



Plasmid-borne *mcr-4.3* in *Acinetobacter nosocomialis* recovered from imported beef cattle: Genomic characterization and one health implications

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ABSTRACT

Background: The emergence of mobile colistin resistance determinants in clinically relevant *Acinetobacter* species poses a growing challenge to antimicrobial stewardship and One Health surveillance. Although *mcr-4.3* has been reported in *Acinetobacter*, its occurrence in imported food animals and its broader phylogenomic context remain poorly defined.

Methods: We investigated fecal samples collected from 150 beef cattle imported from Australia to China. *Acinetobacter* isolates recovered by selective culture were subjected to whole-genome sequencing, and the *mcr-4.3*-positive isolate was further resolved by hybrid assembly using short- and long-read sequencing. Comparative plasmid analysis and global phylogenomic analysis were performed using publicly available *A. nosocomialis* genomes.

Results: Among 54 *Acinetobacter* isolates recovered from imported cattle, one *A. nosocomialis* isolate, NB148, carried *mcr-4.3*. Complete genome sequencing showed that *mcr-4.3* was located on a 28,233-bp plasmid, designated pNB148-*mcr-4.3*, which harbored plasmid maintenance-associated toxin-antitoxin systems and shared substantial backbone similarity with previously reported *mcr-4.3*-bearing plasmids from *Acinetobacter* species. The *mcr-4.3* region was positioned downstream of a putative recombinase and a Tn3-family transposase, suggesting a conserved genetic framework for this resistance determinant. Global phylogenomic analysis incorporating 574 publicly available *A. nosocomialis* genomes placed NB148 within a diverse international population characterized by mixing across animal, environmental, and human sources. Among the global collection, 11 genomes carried *mcr-4.3*, of which 10 belonged to ST279, indicating that this lineage may represent an important genomic background for the persistence and international distribution of *mcr-4.3*-positive *A. nosocomialis*.

Conclusions: This study documents plasmid-borne *mcr-4.3* in *A. nosocomialis* recovered from imported beef cattle and places this finding within a broader international One Health context. Our data expand the known ecological range of *mcr-4.3*-positive *A. nosocomialis* and support strengthened genomic surveillance at animal–human–environment interfaces, particularly in importation and border inspection settings.

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1. Introduction

Acinetobacter nosocomialis has emerged as a significant opportunistic pathogen, especially in healthcare settings and among immunocompromised patients, due to its multidrug resistance [1]. Treatment options are limited, with colistin often serving as a ‘last-resort’ antibiotic. However, the global spread of plasmid-mediated colistin resistance genes (*mcr*) has severely complicated resistance management [2,3]. Initially prevalent in Enterobacteriaceae, *mcr* genes are now also a rising concern in the *Acinetobacter* genus, which is healthcare-associated infections [1,4].

The *mcr-4* gene was first reported on a plasmid in *Salmonella enterica* from swine [5]. The *mcr-4.3* variant is distinguished from *mcr-4* by two specific amino acid substitutions (V179G and V236F) [6]. It was initially detected in 2014 in *Enterobacter cloacae* from human clinical specimens [7]. Notably, the *mcr-4.3* gene was documented on a plasmid in a clinical isolate of *A. nosocomialis* from South Africa in 2017, marking its first report in Africa and in this bacterial species [8]. However, functional studies present a complex picture: while the presence of the *mcr-4.3*-carrying plasmid was linked to colistin resistance in the original *A. nosocomialis* isolate [8], recombinant expression of *mcr-4.3* in *Escherichia coli* did not confer resistance in another study [7], suggesting its functionality may be context-dependent.

The *mcr-4.3* gene has been identified in clinically significant pathogens including *A. nosocomialis*, *Acinetobacter baumannii*, *Enterobacter kobei* and *Leclercia adecarboxylata* [4,8–11]. Its detection in livestock-associated bacteria in China suggests that animals may serve as potential reservoirs, facilitating its environmental dissemination [12,13]. The risk of cross-species transmission is underscored by the discovery of highly similar, large multidrug-resistant plasmids in different *Acinetobacter* species, indicating the potential for such plasmids to transfer across genus boundaries [1]. Critically, the dissemination of such resistance determinants is not confined to hospitals. The “One Health” paradigm recognizes that antimicrobial resistance (AMR) evolves and spreads at the human-animal-environment interface, driven by interconnected factors such as antimicrobial use in veterinary medicine and agriculture. Evidence of *mcr* genes in livestock, food products, and environmental samples confirms their circulation across these reservoirs, creating a pervasive risk of transmission back to humans [14]. Plasmids, especially those harboring complex transposons and stability systems like toxin-antitoxin modules, are key vectors in this cross-boundary gene flow, enabling resistance genes like *mcr-4.3* to transfer between different bacterial species and genera [15]. Despite this understanding, specific data on the role of international live animal trade as a direct pathway for introducing *mcr-4.3*-harboring *A. nosocomialis* into new regions remains scarce.

Despite increasing recognition of the human–animal–environment interface in the dissemination of antimicrobial resistance, the occurrence and genomic features of *mcr-4.3*-positive *A. nosocomialis* in imported food animals remain poorly understood. In particular, it is unclear whether imported livestock may serve as a previously unrecognized reservoir for clinically relevant *Acinetobacter* lineages carrying mobile colistin resistance determinants. In this study, we screened *Acinetobacter* isolates recovered from beef cattle imported from Australia to China and identified an *A. nosocomialis* isolate carrying plasmid-borne *mcr-4.3*. We then combined complete genome sequencing, comparative plasmid analysis, and global phylogenomics to characterize its genetic context and to assess its placement within the international population structure of *A. nosocomialis*. Our findings provide genomic evidence that imported food animals should be considered in One Health surveillance frameworks aimed at tracking the transboundary movement of rare but clinically relevant resistance determinants.

