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Scientific Research

Antimicrobial Resistance and Genotype Analysis of Extended-Spectrum-*β*-Lactamase-Producing Proteus Mirabilis

Ying Huang, Yuanhong Xu*, Zhongxin Wang, Xianghong Lin

Clinical Laboratory, the First Hospital Affiliated to Anhui Medical University, Hefei, China
Email: *xyhong1964@163.com

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Abstract

To analyse the genotypes of clinical isolates of Extended-Spectrum-*β*-Lactamase-Producing (ESBL-producing) *Proteus mirabilis* (*P. mirabilis*) and the mechanisms of antimicrobial resistance, to guide reasonable use of antibiotics and to avoid nosocomial outbreak infections by ESBL-producing *P. mirabilis*. 125 clinical isolates of *P. mirabilis* were collected from the Drug-Resistant Bacteria Surveillance Center of Anhui Province (from Jan 2009 to May 2010). Searching for the genotypes of ESBLs was perfomed by PCR amplification and DNA sequencing, and performed conjugation test simultaneously. Among ESBL-producing strains, CTX-M was the major genotype (3 CTX-M-13 and 1 CTX-M-3). TEM-1b spectrum *β*-lactamase was also prevalence in *P. mirabilis*. The diversity of *β*-lactamases in *P. mirabilis* and the emergency of multi-drug-resistance clinical strains will present serious threat to clinical therapy and even will lead to outbreak of nosocomial infections. Our study emphasizes the need for enhanced supervision of ESBL-producing *P. mirabilis*. Timely and reasonable drug-resistance data are indispensable to clinical therapy.

Keywords

Genotype; Extended-Spectrum-*β*-Lactamase; Antimicrobial Resistance; Proteus Mirabilis

1. Introduction

Proteus mirabilis is one of the most common gram-negative pathogens encountered in clinical specimens and can cause a variety of community- or hospital-acquired illnesses, including urinary tract, wound, and blood stream infections. This organism is intrinsically resistant to nitrofurantoin and tetracycline, but it is naturally

*Corresponding author.

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susceptible to ß-lactams, aminoglycosides, fluoroquinolones, and trimethoprim sulfamethoxazole [1].

Extended-spectrum ß-lactamases (ESBLs) have become increasingly common worldwide and have emerged as a major source of antimicrobial resistance in gram-negative pathogens. Except for Escherichia coli and Klebsiella pneumoniae, Proteus mirabilis is another common ESBL-producing gram-negative pathogen. It has been found that most ESBLs were derivatives of TEM-1 type, TEM-2 type and SHV-1 type of *β*-lactamase, which is composed of one or several point code gene mutation. In recent years, in addition to the TEM-type ESBLs, there also were increasing reports of CTX-M-type ESBLs procuced by Proteus Mirabilis [2]-[8].

ESBLs confer resistance to penicillins, cephalosporins, aztreonam, and also associated with resistance to other classes of nonpenicillin antibiotics, including fluoroquinolones, aminoglycosides, trimethoprimsulfamethoxazole, and ß-lactam/ß-lactamase inhibitor combinations. Thus, ESBL-producing organisms often possess a multidrug resistance phenotype. Detection and susceptibility results of ESBL-producing Proteus mirabilis play an essential role in the treatment of infections caused by this pathogen and also in controlling the spread of ESBLs.

The aim of the present study was to evaluate the prevalence and the molecular distribution of ESBL-producing *Proteus mirabilis* in local area. We examined 125 clinical isolates of *Proteus mirabilis*, detected the existence of ESBLs and the antimicrobial resistance of the ESBL-producing strains.

2. Materials and Methods

2.1. Stains

125 stored isolates were from the Drug-Resistant Bacteria Surveillance Center of Anhui Province (from Jan 2009 to May 2010).

Organism identification was performed by Microscan GN combo card (Dade Behring, West Sacramento, CA, USA.). Pathogens identified as ESBL producers were subjected to PCR amplication and gene sequencing tests. E. coli ATCC 25922 was used as negative control for ESBL production. Standard ESBL-producing strains (TEM-1, TEM-26, SHV-5, CTX-M-3, CTX-M-24, TOHO-1, OXA-1, OXA-2, OXA-10) were used as positive control. All tests were performed according to CLSI guidelines. Minimum inhibitory concentrations (MIC) of 13 antimicrobials(ampicillin, ampicilin/sulbactam, piperacillin, piperacillin/tazobactam, cefoperazone, cefoperazone/sulbactam, ceftazidime, cefotaxime, gentamicin, amikacin, aztreonam, imipenem and ciprofloxacin) were determined by the micro-broth dilution method.

2.2. Polymerase Chain Reaction (PCR)

ESBL genes (blaTEM, blaSHV, blaCTX-M, blaTOHO and blaOXA) were amplified by PCR with the following sets of primers (**Table 1**).

