A Genomic and Bioinformatics View of the Classification and Evolution of *Morganella* Species and Their Chromosomal Accessory Genetic Elements Harboring Antimicrobial Resistance Genes

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**ABSTRACT** In this study, draft-genome sequencing was conducted for 60 Chinese *Morganella* isolates, and furthermore, 12 of them were fully sequenced. Then, a total of 166 global sequenced *Morganella* isolates, including the above 60, were collected to perform average nucleotide identity-based genomic classification and core single nucleotide polymorphism-based phylogenomic analysis. A genome sequence-based species classification scheme for *Morganella* was established, and accordingly, the two conventional *Morganella* species were redefined as two complexes and further divided into four and two genospecies, respectively. At least 88 acquired antimicrobial resistance genes (ARGs) were disseminated in these 166 isolates and were prevalent mostly in the isolates from hospital settings. IS26/IS15DI, IS10 and IS1R, and Tn3-, Tn21-, and Tn7-subfamily unit transposons were frequently presented in these 166 isolates. Furthermore, a detailed sequence comparison was applied to 18 *Morganella* chromosomal accessory genetic elements (AGEs) from the fully sequenced 12 isolates, together with 5 prototype AGEs from GenBank. These 23 AGEs were divided into eight different groups belonging to composite/unit transposons, transposable prophages, integrative and mobilizable elements, and integrative and conjugative elements, and they harbored at least 52 ARGs involved in resistance to 15 categories of antimicrobials. Eleven of these 23 AGEs acquired large accessory modules, which exhibited complex mosaic structures and contained many antimicrobial resistance loci and associated ARGs. Integration of ARG-containing AGEs into *Morganella* chromosomes would contribute to the accumulation and dissemination of ARGs in *Morganella* and enhance the adaption and survival of *Morganella* under complex and diverse antimicrobial selection pressures.

**IMPORTANCE** This study presents a comprehensive genomic epidemiology analysis on global sequenced *Morganella* isolates. First, a genome sequence-based species classification scheme for *Morganella* is established with a higher resolution and accuracy than those of the conventional scheme. Second, the prevalence of accessory genetic elements (AGEs) and associated antimicrobial resistance genes (ARGs) among *Morganella* isolates is disclosed based on genome sequences. Finally, a detailed sequence comparison of eight groups of 23 AGEs (including 19 *Morganella* chromosomal AGEs) reveals that *Morganella* chromosomes have evolved to acquire diverse AGEs harboring different profiles of ARGs and that some of these AGEs harbor large accessory modules that exhibit complex mosaic structures and contain a large number of ARGs. Data presented here provide a deeper understanding of the classification and evolution of *Morganella* species and also those of ARG-containing AGEs in *Morganella* at the genomic scale.
**KEYWORDS** Morganella, accessory genetic elements, antimicrobial resistance, genome sequencing, species classification

*Morganella* is ubiquitously present and belongs to the *Morganellaceae* family (1). *Morganella* includes only two described species, *Morganella morganii* and *Morganella psychrotolerans*, based on DNA–DNA hybridization (2), and *M. morganii* is furthermore divided into two subspecies, *morganii* and *sibonii*, according to trehalose fermentation ability (3). The 16S rRNA gene nucleotide similarity between *M. morganii* and *M. psychrotolerans* isolates is 98.6% (2), which is above the threshold of 97% generally used to separate species (4), indicating that these two species cannot be steadily distinguished by 16S rRNA gene sequences.

*M. psychrotolerans* is occasionally isolated from chilled seafood and is recognized as a rainbow trout pathogen (5). *M. morganii* is frequently isolated from hospital settings and represents an important opportunistic pathogen (6). *M. morganii* is naturally resistant to penicillins, the first/second-generation cephalosporins, nitrofurantoin, tigecycline, macrolides, lincosamides, fusidic acid, polymyxins, and glycopeptides (6). *M. morganii* can acquire diverse accessory genetic elements (AGEs), such as unit transposons (7, 8), integrative and conjugative elements (ICEs) (9), and integrative and mobilizable elements (IMEs) (10, 11). These AGEs carry diverse antimicrobial resistance genes (ARGs), such as *bla*KPC-2 (8), *aadA1* (10), and *qnrD* (12), and thus greatly contribute to the dissemination of antimicrobial resistance in *M. morganii*. Although there are plenty of reports on identifying AGEs and associated ARGs in *M. morganii*, only few of them are devoted to genetically dissecting their modular structures (7, 10).

This study presented a genomic epidemiology analysis on 166 global sequenced Morganella isolates, including 60 sequenced in this study. We established a genome sequence-based species classification scheme for *Morganella* to give a genomic view of *Morganella* species classification and, moreover, disclosed the prevalence of ARG-associated AGEs among *Morganella* isolates. We further performed a detailed sequence comparison of 18 *Morganella* chromosomal AGEs sequenced in this study together with 5 prototype AGEs from GenBank to provide a deeper understanding of *Morganella* AGE diversification.

**RESULTS**

Genomic classification and evolution of *Morganella* species. We determined the draft-genome sequences of 60 Chinese *Morganella* isolates (Fig. 1 and Table S1) and also the complete genome sequences of 12 of these 60 isolates (see Table S2 for quality control results). We then performed the species classification and phylogenomic analysis on a collection of 166 global sequenced *Morganella* isolates, including the above 60 together with the other 106 from GenBank (last accessed February 1 2021). Based on the conventional scheme for classifying *Morganella* species (2), 161 (96.99%) of them were assigned into *M. morganii* while the remaining 5 (3.01%) were assigned into *M. psychrotolerans*, indicating that the overwhelming majority of *Morganella* isolates belonged to *M. morganii*. Given that the absence and presence of trehalose-utilization operon treRBP could be used to distinguish *morganii* and *sibonii* subspecies, respectively (13), it was found here that 147 isolates were assigned into *morganii* subspecies and the remaining 14 were assigned into *sibonii* subspecies (Fig. 2 and Table S1).