2. Materials and methods

2.1. Bacterial isolation and identification

In September 2019, fecal samples were collected from each of the 150 Australian beef cattle in a single batch upon arrival at Ningbo Port, China. Each sample was enriched in Brain Heart Infusion broth (OXOID, United Kingdom) and incubated with shaking at 200 rpm, 37 °C for 20 h. Cultures were then plated on MacConkey agar (OXOID, United Kingdom) with 2 µg/ml cefotaxime to identify cephalosporin-resistant Gram-negative bacteria [16]. The addition of 2 µg/ml cefotaxime was intended to selectively inhibit cephalosporin-susceptible commensal bacteria, thereby enriching for cephalosporin-resistant Gram-negative bacteria, including *Acinetobacter* species, as previously described. We screened multiple colonies per stool sample based on distinct colony morphologies. Selected colonies were purified and identified using MALDI-TOF MS (Vitek MS system, bioMérieux, France) [17]. All 54 *Acinetobacter* sp. isolates underwent whole-genome sequencing for further analysis.

2.2. Antimicrobial susceptibility testing (AST)

The minimum inhibitory concentrations (MICs) of the following antimicrobial agents were determined for the isolate: piperacillin/tazobactam, ceftazidime, cefotaxime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, and polymyxin B. All antibiotics were tested using the standard agar dilution method, except for polymyxin B, for which the broth microdilution method was employed in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines. For each batch of tests, *Escherichia coli* ATCC 25922 was used as the quality control strain, and its MIC results consistently fell within the acceptable ranges defined by CLSI. The MICs were interpreted as susceptible (S), intermediate (I), or resistant (R) according to the CLSI breakpoints.

2.3. Genome sequencing and bioinformatic analysis

Genomic DNA was extracted from 54 *Acinetobacter* sp. isolates using the BeadPure Bacterial DNA Kit B (Zenpio, China). All isolates were subjected to short-read sequencing on an Illumina NovaSeq 6000 platform (San Diego, CA, USA). The isolate carrying the *mcr-4.3* gene (NB148) was selected for complete genome reconstruction via additional long-read sequencing using an Oxford Nanopore MinION system (Oxford Nanopore Technologies, Oxford, United Kingdom) [18]. Hybrid assembly of short and long reads was performed with Unicycler v0.5.1 to generate a circularized genome [19]. Species identification for all isolates was confirmed by Average Nucleotide Identity (ANI) analysis using PyANI v1.33 [20], which classified 16 isolates as *Acinetobacter nosocomialis*. Antimicrobial resistance genes and plasmid replicons were identified using ABRicate v1.4.0 against the ResFinder [21] and PlasmidFinder databases [22], respectively. The genetic context of the *mcr-4.3* gene was analyzed by BLASTn. Circular comparisons of plasmids (Fig. 1A) were visualized using BLAST Ring Image Generator (BRIG) v0.95 [23] and linear comparisons of genetic structures (Fig. 1B) were visualized using Easyfig v2.2.2 [24].

2.4. Comparative genomic and phylogenetic analysis

To place the *mcr-4.3*-positive isolate in a global context, a total of 574 *A. nosocomialis* genome sequences were retrieved from the NCBI Assembly database (accessed on December 18, 2025) using the search term “*Acinetobacter nosocomialis*”. Core-genome single nucleotide polymorphism (SNP) phylogenetic analysis was performed using Snippy

