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Original Article

Characterization of integrons and novel cassette arrays in bacteria from clinical isloates in China, 2000-2014

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Abstract

Rapid dissemination of antibiotic resistance genes among bacterial isolates is an increasing problem in China. Integron, a conserved DNA sequence, which is carried on episomal genetic structures, plays a very important role in development of antibiotic resistance. This systematic analysis was based on MEDLINE and EMBASE databases. We summarized the distribution and proportion of different types of gene cassette arrays of integrons (including class 1, 2, 3 and atypical class 1 integron) from clinical bacteria isolates in China. Fifty-six literatures were included in this study. Most of the strains were Gram-negative bacteria (94.1%, 7,364/7,822) while only 5.9% strains were Gram-positive bacteria. Class 1 integrons were detected in 54.2% (3956/7295) Gram-negative strains. *aadA2* was the most popular gene cassette array detected from 60 Gram-positive bacteria while dfrA17-aadA5 were detected in 426 Gram-negative bacteria. This study identified 12 novel gene cassette arrays which have not been previously found in any species. All the novel gene cassette arrays were detected from Gram-negative bacteria. A regional characteristic of distribution of integrons was presented in this study. The results highlight a need for continuous surveillance of integrons and provide a guide for future research on integron-mediated bacteria resistance.

Keywords: integron, novel cassette arrays, clinical bacteria, China

Introduction

Dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria, thus complicating the treatment of infections. Many studies have shown that integrons play an important role in the development of antibiotic resistance. Integrons are gene capture and expression systems characterised by the presence of an *intI* gene encoding an integrase, a recombination site (*attI*) and a promoter, situated in the bacterial plasmid, chromosome or transposon, which has the capability of site-specific recombination. They can also selectively capture or remove various specific drug resistance box genes, and transfer their drug resistance genes to different strains or different bacterial genera through functions, such as transformation, transduction and conjugation, a mechanism which

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accelerates the spread and dissemination of bacterial drug resistance[1–3]. China has the largest population in the world, and the ratio of unreasonable antibiotics usage in China is higher, so resistance to various antibiotics is common in clinical isolates, often more so than in Western countries[4]. This meta-analysis first reviewed all the researches on integrons and drug resistance in clinical isolates in China.

Till Dec 31, 2014, a total of 56 articles studied on integron and drug resistance of bacteria isolates from patients in China were included. The positive rates of integron were investigated in different species, and the gene cassettes were also measured in some studies. This study mainly review all the literatures, analyzed the data of types (class 1, 2, 3 and atypical integron), positive rates and gene cassette of integrons detected from patients in China.

Materials and methods

Search strategy

Two independent reviewers (WX and BG) performed a systematic literature review of potentially relevant studies on integrons and drug resistance. Studies were identified using the MEDLINE and EMBASE databases (for articles published till Dec 31, 2014) as well as bibliographies of identified papers. The search strategy used the following terms and connectors: "integron" AND "China". The search was not restricted by language. We also attempted to identify potentially relevant articles by checking the references of the germane articles and through personal communications with colleagues.

Inclusion and exclusion criteria

Studies obtained from the literature search were checked by title and citation. If an article appeared relevant, the abstract was reviewed. Relevant abstracts were examined in full text. The criteria for inclusion and exclusion of studies were established by the investigators before the literature was reviewed. Inclusion criteria were as follows: original article, short communication, correspondence or letter that provided sufficient original data; all strains isolated from patients in China. Exclusion criteria were as follows: review and case report; animal, plants, water experiments; strains isolated from healthy people; studies on selected isolates, with certain drug resistance genes, that were already resistant to some antimicrobial agents. Before any studies were excluded, authors of such studies were contacted in an effort to obtain missing data. Differences between reviewers regarding appropriateness for inclusion or exclusion were resolved by consensus.

Validity assessment

Studies were assessed for quality, with only high-quality studies included for analysis. Characteristics of high quality studies were: prospective cohort, retrospective consecutive cohort; provided basic data including study period and area, total tested numbers

