Identification of Carbapenem Resistance genes in the Members of the Family Enterobacteriaceae using in Silico Analysis

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Article Received on 03.12.2016 *Article Published on 05.01.2017*

Abstract

The development of Antimicrobial resistance among the gram negative organisms has been progressive and it is a relentless process. For the past ten years, Carbapenem Resistant Enterobacteriaceae (CRE) members are considered as "Nightmare bacteria" which has disowned the Carbapenem class of antibiotics, the drugs for critically ill patients of last resort. The genes responsible for Carbapenem resistance have been identified in various genera of Enterobacteriaceae family like Escherichia, Salmonella, Enterobacter, Klebsiella and multi drug resistant Pseudomonas aeruginosa (not part of the Enterobacteriaceae). However, there are good chances for the other members of the family Enterobacteriaceae to acquire these genes through horizontal transfer of genomic islands and dissemination of plasmid mediated Carbapenemases. Hence, a study was envisaged to identify various Carbapenem resistance genes in all the members of Enterobacteriaceae to ascertain the possible potential non pathogenic members which might become a threat in the future with the help of In silico tools. BLAST Algorithm was used to identify the similarity in the nucleotide sequences between 13 identified Carbapenem resistance genes among Enterobacteriaceae family. It is found that various non pathogenic genera like Calymmatobacterium, Kluyvera, Leclercia, Morganella, Pantoea, Providencia, Raoultella and Obesumbacterium have been proven to harbor Carbapenem resistance genes like bla AMPc, bla CTX-M, bla GES, bla IMP, bla SHV, bla OXA 48,OMP K, bla VIM, bla VEB, bla KPC, bla NDM etc., This study clearly indicates the presence of carbapenem resistance genes among the Enterobacteriaceae members which may pose a serious threat in the treatment of severe systemic infections and this has also focused on Antiseptic resistance genes in the concerned family.

Keywords: Enterobacteriaceae, *Carbapenem resistance, In silico analysis*

Introduction

Carbapenems are a class of beta-lactam antibiotics that are used primarily to treat infections caused by multi-drug resistant (MDR) bacteria and they are considered to be drugs of last resort. Carbapenems are broad spectrum antibiotics, which means they are able to

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treat both Gram-positive and Gram-negative bacteria, although they are more commonly used to treat Gram-negative bacteria (Papp-Wallace et al., 2011). Carbapenems function by entering the periplasmic space, which is found on Gram-negative bacteria, through porins. These porins are proteins that allow for the diffusion of molecules across the bacterial membrane. Once inside the periplasmic space, carbapenems inhibit penicillin-binding proteins, which are enzymes that aid in bacterial cell wall synthesis by catalyzing the formation of peptidoglycan. The binding of carbapenem to penicillin-binding proteins causes the irreversible loss of catalytic activity, which causes weakening of the peptidoglycan. Ultimately,the bacterial cell ends up lysing due to osmotic pressure. Carbapenems include several clinically-used drugs, such as imipenem,meropenem, ertapenem, and doripenem (Jacob et al., 2013).Compared to other beta-lactams, carbapenems are more likely able to resist extended-spectrum beta-lactamases, making them an effective treatment option for these types of infections (Nicolau,2007); however, carbapenems are not perfect drugs. Recently, Carbapenem-Resistant *Enterobacteriaceae* (CRE) have become a concern in the healthcare industry.

The aim of the present study was to analyse the presence of genes responsible for resistance of carbapenems and antiseptics in all the genera *Enterobacteriaceae* using *In silico* tools.

Materials and Methods

Basic Local Alignment Search Tool is an algorithm for comparing primary biological sequence information, like amino acid sequences of proteins or the nucleotides of DNA sequences. The genes identified to be responsible for Carbapenem resistance are bla KPC -13, bla IMP 14a, bla VIM, bla NDM, bla OXA-48, OMP K 35, OMP K 36,bla TEM, bla SHV, bla CTX-M, bla OKP,bla VEB, bla OXA, bla Amp C, bla GES using NCBI database. A total of 44 genera mentioned in Bergey's manual of Systematic bacteriology second edition (2004) were analysed. *Escherichia, Alterococcus, Arsenophonus, Brenneria, Buchnera, Budvicia, Buttiauxella, Calymmatobacterium, Cedecea, Citrobacter, Edwardsiella, Enterobacter, Erwinia, Ewingella, Hafnia, Klebsiella, Kluyvera, Leclercia, Leminorella, Moellerella, Morganella, Obesumbacterium, Pantoea, Pectobacterium, Phlomobacter, Photorhabdus, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoultella, Saccharobacter, Salmonella, Samsonia, Serratia, Shigella, Sodalis, Tatumella, Trabulsiella, Wigglesworthia, Xenorhabdus, Yersinia* and *Yokenella* are the species analysed using this tool. The genes identified for antiseptic resistance are qac A, qac B, qac C, qac D, qac G, qac H, qac J, qac F, qac Z, smr and the members of the family *Enterobacteriaceae* are analysed.

