactam is attributable to avibactam's ability to inhibit class A, C, and some D β-lactamases, including KPC and OXA-48 carbapenemases **(Papp-Wallace, 2019)**.

Regarding cefepime/enmetazobactam combination; cefepime is a fourth-generation cephalosporin stable against AmpCs and OXA-48 with well-documented efficacy in serious Gram-negative infections. The antibiotic combination cefepime/enmetazobactam has met the European Medicines Agency and US Food and Drug Administration (FDA) pre-specified primary endpoint in the phase 3 clinical trial **(Tselepis *et al*., 2020)**.

References:

Alfei, S., & Schito, A. M. (2022). β-Lactam Antibiotics and β-Lactamase Enzymes Inhibitors, Part 2: Our Limited Resources. *Pharmaceuticals*, *15*(4), 476.

Betrosian, A. P., & Douzinas, E. E. (2009). Ampicillin-sulbactam: An update on the use of parenteral and oral forms in bacterial infections. *Expert Opinion on Drug Metabolism & Toxicology*, *5*(9), 1099–1112.

Bradley, J. S., Garau, J., Lode, H., Rolston, K. V. I., Wilson, S. E., & Quinn, J. P. (1999). Carbapenems in clinical practice: A guide to their use in serious infection. *International Journal of Antimicrobial Agents*, *11*(2), 93–100.

Bush, K., Jacoby, G. A., & Medeiros, A. A. (1995). A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrobial Agents and Chemotherapy*, *39*(6), 1211–1233.

Chase-Topping, M., Gally, D., Low, C., Matthews, L., & Woolhouse, M. (2008). Super-shedding and the link between human infection and livestock carriage of Escherichia coli O157. *Nature Reviews Microbiology*, *6*(12), 904–912.

Christensen, S. B. (2021). Drugs that changed society: History and current status of the early antibiotics: Salvarsan, sulfonamides, and β-lactams. *Molecules*, *26*(19), 6057.

Collins, D., Hogan, A. M., & Winter, D. C. (2011). Microbial and viral pathogens in colorectal cancer. *The Lancet Oncology*, *12*(5), 504–512.

Dabke, G., Le Menach, A., Black, A., Gamblin, J., Palmer, M., Boxall, N., & Booth, L. (2014). Duration of shedding of Verocytotoxin-producing Escherichia coli in children and risk of transmission in childcare facilities in England. *Epidemiology & Infection*, *142*(2), 327–334.

Darmanin Sheehan, A. (2006). *The production of polyclonal, monoclonal and genetically-derived scFv antibody fragments for the detection of the ß-lactam antibiotic, cephalexin, in milk*.

Das, S., Fitzgerald, R., Ullah, A., Bula, M., Collins, A. M., Mitsi, E., Reine, J., Hill, H., Rylance, J., & Ferreira, D. M. (2020). Intrapulmonary pharmacokinetics of cefepime and enmetazobactam in healthy volunteers: Towards new treatments for nosocomial pneumonia. *Antimicrobial Agents and Chemotherapy*, *65*(1), e01468-20.

Denny, J., Bhat, M., & Eckmann, K. (2008). Outbreak of Escherichia coli O157: H7 associated with raw milk consumption in the Pacific Northwest. *Foodborne Pathogens and Disease*, *5*(3), 321–328.

Dowell, S. F., Marcy, S. M., Phillips, W. R., Gerber, M. A., & Schwartz, B. (1998). Otitis media—Principles of judicious use of antimicrobial agents. *Pediatrics*, *101*(Supplement_1), 165–171.

Drawz, S. M., & Bonomo, R. A. (2010). Three decades of β-lactamase inhibitors. *Clinical Microbiology Reviews*, *23*(1), 160–201.

Ferech, M., Coenen, S., Malhotra-Kumar, S., Dvorakova, K., Hendrickx, E., Suetens, C., & Goossens, H. (2006). European Surveillance of Antimicrobial Consumption (ESAC): Outpatient antibiotic use in Europe. *Journal of Antimicrobial Chemotherapy*, *58*(2), 401–407.

Guh, A. Y., Bulens, S. N., Mu, Y., Jacob, J. T., Reno, J., Scott, J., Wilson, L. E., Vaeth, E., Lynfield, R., & Shaw, K. M. (2015). Epidemiology of carbapenem-resistant Enterobacteriaceae in 7 US communities, 2012-2013. *Jama*, *314*(14), 1479–1487.

