Clinical Molecular and Genomic Epidemiology of *Morganella morganii* in China

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**Objectives:** Ongoing acquisition of antimicrobial resistance genes has made *Morganella morganii* a new clinical treatment challenge. Understanding the molecular epidemiology of *M. morganii* will contribute to clinical treatment and prevention.

**Methods:** We undertook a 6-year clinical molecular epidemiological investigation of *M. morganii* from three tertiary hospitals in China since 2014. Antimicrobial susceptibility testing was performed using a VITEK-2 system. All isolates were screened for β-lactam and plasmid-mediated quinolone resistance genes by PCR. Isolates carrying carbapenem-resistant genes were subjected to whole-genome sequencing (WGS). The variation and evolution of these mobile genetic elements (MGEs) were then systematically analyzed.

**Results:** Among all *M. morganii* isolates (*n* = 335), forty (11.9%) were recognized as multidrug resistant strains. *qnrD1*, *aac(6')-Ib-cr*, *blaTEM-104*, and *blaCTX-M-162* were the top four most prevalent resistance genes. Notably, phylogenomic and population structure analysis suggested clade 1 (rhierBAPS SC3 and SC5) associated with multiple resistance genes seemed to be widely spread. WGS showed a *blaOXA-181*-carrying IncX3 plasmid and a *Proteus* genomic island 2 variant carrying *blaCTX-M-3*, *aac(6')-Ib-cr* coexisted in the same multidrug resistant strain zy_m28. Additionally, a *blaIMP-1*-carrying IncP-1β type plasmid was found in the strain nx_m63.

**Conclusion:** This study indicates a clade of *M. morganii* is prone to acquire resistance genes, and multidrug resistant *M. morganii* are increasing by harboring a variety of MGEs including two newly discovered ones in the species. We should be vigilant that *M. morganii* may bring more extensive and challenging antimicrobial resistance issue.

**Keywords:** *Morganella morganii*, molecular epidemiology, *blaOXA-181*, genomic island, *blaIMP-1*
INTRODUCTION

Morganella morganii is emerging as a significant opportunistic pathogen in the hospital settings (Liu et al., 2016). A 6-year study of Gram-negative bacterial infections in Taiwan showed that this pathogen is the ninth most prevalent cause of clinical infections (Chen et al., 2012). It is reported that M. morganii has been involved in a variety of clinical infections, such as peritonitis, septic arthritis, sepsis, infective endocarditis (van Bentum et al., 2019) and bilateral keratitis (Zhang et al., 2017). Invasive M. morganii infections are usually associated with high mortality rates due to lack of appropriate empirical antibiotic treatment (Erlanger et al., 2019).

Morganella morganii has intrinsic resistance to ampicillin, amoxicillin and most of the first- and second-generation cephalosporins because of its intrinsic AmpC resistance gene (Kohlmann et al., 2018). Ongoing resistance genes or virulence factors acquisition via mobile genetic elements (MGEs) including integrative and conjugative elements (ICEs) and mobilizable factors acquisition via mobile genetic elements (MGEs) including (Kohlmann et al., 2018). Ongoing resistance genes or virulence factors acquisition via mobile genetic elements (MGEs) including integrative and conjugative elements (ICEs) and mobilizable genetic islands (MGIs) has promoted M. morganii to become a new clinical treatment challenge (Flannery et al., 2009; Schultz et al., 2017). Resistance genes were mainly plasmid mediated and harbored by various transposons or integrons, such as blaKPC-2-carrying IncP6 plasmid (Kukla et al., 2018), blaOXA-181-carrying IncN plasmid (McGann et al., 2015), blaNDM-1-carrying IncC plasmid (Aires-de-Sousa et al., 2020), blaNDM-5-carrying IncX3 plasmid (Guo et al., 2019), blaIMP-27-carrying Tn7 transposon (Walky et al., 2018), blaCTX-M-3-carrying Tn6741 transposon (Luo et al., 2020), cfr-carrying Tn7 transposon (Chen et al., 2019) and blACES-5-carrying In1390 integron (Moura et al., 2018), which have significantly contributed to the increased levels of resistance in M. morganii.