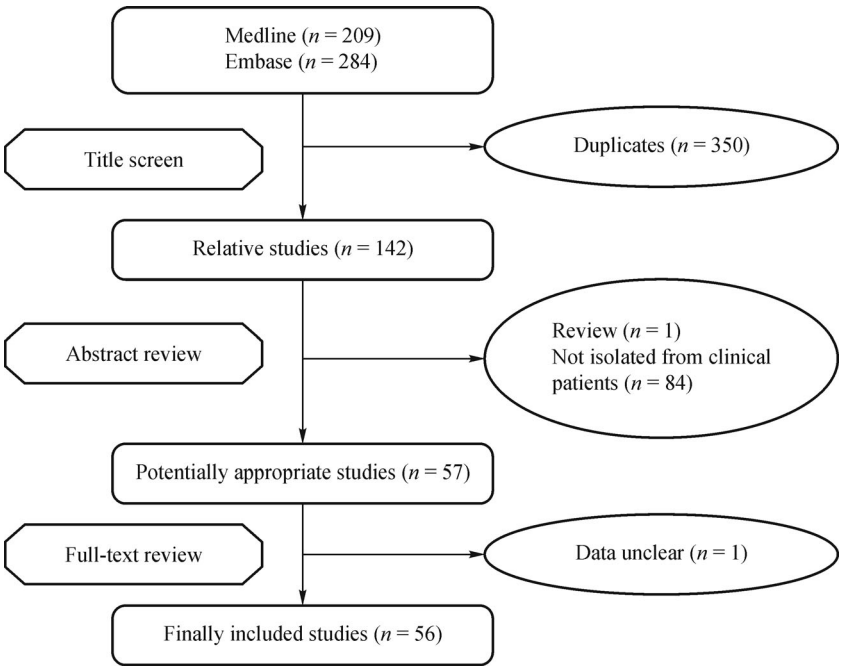

Fig. 1 **Systematic literature search flowchart**

Table 1 **Strains and positive rates of integrons from clinical bacterial isolates in China**

	Class 1 integron	Class 2 integron	Class 3 integron	Atypical class 1 integron
Gram-positive bacteria				
Staphylococcus	44.4% (199/448)	6.1% (11/180)	0 (0/180)	ND
Enterococcus	80.0% (8/10)	20% (2/10)	ND	ND
Gram-negative bacteria				
Escherichia coli	65.4% (1,356/2,072)	4.8% (53/1,096)	0.8%(6/709)	ND
Klebsiella pneumoniae	53.0% (444/838)	0 (0/90)	7.8% (7/90)	ND
Klebsiella oxytoca	50.5% (12/24)	ND	ND	ND
Marcescens	72.2% (13/18)	ND	ND	ND
Salmonella	17.1% (155/909)	0 (0/842)	0 (0/842)	ND
Shigella	75.3% (918/1,219)	79.9% (933/1,168)	0 (0/1,112)	77.5% (213/275)
Enterobacter	39.5% (79/200)	13.3% (2/15)	0 (0/15)	ND
Citrobacter	36.4% (4/11)	ND	ND	ND
Proteus	55.6% (109/196)	66.0% (101/153)	0 (0/153)	ND
Pseudomonas aeruginosa	37.5% (296/789)	1.4% (1/71)	0 (0/71)	ND
Acinetobacter	61.1% (674/1,103)	0.9% (2/229)	0 (0/158)	ND
Stenotrophomonas maltophilia	5.5% (6/109)	ND	ND	ND
Burkholderia cepacia	27.0% (20/74)	ND	ND	ND
Pseudomonas putida	66.7% (8/12)	ND	ND	ND
Other Gram-negative bacteria	71.4% (5/8)	ND	ND	ND

ND: no data.

and resistant numbers; susceptibility test was performed in accordance with guidelines established by the Clinical and Laboratory Standard Institute (CLSI). When studies overlapped, the more recent and larger study was included in the analysis. If the smaller study provided data not reported in the larger study, results were included for that specific variable.

Data extraction

Data extraction was performed by two reviewers using a standardised extraction form. When there was disagreement, the relevant paper was reviewed and differences were resolved by consensus. In this review, microsoft Excel v.1 2.0 was used for data entry and analysis.

Results

Literature search

A total of 519 articles were identified from the initial electronic database search finally, and 56 literatures were finally included (***Fig. 1***)[3–58].

Strains and positive rates of integron

Integron distribution was examined in 7,822 clinical isolates in 56 studies. Most strains (94.1%, 7,364/7,822) were Gram-negative bacteria while only 5.9% strains were Gram-positive bacteria (***Table 1***).

