Characterization of integrons and novel cassette arrays in bacteria from clinical isolates 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...
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

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**Fig. 1** Systematic literature search flowchart
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.12.0 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

| Table 1  | Strains and positive rates of integrons from clinical bacterial isolates in China |
|----------|----------------------------------------------------------------------------------|
| Gram-positive bacteria | Class 1 integron | Class 2 integron | Class 3 integron | Atypical class 1 integron |
| 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% (674/1,013) | 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.
### 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                       | 2.6% (1/16)     |
|                         | dfrA17-aadA5                | 1.3% (1/16)     |
| Staphylococcus hominis  | dfrA12-orfF-aadA2            | 75.0% (6/8)     |
|                         | aacA4-cmlA1                 | 12.5% (1/8)     |
|                         | dfrA17-aadA5                | 6.2% (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)’-Ib-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)’-Ib-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)’-Ib-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)     |
| Strain              | Gene cassette array                                      | Detection rate* |
|---------------------|----------------------------------------------------------|-----------------|
|                     |                                                          |                 |
|                     | aacA4-catB3-dfrA1                                         | 1.0% (1/99)     |
|                     | aac(6')-Ib-catB3-dfrA1                                    | 1.0% (1/99)     |
|                     | aadB-cmlA1                                               | 1.0% (1/99)     |
|                     | aacC4-cmlA1                                              | 1.0% (1/73)     |
|                     | aacC-cmlA1                                               | 0.6% (1/165)    |
|                     | aadA2                                                    | 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-dfrA1                                              | 28.6% (4/14)    |
|                     | dfrA17-aadA4                                             | 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-blaOXA-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-blaOXA-10-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-aar-3-dfrA27-aadA16                         | 28.5% (2/7)     |
|                     | dfrA1-aadA4                                              | 28.5% (2/7)     |
| Marcescens          | dfrA12-hypothesis protein-aadA2                           | 100.0% (10/10)  |
| Salmonella          | dfrXII-orfF-aadA2                                         | 86.5% (32/37)   |
|                     | dfrA12-orfF-aadA2                                         | 50.5% (51/101)  |
|                     | aadA5-dfrA1                                              | 4.7% (5/106)    |
|                     | dfrA1                                                    | 17.8% (18/101)  |
|                     | aadA2                                                    | 15.1% (16/106)  |
|                     | blaOXA-10-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)    |
| 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                                         | 20.0% (3/15)    |
|            | ant(3')–ih–aac(6')–lsd–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')–lb–cr–aar3–dfrA27–aadA16                          | 7.7% (1/13)     |
|            | dfrA7                                                    | 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–blaOXA–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.

| Strain                          | Gene cassette array                                      | Detection rate* |
|---------------------------------|----------------------------------------------------------|-----------------|
| *Pseudomonas aeruginosa*        | aac(6’)-Il–aadA13–cmlA8–blaOXA–10                        | 100.0% (29/29)  |
|                                 | dfr17–aadA5                                              | 81.5% (22/27)   |
|                                 | aacA4–catB8–blaOXA–10                                    | 50.0% (1/2)     |
|                                 | aacA4–blaOXA–10–aacA4                                    | 50.0% (1/2)     |
|                                 | aadA6–orfD                                              | 45.5% (15/33)   |
|                                 | blaOXA–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’)-Il–blaOXA–10                               | 18.2% (6/33)    |
|                                 | aac(6’)-Il–aadA13–cmlA8–blaOXA–10                        | 16.7% (6/36)    |
|                                 | aadB–blaPSE–1                                           | 16.7% (6/36)    |
|                                 | aadB–blaPSE–1–aacA4                                     | 11.1% (1/9)     |
|                                 | dsuI–orfF                                               | 11.1% (1/9)     |
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 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* |
|--------|---------------------|-----------------|
|        |                     |                 |
| Acinetobacter |                     |                 |
|        | aadB                | 11.1% (1/9)     |
|        | aacA4-blaIMP-25-blaOXA-10-catB3 | 8.4% (3/36)   |
|        | dfrA12-orfF-aadA2   | 5.6% (2/36)     |
|        | dfrXII-orfF-aadA2   | 9.1% (3/33)     |
|        | aadB-blaP1          | 3.0% (1/33)     |
|        | dfrA15              | 2.8% (1/36)     |
|        | aadB-aadA2          | 2.8% (1/36)     |
|        | aacA4-aadA2         | 2.8% (1/36)     |
|        | aacA4-catB8-aadA1   | 71.2% (255/358) |
|        | aac(6’)-Id-catB8-aadA1 | 69.6% (16/23) |
|        | aacC1-orfP-orfQ-aadA1 | 27.9% (17/61) |
|        | aacC1-orfX-orfX-orfX’-aadA1 | 16.2% (16/99) |
|        | orfB-aadA1          | 12.8% (12/94)   |
|        | arr3-aacA4          | 10.9% (30/276)  |
|        | dfrXII-orfF-aadA2   | 10.7% (6/56)    |
|        | aadB-catB-like-blaOXA-10-aadA1 | 10.7% (6/56) |
|        | aacC1-orfP-orfP-orfQ-aadA1 | 4.3% (2/46) |
|        | aacA4               | 3.8% (1/26)     |
|        | aadB-blaPSE-1-aacA4 | 3.8% (1/26)     |
|        | dfrA7               | 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-blaPSE-1 | 77.8% (7/9) |
|        | aacA4-catB8-aadA1   | 22.2% (2/9)     |
| Pseudomonas putida |               |                 |
|        | aadB-aac(6’)-II-blaPSE-1 | 60.0% (3/5) |
|        | aadA1               | 20.0% (1/5)     |
| Other Gram-negative bacteria |               |                 |
|        | dfrA7               | 14.3% (1/7)     |
|        | blaPSE-1-aadA2      | 14.3% (1/7)     |
|        | aadB-catB3          | 14.3% (1/7)     |
|        | aadB-catB8-blaOXA-10-aadA1 | 14.3% (1/7) |
|        | aadA2               | 14.3% (1/7)     |

*: 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 Enterobacteriaceae integron (213 strains) were detected from Class 3 integron (13 strains) and atypical class 1 integron. The resistance gene cassettes of aad, dfr, aac, cat, sat, bla, cmu and arr of integrons were detected in China. Figure 2 shows the proportion of those resistance gene cassettes detected in China.

### 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 class 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 blaGES-1-
dfrA12-orfF-aadA2

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 coli, 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.

#### Atypical class 1 integron

All atypical class 1 integrons were detected from Shigella in 3 studies [15,35,42]. The gene cassette array blaOxa-30-aadA1, blaOXA-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-blaP1, aadB-aac(6’)-Ila-blaOXA-25, orfF-oxf-arr-1-aadA1, aadB-cat-like-blaOxa-10-aadA1, dfrA1-aadA5, orfF-HAD-like-aac(6’)-II, orfF-DMSF-1, catB3-qnrVC-like-aacA4, gcuD1-aacA4’-17-gcu38B-catB8::IS10, aacA3c-aadA13-blaOXA-25, and dfrA1-gcu37-aadA5.

### Regional distribution of class 1 integron in China

Based on 56 provinces, 16 cities and municipalities, 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).
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 1 integron 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 phenotype. One integron may carry gene cassettes. The main reasons may be:

Horizontal gene transfer was further suggested by the presence of integrons is closely related to the integrons. The inclusion criteria, the number and type of isolates are different, the results are for reference only.

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