Salmonella genomic island 1 (SGI1), is an integrative MGI that has many variants (Schultz et al., 2017), and can be mobilized by IncA/C conjugative plasmids (Carraro et al., 2014). In addition, some SGI1-related elements, such as Proteus genomic island 2 (PGI2), Acinetobacter genomic island 1 (AGI1) and their variants have been described in various bacteria including diverse serovars of S. enterica, Vibrio cholerae, P. mirabilis, and A. baumannii (Cummings et al., 2020). These GIs consist of a conserved backbone and a highly genetic variable multidrug resistant region derived from one or more complex class 1 integron carrying various resistance gene cassettes (Girlich et al., 2015). The backbone usually integrates into the chromosomes at 3’ end of the trmE gene, and the multidrug resistant region often locates adjacent to the res gene (Lei et al., 2020). Noteworthy, SGI1 variant (SGI1-L) carrying resistance genes dfrA15, floR, tetA(G), blacARB-2 and sul1 has also been identified in M. morganii (Schultz et al., 2017). Besides, carbapenemase gene blaNDM-1 has also been found in the MDR region among SGI1-like sequences (Girlich et al., 2015).

Antimicrobial resistance in M. morganii therefore can be introduced via both resistance plasmid acquisition and genomic island horizontal transfer. However, large-scale and long-term molecular investigations of clinical isolates of M. morganii has rarely been conducted. The aim of this study is to conduct a clinical and molecular epidemiological investigation of M. morganii isolated from three tertiary hospitals in China, and to unravel the molecular mechanisms underlying antimicrobial resistance of M. morganii in China.

MATERIALS AND METHODS

Bacterial Isolates

All M. morganii strains isolated from clinical specimen were collected from three tertiary hospitals in China from June 2014 to June 2020 including The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China (Hospital ZY), Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou, China (Hospital SZY) and General Hospital of Ningxia Medical University, Yinchuan, China (Hospital NX). If more than one strain were isolated from the same patient, only the first isolated strains were included. All the M. morganii strains were identified by VITEK-2 automatic bacterial identification system (BioMérieux, France). Isolates were cultured on Columbia agar with 5% sheep blood (BioMérieux, France) at 37°C in 5% CO2 atmosphere for 16 to 18 h.

Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MICs) of pipacillin-tazobactam, ceftazidime, cefepime, aztreonam, ciprofloxacin, levofloxacin, gentamicin, tobramycin, amikacin and trimethoprim-sulfamethoxazole were analyzed by VITEK-2 drug sensitivity analysis system (BioMérieux, France). MICs of meropenem (ApexBio, United States) and ertapenem (Menlunbio, China) were tested using micro-broth dilution method according to the guidelines of 2019 Clinical and Laboratory Standards Institute (CLSI) criteria (CLSI, 2019). Strain Escherichia coli ATCC 25922 was used as the quality control strain.

Resistance Genes Detection

DNA templates were extracted by the boiling method as previously described (Sun et al., 2014). Polymerase chain reaction (PCR) was performed to detect antimicrobial resistance genes, including β-lactam resistance genes (blaKPC, blaNDM, blaOXA-48, blaVIM, blalMP, blaCTX-M, blaTEM, and blashV), and plasmid-mediated quinolone resistance (PMQR) genes [qnrA, qnrB, qnrC, qnrD, qnrS, acrA(6)-Ib-cr, qepA, and aqxAB] by using primers as described previously (Xiao-Min et al., 2014; Szabó et al., 2018; Cai et al., 2019). Primers synthesis and positive PCR products sequencing were conducted by Sangon company, Shanghai1. BLASTN2 was used to align the sequencing results. Primers and thermal conditions are presented in Supplementary Table 1.