Results

bla Amp C	***bla CTX-M***	***bla GES***	***bla IMP***
Escherichia *Calymmatobacterium* *Citrobacter* *Enterobacter* *Klebsiella*	*Escherichia* *Calymmatobacterium* *Citrobacter* *Enterobacter* *Klebsiella* *Kluyvera* *Leclercia* *Morganella* *Pantoea* *Proteus* *Providencia* *Raoultella* *Salmonella* *Serratia*	*Escherichia* *Calymmatobacterium* *Citrobacter* *Enterobacter* *Klebsiella* *Serratia*	*Citrobacter* *Enterobacter* *Klebsiella* *Leclercia* *Proteus* *Providencia* *Raoultella* *Salmonella* *Serratia* *Shigella*
bla SHV	***bla OXA -48***	***bla VIM***	
Escherichia *Calymmatobacterium* *Citrobacter* *Enterobacter* *Klebsiella* *Kluyvera* *Pantoea* *Proteus* *Providencia* *Raoultella* *Salmonella* *Serratia* *Shigella* *Yersinia*	*Klebsiella* *bla OMP K* *Escherichia* *Arsenophonus* *Calymmatobacterium* *Cedecea* *Citrobacter* *Enterobacter* *Klebsiella* *Kluyvera* *Leclercia* *Lemiorella* *Obesumbacterium*	*Escherichia* *Calymmatobacterium* *Citrobacter* *Edwardsiella* *Enterobacter* *Klebsiella* *Kluyvera* *Leclercia* *Morgaella* *Pantoea* *Plesiomonas* *Proteus* *Providencia* *Raoultella* *Salmonella* *Serratia* *Shigella*	

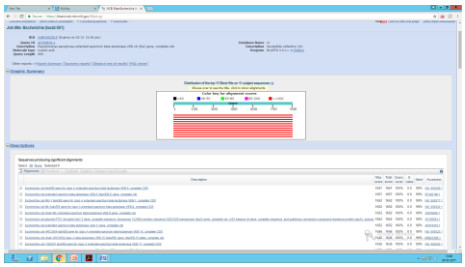
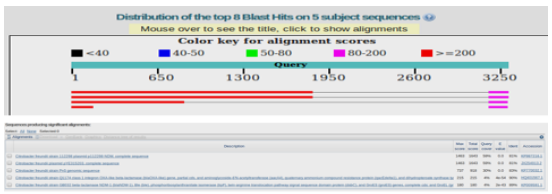


Fig. 1: A picture showing the presence of bla VEB gene in Escherichia



Proteus

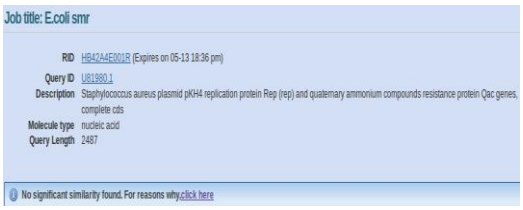


Fig. 2: BLAST results of E.Coli for an antiseptic resistance gene

The antiseptic resistance genes were not found in any of the organisms of *Enterobacteriaceae* family.

Discussion

The genes responsible for carbapenem resistance was previously identified in the genera like *Escherichia, salmonella* and *Klebsiella* spp., But their presence in other species of *Enterobacteriaceae* like *Calymmatobacterium, Citrobcater* and *Kluyvera* suggests the potential of these organisms to carry potent antimicrobial resistance genes in their plasmids. Though these are non pathogenic organisms, they can transfer these resistance genes to other pathogenic members and act as a serious threat in case of patients suffering with systemic infections. In the future there is every possibility for these non pathogenic organisms to become pathogenic and express the resistance to these advanced antibiotics.

Conclusion

Presence of carbapenem and antiseptic resistance genes indicate the possible potential of these organisms to become pathogenic the future through horizontal gene transfer and mutations. Hence there is a need for proper discourse measures to combat the resistance.

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