Molecular Study of Clinical Carbapenem Resistant Providencia Rettgeri

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Abstract: Objective: To investigate the genotyping, drug resistance phenotype and homology of carbapenem resistant Providencia rettgeri. Methods: The strain identification and antibiotic susceptibilities tests of carbapenem-resistant Providencia rettger were caarried out by using VITEK-2 compact system (BioMerieux). The genotypes of genes were screened by PCR. Conjugation experiments were performed to determine the transferability of Plasmids. Clonal relatedness was assessed by pulsed-field gel electrophoresis (PFGE). Results: Modified Hodge tests of 9 strains were all positive. The 9 isolates of carbapenem resistant Providencia rettgeri carried blaNDM-1and blaTEM116, one isolate also harbored aac(6′)-Ⅰb, the drug resistance showed multi-drug resistance. One strain of the NDM-1 gene were successfully transferred to recipient bacteria(E. coli J53AziR) by conjugation. PFGE analysis showed higly homology of the 9 isolates. Conclusion: The NDM-1 producing Providencia rettgeri isolates in this study appeared to be clonal, hospitals should strengthen the monitoring of carbapenem-resistant bacteria.

With the emergence of a large number of carbapenem-resistant enterobacteriaceae bacteria, bacterial infections caused by them maintains an upward trend all over the world [1]. The result on the bacterial drug resistance showed in 2016 that Klebsiella pneumoniae was the main carbapenem-resistant bacteria, with drug resistance rate of >15%.At present, few studies have been reported on Providencia Rettgeri that is drug resistant to carbapeneme. In this study, clinical resistance and molecular epidemiology of the clinically isolated carbapene-resistant Providencia Rettgeri were analyzed.

1 Data and Methods

1.1 The source of strains strains. Carbapenem resistant Providencia Rettgeri was isolated from clinically isolated imipenem and meropenem strains in the laboratory [2]. 7 specimens were obtained from urine and 2 from hydrothorax. The drug susceptibility test and bacterial identification were conducted using the French VITEK 2 Compact system, and the criteria on the judgement of bacterial resistance were carried out according to CLSI 2012.The quality control strains were Escherichia Coli ATCC25922 and Klebsiella pneumoniae ATCC700603.The recipient bacterium was Ecoli J53 AziR.

1.2 Detection of drug-resistant genes. The genome of bacterial strain was extracted by boiling pyrolysis method.The drug resistance genes of carbapenem such as NDM, KPC, IMP, SPM, AIM, VIM, OXA, BIC, GIM, SIM and DIM, the resistance genes of AmpC enzyme such as MOX (MOX-1, MOX-2), DHA (1-2), ACC, EBC (Mir-1t ACT-1), integrase gene intI and intII and ultra-broad spectrum -lactamase genes: SHV, TEM, OXA1 and CTX were detected by PCR method.PCR primers and reaction conditions were referred to the literature [3-9].The sequencing products were sent to Shanghai Sangon Biotech for sequencing, and the sequencing results were compared and analyzed on NCBI website.

1.3 The plasmid conjugation test was based on the literature [10],which is made with appropriate modifications.On the first day, the isolated Providencia Rettgeri(donor bacteria) were inoculated into LB solid medium containing 6ug/ mL ceftazidime pentahydrate, and the recipient bacteria (E.coliJ53Azi) were inoculated into LB medium containing 100ug/ml sodium azide (NaN3) and

cultured overnight at 37℃.On the second day, a single colony was selected from the solid medium and inoculated into the LB liquid test tube containing 6ug/ml ceftazidime, and the recipient bacteria were inoculated into the LB liquid test tube containing 100ug/ml sodium azide (NaN3), and cultured overnight at 37 ° C with oscillations. On the third day, 2% of the donor and receptor bacteria were absorbed and inoculated to the new LB liquid medium, which were cultured at 37 ℃ in oscillation. As the absorbance (A600nm) is about 1.0, 0.5 ml donor and receptor bacteria should be respectively picked to the 4 ml fresh LB liquid medium. At the same time, it should be added into the control group (0.5ml of recipient bacteria and 4.5 ml of fresh LB liquid medium), which is horizontally placed overnight for culture. On the fourth day, the liquid medium was oscillated to end the conjugation, and the conjugation was diluted 10 times and 100 times, and 0.1 mL of each was respectively absorbed and coated to MAC solid medium containing 6ug/ mL ceftazidime, which is cultured overnight at 37℃.On the fifth day, a single pink colonies (strains is pink as the conjugation is well made, the color of Providencia Rettgeri is colorless as they were not succeddfully conjugated , individual recipient bacteria fail to grow on MAC solid medium growth containing 6 ug/ml of ceftazidime) on MAC plate are inoculated into LB liquid medium with 6 ug/ml of ceftazidime for enrichment culture.Besides, template DNA were obtained with boiling method, PCR method was used to verify whether blaNDM-1 genes were carried in zygomycete.

