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Review

Mobile Genetic Elements Associated with Third-Generation Cephalosporin Resistance in *Klebsiella pneumoniae*

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Abstract. *Klebsiella pneumoniae* is the Enterobacteriaceae family can be found in the environment, humans and animals. These bacteria are common causes of hospital, community and healthcare associated infections. Third generation cephalosporin (3GC) is one of the broad spectrum cephalosporin antibiotic that is commonly used to treat this infection. Use of antibiotics without appropriate sensitivity guidance, natural antibiotic resistant bacteria and MGE mediated horizontal gene transfer have led to increased resistance in 3GC. Mobile genetic elements such as insertion sequences (IS), transposons, integrons and resistance plasmids facilitate horizontal gene transfer between bacteria. This element can move between chromosomes or plasmids, transferring genetic material to the recipient bacteria. Horizontal gene transfer can occur by conjugation, transformation, transduction and vesiduction. IS, transposons, integrons and resistance plasmids associated with 3GC resistance are discussed in this article. We analysed the role of these MGEs in 3GC resistance in *K. pneumoniae* using PRISMA methods from different academic sources. We found an association of MGE with ESBL and AmpC betalactamase gene. This element promotes the transfer of resistance genes to other bacteria. Understanding the MGEs that play a role in the spread of antibiotic resistance genes in *K. pneumoniae* is important to control the spread of the gene.

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1. Introduction

Klebsiella pneumoniae is a type of bacteria that is classified as Gram-negative belonging to the Enterobacteriaceae family. This group of bacteria can be isolated from environmental reservoirs and naturally present in the digestive tract of humans or animals [1]. *Klebsiella pneumoniae* is capable of causing nosocomial human infections such as urinary tract infections, bloodstream infections, respiratory tract infections and bacteremia. This bacterial infection acquired from community or healthcare associated [2]. The increasing prevalence of infections caused by *Klebsiella pneumoniae* may occur due to the emergence of strains that are resistant to several antibiotics. The meta analysis study of Mohd Asri et al. (2021) [3], reported that the prevalence of *Klebsiella pneumoniae* resistant to multiple antibiotics used to treat nosocomial infections was estimated to be 32.8% in the world. Furthermore, the inappropriate use of antibiotics with inadequate sensitivity might trigger the emergence of resistance, leading to limited treatment options and an increased risk of patient mortality [4].

The third generation cephalosporin (3GC) is a β-lactam antibiotic commonly used to treat infections caused by Gram positive and Gram negative bacteria and has good stability against β lactamase activity [5]. 3GC is one of the first line antibiotics recommended to treat urinary tract and gastro intestinal tract infections caused by *Klebsiella pneumoniae* [6]. Increased resistance to 3GC of these bacteria has been reported due to the spread of extended spectrum beta lactamases (ESBLs) and overexpression of AmpC beta lactamases in the community and healthcare settings. Research reported by Tekele et al. (2020) [7], provides information on the high ESBL and AmpC betalactamse produced by *E. coli* and *Klebsiella pneumoniae* from urine specimens that cause resistance and multidrug resistance (MDR) on ampicillin, amoxicillin with clavulanic acid, sulfamethoxazole-trimethoprim, cefuroxime and cefotaxime antibiotics.

Horizontal gene transfer mediated by mobile genetic elements (MGE) is one of the important mechanisms in the spread of 3GC resistance genes. MGE can move within or between DNA molecules, facilitating the exchange of genetic material and the spread of resistance genes [8]. The activity of MGEs like insertion sequences (IS), transposons, integrons, integrative conjugative elements (ICE) and plasmids that can move between bacteria plays an important role in horizontal genetic material exchange in the Enterobacteriaceae [9]. The results reported by Wang et al. (2023) [10] helped us to better understand the role of MGEs closely related to resistance genes, where integron class 1 was found in 57.49% of *Klebsiella* isolates encoding 19 types of resistance genes.

Plasmids, a type of MGE can carry genes that confer resistance to antibiotics. The presence of ESBL producing gene groups such as $bla_{\text{CTX-M-15}}$ on plasmid A, can promote the development of 3GC resistance in *K. pneumoniae* and create new ESBL strains [11]. The exchange of plasmids carrying 3GC resistance genes has also been studied by Quan et al. (2023) [12], where the $bla_{\text{CTX-M-14}}$ gene is located on the pKP15255_1 plasmid in *Klebsiella pneumoniae* and the presence of IS*26* plays a role in the exchange of genetic material. In this article, we will review the different type of MGEs associated with 3GC resistance in *Klebsiella pneumoniae* that contribute to the spread antibiotic resistance in the publised literature.

