·研究报告·

The correlation study on antimicrobial resistance and biofilm related genes in clinical isolates of *Acinetobacter baumannii*

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In recent years, the proportion of nosocomial infections caused by *Acinetobacter baumannii* (Ab) strains has increased significantly, and its resistance to antibiotics is rising. The resistance mechanisms of Ab are complex, which include the integron formation, inactivating or deactivating enzyme, outer membrane permeability, biofilm formation, drug exocytosis mechanism and so on. The biofilm formation by bacteria leads to high resistance and immune evasion ability. The aim of this study is to investigate the resistance and distribution patterns of Ab isolates, and the biofilm formation related genes in Ab isolates in our hospital.

MATERIALS AND METHODS

Data sources

The drug resistance profiles of 210 strains of Ab isolated from various specimens in the intensive care unit (ICU) from January 2008 to December 2010 were analyzed retrospectively. Additionally, 72 strains of Ab isolated by clinical laboratory of the hospital from September 2011 to July 2012 were collected (the duplicate isolates from the same source of a patient were excluded). VITEK-2 automatic bacteria identification system was applied for species identification and drug susceptibility test.

Testing and evaluation methods

The antimicrobial sensitivity test was carried out by the Kirby-Bauer method, which was recommended by the World Health Organization (WHO). The result was judged following the standard of Clinical and Laboratory Standards Institute (CLSI). The DNA of 72 strains Ab was extracted by the boiling method. Polymerase chain reaction (PCR) assay was applied to detect biofilm formation related genes—3 genes of the pili assembly system (*csuC*, *csuD* and *csuE*), outer membrane protein A gene (*ompA*), *PER-1* type extended-spectrum beta-lactamases gene (*blaPER-1*) and Autoinducer synthase gene (*abaI*). The primer design and annealing temperatures were followed that of the reported protocols [1-3]. The PCR assay was performed in a final reaction volume of 25 μl [15.3 μl ultrapure water, 2.5 μl 10 × Taq buffer which contains 15 mmol/L $MgCl_2$, 2 μl deoxynucleoside triphosphates (dNTPs), 1 μl (each) reverse and forward primers; 0.2 μl of Taq DNA polymerase and 3 μl of bacterial lysate (supernatant with template DNA)]. Amplifications were performed with the GeneAmp PCR System 9600. The PCR amplification was started with the initial denaturation at 94 ℃ for 10 minutes, then 35 cycles of denaturation at 94 ℃ for 30 seconds, annealing for 30 seconds, extension at 72 ℃ for 60 seconds. A final elongation at 72 ℃ for 10 minutes was conducted. Finally, the PCR amplification products were stored at 4 ℃. The PCR amplification product was separated by gel electrophoresis on 2.0% agarose (product length <500 bp) or 1.2% agarose (product length >500 bp), stained with ethidium bromide, and the gel image was captured digitally with UV transillumination for observation.

Statistical treatment

The data was analyzed by using the SPSS 17.0. The Chi-square test or Fisher exact test was used to analyze the antimicrobial resistance of gene positive strains and gene negative strains. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

The drug resistance of Ab from 2008 to 2010 in ICU increased to 16 kinds of antibiotics, with the exception of gentamicin and sulfamethoxazole. The increase was dramatic for imipenem and meropenem with the increase rate from 4.35% up to over 95%. The nosocomial pan-drug Ab (PDR-Ab) increased from 1 strain (4.35%) in 2008 to 50 strains (39.68%) in 2010. Of the 72 specimens, there were 53 sputum samples, 5 catheter terminal samples, 4 blood samples, 3 throat swabs and 3 urine, 1 each from hydrothorax, ascites, cerebrospinal fluid and bronchoalveolar lavage fluid. Most of the specimens were obtained in the ICU (45.83%), Department of Pneumology (11.11%), Department of Emergency (9.72%) and Cardiac Surgery (8.33%). The PCR results showed that *csuC*, *csuD*, *csuE*, *ompA*, *blaPER-1* and *abaI* gene amplification positive strains were 61 (84.72%), 58 (80.56%), 51 (70.83%), 66 (91.67%), 8 (11.11%) and 54 (75.00%), respectively. Among them, all of the 6 genes were detected in 4 strains of specimens, and no above mentioned genes were detected in other 3 strains. The drug resistance was compared between biofilm related genes positive strains and negative strains. The antimicrobial resistance of *AbaI* positive strains was higher than those in abaI negative strains, and the difference was statistically significant (P <0.01). The antimicrobial resistance of *csuC* positive strains was significantly higher than those in negative strains, but there was no significant

