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Isolation of four carbapenem-resistant gram-negative species from a single fly

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Abstract

The widespread occurrence of carbapenem-resistant organisms has garnered significant public attention. Arthropods, including flies, are important vectors of multidrug-resistant bacteria. In this study, we reported the simultaneous carriage of four carbapenem-resistant isolates from different species, namely, *Escherichia coli* (*E. coli*), *Providencia manganoxydans* (*P. manganoxydan*), *Myroides odoratimimus* (*M. odoratimimus*) and *Proteus mirabilis* (*P. mirabilis*), from a single fly in China. These isolates were characterized through antimicrobial susceptibility testing, conjugation assays, whole-genome sequencing, and bioinformatics analysis. *M. odoratimimus* showed intrinsic resistance to carbapenems. The mechanisms of carbapenem resistance in *E. coli*, *P. manganoxydans*, and *P. mirabilis* were due to the production of NDM-5, NDM-1 and NDM-1, respectively. Genetic context of the bla_{NDM} genes in these three isolates varied. The bla_{NDM-5} gene in *E. coli* was located on an IncHI2/HI2A multidrug-resistant plasmid, which was conjugatively transferable. The bla_{NDM-1} gene in *P. mirabilis* resided on the pPM14-NDM_123k-like nonconjugative plasmid. The bla_{NDM-1} gene in *P. manganoxydans* was found in a nonconjugatively transferable, multidrug-resistant region. The results of this study enhance our understanding of the dissemination of carbapenem-resistant organisms and suggest the need for a more comprehensive approach to antibiotic resistance research encompassing humans, animals, and the environment.

Keywords Fly, Carbapenem-resistant organism, Whole-genome sequencing, Phenotypic characterization, bla_{NDM}

Main text

Carbapenem-resistant organisms (CROs) are a significant concern in the global landscape of infectious diseases and affect both humans and animals. Surveillance networks such as the CHINET and dedicated pet/animal monitoring initiatives highlight the importance of CROs, emphasizing their prevalence and impact (Yang et al. 2023; Sands et al. 2021). Apart from human carriage, the transmission of multidrug-resistant bacteria, including CROs, can also occur through arthropods, particularly flies that feed on human and animal wastes (Hassan et al. 2021). In this study, we collected a fly from a sheep farm in Hubei Province, China, which carried four CROs belonging to different species. This study advocates for a more comprehensive approach to antibiotic resistance research involving not only humans but also animals and the environment.

In April 2023, a fly was collected using flypaper and subsequently homogenized by grinding in sterile saline. The homogenate was transferred into 5 mL LB broth and incubated at 35°C for 18–20 h for amplification.

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A total of four isolates were isolated using China Blue Agar plates supplemented with 0.3 μg/mL meropenem (Zhang et al. 2022). Species identification was performed using matrix-assisted laser desorption and ionization–time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany). Among the four isolates, 36-1-1, 36-1-2, 36-2-1 and 36-2-2 were identified as *Escherichia coli* (*E. coli*), *Providencia manganoxydans* (*P. manganoxydans*), *Myroides odoratimimus* (*M. odoratimimus*) and *Proteus mirabilis* (*P. mirabilis*), respectively. The minimum inhibitory concentrations (MICs) for the four isolates were determined by broth microdilution with *E. coli* ATCC 25922 as the control, and the results were interpreted according to CLSI standards (Clinical & Laboratory Standards Institute 2020). All four isolates were resistant to imipenem, meropenem, cefmetazole, ceftazidime, cefotaxime, piperacillin-tazobactam, cefoperazone-sulbactam, cefepime and ceftazidime-avibactam. All isolates, except for *E. coli* 36-1-1, were resistant to polymyxin B (Table 1).

Genomes of the four isolates were sequenced using the Illumina HiSeq 2500 platform with a 2 × 150 bp paired-end strategy. *De novo* sequence assembly was performed using SPAdes V.3.15.5 (Bankevich et al. 2012). *M. odoratimimus* exhibited intrinsic resistance to carbapenem, partly due to the production of the chromosome-encoded β-lactamase MUS (Yang et al. 2020). In the *M. odoratimimus* isolate 36-2-1, only one antibiotic resistance gene (ARG), $bla_{\text{MUS-1}}$, was identified. Among the other three isolates, 10-14 had acquired ARGs conferring resistance to various classes of antibiotics. Carbapenem resistance in the *E. coli* 36-1-1, *P. manganoxydans* 36-1-2, and *P. mirabilis* 36-2-2 isolates was associated with the production of NDM-5, NDM-1 and NDM-1, respectively (Fig. 1A).

