Current Research in Bioinformatics

Original Research Paper

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

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Article history
Received: 25-12-2019
Revised: 11-02-2020
Accepted: 12-02-2020

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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 Enterobacterales (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 Enterobacterales 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 Enterobacterales 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 *bla*OXA-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 Enterobacterales 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 $bla_{\text{OXA-48}}$ gene encoding for carbapenemase except four plasmids wich encoded β-lactamases $bla_{\text{IMI-4}}$, $bla_{\text{KPC-4}}$, $bla_{\text{IMP-34}}$ and bla_{FOX}. In addition, one plasmid (pJEG011) harbored both of $bla_{\text{OXA-48}}$ and $bla_{\text{CTX-M-14}}$. None of the analyzed plasmids contained resistance genes other than β-lactamases genes reported above.

When compared using the neighbor-joining algorithm, the plasmids compared using *repA* gene were divided into three clusters (Fig. 1): Cluster 1 contained all plasmids harboring the $bla_{\text{OXA-48}}$ gene (n = 23), the cluster 2 contained the two plasmids encoding IMP-like carbapenemase and the cluster 3 contained the remaining two plasmids carrying respectively the $bla_{\text{KPC-4}}$ and bla_{FOX} genes. However, when we use the *excA* gene for comparison, the neighbor-joining algorithm showed three clusters in which the plasmid pOXA48-Pm encoding $bla_{\text{OXA-48}}$ is assigned in the same cluster of the plasmid encoding $bla_{\text{KPC-4}}$ (Fig. 2). Thus, these two plasmid analyses showed different results depending on the gene used for comparison. It's interesting to highlight these all of these plasmids are assigned to the same incompatibility group IncL/M. Our aim to compare plasmids on the basis of conserved genes, her the *repA* and *excA* genes, is to give a useful simplest tool to track the evolution of plasmids.

One limitation of our study is the number of plasmids tested and the number of gene candidates to analyze. It's recommended to validate our results using other types of plasmids and other genes to get a correct view of the plasmids' evolution.

In conclusion, this study suggested that IncL/M plasmids harboring different antibiotic resistance genes have evolved differently.

Table 1: Characteristics of the plasmids used in this study

Accession number	*Species*	Plasmid name	Size of plasmid (pb)	*repA* gene		*excA* gene		Blase
				size (pb)	Position	Size (pb)	Position	
LN864821.1	*Raoultella planticola*	pRA35	63 434	1056	7938-5900	654	56690-57343	OXA-48
JX101693.1	*Enterobacter cloacae*	pEl1573	87 731	1056	51866-52921	654	50610-51263	IMP-4
JQ837276.1	*Enterobacter cloacae*	pNE1280	66 531	1056	23131-24186	654	21877-22530	KPC-4
KC354801.1	*Klebsiella pneumoniae*	pJEG011	71 446	1056	65962-67017	654	64702-65355	OXA-48+CTX-M-14
KM877517.1	*Enterobacter cloacae*	pIMP-HB623	133 208	1056	547-1602	654	132499-133152	IMP-34
KX524525.1	*Klebsiella pneumoniae*	pRAY	49 257	1056	16098-17153	654	14838-15491	OXA-48
HG934082.1	*Klebsiella pneumoniae*	pFOX-7a	90 439	1056	c2428-1373	654	7982-8635	FOX
KX523901.1	*Klebsiella pneumoniae*	pOXA-48_30715	65 488	1056	60004-61059	654	58744-59397	OXA-48
KC335143.1	*Klebsiella pneumoniae*	E71T	63 578	1056	58552-59607	654	57292-57945	OXA-48
KM406491.1	*Klebsiella pneumoniae*	pKpn-E1.Nr7	63 581	1056	32334-33389	654	31074-31727	OXA-48
KP659188.1	*Klebsiella pneumoniae*	pOXA-48E1	62 014	1068	595-1662	654	61361- 62014	OXA-48
JN626286.1	*Klebsiella pneumoniae*	pOXA-48_Ref	61 881	1056	56394-57449	654	55134-55787	OXA-48
KP025948.1	*Proteus mirabilis*	pOXA48-Pm	72 127	1056	527-1582	654	71401-72054	OXA-48
MK121456.1	*Cronobacter malonaticus*	pTr94	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121455.1	*Raoultella ornithinolytica*	pTr76	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121454.1	*Pluralibacter gergoviae*	pTr48	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121453.1	*Citrobacter werkmanii*	pTr43	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121452.1	*Klebsiella pneumoniae*	pTr103	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121451.1	*Enterobacter cloacae*	pTr67B	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121448.1	*Escherichia coli*	pTr73	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121447.1	*Klebsiella pneumoniae*	pTr68A	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121446.1	*Klebsiella pneumoniae*	pTr66A	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121445.1	*Klebsiella pneumoniae*	pTr47	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121444.1	*Escherichia coli*	pTr77	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121443.1	*Escherichia coli*	pTr92	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121450.1	*Escherichia coli*	pTr90	61 881	1056	56394-57449	654	55134-55787	OXA-48
MK121449.1	*Escherichia coli*	pTr78A	61 881	1056	56394-57449	654	55134-55787	OXA-48

