

Analysis of Genetic Determinants Involved in Multiresistance in Clinical Strains Isolated from Renal Transplantation Recipients in Guangzhou, China

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In the present study, we examined the antibiotic sensitivity of 19 bacterial strains [5 coagulase-negative *Staphylococcus*, 2 methicillin-resistant *Staphylococcus aureus* (*S. aureus*), 2 *Enterococcus faecium* (*E. faecium*), 5 *Escherichia coli* (*E. coli*), 3 *Cedecea* sp., 1 *Klebsiella pneumoniae* (*K. pneumoniae*), and 1 *Burkholderia cepacia* (*B. cepacia*)], which were isolated from renal transplantation patients using the Kirby-Bauer method. We also investigated the production of β -lactamase and extended-spectrum β -lactamase (ESBL), and the presence of the integrase gene (*intI1*) and resistance gene cassette. Among the 19 strains tested, all displayed severe multiresistance, and 12 strains produced β -lactamase, in which 6 strains were ESBL positive. Eleven strains were revealed to possess the class 1 integron; however, neither class 2 nor 3 was detected. Additionally, 3 drug resistance genes, *aadA2*, *dfxA17*, and *aadA5*, were found in some strains. The results indicate that the horizontal transfer of the β -lactamase gene and/or the class 1 integron may contribute significantly to the spread of multiresistant bacteria among renal transplantation patients.

Key words — renal transplantation, multiresistance, β -lactamase, integron

INTRODUCTION

Continued improvements in graft survival have led to widespread acceptance of renal transplantation as the preferred treatment for the majority of patients with end-stage renal disease.^{1,2)} However, because of the administration of immunosuppressants or other drugs, it often causes a series of short- or long-term side effects, of which bacterial infections are particularly important. Nearly 80% of renal transplant recipients have at least one episode of infection during the first year after transplantation, and the infection is the highest during the first 3 months.^{3–7)} The occurrence of drug-resistant pathogens makes the situation even worse.

The efficient acquisition and dissemination of resistance determinants is carried out through a mobile genetic element such as a plasmid, transposon or integron.⁸⁾ Integrons harboring the integrase gene (*intI1*) and resistance gene(s) are known to

be responsible for site-specific recombination, capture, and mobilization of the gene cassette, and this genetic element is presently divided into several classes based on variation of the *intI1* gene.^{9–11)} On the other hand, the β -lactamase gene contributes another mechanism of dissemination of the resistance determinants. Since this gene is frequently located on a plasmid, it may be transferred horizontally from strain to strain by conjugation.¹²⁾ The emergence of bacteria producing extended-spectrum β -lactamase (ESBL) has posed a serious threat to the continued use of this family of antibiotics.¹³⁾

In this study, we investigated the antibiotic sensitivity of 19 strains isolated from renal transplantation recipients and evaluated the presence of resistance determinants such as the β -lactamase gene and integron to assess the contribution of horizontal gene transfer to the spread of multiresistance bacteria.

MATERIALS AND METHODS

Bacterial Strains — Within a period of 15 months (June 2004–August 2005), collected 19 bac-

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terial strains from 19 clinical renal allograft recipients aged between 13 and 84 years in a single local hospital. The patients fulfilled the following criteria: had indwelling urinary catheters or wound drainage tubes; infection occurred during the first 3 months after renal transplantation; had at least one of the symptoms of fever over 38°C, chills, White Blood Cell (WBC) > 10 × 10⁹/l, X-ray indicative of pulmonary infection, frequent micturition, or urgent micturition and dysuria. The strains were isolated from various specimens including sputum and urine (Table 1). Sputum samples were gathered in the morning by deep cough or by using one-off sputum aspiration tubes, and the urine was middle segment. The specimens were not cultured in broth before colony selection. All these operations were carried out sterilely, and the bacteria were identified with an automatic microbial analyzing system (Vitek-32, BioMerieux, Lyon, France).

