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A survey of five broad-host-range plasmids in gram-negative bacilli isolated from patients

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ABSTRACT

Objectives: To learn the prevalence of the primary classical broad-host-range (BHR) IncA/C, IncN, IncP, IncQ, and IncW plasmids in dominant gram-negative bacilli from inpatients in a teaching hospital in southern China.
Methods: A multiplex polymerase chain reaction based on the replicons of BHR IncA/C, IncN, IncP, IncQ, and IncW plasmids was developed and used to determine these BHR plasmids. The difference in prevalence rates among the different species from three specimens was evaluated by a binary logistic regression model and the differences between multidrug-resistant organisms (MDRO) and non-MDRO were assessed using a chi-square test.
Results: The average positive detection percentages of the replicons were 4.3%, 3.7%, 3.0%, 2.6%, and 1.9%, respectively, for IncN, IncP, IncQ, IncW, and IncA/C in descending order. The distribution of all five BHR plasmids did not differ significantly between specimens collected from wounds and urine, although both were significantly higher than those of sputum. The prevalence rates of all five BHR plasmids in MDROs were significantly higher than those in non-MDRO for Enterobacteriaceae; however, no significant difference was seen in non-fermenting gram-negative bacilli (NFGNB).
Conclusions: BHR IncA/C, IncN, IncP, IncQ, and IncW plasmids, which occur more often in bacilli from wound and urine specimens than those of sputum, are widespread in *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* strains isolated from inpatients. The prevalence rates in MDRO are higher than those in non-MDRO for Enterobacteriaceae but not significantly different for NFGNB.

1. Introduction

Broad-host-range (BHR) plasmids play a more important role in horizontal gene transfer (HGT) than narrow-host-range (NHR) plasmids because they can transfer and replicate in at least two distinct phylogenetic groups of bacteria such as different subdivisions of the Proteobacteria (Dronen et al., 1998). Resistance genes are usually transferred on plasmids via conjugation among *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae* of Enterobacteriaceae, and *Acinetobacter baumannii*, *Pseudomonas aeruginosa* of non-fermenting gram-negative bacilli (NFGNB) (Bertini et al., 2010; Carattoli, 2009; Pfeifer et al., 2010). In recent years, multidrug resistance in clinically dominant gram-negative bacilli is an emerging concern among health care practitioners worldwide, including often isolated members of Enterobacteriaceae and NFGNB (Falagas and Bliziotis, 2007; Ho et al., 2010, 2011; Yong et al., 2009). IncP, IncA/C, IncN, IncQ, and IncW

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plasmids are the primary classical BHR plasmids in these bacilli that can transfer between the different species among Enterobacteriaceae and NFGNB. An epidemiological investigation of the BHR plasmids in gram-negative bacilli can help us to learn about how important these BHR plasmids are in HGT among these bacilli.

Inc typing is a technique that classifies plasmids by their ability to stably coexist with other plasmids within the same bacterial strain, a trait that is dependent on their replication machinery. Inc typing initially relied on the introduction of a plasmid into a strain carrying another plasmid and the determination of whether both plasmids were stably maintained in the progeny (Lubeck and Hoorfar, 2003). Unfortunately, physical Inc typing proved to be a time-consuming task that was fraught with shortcomings. Couturier et al. developed a classification scheme based on identification of basic replicons using DNA:DNA hybridization (Couturier et al., 1988). However, the use of this technique was still extremely laborious.

The advent of polymerase chain reaction (PCR) enabled novel time-saving methods of plasmid typing (Carattoli et al., 2005; Götz et al., 1996). Carattoli et al. designed a PCR-based method with a total of eight reactions (five multiplex, three simplex reactions) to identify a total of 18 different broad or narrow host range plasmids found in Enterobacteriaceae (Carattoli et al., 2005). However, the detection of BHR IncP, IncA/C, IncN, and IncW plasmids was carried out in four different multiplex PCR, and the group of 18 plasmids does not include the BHR IncQ plasmid. The aim of this work was to develop a multiplex PCR-based replicon typing method for the five BHR plasmids and study their prevalence in *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. cloacae*, *P. aeruginosa*, and *A. baumannii* strains isolated from inpatients.