Genome typing with ClermonTyping, mlst, and ECTyper suggested that *E. coli* 36-1-1 belongs to phylogroup A, which represents a novel sequence type (MLST alleles: *adk* (10)-*fumC* (7)-*gyrB* (5)-*icd* (8)-*mdh* (8)-*purA* (18)-*recA* (2)) and serotype O128:H19 (Seemann 2016; Beghain et al. 2018; Bessonov et al. 2021). Genetic alignment suggested that the $bla_{\text{NDM-5}}$ gene in *E. coli* 36-1-1 was located on a pNDM33-1-like multidrug-resistant plasmid. pNDM33-1 (GenBank accession: MN915011) is an IncHI2/HI2A plasmid that is 266,777 bp in length and encodes 330 ORFs with a G+C content of 47.1% (Fig. 1B). In addition to $bla_{\text{NDM-5}}$, pNDM33-1 also carried other ARGs, which were detected in the genome of *E. coli* 36-1-1, including *aph(3')-I*, *aph(4)-I*, $bla_{\text{OXA-10}}$, *sul3*, *lnu*(F), *aac(3)-IVa*, *aadA2*, *dfrA14*, and *floR*.

In *P. mirabilis* 36-2-2, the $bla_{\text{NDM-1}}$ gene was located on the pPM14-NDM_123k-like plasmid. The pPM14-NDM_123k plasmid (GenBank accession: CP137087) is 123,188 bp in length and encodes 136 ORFs with a G+C content of 40.6% (Fig. 2). It also harbors ARGs, including *dfrA1*, *mph*(E), *msr*(E), *sul*, *aadA1*, and *lnu*(F).

The $bla_{\text{NDM-1}}$ gene in *P. manganoxydans* 36-1-2 was located on an 8.9 kb contig that also carried ARGs such as *sul1*, $bla_{\text{OXA-1}}$, *catB3*, *aac(6')Ib-cr* and *arr-3*. This contig was 100% identical to the corresponding chromosome fragment bordered by the mobile elements IS*26* and IS*CR1* from *P. mirabilis* XH1653 (GenBank accession: CP065039), with 98% coverage. The genetic context of $bla_{\text{NDM-1}}$ in *P. manganoxydans* 36-1-2, *qacE*Δ*1-sul1*-IS*CR1-hp-*$bla_{\text{NDM-1}}$-ΔIS*Aba125*, was similar to that in *P. mirabilis*, which was *qacE*Δ*1-sul1*-IS*CR1-hp-hp-*$bla_{\text{NDM-1}}$-IS*Aba125* (Fig. 3). This evidence collectively suggested the mobility of such a $bla_{\text{NDM-1}}$-carrying fragment.

To test the transferability of the carbapenem-resistant phenotype, we conducted a conjugation experiment with the filter-mating method using rifampicin-resistant *E. coli* EC600 as the recipient. Transconjugants

Table 1 Minimum inhibitory concentration (μg/mL) profiles of isolates in this study

Isolates[a]	Species	IPM	MEM	CMZ	CAZ	CTX	TZP	SCF	CAV	FEP	PB	TGC	CIP	AK	ATM
36-2-1	*Myroides odoratimimus*	64	8	128	64	64	32/4	> 256/128	64/4	16	> 8	0.5	≤ 1	> 128	> 128
36-2-2	*Proteus mirabilis*	> 128	128	64	> 128	> 128	32/4	256/128	> 64/4	> 64	> 8	4	≤ 1	> 128	> 128
36-1-1	*Escherichia coli*	8	16	> 128	> 128	> 128	> 256/4	> 256/128	> 64/4	64	1	1	≤ 1	≤ 4	≤ 4
36-1-2	*Providencia manganoxydans*	128	64	16	> 128	> 128	> 256/4	> 256/128	> 64/4	> 64	> 8	≤ 0.25	≤ 1	≤ 4	≤ 4
EC600	*Escherichia coli*	≤ 1	≤ 1	≤ 2	≤ 2	≤ 4	≤ 8/4	≤ 8/4	≤ 0.5/4	≤ 4	≤ 0.5	≤ 0.25	≤ 1	≤ 4	≤ 4
36-1-1-TC	*Escherichia coli*	8	16	8	> 128	> 128	128/4	256/128	> 64/4	32	≤ 0.5	≤ 0.25	2	≤ 4	≤ 4