2. Experimental Section

Between August and December 2023, the authors of this review searched several databases of scientific articles and research results such as Pubmed, Science Direct and Google Scholar. Only studies that met the above search criteria were included in the systematic review. The search included the terms (1) *Klebsiella pneumoniae*, (2) 3GC mechanism of action, (3) 3GC resistance mechanisms, (4) antibiotic resistance genes (ARGs) and (5) MGEs associated with 3GC resistance. Exclusion criteria included duplicate articles with a publication year ranging from 2019 to 2023.

We analysed recent research studies to understand the latest findings on MGEs associated with 3GC resistance in *Klebsiella pneumoniae*. Keywords were used to search all databases, and a total of 311 articles were found and 50 duplicate articles were removed. Articles with screening potential were identified from abstract to full text publication. 199 articles were excluded because they did not fulfil

the criteria. Therefore, 62 journals fulfil the criteria and were available for review. Figure 1 illustrates the workflow for the article selection process.

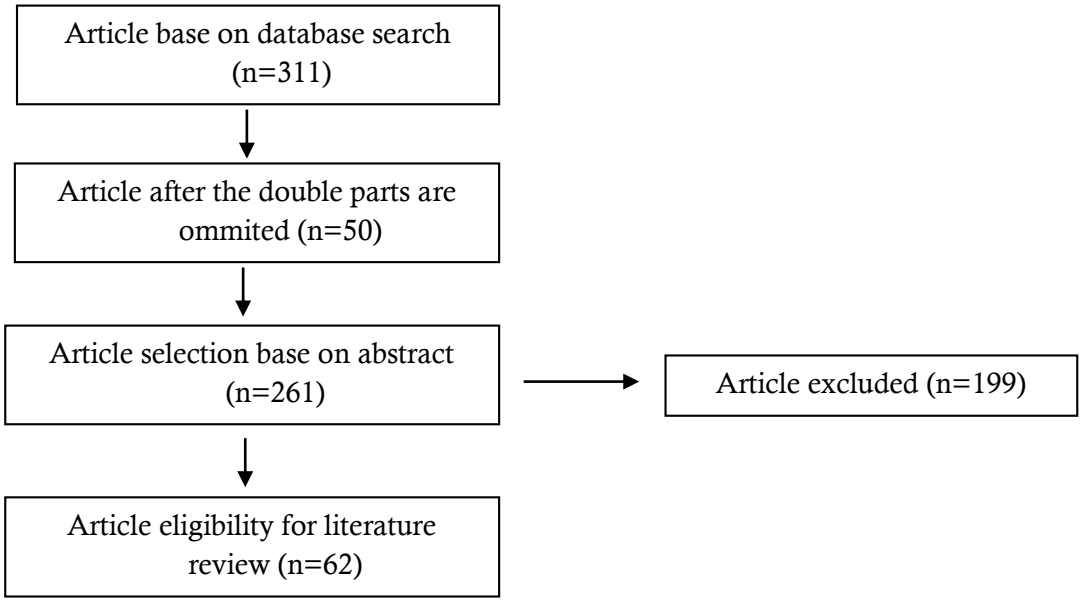

Figure 1. PRISMA workflow

3. Results and Discussion

3.1 *Klebsiella pneumoniae*

Klebsiella pneumoniae is rod shaped, with 0.3-1.0 µm in diameter and 0.6-6.0 µm in size. It is a Gram negative bacteria of the Enterobacteriaceae family, capsule, non-spore-forming, non-motile, and facultative anaerobe [13]. The morphology of the colonies of this bacteria on sheep blood agar is grayish white, slimy, and non-hemolytic. On Eosin Methylene Blue (EMB) agar, the colonies are dark brown and slimy, indicating the presence of lactose fermentation and acid production, without a metallic green sheen distinguishable from *E. coli* colonies [14].