(1) Gram-positive bacteria

In all of the studies collected, a total of 458 Gram-positive bacteria were detected of which 448 strains were Staphylococcus. Among these Gram-positive

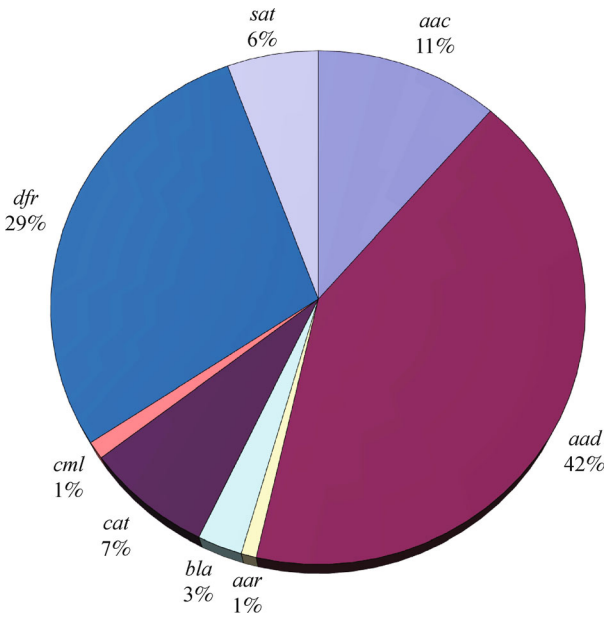

Fig. 2 **The proportions of the resistance genes of integrons in China**

Table 2 (a). **Gene cassette arrays of class 1 integrons in Gram-positive bacteria**

Strain	Gene cassette array	Detection rate*
Staphylococcus aureus	*aadA2*	59.2% (58/98)
	dfrA12-orfF-aadA2	48.7% (37/76)
	aacA4-cmlA1	2.6% (2/76)
	dfrA17-aadA5	1.3% (1/76)
Staphylococcus epidermidis	*dfrA12-orfF-aadA2*	75.0% (12/16)
	aacA4-cmlA1	12.5% (2/16)
	aadA2	6.2% (1/16)
	dfrA17-aadA5	6.2% (1/16)
Staphylococcus hominis	*dfrA12-orfF-aadA2*	75.0% (6/8)
	aacA4-cmlA1	12.5% (1/8)
	dfrA17-aadA5	12.5% (1/8)
Staphylococcus haemolyticus	*dfrA12-orfF-aadA2*	100% (5/5)
Staphylococcus warneri	*dfrA12-orfF-aadA2*	100% (1/1)
Enterococcus	*dfrA12-orfF-aadA2*	75.0% (6/8)
	dfrA17-aadA5	25.0% (2/8)
	aadA2	12.5% (1/8)

*: The denominator is the number of the detected integrons in the study.

Table 2 (b). **Gene cassette arrays of class 1 integrons in *Enterobactericeae***

Strain	Gene cassette array	Detection rate*
Escherichia coli	*aacA4-catB8-aadA1*	60.7% (17/28)
	dfrA17-aadA5	41.9% (284/678)
	orfD-aacA4-catB8	25.0% (7/28)
	dfrA12-orfF-aadA2	16.9% (89/526)
	aac(6')-1b-cmlA1	11.1% (11/99)
	aadA1-dfrA12	11.0% (12/109)
	dfrA1-aadA1	9.1% (9/99)
	dfrA12-aadA2	9.0% (16/178)
	dfr2d	8.1% (14/172)
	aadA23b	7.3% (8/109)
	dfrA17	5.1% (5/99)
	aadA1	4.4% (13/298)
	aac(6')-1b-catB8-aadA1	4.0% (4/99)
	dfrA7	3.8% (1/26)
	arr3-aacA4	3.8% (3/79)
	aacA4-cmlA1	3.8% (14/373)
	dfrA1	3.4% (6/178)
	aadA2	2.0% (2/99)
	dfrA5	2.0% (2/99)
	aac(6')-1b-cr-arr3-dfrA27-aadA16	2.0% (2/99)
	dfrA12	2.0% (2/99)
	dfrA1-orfC	1.2% (2/165)
	dfrv	1.2% (2/26)
	aadB-aadA2	1.1% (3/264)
	aadB-orf1-cmlA1	1.0% (1/99)

Table 2 (b). **Gene cassette arrays of class 1 integrons in *Enterobactericeae*** (continued)

