THE EXPRESSION OF GENES ADEB AND ADEJ IN PATIENTS RESISTANT TO DRUG TIGECYCLINE

HONGBO LIU, SHENGQI LI
[1]Department of Respiratory Medicine, Shengjing Hospital of China Medical University, Shenyang, Liaoning 110004, P.R. China

ABSTRACT

Objective: *To investigate the effect of overexpression of efflux pump genes adeB and adeJ on the sensitivity of tigecycline resistance in carbapenems-resistant Acinetobacter baumannii strains. Methods: A total of 137 strains of meropenem-and imipenem-resistant Acinetobacter baumannii isolates from in-patient samples from our hospital within the period May 2016 to November 2017 were selected. The ATCC25922 E. coli quality control strain was used to determine the minimal inhibitory concentrations (MIC) of tigecycline against Acinetobacter strains, with broth microdilution method and efflux pump inhibition experiments. The relative expression levels of adeB and adeJ, were determined with RT-PCR, and their differences were analyzed.*

Results: *A total of 137 strains of carbapenems-resistant Acinetobacter baumannii were collected. They comprised 25 (18.25 %) tigecycline non-susceptible A. baumannii (TNAB) strains and 112 (81.75 %) tigecycline-susceptible A. baumannii (TSAB) strains. After exposing the strains to efflux pump inhibitors, the MIC of tigecycline non-susceptible A. baumannii was significantly decreased ($p<0.05$). There were no statistically significant differences between frequencies of adeB and adeJ between tigecycline non-susceptible A. baumannii strains and tigecycline susceptible A. baumannii strains ($p>0.05$). However, the relative expression levels of adeB and adeJ in tigecycline-susceptible A. baumannii strains were lower than those in the tigecycline non-susceptible A. baumannii ($p<0.05$).*

Conclusion: *Overexpressions of efflux pump genes adeB and adeJ reduce the sensitivity of tigecycline resistance in carbapenems-resistant Acinetobacter baumannii strains.*

Keywords: *Acinetobacter baumannii, Tigecycline, Efflux pump gene, Drug sensitivity.*

DOI: 10.19193/0393-6384_2019_2_160

Received *November 30, 2018;* ***Accepted*** *January 20, 2019*

Introduction

Acinetobacter baumannii is a gram-negative bacillus without fermentation and motivation[1]. As a conditional pathogen, *Acinetobacter baumannii* often infects people with low resistance, causing meningitis, endocarditis, wound infections and genito-urinary tract infections. In severe cases, it also results in sepsis, eventually leading to death. It is one of the pathogens responsible for nosocomial infections[2-3]. Tigecycline, a glycocycline antibiotic with broad-spectrum antibacterial activity, is effective against various gram-negative and positive bacteria, as well as anaerobic bacteria. In recent years, carbapenem-resistant *Acinetobacter baumannii* has become a common pathogen in hospitals due to excessive use of antibiotics, and tigecycline is one of the few effective antibacterial drugs used for eliminating it[4].

Many studies have demonstrated that efflux pump genes are closely related to drug-resistant Acinetobacter baumanniìi[5-6]. The present study was carried out to investigate the effect of overexpression of the efflux pump genes adeB and adeJ on sensitivity of carbapenem-resistant *Acinetobacter baumannii* to tigecycline, using *Acinetobacter baumannii* strains isolated from in-patients from our hospital.

Materials and Methods

Bacterial strains

A total of 137 strains of meropenem- or imipenem-resistant *Acinetobacter baumannii* isolated from in-patients in our hospital from May 2016 to November 2017 were selected. All strains were identified as *Acinetobacter baumannii*. The quality control strains were Escherichia coli strain ATCC25922, which was provided by Guangdong Huankai Microbial Sci. & Tech. Co., Ltd.