Klebsiella consists of various species with seven phylogroups are included in the *Klebsiella pneumoniae* species complex (KpSC), while other *Klebsiella* species are included in genetically different groups. The KpSC group of bacteria is widely reported to be responsible for the majority of cases of community-acquired and healthcare-associated infections [15]. Based on its pathogenicity in host cells, *Klebsiella pneumoniae* has several pathotypes, namely classical *Klebsiella pneumoniae* (cKp), hypervirulent *Klebsiella pneumoniae* (hvKp), and multidrug resistant and hypervirulent *Klebsiella pneumoniae* (MDR-hv) [16].

The cKp strain can pick up mutations that lead to the formation of many clones. This strain very widespread and can pick up many genetic elements that make it resistant to multiple drugs, such as spectrum β-lactamase (ESBL) [17]. The hvKp strain is an invasive variant of cKp that shows hypermucoviscosity, hypervirulence, and has many siderophores as virulence factors. The emergence of multidrug resistant and hypervirulent (MDR-hv) strains can occur through horizontal plasmid-mediated transfer of resistance genes or virulence factors [18].

The ability of *Klebsiella pneumoniae* to infect is associated with several virulence factors that enable it to survive and overcome the host cell defence system, including the capsule, lipopolysaccharide, adhesin, pili, type VI secretion proteins and biofilm formation [19]. The capsule that surrounds the

surface of these bacteria has functions to protect the outside of the bacteria, inhibit phagocytosis and contribute to biofilm formation [20]. Research conducted by Haudiquet et al. (2021) [21], found that the capsule facilitates the movement of MGE carried by *Klebsiella pneumoniae* of the same serotype that causes nosocomial infections and is associated with ARG acquisition.

3.2 Third Generation Cephalosporins (3GC) Antibiotics and Mechanism of Action

3GC is a β-lactam antibiotic cephalosporin group with a semisynthetic analog consists of a β-lactam and a dihydrothiazine ring with different chemical structures in the C7. 3GC consists of cefotaxime, ceftazidime, cefdinir, ceftriaxon, cefodoxime, cefoperazone, cefixime, and several other types [22]. 3GC is one of the antibiotics widely use to treat infections caused by Gram-positive and Gram-negative bacteria that are resistant to first and second generation cephalosporins. These antibiotics used to treat meningitis caused by *H. influenzae, Neisseria meningitidis,* and *Streptococcus pneumoniae* infections, and *Pseudomonas aeruginosa* infections[23].

3GC works like other generations of cephalosporins by binding to penicillin binding protein (PBP) and preventing the formation of cross links with peptidoglycan strands, thus inhibiting cell wall formation. Changes in cell integrity cause osmotic pressure to increase, causing bacterial cells to swell. This leads to cell lysis and bacterial death [24]. 3GC antibiotics are widely used in medical circles to treat infections because they have good effectiveness to overcome the β latamase enzyme, especially those produced by *E. coli* and *Klebsiella pneumoniae* [25].

In Indonesia, a study by Asmarawati et al. (2023) [26] reported that ceftriaxone is one of the 3GC antibiotics widely used to treat urinary tract infections by 69.3% in 2019-2020, with the dominant bacteria in this study being *E. coli* and *Klebsiella pneumoniae*.The overuse of 3GC to treat infections without proper sensitivity guidelines and inadequate disease diagnosis is one of the triggers for the emergence of resistance to 3GC and treatment failure [27].

3.3 Mechanisms of 3GC Resistance in *K. pneumoniae*

ESKAPE pathogens, consisting of six microorganisms, namely *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter*, are a group of pathogenic bacteria that cause resistance to antibiotics [28]. *K. pneumoniae* is one of the ESKAPE pathogenic bacteria that has been reported to have increased resistance to β-lactam antibiotics [29].

Extended-spectrum cephalosporin-resistant *Klebsiella pneumoniae* (ESCR-KP) has resistance activity to third and fourth generation cephalosporins, which is a threat to hospitalized patients because it can cause blood, intra-abdominal, urinary tract, and pneumonia infections [30].The mechanism of resistance of *Klebsiella pneumoniae* to 3GC can occur presence of β-lactamase enzyme produced by *Klebsiella pneumoniae*. This enzyme can hydrolyze the β-lactam ring in the periplasmic region of the cell, thus inhibiting the binding of PBPs [31].

Cephalosporinase or AmpC-lactamase enzyme production, which can occur through chromosomes or plasmids, is the mechanism of resistance to first-generation to third-generation cephalosporin antibiotics (cefazidime, cefotaxime, and ceftriaxon). There are more than 40 genotypes of AmpC enzymes (class C ambler) that can spread rapidly between strains through plasmids [32]. Modification of the 3GC target site and mutation of the target gene in *Klebsiella pneumoniae* results in the inability to bind to the target site, causing the process of bacterial cell lysis not to occur [33].