To perform genomic classification and phylogeny of *Morganella*, the pairwise average nucleotide identity (ANI) values of these 166 isolates were calculated (Fig. S1 and Table S3). Based on the threshold of 95% ANI for genospecies delineation (14), a total of six genospecies could be classified and then designated *M. morganii*, *M. chanii*, *M. sibonii*, *M. laugraudii*, *M. psychrotolerans*, and *M. kristinii*, respectively. The former four genospecies were assigned into *M. morganii* complex, while the latter two were assigned into *M. psychrotolerans* complex. These two complexes displayed ≈84.4% ANI with each other, while the genospecies within each complex displayed ≈90.5% ANI.
The 60 isolates sequenced in this study could be assigned into the two genospecies \textit{M. morganii} (\(n = 59\)) and \textit{M. sibonii} (\(n = 1\)) of the \textit{M. morganii} complex.

For further phylogenomic analysis, a total of 3,538 core single nucleotide polymorphisms (SNPs) were identified from these 166 chromosome sequences. The recombination relative to point mutation (\(r/m\)) value was calculated to evaluate the impact of homologous recombination on sequence diversification (15). An \(r/m\) value of 2.6 was inferred at the genome level, indicating that homologous recombination introduced 2.6 times more nucleotide substitution than point mutation and, thereby, recombination events frequently occurred during genomic evolution and classification of \textit{Morganella} (15). To avoid the influence of homologous recombination on phylogenetic reconstruction, a collection of 1,299 recombination-free SNPs was generated, and a maximum-likelihood phylogenetic tree was constructed using these recombination-free SNPs (Fig. 2). Almost all of the branches in this tree had bootstrap values of \(\geq 70\%\), suggesting that this recombination-free tree could accurately reflect the evolutionary relatedness and population structure of \textit{Morganella} (16). In this tree, the isolates from \textit{M. morganii} and \textit{M. psychrotolerans} complexes were clustered into two primary phylogroups that split earliest and emerged independently, indicating very distinct evolutionary histories of these two complexes. These two primary phylogroups could be further divided into four and two sub-phylogroups, respectively; as expected, they showed perfect correspondence to the above six genospecies, illustrating the consistency between ANI-based genospecies classification and phylogenomic analysis. The population (\(n = 147\)) of \textit{M. morganii}, much larger than that of the other five genospecies, exhibited a highly clonal structure independent of geographic locations, time, and specimens of these isolates (Fig. 2 and Table S1).

\textbf{Distribution of acquired ARGs among \textit{Morganella} isolates.} At least 88 kinds of acquired ARGs, involved in resistance to 16 different categories of antimicrobials, were identified in these 166 \textit{Morganella} isolates (Fig. S2). All these ARGs were distributed in \textit{M. morganii} complex (116/166, 67.47%), including genospecies \textit{M. morganii} (103/166, 62.04%), \textit{M. sibonii} (10/166, 6.02%), and \textit{M. chanii} (3/166, 1.81%) (Fig. S3 and Table S4).
The most prevalent acquired ARGs were tetracycline-resistance genes (99/166, 59.64%), followed by aminoglycoside-resistance genes (70/166, 42.17%), sulfonamide-resistance genes (62/166, 37.35%), trimethoprim-resistance genes (56/166, 33.73%), and \(\beta\)-lactam-resistance genes (54/166, 32.53%) (Fig. S3 and Table S4).

These 88 acquired ARGs were further assigned into the reservoirs (humans, animals, and the environment) of the 149 isolates with source information (Table S1). Morganella isolates from humans contained many more acquired ARGs than those from animals and the environment (Fig. 3A), and moreover, 80 of 88 acquired ARGs (especially including aminoglycoside-resistance genes \(n = 23, P < 0.0186\) and \(\beta\)-lactam-resistance genes \(n = 15, P < 0.0264\)) could be found in human reservoirs (Fig. 3B), indicating that Morganella from hospitalized patients had evolved to acquire many more ARGs to encounter complex and high selection of antimicrobials in hospital settings. A total of 18 acquired ARGs, involved in resistance to nine different categories of antimicrobials, were shared by Morganella isolates from all the three reservoirs, denoting a long history of acquisition and wide dissemination of these ARGs in Morganella.

Forty-nine of the 60 sequenced isolates harbored 63 of the above-mentioned 88 ARGs, and these 63 ARGs were involved in resistance to 15 (except for polymyxin) of the above-mentioned 16 antimicrobials (Fig. S2). These 63 ARGs were found in...
Distribution of acquired ARGs among Morganella isolates. (A) Boxplot displays the number of kinds of acquired ARGs in three reservoirs. (B) Venn diagram shows the distribution of different classes of acquired ARGs in three reservoirs.
genospecies *M. morganii* (48/60, 90%) and *M. sibonii* (1/60, 1.67%). The top 5 ARGs in these 60 isolates were tetracycline-resistance genes (46/60, 81.67%), aminoglycoside-resistance genes (42/60, 70%), sulfonamide-resistance genes (34/60, 56.67%), chloramphenicol-resistance genes (30/60, 50%), and β-lactam-resistance genes (29, 48.33%); this observation was highly similar to the prevalence of 88 acquired ARGs in the 166 isolates described above.