Strain	Gene cassette array	Detection rate*
	aacA4-catB3-dfrA1	1.0% (1/99)
	aac(6')-1b-catB3-dfrA1	1.0% (1/99)
	aadB-cmlA1	1.0% (1/99)
	aacC4-cm1A1	1.0% (1/73)
	aacC-cmlA1	0.6% (1/165)
	aadA22	0.6% (1/165)
	aadB-aadA-cmlA6	0.6% (1/165)
	arr3-dfrA27	0.6% (1/165)
	dfrA5	0.6% (1/165)
	dfrA27	0.6% (1/165)
Klebsiella pneumoniae	*orfD-aacA4*	71.4% (10/14)
	dfrA12-orfF-aadA2	32.7% (55/168)
	aadA5-dfrA17	28.6% (4/14)
	dfrA17-aadA5	22.0% (37/168)
	dfrA1-orfC	19.5% (30/154)
	dfrA27-aac(6')-Ib-cr	14.1% (10/71)
	arr2-ereC-aadA1-cmlA7	7.1% (1/14)
	aadB-catB8-bla$_{OXA-10}$*-aadA1*	7.1% (1/14)
	aacC1/aacA1-orfP-orfQ-aadA1	7.1% (1/14)
	aadA2	5.6% (4/71)
	dfrA1-aadA1	5.6% (4/71)
	aacA4-catB8-aadA1	4.8% (4/83)
	aac(6')-Ib-cr-aar-3	4.2% (3/71)
	dfrA25	3.5% (3/85)
	accC4-cmlA	2.4% (2/83)
	aadA1	1.4% (1/71)
	ORF for hypothetical protein-mfs-1	1.4% (1/71)
	aacA4-bla$_{OXA-4}$*-aadA2*	1.2% (1/83)
	dfrA12-orfF	1.2% (1/83)
	dfrA5	1.2% (1/83)
Klebsiella oxytoca	*dfrA17-aadA5*	42.9% (3/7)
	aac(6')-Ib-cr-aar3-dfrA27-aadA16	28.5% (2/7)
	ddfrA1-aadA5	28.5% (2/7)
Marcescens	*dfrA12-hypothesis protein-aadA2*	100.0% (10/10)
Salmonella	*dhfrXII-orfF-aadA2*	86.5% (32/37)
	dfrA12-orfF-aadA2	50.5% (51/101)
	aadA5-dfrA1l	4.7% (5/106)
	dfrA1	17.8% (18/101)
	aadA2	15.1% (16/106)
	bla$_{OXA-30}$*-aadA1*	13.5% (5/37)
	blaP1	5.0% (5/101)
	dfrA1-aadA1	5.0% (5/101)
	aadA22	4.0% (4/101)
	aadA1	1.0% (1/101)
	dfrA12-unknown-aadA1	1.0% (1/101)

Table 2 (b). **Gene cassette arrays of class 1 integrons in *Enterobactericeae*** (continued)

Strain	Gene cassette array	Detection rate*
Shigella	*dfrA17- aadA5*	17.5% (73/417)
	aar-3-aacA4	0.8% (2/249)
	dfrA12-orfF-aadA2	0.5% (2/417)
Enterobacter	*dfrA17-aadA5*	61.5% (8/13)
	dfrA12-hypothesis protein-aadA2	23.1% (3/13)
	dfrA12–orfF–aadA2–orfII–orfIII	20.0% (3/15)
	ant(3'')-Ih–aac(6')-Iid–catB8	16.7% (1/6)
	aacA4–catB8–aadA1	9.5% (2/21)
	dfrA12–orfF–aadA2	9.5% (2/21)
	dfrA15	13.3% (2/15)
	aac(6')-Ib-cr-aar3-dfrA27-aadA16	7.7% (1/13)
	drfA7	7.7% (1/13)
	dfrA15-aadA2	6.7% (1/15)
Citrobacter	*aadA2*	66.7% (2/3)
	dfrA12-hypothesis protein-aadA2	33.3% (1/3)
Proteus	*aadB-aadA2*	28.0% (37/132)
	dfrA17-aadA5	15.8% (22/139)
	dfrA12-hypothesis protein-aadA2	14.3% (1/7)
	dfrA1–orfF	14.3% (1/7)
	aadB-catB8-bla$_{OXA-10}$-aadA1	14.3% (1/7)
	dfrA12-orfF-aadA2	7.9% (11/139)
	aadA2	2.2% (3/139)
	dfrA1-orfC	1.5% (2/132)
	aacA4-cmlA1	0.8% (1/132)
	aadB	0.8% (1/132)
	dfrA1-sat2	0.8% (1/132)

*: The denominator is the number of the detected integrons in the study.

