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DRUG RESISTANCE AND MOLECULAR EPIDEMIOLOGY OF ACINETOBACTER BAUMANNII ISOLATED FROM PATIENTS WITH INTRACRANIAL INFECTION AFTER NEUROSURGERY

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Abstract: To study the drug resistance and molecular epidemiological characteristics of Acinetobacter baumannii (AB) isolated from patients with intracranial infection (ICI) after neurosurgery. From March 2019 to April 2021, 107 ICI patients and 102 non-ICI patients with AB detected in the cerebrospinal fluid culture of the Neurosurgery Department of Critical Care Medicine, Changjiang Shipping General Hospital were selected as the research objects. The clinical data of the two groups were collected, and the cerebrospinal fluid of the ICI patients was collected. Bacterial culture and drug susceptibility test were carried out, and the molecular epidemiological characteristics of AB were analyzed by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) techniques. The age and proportion of diabetes in the ICI group were higher than those in the non-ICI group (P <0.05). A total of 107 AB strains were detected in 107 ICI patients, and all of them had different degrees of resistance to commonly used antibacterial drugs. PFGE showed that 107 AB strains were divided into 7 clonotypes, including 91 strains of type A, 6 strains of type B, 4 strains of type C, 2 strains of type D, 2 strains of type E, 1 strain of type F and 1 strain of type G. MLST typing results showed that 107 strains of AB could be divided into 14 ST genotypes, among which the more common genotypes were ST208, ST195, ST75, ST92 and ST1696, and the number of strains were 64, 17, 9, 5 and 3, respectively. All other ST genotypes were 1 strain. Homology analysis by eBUST software showed that there were 97 strains (90.65%) of genotypes ST75, ST92, ST136, ST138, ST195 and ST208, all of which belonged to clonal complex 92 (CC92) and only the gpi allele locus existed The remaining 10 strains belonged to 8 ST genotypes. Patients with ICI after neurosurgery are older and often complicated with diabetes mellitus. The AB flora has certain resistance to the commonly used antibacterial drugs at the present stage, and the results of PFGE and MLST both show the prevalence of dominant clones.
Keywords: Neurosurgery; Intracranial infection; Acinetobacter baumannii; Drug resistance; Epidemiology

1 OBJECTS AND METHODS

Intracranial infection (ICI) is a common complication after neurosurgery. Studies [1] have shown that Acinetobacter baumannii (AB) can easily cause widespread epidemics in hospitals through cross-infection. It is common in critically ill patients and adversely affect prognosis. Literature [2] reported that AB accounted for 13.5% of the pathogenic bacteria in cerebrospinal fluid in my country, and the resistance rate to carbapenems gradually increased from 31.0% to 66.7% in recent years. With the widespread use of broad-spectrum antimicrobials and the increase in interventional procedures, multidrug resistance (MDR) and extensive drug resistance (XDR) have increased significantly, and even pan -drug resistance (PDR) has emerged.) strains, which pose a serious challenge to anti-infection therapy[3-4]. Chen et al[5] believed that clonal dissemination is an important reason for the increase of CRAB infection rate, pulsed field gel electrophoresis (Pulsed field gel electrophoresis, PFGE) and multilocus sequence typing (Multilocus sequence typing, MLST) are now A common method for detecting the homology and prevalence characteristics of strain distribution at different stages[6]. This paper analyzes and cites AB drug resistance, PFGE and MLST classification in neurosurgical ICI patients.

1.1 Design Ideas

Subjects 107 ICI patients with AB detected in the cerebrospinal fluid culture of the Department of Neurosurgery, Department of Intensive Medicine, Changjiang Shipping General Hospital from October 2020 to April 2021 were selected as the research subjects. Simple random sampling was used to select 102 cases as the control group. Inclusion criteria: (1) All patients met the diagnostic criteria established by the Infectious Diseases Society of America in 2017 [7]. (2) All received craniotomy or cerebrospinal fluid drainage within 30 days, or received artificial material implantation within 1 year. (3) Aged 18~80 years old. (4) The cerebrospinal fluid culture was completed and the pathogen was confirmed to be AB. (5) Patients and their family members are aware of the detailed content of the study and sign the consent form. Exclusion criteria: (1) Meningitis, brain abscess or intracranial infection existed before operation. (2) Patients who have not broken the blood-brain barrier such as vascular intervention, cranioplasty or spinal surgery. (3) Combined with bloodstream infection, sepsis or other systemic infection. This study has been reviewed and approved by the Hospital Ethics Committee.