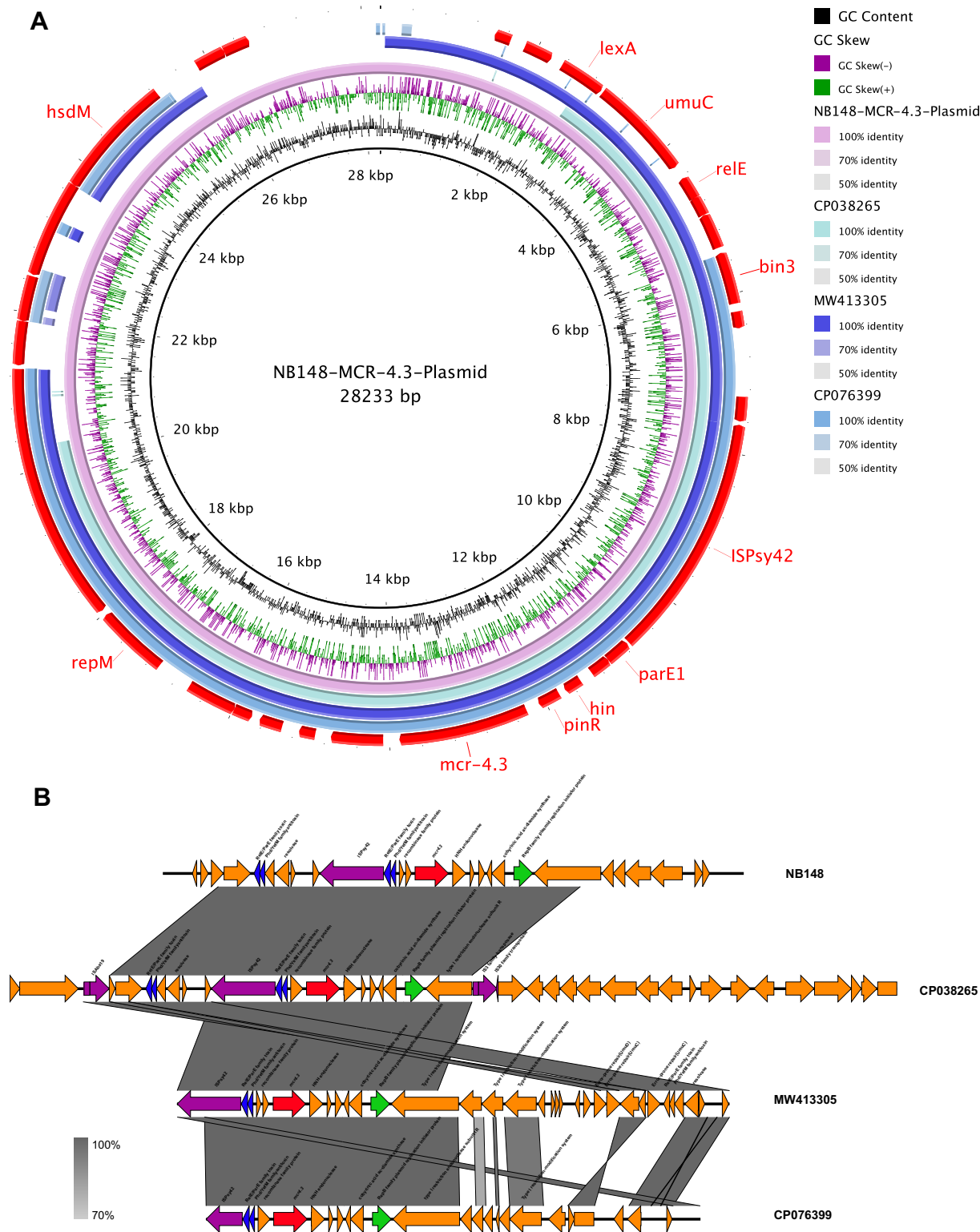


Fig. 1. Genomic features of the *mcr-4.3*-bearing plasmid in *Acinetobacter nosocomialis* NB148. (A) Circular comparison of plasmid pNB148 *mcr-4.3* identified in this study with representative *mcr-4.3*-bearing plasmids from *Acinetobacter* species based on BLASTn analysis. The outer circle represents pNB148 *mcr-4.3*, and the inner circles represent reference plasmids with GenBank accession numbers CP038265, MW413305, and CP076399. Shaded regions indicate homologous nucleotide similarity between plasmids. (B) Linear comparison of the major structural features and *mcr-4.3* genetic contexts of pNB148 *mcr-4.3* and representative related plasmids. Open reading frames are shown as arrows indicating gene orientation, with selected genes labeled according to annotated function.

v4.6.0 (<https://github.com/tseemann/snippy>) with the *Acinetobacter nosocomialis* strain XH1679 as the reference. Recombination regions were filtered out using Gubbins v3.4.3 [25], and a maximum-likelihood phylogenetic tree was constructed with Fasttree v2.1.11 [26] and visualized in iTOL (<https://itol.embl.de>). Sequence types (STs) were determined via PubMLST according to the multilocus sequence typing (MLST) scheme (<https://pubmlst.org/organisms>). A mutation in the *fusA* gene was identified by sequence alignment. Clonal relatedness was defined as isolates differing by ≤ 200 core-genome SNPs, a threshold used in similar studies of bacterial transmission,

3. Results and discussion

3.1. Recovery of *Acinetobacter* isolates from imported beef cattle and identification of an *mcr-4.3*-positive *A. nosocomialis* isolate

In September 2019, 150 fecal samples from Australian beef cattle imported via Ningbo Port were analyzed for bacteria. After enrichment and plating, 54 potential *Acinetobacter* sp. colonies were identified. MALDI-TOF MS, whole-genome sequencing, and ANI analysis confirmed 11 *A. nosocomialis* isolates and 5 subspecies highly similar to *A. nosocomialis* (Fig. S1). Whole genomic sequences showed one isolate, NB148, had the plasmid-borne colistin resistance gene *mcr-4.3*. Antimicrobial susceptibility testing of isolate NB148 (Table 2) showed susceptibility to a range of antibiotics including ceftazidime, cefotaxime, carbapenems (imipenem and meropenem), aminoglycosides (amikacin and gentamicin), fluoroquinolones (ciprofloxacin and levofloxacin), and trimethoprim/sulfamethoxazole. In contrast, it was resistant to piperacillin/tazobactam and, notably, exhibited resistance to polymyxin B with a minimum inhibitory concentration (MIC) of 8 mg/l, which aligns with the presence of the acquired *mcr-4.3* gene.

3.2. Complete genomic characterization of NB148 and structural features of plasmid pNB148_ *mcr-4.3*

Based on the genomic data from the *mcr-4.3*-positive *A. nosocomialis* strain NB148, hybrid assembly of Illumina and Nanopore sequencing reads produced a complete genome consisting of a single circular chromosome (3,898,347 bp) and three plasmids. The *mcr-4.3* gene was identified on a 28,233 bp plasmid, designated pNB148_ *mcr-4.3*. This plasmid could not be assigned to any known replicon type using the PlasmidFinder database. BLASTn analysis revealed that pNB148_ *mcr-4.3* shared greater than 62% coverage and 98.06% nucleotide identity with previously reported *mcr-4.3*-harboring plasmids from *Acinetobacter* species, such as pAb-MCR4.3 from a clinical *A. baumannii* isolate (Fig. 1A) [4]. The plasmid carries a RepM protein of the RepB family (Rep-3 superfamily), which is consistent with the type of *mcr-4.3* plasmid pCAC13a found in the clinical *A. nosocomialis* isolate from South Africa [8]. It contains two type II toxin-antitoxin (TA) systems (PhD/YefM and RelE/ParE), which are associated with plasmid stability and persistence [2], this might clarify why the elimination of these plasmids can reinstate the strain's sensitivity to colistin [8]. Genetic context analysis showed the *mcr-4.3* gene is located downstream of a putative recombinase and a Tn3 family transposase (ISPsy42), while no mobile genetic elements were identified immediately downstream of the gene, forming a structure consistent with other characterized *mcr-4.3* genetic environments (Fig. 1B) [4,8]. The conserved genetic context of *mcr-4.3* across different *Acinetobacter*-associated plasmids suggests that this determinant may persist across multiple ecological settings, underscoring the need for continued public health surveillance [8,27].