Klebsiella pneumoniae acquires resistance to 3GC by reducing the entry of antimicrobial agents into the bacteria through the reduction or elimination of outer membrane porins such as OmpK35 and OmpK36, which can reduce the permeability of the cell membrane. The non-ESBL *Klebsiella pneumoniae* express OmpK35 and OmpK36, ESBL-producing *Klebsiella pneumoniae* widely express the OmpK36 porin [34]. Overexpression of efflux pumps also plays a role in antibiotic resistance mechanisms in many Gram-negative and MDR bacterial pathogens [35].

Klebsiella pneumoniae have the ability to form biofilms, which not only protect the bacteria but also act as an osmotic barrier that 3GC can't break down. The biofilm matrix provides mechanical and biochemical protection that can weaken antibiotic activity. Bacteria that form biofilms are more difficult to remove with antibiotics such as 3GC [36]. In addition, the horizontal gene transfer mediated by MGE facilitates the movement of virulence factor causing infection and resistance genes.

3.4 MGE in *Klebsiella pneumoniae*

Bacteria can acquire antimicrobial resistance genes through mutations, plasmids, and transferable genetic elements, resulting in resistant strains. MGEs are mobile genetic elements that can be found in all organisms, and the collection of MGEs in an organism is called the mobilome. The mobilome consists of a large number of plasmids, transposons, and prophages [37].

MGEs are very important in microbial ecology because they can transfer genes with different functions between microbial populations. The collection of all ARG genes and their precursors in pathogenic and non-pathogenic bacteria derived from intrinsic or acquired resistance is referred to as the resistome [38]. The study by Decano et al. (2021) [39] reported that urapatogenic *E. coli* and *K. pneumoniae* isolated from patients in Uganda and Kenya have distinct resistomes that may mediate MDR.

Horizontal gene transfer is one of the most important mechanisms that play a role in the spread of resistome [40]. The study by Zhou et al. (2021) [41] reported that the high probability of resistome transfer can occur in human gut microbiome groups or phylogenetically related groups of pathogenic bacteria. *Klebsiella pneumoniae* strains can transfer to the environment, humans, or animals by carrying acquired antibiotic resistant genes or plasmids [32].

Resistance in *Klebsiella pneumoniae* can occur through intercellular genetic exchange mechanisms, including plasmid-mediated conjugation, bacteriophage-mediated transduction, transformation by extracellular DNA uptake and vesiduction [42]. Plasmid conjugation and interactions between different types of MGEs have the potential to create a variety of MDRs in this bacteria [43].

Some MGEs that play a role in horizontal gene transfer and contribute to 3GC resistance in *Klebsiella pneumoniae* include IS, transposon, integron and resistance plasmid. Figure 2 illustrates the movement of the mobile gene cassette into the integron and transposon flanked by two ISs, and the insertion process into a conjugative plasmid allowing horizontal gene transfer.

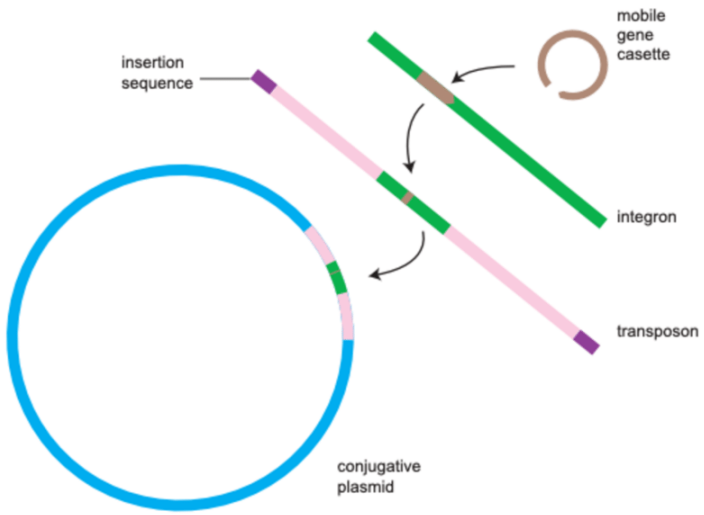

Figure 2. MGEs associated with horizontal gene transfer [44]

3.5 IS *(Insertion Sequences*) Associated With 3GC Resistance in *K. pneumoniae*

IS are pieces of DNA with mobile terminal repeat sequences and open reading frame (ORF) that can change the structure of genomes. IS can carry one or two ORFs containing transposase genes (tnp). Transposition in IS occurs by adding, deleting, duplicating genes in a cut and paste mechanism. This process can be site specific or random and can occur independently. Two copies of the same IS that can move as a unit and transfer resistance genes form composite transposons [45-46].