2. Materials and methods

2.1. In silico analysis of IncA/C, IncN, IncP, IncQ, and IncW replicons

Multiple-sequence alignments of the replication initiation protein genes of BHR IncP, IncA/C, IncN, IncQ, and IncW plasmids were performed using multiple alignment program of DNAMAN software (Lynnon BioSoft, Vaudreuil, Quebec, Canada) set for DNA quick alignment trying both strands with a gap penalty of 7, a K-tuple of 3, 5 top diagonals, and a window size of 5. BHR IncP plasmids included RK1, pTB11, pBS228, pRK415, PB5, pB11, pRKNH3, R751, pTP6, pUO1, pJP4, pB10, pB8, pAKD14, pAKD33, pAKD29, pAKD17, pAKD15, pAKD1, pBP136, pB4, pAKD26, pA1, pKJK5, pAKD34, pAKD16, and pAKD25. IncA/C plasmids included pAM04528, pAR060302, peH4H, pIP1202, pSN254, pP91278, pAPEC1990_61, pPG010208, YUHS07-18, pYR1, pNDM-1_Dok01, pRA1, and pRAx. IncN plasmids included R46, pKM101RC, pKP96, pN3RC, pKOX105RC, pKC394, pKC396, and pNL194. IncQ plasmids included RSF1010, pSRC26, pIE1130, pIE1115, pIE1107, pGNB2RC, pQ7RC, and pAHH04RC. IncW plasmids included pSa, R388RC, pIE321, and R7K.

2.2. Multiplex PCR based on replicon typing

A multiplex PCR was devised to detect five replicons. Specific primer pairs were designed for five different replicons (Table 1) on the basis of sequence alignments of the replication initiation protein genes. Template DNA was prepared from lysis buffer cultures using a boiled lysate method (Johnson and Stell, 2000). The multiplex PCR reaction mixture contained (final concentrations) 1.25 U of Taq polymerase (Fermentas, CA), 0.2 mM deoxynucleoside triphosphates (Fermentas, CA), 1.5 mM $MgCl_2$, and 0.2 μM of each multiplex primers. Thermal cycling conditions for multiplex PCR were as follows: initial denaturation at 95 °C for 3 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and elongation at 72 °C for 60 s. The amplification was concluded with an extension program of one cycle at 72 °C for 5 min.

2.3. Strains and plasmids

IncP plasmid R751 in *E. coli* CM544, IncN plasmid RN3 in *E. coli* j53, IncQ plasmid RSF1010 in *E. coli* DH5α, IncA/C plasmid pAR060302 in *E. coli* DH5α, and IncW plasmid R388 in *E. coli* j53 were kindly provided by Dr. Kornelia Smalla (Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Epidemiology and Pathogen Diagnostics, Braunschweig, Germany). A total of 300 *E. coli*, 390 *K. pneumoniae*, 305 *P. mirabilis*, 296 *E. cloacae*, 310 *P. aeruginosa*, and 407 *A. baumannii* isolates were collected between June 1, 2011 and March 15, 2012 from patients hospitalized in medical (820 patients), surgical (382 patients), gynecological (110 patients), obstetric (80 patients), pediatric (68 patients), and intensive care unit (390 patients) wards of Xiamen University-affiliated Zhongshan Hospital, a teaching hospital in southern China. The 1000 male and 850 female patients were 0–98 years of age (mean, 42 years). Specimens were taken of their wounds, urine, and sputum. Isolates were non-repetitive and only one isolate of the same species per patient was collected. And, only one isolate for a type of specimen from the same department was adopted for the same species of strains with identical antibiograms within a week. The strains were identified using Vitek 32 system (bioMérieux, France). According to Clinical and Laboratory Standards Institute 2011 criteria, antimicrobial susceptibility testing was performed using the disk-diffusion method (Kirby-Bauer). The *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853 strains were used for quality control. An isolate was defined as a multidrug-resistant organism (MDRO) if it was resistant to representative antibiotics of at least three different antimicrobial classes (Magiorakos et al., 2012).

2.4. Positive controls

BHR R751, RN3, RSF1010, pAR060302, and R388 plasmids were initially used as positive controls. The wild-type strains harboring five different BHR plasmids were used as positive controls for the multiplex PCR. All of the amplicons on the wild-type control strains were cloned into a TA-cloning vector (TaKaRa pMD® 19-T Vector, Dalian, China) and transformed into competent *E. coli* DH5α cells