3.3. Global phylogenomic placement of NB148 within the international *A. nosocomialis* population

To assess the phylogenetic context of the imported *mcr-4.3*-positive strain, we analyzed 574 *A. nosocomialis* genome assemblies from NCBI and constructed a core-genome SNP-based maximum-likelihood tree

with 16 strains from our study (Table 1, Fig. 2). The resulting phylogeny revealed extensive mixing of isolates from animals, the environment, and humans across countries, with no clear geographic clustering. This pattern suggests that the global spread of *A. nosocomialis* lineages is driven less by the expansion of specific geographic clones and more by the frequent horizontal transfer of mobile genetic elements (MGEs) across ecological boundaries, which aligns with the known role of broad-host-range plasmids in facilitating the cross-sectoral spread of resistance genes within the *Acinetobacter* genus [1]. Strain NB148, isolated from imported cattle in China, grouped closely with a human-derived strain from Malaysia (2015) and was phylogenetically distinct from most Chinese isolates. Its placement within a mixed clade containing isolates from multiple countries and source types is consistent with circulation across interconnected ecological settings indicating that related lineages or associated mobile genetic elements may circulate internationally. Although the precise transmission routes cannot be resolved from our data, this finding echoes previous reports where identical or highly similar plasmids carrying critical resistance genes (*mcr-4.3*) have been identified in both animal and human *Acinetobacter* isolates from disparate regions, underscoring a connected One Health transmission cycle [4,8]. The observed phylogenetic mixing across sectors underscores that *A. nosocomialis* transmission is not confined to specific hosts or environments, but rather facilitated by mobile genetic elements like plasmids, which can rapidly disseminate resistance genes globally. This highlights an urgent need for coordinated surveillance across human, animal, and environmental sectors to track and mitigate the spread of high-risk resistance determinants. Therefore, the phylogenetic position of NB148 supports the view that imported livestock may intersect with the international circulation of resistance-associated *Acinetobacter* lineages, underscoring the value of enhanced genomic surveillance at ports of entry within integrated One Health strategies.

3.4. Global distribution of *A. nosocomialis* across source, geographic regions, and sequence types

A Sankey diagram analysis of 590 *A. nosocomialis* isolates (16 from this study, 574 from NCBI) highlighted intricate epidemiological links among geographic origin, sequence type (ST), and isolation source. The strains came from 23 countries, mainly the USA (184), China (179), and Japan (48). MLST analysis revealed diverse STs, including ST410, ST433, ST71, ST768, ST279, ST68, ST217, ST1264, ST395, and ST501, with 255 strains in other STs. Notably, ST279, prevalent among *mcr-4.3*-positive strains, was found in animal, environmental, and human sources, indicating a broad host range. Isolation sources were human (306), environmental (204), animal (37), and unknown (43). The diagram showed extensive cross-linking among all sources, STs, and regions, with no source-specific lineage segregation (Fig. 3). This interconnected distribution underscores the relevance of *A. nosocomialis* to the human-animal-environment interface and supports its inclusion in One Health surveillance frameworks. The clinical relevance of human-derived strains is well-documented, as *A. nosocomialis* is a recognized cause of nosocomial bloodstream infections [28,29]. Recent studies corroborate these epidemiological links, showing that *A. nosocomialis* exhibits diverse sequence types (STs) across different reservoirs [30] and is a significant cause of nosocomial bloodstream infections [31]. Furthermore, environmental surveillance has detected *Acinetobacter baumannii* complex in healthcare facility wastewater [32], while genomic analysis of *A. pittii* demonstrates the transfer of carbapenem resistance via plasmids [33], underscoring the role of mobile genetic elements in cross-sectoral spread.

3.5. International occurrence and close relatedness of *mcr-4.3*-positive *A. nosocomialis* isolates

Including the NB148 strain characterized in this study, a total of twelve *mcr-4.3*-positive *A. nosocomialis* genomes were identified among