The antimicrobial susceptibility/resistance profiles of these 60 isolates were determined using 15 different antimicrobials (Fig. 4 and Table S1). As expected, all 60 of these isolates were highly resistance to ampicillin, cefazolin, cefuroxime, and nitrofurantoin due to intrinsic resistance. These 60 isolates displayed nonsusceptibility rates of >50% for three antimicrobials, 50% to 20% for four antimicrobials, and <20% for the remaining four antimicrobials, including aztreonam (18.34%, 11/60), cefepime (16.67%, 10/60), meropenem (10%, 6/60), and amikacin (5%, 3/60). *Morganella* isolates in China showed the highest nonsusceptibility rate (*n* = 40, 66.67%) for fluoroquinolones, including levofloxacin and ciprofloxacin. Meropenem and amikacin could be the first choice for experiential treatment of *Morganella*-induced infections in China because they have the lowest detected nonsusceptibility rates (≤10%). All six of the meropenem-resistance *Morganella* isolates discussed herein acquired the carbapenemase gene *bla*KPC-2 (*n* = 4) or *bla*NDM-1.

![Antimicrobial susceptibility data of 60 Morganella isolates.](image-url) FIG 4 (A) Shown are the antimicrobial resistance profiles of 60 *Morganella* isolates collected in this study. (B) Shown are the nonsusceptibility rates [(resistant + intermediate)/(sensitivity + intermediate + resistant)] of 60 *Morganella* isolates for each antimicrobial. Original data are shown in Table S1.
TABLE 1 The distribution of 17 major AGE groups in 166 global Morganella isolates

| Family and subfamily | Core transposition determinant | Genospecies          | No. of positive strains | %  |
|----------------------|--------------------------------|----------------------|-------------------------|----|
| IS                   | tnpA                            | Morganella. morganii  | 67                      | 41.75 |
| IS26/IS15DI          | tnpA                            | M. sibonii           | 1                       |    |
| IS26/IS15DI          | tnpA                            | M. kristinii         | 1                       |    |
| IS10                 | tnpA                            | M. morganii          | 65                      | 39.16 |
| IS1R                 | tnpA                            | M. morganii          | 38                      | 23.49 |
| IS1R                 | tnpA                            | M. sibonii           | 1                       |    |
| Tn3                  | tnpAR                           | M. morganii          | 12                      | 7.23 |
| Tn21                 | tnpAR                           | M. morganii          | 32                      | 19.88 |
| Tn21                 | tnpAR                           | M. sibonii           | 1                       |    |
| Tn163                | tnpAR                           |                      | 0                       |    |
| Tn4430               | tnpAR                           |                      | 0                       |    |
| Tn4651               | tnpAR                           |                      | 0                       |    |
| Tn4401               | tnpAR                           |                      | 0                       |    |
| Tn7                  | tnsABCDE                         | M. morganii          | 23                      | 13.86 |
| Tn6230               | tnsABCD                         |                      | 0                       |    |
| Tn552                | tnsCBR                          |                      | 0                       |    |
| Tn6022               | tnsABCDE                         |                      | 0                       |    |
| Tn5053               | tniABQR                          |                      | 0                       |    |
| Tn554                | tnpABC                           |                      |                        |    |
| Tn6488               | ginABCD                          |                      | 0                       |    |
| Tn6571               | ginABCD                          |                      | 0                       |    |

(n = 2) (Table S1) and were confirmed to have carbapenemase activity in bacterial cell extracts. There were 25 of these 60 isolates that carried multiple aminoglycoside-modifying enzyme genes and thereby displayed resistance to gentamicin and tobramycin, but only 3 of these 25 isolates were nonsusceptible to amikacin due to the following two reasons: (i) amikacin was insensitive against these enzymes (17) and (ii) these 3 amikacin-resistant isolates additionally acquired the 16S rRNA methyltransferase gene rmtB, therefore mediating high-level amikacin resistance (18).

A global view of AGEs in the 166 sequenced Morganella isolates. AGEs acted as the vectors of ARGs and thus were responsible for the accumulation and dissemination of ARGs in different bacterial isolates by intracellular/intercellular transfer (19). To understand the prevalence of ARG-containing AGEs in Morganella, we screened the 166 sequenced Morganella isolates for the prevalence of the 17 major AGE groups frequently found in Gram-negative bacteria (Table 1). Detected were 6 of the above 17 groups: IS26/IS15DI, IS10, and IS1R and Tn3-, Tn21-, and Tn7-subfamily unit transposons were found in 69 (41.75%), 65 (39.16%), 39 (23.49%), 12 (7.23%), 33 (19.88%), and 23 (13.86%) of these 166 isolates. These six groups of AGEs were identified in the genospecies Morganella. morganii (n = 82) and M. sibonii (n = 3) belonging to Morganella. morganii complex and the genospecies M. kristinii (n = 1) belonging to Morganella. psychrotolerans complex. Accordingly, the selection of 12 nonredundant isolates for whole-genome sequencing (see above) was based on the reason that they probably carried at least one of IS26/IS15DI-, IS10-, or IS1R-composite transposons and Tn21- and Tn7-subfamily unit transposons.

A collection of 23 AGEs for detailed sequence comparison. Each of these fully sequenced 12 isolates harbored 1 to 3 kinds of chromosomal AGEs, giving a total of 18 identified (Table S5). Additionally, a total of 11 plasmids were identified from 7 of these 12 isolates (see Table S6 for details). Subsequent analysis was then focused on these 18 chromosomal AGEs, further dividing into eight distinct groups: (i) two IS26/IS15DI-composite transposons, Tn6759 and Tn6760 from strains 11759 and 621164, respectively, (ii) three IS10-composite transposons, Tn10, Tn6798, and Tn6799 from strains
**TABLE 2** Major features of AGEs characterized in this study