Conjugation Experiments

Conjugation experiment was performed to determine the transmissibility of carbapenem-resistant genes. The same
amount \((1 \times 10^7 \text{ CFU/mL})\), counted using the Sysmex UF-1000i™ Automated Urine Particle Analyzer; Tokyo, Japan) of mid-logarithmic phase donor (strain zy\_m28 and nx\_m63) and recipient cells \((E. \text{ coli C600})\) were mixed in 200 \(\mu\text{L}\) LB in 96-well plates. After mating for 6 h at 37°C, 20 \(\mu\text{L}\) mixed cultures were spread on LB agar containing 1 \(\mu\text{g/mL}\) meropenem plus 100 \(\mu\text{g/mL}\) rifampin. The conjugation frequency was calculated as transconjugants divided by number of donors. All experiments were carried out three times. Species identification, antimicrobial susceptibility testing and resistance genes detection were further performed on the transconjugants.

**Whole-Genome Sequencing, Assembly and Phylogenomic Analysis**

Two strains harboring carbapenem-resistant genes were whole-genome sequenced. Genomic DNA was extracted using a MiniBEST Bacteria Genomic DNA Extraction Kit (TaKaRa, Dalian, China). Chromosomal libraries with a 300 bp insert size were prepared as previously described (Cai et al., 2019) and all barcoded libraries were sequenced on a NextSeq 500 platform (Illumina Inc., San Diego, CA, United States). To construct a current phylogenomic tree, raw reads in fastq format or pre-assembled sequences in fasta format were downloaded from NCBI database for all publicly available \(M. \text{ morganii}\) isolates (Supplementary Figure 2). Paired-end raw reads were filtered using FASTQ preprocessor Fastp v0.12.5 to exclude library adapter and low quality reads (Chen et al., 2018) and de novo assembled with Unicycler v0.4.9b (Wick et al., 2017). Then, the scaffolds were annotated with Prokka v1.4.6 (Seemann, 2014). Antimicrobial resistance genes and plasmid types were identified by ABRicate v0.8.13\(^4\). ICEs and MGIs were identified ICEberg 2.0 (Liu et al., 2018). Pan-genome analysis was done using Roary v3.11.2 (Page et al., 2015) and core genome single-nucleotide polymorphisms (cgSNPs) were extracted using SNP-sites (Page et al., 2016). cgSNPs were filtered using VCFtools v0.1.17 (Danecek et al., 2011). After filtering, 20663 cgSNPs out of a possible 32774 cgSNPs from 1425 core orthologous genes were kept. A maximum likelihood phylogenomic tree of 246 \(M. \text{ morganii}\) genomes was constructed by RAxML v8.2.12 (Stamatakis, 2014) using the filtered cgSNPs. Population structure based on Bayesian analysis was then identified using rhierBAPS v1.0.1 (Tonkin-Hill et al., 2018) using the filtered cgSNPs. Besides, population structure analyzed by PopPUNK is also provided as a supplement (Lees et al., 2019). The phylogenetic tree is displayed and annotated using iTOL v5 (Letunic and Bork, 2021).

**Plasmids Assembly, Comparation and Phylogenetic Analysis**

Plasmids were assembled using plasmidSPAdes (Antipov et al., 2016). The scaffolds were reordered using Ragout v2.3 (Kolmogorov et al., 2014) with reference to the most similar GI sequence recognized by BLASTN. The gaps were filled using PCR method (Primers and thermal conditions are presented in Supplementary Table 1). Then, the complete GI sequence was annotated with Prokka v1.14.6 (Seemann, 2014). To compare the variations of current SG1-related GIs, single-copy orthogroups of GI sequence found in this study and other pre-assembled GIs from NCBI database were identified by OrthoFinder v2.3.7 (Emms and Kelly, 2019), and a maximum likelihood phylogenetic tree is then done from the concatenated alignment using RAxML v8.2.12 (Stamatakis, 2014). The phylogenetic tree is displayed and annotated using iTOL v5 (Letunic and Bork, 2021). Genetic elements of PGI2 family were visualized and compared using Easyfig v2.2.2 (Sullivan et al., 2011).

**Data Availability**

Newly sequenced genomic island and plasmids were deposited in GenBank under the accession numbers, MW080367.1, MW080368.1, and MW150990.1, respectively.

