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Original Article

# Multicenter comparative genomic study of *Klebsiella oxytoca* complex reveals a highly antibiotic-resistant subspecies of *Klebsiella michiganensis*

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**Abstract** *Background:* The *Klebsiella oxytoca* complex is an opportunistic pathogen that has been recently identified as an actual complex. However, the characteristics of each species remain largely unknown. We aimed to study the clinical prevalence, antimicrobial

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*Klebsiella oxytoca* complex;
*Klebsiella michiganensis*;
Pan genome

profiles, genetic differences, and interaction with the host of each species of this complex.
*Methods:* One hundred and three clinical isolates of the *K. oxytoca* complex were collected from 33 hospitals belonging to 19 areas in China from 2020 to 2021. Species were identified using whole genome sequencing based on average nucleotide identity. Clinical infection characteristics of the species were analyzed. Comparative genomics and pan-genome analyses were performed on these isolates and an augmented dataset, including 622 assemblies from the National Center for Biotechnology Information. *In vitro* assays evaluating the adhesion ability of human respiratory epithelial cells and survivability against macrophages were performed on randomly selected isolates.
*Results: Klebsiella michiganensis* (46.6%, 48/103) and *K. oxytoca* (35.92%, 37/103) were the major species of the complex causing human infections. *K. michiganensis* had a higher genomic diversity and larger pan-genome size than did *K. oxytoca*. *K. michiganensis* isolates with $bla_{oxy\text{-}5}$ had a higher resistance rate to various antibiotics, antimicrobial gene carriage rate, adhesion ability to human respiratory epithelial cells, and survival rate against macrophages than isolates of other species.
*Conclusion:* Our study revealed the genetic diversity of *K. michiganensis* and firstly identified the highly antimicrobial-resistant profile of *K. michiganensis* carrying $bla_{oxy\text{-}5}$.


## Introduction

The *Klebsiella oxytoca* complex can cause antibiotic-associated hemorrhagic colitis (AAHC)[1] and other infections.[2—5] Fifteen outbreaks of healthcare-associated infections due to the complex have been reported,[2] and most of these outbreaks occurred in the neonatal intensive care unit (NICU).[6—12] In addition, a recent study[13] found that infections by the *K. oxytoca* group were more invasive than those by the *Klebsiella pneumoniae* group, thereby highlighting the clinical importance of this underestimated group.

The *K. oxytoca* complex comprises six known species, *K. oxytoca*, *Klebsiella michiganensis*, *Klebsiella grimontii*, *Klebsiella huaxiensis*, *Klebsiella pasteurii*, and *Klebsiella spallanzanii*, and three new unnamed species. Sequence variations of the chromosomally encoded β-lactamase gene $bla_{oxy}$ can be used to categorize the *K. oxytoca* complex into phylogroups.[2] The bacterial species can be categorized into 12 genotypes ($bla_{OXY\text{-}1}$ to $bla_{OXY\text{-}12}$) corresponding to different designated species within the complex. For instance, *K. oxytoca* carries $bla_{oxy\text{-}2}$, and *K. michiganensis* carries $bla_{oxy\text{-}1}$ or $bla_{oxy\text{-}5}$. The prevalence of each species of the complex in the clinical setting and their infection characteristics, antimicrobial profile, and clinical relevance remain largely unknown. Identifying species-specific clinical characteristics and antimicrobial profiles paves the way for effective control of risk species in a clinical setting.

## Methods

### Bacterial isolates

Based on the Special Foundation for National Science and Technology Basic Research Program of China, 11,486 bacterial isolates were collected from 69 hospitals from January 2020 to November 2021. After species identification using VITEK MS (bioMérieux, Marcy l'Etoile, France), 103 clinical isolates of the *K. oxytoca* complex were enrolled. The species was determined by comparing the genome to the type strains of the *K. oxytoca* complex (Table S1) using ANIm[14] with a threshold of 97%. The gene type of $bla_{oxy}$ was used for confirming the results of ANI analysis. Nine isolates, which shared ANIm >99.9% with another isolate from the same region, were considered to have undergone clonal spreading (Table S2) and were excluded when comparing the antimicrobial profile and genomic differences between species.

### Ethical statement

The study was approved by the human research ethics committee of Peking Union Medical College Hospital (No. JS-2581).

### Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted in a central monitoring laboratory. Twenty antimicrobial agents were tested using the broth microdilution method (gram-negative bacteria susceptibility test card; Dier, China) specified by the Clinical and Laboratory Standards Institute (CLSI) standards.[15] The susceptibilities of the antimicrobial agents were interpreted according to the current CLSI guidelines.[16]

### NCBI data

After filtering out low-quality sequences and those collected from the non-human host, 622 genome assemblies

of *K. michiganensis* and *K. oxytoca* were downloaded from the NCBI on July 16, 2022 (Table S3). Precise species were identified based on the ANI and the gene type of $bla_{oxy}$.

### Genomic DNA extraction and sequencing

Genomic DNA was extracted using a TIANamp bacteria genomic DNA kit (Tiangen Biotech Co. Ltd., Beijing, China) and subjected to shotgun sequencing using the Illumina genome analyzer IIx (San Diego, CA, USA). The isolated genomic DNA (5 μg) was used to prepare paired-end libraries using the TruSeq DNA sample prep kit A (Illumina Inc.), according to the manufacturer's instructions. Genomic paired-end libraries were sequenced with a read length of 2 × 150 nucleotides using Illumina GAIIx. The raw Illumina sequencing reads were trimmed at a threshold of 0.01 (with a Phred score of 20) using the Fastx-toolkit (http://hannonlab.cshl.edu/fastx_toolkit/commandline.html).