1.4 Pulsed Field Gel Electrophoresis(PFGE) was operated according to literature[11]. SfiI enzyme was used for digestion. Electrophoresis parameters were set as follows: voltage 6V/cm with 120°of included angle, fragment size 50-300K, 25.5h.Homology between strains was interpreted according to Tenover rules.

2 Results

2.1 The results of plasmid conjugation test and bacterial drug resistance showed that only one of the 9 strains of Providencia Rettgeri was successfully conjugated to finally obtain zygotes. PCR method was adopted to verify that the drug resistance genes of zygotes are consistent with the primary bacteria, which all carry blaNDM - 1, aac (6 ') - Ⅰ b, TEM116 gene. Are shown in table 2.Results by drug susceptibility test are shown in Table 1.

Table 1. Results of drug susceptibility of primary bacteria of Providencia Rettgeri, zygote and recipient bacteria (J53AziR) (g/ml)

Strain No.	TZP	CTX	CAZ	CRO	FEP	ATM	ETP	IPM	MEM	AK	CN	TOB	CIP	LE	SXT
P01	64	≥64	≥64	≥64	≥64	≥64	≥8	≥16	≥16	≥64	≥16	≥16	≥4	≥8	40
P01-C	64	≥64	≥64	≥64	8	4	≥8	≥16	≥16	≥64	≥16	≥16	≤0.25	≤0.25	≤20
P173	64	≥64	≥64	≥64	≥64	≥64	≥8	≥16	≥16	4	≥16	8	≥4	4	≤20
P174	64	≥64	≥64	≥64	≥64	2	≥8	≥16	≥16	8	≥16	8	≥4	≥8	80
P175	64	≥64	≥64	≥64	≥64	≤1	≥8	≥16	≥16	16	≥16	8	≥4	≥8	40
P176	64	≥64	≥64	≥64	≥64	2	≥8	≥16	≥16	16	≥16	8	≥4	≥8	160
P177	64	≥64	≥64	≥64	≥64	≥64	≥8	≥16	≥16	4	≥16	8	≥4	4	≤20
J53AziR	≤4.0	0.047	≤1.0	≤1.0	≤1.0	≤1.0	≤0.5	≤1.0	≤1.0	≤2.0	≤1.0	≤1.0	≤0.25	≤0.25	≤20

Note: TZP (Piperacillin/tazobactam) CTX (Cefotaxime), CAZ (ceftazidime), CRO (ceftriaxone), FEP (cefepime), ATM (Aztreonam), ETP (ertapenem), IPM (imipenem), MEM (meropenem), AK (amikacin), CN (gentamycin), TOB (tobramycin), CIP(ciprofloxacin) LE (levofloxacin), SXT (sulfamethoxazole/trimethoprim).

2.2 Characterization of isolated strains and detection of drug resistance genes. 9 strains of Providencia Rettgeri were NDM 1 type, which all carry TEM116 gene with one containing aac (6 ') - Ⅰ b and the zygote carries the completely identical genes, which is shown in Table 2.

Table 2. Providencia Rettgeri and genes carried in zygot and the characteristics

Bacteria No	Department	Specimen	Bacteria	Hodge	MBL	Genes carried
P01	ICU 1	Urine	Providencia Rettgeri	Positive	Positive	blaNDM-1、aac(6′)-Ⅰb、TEM116
P01-C			P01Zygomycetes	Positive	Positive	blaNDM-1、aac(6′)-Ⅰb、TEM116
P173	ICU 2	hydrothorax	Providencia Rettgeri	Positive	Positive	blaNDM-1、TEM116
P174	neurology department	Urine	Providencia Rettgeri	Positive	Positive	blaNDM-1、TEM116
P175	Emergency Care Department	Urine	Providencia Rettgeri	Positive	Positive	blaNDM-1、TEM116
P176	Emergency Care Departme	Urine	Providencia Rettgeri	Positive	Positive	blaNDM-1、TEM116
P177	ICU 2	hydrothorax	Providencia Rettgeri	Positive	Positive	blaNDM-1、TEM116