1.2 Method

1.2.1 Data collection
Basic information such as gender, age, smoking and alcohol history, and body mass index (BMI), preoperative primary diseases, and comorbidities were collected by reviewing medical records.
1.2.2 Cerebrospinal fluid culture and drug sensitivity test

The cerebrospinal fluid of ICI patients was collected for bacterial culture, the type of pathogenic bacteria was analyzed by VITEK-2Compact automatic microbial identification instrument (BioMérieux, France), and the drug susceptibility test was carried out by Kriby-Bauer disc diffusion method. Clinical Laboratory Center) are Staphylococcus aureus ATCC 29213, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853. All operations are completed in accordance with the "National Clinical Laboratory Operation Procedures" [8], and the results are interpreted with reference to American clinical laboratories Contents of the 2018 (M100-S28) edition of the Institute of Standardization [9].

1.2.3 PFGE classification

Select the cultured fresh colony and mix it with 1% Seakem Gold to make a gel, add cell lysate and proteinase K to lyse, and then use the restriction endonuclease APAI (Dalian Bao Biological Engineering Co., Ltd.) to keep the temperature at 37°C After bathing in water for 2 hours, the product was subjected to gel electrophoresis and stained with GelRed, observed and photographed by a gel imaging system (Bio-Rad, USA), and clustered and analyzed by BioNumerics software. Clones, more than 3 are different clones[10].

1.2.4 MLST classification

The separated AB was inoculated into Columbia blood plate and incubated at 37 ℃ for 16h, and the extracted DNA solution was stored at -20 ℃. The seven housekeeping genes cgltA, gyrB, gdhB, recA, cpn60, gpi and rpoD in the AB genome were detected by polymerase chain reaction (PCR) amplification method. The reaction system included 22 μl of distilled water, 1 μl of upstream and downstream primers, 1 μl of genomic DNA μl and 2×Es Taq Master Mix (Jiangsu Kangwei Century Biotechnology Co., Ltd.) 25 μl, a total of 50 μl, the reaction conditions were pre-denaturation at 94 °C for 5 min, and then 94 °C/30s, 57 °C/30s, 72 °C/ Cycled 34 times in 45s, and finally extended at 70°C for 8 min. The primer sequences are shown in Table 1. Upload the reaction results to the Pubmlst database for online comparison, obtain the allelic sequence numbers of each housekeeping gene and determine the strain type and ST type.

Table 1 Primer sequences of housekeeping genes in AB genome

Gene loci	upstream primer	downstream primer	Fragment length (bp)
cgltA	5'-AATTTACAGTGGCACATTAGGTCCC-3'	5'-GCAGAGATACCAGCAGATACACG-3'	722
gyrB	5'-TAAAGGCGGATTATCTAGGT-3'	5'-GCTGGGTCTTTTTCCTGACA-3'	594
wxya	5'-GCTACTTTTTATGCAACAGAGCC-3'	5'-GTTGAGTTGGCGTATGTTGTGC-3'	774
recA	5'-CCTGAATCTTCTGGTAAAAC-3'	5'-GTTTCTGGGCTGCCAAACATTAC-3'	425
cpn60	5'-GGTGCTCAACTTGTTCGTGA-3'	5'-CACCGAAACCAGGAGCTTTA-3'	640
gpi	5'-GAAATTTCCGGAGCTCACAA-3'	5'-TCAGGAGCAATACCCCACTC-3'	456
wxya	5'-ACCCGTGAAGGTGAAATCAG-3'	5'-TTCAGCTGGAGCTTTAGCAAT-3'	672

1.3 Statistical Analysis

SPSS 22.0 software was used, and counting data were expressed as [n(%)], and $\chi 2$ test was used for comparison between groups, and measurement data were expressed as (x'±s) in line with normal distribution by Kolmogorov-Smirnov test, and independent comparisons between two groups were performed. Sample t test, $P < 0.05$ was considered statistically significant.

2 RESULTS

2.1 Analysis of Basic Clinical Data of Neurosurgery AB Patients with ICI

After neurosurgery, the age and proportion of diabetes in the ICI group were higher than those in the non-ICI group ($P < 0.05$). There was no statistical difference in gender, BMI and other basic data of the primary disease between the two groups. See Table 2.