### Comparative genomic analysis

Whole genome sequencing data were assembled using SPAdes.[17] Prokka (v1.14)[18] was used for annotation. Orthologous groups were constructed using Roary (v3.13.0).[19] The core genome alignment was used to construct an ML phylogenetic tree using FastTree (v2.1).[20] Average pairwise genomic distances were calculated using the Tamura 3-parameter method in MEGAX.[21] Multilocus sequence typing was performed using the MLST software (https://github.com/tseemann/mlst) and was further confirmed by searching the assemblies on PubMLST website. The sizes of the core and pan-genomes were calculated using the Python script reported previously.[13] The O- and K-loci were determined using KLEBORATE (v2.2.0).[22] Virulence factors and AMR determinants were identified by comparing the genomes with the virulence factor[23] and a comprehensive antibiotic resistance database (3.2.3),[24] respectively. Potential plasmids were identified using PlasmidFinder database.[25] Mobile genetic elements were identified using MobileElementFinder.[26]

### Growth analysis

Strains were grown overnight and diluted to 1 × $10^6$ CFU/mL. Then the suspension was cultured for 24 h at 37 °C in a 96-well plate. Growth was monitored by measuring the absorbance at 600 nm every 30 min using Epoch™ 2 Microplate Spectrophotometer (BioTek Instruments) with orbital shaking for 15s before each measurement.

### Adhesion and macrophage survival assay

The adhesion assay was performed as previously described.[27] For the macrophage survival assay, the murine macrophage RAW264.7 cells (5 × $10^6$ cells/well) were seeded in 12-well cell culture plates and incubated with an appropriate MOI (50) of bacteria for 2.5 h. Non-phagocytosed bacteria were removed by washing with PBS (pH 7.2–7.4), and cells were lysed with 1 mL of 0.2% Triton X-100 for 20 min. Surviving bacteria were plated on blood agar plates (100 μL/plate) containing 5% sheep blood. Data are expressed as $\log_{10}$ CFU/mL after the incubation period compared with the starting inoculum.

### Statistical analyses

Categorical variables were examined using the $\chi^2$/Fisher's exact test and are expressed as % (m/n). Non-normally distributed data were compared using the Mann–Whitney *U* test and are expressed as the median and IQR. All *P* values were two-tailed, and statistical significance was set at $P < 0.05$. All statistical analyses were performed and graphs were plotted using R (v4.2.1; https://cran.r-project.org).

## Results

### Sample information and species identification

Among the 103 isolates, 48 (46.60%) were identified as *K. michiganensis*, the proportion of which was higher than that of *K. oxytoca* (35.92%), *K. grimontii* (12.62%), and *K. pasteurii* (4.85%; Fig. 1A). Nine isolates, including two *K. michiganensis* isolates and seven *K. oxytoca* isolates, which shared ANIm >99.9% with another isolate from the same region, were considered to have undergone clonal spreading (Table S2) and were excluded when comparing the antimicrobial profile and genomic differences between species. Clinical information was similar among these species (Table S4). The median age of patients infected with *K. grimontii* (38 [0.25–60]) was significantly lower than that of patients infected with non-*K. grimontii* species (60 [49–72.25], $P = 0.036$), and 5 of the 13 patients were younger than 1 year. As these five *K. grimontii* genomes belong to five different sequence types (STs), clonal spreading could be excluded (Table S2). The 103 genomes could be assigned to 71 STs (Table S5), which suggests the very diverse clonal background of the complex.

### Pan-genome and comparative genomics analyses reveal a higher genomic diversity in *K. michiganensis* than in other species

The plasmid replicons detected in *K. michiganensis* differed from those detected in the *K. oxytoca*, *K. grimontii* and *K. pasteurii* (Fig. 1B). We detected a significantly lower median number of plasmid replicons per isolate in *K. oxytoca* than in *K. michiganensis* and *K. grimontii* (Fig. 1C). The diversity of capsular polysaccharide (K locus) and lipopolysaccharide (containing O locus) between different species were analyzed (Fig. 1D). We found a higher diversity of the K_loci in *K. michiganensis* than in the other species (Fig. 1D). There were 21 types of K_loci were identified in *K. michiganensis* while only 8 types of K_loci were identified in *K. oxytoca* (Fig. 1D). In contrast, a higher diversity with three types of O_loci was detected in *K. oxytoca*. The other three species only carried the O_locus O1/O2v1 (Fig. 1D). The core genome of these assemblies comprised 3581 genes, which were shared among 99% of these isolates. Corresponding to four species of the *K. oxytoca* complex, a maximum likelihood (ML) phylogenetic