Susceptibility to Antimicrobial Agents— Susceptibility to antibiotics was routinely tested using the Kirby-Bauer disk diffusion method. The disks and Mueller-Hinton agar plates were purchased from Oxoid (Basingstoke, U.K.), and the results were evaluated according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS).¹⁴ Antimicrobial agents

were chosen according to clinical usage. *Staphylococcus aureus* (*S. aureus*) strain ATCC 25923, *Escherichia coli* (*E. coli*) strain ATCC 25922, and *Pseudomonas aeruginosa* strain ATCC 27853 were used as controls.

Production of β -Lactamase and ESBL— The production of β -lactamase was assessed with BBL Cefinase nitrocefin disks (Becton Dickinson, Franklin Lakes, NJ, U.S.A.). Each disk impregnated with nitrocefin was moistened with sterile distilled water, and a loopful of bacterial cells on Mueller-Hinton agar was inoculated on the disk surface. The disk was protected from drying and examined if pink-red coloration appeared within 10 min. *S. aureus* strain ATCC 29213 and strain ATCC 25923 were used as the positive and negative control, respectively.

ESBL production was examined in the phenotypic confirmatory test as recommended by the NCCLS.¹⁴ Antibiotic disks (cefotaxime 30 μ g, cefotaxime 30 μ g plus clavulanic acid 10 μ g, ceftazidime 30 μ g, ceftazidime 30 μ g plus clavulanic acid 10 μ g) were supplied by Oxoid. The diameter of the bactericidal zone of the combination disk was compared with that of the cephalosporin disk, and a difference of ≥ 5 mm was defined as ESBL positive. *Klebsiella pneumoniae* (*K. pneumoniae*) strain ATCC 700603

Table 1. Source and β -Lactamase and Integron Determinants in 19 Strains Obtained from Renal Transplantation Recipients in Guangzhou, China

Strain	Species	Source	β -Lactamase	Integrase	Gene cassette
1	MRSA	Sputum	+	-	
2	MRSA	Sputum	+	-	
3	MSSCON	Bile	-	+ (<i>IntI1</i>)	-
4	MRSCON	Renal drainage	+	+ (<i>IntI1</i>)	-
5	MRSCON	Sputum	+	-	
6	MRSCON	Bile	+	-	
7	MRSCON	Urine	+	-	
8	<i>E. faecium</i>	Urine	-	-	
9	<i>E. faecium</i>	Renal drainage	-	+ (<i>IntI1</i>)	-
10	<i>E. coli</i>	Urine	+ (ESBL)	-	
11	<i>E. coli</i>	Sputum	+ (ESBL)	+ (<i>IntI1</i>)	<i>aadA2</i>
12	<i>E. coli</i>	Renal drainage	+ (ESBL)	+ (<i>IntI1</i>)	-
13	<i>E. coli</i>	Renal drainage	+ (ESBL)	+ (<i>IntI1</i>)	-
14	<i>E. coli</i>	Urine	+ (ESBL)	-	
15	<i>K. pneumoniae</i>	Sputum	+ (ESBL)	+ (<i>IntI1</i>)	-
16	<i>Cedecea</i> sp.	Trachea secretion	-	+ (<i>IntI1</i>)	<i>dfrA17-aadA5</i>
17	<i>Cedecea</i> sp.	Sputum	-	+ (<i>IntI1</i>)	<i>aadA2</i>
18	<i>Cedecea</i> sp.	Sputum	-	+ (<i>IntI1</i>)	<i>aadA2</i>
19	<i>B. cepacia</i>	Sputum	-	+ (<i>IntI1</i>)	<i>aadA2</i>

MRSA: methicillin-resistant *Staphylococcus aureus*, MSSCON: methicillin-susceptible coagulase-negative *Staphylococcus*, MRSCON: methicillin-resistant coagulase-negative *Staphylococcus*.

and *E. coli* strain ATCC 25922 were used as the positive and negative control, respectively.

Template DNA Preparation—DNA used as a template for PCR amplification was prepared from overnight cultures grown in Luria-Bertani broth at 37°C. The bacterial cultures were diluted 10-fold with Tris-HCl buffer 10 mM (pH 8.0) containing EDTA 1 mM, boiled for 10 min, and cooled for 10 min in an ice-bath. Thereafter, the sample was centrifuged at 12000 × *g* for 2 min, and the supernatant containing DNA was collected.