| Group                        | AGEa                           | Accession no. | Chromosomal nucleosomal position | Length (bp) | Host bacterium                  | Reference   |
|------------------------------|--------------------------------|---------------|----------------------------------|-------------|---------------------------------|-------------|
| IS26/IS1SDI-composite transposons | Tn6759                        | CP059986      | 3662767–3668698                  | 5,932       | Morganella morganii             | This study  |
| Tn10-related elements        | Tn6760                        | CP064829      | 2991196–3002147                  | 10,952      | M. morganii 621164              | This study  |
|                             | Tn10JZC25                     | CP064828      | 1486140–1495286                  | 9,147       | M. morganii ZJC25               | This study  |
|                             | Tn10J229813                    | CP043955      | 1519262–1528408                  | 9,147       | M. morganii 229813              | This study  |
|                             | Tn10h1799                      | CP059986      | 1720225–1729371                  | 9,147       | M. morganii 11759               | This study  |
|                             | Tn6798                        | CP064829      | 1802073–1811996                  | 9,924       | M. morganii 621164              | This study  |
|                             | Tn6799                        | CP064833      | 1990256–1990256                  | 38,672      | M. morganii 715304              | This study  |
|                             | T10RE<sub>GN28</sub>         | CP064055      | 2305754–2348095                  | 42,342      | M. morganii GN28                | This study  |
| Tn7-related elements         | Tn7<sub>JZC25</sub>          | CP064828      | 19506–33572                     | 14,067      | M. morganii ZJC25               | This study  |
|                             | Tn7<sub>ZJC25</sub>          | CP043955      | 19469–33535                     | 14,067      | M. morganii 229813              | This study  |
|                             | Tn6800                        | CP064830      | 22578–37741                     | 15,164      | M. morganii 81703               | This study  |
|                             | T7RE<sub>11759</sub>         | CP064829      | 20656–62976                     | 42,321      | M. morganii 621164              | This study  |
| Tn1696-related elements      | Tn1696                        | U12338        | Not applicable                   | 16,318      | Pseudomonas aeruginosa R1033    | 23          |
| Tn6963-related transposable prophages | Tn6963                        | CP030356      | 2191792–2236217                  | 44,426      | M. morganii L241                | 24          |
| Tn6872-related IMEs          | Tn6964                        | CP064832      | 2277663–2371532                  | 93,870      | Providencia rustigianii         | This study  |
|                             | Tn6872                        | LR134189      | 3025117–3045606                  | 20,490      | Not applicable                  | NCTC6933    |
| Tn6397-related ICEs          | Tn6966                        | CP064830      | 3371703–3442289                  | 71,127      | M. morganii 81703               | This study  |
|                             | Tn6397                        | CP021851      | 386444–510401                    | 123,958     | Enterobacter cloacae            | A1137       |
| Tn2670-related elements      | Tn6967                        | CP064827      | 628561–738154                    | 109,594     | M. morganii ZJG944              | This study  |
|                             | Tn2670                        | AP000342      | Not applicable                   | 22,760      | Shigella flexneri R100          | 28          |
|                             | T2670RE<sub>11759</sub>      | CP059986      | 2289505–2323566                  | 35,862      | M. morganii 11759               | This study  |

<sup>a</sup>T10RE<sub>GN28</sub>, T2670RE<sub>11759</sub>, T7RE<sub>621164</sub>, and T1696RE<sub>229813</sub>. Would lose their intracellular mobility due to the lesion of their core transposition determinants IS10, IS1R, tnsABCDE, and tnpAR, respectively. The remaining AGEs are intact and would have intracellular or intercellular mobility.

ZJC25/229813/516602/11759, 621164, and 715304, respectively, together with a 43.1-kb Tn10-related element from strain GN28 designated T10RE<sub>GN28</sub> (iiii) two Tn7-related unit transposons, Tn7 and Tn6800 from strains 229813 and ZJC25, respectively, together with a 42.3-kb Tn7-related element T7RE<sub>229813</sub> from strain 229164, (iv) four Tn1696-related unit transposons, Tn6913a, Tn6913b, Tn6914, and Tn6915 from strains ZJG812, ZJG944, ZJC25, and 229813, respectively, together with a 63.8-kb Tn1696-related element T1696RE<sub>ZJC25</sub> from strain ZJDS81, (v) a Tn6963-related transposable prophage Tn6964 from strain 12304, (vi) a Tn6872-related IME Tn6966 from strain 81703, (vii) a Tn6397-related ICE Tn6967 from strain ZJG944, and (viii) a 35.8-kb Tn2670-related element T2670RE<sub>11759</sub> from strain 11759. All of these T10RE, T7RE, T1696RE, and T2670RE elements could not be recognized as intact transposons due to truncation of relevant core transposition modules. A detailed sequence comparison was applied to these 18 AGEs together with five prototype AGEs, Tn1696, Tn6963, Tn6872, Tn6397, and Tn2670 from GenBank (Table 2). At least 52 ARGs, involved in resistance to 15 different categories of antimicrobials, were identified in these 23 AGEs (Table S5).

**Two IS26/IS1SDI-composite transposons, Tn6759 and Tn6760.** Tn6759 and Tn6760 (Fig. 5) from two Morganella isolates were inserted at different chromosomal locations and bracketed by 8-bp direct repeats (DRs; target site duplication signals for transposition). Tn6759 was bound by two copies of IS26, while Tn6760 was bound by two copies of IS1SDI, and these two IS elements belonged to IS26 family and had only three point variation sites on their nucleotide sequences. Tn6759 and Tn6760 carried completely different antimicrobial resistance loci (ARLs): (i) a disrupted ISCR2-flor unit in Tn6759 and (ii) a type A In37-like element with a truncated gene cassette.
array (GCA) aacA4cr–blaOXA-1–catB3–arr-3, plus a concise class 1 integron In54 with a GCA dfrA17–aadA5 in Tn6760.

**Tn10 and its derivatives Tn6798, Tn6799, and T10REGN28.** Tn10 was initially described in *Shigella flexneri* plasmid R100, and it was a prototype IS10-composite transposon carrying a class B tetracycline-resistance module tetRACD (20). Here, Tn10 and its three derivatives underwent two major insertion events: (i) IS1R was inserted downstream of ydhA in Tn6798 and (ii) Tn2670-related transposon Tn6970 (see below) and 34.9-kb T2670REGN28 (see below) were inserted at the same site within ydhA in Tn6799 and T10REGN28, respectively, leading to truncation of ydhA in Tn6799 and that of ydhA plus IS10L in T10REGN28.