Major instruments and reagents

The instruments and reagents used and their sources (in brackets) were: automatic bacteria identification apparatus (Bio Mérieux, France, model: VITEK 2 Compact); low temperature-high-speed centrifuge (Ai Bend, Germany, model: 524r); carbon dioxide constant-temperature incubator (Jinan Xin Beisi Biotechnology Co., Ltd., model: QP- 80); fluorescence quantitative PCR apparatus (BIO-Rad, USA. model: CFX 96Touch), and gel electrophoresis imaging analyzer (Shanghai JiaPeng Technology Co., LTD., model: ZF-288). Others were super-clean worktable (Shanghai Precision Instrumentation Co., Ltd., model: VD-650); reverse transcription kit (Fermentas, USA, model: K1622); real-time PCR kit (BIO-Rad, USA); bacterial lysate (Shanghai Times) Biotechnology Co., Ltd.; injectable tigecycline (Zhengda Tianqing Pharmaceutical Group Co., Ltd., specification: 5 mg/bottle, batch number: 2016010732); nutrient broth (BD Difco, USA), and nutrient agar (BD Difco, USA).

Methods

The meropenem- or imipenem-resistant *Acinetobacter baumannii* isolated from the in-patient specimens and identified using the automatic bacteria identification apparatus was incubated in a 37 °C carbon dioxide incubator for 24 h. *Acinetobacter baumannii* and Escherichia coli were inoculated aseptically on agar medium and cultured at 37 °C for 24 h. The sensitivity of carbapenem-resistant *Acinetobacter baumannii* to tigecycline was tested with micro-broth dilution method, to determine the MIC against *Acinetobacter baumannii*. The criteria of tigecycline sensitivity was consistent with the interpretation criteria issued by the European Antibacterial Sensitivity Test Committee as adopted by Zheng et al.[7]. Changes in the MIC of tigecycline-sensitive and -insensitivity strains were determined by efflux pump inhibition experiments. The strains were incubated on agar medium in a germ-free condition and cultured in environment of 37 °C overnight. Single bacterial colonies were selected and configured into bacterial liquid. After complete dissociation and centrifugation, the supernatant was used to isolate total RNA, and the RNA was subjected to RT-PCR to determine the relative expression levels of efflux pump genes adeB and adeJ in tigecycline-sensitive and -insensitive strains.

Observation indicators

The observation indices were sensitivity of carbapenem-resistant *Acinetobacter baumannii* to tigecycline, changes in the MIC of tigecycline in each strain after efflux pump inhibition test, positive expressions of the efflux pump genes adeB and adeJ, and the relative expression levels of the efflux pump genes adeB and adeJ.

Statistical Analyses

All data were entered into the computer by two persons working independently, so as to ensure accuracy of data entry. The data were analyzed with SPSS software version 21.0. Measurement data were analyzed with t-test, while enumeration data were analyzed with chi square test. $p<0.05$ were taken as indicating statistically significant differences.

Results

Sensitivity of carbapenem-resistant Acinetobacter baumannii to tigecycline

The results of micro-broth dilution test showed that there were 112 tigecycline-sensitive strains of carbapenem-resistant *Acinetobacter baumannii* in 137 strains, accounting for 81.75 %, and 25 strains of tigecycline-insensitive strains, accounting for 18.25 %.

Changes in the MICs of tigecycline-sensitive and tigecycline–insensitive strains after efflux pump inhibition test

The growth of two tigecycline-sensitive *Acinetobacter baumannii* strains and 11 tigecycline-insensitive *Acinetobacter baumannii* strains was well after culturing with efflux pump inhibitors. The MIC of tigecycline against tigecycline-sensitive *Acinetobacter baumannii* strains was slightly lower than that before culture ($p > 0.05$). On the other hand, the MIC of tigecycline against tigecycline -insensitive *Acinetobacter baumannii* strain to tigecycline was much lower than that before culture ($p<0.05$). These results are shown in Table 1.

MIC (μg/ml)	Tigecycline-sensitive strain (n=2)	Tigecycline-insensitive (n=11)
Before culture	0.47±0.11	7.52±1.06
After culture	0.42±0.13	1.23±0.39
T	0.415	18.470
P	0.718	<0.0001

Table. 1: Comparison of the MICs of tigecycline in the two strains before and after efflux pump inhibition test.

Comparison of the presence of efflux pump genes adeB and adeJ in tigecycline-sensitive and non-sensitive strains

As shown in Table 2, in the tigecycline-sensitive and -insensitive *Acinetobacter baumannii* strains, the levels of efflux pump gene adeB were 92.86 % (104/112) and 100.00 % (25/25), respectively, and those of the efflux pump gene adeJ were 95.54 % (107/112) and 96.00 % (24/25), respectively. The differences were not statistically significant ($p>0.05$).

