Letter to the Editor

Characterization of Class 1 Integron Gene Cassettes among Clinical Bacteria Isolated from One Large Hospital in Northern China*

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The class 1 integron and complex gene cassettes among different species of clinical isolates in northern China were characterized in this study. 383 clinical isolates were obtained from northern China, and class 1 integrons containing gene cassettes widely distributed among gram negative clinical isolates was observed. We find that the class 1 integron showed positive correlation with multidrug resistance phenotype of gram negative bacteria. In addition, we find that isolates belonged to one species harbored different types of gene cassette arrays, while same types of gene cassette arrays were observed in different species of isolates. The diversity of gene cassette arrays among the isolates indicated the complexity of multidrug resistance in clinical isolates in northern China.

The dissemination of numerous antimicrobial resistance genes among bacteria has become an increasingly serious problem, which got more attention worldwide in recent decade[1-2]. Integrons have been identified as important factors resulting in bacteria multidrug resistance and as the capture and dissemination of antimicrobial resistance genes both in humans and animals. However, little has been known about the characters of the gene cassette arrays among different species of clinical bacteria isolates from the same region. In this study, class 1 integron and gene cassettes among 383 isolates belonging to 35 species/genus from one large hospital in northern China were characterized by sequence analysis.

Among the 383 clinical isolates, 335 were gram negative and 48 were gram positive (Table 1). Nearly half of the clinical isolates were *int1* gene positive (*n*=184, 48.0%), indicating that *int1* gene was prevalent in common clinical gram negative bacteria in northern China. The *int1* gene was prevalent among the isolates belonging to 20 speices/genus. The predominant isolates containing *int1* gene were *Escherichia* coli (*n*=49), and more than one *int1* gene positive isolates were detected in *Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Enterobacter cloacae, Klebsiella oxytoca, Serratia marcescens*, *Enterobacter aerogenes*, *Morganella morganii*, *Serratia liquefaciens*, *Proteus mirabilis, Citrobacter freundii*, *Citrobacter braakii,* and *other species of Acinetobacter* spp. We also detected that only one *int1* gene isolate was found in six species. However, No *int1* gene isolates were detected among gram positive isolates and some species of *Enterobacteriaceae* isolates. These results support the viewpoint that integrons were widely distributed among many species of bacteria isolates worldwide[2-4].

It was confirmed that gene cassette arrays of class 1 integron have close relationship with bacteria antimicrobial resistance phenotype[1]. In this study, 106 isolates (belonging to 20 species/genus) were selected for gene cassette array detecting according to their antimicrobial resistance phenotypes. 21 types of gene cassette arrays were observed among these isolates. The gene cassettes included many kinds of resistance genes. Aminoglycoside modifying enzymes encoding genes (*aacA4*, *aacC1*, *aadA1*, *aadA2*, *aadA5*, *aadA14,* and *aadB*), dihydropteroate synthase gene (*sul3*), and trimethoprim resistant genes (*dfrA1*, *dfrA7*, *dfrA17*, *dfrA12*, and *dfrA27*) were distributed widely, which was similar with the results in other former researches[1,5-6]. Chloramphenicol resistance encoding genes (*catB3*, *catB8,* and *cmlA*) were detected in many *Enterobacteriacae* isolates, which indicated that chloramphenicol resistance was not disappear as the clinical use of chloramphenicol reduced, and could be transmitted to humans via food chain and widely disseminated by inserting in class 1 integrons as

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gene cassettes[7]. The detection of extended-spectrum β-lactamase (ESBL) genes (bla_{PSE-1}, bla_{OXA-10}, bla_{OXA-30}) indicated that extensive use of the third generation of cephalosporins might contribute to the capture capability and selection pressure of dissemination of ESBLs gene cassettes. Rifampin ADP-nucleotydyltransferase encoding gene (*aar3*), and *aac(6')-Ib-cr* gene conferring ciprofloxacin and aminoglycosides drug-resistance were also found in this study.

Variety of gene cassettes arrays were observed even in one species of isolates, indicating the diversity of class 1 integron. As shown in Table 2, *P. aeruginosa*, which included six kinds of gene cassettes arrays, was the most important gene cassettes arrays carrier. Four kinds of gene cassettes arrays were detected in *E. coli*, *A. baumannii*, and *K. pneumoniae* isolates, respectively. There were three kinds of gene cassette arrays in *K. oxytoca* and *M. morganii*, respectively. Two kinds of gene cassette arrays existed in other *Acinetobacter* spp., *E. cloacae* and *C. freundii*, respectively. The results from our observation were similar to those in reports from Sudan and southern China[4,8].