**Tn7 and its derivatives Tn6800 and T7REg21164.** The prototype unit transposon Tn7 was initially found in *Escherichia coli* plasmid R483 and composed of the core transposition module tnsABCDE and a class 2 integron In2-4 (GCA: dfrA1–sat2–aadA1) (21). Here, Tn7 and its two derivatives (Fig. 7) from four *Morganella* isolates were integrated at the same chromosomal location and bracketed by 5-bp DRs. Tn6800 or T7REg21164 differed from Tn7 by acquisition of In2-77 with GCA Inv(F)1b–catB2–sat2–aadA1 or In2-16 with GCA Inv(F)1b–catB2–sat2–aadA1, respectively, instead of In2-4 (Fig. 5A). T7REg21164 underwent an additional insertion event: a 31.1-kb multidrug resistance (MDR) region was inserted within tnsD (Tn7 target-site selection protein), leading to truncation of tnsABCDE (Fig. 7A). This MDR region (Fig. 7B) harbored two ARLs: IS26–mph(E)–IS26 unit and In1684. In1684 was a complex class 1 integron carrying aacA4–blaOXA-1–catB3–arr-3 (GCA/VR1: variable region 1), a disrupted Tn2 containing blaTEM-1, ΔTn6502a containing blaCTX-M-3, and VR2 (ISCR1–qnrVC1 unit plus truncated ISCR1–rmtB unit).

**Five Tn1696 derivatives, Tn6913a, Tn6913b, Tn6914, Tn6915, and T1696tEz3981.** Tn1696 was initially found in *Pseudomonas aeruginosa* plasmid R1033 (22). It was one of the Tn21-subfamily prototype unit transposons and had a backbone structure, IRL (inverted repeat left)–tnpA (transposase)–tnpR (resolvase)–res (resolution site)–mer...
(mercury resistance operon)–IRR (inverted repeat right), with integration of a class 1 integron In4 (GCA: \texttt{aacC1–gcuE–aadA2–cmlA1}) into \textit{res} (23). Here, the five Tn1696 derivatives (Fig. 8) from five \textit{Morganella} isolates were inserted into three different chromosomal locations and bracketed by 5-bp DRs. Each of these five derivatives acquired a unique ARL instead of In4 in Tn1696: 38.9-kb T2670REZJG812 (see below) in Tn6913a, 38.5-kb T2670REZJG944 (see below) in Tn6913b, In1396 (GCA: aadA13–gcuD) in Tn6914, In1396 plus a 37.9-kb MDR region (see below) in Tn6915, and In1785 (VR1: aadB–catB5f–bla\texttt{OXA-10}–aadA1dx; VR2: IS\texttt{CR1}–aphA6 unit) plus 39.6-kb MDR region (see below) in T1696REZJD581. Additionally, \textit{tnpA} of T1696REZJD581 was interrupted by Tn6260:IS\texttt{Pmi3}.

Two transposable proviruses, Tn6963 and Tn6964. The prototype transposable provirus Tn6963 was initially found in \textit{M. morganii} L241 (24). Tn6963 and Tn6964 (Fig. 9) shared conserved \lambda phage life cycle-related markers \textit{attL/attR} (attachment sites at left/right ends), \textit{int-xis} (integration and excision), \textit{bet}–to–\textit{cll} (lysogeny), \textit{repO}–\textit{dnaC} (DNA replication), \textit{hol–lys} (lys), \textit{terS}–\textit{gpB}–\textit{cap–Fl–FII} (head assembly), and \textit{gpZVUGTHMLKJ} (tail assembly) (25). Tn6963 contained no accessory modules, while Tn6964 acquired two: IS10-composite transposon Tn6965 that harbored truncated Tn10 carrying \textit{tetRACD}(B) plus 39.4-kb MDR region (see below), and so called “inserted region” (26) that was cryptic and bracketed by 2-bp DRs.

Two IMEs, Tn6872 and Tn6966. The prototype IME Tn6872 was initially found in \textit{Providencia rustigianii} NCTC6933 (accession number LR134189). Tn6872 and Tn6966 (Fig. 10) shared core IME backbone markers \textit{attL/attR}, \textit{int}, and \textit{oriT} (origin of conjugal replication), but they displayed dramatic modular variations across the backbones: Tn6872 had its unique regions \textit{orf201} to \textit{orf207}, \textit{orf627} to \textit{orf1068}, and \textit{orf1338} to \textit{uvrD},

FIG 6 Comparison of Tn10 and its three derivatives. Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity ≥ 95%). Numbers in brackets indicate nucleotide positions within the chromosomes of strains ZJC25, 229813, 516602, 11759, 621164, 715304, and GN28, respectively.
FIG 7 Comparison of Tn7 and its two derivatives. (A) Organization of Tn7 and its two derivatives. (B) Organization of 31.1-kb MDR region from 42.3-kb Tn7. Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity \( \geq 95\% \)). Numbers in brackets indicate nucleotide positions within the chromosomes of strains ZJC25, 229813, 81703, and 621164, respectively. Accession numbers of ISCR1–rmtB unit, Tn6502a (54), and Tn1722 (55) are CP059348, KF914891, and X61367, respectively.
FIG 8 Comparison of Tn1696 and its five derivatives. Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity ≥ 95%). Numbers in brackets indicate nucleotide positions within the chromosomes of strains ZJG944, ZJG812, ZJC25, 229813, and ZJD581, respectively. Accession numbers of Tn1696 (22) and ISAba14–aphA6–ISABA14 unit (56) used as reference are U12338 and CP046406, respectively.
while Tn6966 contained the counterparts hasdMSR–mrr–orf411, orf597 to orf1107, and orf1311 to hnhc, respectively. Tn6872 carried no accessory modules, while Tn6966 acquired two: IS10R and a region composed of 23.2-kb MDR region (see below) plus Tn21-related transposon Tn6971 (see below).