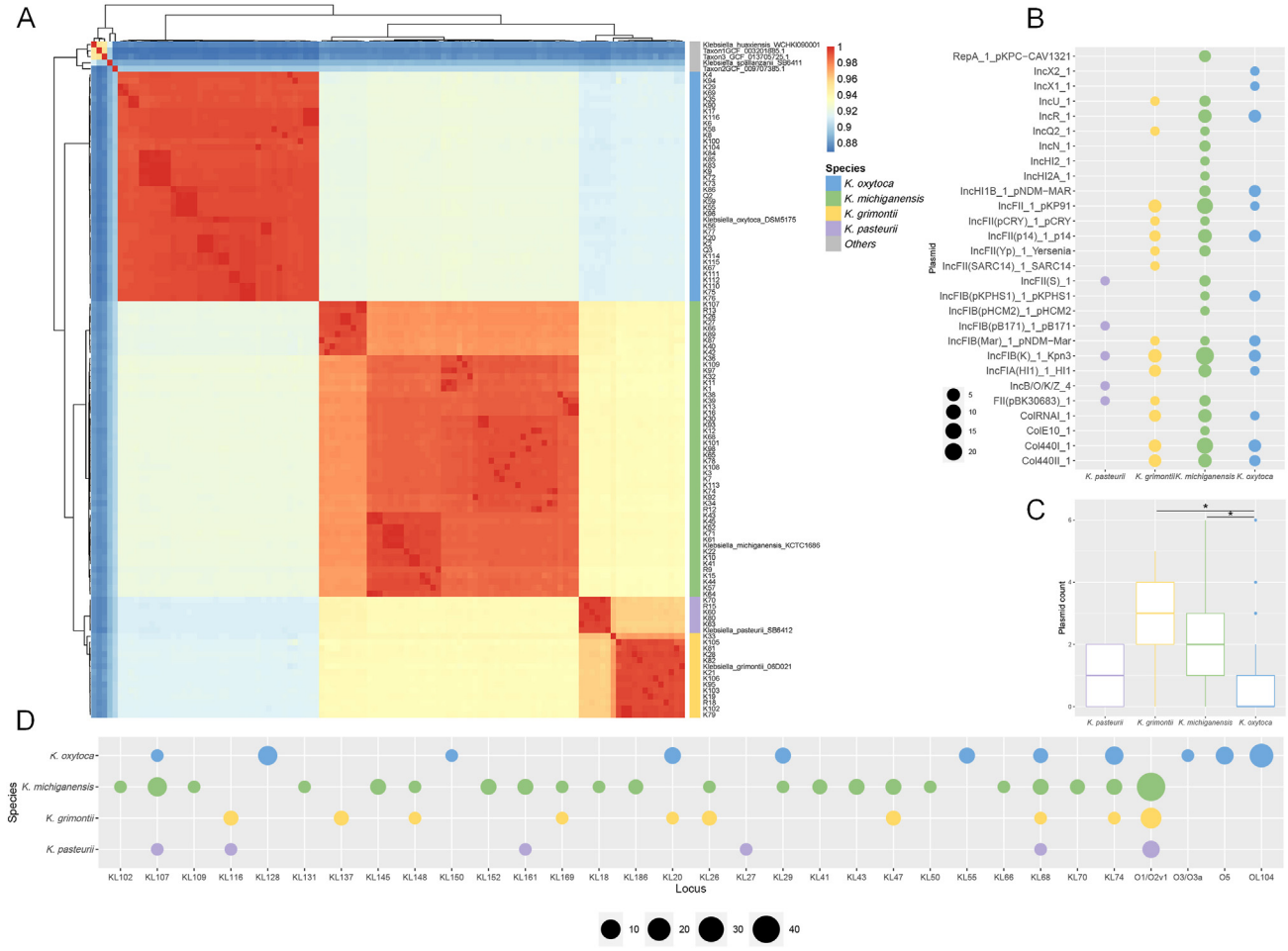


**Figure 1. Species identification and genomic content analysis of the isolates. A.** Heatmap depicting the average nucleotide identity results which were further confirmed by the *bla*$_{oxy}$ gene type (see Fig. 2). **B.** Plasmid replicons identified in all isolates. **C.** Number of plasmid replicons identified in different species. **D.** Predicted polysaccharide (K-locus) and lipopolysaccharide (O-locus) serotypes in different species. * represents $P < 0.05$.

analysis of single-nucleotide polymorphisms (SNPs) of the core genome identified five phylogroups, with *K. michiganensis* clustered into two subgroups (Fig. 2, left panel). The average pairwise genetic distance of the core genome of each species showed a higher diversity in *K. michiganensis* (distance ± SE: $9.881 \times 10^{-3} \pm 2.8 \times 10^{-5}$) than in *K. oxytoca* ($4.722 \times 10^{-3} \pm 2.1 \times 10^{-5}$), *K. grimontii* ($6.559 \times 10^{-3} \pm 2.2 \times 10^{-5}$), and *K. pasteurii* ($2.761 \times 10^{-3} \pm 1.9 \times 10^{-5}$). These results indicate that *K. michiganensis* was the most genetically diverse species in this complex.

## *K. michiganensis* with *bla*$_{oxy-5}$ has higher antimicrobial resistance than other species

Each species carried distinct variants of *bla*$_{oxy}$, a β-lactamase-encoding gene intrinsic to the *K. oxytoca* complex. Specifically, *K. oxytoca*, *K. grimontii*, and *K. pasteurii* carried *bla*$_{oxy-2}$, *bla*$_{oxy-6}$, and *bla*$_{oxy-4}$, respectively. The *K. michiganensis* isolates carried *bla*$_{oxy-1}$ or *bla*$_{oxy-5}$. The *bla*$_{oxy}$ gene was absent in one isolate (Fig. 2). The multidrug resistance transporter homolog from *Escherichia coli msbA* was detected in all isolates of *K. michiganensis*, *K. grimontii*, and *K. pasteurii*, except *K. oxytoca*, wherein only two isolates carried it. Based on the whole AMR gene spectrum, isolates of *K. michiganensis* carried a higher number of AMR genes than the isolates of the other three species. Isolates of *K. michiganensis* with *bla*$_{oxy-5}$ showed the highest antimicrobial resistance and contained many AMR genes, which were also supported by antimicrobial susceptibility test (AST) (Fig. 2). As these seven isolates belonged to seven STs and were collected from six regions of China, the possibility of outbreaks can be ignored.