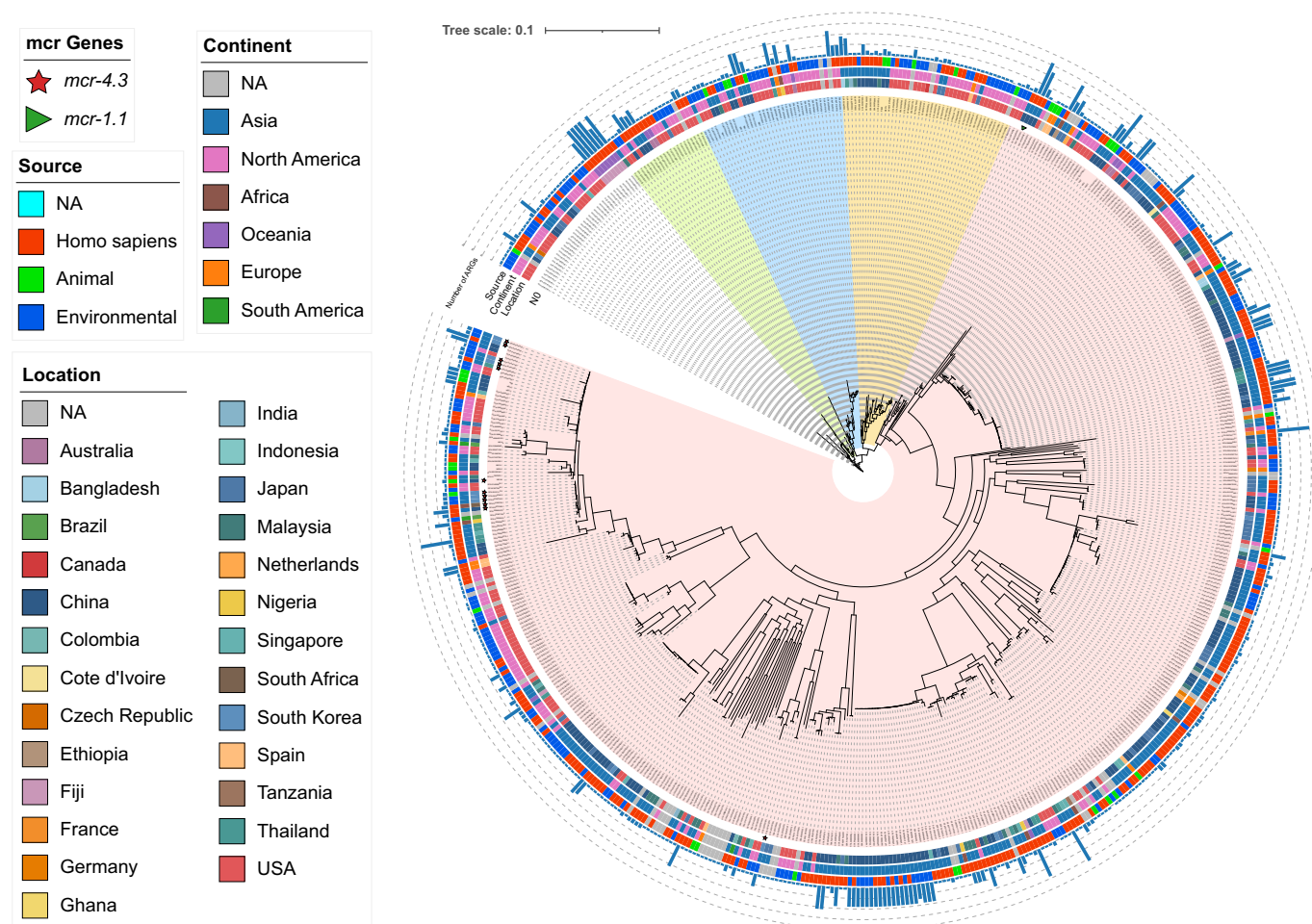


Fig. 2. Global phylogenomic context of *Acinetobacter nosocomialis*, including NB148 recovered from imported beef cattle.

Maximum-likelihood phylogenetic tree inferred from core-genome SNPs of *A. nosocomialis* genomes included in this study together with publicly available genomes from the NCBI database. Metadata displayed alongside the tree include geographic origin, continent, isolation source, and number of antimicrobial resistance genes (ARGs). The presence or absence of *mcr* genes is indicated in the annotation panel.

the 574 global genomes analyzed. These twelve strains originated from four geographic locations (South Korea, Thailand, South Africa and China) and three source types (environmental, animal, and human). The *mcr-4.3*-positive *A. nosocomialis* strain NB148, recovered from imported beef cattle in this study, was identified as sequence type ST279, consistent with the predominant lineage observed among global *mcr-4.3*-positive isolates. Eight of these strains originated from South Korea (seven environmental, one animal) (Fig. 4), while the remaining three were human-associated cases from Thailand, South Africa, and Taiwan area. This indicates that these *mcr-4.3*-positive *A. nosocomialis* have been detected across multiple continents. Notably, the presence of *mcr-4.3* in a clinical *A. nosocomialis* isolate from South Africa has been independently confirmed, with the gene located on a RepB plasmid (pCAC13a) [8]. This finding documents the first detection of plasmid-mediated *mcr-4.3* in Africa and underscores its establishment in clinical settings. Core-genome SNP analysis revealed that the three human-associated strains from Thailand, South Africa, and Taiwan area differed by ≤ 200 SNPs from the major Korean cluster (Table 1). This degree of genetic relatedness supports the presence of a closely related international cluster, although the timing and direction of spread cannot be resolved from the available data. Although NB148 belonged to the same sequence type (ST279) as the majority of *mcr-4.3*-positive isolates, core-genome SNP analysis revealed that NB148 was genetically more distant, differing by approximately 2000 SNPs from the major Korean environmental cluster and the human-associated strains (Table 1). This degree of divergence suggests that, while ST279 may represent a common

genomic background for *mcr-4.3* carriage across different reservoirs, the global dissemination of this determinant is not driven by a single highly clonal lineage but rather by the horizontal transfer of *mcr-4.3*-bearing plasmids across genetically diverse ST279 strains. The identification of *mcr-4.3* on a plasmid in the South African isolate [8] provides a plausible genetic mechanism for the observed clonal spread, suggesting that South Korea is currently overrepresented among available genomes in this cluster, although this pattern may also reflect sampling bias. Multilocus sequence typing (MLST) revealed that, among the eleven *mcr-4.3*-positive strains from Korea, Thailand, South Africa, and Taiwan, ten belonged to ST279 and one (from Korea) to ST322. Including the NB148 strain (also ST279) characterized in this study, a total of 11 out of 12 *mcr-4.3*-positive *A. nosocomialis* genomes belonged to ST279. The predominance of ST279 among currently available *mcr-4.3*-positive genomes suggests that this lineage may represent an important genomic background for the persistence or wider distribution of this determinant, although additional genomic and functional data are needed to define its clinical impact [8]. Further analysis identified a unique mutation in the *fusA* gene within the strain from Taiwan area. This mutation may represent a lineage-associated genomic feature, although its functional relevance to antibiotic resistance remains to be determined. Compared with prior reports focused mainly on *mcr-4.3* carriage, this observation suggests that additional lineage-associated genomic variation may accompany the broader distribution of ST279-related isolates [8].