**Statistical Analysis**

All the statistical analysis were performed by SPSS 19.0 (IBM Corp., Armonk, United States). Chi-square test or Fisher’s exact test was applied to evaluate the differences of antibiotic resistance rates and resistance genes prevalence among the three hospitals. Mann–Whitney \(U\) rank sum test was applied to evaluate the differences of antimicrobial resistance genes distribution between different phylogenomic clades. \(P < 0.05\) was considered statistically significant.

**Ethical Considerations**

This study was approved by Institutional Review Board of The First Affiliated Hospital of Sun Yat-sen University. The study was retrospective and all clinical data were anonymized.

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\(^1\)https://github.com/tseemann/abrice

\(^2\)http://www.pathogenomics.sfu.ca/islandviewer/

\(^3\)https://github.com/wilkoz/gggenes

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RESULTS

Isolation and Characterization of Morganella morganii

A total of 335 M. morganii clinical isolates were collected from two hospitals in Guangzhou, Southeast China and one hospital in Yinchuan, Northwest China during June 2014 to June 2020. In general, urine (26.6%, 89/335), wound secretion (14.0%, 47/335), sputum (11.9%, 40/335) and shunt fluid (10.2%, 34/335) were the top four most frequent sample types of M. morganii clinical isolates, while the sample-type distributions among the three hospitals were statistically different ($\chi^2 = 85.0, P < 0.001$) (Supplementary Table 2). These strains were mainly isolated from hepatic-biliary-pancreatic surgery (14.0%, 47/335), intensive care unit (11.0%, 37/335), general surgery (9.6%, 32/335), and burn center (6.0%, 20/335) (Supplementary Table 3).

Antibiotic Resistance Rates of Morganella morganii

Common antibiotics had different MIC distributions. Ceftazidime, gentamicin, and trimethoprim-sulfamethoxazole showed classic bimodal distributions. Meropenem, ertapenem, amikacin, etc. showed clear distributions for the sensitive cases. Ciprofloxacin, levofloxacin and tobramycin showed more spread distributions around all the concentration ranges (Supplementary Figure 1). The susceptibility testing results showed that 130 (38.81%), 66 (19.70%), 61 (18.21%), and 47 (14.0%) strains were resistant to trimethoprim-sulfamethoxazole, ceftazidime, gentamicin, ciprofloxacin and ceftriaxone, respectively (Supplementary Table 4). Among them, strain zy_m28 from Hospital ZY and strain nx_m63 from Hospital NX showed intermediate resistant to meropenem (MIC = 2 µg/mL). There was no statistical difference of the resistance rates between the two hospitals in Guangzhou (Hospital ZY and Hospital ZSY). However, the resistance rates of the ceftriaxone, ceftazidime, cefepime, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole were significantly higher in Hospital NX compared to Hospital ZY and ZSY. Forty (11.9%) M. morganii isolates were recognized as multidrug resistance (MDR) strains as they were resistant to three or more classes of antibiotics, and the MDR M. morganii rates were also statistically different among the three hospitals ($\chi^2 = 10.5, P < 0.05$). The MDR rate in Hospital NX was higher than in Hospital ZY. The strain zy_m28 was a MDR strain resistant to ceftriaxone, ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole.