Table 2 (c) **Gene cassette arrays of class 1 integrons in non-fermentative bacteria and other Gram-negative bacteria**

Strain	Gene cassette array	Detection rate*
Pseudomonas aeruginosa	*aac(6')-II-aadA13-cmlA8-oxa-10*	100.0% (29/29)
	dfr17-aadA5	81.5% (22/27)
	aacA4-catB8a-bla$_{OXA-10}$	50.0% (1/2)
	aacA4-bla$_{IMP-9}$-aacA4	50.0% (1/2)
	aadA6-orfD	45.5% (15/33)
	bla$_{IMP-9}$-aacA4-bla$_{OXA-10}$-aadA2	44.4% (16/36)
	aadA2	24.2% (8/33)
	dfrA17-aadA5	22.2% (2/9)
	aadB-aacA4	22.2% (2/9)
	aadB-aadA1	22.2% (2/9)
	aadB-aac(6')-IIa-bla$_{CARB-8}$	18.2% (6/33)
	aac(6')-II–aadA13–cmlA8–bla$_{OXA-10}$	16.7% (6/36)
	aadB-bla$_{PSE-1}$	16.7% (6/36)
	aadB-bla$_{PSE-1}$-aacA4	11.1% (1/9)
	dsul3-Δorf5	11.1% (1/9)

Table 2 (c) **Gene cassette arrays of class 1 integrons in non-fermentative bacteria and other Gram-negative bacteria** (continued)

Strain	Gene cassette array	Detection rate*
	aadB	11.1% (1/9)
	aacA4-bla$_{IMP-25}$-bla$_{OXA-30}$-catB3	8.4% (3/36)
	dfrA12-orfF-aadA2	5.6% (2/36)
	dfrXII-orfF-aadA2	9.1% (3/33)
	aadB-bla$_{P1}$	3.0% (1/33)
	dfrA15	2.8% (1/36)
	aadB-aadA2	2.8% (1/36)
	aacA4-aadA2	2.8% (1/36)
Acinetobacter	*aacA4-catB8-aadA1*	71.2% (255/358)
	aac(6')-IId-catB8-aadA1	69.6% (16/23)
	aacC1-orfP-orfQ-aadA1	27.9% (17/61)
	aacC1-orfX-orfX-orfX'-aadA1	16.2% (16/99)
	orfI-aadA1	12.8% (12/94)
	arr3-aacA4	10.9% (30/276)
	dfrXII-orfF-aadA2	10.7% (6/56)
	aadB-catB-like-bla$_{OXA-10}$/aadA1	10.7% (6/56)
	aacC1-orfP-orfP-orfQ-aadA1	4.3% (2/46)
	aacA4	3.8% (1/26)
	aadB-bla$_{PSE-1}$-aacA4	3.8% (1/26)
	drfA7	3.8% (1/26)
	dfr17-aadA5	3.6% (2/56)
	aacC1-orfA-orfB-aadA1	1.4% (2/139)
	dfrA15	1.4% (2/139)
	aadB-aadA2	1.4% (2/139)
	aadA2	1.3% (2/155)
Stenotrophomonas maltophilia	*dfrA15*	33.3% (1/3)
	aadB–aadA2	33.3% (1/3)
	aadB–aadA4	14.3% (3/21)
Burkholderia cepacia	*aadB-aac(6')-II-bla$_{PSE-1}$*	77.8% (7/9)
	aacA4-catB8–aadA1	22.2% (2/9)
Pseudomonas putida	*aadB-aac(6)-II-bla$_{PSE-1}$*	60.0% (3/5)
	aadA1	20.0% (1/5)
Other Gram-negative bacteria	*drfA7*	14.3% (1/7)
	bla$_{PSE-1}$-aadA2	14.3% (1/7)
	aadB-catB3	14.3% (1/7)
	aadB-catB8-bla$_{OXA-10}$-aadA1	14.3% (1/7)
	aadA2	14.3% (1/7)

*: The denominator is the number of the detected integrons in the study.

bacteria, class 1 integron was detected in 207 strains while class 2 integron in 13 strains. No class 3 integron and atypical class 1 integron was detected.

(2) Gram-negative bacteria:

Among 56 included studies, 47 studies focused on Gram-negative bacteria. A total of 7,295 Gram-negative strains were detected in these studies. Based on data, class 1 integron positive were detected in 3,956 strains, 1,037 strains positive in class 2 integron, and 13 strains positive in class 3 integron. It is worth noting that atypical class 1 integrons were detected in 213 Gram-negative strains.