PCR Amplification of the Integrase Gene and Resistance Gene Cassette—PCR amplification for three classes of the integrase gene and for the resistance gene cassette was performed as described previously.¹⁵⁾

PCR-Restriction Fragment Length Polymorphism (PCR-RFLP)—The amplicons from PCR using the primer set in-F/in-B were digested for 1 hr with *Mva*I, *Eco*RII, or *Pst*I (TaKaRa Bio, Otsu, Japan) and analyzed using 2.0% agarose gel electrophoresis, followed by ethidium bromide staining.

RESULTS

Antimicrobial Resistance and Production of β -Lactamase and ESBL

The antimicrobial susceptibility test of 19 strains showed that all strains were resistant to at least two of the antibiotics used, and 9 strains were resistant to more than four antibiotics. On the other hand, only a few isolates were susceptible to the antibiotics. As shown in Table 2, the antibiotic-resistant percentage was mainly greater than 60%, whereas none of the strains showed resistance to

imipenem.

Among the 19 strains tested, 12 isolates produced β -lactamase, in which 6 isolates showed ESBL activity (Table 1).

Multiple PCR Amplification of the Integrase Gene

In multiple PCR amplification, 11 strains yielded a single 565 bp PCR product, suggesting that they have the class 1 *intI1*; however, the class 2 or 3 *intI1* was not detected (Table 1).

PCR-RFLP

The *intI1*-positive strains were subjected to PCR using the primers in-F and in-B to detect the antibiotic resistance gene cassette. Four strains produced an amplicon of about 1.0 kb, while strain 16 produced an amplicon of about 1.7 kb. When digested, all of the 1.0 kb amplicons had an identical RFLP pattern with the 1009 bp PCR product of the class 1 integron present in *Salmonella enterica* serovar Hadar strain S87 (Table 3), which contained an aminoglycoside gene *aadA2* (GenBank accession no. AY258269).¹⁶⁾ On the other hand, the 1.7 kb amplicon showed an identical RFLP pattern with the 1664 bp PCR product of the class 1 integron present in *E. coli* strain D20 (Table 3), which harbors the *dfr17* and *aadA5* genes (GenBank accession no. AB189264) conferring resistant to trimethoprim and aminoglycoside.¹⁵⁾

DISCUSSION

Organ transplantation is often chosen for patients with end-stage renal disease.¹⁷⁾ Although significant advances have been made in surgical tech-

Table 2. Frequency of Antibiotic Resistance and Multiple Resistance in the 19 Strains

Species	Resistance to antibiotic agents																
	PEN	OXA	AMP	CZO	FOX	CAZ	CTX	CPS	FEP	IPM	GEN	SXT	CIP	CLI	ERY	TBT	VAN
MRSA (<i>n</i> = 2)	2/2	2/2									2/2	2/2	2/2	2/2	2/2	1/2	0/2
Coagulase-negative <i>Staphylococcus</i> (<i>n</i> = 5)	5/5	4/5									5/5	3/5	5/5	2/5	5/5	0/5	0/5
<i>E. faecium</i> (<i>n</i> = 2)	2/2		2/2								1/2		2/2			1/2	1/2
<i>E. coli</i> (<i>n</i> = 5)			5/5	5/5	2/5	2/5	5/5	0/5	3/5	0/5	3/5		4/5				
<i>K. pneumoniae</i> (<i>n</i> = 1)			1/1	1/1	1/1	1/1	1/1	0/1	0/1	0/1	1/1		1/1				
<i>Cedecea</i> sp. (<i>n</i> = 3)			3/3	3/3	1/3	1/3	2/3	1/3	2/3	0/3	1/3		2/3				
<i>B. cepacia</i> (<i>n</i> = 1)			1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1		0/1				

MRSA: methicillin-resistant *Staphylococcus aureus*, PEN: penicillin, OXA: oxacillin, AMP: ampicillin, CZO: cefazolin, FOX: ceftaxime, CAZ: ceftazidime, CTX: cefotaxime, CPS: cefoperazone/sulbactam, FEP: cefepime, IPM: imipenem, GEN: gentamicin, SXT: sulfamethoxazole/trimethoprim, CIP: ciprofloxacin, CLI: clindamycin, ERY: erythromycin, TBT: tetracycline, VAN: vancomycin.