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Table1　Analysis of the correlation between antimicrobial resistance and *abaI*, *csuC* in 72 strains of *Acinetobacter baumannii*

antimicrobial	resistance (%)	*abaI* resistance(%)			*csuC* resistance(%)		
		positive	negative	*P* value	positive	negative	*P* value
ampicillin	77.78	88.89	44.44	<0.001	78.69	72.73	>0.05
cefepime	68.06	79.63	33.33	<0.001	73.77	36.36	0.036
ampicillin-sulbactam	65.28	77.78	27.78	<0.001	70.49	36.36	>0.05
aztreonam	81.94	90.74	55.56	0.003	81.97	81.82	>0.05
cefazolin	100.00	100.00	100.00	>0.05	100.00	100.00	>0.05
cefotetan	100.00	100.00	100.00	>0.05	100.00	100.00	>0.05
ceftazidime	69.44	83.33	27.78	<0.001	75.41	36.36	0.026
ceftriaxone	73.61	85.19	38.89	<0.001	80.33	36.36	0.008
ciprofloxacin	69.44	81.48	33.33	<0.001	75.41	36.36	0.026
gentamicin	68.06	81.48	27.78	<0.001	73.77	36.36	0.036
imipenem	65.28	77.78	27.78	<0.001	70.49	36.36	>0.05
furadantin	98.61	98.15	100.00	>0.05	98.36	100.00	>0.05
tobramycin	66.67	81.48	22.22	<0.001	72.13	36.36	0.049
sulfamethoxazole	68.06	81.48	27.78	<0.001	73.77	36.36	0.036
cefnixime	100.00	100.00	100.00	>0.05	100.00	100.00	>0.05
piperacillin	65.28	77.78	27.78	<0.001	70.49	36.36	>0.05

difference among most of the antibiotics (table 1).

DISCUSSION

The antimicrobial resistance of Ab in ICU is increasing during the period of 2008 to 2010, and so was the isolation rate of PDR-Ab. The United States Centers for Disease Control and Prevention experts estimated that 65% of the human bacterial infections was related to biofilms [4]. Many chronic and refractory infections are associated with biofilm formation of the microorganisms. Along with the new invasive biological materials being increasingly used, the rate of biological materials associated infections was also increased year by year. The main pathogen of apparatus-associated infections is Ab. In our study most of the specimens were obtained from ICU, Department of Pneumology, Department of Emergency, Cardiac Surgery and other departments where medical materials and devices (all kinds of catheters, artificial valves and pacemakers, etc.) were widely used. Over 90% of specimens were also closely related with the application of invasive materials, for example, sputum, catheter tip, blood, urine and so on.

Biofilm formation is one of the mechanisms of bacterial resistance. Few studies have been done on biofilm in Ab. Adhesion is the initial stage of biofilm formation, and it is crucial in the process. CsuA/BABCDE pili assembly system plays an important role in Ab pili formation, adhesion to plastic surface, and subsequent formation of biofilm. OmpA is the main component of Ab outer membrane proteins, main role of which is to maintain the integrity of the outer membrane. Gaddy et al [5] showed that OmpA played a part of role in biofilm formation, but it was absolutely necessary for bacteria and fungal filaments to adhere to the epithelial cells. These processes did not depend on CsuA/BABCDE mediating pilus synthesis. Niu et al [6] identified the important gene abaI in Ab Quorum System, which was found to play an important role in the maturation stage of biofilm formation process. The gene inactivation led to the damage of biofilm formation. Another study showed that Ab strains with *blaPER-1* possessed stronger epithelial cells adhesion and biofilm formation ability, as compared with negative ones. We studied these biofilm formation related genes simultaneously for the first time, and found that all of them possessed high positive rates, except *blaPER-1*. *AbaI* was significantly associated with the drug resistance of Ab. Because we could not detect the biofilm formation ability at the same time, the role of *abaI* in Ab biofilm formation could not be assessed unfortunately. The presence of *blaPER-1* in our hospital gave us an alarm to guard against potential spread of these resistant strains. In conclusion, most studies on Ab have focused on the prevalence of drug resistance and resistant genes up to the present. There has been little research on biofilm and related genes. This study could contribute to the further elucidation of the mechanisms of Ab resistance. Attention should be paid to biofilm resistance mechanism of Ab by clinicians. Many factors affect Ab biofilm formation and further studies are necessary to look for novel strategies and therapy against infections produced by biofilm forming micro-organisms.

REFERENCES

[1] Wei X, Shen DX, Luo YP, et al. Molecular mechanism of biofilm formation in *Acinetobacter baumannii*. Chin J Nosocomiol, 2010, 20: 2735-2738.

[2] Turton JF, Gabriel SN, Valderrey C, et al. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of *Acinetobacter baumannii*. Clin Microbiol Infect, 2007, 13: 807-815.

[3] Anbazhagan D, Mansor M, Yan GO, et al. Detection of quorum sensing signal molecules and identification of an autoinducer synthase gene among biofilm forming clinical isolates of *Acinetobacter* spp. PLoS One, 2012, 7: e36696.

[4] Potera C. Forging a link between biofilms and disease. Science, 1999, 283: 1837, 1839.

[5] Gaddy JA, Tomaras AP, Actis LA. The *Acinetobacter baumannii* 19606 *OmpA* protein plays a role in biofilm formation on abiotic surfaces and in the interaction of this pathogen with eukaryotic cells. Infect Immun, 2009, 77: 3150-3160.

[6] Niu C, Clemmer KM, Bonomo RA, et al. Isolation and characterization of an autoinducer synthase from *Acinetobacter baumannii*. J Bacteriol, 2008, 190: 3386-3392.

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