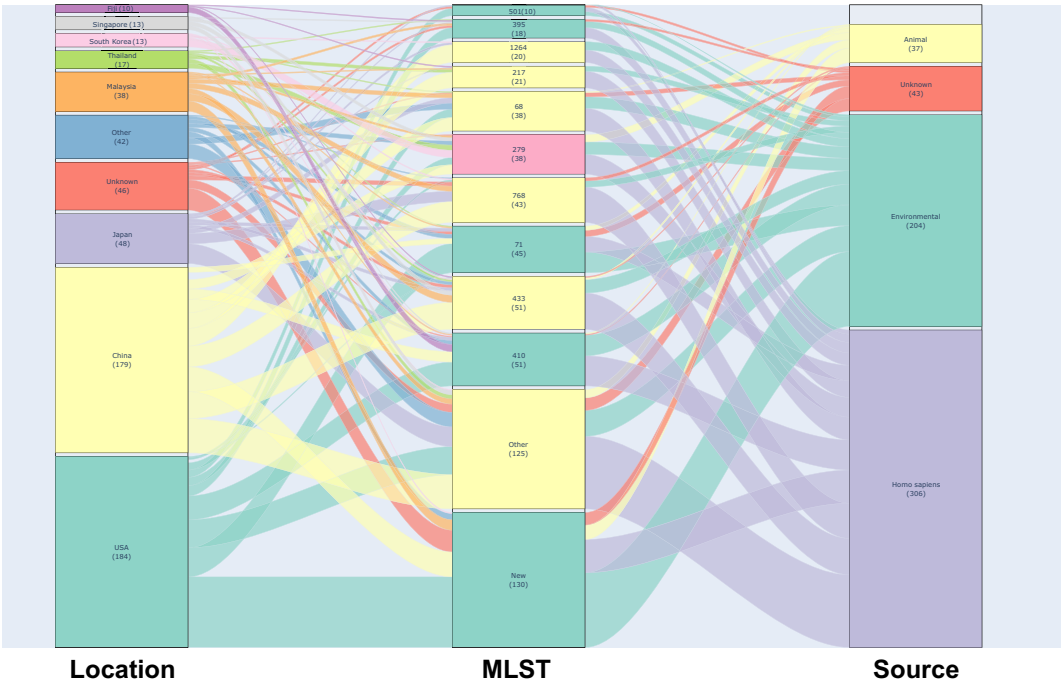


Fig. 3. Global distribution of *Acinetobacter nosocomialis* across geographic regions, sequence types, and isolation sources. Sankey diagram summarizing the relationships among geographic origin, multilocus sequence type (MLST), and isolation source for 590 *A. nosocomialis* genomes analyzed in this study, including strains generated here and genomes retrieved from public databases. Flows illustrate the distribution of major sequence types across different regions and ecological source categories.

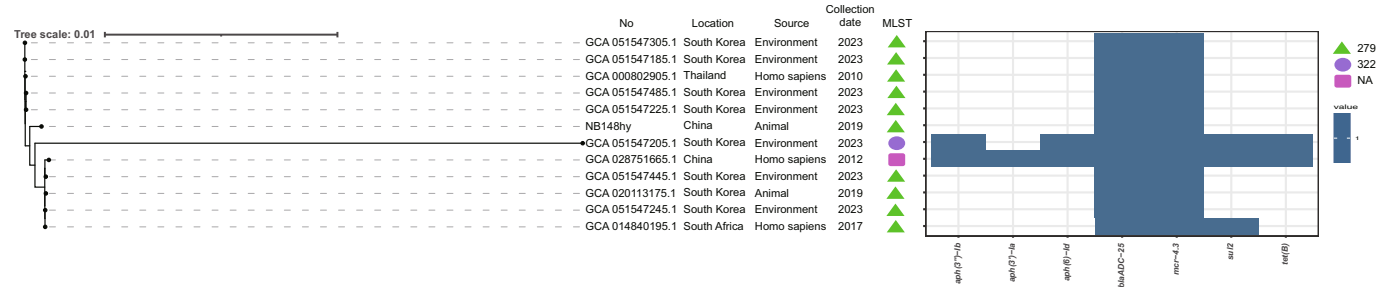


Fig. 4. Phylogenomic relatedness of *mcr-4.3*-positive *Acinetobacter nosocomialis* isolates. Phylogenetic tree of *mcr-4.3*-positive *A. nosocomialis* isolates identified from the NCBI database together with the isolate characterized in this study. Metadata shown alongside the tree include geographic origin, isolation source, collection date, and MLST type. Associated resistance gene profiles are displayed in the annotation panel.

4. Conclusion

In conclusion, this study documents plasmid-borne *mcr-4.3* in an *A. nosocomialis* isolate recovered from imported beef cattle in China and places this finding within a broader international genomic context. Complete genome sequencing showed that *mcr-4.3* was located on a 28,233-bp plasmid carrying maintenance-associated toxin-antitoxin systems and a conserved transposition-linked genetic environment, supporting its persistence in *Acinetobacter* plasmid backgrounds. Comparative phylogenomics further indicated that *mcr-4.3*-positive *A. nosocomialis* remains uncommon but is distributed across multiple regions and source types, with most currently available genomes belonging to ST279. Although our data do not establish direct trade-mediated transmission, they expand the known ecological range of this resistance determinant and support the inclusion of imported food animals in border-associated One Health surveillance aimed at detecting the transboundary movement of clinically relevant antimicrobial resistance genes.

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CRediT authorship contribution statement

Hao Xu: Writing – original draft, Formal analysis. Yaling Li: Writing – original draft, Formal analysis. Shaohua Hu: Visualization, Data curation. Ruishan Liu: Visualization, Data curation. Kun Wang: Visualization, Data curation. Beiwen Zheng: Writing – review & editing, Resources, Conceptualization. Yonghong Xiao: Writing – review & editing, Resources, Conceptualization.

Ethical approval

Not applicable.