Heaton, J. C., & Jones, K. (2008). Microbial contamination of fruit and vegetables and the behaviour of enteropathogens in the phyllosphere: A review. *Journal of Applied Microbiology*, *104*(3), 613–626.

Huemer, M., Mairpady Shambat, S., Brugger, S. D., & Zinkernagel, A. S. (2020). Antibiotic resistance and persistence—Implications for human health and treatment perspectives. *EMBO Reports*, *21*(12).

Huttner, A., Bielicki, J., Clements, M. N., Frimodt-Møller, N., Muller, A. E., Paccaud, J.-P., & Mouton, J. W. (2020). Oral amoxicillin and amoxicillin–clavulanic acid: Properties, indications and usage. *Clinical Microbiology and Infection*, *26*(7), 871–879.

Isler, B., Harris, P., Stewart, A. G., & Paterson, D. L. (2021). An update on cefepime and its future role in combination with novel β-lactamase inhibitors for MDR Enterobacterales and Pseudomonas aeruginosa. *Journal of Antimicrobial Chemotherapy*, *76*(3), 550–560.

Johnson, A., McEntee, L., Farrington, N., Kolamunnage-Dona, R., Franzoni, S., Vezzelli, A., Massimiliano, M., Knechtle, P., Belley, A., & Dane, A. (2020). Pharmacodynamics of cefepime combined with the novel extended-spectrum-β-lactamase (ESBL) inhibitor enmetazobactam for murine pneumonia caused by ESBL-producing Klebsiella pneumoniae. *Antimicrobial Agents and Chemotherapy*, *64*(6), e00180-20.

Kadry, A. A., Serry, F. M., El-Ganiny, A. M., & El-Baz, A. M. (2017). Integron occurrence is linked to reduced biocide susceptibility in multidrug resistant Pseudomonas aeruginosa. *British Journal of Biomedical Science*, *74*(2), 78–84.

Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Nature reviews. Microbiology. *Nat Rev Microbiol*, *2*(2), 123–140.

Kazmierczak, A., Cordin, X., Duez J.M., Siebor E., Pechinot A., Sirot J. (1990). Differences between clavulanic acid and sulbactam in induction and inhibition of cephalosporinases in *Enterobacteria. Journal of International Medical Research,18,* 67D-77D.

Khanna, N. R., & Gerriets, V. (2021). Beta lactamase inhibitors. In *StatPearls [Internet]*. StatPearls Publishing.

Kotra, L. P., Samama, J., & Mobashery, S. (2002). Beta-lactamases and resistance to beta-lactam antibiotics. *Bacterial Resistance to Antimicrobials*, 123–160.

Koyama, T., Kirjavainen, P. V., Fisher, C., Anukam, K., Summers, K., Hekmat, S., & Reid, G. (2010). Can bacterial interference prevent infection? *Canadian Journal of Microbiology*, *56*(9), 730.

Ku, Y.-H., & Yu, W.-L. (2021). Cefoperazone/sulbactam: New composites against multiresistant gram negative bacteria? *Infection, Genetics and Evolution*, *88*, 104707.

Lima, L. M., da Silva, B. N. M., Barbosa, G., & Barreiro, E. J. (2020). β-lactam antibiotics: An overview from a medicinal chemistry perspective. *European Journal of Medicinal Chemistry*, *208*, 112829.

Livermore, D. M. (1996). β-Lactams: Mode of action and mechanisms of bacterial resistance. *Antibiotics in Laboratory Medicine*, 502–578.

Loder, B., Newton, G. G. F., & Abraham, E. P. (1961). The cephalosporin C nucleus (7-aminocephalosporanic acid) and some of its derivatives. *Biochemical Journal*, *79*(2), 408.

Malik, F., & Figueras, A. (2019). Continuous rise in cephalosporin and fluoroquinolone consumption in Pakistan: A 5 year analysis (2014–18). *JAC-Antimicrobial Resistance*, *1*(3), dlz063.

Mogasale, V. V., Saldanha, P., Pai, V., & Rekha P.D. (2021). A descriptive analysis of antimicrobial resistance patterns of WHO priority pathogens isolated in children from a tertiary care hospital in India. *Science Reports*, *11*(1), 1-7.

Mohawk, K. L., & O'Brien, A. D. (2011). Mouse models of Escherichia coli O157: H7 infection and shiga toxin injection. *Journal of Biomedicine and Biotechnology*, *2011*.