The AST results among four species showed relatively higher antimicrobial resistant rates of *K. michiganensis* than that of the other three species (Fig. 3A). Nine (19.57%) isolates of *K. michiganensis* carried extended-spectrum β-lactamases (ESBL) genes; this number was higher than that of *K. oxytoca* (6.67%), *K. grimontii* (7.69%), and *K. pasteurii* (20.00%) (Fig. 3B). The carriage rate of ESBL genes in *K. michiganensis* with *bla*$_{oxy-5}$ (42.86%) was significantly higher than that in *K. oxytoca* isolates ($P = 0.037$; Fig. 3B). Consistently, the resistance rate of second-, third-, and fourth-generation cephalosporins was significantly higher in

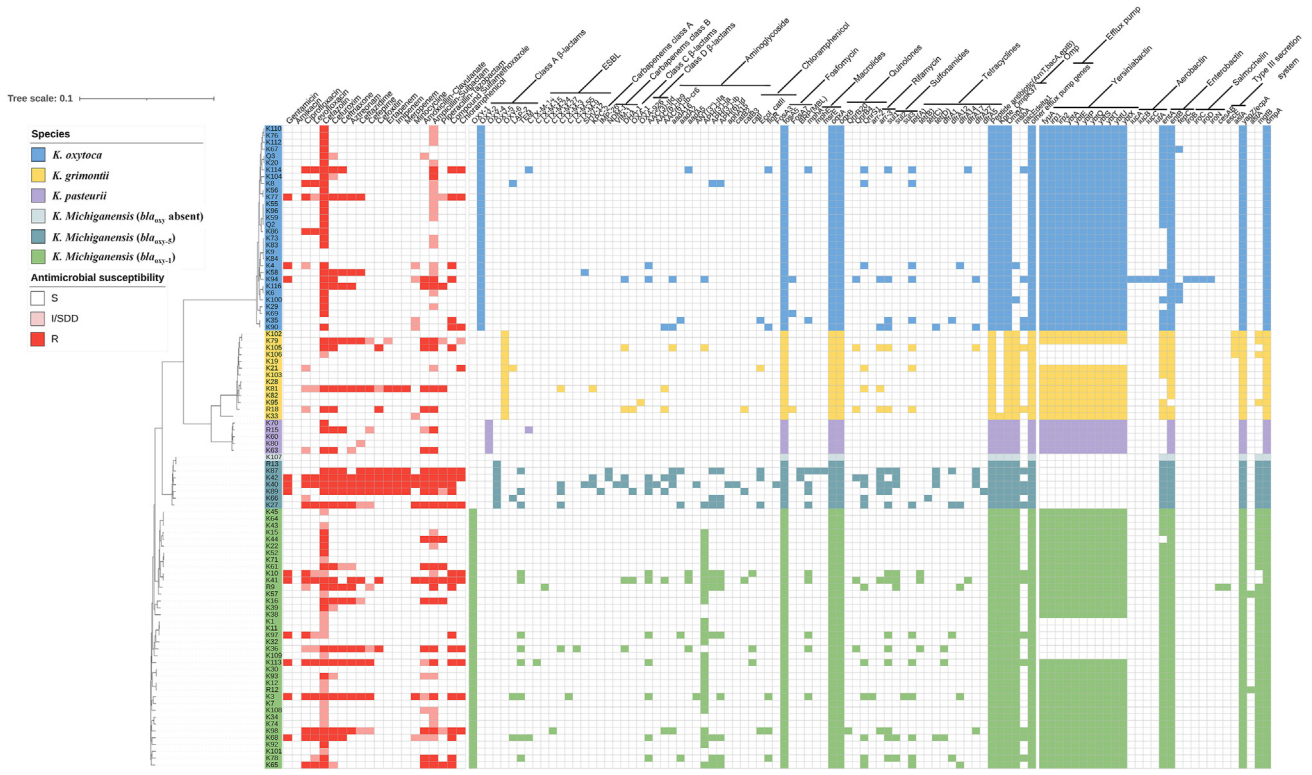


**Figure 2.** The maximum likelihood phylogenetic tree of single-nucleotide polymorphisms of the core genome of isolates, annotated with antimicrobial susceptibility testing results, antimicrobial resistance determinants, and virulence factors.

