**Comparison of IncL/M Plasmids Using the Neighbor-Joining Method on Basis repA and excA Genes**

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**Abstract:** The aim of this study is to make comparison of IncL/M groups on basis of two genes candidates’ repA and excA genes. The sequences of 27 plasmids were compared using the neighbor-joining method. This method was used to construct a phylogenetic tree for the nucleotide sequences of two genes (repA and excA), using the program MEGA X software. The evolutionary distances were computed using the maximum composite likelihood method. The neighbor-joining method analysis showed different results based on the gene used for comparison. The repA gene was more accurate than excA gene to distinguish between different IncL/M plasmids. This study suggested that IncL/M plasmids harboring different antibiotic resistance genes have evolved differently.

**Keywords:** Plasmids, IncL/M Type, Phylogenetic Analysis, repA Gene, excA Gene

**Introduction**

The dissemination of antimicrobial resistance in Gram-negative bacteria has been largely attributed to the horizontal transfer of plasmid-located resistance genes (Carattoli, 2013). A plasmid is defined as a double-stranded, circular DNA molecule able of autonomous replication. By definition, plasmids do not carry genes essential for the growth of host cells under no stressed conditions (Carattoli, 2009).

Carbapenem antibiotics are generally considered the most effective antibacterial agents for the treatment of multidrug-resistant bacterial infections. However, with the widespread use of carbapenem, the prevalence of Carbapenem-Resistant Enterobacteriales (CRE) has increased rapidly and has become a serious threat to public health. The production of carbapenemases is the major mechanism underlying carbapenem resistance in CRE throughout the world (Cui et al., 2019). Carbapenemases belong to Ambler class A (i.e., KPC types), class B (i.e., MBLs: VIM, IMP and NDM types) and class D (i.e., OXA-48). The KPC, NDM, IMP, VIM and OXA-type enzymes are the most common global carbapenemases among Gram-negative bacteria (Pitout et al., 2019). OXA-48-type carbapenem-hydrolyzing class D β-lactamases are widely distributed among Enterobacteriaceae, with significant geographical differences (Mairi et al., 2018).

Genes encoding carbapenemases are mostly located on conjugative plasmids that allow their efficient dissemination among Enterobacteriales species. Although the prevalence of particular plasmids may vary depending on the source and geographical site, they have been increasingly isolated from bacteria of human, animal and environmental origin (Rozwandowicz et al., 2018; Touati and Mairi, 2019). In a recent review, Touati and Mairi concluded that the diffusion of OXA-48 in Algeria is probably linked to plasmid diffusion. This plasmid is largely conjugative between Enterobacteriales members and assigned to IncL/M group (Touati and Mairi, 2019).

The aim of this study was to compare plasmids of IncL/M group encoding different β-lactamases on the basis of two genes repA and excA.

**Methods**

**Plasmid Characterization**

The sequences of 14 plasmids carrying the blaOXA-48 gene were already published (Mairi et al., 2019) and served as a matrix for bioinformatics comparison. Briefly, these plasmids were originated from different species of Enterobacteriales strains obtained from different ecological niches in Algeria. They were sequenced on the Illumina genome analyser IIX system by GenoScreen SA (Lille, France). The sequences of these plasmids were deposited in the GenBank (MK121443.1 to MK121456.1).

**Bioinformatic Analysis**

To generate comparisons, 13 sequenced plasmids carrying different β-lactamases genes were obtained from...
the NCBI nucleotide database (www.ncbi.nlm.nih.gov/nucleotide). Results were filtered to exclude plasmid sequences that did not belong to the IncL/M group. Antimicrobial resistance genes were identified using ResFinder tool 3.2 (https://cge.cbs.dtu.dk/services/ResFinder/). The sequence of two genes was extracted from these plasmid sequences including repA and excA genes.

Complete sequence alignments of these two genes were then undertaken using CLUSTALW on the CLUSTAL Omega website of the EMBL-EBI (www.ebi.ac.uk/Tools/service/web). The neighbor-joining method was used to construct a phylogenetic tree for the nucleotide sequences of these two genes, using the program MEGA X software. The evolutionary distances were computed using the Maximum Composite Likelihood method.