Mortazavi-Tabatabaei, S. A. R., Ghaderkhani, J., Nazari, A., Sayehmiri, K., Sayehmiri, F., & Pakzad, I. (2019). Pattern of antibacterial resistance in urinary tract infections: A systematic review and meta-analysis. *International Journal of Preventive Medicine*, *10*.

Ozbek, B., & Otuk, G. (2010). Activities of fourth generation cephalosporins alone and in combination with gentamicin, amikacin, ciprofloxacin and levofloxacin against bloodstream Pseudomonas aeruginosa isolate. *Indian Journal of Medical Microbiology*, *28*(1), 84.

Pallett, A., & Hand, K. (2010). Complicated urinary tract infections: Practical solutions for the treatment of multiresistant Gram-negative bacteria. *Journal of Antimicrobial Chemotherapy*, *65*(suppl_3), iii25–iii33.

Papp-Wallace, K. M. (2019). The latest advances in β-lactam/β-lactamase inhibitor combinations for the treatment of Gram-negative bacterial infections. *Expert Opinion on Pharmacotherapy*, *20*(17), 2169–2184.

Perez, F., & Van Duin, D. (2013). Carbapenem-resistant Enterobacteriaceae: A menace to our most vulnerable patients. *Cleveland Clinic Journal of Medicine*, *80*(4), 225.

Pitout, J. D. D. (2012). Extraintestinal pathogenic Escherichia coli: A combination of virulence with antibiotic resistance. *Frontiers in Microbiology*, *3*(JAN).

Podschun, R., & Ullmann, U. (1998). Klebsiella spp. As nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews*, *11*(4), 589–603.

Rafailidis, P. I., Ioannidou, E. N., & Falagas, M. E. (2007). Ampicillin/sulbactam. *Drugs*, *67*(13), 1829–1849.

Sargeant, J. M., Sanderson, M. W., Smith, R. A., & Griffin, D. D. (2004). Associations between management, climate, and Escherichia coli O157 in the faeces of feedlot cattle in the Midwestern USA. *Preventive Veterinary Medicine*, *66*(1–4), 175–206.

Shirley, M. (2018). Ceftazidime-avibactam: A review in the treatment of serious gram-negative bacterial infections. *Drugs*, *78*(6), 675–692.

Steed, M. E., & Rybak, M. J. (2010). Ceftaroline: A new cephalosporin with activity against resistant Gram-positive pathogens. *Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy*, *30*(4), 375–389.

Tehrani, K. H., & Martin, N. I. (2018). β-lactam/β-lactamase inhibitor combinations: An update. *Medchemcomm*, *9*(9), 1439–1456.

Thompson, S., Yacomeni, S., & Townsend, R. (2010). Antibiotics for uncomplicated urinary tract infection in women. *Prescriber*, *21*(7), 15–28.

Tooke, C. L., Hinchliffe, P., Bragginton, E. C., Colenso, C. K., Hirvonen, V. H., Takebayashi, Y., & Spencer, J. (2019). β-Lactamases and β-Lactamase Inhibitors in the 21st Century. *Journal of Molecular Biology*, *431*(18), 3472–3500.

Tselepis, L., Langley, G. W., Aboklaish, A. F., Widlake, E., Jackson, D. E., Walsh, T. R., Schofield, C. J., Brem, J., & Tyrrell, J. M. (2020). In vitro efficacy of imipenem-relebactam and cefepime-AAI101 against a global collection of ESBL-positive and carbapenemase-producing Enterobacteriaceae. *International Journal of Antimicrobial Agents*, *56*(1), 105925.

Uto, L. R., & Gerriets, V. (2022). Clavulanic acid. In *StatPearls [Internet]*. StatPearls Publishing.

Wang, D. Y., Abboud, M. I., Markoulides, M. S., Brem, J., & Schofield, C. J. (2016). The road to avibactam: The first clinically useful non-β-lactam working somewhat like a β-lactam. *Future Medicinal Chemistry*, *8*(10), 1063–1084.

Williams, K. P., Gillespie, J. J., Sobral, B. W. S., Nordberg, E. K., Snyder, E. E., Shallom, J. M., & Dickerman, A. W. (2010). Phylogeny of gammaproteobacteria. *Journal of Bacteriology*, *192*(9), 2305–2314.

Yahav, D., Giske, C. G., Grāmatniece, A., Abodakpi, H., Tam, V. H., & Leibovici, L. (2020). New β-lactam–β-lactamase inhibitor combinations. *Clinical Microbiology Reviews*, *34*(1), e00115-20.