**Two ICEs, Tn6397 and Tn6967.** The prototype ICE Tn6397 was initially found in *Enterobacter* spp. A1137 (27). Tn6397 and Tn6967 (Fig. 11A) shared conserved ICE backbone markers attL/attR, int, xis, rix (relaxase), oriT, cpl (coupling protein), and F (TivF)-type type IV secretion system gene set (mating pair formation). Tn6397 and Tn6967 each harbored a unique large accessory module (LAM) inserted at the same site within ICE backbones. These two LAMs (Fig. 11B) had similarity in gene organizations but exhibited totally different profiles of ARLs: (i) Tn1696-related transposon Tn6378 carrying In73 (GCA: bldIMP-8–aacA4'-3) plus macrolide-resistance locus macAB-tolC in 63.7-kb LAM of Tn6397 and (ii) a 20.9-kb MDR region (see below) plus a Tn21-related transposon Tn6972 (see below) in 48.5-kb LAM of Tn6967.

**Two Tn21-subfamily transposons, Tn6971 and Tn6972.** The sequence comparison (Fig. 12) was also applied to the two Tn21 derivatives Tn6971 and Tn6972 (identified as the inner components of Tn6966 and Tn6967, respectively; see above), together with Tn21 (28). Tn21, initially found in *Shigella flexneri* plasmid R100 (28), was another Tn21-subfamily prototype transposon, and it displayed the backbone structure IRL–tnpAR–res–mer–IRR with the integration of In2. Tn6971 and Tn6972 (Fig. 12) harbored Tn21 core transposition determinants tnpAR and IRL/IRR, but Tn1696 mer locus, instead of that in Tn21, was found in Tn6971, while Tn6972 did not contain mer. Tn6971 and Tn6972 each acquired a unique ARL: In1086 [which had GCA aacA4cr–blaOXA-1–catB3–

![FIG 9 Comparison of two Tn6963-related transposable prophages. Genes are denoted by arrows. Genes, AGEs, and other features are colored based on their functional classification. Shading denotes regions of homology (nucleotide identity ≥ 95%). Numbers in brackets indicate nucleotide positions within the chromosomes of strains L241 and 12034, respectively. Accession number of Tn6963 (24) used as reference is CP033056.](image-url)
arr-3–dfra27–aadA16 and was additionally inserted with tetRACD(B)-carrying ΔTn10 in Tn6971 and macAB-tolC in Tn6972.

**Five Tn2670 derivatives, Tn6970, T2670RE11759, T2670RE1211759, T2670RE1211759, and T2670RE1211759.** The sequence comparison (Fig. 13) was also applied to five Tn2670 derivatives, Tn6970, T2670RE11759, T2670RE1211759, T2670RE1211759, and T2670RE1211759 together with Tn6970 (28). T2670RE11759 was directly integrated into the chromosome, while the remaining four were recognized as the inner components of Tn10 derivatives Tn6799 and T10RE12, and Tn696 derivatives Tn6913a and Tn6913b (see above). Tn6970 was an IS1R-composite transposon composed of a Tn9-like backbone (29) with the integration of Tn21 (30), and it was initially found in Shigella flexneri plasmid R100 (28). Compared to Tn2670, Tn6970, T2670RE1211759, and T2670RE1211759 harbored the same intact Tn9-like backbone but contained different versions of truncated Tn21 with integration of two different integrons: (i) In399 [GCA: Inu(F)1–catB2–sat2–aadA1] inserted with In313 (VR1: blaCARB2–aadA2 and VR2: ISCR1–ISCR1–lqg-dfrA19) in Tn6970 and (ii) In252 (VR1: aad8–catB5–blaOXA–aadA1a; VR2: ISCR1–qnrVC1 unit) in T2670RE1211759 and T2670RE1211759. T2670RE1211759 contained the whole S′-terminal Tn9-like backbone and a very small Tn21 remnant; moreover, each of them acquired a unique LAM, the resulting two LAMs shared one ARL [IS26–mph (A)–Is6100 unit], and each LAM further acquired five or four different ARLs: (i) In354, 1.4-kb truncated aacC2–tmb region, Tn6029 (containing blasTM–sul2, strA, and strB) interrupted by Tn4352 (containing apha1), truncated type A IS26–fosA3–IS26 unit, and type A In37-like element in T2670RE1211759 and (ii) type B In37-like element (GCA: aacA4cr–blaOXA1–catB3–arr-3), 3.3-kb truncated aacC2–tmb region, In313 (GCA: blasCARB2–aadA2), and ISCR3–ereB unit in T2670RE1211759. Notably, eight and five copies of IS26/IS150I and IS6100 were presented in T2670RE1211759 and T2670RE1211759, respectively; all these IS elements belonged to Is6 family and possessed almost identical 14-bp IR sequences, and thus they would be together involved in complex homologous recombination events, promoting the assembly of LAMs in T2670RE1211759 and T2670RE1211759.

**Five aacC4/aph(4)-Ia/sul2/floR-carrying MDR regions.** The sequence comparison (Fig. 14) was also applied to five different MDR regions (identified as the inner components of Tn6967, Tn6915, T1696RE1211759, Tn6966, and Tn6965, respectively; see above), which shared 15.5/16.0-kb aacC4/aph(4)-Ia/sul2/floR-carrying region. All these 15.5/16.0-kb regions comprised three major ARLs: IS26–aacC4–aph(4)-Ia–IS150I, ISCR2–sul2 unit, and truncated ISCR2–floR unit. In addition, each of these five MDR regions integrated one or more additional ARLs: (i) In37, type C In37-like element, and type D (all contained the intact GCA aacA4cr–blaOXA1–catB3–arr-3) in MDR regions from T1696RE1211759, Tn6967, and Tn6915, respectively, (ii) In27 (GCA: dfrA12–aadA2) plus apha1-containing ΔTn4352 as shared by MDR regions from T1696RE1211759, Tn6966, and Tn6965, (iii) truncated IS26–mefB–sul3–IS440 unit and In641 (GCA: aadA2–cmlA1a–
aadA1–qacH2) in MDR region from Tn6915, and (iv) In1787 (GCA: aacA4cr12–blaOXA-1–catB3–arr-3–dfrA27–aadA16) in MDR region from Tn6965.