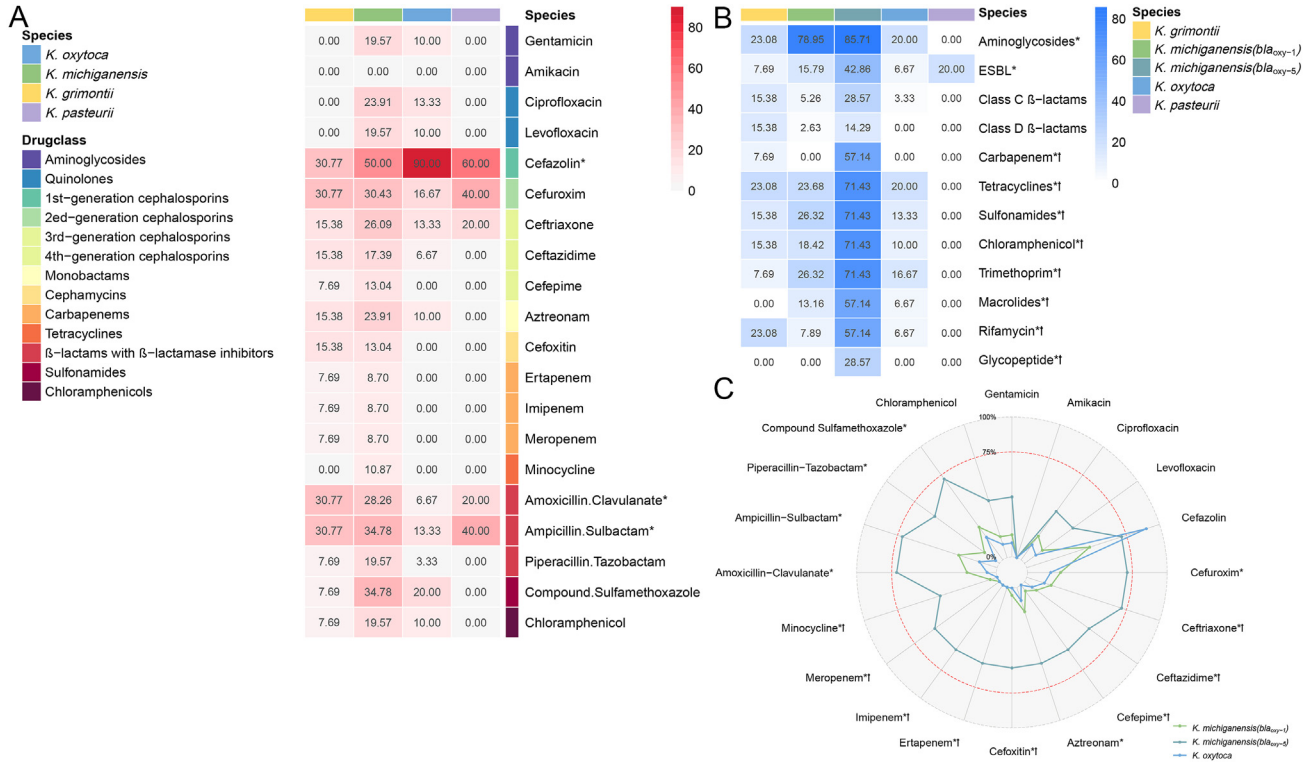


**Figure 3.** **Antimicrobial profile of each species. A.** Antimicrobial resistance rates * represents $P < 0.05$ between *K. michiganensis* and *K. oxytoca*. **B.** Antimicrobial resistance gene carriage rates. **C.** Antimicrobial resistance rates of *K. oxytoca*, *K. michiganensis* with $bla^{oxy\text{-}1}$, and *K. michiganensis* with $bla_{oxy\text{-}5}$. * represents $P < 0.05$ between *K. michiganensis* with $bla^{oxy\text{-}5}$ and *K. oxytoca*. † represents $P < 0.05$ between *K. michiganensis* with $bla_{oxy\text{-}5}$ and *K. michiganensis* with $bla_{oxy\text{-}1}$.

*K. michiganensis* with $bla_{oxy-5}$ than in *K. michiganensis* with $bla_{oxy-1}$ and *K. oxytoca*, and resistance was found in addition to β-lactamase inhibitors (Fig. 3C).

Four (8.70%) isolates of *K. michiganensis* carried carbapenemase genes, and all of them were *K. michiganensis* with $bla_{oxy-5}$ (57.14%); this proportion was significantly higher than that of *K. michiganensis* with $bla_{oxy-1}$ (0; $P < 0.001$), *K. oxytoca* (0; $P < 0.001$), and *K. grimontii* (7.69%; $P = 0.031$) (Fig. 3B). Consistently, a significantly higher resistance rate against carbapenem was found in *K. michiganensis* with $bla_{oxy-5}$ than in *K. michiganensis* with $bla_{oxy-1}$ and *K. oxytoca* (Fig. 3C). *K. michiganensis* with $bla_{oxy-5}$ showed higher antimicrobial resistance than the other species of the *K. oxytoca* complex.

## *K. michiganensis* with $bla_{oxy-5}$ carries lesser virulence factors than the other species

The balance between virulence and AMR was found in *K. michiganensis* with $bla_{oxy-5}$. Yersiniabactin, a phenolate siderophore produced by *Yersinia enterocolitica*,[28] is well known for scavenging iron *in vivo*[29] and reducing reactive oxygen species formation in phagocytes. According to the virulence factor database,[23] *Klebsiella* species have 11 genes within the Yersiniabactin gene cluster and they were all searched in our isolates. Yersiniabactin genes were detected in all the isolates of *K. oxytoca*, *K. pasteurii*, 76.92% isolates of *K. grimontii*, and 84.62% isolates of *K. michiganensis* with $bla_{oxy-1}$. However, these were absent in all isolates of *K. michiganensis* with $bla_{oxy-5}$. Notably, *mgtB*, a crucial magnesium transporter gene that is related to survival inside macrophages,[30] was detected in 95.83% of isolates of *K. michiganensis* (100% in *K. michiganensis* with $bla_{oxy-5}$) and 30.77% of *K. grimontii* (Fig. 2). However, it was absent in *K. oxytoca* and *K. pasteurii* isolates, indicating a better intramacrophage survival capability of *K. michiganensis*.

## Global data support the diversity of *K. michiganensis* and genetic characteristics of *K. michiganensis* with $bla_{oxy-5}$

To support our founding on a global scale, 355 assemblies of *K. michiganensis* and 212 assemblies of *K. oxytoca* were downloaded from the NCBI after species identification (Fig. S1). They were merged with our dataset to investigate the pan-genome diversity and the presence of AMR and virulence factor. A ML phylogenetic analysis of core-genome SNPs showed a higher diversity of *K. michiganensis* than of *K. oxytoca*, as more clusters were found in *K. michiganensis* (Fig. 4A). The pan-genomes were determined by comparing the number of unique orthologous clusters with the core genomes. Consistent with the ML phylogeny results, we found a higher pan-to core-genome ratio in *K. michiganensis* than in *K. oxytoca*, suggesting a larger pan-genome and higher diversity of *K. michiganensis* (Fig. 4B). In addition, the number of orthologous clusters in *K. michiganensis* reached 50,000 when 400 strains were added to the analysis, which was similar to *Klebsiella pneumonia* reported previously.[13] Within *K. michiganensis*, the pan-genome size of *K. michiganensis* with $bla_{oxy-1}$ was relatively larger than that of *K. michiganensis* with $bla_{oxy-5}$, although the result was limited by the sample size of *K. michiganensis* with $bla_{oxy-5}$. A significantly higher number of insertion sequences and plasmid replicons was detected in *K. michiganensis* with $bla_{oxy-5}$ than in *K. michiganensis* with $bla_{oxy-1}$ and *K. oxytoca* (Fig. 4C and D). A higher diversity of K_ and O_loci was found in *K. michiganensis* and *K. oxytoca*, respectively. We found that KL128, KL150, KL27, KL55, KL62, O3/O3a, O3b, O5 and OL104 were exclusively identified in assemblies of *K. oxytoca* (Fig. 4E). KL102, KL103, KL109, KL103, KL131, KL149, KL152, KL164, KL179, KL18, KL181, KL25, KL43, KL80, and O1/O2v2 were solely identified in assemblies of *K. michiganensis* carrying the $bla_{oxy-1}$. The loci KL156-D1 was specific to *K. michiganensis* with $bla_{oxy-5}$ (Fig. 4E).