Table 2 Analysis of clinical basic data of patients with AB infection ICI in neurosurgery

basic information	ICI group (n=107)	Non-ICI group (n=102)	Statistics	p-value
Gender (example) Male	62	53	0.755	0.385
female	45	49		
age)	52.63±8.94	50.17±8.46	2.041	0.043
BMI(kg/m2)	23.74±4.06	23.18±3.52	1.063	0.289
primary cerebrovascular disease	49	41	0.689	0.708
(Example) Brain tissue trauma	35	36		
brain tumor	twenty three	25		
Smoking history (example)	32	27	0.304	0.581
Drinking history (example)	26	twenty one	0.413	0.521
comorbid diabetes	twenty four	11	5.080	0.024

(Example) Hypertension	19	15	0.357	0.550
Hyperlipidemia	12	13	0.116	0.733
coronary heart disease	10	8	0.150	0.699

2.2 Analysis of AB Drug Resistance in ICI Infection after Neurosurgery

A total of 107 AB strains were detected in 107 ICI patients. The results of drug susceptibility tests showed that AB had different degrees of resistance to commonly used antibacterial drugs. See Table 3.

Table 3 Analysis of AB drug resistance in ICI infection after neurosurgery

antibacterial drugs	Number of resistant strains (n=107)	Drug resistance rate (%)
Cefoperazone/Sulbactam	74	69.16
Piperacillin/Tazobactam	71	66.36
Ampicillin	86	80.37
Nitrofurantoin	104	97.20
Levofloxacin	63	58.88
Ciprofloxacin	68	63.55
Ceftazidime	72	67.29
Cefotaxime	105	98.13
Ceftriaxone	65	60.75
cefotetan	89	83.18
Cefazolin	94	87.85
Cefepime	96	89.72
Meropenem	64	59.81
imipenem	61	57.01
Aztreonam	97	90.65
Gentamicin	70	65.42
tetracycline	95	88.79
Minocycline	34	31.78
Tigecycline	41	38.32
Amikacin	46	42.99

2.3 PFGE Typing Results of AB Patients with ICI in Neurosurgery

PFGE fingerprints showed that 107 AB strains were divided into 7 clonotypes, namely 91 strains of type A, 6 strains of type B, 4 strains of type C, 2 strains of type D, 2 strains of type E, 1 strain of type F and 1 strain of type G. strains, the percentages of each clonotype were 85.05%, 5.61%, 3.74%, 1.87%, 1.87%, 0.93% and 0.93%, of which type A includes type A1 58 strains (54.21%), 29 strains (27.10%) of type A2 and 4 strains of type A3 (3.74%).

2.4 Results of MLST Classification in Neurosurgery Patients with AB Infection and ICI

MLST typing results showed that 107 AB strains could be divided into 14 ST genotypes, among which the more common genotypes were 64 ST208 strains (59.81%), 17 ST195 strains (15.89%), 9 ST75 strains (8.41%), 5 (4.67%) ST92 and 3 ST1696 (2.80%), and 1 other ST genotypes accounted for 0.93%.

2.5 Homology Analysis of AB Gene in Patients with ICI after Neurosurgery

As of 2019, AB in the MLST database includes a total of 1144 ST genotypes. Homology analysis using eBUST software shows that there are 97 strains (90.65%) of genotypes ST75, ST92, ST136, ST138, ST195 and ST208, all Belonging to clonal complex 92 (CC92) with only differences in the gpi allele locus, and having obvious gene homology in evolution, it is the dominant clonal group of AB in patients with ICI infection after neurosurgery in the hospital, and the remaining 10 strains belong to 8 A ST genotype is a sporadic clonal group. see Figure 1.

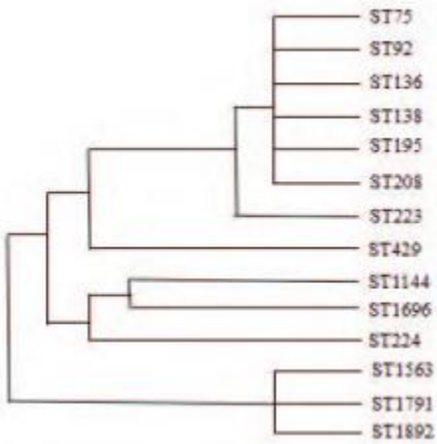

Fig. 1 The phylogenetic tree of different genotypes of AB in patients with ICI infection after neurosurgery

3 DISCUSSION

AB is a common pathogenic bacteria in ICI after neurosurgery. Because it is widely distributed in the air of wards, medical equipment, and fingers of medical staff, it is easy to cause mutual transmission and cross-infection. In severe cases, it can cause bacteremia and threaten the lives of patients[11]. Gao Jianguo et al[12] reported that the poor prognosis rate of AB-induced ICI patients was 58%, and increased drug resistance was the main reason for the poor efficacy. Therefore, it is of great significance to clarify AB drug resistance and genotyping.