Abbreviations: *IPM* Imipenem, *MEM* Meropenem, *CMZ* Cefmetazole, *CAZ* ceftazidime, *CTX* Cefotaxime, *TZP* Piperacillin/tazobactam, *SCF* Sulbactam/cefoperazone, *CAV* Ceftazidime/avibactam, *FEP* Cefepime, *PB* Polymyxin B, *TGC* Tigecycline, *CIP* Ciprofloxacin, *AK* Amikacin, *ATM* Aztreonam

[a] Isolates 36-2-1, 36-2-2, 36-1-1 and 36-1-2 were isolated from a single fly in this study; *E. coli* EC600 was the recipient used in the conjugation assay; *E. coli* 36-1-1-TC was the transconjugant that acquired the $bla_{\text{NDM-5}}$ gene from the donor isolate *E. coli* 36-1-1

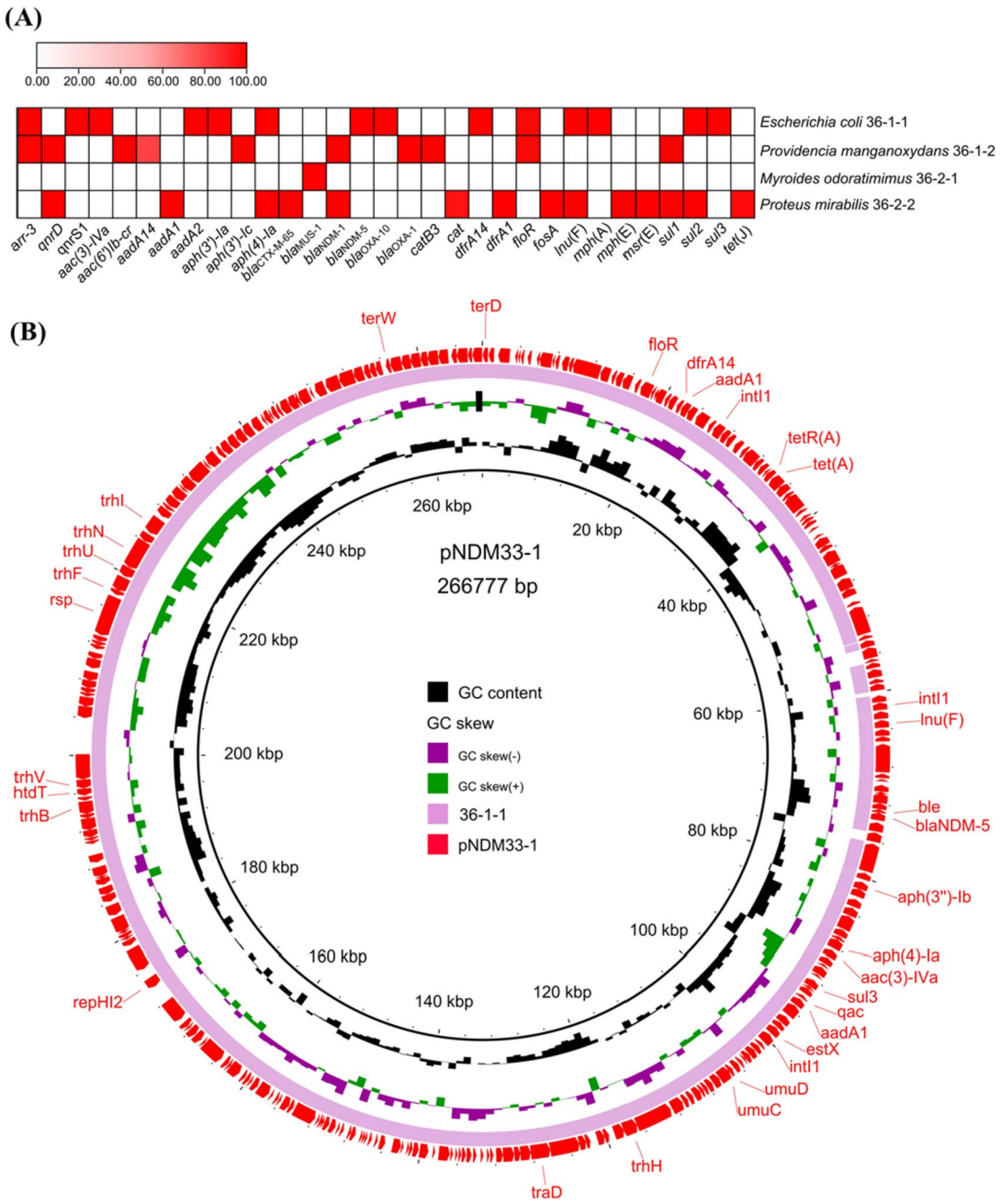

Fig. 1 **A** Heatmap of antimicrobial resistance genes carried by carbapenem-resistant isolates from a fly in this study. The horizontal axis represents the antimicrobial resistance genes, and the vertical axis represents the isolate IDs. The red boxes represent the presence of the corresponding items among the sequenced isolates, and the white boxes represent their absence. The gradient identity bar indicates the percentage similarity of related genes. **B** Circular alignment of the reference plasmid sequence pNDM33-1 (GenBank accession: MN915011) with homologous bla_{NDM-5}-carrying contigs from the *E. coli* isolate 36-1-1 in this study. Representative genes, such as antimicrobial resistance genes and conjugation-associated genes, are labeled in the outermost circle