We found a significantly higher gene carriage rate of *K. michiganensis* with $bla_{oxy-5}$ in multiple drug classes (Fig. 5A). Specific genes that were significantly different among *K. michiganensis* with $bla_{oxy-5}$, *K. michiganensis* with $bla_{oxy-1}$, and *K. oxytoca* are shown in Fig. 5B–D and Table S6. Consistently, a significantly higher carriage rate of ESBL and carbapenemase genes was observed in the assemblies of *K. michiganensis* with $bla_{oxy-5}$ (Fig. 5A). Specifically, the carriage rates of CTX-M-1 and CTX-M-14 were higher in *K. michiganensis* with $bla_{oxy-5}$ (3.3% and 5%, respectively) than in *K. oxytoca* (0% and 1.24%, respectively) and *K. michiganensis* with $bla_{oxy-1}$ (0% and 0.29%, respectively) (Fig. 5B–D, Table S6). The carbapenemase genes KPC-2 and NDM-1 were found in 20.00% and 15.00% of *K. michiganensis* isolates with $bla_{oxy-5}$, respectively (Fig. 5B); this proportion was significantly higher than that in *K. michiganensis* with $bla_{oxy-1}$ (9.12% and 3.24%, respectively) and *K. oxytoca* (1.65% and 1.24%, respectively). To eliminate the influence of our local data, similar comparison using solely NCBI dataset were performed and found the same trend (Table S7). Specific mobile genetic elements that were significantly different among *K. michiganensis* with $bla_{oxy-5}$, *K. michiganensis* with $bla_{oxy-1}$, and *K. oxytoca* were clustered with significant and different AMR genes (Fig. S2). We found a correlation between KPC-2 and an insertion sequence named ISKpn6, with 74.47% of assemblies harboring KPC-2 carrying ISKpn6. Differences in virulence factors among these three species were consistent with abovementioned results. Overall, this augmented dataset confirmed that assemblies of *K. michiganensis* with $bla_{oxy-5}$ carried a higher number of AMR genes but lesser virulence factors than did *K. michiganensis* with $bla_{oxy-1}$ and *K. oxytoca*.

## *K. michiganensis* with $bla_{oxy-5}$ exhibits higher adhesion ability to human epithelial cells and survivability against macrophages

Given the discrepancy found in the virulence factors of *K. oxytoca*, *K. michiganensis* with $bla_{oxy-1}$, and *K. michiganensis* with $bla_{oxy-5}$, three clinical isolates were randomly selected from each species to explore their interaction with human respiratory epithelial cells and macrophages. However, no significant differences were found in the growth curve and generation time (Fig. 6A and B). Isolates of *K. michiganensis* with $bla_{oxy-5}$ showed better adhesion ability