Table 1
A table of SNP differences, based on core genome alignment, between the 11 *Acinetobacter nosocomialis* strains carrying mcr-4.3 downloaded from the NCBI database and strain NB148 from this study.

snp-dists	GCA_000802905.1	GCA_014840195.1	GCA_020113175.1	GCA_028751665.1	GCA_051547185.1	GCA_051547205.1	GCA_051547225.1	GCA_051547245.1	GCA_051547305.1	GCA_051547445.1	GCA_051547485.1	NB148hy
GCA_000802905.1	0	2018	2089	2180	69	55,720	138	2020	79	2094	137	1652
GCA_014840195.1	2018	0	145	431	2081	56,307	2125	40	2091	150	2124	2757
GCA_020113175.1	2089	145	0	506	2152	56,235	2196	147	2162	197	2195	2828
GCA_028751665.1	2180	431	506	0	2238	52,574	2286	436	2249	441	2285	2761
GCA_051547185.1	69	2081	2152	2238	0	55,939	132	2083	12	2156	131	1714
GCA_051547205.1	55,720	56,307	56,235	52,574	55,939	0	56,007	56,286	55,976	56,136	55,927	56,444
GCA_051547225.1	138	2125	2196	2286	132	56,007	0	2127	142	2200	1	1758
GCA_051547245.1	2020	40	147	436	2083	56,286	2127	0	2093	151	2126	2758
GCA_051547305.1	79	2091	2162	2249	12	55,976	142	2093	0	2166	141	1724
GCA_051547445.1	2094	150	197	441	2156	56,136	2200	151	2166	0	2199	2779
GCA_051547485.1	137	2124	2195	2285	131	55,927	1	2126	141	2199	0	1757
NB148hy	1652	2757	2828	2761	1714	56,444	1758	2758	1724	2779	1757	0

Table 2
Antimicrobial drug susceptibility profiles of L3859.

Antibiotic	MIC (mg/l) / antimicrobial susceptibility
Piperacillin/Tazobactam	>128/4//R
Ceftazidime	4/S
Cefotaxime	8/S
Imipenem	0.5/S
Meropenem	0.25/S
Amikacin	4/S
Gentamicin	2/S
Ciprofloxacin	0.25/S
Levofloxacin	0.125/S
Trimethoprim/Sulfamethoxazole	0.125/2.375/S
Polymixin B	8/R

S, susceptible; R, resistant.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Sequences mentioned in the present study were submitted to the GenBank nucleotide database with BioProject Accession Number: PRJNA1434333.

References

[1] A.G. Alattraqchi, F. Mohd Rani, A.R. Ni, S. Ismail, D.W. Cleary, S.C. Clarke, C. C. Yeo, Complete Genome Sequencing of *Acinetobacter baumannii* AC1633 and *Acinetobacter nosocomialis* AC1530 Unveils a Large Multidrug-Resistant Plasmid Encoding the NDM-1 and OXA-58 Carbapenemases, *mSphere* 6 (2021), <https://doi.org/10.1128/mSphere.01076-20>.

[2] S. Lee, J.U. An, W.H. Kim, S. Yi, J. Lee, S. Cho, Different threats posed by two major mobilized colistin resistance genes - mcr-1.1 and mcr-3.1 - revealed through comparative genomic analysis, *J. Glob. Antimicrob. Resist.* 32 (2023) 50–57, <https://doi.org/10.1016/j.jgar.2022.12.007>.

[3] Y. Li, X. Dai, J. Zeng, Y. Gao, Z. Zhang, L. Zhang, Characterization of the global distribution and diversified plasmid reservoirs of the colistin resistance gene mcr-9, *Sci. Rep.* 10 (2020) 8113, <https://doi.org/10.1038/s41598-020-65106-w>.

[4] N. Martins-Sorenson, E. Snesrud, D.E. Xavier, L.C. Cacci, A.T. Iavarone, P. McGann, L.W. Riley, B.M. Moreira, A novel plasmid-encoded mcr-4.3 gene in a colistin-resistant *Acinetobacter baumannii* clinical strain, *J. Antimicrob. Chemother.* 75 (2020) 60–64, <https://doi.org/10.1093/jac/dkz413>.

[5] A. Carattoli, L. Villa, C. Feudi, L. Curcio, S. Orsini, A. Luppi, G. Pezzotti, C. F. Magistrali, Novel plasmid-mediated colistin resistance mcr-4 gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016, *Euro Surveill.* 22 (2017), <https://doi.org/10.2807/1560-7917.ES.2017.22.31.30589>.

[6] S.R. Partridge, V. Di Pilato, Y. Doi, M. Feldgarden, D.H. Haft, W. Klimke, S. Kumar-Singh, J.H. Liu, S. Malhotra-Kumar, A. Prasad, G.M. Rossolini, S. Schwarz, J. Shen, T. Walsh, Y. Wang, B.B. Xavier, Proposal for assignment of allele numbers for mobile colistin resistance (mcr) genes, *J. Antimicrob. Chemother.* 73 (2018) 2625–2630, <https://doi.org/10.1093/jac/dky262>.

[7] J.W.P. Teo, M. Kalisvar, I. Venkatachalam, O.T. Ng, R.T.P. Lin, S. Octavia, Mcr-3 and mcr-4 variants in Carbapenemase-producing clinical Enterobacteriaceae do not confer phenotypic Polymyxin resistance, *J. Clin. Microbiol.* 56 (2018), <https://doi.org/10.1128/JCM.01562-17>.

[8] Y. Snyman, S. Reuter, A.C. Whitelaw, L. Stein, M.R.B. Maloba, M. Newton-Foot, Characterisation of mcr-4.3 in a colistin-resistant *Acinetobacter nosocomialis* clinical isolate from Cape Town, South Africa, *J. Glob. Antimicrob. Resist.* 25 (2021) 102–106, <https://doi.org/10.1016/j.jgar.2021.03.002>.