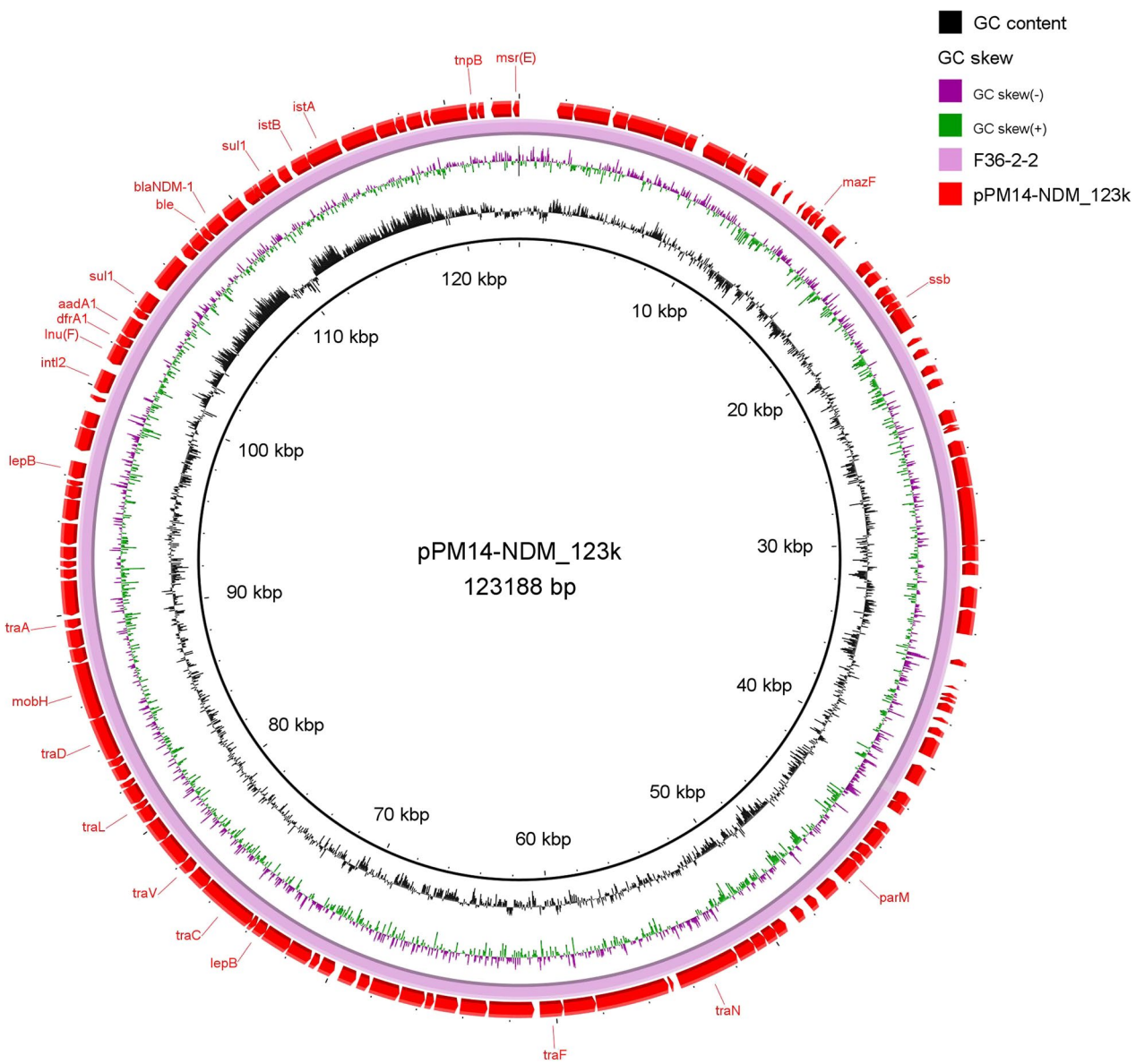

Fig. 2 Circular alignments of the reference plasmid sequence pPM14-NDM_123k (GenBank accession: CP137087) with homologous bla_{NDM-1}-carrying contigs from the *Proteus mirabilis* strain 36-2-2 in this study. Representative genes, such as antimicrobial resistance genes and conjugation-associated genes, are labeled in the outermost circle

were selected on agar plates containing 0.5 μg/mL meropenem. The carbapenem resistance of *E. coli* 36-1-1 was transferable, whereas that of the other three isolates was nonconjugative. The nonconjugative nature of the three isolates suggested that there are other mechanisms involved in the transmission of carbapenem resistance in these bacteria.

Conclusions

In summary, this study reported the simultaneous carriage of four carbapenem-resistant isolates from different species, namely, *E. coli*, *P. manganoxydans*, *M. odoratimimus* and *P. mirabilis,* from one fly in China. *M. odoratimimus* was intrinsically resistant to carbapenems, and the mechanism of carbapenem resistance in the other isolates involved the production of NDM carbapenemases. The bla_{NDM-5} gene in *E. coli* was plasmid-borne

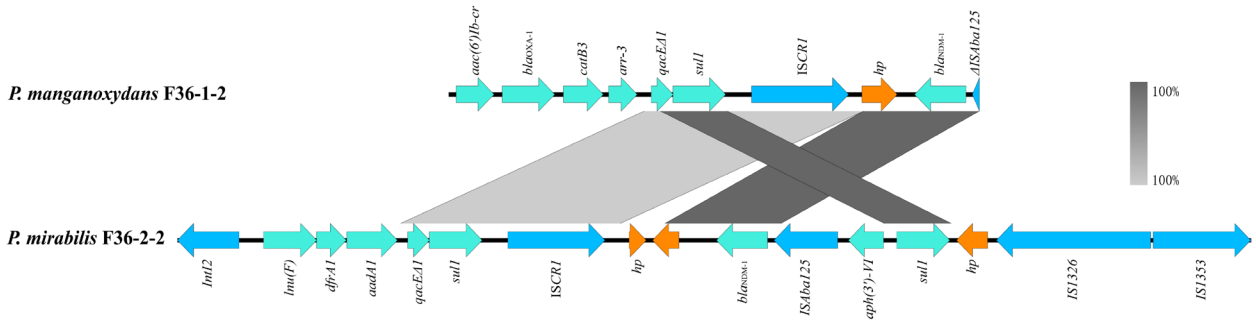

Fig. 3 Genetic context of blaNDM-1 in *P. mirabilis* F36-2-2 and *P. manganoxydans* F36-1-2. The cyan, blue and orange arrows represent resistance genes, mobile genetic elements and other proteins, respectively

and conjugatively transferable. The $bla_{\text{NDM-1}}$ genes in the *P. manganoxydans* and *P. mirabilis* isolates were both nontransferable. Our findings contributed valuable insights to understanding the dissemination of CRO and posed critical questions about the correlations among antibiotic resistance in livestock, flies, and humans.