Group	N	adeB	adeJ
Tigecycline-sensitive strain	112	104	107
Tigecycline-insensitive strain	25	25	24
χ^2		0.820	0.011
P		0.365	0.918

Table. 2: Distribution of efflux pump genes adeB and adeJ in tigecycline-sensitive and non-sensitive strains (n).

Comparison on the relative expression levels of efflux pump genes adeB and ade J in tigecycline-sensitive and -insensitive strains

The relative expression levels of efflux pump gene adeB and adeJ were much lower in the tigecycline-sensitive *Acinetobacter baumannii* strain than in the tigecycline-insensitive *Acinetobacter baumannii* strain ($p<0.05$), as shown in Table 3.

Group	N	adeB	adeJ
Tigecycline-sensitive strain	112	2.23±0.81	4.97±0.68
Tigecycline-insensitive strain	25	41.02±10.24	27.49±6.93
T		-18.927	-16.231
P		<0.001	<0.001

Table. 3: Relative expression levels of efflux pump genes adeB and ade J in tigecycline-sensitive and -insensitive strains.

Discussion

With strong clonal transmission, *Acinetobacter baumannii* often gives rise to infection when there is a decline in immunity, resulting in urinary tract, soft tissue, skin and wound infections, thereby prolonging hospital stay, and posing a threat to survival of the patient[8]. Due to unreasonable use of antibacterial drugs and the drug resistance of *Acinetobacter baumannii*, carbapenem-resistant *Acinetobacter baumannii* strains have continued to evolve, and drugs effective against carbapenem-resistant *Acinetobacter baumannii* are becoming fewer, thus increasing the incidence of carbapenem-resistant *Acinetobacter baumannii* infection[9].

Tigecycline is a glycyltetracycline antibiotic which is also known as diglyconin. Intravenous injection of tigecycline down-regulates bacterial protein synthesis, thereby inhibiting bacterial growth[10]. Tigecycline has strong antibacterial activity against gram-negative, gram-positive and anaerobic bacteria. Since it was introduced in the United States in 2005, it has been effectively used for treating bacterial, pneumonia, abdominal cavity and skin infections in adults aged 18 years and above[11]. The adverse reactions associated with tigecycline include mild-to-moderate nausea and vomiting and other gastrointestinal symptoms, which can be treated without special interventions. In addition, it has no obvious deleterious effects on the liver. Thus, the dose can be adjusted based on state of the patient's kidney, and the drug is more convenient than other antibiotics[12-13]. Currently, tigecycline is the drug of choice against Acinetobacter baumannii, but the number of tigecycline-resistant *Acinetobacter baumannii* has gradually increased in recent years. Therefore, there is need to unravel the mechanism underlying the resistance of *Acinetobacter baumannii*[14].

Findings from several studies have shown that multiple types of pathogens exist in efflux pump system, which is closely related to the drug resistance of pathogens[15-16]. Being the main efflux pump genes in *Acinetobacter baumannii*, adeB and adeJ express their corresponding proteins and discharge various antibiotics from bacteria with the help of Na+/proton coupling exchange to reduce or eliminate the effect of antibiotics, resulting in drug resistance by *Acinetobacter baumannii*[17].

Results from micro-broth dilution method revealed that there were 25 tigecycline-insensitive carbapenem-resistant *Acinetobacter baumannii*

strains, accounting for 18.25 % of the total isolates. After the intervention of efflux pump inhibitors, the MIC of tigecycline-insensitive carbapenem-resistant *Acinetobacter baumannii* strain was significantly reduced, while there was no significant change in the MIC of the tigecycline-sensitive strains. This suggests that the expressions of the efflux pump genes are related to the sensitivity of carbapenem-resistant *Acinetobacter baumannii* to tigecycline. The efflux pump genes adeB and adeJ were present in tigecycline-sensitive and -insensitive carbapenem-resistant *Acinetobacter baumannii* strains, but the relative expression levels of adeB and adeJ were much higher in the tigecycline-insensitive strains than in the tigecycline-sensitive strains. This suggests that the higher the relative expression levels of efflux pump genes adeB and adeJ, the lower the sensitivity of carbapenem-resistant *Acinetobacter baumannii* to tigecycline.