Resistance Genes Prevalence in Morganella morganii

All isolates were detected by PCR for β-lactam resistance genes and PMQR genes. PCR results showed that 41 (12.2%), 27 (8.1%), 80 (23.9%), and 40 (12.0%) M. morganii strains carried blaTEM, blaCTX-M, aac(6′)Ib-cr and qnrD1, respectively (Table 1). Among blaTEM and blaCTX-M, blaTEM-104 and blaCTX-M-162 were the most popular subtypes. Two carbapenem-resistant genes blaOXA-181 and blaIMP-1 were detected in strain zy_m28 and nx_m63, respectively. MDR strain zy_m28 carried blaOXA-181, blaTEM-1, blaCTX-M-3, qnrS1 and aac(6′)Ib-cr at the same time. Other PMQR genes qnrA1, qnrB1, oqxA, and oqxB were also detected. Sixty (17.9%) M. morganii strains carried at least one of the extended-spectrum β-lactamase (ESBL) resistance genes, and 103 (30.8%) carried at least one of the PMQR genes. There was no statistical difference in ESBL resistance genes prevalence among the three hospitals, while prevalence rates of PMQR genes among the three hospitals were statistically different ($\chi^2 = 9.3, P < 0.05$). PMQR genes were more prevalent in Hospital NX than in Hospital ZY and ZSY. Thirty-four (10.1%) M. morganii strains carried both ESBL and PMQR resistance genes, and blaTEM-104 coexistence with qnrD1 or aac(6′)Ib-cr were the most common combinations (Supplementary Table 5).

Conjugation Experiments

Carbapenem-resistant genes blaOXA-181 and blaIMP-1 could be successfully transferred from strain zy_m28 and nx_m63 to the E. coli C600. Conjugation frequencies of blaOXA-181 and blaIMP-1 plasmids were $(2.4 \pm 7.9) \times 10^{-4}$ per donor cell and $(1.2 \pm 3.8) \times 10^{-4}$ per donor cell, respectively. Susceptibility testing results showed that transconjugants zy_m28-E. coli C600 were resistant to meropenem, ciprofloxacin, and transconjugants nx_m63-E. coli C600 were resistant to meropenem (Supplementary Table 6). Transconjugants zy_m28-E. coli C600 were found to contain blaOXA-181, qnrS1 but without blaTEM-1, blaCTX-M-3 and aac(6′)Ib-cr. Transconjugants nx_m63-E. coli C600 were found to contain blaIMP-1 as expected. Conjugative transfer of carbapenem-resistant genes blaOXA-181 and blaIMP-1 could be a threat to public health worldwide.

Phylogenomic Analysis and Population Structure of Morganella morganii

Two strains zy_m28 (GCA_014333515.1) and nx_m63 (GCA_014283905.1) harboring carbapenem-resistant genes were whole-genome sequenced. Other M. morganii genomes were accessed from NCBI assembly database and SRA database (Supplementary Figure 2). A total of 20663 filtered sgSNPs were generated by Roary v3.11.2 (Page et al., 2015). Phylogenomic analysis of the two and current public M. morganii genomes showed that these M. morganii clustered into multiple clades. It is interesting that clade 1 (showed in blue) associated with multiple resistance genes containing blaKPC-2, blaIMP-10, blaIMP-27, blaOXA-48, blaOXA-181, blaNDM-1, blaNDM-7, blaqE-S-5, mcr-1, and mcr-5, seemed to be widely spread in multiple global regions, including Asia, North America, South America, Europe, Australia and South Africa (Figure 1 and Supplementary Table 7). Mann–Whitney U rank sum test showed that antimicrobial resistance genes (ARGs) in clade 1 was more than ARGs in non-clade 1 ($P < 0.001$) (Supplementary Figure 3A). Some noticeable MGES, such as blaKPC-2-carrying IncP-6 plasmid, blaNDM-1-carrying IncN2 plasmid, blaNDM-7-carrying IncX3 plasmid, blaqOXA-48-carrying IncI/M plasmid, blaqKPC-2-carrying IncR plasmid, mcr-1-carrying IncX4 plasmid, and MGIVflnld1 were identified in M. morganii for the first time in
TABLE 1 | Prevalence of resistance genes in Morganella morganii clinical isolates.