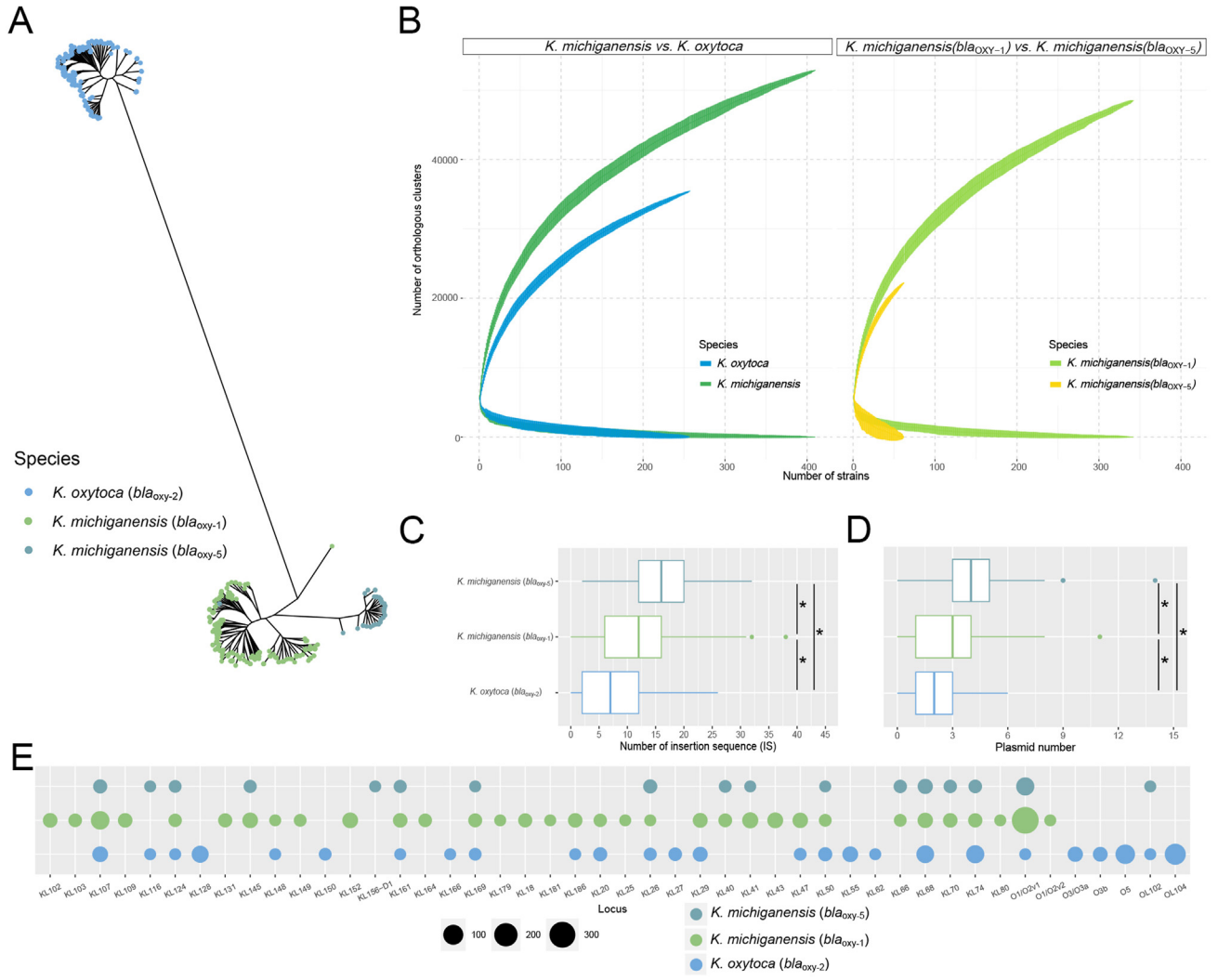


**Figure 4. Genomic content of assemblies of *Klebsiella oxytoca* and *Klebsiella michiganensis*. A.** Core-genome maximum likelihood phylogenetic tree. **B.** Comparison of the pan- (upper) and core- (lower) genome accumulative curves. **C.** Number of insertion sequence. **D.** Number of plasmid replicons. **E.** Predicted polysaccharide (K-locus) and lipopolysaccharide (O-locus) serotypes. * represents $P < 0.05$.

to the human respiratory epithelial A549 cells (Fig. 6C) and higher survival rate against macrophages than the isolates of the other two species (Fig. 6D).

## Discussion

*K. michiganensis* and *K. oxytoca* constituted the major species of the complex, causing human infections in China. The higher isolation rate of *K. michiganensis* could be attributed to the higher diversity of its pan-genome. We identified a higher number of plasmid replicons, insertion sequences, and K_loci in *K. michiganensis* than in *K. oxytoca*. Additionally, the core genome of *K. michiganensis* showed a higher diversity than that of *K. oxytoca*. Therefore, *K. michiganensis* is the most diverse species in the *K. oxytoca* complex.

According to SENTRY (https://www.jmilabs.com/sentry-surveillance-program/) data, the vast majority of *K. oxytoca* isolates are susceptible to tigecycline, colistin, aminoglycosides especially amikacin. Clinical isolates of the *K. oxytoca* complex in China (n = 30,781, from 1375 hospitals in 2019; http://www.carss.cn/) had higher rates of nonsusceptibility to most antimicrobial agents than those in SENTRY and Japan, in particular to carbapenems, ceftazidime, cefepime, and fluoroquinolones.[2] The results of our study aligned with the CARSS data in general. More importantly, our study provided more precise antimicrobial profile into species level and further found in the first time that *K. michiganensis* isolates carrying the $bla_{oxy-5}$ gene exhibited a higher resistance rate to various antibiotics. Consistently, *K. michiganensis* isolates with $bla_{oxy-5}$ carried a higher number of antimicrobial