Table 1. Distribution of *int1* Gene among 383 Clinical Isolates

Bacterial Name	No. of Isolates	No. *of int1* Positive	*int 1* Positive Percentage (%)	No. of Gene Cassettes Detection
Acinetobacter baumannii	41	20	48.8	14
Other *Acinetobacter* spp.	33	12	36.4	12
Klebsiella oxytoca	24	12	50	7
Pseudomonas aeruginosa	46	25	54.4	9
Pseudomonas mendocina	1	0	0	0
Stenotrophomonas maltophilia	3	1	33.3	1
Sphingomonas paucimobilis	3	0	0	0
Burkholderia cepacia	1	0	0	0
Shewanella putrefaciens	1	0	0	0
Serratia marcescens	13	10	76.9	7
Serratia liquefaciens	4	2	50	2
Serratia plymithica	1	1	100.0	1
Escherichia coli	57	49	86.0	20
Klebsiella pneumoniae	49	19	38.8	11
Proteus mirabilis	6	2	33.3	1
Proteus vulgaris	3	1	33.3	1
Enterobacter cloacae	21	15	71.4	8
Enterobacter aerogenes	6	5	83.3	3
Enterobacter intermedius	1	0	0	0
Enterobacter amnigenus	1	0	0	0
Enterobacter hormaechei	1	1	1	1
Morganella morganii	5	3	60	3
Citrobacter freundii	8	2	25	2
Citrobacter braakii	2	2	100	1
Citrobacter koseri	1	0	0	0
Pantoea agglomerans	1	0	0	0
Providencia rettgeri	1	1	100	1
Aeromonas sobria	1	1	100	1
Staphylococcus aureus	28	0	0	0
Staphylococcus epidermidis	5	0	0	0
Staphylococcus haemolyticus	1	0	0	0
Staphylococcus saprophyticus	1	0	0	0
Enterococcus faecium	9	0	0	0
Enterococcus faecalis	3	0	0	0
Streptococcus pneumoniae	1	0	0	0
Total	383	184	48.0	106

Table 2. Content of Class 1 Integron Gene Cassettes and Resistant Phenotypes among 106 Selected Gram Negative Isolates

Bacterial Name	Gene Cassette Array	No. of Isolates	Resistant Phenotypes (abbreviations)
A. baumannii	*aacA4-catB8-aadA1*	11	GMXFPTSC; GMXFPTSCE
	drfA7	1	GMXFPTSCE
	aadB-bla$_{PSE-1}$*-aacA4*	1	GFPTSC
	aacC1-aacC1-orfP-orfP-orfQ-aadA1	1	GMXFPTSCE
Other	*aacA4-catB8-aadA1*	11	GMXFPSE; GMXFPTSC; GMXFPTSCE
Acinetobacter spp.	*aacA4*	1	GMXFPTSCE
K. oxytoca	*aac(6')-Ib-cr-aar3-dfrA27-aadA16*	2	XFAPSCESEH
	dfrA17-aadA5	3	GXA PSEH; GXFAPTSEH; GFAPSCEH
	dfrA1-aadA5	2	GXFAPS; GXFAPSH
P. aeruginosa	*aadB-aacA4*	2	GSC; GMFASC
	aadB-aadA1	2	GS
	aadB-bla$_{PSE-1}$*-aacA4*	1	GPTSC
	dfrA17-aadA5	2	P; GPC
	aadB	1	GXFAPSC
	sul3-Δorf5	1	GFASC
S.maltophilia	fused *aadA16/aacA4*	1	GXSE
S. marcescens	*dfrA12*-hypothesis protein-*aadA2*	7	GFAPSH; GFAPSEH
S.liquefaciens	*dfrA12*-hypothesis protein-*aadA2*	2	GFAPSH; GFAPSEH
S. plymithica	*dfrA12*-hypothesis protein-*aadA2*	1	G FAPSH
E.coli	*aadB-aadA1-cmlA*	1	GXFAPSCEH
	dfrA17-aadA5	16	SCE; GPSE; GPSCE; GFPSCE; G XFAPSCE; GFAPSCEH; XFAPSCEH; GXFA PSCEH; GXFAPTSCE
	aadA4-cmlA variant	1	GFPSCE
	dfrA12-hypothesis protein-*aadA2*	2	GFPSC
K. pnenmoniae	*aac(6')-Ib-cr-aar3-dfrA27-aadA16*	5	GSCEH; GXFAPSCEH; GXAPSCEH; G XFAPSEH
	*dfrA12-h*ypothesis protein-*aadA2*	4	GEFPAPSC; GXFAPSC; GXFAPSCH; GXFAPTSCE;
	aacA4-bla$_{OXA30}$*-catB3-arr3*	1	GXAPSC
	dfrA17-aadA5	1	GXAPSEH
P. mirabilis	*aadB-catB8-bla*$_{OXA10}$*-aadA1*	1	GMXFA PSCEH
P. vulgaris	*dfrA12*-hypothesis protein-*aadA2*	1	GSEH
E. cloacae	*dfrA17-aadA5*	5	GXAPSE; GXAPSEH; GXFAPSEH; GXFAPSE; GXFA PSEH;
	dfrA12-hypothesis protein-*aadA2*	3	GXFAPTSC
E. aerogenes	*dfrA17-aadA5*	3	GXAPSEH; GXFAPSEH
E.hormaechei	*aac(6')-Ib-cr-aar3-dfrA27-aadA16*	1	FPSEH
M. morganii	*drfA7*	1	ASEH
	bla$_{PSE1}$*-aadA2*	1	GSCEH
	aadB-catB3	1	GPSCEH
C.freundii	*dfrA12*-hypothesis protein-*aadA2*	1	GAPTSCEH
	aadA2	1	GFPSH
C.braakii	*aadA2*	1	XAPTSCE
P. rettgeri	*aadB-catB8-bla*$_{OXA10}$*-aadA1*	1	G PTSCEH
A. sobria	*aadA2*	1	GXFASH