| Resistance genes | Hospital ZY (n = 82) | Hospital SZY (n = 112) | Hospital NX (n = 141) | Total (n = 335) | Three hospitals comparison |
|------------------|----------------------|------------------------|-----------------------|----------------|--------------------------|
|                  | Prevalence (%)       | Prevalence (%)         | Prevalence (%)        | Prevalence (%) |                          |
| blßIMP           | 0.0                  | 0.0                    | 0.7                   | 0.3            | \                        |
| blßOXA-48        | 1.2                  | 0.0                    | 0.0                   | 0.3            | \                        |
| blßTEM           | 9.8                  | 10.7                   | 14.9                  | 12.2           | \                        |
| blßCTX-M         | 11                   | 3.6                    | 9.9                   | 8.1            | \                        |
| ESBL             | 18.3                 | 11.6                   | 22.7                  | 17.9           | \                        |
| qnrD             | 1.2                  | 0.0                    | 2.1                   | 0.9            | \                        |
| qoxD             | 19.5                 | 19.6                   | 29.8                  | 23.9           | \                        |
| aac(6')-Ib-cr    | 8.5                  | 9.8                    | 15.6                  | 11.9           | \                        |
| qoxA             | 0.0                  | 0.0                    | 0.0                   | 10.7           | \                        |
| qoxB             | 6.1                  | 7.1                    | 14.9                  | 10.2           | \                        |
| PMQR             | 23.2                 | 25.0                   | 39.7                  | 30.8           | \                        |
| blßTEM, qucrD    | 1.2                  | 6.3                    | 5.7                   | 4.8            | \                        |
| blßTEM, aac(6')-Ib-cr | 2.4               | 4.5                    | 6.4                   | 4.8            | \                        |
| blßCTX-M, qucrD  | 2.4                  | 2.7                    | 6.4                   | 4.2            | \                        |
| ESBL, PMQR       | 6.1                  | 7.1                    | 14.9                  | 10.2           | \                        |

ESBL, extended-spectrum ß-lactamase; PMQR, plasmid mediated quinolone resistance. Hospital ZY, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, China; Hospital SZY, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou, China; Hospital NX, General Hospital of Ningxia Medical University, Yinchuan, China.

* Using Fisher’s exact test.

** Prevalence of resistance gene in Hospital NX was higher than Hospital ZY (chi-square test pairwise comparison, adjusted α = 0.017).

* Prevalence of resistance gene in Hospital NX was higher than Hospital SZY (chi-square test pairwise comparison, adjusted α = 0.017).

\ Dissatisfaction the preconditions of chi-square test.

this interesting clade (Supplementary Tables 7, 8). rhierBAPS population structure analysis showed that M. morganii could be classified into ten sequence clusters (SCs) based on the cgSNPs. The interesting clade 1 associated with multiple resistance genes (Figure 1, blue clade) was classified as SC3 and SC5. PopPUNK population structure analysis showed that clade 1 could be classified as combined cluster 2, 7, 8, and 12 (Supplementary Table 7). PopPUNK offered more detailed population structure groups than rhierBAPS, which may be resulted from PopPUNK considered both the accessory distance and core distance (Supplementary Figure 3B). MDR strain zy_m28 was classified as SC3. Growth curve showed its greater fitness advantage in LB medium and M9CA minimal medium than strain nx_m63 classified as SC1 and larger maximum population capacity (Ym) than a plasmid-free control strain zy_m3 (Supplementary Figures 3C, D). There was no statistical relationship between population structure and geographical origin (χ²-test).

Genetic Contexts of Mobile Genetic Elements in Morganella morganii Strain zy_m28

Strain zy_m28 was isolated from the right pelvic drainage fluid of a 28-year-old Korea patient with Crohn’s disease and intestinal infection, and Enterococcus raffinosus strain was also cultured from the same sample. WGS analysis showed that two MGEs, PGI2 variant PGI2-zym28 (MW080367.1) and blßOXA-181-carrying IncX3 plasmid pZYM28-OXA-181 (MW080368.1) coexisted in zy_m28.

Bioinformatics analysis showed that PGI2-zym28 integrated into the chromosome between the trmE and EamA-like transporter family genes. The MDR region in PGI2-zym28 contained one complete class 1 integron carrying dfrA16, blßOXA-181, adaA2, cmlA1 and adaA1 gene cassettes at the left side, and one partial class 1 integron missing the 5'-CS carrying aac(6')-Ib-cr, blßOXA-181, catB3 and arr-3 gene cassettes at the right side, as well as various additional resistance genes including blßTEM, blßCTX-M, fosA3, tet(A), floR, mph(E) and mssR connected by IS26 (Figure 2A).