Table 3 **Gene cassette arrays of class 2 integrons in China**

Strain	Gene cassette array	Detection rate*
Enterococcus	*dfrA1-sat1–aadA1*	100.0% (2/2)
Escherichia coli	*dfrA1-sat2-aadA1*	100.0% (3/3)
	dfrA1-sat1-aadA1	2.8% (3/109)
Shigella	*dfrA1-sat1-aadA1*	40.0% (167/417)
Proteus	*dfrA1-sat2-aadA1*	45.5% (60/132)

*: The denominator is the number of the detected integrons in the study.

A total of 5,201 *Enterobacteriaceae* were detected in these 41 studies, class 1 integrons were detected in 2,947 strains, and class 2 integron in 1,034 strains. the Class 3 integron (13 strains) and atypical class 1 integron (213 strains) were detected from *Enterobacteriaceae*. A total of 2,087 non-fermenting bacteria strains were detected of which 1,004 strains were positive in class 1 integron while only 3 strains positive in class 2 integron.

Gene cassettes of integrons in China

Based on the 56 including studies, we summarized the distribution of gene cassettes of integrons detected from isolates. ***Table 2*** and ***Table 3*** show the proportions of class 1 integrons and classs 2 integrons from clinical strains. *aadA2* was the most popular gene cassette array detected from 60 Gram-positive bacteria while 426 Gram-negative bacteria were detected *dfrA17-aadA5*. Only 1 study detected class 3 integron in *Klebsiella pneumoniae*, and the gene cassette array of all the 6 isolates were the same as those comprising *bla*$_{\text{GES-1}}$-*bla*$_{\text{OXA-10}}$-*aac*(6')-*Ib*.

Besides the proportion of gene cassette arrays in isolates, we also analyzed the different types of the gene cassette arrays of integrons. We found that a total of 19 kinds of arrays composed by only one gene cassette were detected in China. The array *aadA2* was the most common among the 19 kinds of arrays, which was detected from 95 strains, mainly from *Staphylococcus aureus, Proteus* and other Gram-negative bacteria. *aacC* and *dfr2d* took the second place among the one gene cassette arrays, which were all detected from 17 *Escherichia coli* strains. There were 33 kinds of arrays composed by 2 gene cassettes in which *dfrA17-aadA5* was the most array from 431 strains including *Escherichia col*i, *Klebsiella pneumoniae* and *Shigella*, while *aadB-aadA2 was* detected from 43 clinical isolates mainly from *Proteus* and 35 clinical strains, mainly from *Acinetobacter*, detected *arr-3-aacA4*. The type of arrays composed by 3 gene cassettes were detected in 22 different kinds in China. *aacA4-catB8-aadA1* followed by *dfrA12-orfF-aadA2* and *dfrA1-sat1-aadA1* occupied the top 3 which were detected from 280, 227, and 172 strains mainly from *Acinetobacter*, *Escherichia coli*, *Klebsiella*, and *Shigella*, respectively.

The resistance gene cassettes of *aad*, *dfr*, *aac*, *cat*, *sat*, *bla*, *cml* and *aar* of integrons were detected in China. ***Figure. 2*** shows the proportion of those resistance gene cassettes detected in China.

Atypical class 1 integron

All atypical class 1 integrons were detected from *Shigella* in 3 studies[15,35,42]. The gene cassette array *bla*$_{\text{oxa-30}}$-*aadA1*, *bla*$_{\text{OXA-1}}$-*aadA1* and *dfrA1-sat1-aadA1* were detected in these studies.

Novel gene cassette array

Six studies claimed they found the novel gene cassette array which was not found in any species previously[4,7–8,20,33,54]. All the novel gene cassette arrays were detected from Gram-negative bacteria. After excluding the unknown gene cassette arrays, the 12 novel gene cassette arrays are listed as follows: *aadA6-orfD, aadB-bla*$_{P1}$, *aadB-aac(6')-IIa-bla*$_{\text{CARB-8}}$, *orfI-aadA1, aadB-catB*-like-*bla*$_{\text{oxa-10}}$/*aadA1, dfrA1-aadA5, orfF-HAD*-like-*aac(6')-II, orfF-*Δ*MFS*-1, *catB3-qnrVC*-like-*aacA4, gcuD1-aacA4'-17-gcu38B-catB8::IS10*, *aacA3c-aadA13*-bla$_{\text{OXA-25}}$, and *dfrA1-gcu37-aadA5*.