Intracranial infection in patients after neurosurgery is related to decreased body resistance and invasive operations. Some studies suggest that age and diabetes can increase the risk of ICI[13]. The results of this study showed that age and the proportion of patients with diabetes were significantly higher in the ICI group, suggesting that age and diabetes may be important factors affecting the occurrence of ICI after neurosurgery in the hospital. Bacterial drug resistance is an important reason for the efficacy of AB antimicrobial therapy. Foreign reports [4-6] also show that AB drug resistance has increased significantly in recent years, and MDR, XDR and PDR strains have been widely colonized in medical institutions. In this study, the drug susceptibility test of 107 AB strains in neurosurgery patients with ICI showed that AB had different degrees of drug resistance to commonly used antibacterial drugs, among which the drug resistance rate was higher in order of cefotaxime, nitrofurantoin and aztreonam, etc. Those with lower drug rates were minocycline, tigecycline, and amikacin, etc. Compared with the results reported by Zou Dewei et al. [14], AB drug resistance showed a clear upward trend. Minocycline and tigecycline are new broad-spectrum tetracycline antibacterial drugs, which were commonly used in the treatment of Gram-positive bacterial infections in the past, and were considered relatively weak against Gram-negative bacteria. In recent years, studies have shown that colistin combined with replacement Gacycline has a high sensitivity to MDR-AB[15], but colistin has severe renal and neurotoxicity, so it is often used as the last choice when other combined anti-infection treatment regimens have no obvious effect, which is beneficial to ensure anti-infection Treatment effect, while reducing adverse drug reactions and preventing further increase of AB drug resistance In addition to host factors, AB drug resistance is also related to genotype and molecular characteristics, and there are certain differences in the prevalence of strains in different regions. Homology has a positive effect on the prevention and treatment of infection. At present, common analysis methods include PFGE, MLST and high-throughput whole gene sequencing technology, etc. [16]. The results of PFGE detection in this study showed that 107 strains of AB could be divided into 7 clonotypes from A to G, among which type A includes 3 subtypes, namely A1, A2 and A3, which shows that there are obvious dominant strains in the distribution of AB in the neurosurgery department of the hospital. Previous studies[17] believed that the long-term prevalence of mainstream clones is an important reason for the increase in AB infection rate, and the drug resistance rate of dominant clones can also be significantly increased, so monitoring their genotypes is important to prevent the spread of AB in endemic areas and reduce the fatality rate. is of great significance. PFGE typing has good resolution and stable results, but multiple genetic events in bacteria may cause PFGE to judge strains with original homology as irrelevant strains, so PFGE is often used in combination with other typing methods[18]. MLST is a typing method for judging the variation of AB strains by measuring the partial DNA sequence of 7 housekeeping genes and generating a specific number. It can upload and count AB genotypes around the world and generate an AB gene map to discover the molecular epidemiological characteristics of AB. and propagation laws [19]. As of 2019, AB in the MLST database includes a total of 1144 ST genotypes, of which CC92 is the largest, containing about 30 ST genotypes, and most of the widely prevalent ABs in the world belong to this clonal group[20 -21]. The results of this study showed that 97 (90.65%) of the genotypes ST75, ST92, ST136, ST138, ST195 and ST208 among the 107 AB strains in the neurosurgery department of the hospital belonged to the CC92 clonal group and only the gpi allele loci were different. It has obvious gene homology in evolution, and belongs to the CC92 clone group with the AB strain reported by Liu Lijuan et al[20], but there are differences in specific subtypes, suggesting that there is a certain degree of variation in the carbapenemase gene of AB strains in different regions and hospitals in my country Therefore, the clinical application of antibacterial drugs should be combined with the local actual situation and the drug response of patients, and strive to achieve individualized treatment. In addition, the remaining 10 AB strains in this study belonged to 8 ST genotypes respectively, indicating that CC92 was the dominant clonal group of AB bacteria in patients with ICI infection after neurosurgery in the hospital, and the rest of the individuals were sporadic clonal groups, suggesting that the AB strains

crossed in the ward. The risk of infection is high, and it is necessary to strengthen sterilization in the ward and strictly implement aseptic operation during treatment.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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