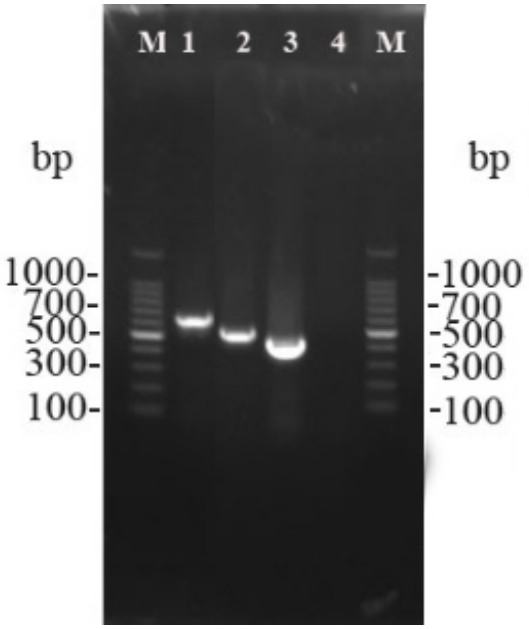
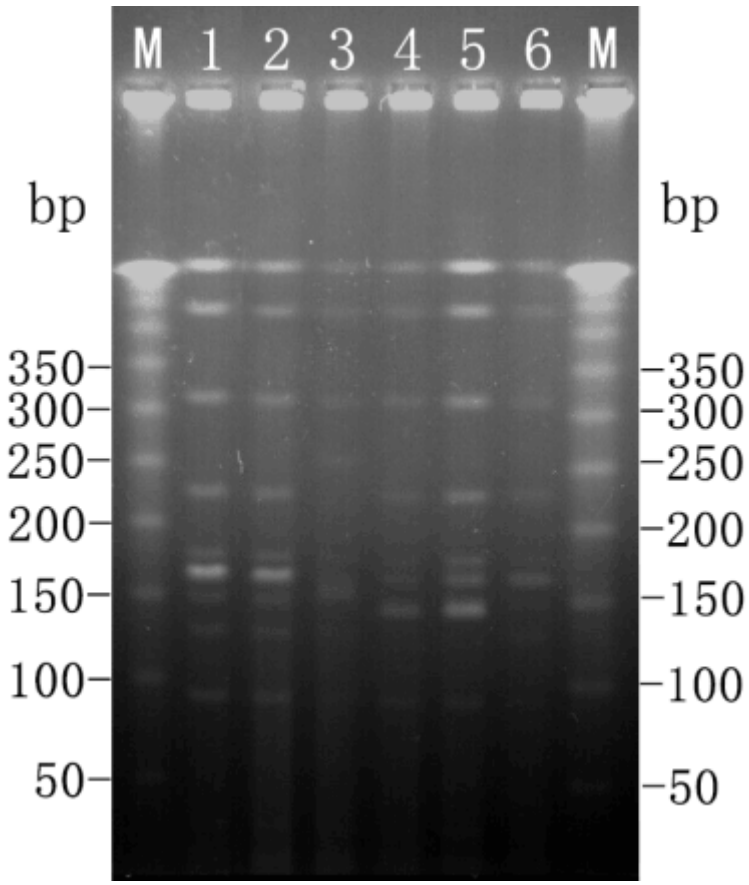


Figure 1. PCR amplification and electrophoretogram of the drug-resistant gene of the clinical strain of Providencia Rettgeri

Note: M, 100bp ladder marker; 1 ~ 3, are respectively blaNDM-1, blaTEM-116, and aac (6 ') - Ⅰ b gene PCR amplification products; 4. Negative control.

2.3 The results of pulsed field gel electrophoresis showed that 9 strains of Providencia Rettgeri

P01, P173 and P177 were exactly the same in the number of electrophoretic band and position, and they were considered as the main outbreak strains according to Tenover rule. Only one band of P175 and P176 was different from the outbreak strain, and they were believed to be closely related to the outbreak strain. Two bands of P174 were different from the outbreak strain and were considered to have a great correlation with the outbreak strain. See Figure 2 for details.



Note: Lane 1~6 refer to Providencia Rettgeri P1, P173, P174, P175, P176, and P177, respectively. M, ProMega - Marker ® Lambda Ladders

Figure 2. SfiI enzymatic digestion PFGE diagram of Providencia Rettgeri genome

3 Discussion

Providencia Rettgeri is opportunistic pathogens in human body, which can cause catheter-related bacteremia, urinary tract infection, gastroenteritis and abscess [11, 12]. With the gradual enhancement of bacterial drug resistance in recent years, carbapenems antibiotics become the last defense line of gram-negative bacteria. However, the number of carbapenems antibiotic-resistant enterobacteriaceae isolated from hospitals has been increasing year by year.

Since the NDM-1 gene was reported, a variety of enterobacteriaceae bacteria with blaNDM-1 have been found, which were mainly Klebsiella pneumoniae and escherichia coli [13-15]. In this study, the blandm-1 carrying strain isolated from the clinic was Providencia Rettgeri, which has been rarely reported at home and abroad.

In this study, most of the pathogenic bacteria were isolated from intensive care units, which may be related to more invasive operations in intensive care units. The Providencia Rettgeri isolated in this study are multidrug-resistant bacteria. Due to the inherent drug resistance of the bacteria, they are resistant to the first-generation cephalosporin, tigacycline, ampicillin and polymyxin B, etc. Therefore, such infections pose great challenges to clinical treatment. The Providencia Rettgeri isolated in this study is endowed with high homology, which can be speculated to have a great epidemiological correlation. Nosocomial infection outbreaks caused by epidemics occur frequently and should be paid close attention to.It is urgent to strengthen the monitoring of carbapenem-resistant enterobacter SPP and antibiotics should be rationally used.

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