Table 3. Classification of PCR Products Carrying the Gene Cassette by RFLP

Strain	Restriction enzymes and fragment sizes (bp)			Uncut PCR amplicon size (bp)	Identical RFLP pattern with
	<i>MvaI</i>	<i>EcoRII</i>	<i>PstI</i>		
11	336, 673	334, 675	429, 580	1009	<i>Salmonella enterica</i> Serovar Hadar strain S87
17	336, 673	334, 675	429, 580	1009	
18	336, 673	334, 675	429, 580	1009	
19	336, 673	334, 675	429, 580	1009	
16	248, 464, 952	250, 464, 950	142, 1522	1664	<i>Escherichia coli</i> strain D20

niques, microbial infectious disease is still a serious problem. Posttransplant bacterial infections are important because of the serious effects on patients as well as graft outcomes.¹⁸⁾ In this study, we investigated 19 patients for posttransplant bacterial infection. Among 19 strains isolated from each of the patients, 9 strains were gram-positive cocci including methicillin-resistant *S. aureus* (2 strains), coagulase-negative *Staphylococcus* (5 strains), and *Enterococcus faecium* (*E. faecium*) (2 strains), and 10 strains were gram negative, which consisted of *E. coli* (5 strains), *K. pneumoniae* (1 strain), *Cedecea* sp. (3 strains), and *Burkholderia cepacia* (*B. cepacia*) (1 strain). The infection was severe, and the indwelling catheters might have provided the opportunity for the bacteria to invade the body. A total of 7 bacterial species were isolated herein, suggesting that a broad range of microbial pathogens are associated with the infection of renal transplantation recipients, as reported previously.^{18,19)} Patients from whom *E. coli*, coagulase-negative *Staphylococcus*, and *E. faecium* were isolated had urinary tract infections, and *S. aureus* was isolated from pulmonary infections. Therefore the clinical symptoms might be mainly due to these isolates.

In this study, integron- and/or β -lactamase-positive strains were isolated in a considerably high percentage. The production of β -lactamase was observed in 4 species and the integron was present in at least 6 species. These results suggest horizontal transfer of the genes. All 6 gram-positive cocci producing β -lactamase were resistant to penicillin and oxacillin, and the 6 ESBL-positive strains were resistant to ampicillin, cefazolin, and cefotaxime. Therefore it is evident either of the β -lactamases accounts for resistance to β -lactam antibiotics. *E. coli*, *Cedecea* sp., or *B. cepacia* that possesses the class 1 integron harboring the *aadA2* cassette was resistant to gentamicin, suggesting this integron might be responsible for resistance to aminoglycosides. However, it should be noted that *E. faecium* with

neither β -lactamase nor the integron was also multiresistant. Therefore, although β -lactamase and the class 1 integron may play important roles in drug resistance, other genetic element(s) also contribute to bacterial multiresistance.

In contrast to the gram-positive bacteria, the gram-negative bacteria were more frequently integrase positive. In agreement with this finding, others have reported many gram-negative isolates have the integron(s) and are multiresistant.^{20–22)} Bush²³⁾ recently documented that ESBL might be the most important resistance mechanism for penicillins and cephalosporins in *E. coli* and *Klebsiella* sp. However, the present study showed that *E. coli* and *K. pneumoniae*, which apparently produce ESBL, were sensitive to cefoperazone/sulbactam. Further investigations are required to resolve this discrepancy.

In conclusion, the present study demonstrated that the horizontal transfer of the class 1 integron and the β -lactamase gene might contribute to the spread of multiresistant bacteria among renal transplantation patients.

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