Regional distribution of class 1 integron in China

Based on 56 studies, 16 provinces and municipalities had data on distribution of class 1 integron. Beijing, Guangzhou and Henan province had data on not only Gram-positive bacteria but also Gram-negative bacteria, while only Gram-negative bacteria were detected in other areas including Anhui, Gansu, Hubei, Hunan, Jiangsu, Shandong, Shanghai, Shenzhen, Sichuan, Tianjin, Zhejiang, Taiwan, and Hong Kong. Among Gram-negative bacteria, the highest rate is 91.5% (65/71) in Tianjin; on the contrast, the lowest rate is 12.6% (105/834) in Hong Kong (***Fig. 3***).

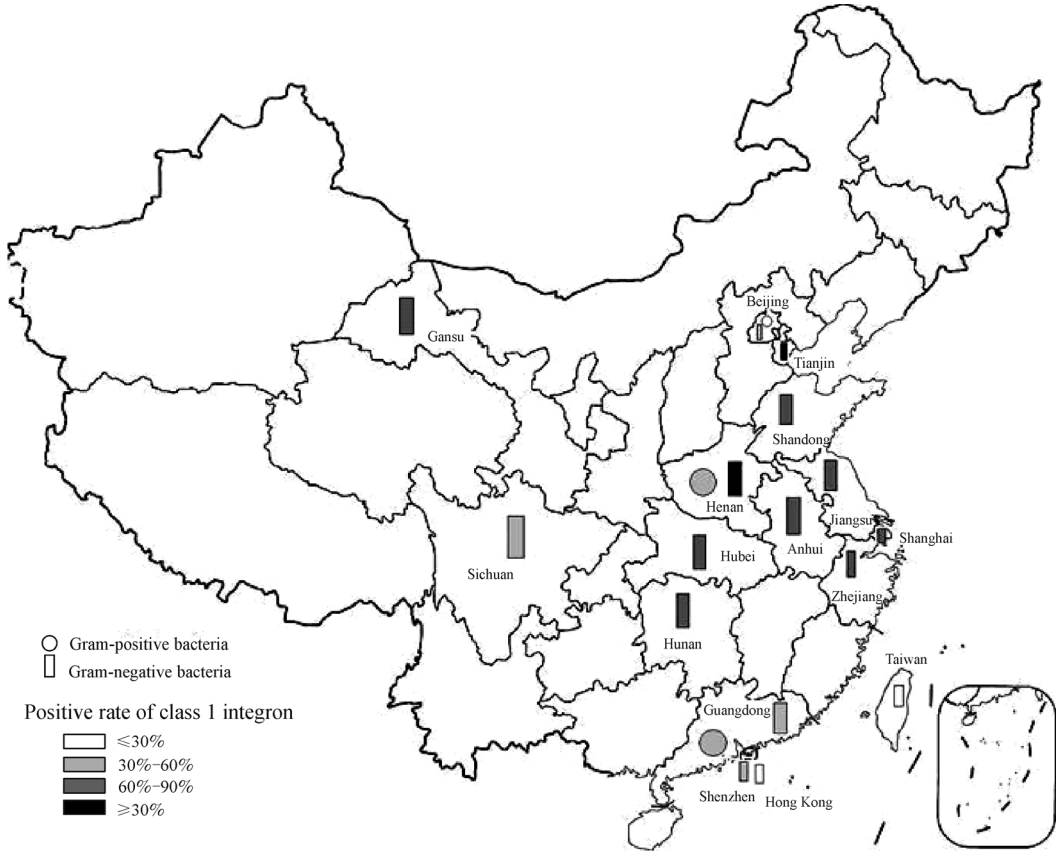

Fig. 3 **Distribution of class 1 integron in China**

Discussion

Integrons play an important role in the dissemination of antimicrobial resistance through horizontal transmission. Their contribution to the prevalence of multi-drug resistance Gram-negative bacteria has been demonstrated. This study comprehensively analyzed integron distribution in China based on published articles.