Abbreviations

CRO	Carbapenem-resistant organism
CHINET	China Antimicrobial Surveillance Network
MALDI-TOF MS	Matrix-assisted laser desorption and ionization–time-of-flight mass spectrometry
CLSI	Clinical and Laboratory Standards Institute
ARG	Antibiotic resistance gene

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Not applicable.

Authors' contributions
HW performed strain isolation and phenotypic characterization and wrote the first draft. HZ and GC performed the genomic characterization. ND conceived the study, participated in its design and coordination, and edited the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The assembled genome sequences of isolates in this study have been deposited in the National Center for Biotechnology Information (NCBI) database under Bioproject PRJNA1039261.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
Bankevich, A., S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, V.M. Lesin, S.I. Nikolenko, S. Pham, and A.D. Prjibelski. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19 (5): 455–477. https://doi.org/10.1089/cmb.2012.0021.

Beghain, J., A. Bridier-Nahmias, H. Le Nagard, E. Denamur, and O. Clermont. 2018. ClermonTyping: An easy-to-use and accurate in silico method for *Escherichia genus* strain phylotyping. *Microbial Genomics* 4 (7): e000192. https://doi.org/10.1099/mgen.0.000192.

Bessonov, K., C. Laing, J. Robertson, I. Yong, K. Ziebell, V.P. Gannon, A. Nichani, G. Arya, J.H. Nash, and S. Christianson. 2021. ECTyper: In silico *Escherichia coli* serotype and species prediction from raw and assembled whole-genome sequence data. *Microbial Genomics* 7 (12): 000728. https://doi.org/10.1099/mgen.0.000728.

Clinical and Laboratory Standards Institute. *Standards for Antimicrobial Susceptibility Testing—Thirty-Third Edition: M100*. 2023.

Hassan, B., M. Ijaz, A. Khan, K. Sands, G.-I. Serfas, L. Clayfield, M.M. El-Bouseary, G. Lai, E. Portal, and A. Khan. 2021. A role for arthropods as vectors of multidrug-resistant enterobacterales in surgical site infections from South Asia. *Nature Microbiology* 6 (10): 1259–1270. https://doi.org/10.1038/s41564-021-00965-1.

Sands, K., M.J. Carvalho, E. Portal, K. Thomson, C. Dyer, C. Akpulu, R. Andrews, A. Ferreira, D. Gillespie, and T. Hender. 2021. Characterization of antimicrobial-resistant gram-negative bacteria that cause neonatal sepsis in seven low-and middle-income countries. *Nature Microbiology* 6 (4): 512–523. https://doi.org/10.1038/s41564-021-00870-7.

Seemann, T. 2016. MLST—Scan contig files against PubMLST typing schemes. https://github.com/tseemann/mlst. Accessed 1 Nov 2023

Yang, S., Q. Liu, Z. Shen, H. Wang, and L. He. 2020. Molecular epidemiology of *Myroides odoratimimus* in nosocomial catheter-related infection at a general hospital in China. *Infection and Drug Resistance* 13: 1981–1993. https://doi.org/10.2147/IDR.S251626.

Yang, W., L. Ding, R. Han, D. Yin, S. Wu, Y. Yang, D. Zhu, Y. Guo, and F. Hu. 2023. Current status and trends of antimicrobial resistance among clinical isolates in China: A retrospective study of CHINET from 2018 to 2022. *One Health Advances* 1 (1): 8. https://doi.org/10.1186/s44280-023-00009-9.

Zhang, Y., D. Gu, X. Yang, Y. Wu, C. Liu, Z. Shen, and R. Zhang. 2022. Emergence and genomic characterization of a KPC-2-, NDM-1-, and IMP-4-producing *Klebsiella michiganensis* isolate. *Frontiers in Microbiology* 12: 762509. https://doi.org/10.3389/fmicb.2021.762509.

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