IS*Ecp1* is one of the IS types first identified in *E. coli*. Study Perdigao et al. (2021) [47] reported presence of IS*Ecp1* in upstream of the $bla_{\text{CTX-M-15}}$ gene in *Klebsiella pneumoniae,* and this element can be found in other bacteria that play a role in 3GC resistance. Another studies conducted by Kieffer et al. (2020) [48] reported insilico tests on *K. pneumoniae* isolates from urine samples in Switzerland showed an association between the $bla_{\text{CTX-M-14}}$ gene. This element as a promoter of resistance genes to broad-spectrum cephalosporin antibiotics. IS*Ecp1* is a member of the IS*1380* family and has been reported to be associated with plasmid or chromosome mediated spread of the $bla_{\text{CTX-M}}$ ESBL gene in the Enterobacteriacea group [49].

IS*26* is a member of the IS6 family found in *Klebsiella pneumoniae* and other Gram negative bacteria. This element encodes a single transposase and undergoes transpositional replication that generates composite transposons. IS26 has been reported to be associated with several resistance determinants, including $bla_{\text{SHV-11}}$ [50]. Research Du et al (2022) [51], reported IS26 located on the *K. pneumoniae* MDR-ST23-hvKp plasmid is involved in horizontal gene transfer and ARG acquisition. Some ESBL resistance genes mediated by IS*26* include $bla_{\text{CTX-M-15}}$ and $bla_{\text{TEM-1B}}$ which are associated with β lactam antibiotic resistance, including 3GC [51].

3.6 Transposons Associated With 3GC Resistance in *Klebsiella pneumoniae*

Resistance genes in bacteria can move between plasmids or between genomes by jumping genetic elements such as transposons. These elements are DNA structures that are part of plasmids, bacteriophages, bacterial chromosomes, IS or complex transposons. There are two classes of transposons, class 1 and class 2. Class 1 transposons consist of ISs and IS dependent elements and class 2 transposons consist of non complex transposons [52].

Transposons carrying resistance genes are inserted into plasmids or chromosomes and travel to other cells where they replicate and become resistant to antibiotics. Tn1, Tn2 and Tn3 are transposon types that have been widely identified in Gram-negative bacteria, where bla_{TEM} genes encoding extended-spectrum β-lactamases (ESBLs) are found in all three transposons. Tn1 is known to carry the $bla_{\text{TEM-2}}$ gene, while Tn2 and Tn3 carry the $bla_{\text{TEM-1}}$ gene encoding the TEM-1 β-lactamase [53].

The study of Altayb et al., (2022) [54] reported that five clinical isolate and one environmental isolate *Klebsiella pneumoniae* from Sudan detect $bla_{\text{CTX-M-15}}$ that appeared by by IS*Ec9* and Tn3 transposases. This research showed association between presence of $bla_{\text{CTX-M-15}}$ and fenotif resistance 3GC (cefatzidime and cefotaksim). The presence of ESBL genes in *Klebsiella pneumoniae* flanked by transposons can promote horizontal gene transfer. This can trigger the spread of these genes.

3.7 Integron Associated With 3GC Resistance in *Klebsiella pneumoniae*

The integron contains a gene cassette, which is a mobile element consisting of one or two genes with a promoter site (Pc), *intI* (integron integrase), and *attI* recombination site. The cassete gene on the integron can be a free loop which is non replicative and can be obtained exogenously. Integrons can attach to different DNA molecules in chromosomes and plasmids. This element can capture and control various antibiotic resistance genes and play a role in the mechanism of MDR spread [55].

Integrons divide into three groups that have been widely studied and have different types of integrase (intI). Integron class 1 (*intI*1) consists of three recombinant sites and associated with the Tn3 family. This group is commonly found in *Klebsiella pneumoniae*. Integron class 2 (*intI*2) consist of *attI*2 recombinan site associated with Tn7 family. Integron class 3 (*intI*3) associated with metallo-betalactamse and aminoglikoside resistance gene [55-56].