From the data of integron distribution, we can easily conclude that integrons in both Gram-positive bacteria and Gram-negative bacteria can be detected, but integrons were widely distributed among clinically isolated Gram-negative bacteria while only seldom integrons can be detected in Gram-positive bacteria (mostly in *Staphylococcus*).We also found that only 5 studies were about integron in *Staphylococcus*[3,12,27,30–31], and wherein 4 were by the same author, so we have reason to suspect that the detection results are representative. Among Gram-negative bacteria, *Enterobacteriaceae* had a higher positive rate of integron than non-fermenting bacteria. From the results, it is evident that class 1 integron positive rate is the highest, much higher than class 2 integron, class 3 integron and atypical class 1 integron. The three highest positive rates of class 1integron occurred in *Shigella*, *Escherichia coli* and *Marcescens*. Class 2 integrons were detected most frequently in *Shigella*, and the rest were detected in *Proteus*, *Staphylococcus*, and *Escherichia coli*, etc. Class 3 integrons were detected in multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli*. Atypical class 1 integrons were only detected in *Shigella*. In our previous study, we detected integron distribution in different kinds of Gram-negative bacteria, including *Shigella*, *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter*[4,33,44]. Our results showed that the positive rates of class 1,2 and atypical class 1 integron in *Shigella* were 68.5% (660/964), 85.2% (821/964) and 73.0% (704/964), respectively, the positive rates of class 1 and 2 integron in *Enterobacteriaceae* were 58.5% (83/142), 14.1% (20/142), respectively, and 40.8% (40/98) *Pseudomonas aeruginosa* and 52.8% (56/106) *Acinetobacter* were detected class 1 integron. These results are similar to the review results. Furthermore, in our previous studies, we also found 5 novel gene cassette arrays in the same bacteria. These phenomena give us important information about the characteristics of integron distribution that may provide a basis for future study.

According to our review results, many types of gene cassettes were observed in the same species/genus of

isolates, while the same gene cassette array was found in different species/genus of isolates, which might contribute to genes capture capacity and dissemination capacity of integrons. Most isolates carrying class 1 integron contained gene cassettes. However, a few isolates among class 1 integron positive strains did not contain gene cassettes. The main reasons may be: defects or mutations at the 3'CS; gene cassette array in novel, complex or unusual class 1 integrons or the variable region was too long to be amplified.

In this study, we also found that the class 1 integron positive rate in mainland China was higher than Taiwan and Hong Kong. We speculate that whether the different antibiotic policies lead to different situations of bacteria resistance. But because the inclusion criteria, the number and type of isolates are different, the results are for reference only.

Among 56 studies, many studies also showed that the presence of integrons is positively correlated with multi-drug resistance phenotype. One integron may carry several box genes. The production of bacterial multi-drug resistance is closely related to the integrons. The gene cassettes included those encoding resistance to trimethoprim (*dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA15*, *dfrA17* and *dfrA27*), aminoglycosides (*aadA*, *aadA1*, *aadA2*, *aadA5*, *aadA12*, *aadA13*, *aadA16*, *aadA22*, *aadB*, *aac(6')-II*, *aac(6')-Iid*, *aac(6')-1b*, *aacA4*, *aacC*, *aacC1*, *aacC4* and *ant(3')-Ih*), the β-lactamase ($bla_{\text{PSE-1}}$, $bla_{\text{OXA-4}}$, $bla_{\text{OXA-10}}$, $bla_{\text{OXA-30}}$, $bla_{\text{CARB-8}}$,$bla_{\text{IMP-9}}$ and $bla_{\text{IMP-25}}$), chloramphenicol (*cmlA1*, *cmlA6*, *cmlA7*, *cmlA8*, *catB3* and *catB8*), quinolones (*qnrVC*-like) and rifampicin (arr2, arr3). Horizontal gene transfer was clearly evident among the Gram-negative bacteria in some studies, as gene arrays, including *aacA*4-*catB8-aadA1*, *dfrA12-orfF-aadA2*, *dfrA15* and *aadB-aadA2* were found in different species of *Enterobacteriaceae* and non-fermentative bacteria. Horizontal gene transfer was further suggested by the discovery of arrays (*dfrA5*, *dfrA1-orfC* and *dfrA1-aadA5*) in different *Enterobacteriaceae* species. 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. It is urgent to conduct consecutive surveillance of class 1 integron related antimicrobial resistance in China. Meanwhile, 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.

It is worth noting that 12 novel gene cassette arrays were discovered in several studies. It indicated that integrons can efficiently capture and integrate genes. For example, the *qnrVC*-like gene, which was included in the *catB3-qnrVC*-like-*aacA4* array, showed 98% identity with the functional qnrVC genes, which differed by 14 and 15 nucleotides compared with qnrVC1 and qnrVC3, respectively. The presence of novel integron structures in clinical isolates suggests that hospital environments may favor the formation of novel combination of gene cassettes. Moreover, the high prevalence of integrons in multi-drug resistant isolates highlights the urgent need to employ effective means to avoid dissemination of drug-resistant bacteria.

Overall, this study is a statistical study on the integron types (including class 1 integron, class 2 integron, class 3 integron and atypical class 1 integron) , the positive rates and the gene cassettes detected from clinical isolates in China. This study may provide a reference for future study on integron-mediated bacteria resisteance.

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