**Results and Discussion**

The data concerning the 27 analyzed plasmids are presented in Table 1. The size of the plasmids ranged from 49257 pb (pRAY) to 133208 pb (pIMP-HB623). The repA gene has 1056 pb in size while the excA gene has 654 pb in size. All the 14 plasmids reported in our study had the same size (61881).

All plasmids carried the blaOXA-48 gene encoding for carbapenemase except four plasmids wich encoded β-lactamas

| Accession number | Species                        | Plasmid name | Size of plasmid (pb) | repA gene size (pb) | Position | excA gene size (pb) | Position | Blaste |
|------------------|--------------------------------|--------------|----------------------|---------------------|----------|---------------------|----------|--------|
| LN064821.1       | Raoultella planticola          | pRA35        | 63 434               | 1056 7938-5900      | 65       | 56690-57343         | OXA-48   |
| JX048939.4       | Enterobacter cloacae           | pECl973      | 87 731               | 1056 51866-52921    | 65       | 50610-51263         | IMP-4    |
| JQ837276.1       | Enterobacter cloacae           | pNE1280      | 66 531               | 1056 23131-24186    | 65       | 21877-22530         | KPC-4    |
| KC534801.1       | Klebsiella pneumoniae          | pEG011       | 71 446               | 1056 65962-67017    | 65       | 64702-65355         | OXA-48+CTX-M-14 |
| KM877517.1       | Enterobacter cloacae           | pIMP-HB623   | 133 208              | 1056 547-1602       | 65       | 132499-131352       | IMP-34   |
| KX224252.1       | Klebsiella pneumoniae          | pRay         | 49 257               | 1056 16098-17153    | 65       | 14838-15491         | OXA-48   |
| HG934082.1       | Klebsiella pneumoniae          | pFOX-7a      | 90 439               | 1056 2428-1373      | 65       | 7982-8635           | FOX      |
| KX523901.1       | Klebsiella pneumoniae          | pOXA-48_3017 | 65 488               | 1056 60004-61059    | 65       | 58744-59397         | OXA-48   |
| KC335143.1       | Klebsiella pneumoniae          | E71T         | 63 578               | 1056 58552-59607    | 65       | 57292-57945         | OXA-48   |
| KM406049.1       | Klebsiella pneumoniae          | pKrp-E1 N7   | 63 581               | 1056 32334-33389    | 65       | 31074-31727         | OXA-48   |
| KP695888.1       | Klebsiella pneumoniae          | pOXA-48E1    | 62 014               | 1068 595-1662       | 65       | 61361-62014         | OXA-48   |
| JN626286.1       | Klebsiella pneumoniae          | pOXA-48_Ref  | 61 681               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| KJ1021994.1      | Proteus mirabilis              | pOXA-48_Pm   | 72 127               | 1056 527-1582       | 65       | 71401-72004         | OXA-48   |
| MK124156.1       | Cronobacter malonicatis        | pTR94        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124185.1       | Raoultella ornithinolytica     | pTR76        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124141.1       | Pluralibacter gergoviae        | pTR48        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124143.1       | Citrobacter werkmani           | pTR43        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124145.1       | Klebsiella pneumoniae          | pTR103       | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124145.1       | Enterobacter cloacae           | pTR67B       | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124148.1       | Escherichia coli               | pTR73        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124147.1       | Klebsiella pneumoniae          | pTR69A       | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124146.1       | Enterobacter cloacae           | pTR66A       | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124145.1       | Klebsiella pneumoniae          | pTR47        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124144.1       | Escherichia coli               | pTR77        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124143.1       | Escherichia coli               | pTR92        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124150.1       | Escherichia coli               | pTR90        | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
| MK124149.1       | Escherichia coli               | pTR78A       | 61 881               | 1056 56394-57449    | 65       | 55134-55787         | OXA-48   |
Fig. 1: The evolutionary history inferred using the Neighbor-joining method on basis of repA gene

Fig. 2: The evolutionary history inferred using the Neighbor-Joining method on basis of excA gene
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Author’s Contributions

Mairi Assia: Has characterized the plasmids and written the article.

Touati Abdelaziz: Has made the bioinformatic analysis of plasmids and reviewed the article

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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