To compare the variations of current SGII-related GIs, we then compared the genetic structure and conducted a phylogenetic analysis for SGII-like sequences (Supplementary Figure 4). In general, these GIs could be divided into three clades related to SGII family, AGII family and PGI2 family, respectively. The backbone regions in all GIs were relatively conservative, however, deletion, insertion and inversion events still happened (SGII family). Notably, most SGII-like sequences were found in S. enterica and P. mirabilis, but it has now also been described in A. baumannii, E. coli and E. cloacae (AGII and PGI2 family). PGI2 family showed significant variations in MDR regions, bringing challenges to the control of antimicrobial resistance (Figure 2B). PGI2-zym28 showed high identities with PGI2-C55 of animal origin (MK847915.1), suggesting...
horizontal transfer of PGI2 variants facilitated the dissemination of antimicrobial resistance.

pZYM28-OXA-181 was an IncX3 type plasmid carrying blaOXA-181 and qnrS1. Resistance genes blaOXA-181 and qnrS1 were flanked by two same oriented IS26. The same structure was also found in IncF and IncN plasmids (Figure 3). Though the fragment (IS26-IS3000-blaOXA-181-ISKpn19-Tn3-qnrS-IS26) was relatively conservative, variations still happened. In plasmid pOXA-484_EC-JS316 (CP058621.1), blaOXA-181 was replaced with blaOXA-484. While in plasmid pEC2-1 (CP041956.1), the
transposon was flanked by IS6 on the 3' side, joining aac(6')-Ib-cr, blaCTX-M-3 and blaTEM-1, with the help of some transposons. IncX3 plasmids are prevalent worldwide (Roer et al., 2018). Genetic structures of IncX3 plasmids in different species were compared, and a phylogenetic tree was also constructed. Phylogenetic tree of IncX3 plasmids showed minor genetic variabilities. There were three main types of transposons disseminated in IncX3 type plasmids, with one mainly associated with blaNDM, one with blaOXA-181, and the other linked to blaKPC and ESBL-encoding genes (Figure 4). blaNDM genes were located on Tn125-like transposons (ISCR21-groL-groS-cutA-dsbD-trpF), while blaOXA-181 genes were located on the IS26-flanked transposons as described above. These transposons always integrated into the IncX3 backbone adjacent to umuD gene and flanked by Tn2 at the other side. IncX3 plasmids harboring carbapenemase genes were frequently described in different species, suggesting it is an important medium for the spread of carbapenemase genes. This poses a challenge for antimicrobial resistance control.

**DISCUSSION**

In this study, we collected all the *M. morganii* strains isolated from June 2014 to June 2020 in three tertiary hospitals in China, regardless of sample types and department sources, to provide an overview of molecular epidemiology of *M. morganii*. The antibiotic resistance rates and prevalence of resistance genes
from Hospital NX in Northwest China were generally higher than those of Hospital ZY and SZY in Southeast China, which may be resulted from the differences in economic and medical conditions, prescribing behaviors and differences in knowledge of antimicrobial clinicians (Zhen et al., 2019). We also found that PMQR and ESBL-encoding genes usually coexisted, causing multidrug resistance, consistent with the research of Briales et al. (2012). Conjugation experiment confirmed the co-transfer of \(\text{bla}_{\text{OXA-181}}\) and \(\text{qnrS1}\) in our study.

Phylogenomic and population structure analysis of public \(M.\ morganii\) genomes showed that clade 1 (rhiERAPS SC3 and SC5) associated with multiple resistance genes seemed to be widely spread in multiple global regions. The resistance genes pattern of this clade was largely divergent from other clades. Various putative IMEs or ICEs, and noticeable carbapenem and polymyxin-resistant plasmids were identified in the clade. Growth curve showed that strain zy_m28 belonging to rhiERAPS SC3, had greater fitness advantage than strain nx_m63 belonging to rhiERAPS SC1 and even had larger maximum population capacity \((Y_m)\) than a plasmid-free control strain zy_m3. A reasonable guess is that these subclusters may be more suitable hosts to hold acquired resistance genes than other clusters because of low fitness cost and may cause resistance issue in the future. Further studies and more samples are needed to demonstrate this hypothesis.