The study by Wang et al., (2023) [57] reported *intI*1 was detected in 93 clinical isolates of *Klebsiella pneumoniae* from Eastern China, where the *intI*1 group was positive for beta-lactamase resistance gene *bla* $_{SHV-1}$ 73,96%. This group was highly resistant to several of the antibiotics tested, including ceftazidime. Previous studies by Delarampour et al (2020) [58] also reported *intI*1 positivity detected in clinical isolates of *Klebsiella pneumoniae* from Iran, showing that 29.6% of isolates were resistant to one of the 3GC antibiotics, ceftriaxone. Based on the literature review, there have been many studies and review articles on class 1 integrons. The presence of class 1 integrons in clinical isolates of *Klebsiella pneumoniae* is associated with MDR because they can form different gene cassettes for each type of antibiotic.

3.8 Resistance Plasmid Associated With 3GC Resistance in *Klebsiella pneumoniae*

Plasmid transmission is one of mechanism in *Klebsiella pneumoniae* resistance genes can be passed on to other Gram-negative bacteria. This process can happen between ESKAPE and *E. coli* pathogenic bacteria, which have a lot of different acquired ARGs. Resistance plasmids generally consist of one or more resistance genes associated with MGEs. It is also possible for *Klebsiella pneumoniae* to add β-lactamase AmpC genes to plasmids, especially broad-spectrum cephalosporins. AmpC β-lactamase genes mediated by plasmids (pAmpCs) include bla_{CMY}, bla_{DHA}, bla_{FOX}, and bla_{MOX} [59].

Other plasmids mediate resistance to 3GC is ESBL-carrying plasmids, are located on large plasmids, one of which is plasmid A, which carries the *bla* $_{CTX-M-15}$ gene [60]. Study by Pedersen et al., (2020) [61] also reported the existence of IncFIIK and IncR plasmids that carry the $bla_{CTX-M-15}$ gene, which allows horizontal gene transfer. In addition to $bla_{CTX-M-15}$, another ESBL gene detected in *K. pneumoniae* is bla_{TEM-1}, which is located on plasmids pNJST258N5 and pJHCMW1 in Tn 1331 [62].

The ability of plasmids and other MGEs to carry ESBL and AmpC beta-lactamase resistance genes by horizontal gene transfer contributes to the rapid spread of antibiotic resistance. Some of the MGEs associated with 3GC resistance in *Klebsiella pneumoniae* based on the results of the literature review are listed in Table 1.

Table 1. MGEs Associated with 3GC resistance in *Klebsiella pneumoniae*

MGE	Resistance Determinan	Reference
ISEcp1	$bla_{CTX-M-14}$, $bla_{CTX-M-15}$	47, 48, 49
IS26	bla_{SHV-11}, $bla_{CTX-M-15}$, *bla* $_{TEM-1B}$	50, 51
Tn1	bla_{TEM-2}	53
Tn2	bla_{TEM-1}	53
Tn3	bla_{TEM-1}, $bla_{CTX-M-15}$	53, 54
intI1	bla_{SHV-1}	57
Plasmid A	$bla_{CTX-M-15}$	11, 60
pKP15255_1	$bla_{CTX-M-14}$	12
pAmpC	bla_{CMY}, bla_{DHA}, bla_{FOX}, bla_{MOX}	59
pIncFIIK, pIncR	$bla_{CTX-M-15}$	61
pNJST258N5, pJHCMW1	bla_{TEM-1}	62

4. Conclusion

Klebsiella pneumoniae is a member of the Enterobacteriaceae family of bacteria that can cause nosocomial, community and healthcare-acquired infections. 3GC is a broad-spectrum antibiotic in the cephalosporin group, which is widely prescribed to treat a variety of infections caused by this bacteria. The high use of drugs and the presence of factors that trigger the emergence of resistance lead to high resistance to 3GC. MGEs is celular componet can mediate horizontal gene transfer, which is a factor in the emergence of resistance. Base on literature review, MGEs like IS, transposon, integron and plasmid mediated ESBL and AmpC beta lactamase gene in 3GC resistance. The presence of MGE plays an important role in the transmission of resistance genes in *Klebsiella pneumoniae* and increases the likelihood of their spread to another Enterobectericea family.

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