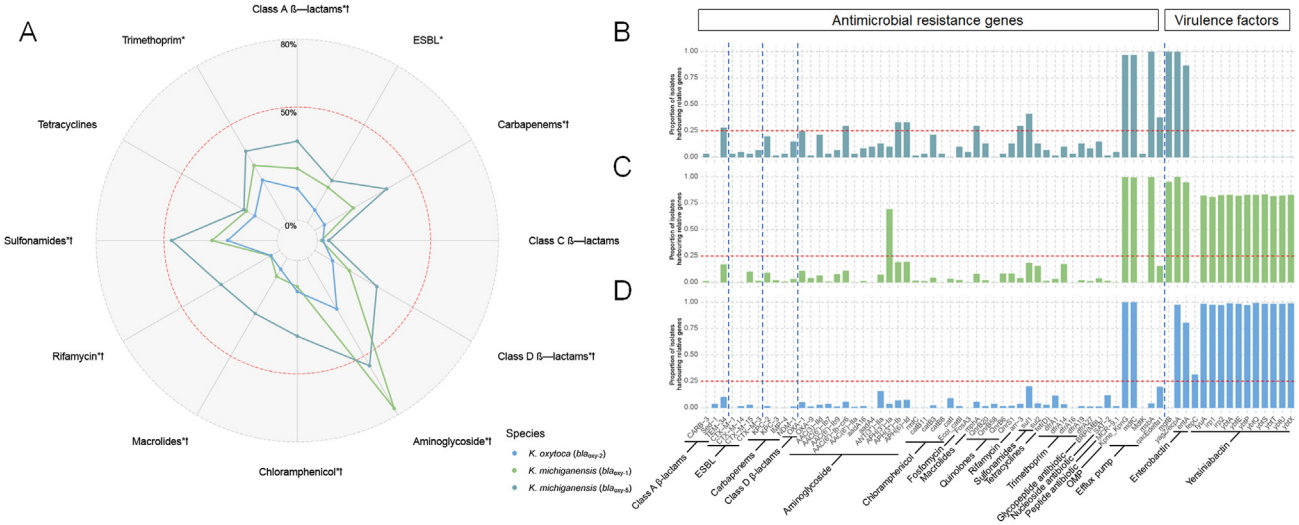


**Figure 5. The antimicrobial profile of *Klebsiella oxytoca* and *Klebsiella michiganensis* on a global scale. A.** Antimicrobial resistance (AMR) gene carriage rates in assemblies of NCBI and our dataset (N = 642). * represents $P < 0.05$ between *K. michiganensis* with *bla*$^{oxy\text{-}5}$ and *K. oxytoca*. † represents $P < 0.05$ between *K. michiganensis* with *bla*$_{oxy\text{-}5}$ and *K. michiganensis* with *bla*$_{oxy\text{-}1}$. **B—D.** Significantly different and specific AMR genes and virulence factors among *K. michiganensis* with *bla*$_{oxy\text{-}5}$ (B), *K. michiganensis* with *bla*$_{oxy\text{-}1}$ (C), and *K. oxytoca* (D).

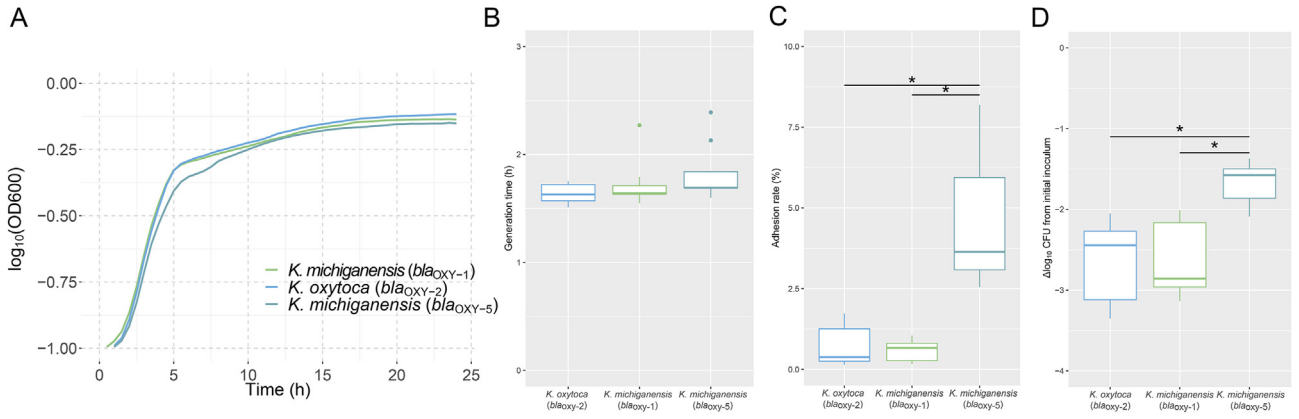


**Figure 6. Growth and infection features of clinical isolates. A.** Growth curves of clinical isolates of the three species. For each species, a growth curve was generated by the mean $\log_{10}(OD_{600})$ of three replicates of three randomly selected samples. **B.** Generation time of the three species. **C.** Adhesion rate of the selected clinical isolates to human respiratory epithelial cells. **D.** Cfus recovered from macrophages infected by the selected clinical isolates.

genes than other species. The augmented dataset analysis also revealed a higher genetic diversity of *K. michiganensis* and higher carriage rates of genes against multiple drug classes in *K. michiganensis* with *bla*$_{oxy\text{-}5}$. The high carriage rates of the carbapenemase genes KPC-2 and NDM-1 in *K. michiganensis* with *bla*$_{oxy\text{-}5}$ are concerning.

A relatively higher AMR capability may have compromised the virulence of *K. michiganensis* with *bla*$_{oxy\text{-}5}$ during evolution. A huge discrepancy concerning the presence of yersiniabactin genes in *K. michiganensis* with *bla*$_{oxy\text{-}1}$ and *K. michiganensis* with *bla*$_{oxy\text{-}5}$ was observed. Yersiniabactin, a phenolate siderophore produced by *Y. enterocolitica*,[28] is well known for scavenging iron *in vivo*[29] and reducing reactive oxygen species formation in phagocytes. However, *K. michiganensis* isolates with *bla*$_{oxy\text{-}5}$ exhibited a higher survival rate against macrophages than did *K. michiganensis* with *bla*$_{oxy\text{-}1}$. The reasons behind this need further investigation.

In conclusion, we described the prevalence of species within the *K. oxytoca* complex and compared the antimicrobial and clinical infection profiles between species. We further present the genomic diversity of *K. michiganensis* and identify a highly antimicrobial-resistant subgroup, *K. michiganensis* with *bla*$_{oxy\text{-}5}$. The novel contribution of the present study is that we identified the highly antimicrobial-resistant profile of *K. michiganensis* carrying *bla*$_{oxy\text{-}5}$ and further confirmed it using NCBI dataset. Although *K.*

*michiganensis* with $bla_{oxy-5}$ exhibits higher adhesion ability to human respiratory epithelial cells and survivability against macrophages *in vitro*, the underlying mechanism requires further investigation.

## Contributors

DL, XC, RM, LD, TW, JL, GZ, HS, XW, MH, XC, LZ, RW, XW, and WK collected the isolates and performed the experiments. XJ helped in data analysis. YL analyzed the data, prepared the plots, and drafted the manuscript. YW performed *in vitro* assays. YL, HS, and YX obtained funding and designed the study.

## Data sharing statement

All short-read data (n = 103) were uploaded to the NCBI under the project number PRJNA878595. The remaining sequences (n = 622) were downloaded from the NCBI with ftp links provided in Table S6.

## Declaration of competing interest

The authors declare no potential conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2023.10.014.