**Newly identified or designated AGEs.** There were 19 newly identified AGEs in total: (i) 12 of them directly integrated into the chromosomes and included 4 composite transposons, Tn6759, Tn6760, Tn6798, and Tn6799, 5 unit transposons, Tn6800, Tn6913a, Tn6913b, Tn6914, and Tn6915, 1 transposable prophage, Tn6964, 1 IME, Tn6966, and 1 ICE, Tn6967, and (ii) the remaining 7 were the inner components of the above 12 and comprised 2 composite transposons, Tn6965 and Tn6970, 2 unit transposons, Tn6971 and Tn6972, and 3 integrons, In2-77, In1785, and In1787. Additionally, there were two newly designated (first designated in this study but with previously determined sequences)
AGEs: 1 putative resistance unit, ISCR3-ere(B) unit, and 1 transposable prophage, Tn6963. All these 21 newly identified or designated AGEs were first identified in the 166 global Morganella isolates. Additionally, Tn6872-related IME, Tn6397-related ICE, and ARG-harbor- ing Tn6963-related transposable prophage were reported for the first time in Morganella.

DISCUSSION

There are few reports on characterizing ARGs in individual Morganella isolates or a small collection of Morganella isolates (n ≤ 22 in each study) (31–33). This study provides a genomic and bioinformatics view of the classification and evolution of Morganella species and the global prevalence of AGEs and associated ARGs in Morganella, based on a collection of 166 sequenced isolates, including 60 sequenced in this study.

We establish a systematic genome sequence-based species classification scheme for Morganella based on ANI analysis plus phylogenomic analysis. The two conventional Morganella species, M. morganii and M. psychrotolerans, should be redefined as two complexes: M. morganii and M. psychrotolerans, which can be further divided into four and two genospecies, respectively. These two complexes display a very long-distance phylogenomic relationship, being consistent with a previous phylogenetic analysis based on seven Morganella housekeeping genes (2). Moreover, the six genospecies display the obvious segregation at genome scale between each other, as revealed by ANI analysis and further confirmed by phylogenomic assay. Notably, all these genospecies cannot be distinguished based on the sequence variation of 16S rRNA genes or that of the seven housekeeping genes (2, 34). Isolates of M. morganii genospecies can be mostly frequently recovered from patients, animals, and the environment, accounting for 147 (91.9%) of the total 160 strains studied. Our phylogenomic analysis on these 147 isolates of M. morganii genospecies shows that this genospecies exhibits a highly clonal population disseminated across at least 16 countries of the five continents (Table S1). These 147 isolates gather at the farthest position from the root in the phylogenetic tree, and therefore M. morganii genospecies represents the latest differentiated clone among all the six genospecies.

Of these 88 acquired ARGs, the most prevalent are tetracycline-resistance tetAR genes, which are found in 99 (59.64%) of the 166 isolates studied. Tetracycline antibiotics have been extensively used in treatment of human and animal infections for at least 60 years (35). The long-term use of tetracycline antibiotics promotes the acquisition...
and dissemination of tetracycline-resistance genes in various bacteria, such as Acinetobacter, Escherichia, and Salmonella (36), and also Morganella, here. In addition, the wide prevalence of β-lactam-resistance genes is also observed in Morganella isolates (n = 58, 34.93%), which might be due to the frequent empirical use of cephalosporins for clinical therapy of M. morganii-induced infections (37).

The 60 isolates sequenced in this study exhibit the highest nonsusceptibility rate (n = 40, 66.67%) for levofloxacin and ciprofloxacin. There are three major mechanisms of fluoroquinolone resistance in Morganella: (i) acquisition of quinolone-resistance genes qnr (12), qepA (38), and aacA4cr (39), (ii) mutation of type II topoisomerases genes gyrAB and parCE (40, 41), and (iii) enhancement of proton-dependent active effluxes (42). A total of 31 of these 60 isolates carry acquired qnr and/or aacA4cr genes, but only 17 of these 31 isolates are nonsusceptible to fluoroquinolones (Table S1). Commonly, the carriage of qnr, qepA, and aacA4cr genes only mediates limited decreased susceptibility to fluoroquinolones but cannot guarantee that the isolates exhibit the fluoroquinolone resistance with a breakpoint of 0.5 μg/mL provided by Clinical and Laboratory Standards.
It is speculated that the extensive nonsusceptibility for fluoroquinolones in *Morganella* isolates is most likely caused by the combination of the above multiple resistance mechanisms.

The lowest nonsusceptibility rates were observed for meropenem (n = 6, 10%) and amikacin (n = 3, 5%) in these 60 Chinese isolates. Similar results were found for the 692 *Morganella* isolates from China Antimicrobial Surveillance Network (Table S7). These denote that carbapenems and amikacin will be the most effective antimicrobials against *Morganella* in China. The observed meropenem resistance and amikacin resistance in the above six and three *Morganella* isolates are mediated by the acquisition of carbapenemase gene (\(\text{bla}_{KPC-2}\) [n = 4] or \(\text{bla}_{NDM-1}\) [n = 2]) and 16S rRNA methyltransferase gene (\(\text{rmtB}\)), respectively. The presence of carbapenemase genes or \(\text{rmt}\) gene can be identified in only 21 of these 166 global *Morganella* isolates (Table S1). Taken together, meropenem resistance or amikacin resistance is still not widely disseminated in *Morganella*.