Conclusion

The expression levels of the efflux pump genes adeB and adeJ are closely associated with the drug resistance of carbapenem-resistant *Acinetobacter baumannii*. The overexpressions of adeB and adeJ may reduce the sensitivity of carbapenem-resistant *Acinetobacter baumannii* to tigecycling.

References

1) Meshkat Z, Salimizand H, Amini Y, Khakshoor M, Mansouri D, et al. Molecular characterization and genetic relatedness of clinically Acinetobacter baumanii isolates conferring increased resistance to the first and second generations of tetracyclines in Iran. Ann Clin Microbiol Antimicrob 2017; 16: 51.
2) Jia X, Dai W, Ma W, Yan J, He J, et al. Carbapenem-Resistant E. cloacae in Southwest China: Molecular Analysis of Resistance and Risk Factors for Infections Caused by NDM-1-Producers. Front Microbiol 2018; 9: 658.
3) Li N, Huang YF, Tang YY, Li F, Liu L, et al. Meta-analysis on risk factors for healthcare-associated infection with mul-tidrug-resistant Acinetobacter baumannii. Chin J Infect Control 2017; 16: 115-120.
4) Lin W. Clinical study of tigecycline in the treatment of severe pneumonia caused by multiple or pan-resistant Acinetobacter baumannii. Nat Chronic Dis Treat Forum 2016; 11: 53-53.
5) Bhattacharyya T, Sharma A, Akhter J, Pathania R. The small molecule IITR08027 restores the antibacterial activity of fluoroquinolones against multidrug-resistant acinetobacter baumannii by efflux inhibition. Int J Antimicrob Agents 2017; 50: 219-226.
6) Wu WQ, Zhang YP, Li ZC. Study on efflux pump resistance mechanism of carbapenem resistant acinetobacter baumannii. Guangdong Med J 2017; 38: 519-521.
7) Zheng W, Xu Y, Ling BD. Comparison of three methods in vitro Susceptibility test of tigecycline aganist acinetobacter baumannii. J Food Sci Biotechnol 2016; 35: 734-738.
8) Li JP. Resistance to β-Lactam Antibiotics and Relative Genes of Acinetobacter baumanii. Amino Acids Biotic Res 2016; 38: 32-36.
9) Guth C, Cavalli Z, Pernod C, Lhopital C, Wey P, et al. Prompt control of an imported carbapenem-resistant Acinetobacter baumanii outbreak in a French intensive care unit. Med Et Sante Trop 2016; 26: 110.
10) Rosa FGD, Silvia C, Giovanni DP, Scaglione F. Re-defining tigecycline therapy. New Microbiol 2015; 38: 121-36.
11) Liebenstein T, Schulz LT, Viesselmann C, Bingen E, Musuuza J, et al. Effectiveness and Safety of Tigecycline Compared with Other Broad-Spectrum Antimicrobials in Abdominal Solid Organ Transplant Recipients with Polymicrobial Intraabdominal Infections. Pharmacother 2016; 37: 151.
12) Wu SL, Liu YH, Huang YF, Chen C, Zhou H, et al. Centralizd monitoring and analysis on the clinic use of tigecycline based on the real world. Chin J Hospital Pharm 2018; 5: 468-473.
13) Zhong X, Chen DK, Xu HT, Chen G, Hu X. New advance in studies on tigecycline. Chin J Antibiotics 2015; 40: 870-875.
14) Alfizah H, Sarah OZS, Noraziah MZ. Interaction of imipenem and tigecycline against carbapenem-resistant Acinetobacter baumanii: The relativity of synergism. Sains Malaysiana 2015; 44: 1061-1065.
15) Kincses A, Szabó Á M, Saijo R, Watanabe G, Kawase M, et al. Fluorinated Beta-diketo Phosphorus Ylides Are Novel Efflux Pump Inhibitors in Bacteria. Vivo 2016; 30: 813.
16) Dou Q, Zou M, Li J, Wang H, Hu Y, et al. AdeABC efflux pump and resistance of Acinetobacter baumannii against carbapenem. J Central South Univ 2017; 42: 426-433.
17) Bao QL, Ke J, Hu F, Zhang LH, Huang Y, et al. Study on Aminoglycoside Pan Resistance Related Genes of Acinetobacter Baumannii. Med Inf 2018; 31: 378.

Corresponding Author:
Shengqi Li
Email: xr1185@163.com
(China)