Note. G, gentamicin; M, imipenem; X, cefotaxime; F, cefepime; P, piperacillin; T, piperacillin-tazobactam; S, trimethoprim-sulfamethoxazole; C, ciprofloxacin; A, aztreonam; H, chloramphenicol; E, tetracycline.

Horizontal gene transfer was evident among the gram negative isolates in this study, and same type of gene cassette was detected among different species of isolates. Gene cassettes arrays *dfrA17-aadA5* and *dfrA12*-hypothesis protein-*aadA2* were detected in *Enterobacteracea*, *P. aeruginosa* isolates while these gene cassettes were often found in *E. coli* isolates[1,8]. Some gene cassette arrays were reported for the first time in some species, such as gene cassette array *aac*(*6'*)*-Ib-cr-aar3-dfrA27-adA16* was found in *K. pneumoniae* and *E. hormaechei* isolates in this study, and it was also found in *K. pneumoniae* strain C2367 (JF775514). Gene cassette array *aadB-catB8-bla*$_{OXA10}$*-aadA1-dfrA1* was observed in *P. vulgaris* and *P. rettgeri* isolates, as well as in *Proteus* spp. isolate (HQ386837). Gene cassette array *aadB-bla*$_{PSE-1}$*-aacA4* was already found in *E. coli* and *Pseudomonas* spp. reported by previous study[8], and its existence in *P. aseudomonas* and *A. baumannii* isolates in this study indicated that this type of gene cassette array had broader hosts.

The correlation between *int1* gene and multidrug resistance in clinical isolates was showed in Table 3. There was a significant correlation between *int1* gene and multidrug resistance (P<0.01, χ^2=92.81). This result was similar to a previous study[9]. The resistance phenotypes have close relationship with gene cassette arrays in many isolates, while some phenotypes were not related to class 1 integron. Some *K. oxytoca* and *E. hormaechei* isolates carried *aac*(*6'*)*-Ib-cr* gene were not resistant to gentamicin and ciprofloxacin; some *E. coli* and *P. aeruginosa* isolates containing *dfrA17-aadA5* were not resistant to gentamicin; and some *C. braakii* isolates carriying *aadA* gene were sensitive to gentamicin. These results might be due to the presence of transcriptional repressors or weak promoters[9-10]. Some isolates show more kinds of resistant phenotypes than gene cassette array, which might be due to other resistance mechanisms such as resistance plasmids, transposons, biomembrane, IS*CR*s, and natural resistant mechanisms, further studies are needed to be conducted.

Table 3. Information of *int1* Gene Prevalence and Multidrug Resistance among 335 Gram Negative Clinical Isolates

int1	Non-multidrug Resistant Isolates		Multidrug Resistant Isolates		Total
	No.	%	No.	%	
Negative	71	47.0	80	53.0	151
Positive	5	2.7	179	97.3	184

Note. P=0.000, χ^2=92.81.

The study demonstrated that class 1 integrons containing gene cassettes were distributed widely among gram negative clinical isolates in northern China, and the presence of class 1 integrons is positively correlated with multidrug resistance phenotype. Many types of gene cassettes were observed in same species/genus of isolates, while same gene cassette array was found in different species/genus of isolates, which might contribute to genes capture capacity and dissemination capacity of class 1 integrons. The research on class 1 integrons and related gene cassettes may provide evidence and information to understand the evolutionary changes of class 1 integrons and gene cassettes. Furthermore, It is urgent to conduct consecutive surveillance of 1 integron related antimicrobial resistance in China.

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