Molecular and clinical epidemiology of carbapenem-resistant Enterobacter aerogenes strains isolated from a tertiary hospital in China

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Abstract

Purpose: The frequently reported carbapenem-resistant Enterobacteriaceae worldwide called for alarm. In this study, the resistance mechanism and epidemiology of carbapenem-resistant Enterobacter aerogenes (CREA) which isolated in a Chinese tertiary hospital were investigated. Methods: Antimicrobial susceptibility testing of 17 antibiotics were performed. β-lactamase genes were detected. Efflux pump phenotype test and outer membrane porin SDS-PAGE were performed. Clonal relatedness was investigated by PFGE (pulsed field gel electrophoresis). S1 nuclease-PFGE and plasmid incompatibility groups analysis were performed to analyze blaKPC-2 carrying plasmids. Epidemiological data were collected via chart review. Results: Only tigecycline and colistin maintained high level susceptibility to 14 CREA isolates. Five distinct groups (PFGE types A-E) were observed. All 14 isolates carried the blaKPC-2 gene. Only one isolate lost Omp36 porin. Efflux pump had no contribution to these 14 isolates. S1 nuclease-PFGE indicated that the size of blaKPC-pasmids were approximately 20kb to 200kb. 14 blaKPC carrying plasmids belonged to variant inc groups. Epidemiological and molecular investigations showed that CREA type A, including 11 KPC-2 producing clinical isolates, was primarily responsible for the dissemination. Conclusion: Our findings suggest that healthcare facility should focus on judicious use of available antibiotics and strict implementation of infection-control measures to avoid the rapid spread or clonal dissemination caused by CREA in healthcare facilities.

Methods

2.1 Antimicrobial Susceptibility

Antimicrobial susceptibility testing was performed by disc diffusion and broth microdilution method based CLSI guidelines. In addition, MICs of imipenem were determined in the presence of 25 μg/mL efflux pump inhibitor PAJN in order to investigate the role of efflux pump in carbapenem-resistant isolates.

2.2 PFGE

Pulsed-field gel electrophoresis (PFGE) of XbaI-digested genomic DNA samples of Enterobacter aerogenes isolates was performed [1].

2.3 Detection of carbapenem resistance

a. Detection of β-lactamase genes: All Enterobacter aerogenes isolates were screened for carbapenemases, plasmid-mediated AmpC β-lactamases and β-lactamases by PCR [2].

b. Analysis of outer-membrane proteins

2.4 characteristics of resistance plasmids

a. Transformation and S1 nuclease-PFGE

14 CREA strains producing blaKPC-2 served as the donors. S1 nuclease-PFGE was performed to analyze the location of blaKPC-2 gene [3]. b. Incompatibility groups analysis of blaKPC-2 carrying plasmids

The potential plasmids were identified by PlasmidFinder and plasmid typing was performed through pMSI software [4].

2.6 Clinical epidemiology

Epidemiological data were collected via chart review for each patient from the hospital’s uniform electronic database. The following parameters were assessed: (1) general demographics, such as age, sex and other background information; (2) the ward to which the patient was assigned after admission; (3) previous use of antibiotics, particularly carbapenams. Results

3.1 Susceptibility testing

The MICs of the 14 isolates to 17 antimicrobial agents are listed in Table 1. Efflux pump inhibitor PAJN did not down-regulate MICs of carbapenams in any of the CREA isolates tested.

3.2 PFGE

Five distinct PFGE groups (types A–E) were observed among the 14 CREA isolates; type A respectively included 71.4% (10/14) of the isolates. The epidemiological data of three patients (A25, A31 and A32) were not available, because these patients were hospitalized in branch hospital. Other 11 CREA isolates were detected in some isolates (Table 2).

3.3 β-Lactamase characterization and clinical epidemiology

Among 14 isolates, all the strains carried the blaKPC-2 gene, blaSHV-11, blaCTX-M-15, and blaTEM-14 were detected in some isolates (Table 2). The epidemiological data of three patients (A25, A31 and A32) were not available, because these patients were hospitalized in branch hospital. Other 11 CREA isolates were identified from 11 patients (8 male and 3 female, ages 14-67 years) hospitalized on the surgery ward of Huashan Hospital, except one (A61) hospitalized on the ICU ward.

3.4 Analysis of outer-membrane proteins

SDS-PAGE analysis revealed A52 lost Omp36 porin, and no porin loss was found in other 13 CREA isolates (Figure 2)

5.3 plasmid analysis

S1 nuclease-PFGE indicated that the size of blaKPC-pasmid in 14 isolates were approximately among 20kb to 200kb IncFII and IncN are two preponderant plasmid groups detected in blaKPC-2 plasmids (Table2).

Discussion

Enterobacteriaceae spread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons. The characteristics of easy-to-spread and hard-to-control made it a great challenge to clinical treatment. In our research, KPC-2 producing was the major carbapenem resistance mechanism of CREA. KPC-producing isolates were easy to cause clonal spread in the hospital. As only few novel antimicrobials are in development for the treatment of these extensively drug-resistant infections, judicious use of available antibiotics and implementation of strict infection-control measures to avoid the rapid spread of CRE in hospital is extremely urgent.

Reference