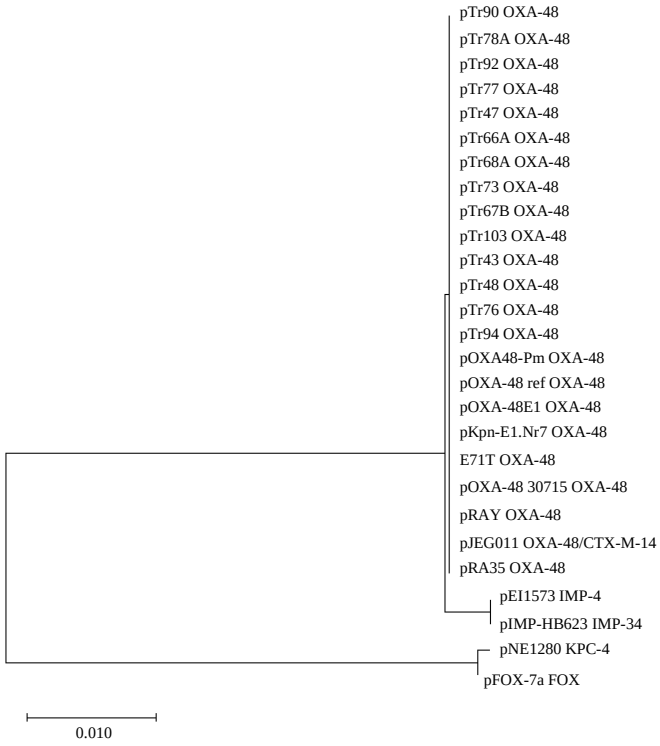

Fig. 1: The evolutionary history inferred using the Neighbor-joining method on basis of *repA* gene

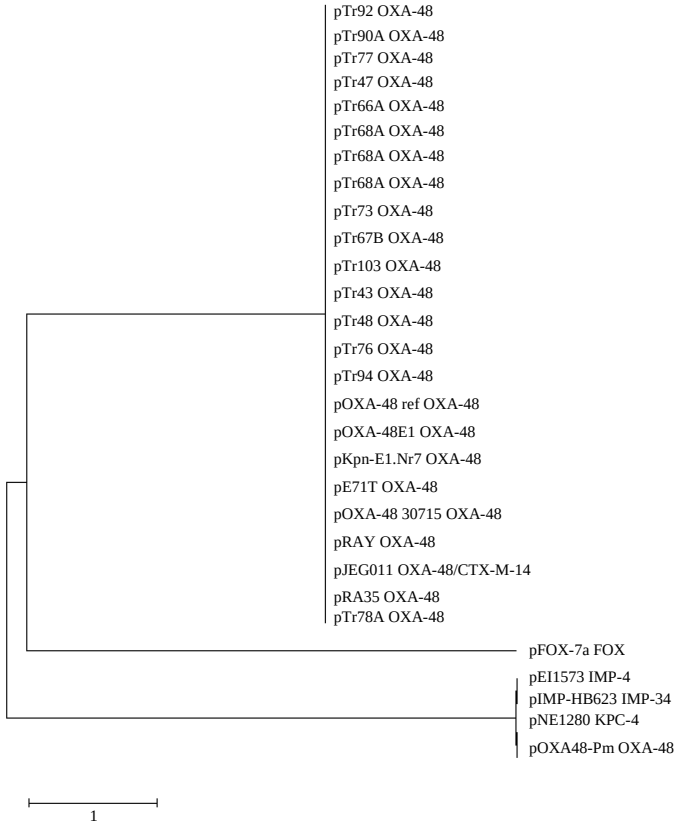

Fig. 2: The evolutionary history inferred using the Neighbor-Joining method on basis of *excA* gene

Acknowledgement

We thank the "*Direction Générale* de la *Recherche Scientifique* et du *développement technologique* (DGRSDT) » *of* Algerian Ministry of Higher Education and Scientific Research"

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.

References

Carattoli, A., 2009. Resistance plasmid families in Enterobacteriaceae. Antimicrob Agents Chemother, 53: 2227-2238. DOI: 10.1128/AAC.01707-08

Carattoli, A., 2013. Plasmids and the spread of resistance. Int. J. Med. Microbiol., 303: 298-304. DOI: 10.1016/J.IJMM.2013.02.001

Cui, X., H. Zhang and H. Du, 2019. Carbapenemases in Enterobacteriaceae: Detection and antimicrobial therapy. Front Microbiol., 10: 1823-1823. DOI: 10.3389/FMICB.2019.01823

Mairi, A., A. Pantel, A. Sotto, J.P. Lavigne and A. Touati, 2018. OXA-48-like carbapenemases producing Enterobacteriaceae in different niches. Eur. J. Clin. Microbiol. Infect. Dis., 37: 587-604. DOI : 10.1007/S10096-017-3112-7

Mairi, A., A. Pantel, F. Ousalem, A. Sotto and A. Touati, 2019. OXA-48-producing Enterobacterales in different ecological niches in Algeria: Clonal expansion, plasmid characteristics and virulence traits. J. Antimicrob Chemother, 74: 1848-55. DOI: 10.1093/JAC/DKZ146

Pitout, J.D.D., G. Peirano, M.M. Kock, K.A. Strydom and Y. Matsumura, 2019. The global ascendency of OXA-48-type carbapenemases. Clin. Microbiol. Rev.

Rozwandowicz, M., M.S.M. Brouwer, J. Fischer, J.A. Wagenaar and B. Gonzalez-Zorn *et al*., 2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J. Antimicrob Chemother, 73: 1121-37. DOI: 10.1093/JAC/DKX488

Touati, A. and A. Mairi, 2019. Carbapenemase-producing Enterobacterales in Algeria: A systematic review. Microb. Drug Resist.