2.3. Agar Gel Electrophoresis Test

Agar gel electrophoresis test (DYY-10C, Beijing Six-one Instrument Company) was used to compare PCR products with the standard ESBL-producing strains.

2.4. Gene Sequencing Test

Sequencing of PCR amplicons were performed twice on both strands with an ABI Prism 3730 DNA Squencer (Perkin-Elmer, Applied Biosystems Division), by the dideoxy chain termination method of Sanger method. The nucleotide sequences were analysed with the BLAST.

3. Results

3.1. PCR and Agar Gel Electrophoresis Test Results

125 stains of Proteus mirabilis were amplified by PCR with the former primers. There were 9 positive strains, including 5 blaTEM, 3 blaCTX-M-13 and 1 blaCTX-M-3. No positive strain was detected in the types of SHV, OXA-1, OXA-2, OXA-10, and TOHO-1.

3.2. Gene Sequencing Test

Sequencing analysis identified that 5 stains harboured blaTEM-1b, 3 harboured blaCTX-M-14(1 together with

blaTEM-1b), and 1 harboured blaCTX-M-3 (**Table 2**).

3.3. Antimicrobial Susceptibility Tests

All the PCR positive strains expressed high resistance to ampicillin, piperacillin and cefoperazon, which MICs were >256, >256 and >64 respectively. But all the strains were sensitive to Imipenem, and the MICs ranged from 1 to 4. As showed in **Table 3**, the 4 CTX-M-positive *P. mirabilis* were all resistant to cefotaxime and ceftazidime, and, at the same time showed high resistance to the other antimicrobials listed in the table. On the contrary, the 5 TEM-1b-positive strains were all sensitive to cefotaxime and ceftazidime, which are one group of

Table 1. Primers and conditions of PCR.

Primer name	Oligonucleotide sequence (5'–3')	Annealing temp. (˚C)
TEM-A	TGCGGTATTATCCCGTGTTG	54
TEM-B	TCGTCGTTTGGTATGG CTTC	
SHV-A	TCTCCCTGTTAGCCACCCTG	62
SHV-B	CCACTGCAGCAGCTGCCGTT	
CTXM1-A	ACAGCGATAACGTGGCGATG	55
CTXM1-B	TCACCCAATGCTTTACCCAG	
CTXM13-A	CTGCTTAATC-AGCCTGTCGA	60
CTXM13-B	TCAGTGCGATCCAGACGAA A	
TOHO1-A	TGGAAGCCCTGGAGAAAAGT	62
TOHO1-B	CTTATCGCTCTCGCTCTGTT	
OXA-1A	TTTTCTGTTGTTTGGGTTTT	56
OXA-1B	TTTCTTGGCTTTTATGCTTG	
OXA-2A	CGCTGTTCGTGATGAGTTCC	54
OXA-2B	ATCGGCGTTGCCATAGTC	
OXA-10A	ATGGTGTCTTCGTGCTTT	54
OXA-10B	TCTTACTTCGCCAACTTCT	
TEM-A(entire)	TTGAATTCCCCTGGTAAATGCTTC	58
TEM-B(entire)	TGGATCCGAGTAAACTTGGT CTG	
CTX-Q1-A(entire)	CGGAATTCCGTCGTCTCTTCCAGA	62
CTX-Q1-B(entire)	CGGGATCC-CGC AGCGCTTTTGCCGTCTAAG	
CTX-Q13(entire)	CGGAATTCCGGAAGCAGTCTAAATTCTTCGTGAAATAG	63
CTX-Q13-B(entire)	CGGGATCCCGGGGCC-AGTTGGTGATTTGA	

Table 2. Genotype of PCR positive strains.

Stains	Genetypes of β-lactemases
PM07	TEM-1b
PM12	CTX-M-14
PM48	TEM-1b
PM55	CTX-M-3
PM60	TEM-1
PM61	TEM-1b
PM67	CTX-M-14
PM72	TEM-1b
PM98	CTX-M-14TEM-1b

Table 3. MICs results of the PCR positive strains.