Mobile genetic elements are essential in horizontal transfer of resistance genes. In this study, we identified a PGI2 variant PGI2-zym28 carrying \(\text{bla}_{\text{CTX-M-3}}\) and \(\text{aac(6’)-Ib-cr}\). To the best of our knowledge, this is the first report of PGI2 variant in \(M.\ morganii\). PGI2-zym28 showed high identities with PGI2-C55 initially found in a \(P.\ mirabilis\) strain isolated from a Chicken in 2018 from Shandong, China (Lei et al., 2020). The result suggests that
PGI2-like sequences may be transferred horizontally between *P. mirabilis* and *M. morganii* among humans and animal sources. Further studies are needed to monitor the spread of PGI2-like sequences in clinical settings and understand their effects on clinical antimicrobial resistance.

An IncX3 plasmid pZYM28-OXA-181 harboring *bla*OXA-181 and *qnrS1* was found to coexist with PGI2-zym28 in strain *zy_m28*. *bla*OXA-181, a variant of *bla*OXA-48 family, is showing an increasing prevalence since first reported in India in 2007 (Qin et al., 2018). While *bla*OXA-181 has been found in different plasmid types, such as IncN (McGann et al., 2015), IncT (Villa et al., 2013) and IncX3 (Qin et al., 2018), *bla*OXA-181 -harboring IncX3 plasmids are the most prevalent (Roer et al., 2018; Feng et al., 2019). There are two main epidemic types of IncX3 plasmids, one spreading *bla*OXA-181 and *qnrS* together on an IS26-flanked composite transposon and the other spreading *bla*NDM and *bla*SHV-12 together on a Tn125-like transposon. To the best of our knowledge, this is the first report of IncX3 type plasmid carrying *bla*OXA-181 in *M. morganii*. The spread of *bla*OXA-181-harboring IncX3 plasmid in clinical *M. morganii* strains may further limit clinical therapeutic solutions.

Another noticeable plasmid, an IncP-1β type plasmid pNXM63-IMP carrying *bla*IMP-1 on a Tn402-like class 1 integron, was found in the *M. morganii* strain nx_m63 for the first time. The Tn402-like integrons carrying *bla*IMP-1 were found in multiple species including *P. aeruginosa* (Xiong et al., 2013), *E. cloacae* (Wang et al., 2015), and *K. pneumoniae* (Jiang et al., 2017). Interestingly, they were often reported in Asia especially in China. Although the *bla*IMP-1-carrying Tn402-like integron found in this study did not carry other resistance gene cassettes, it is worth noting that with the help of various transposases, more complex multidrug resistant Tn402-like integrons carrying both *bla*IMP-45 and *bla*VIM-1 are emerging (unpublished data, CP040126.1).

This study demonstrates that a clade of *M. morganii* is prone to acquire resistance genes, and multidrug resistant *M. morganii* are increasing by harboring a variety of MGEs including two newly discovered ones in the species. The variation...
and evolution of these MGEs may bring more extensive and challenging antimicrobial resistance issue. Horizontal infection control strategies should be considered in tackling antimicrobial resistance in _M. morganii_ and other pathogens.

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/genbank/, under the accession numbers, MW080367.1, MW080368.1, and MW150990.1.

**AUTHOR CONTRIBUTIONS**

BH, WJ, CC, and KLi designed the experiments. GX, YC, MZ, JT, YM, WZ, ZW, XY, and YLi collected the isolates. GX, YC, and YM completed the experiments. GX, YC, and JZ completed the data analysis. GX, KLa, YC, and KLi completed the draft of the manuscript. YL, CX, LC, and Y-WT revised the manuscript. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.744291/full#supplementary-material

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