This study presents the full sequences of 18 AGEs located within *Morganella* chromosomes. Subsequently, a detailed sequence comparison was applied to these 18 AGEs, together with 5 additional prototype AGEs from GenBank. These 23 AGEs could be divided into eight distinct groups: two IS26–\(\text{mef}(B)–\text{sul3}–\text{IS440}\) unit (60), \(\text{chrA}–\text{orf98}\) unit (53), IS\(\text{Ec}29–\text{mph}(E)–\text{IS26}\) unit (61), Tn\(\text{n}3\)S\(\text{L}\)2c, Tn\(\text{n}3\)S\(\text{L}\)2 (62), Tn\(\text{n}539\)c (63), and IS\(\text{CR}2–\text{floR}\) unit (53) are FJ196385, CP042858, AF550415, CP042857, CP042858, AF262622, and CP042857, respectively.
derivatives, Tn7 and its two derivatives, Tn1696 and its five derivatives, Tn6963 and its one derivative, Tn6872 and its one derivative, Tn6397 and its one derivative, and Tn2670 and its one derivative. Eleven of these 23 AGEs each carries a distinct LAM (~29.5 kb) with complex mosaic structure: (i) LAMs of Tn6799, T10RE621164, Tn6913a, and Tn6913b manifest as distinct Tn2670-related elements, (ii) those of T7RE421750, Tn6915, and T1696RE7500 contained different MDR regions, (iii) that of Tn6964 manifests as Tn6965 integrated with MDR region, (iv) that of Tn6966 comprises Tn6971 and MDR region, (v) that of Tn6967 contains Tn6972 and MDR region, and (vi) T2670RE1750 as a LAM directly integrates into the chromosome. These LAMs are likely assembled from complex transposition and homologous recombination and, notably, comprise various ARLs, including composite/unit transposons, integrons, and putative resistance units, resulting in accumulation of at least 44 ARGs in Morganella chromosomes.

In summary, a genomic epidemiology analysis on 166 global sequenced Morganella isolates, including 60 sequenced here, was conducted in this study. First, a genome sequence-based species classification scheme for Morganella was established, and the two conventional Morganella species were redefined as two complexes, which were further divided into four and two genospecies, respectively. Second, the prevalence of acquired ARGs was screened based on genome sequences, demonstrating that at least 88 acquired ARGs are accumulated and disseminated in Morganella. Finally, a detailed sequence comparison of eight groups of 23 AGEs (including 18 Morganella chromosomal AGEs sequenced in this study) was performed. There are LAMs in 11 of these 23 AGEs, and these LAMs have complex mosaic structures and contain many ARLs and associated ARGs. Integration of these ARG-containing AGEs into Morganella chromosomes would contribute to the accumulation and dissemination of ARGs in Morganella and enhance the adaption and survival of Morganella under complex and diverse antimicrobial selection pressures.

MATERIALS AND METHODS

Bacterial isolates and identification. A total of 60 Morganella isolates were collected from 2013 to 2019, including 56 from hospitalized patients in seven Chinese public hospitals and 4 from animals in four Chinese farms (Table S1). The 16S rRNA genes and the carbapenemase genes blaNDM1, and blaKPC2 were detected as described previously (45). The activity of class A/B/D carbapenemases in bacterial cell extracts was detected by a modified CarbaNP test (45). The bacterial antimicrobial susceptibility was tested using bioMérieux Vitek 2 and interpreted as per the 2020 CLSI guidelines (44).

Genomic DNA sequencing and sequence assembly and annotation. All these 60 isolates were subjected to draft-genome sequencing using a paired-end library with an average insert size of 350 bp (ranged from 150 bp to 600 bp) on a HiSeq sequencer (Illumina, CA, USA). In addition, 12 (Table S1) of these subjects were subjected to complete-genome sequencing with a shared DNA library with an average size of 15 kb (ranged from 10 kb to 20 kb) on a PacBio RSII sequencer (Pacific Biosciences, CA, USA). The quality control analysis of sequencing data was conducted using NanoPack (46) and FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc). Sequence assembly and annotation were performed as described previously (47).

ANI analysis and phylogenetic analysis. The pairwise ANI values of Morganella genome sequences were calculated using FastANI (48). Morganella genome sequences were aligned to the complete chromosome sequence (accession number CP004345) of M. morganii subsp. morganii isolate KT used as reference, and the SNPs were identified by Mummer v3.25 (49). All the SNPs in the repetitive DNA regions were identified and filtered by RepeatMasker (http://www.repeatmasker.org/). Homologous recombination at a genome-wide level was predicted using ClonalFrameML (50), followed by removal of all putative recombinant SNP sites. Based on the final recombination-free core SNPs, a maximum-likelihood phylogenetic tree was constructed using RAxML (51) with a bootstrap iteration of 1,000 and displayed using iTOL (https://itol.embl.de).

In silico analysis of prevalence of AGEs. We collected the core transposition determinants (encoding transposases and their auxiliary factors) of the 17 major AGE groups, which were frequently found in Gram-negative bacteria (19). These 17 groups included IS26/IS5/SAI, IS10, and IS401 subfamilies), Tn7-family (composed of Tn7, Tn6820, Tn552, Tn6022, and Tn5035 subfamilies), and Tn554-family (comprising Tn554, Tn6488, and Tn6571 subfamilies) unit transposons, as shown in our unpublished DANNEL database (https://39.100.87.11/dannmel_V1.0/index.php). The sequence alignment of these core transposition determinants was conducted on the draft-genome sequences of these 166 Morganella isolates, screening for the prevalence of these 17 major group AGEs in Morganella.

Statistical analysis. The statistical differences for ARGs among reservoirs were tested by Pearson’s \( \chi^2 \) test. Statistical computations and figures were plotted with R package v.3.2.71 (http://www.r-project.org) and visualized with Adobe Illustrator.
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