Stains	MIC of antimicrobial agent (μg/ml)												
	AMP	SAM	PIP	TZP	CPZ	CPS	CTX	CAZ	ATM	IPM	AMK	GEN	CIP
PM07	>256	4/2	>256	8/4	>64	4/2	2	2	2	0.5	4	8	16
PM12	>256	>32/16	>256	64/4	>64	32/16	>64	64	4	0.5	8	16	>32
PM48	>256	4/2	>256	16/4	>64	8/4	4	4	4	0.5	2	32	4
PM55	>256	>32/16	>256	64/4	>64	32/16	>64	>64	128	1	16	64	>32
PM60	>256	8/4	>256	8/4	>64	4/2	1	8	8	1	4	64	0.5
PM61	>256	8/4	>256	32/4	>64	4/2	1	4	2	0.5	4	4	1
PM67	>256	>32/16	>256	64/4	>64	32/16	>64	>64	>128	1	8	32	>32
PM72	>256	8/4	>256	8/4	>64	8/4	4	8	2	0.5	4	2	1
PM98	>256	>32/16	>256	64/4	>64	32/16	>64	>64	>128	2	64	128	>32

Note: Abbreviations for antimicrobial agents follow (CLSI2012 breakpoints for susceptibility [S] and resistance [R] in micrograms per milliliter] are given in parentheses): AMP, ampicillin (S ≤ 8, R ≥ 32); SAM, ampicillin plus sulbactam (S ≤ 8/4, R ≥ 32/16); PIP, piperacillin (S ≤ 16, R ≥ 128); TZP, piperacillin plus tazobactam (S ≤ 16/4, R ≥ 128/4); CPZ, cefoperazone (S ≤ 16,R ≥ 64); CPS, cefoperazone plus sulbactam (S ≤ 16/8, R ≥ 64/32,t); CTX, cefotaxime (S ≤ 8, R ≥ 64); CAZ, ceftazidime (S ≤ 8, R ≥ 32); ATM, aztreonam (S ≤ 8, R ≥ 32); IPM, imipenem (S ≤ 4, R ≥ 16); AMK, amikacin (S ≤ 16, R ≥ 32); GEN, gentamicin (S ≤ 4, R ≥ 8); CIP, ciprofloxacin (S ≤ 1, R ≥ 4).

β-lac-temases and not belong to the family of ESBL. Also they were widely susceptible to ampicillin-sulbactam, pi-peracillin-tazobactam, cefoperazone-sulbactam, aztreonam, amikacin, gentamicin and ciprofloxacin.

4. Discussion

The present study revealed that the prevalent genetypes in ESBL-producing *Proteus mirabilis* in local area was CTX-M type (3 were CTX-M-14 type, 1 was CTX-M-13 type, and 1 was CTX-M-3 type). Besides, TEM-type *β*-lactemases were ubiquitous in clinical original Proteus mirabilis.

ESBLs have emerged as a major source of antimicrobial resistance in gram-negative pathogens and generally encoded by plasmid-borne genes. ESBL-producing organisms often possess a multidrug resistance phenotype. In spite of the worldwide use of *β*-lactam antimicrobial agents, which is considered as the main reason for the emergence of ESBLs [9], the distributions of ESBLs are far from uniform. Some literatures reported that TEM-type ESBLs was prevalent in Proteus mirabilis [7] [10]-[13]. In 2000, TEM-72 ESBL was identified in *Proteus mirabilis* in Italy, who had 4 substituents in amino acid sequence: Q39K, M182T, G238S and E240K compared to TEM-1 type [14]. TEM-92 ESBL was isolated from *Proteus mirabilis* by French researchers in 2001 [15]. In a study of Enterobacteria, 76 stains were detected to produce ESBLs in 106 stains of *Proteus mirabilis*, and their genotypes were TEM-20, TEM-26, TEM-47, TEM-52 and TEM-87 [16]. In the current study, we only detected the existent of TEM-1 *β*-lactamase in *P. mirabilis*.

But the emergence of more and more CTX-M type ESBLs in Proteus mirabilis had aroused wide concern [17] [18]. 2003, Japanese researchers detected 19 stains of CTX-M-2 ESBL-producing Proteus mirabilis in a out-break of nosocomial infection, which are all presented as multi-drug resistant phenotype [19]. Researchers in Hong Kong area detected CTX-M-13 and CTX-M-14 types of ESBLs from *P. mirabilis*. 13 genotype positive strains were analyzed. There were 8 CTX-M-14 ESBLs, which all simultaneous with TEM-2 gene. And 3 strains were CTX-M-13 type ESBLs and one of them was together with TEM-2 gene. There still were one TEM-11 and one TEM-1 strains [20].

In the century, CTX-M-type ESBLs develop quickly and they have higher potentiality to hydrolyze cefotax-ime than ceftazidime. Even they has the lower prevalence ratio than TEM-type ESBLs in Proteus mirabilis, they should receive more attention to their wide spectrum of substrate specificity. ESBL production was associated with severe adverse outcomes, including higher overall and infection-related mortality, increased length of stay, delay in appropriate therapy, discharge to chronic care, and higher costs [21].

The diversity of distribution of ESBLs in *P. mirabilis* remind us it's important to detect the genotype and the antimicrobial susceptibility pattern which are critical in the therapy of infections caused by ESBL-positive bacteria. Our results showed that except for imipenem, amikacin and piperacillin/tazobactam might be effective drugs *in vitro* too.

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