[9] I. Bitar, M. Medvecky, T. Gelbicova, V. Jakubu, J. Hrabak, H. Zemlickova, R. Karpiskova, M. Dolejska, Complete nucleotide sequences of mcr-4.3-carrying plasmids in *Acinetobacter baumannii* sequence type 345 of human and food origin

- from the Czech Republic, the first case in Europe, *Antimicrob. Agents Chemother.* 63 (2019), <https://doi.org/10.1128/AAC.01166-19>.
- [10] V.M. Marchetti, I. Bitar, M. Sarti, E. Fogato, E. Scaltriti, C. Bracchi, J. Hrabak, S. Pongolini, R. Migliavacca, Genomic characterization of VIM and MCR co-producers: the first two clinical cases, in Italy, *Diagnostics (Basel)* 11 (2021), <https://doi.org/10.3390/diagnostics11010079>.
- [11] Q. Sun, H. Wang, L. Shu, N. Dong, F. Yang, H. Zhou, S. Chen, R. Zhang, Leclercia adecarboxylata from human gut Flora carries mcr-4.3 and Bla (IMP-4)-bearing plasmids, *Front. Microbiol.* 10 (2019) 2805, <https://doi.org/10.3389/fmicb.2019.02805>.
- [12] C.Y. Mei, Y. Jiang, Q.C. Ma, M.J. Lu, H. Wu, Z.Y. Wang, X. Jiao, J. Wang, Chromosomally and plasmid-located mcr in Salmonella from animals and food products in China, *Microbiol. Spectrum* 10 (2022) e0277322, <https://doi.org/10.1128/spectrum.02773-22>.
- [13] F. Ma, C. Shen, X. Zheng, Y. Liu, H. Chen, L. Zhong, Y. Liang, K. Liao, Y. Xia, G. B. Tian, Y. Yang, Identification of a novel plasmid carrying mcr-4.3 in an Acinetobacter baumannii strain in China, *Antimicrob. Agents Chemother.* 63 (2019), <https://doi.org/10.1128/AAC.00133-19>.
- [14] J. Li, J. Chang, J. Ma, W. Zhou, Y. Yang, J. Wu, C. Guan, X. Yuan, L. Xu, B. Yu, F. Su, S. Ye, Y. Chen, G. Zhao, B. Tang, Genome-based assessment of antimicrobial resistance of Escherichia coli recovered from diseased swine in eastern China for a 12-year period, *mBio* 16 (2025) e0065125, <https://doi.org/10.1128/mbio.00651-25>.
- [15] R. Wang, L. van Dorp, L.P. Shaw, P. Bradley, Q. Wang, X. Wang, L. Jin, Q. Zhang, Y. Liu, A. Rieux, T. Dorai-Schneiders, L.A. Weinert, Z. Iqbal, X. Didelot, H. Wang, F. Balloux, The global distribution and spread of the mobilized colistin resistance gene mcr-1, *Nat. Commun.* 9 (2018) 1179, <https://doi.org/10.1038/s41467-018-03205-z>.
- [16] B. Zheng, H. Xu, C. Huang, C. Yu, L. Guo, H. Han, J. Zhang, X. Jiang, C. Chen, Y. Xiao, Occurrence and genomic characterization of two MCR-1- producing Escherichia coli isolates from the same mink farmer, *mSphere* 4 (2019), <https://doi.org/10.1128/mSphere.00602-19>.
- [17] H. Jia, Q. Tong, L. Wang, Y. Wu, X. Li, S. Li, Y. Kong, Y. Zhang, J.P.R. Furlan, N. O. Khine, P. Butaye, J. Zhang, Q. Yang, Z. Ruan, Silent circulation of plasmid-borne tet(X6) and Bla(OXA-58) genes in a community-acquired Acinetobacter baumannii strain, *Drug Resist. Updat.* 79 (2025) 101194, <https://doi.org/10.1016/j.drug.2024.101194>.
- [18] Q. Wang, S. Salman, Y. Luo, X. Tong, Coexistence of Bla(MIR-1) and Bla(NDM-1) resistance genes with a novel ST type in Enterobacter rogerskampii from a stool sample: a genome sequencing study, *J. Glob. Antimicrob. Resist.* 40 (2025) 37–41, <https://doi.org/10.1016/j.jgar.2024.11.006>.
- [19] R.R. Wick, L.M. Judd, C.L. Gorrie, K.E. Holt, Unicycler: resolving bacterial genome assemblies from short and long sequencing reads, *PLoS Comput. Biol.* 13 (2017) e1005595, <https://doi.org/10.1371/journal.pcbi.1005595>.
- [20] M. Richter, R. Rossello-Mora, Shifting the genomic gold standard for the prokaryotic species definition, *Proc. Natl. Acad. Sci. USA* 106 (2009) 19126–19131, <https://doi.org/10.1073/pnas.0906412106>.
- [21] V. Bortolaia, R.S. Kaas, E. Ruppe, M.C. Roberts, S. Schwarz, V. Cattoir, A. Philippon, R.L. Allesoe, A.R. Rebelo, A.F. Florensa, L. Fagelhauer, T. Chakraborty, B. Neumann, G. Werner, J.K. Bender, K. Stingl, M. Nguyen, J. Coppens, B.B. Xavier, S. Malhotra-Kumar, H. Westh, M. Pinholt, M.F. Anjum, N. A. Duggett, I. Kempf, S. Nykasenoja, S. Olkkola, K. Wiecek, A. Amaro, L. Clemente, J. Mossong, S. Losch, C. Ragimbeau, O. Lund, F.M. Aarestrup, ResFinder 4.0 for predictions of phenotypes from genotypes, *J. Antimicrob. Chemother.* vol. 75 (2020) 3491–3500, <https://doi.org/10.1093/jac/dkaa345>.
- [22] A. Carattoli, E. Zankari, A. Garcia-Fernandez, M. Voldby Larsen, O. Lund, L. Villa, F. Moller Aarestrup, H. Hasman, In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing, *Antimicrob. Agents Chemother.* 58 (2014) 3895–3903, <https://doi.org/10.1128/AAC.02412-14>.
- [23] N.F. Alikhan, N.K. Petty, N.L. Ben Zakour, S.A. Beatson, BLAST ring image generator (BRIG): simple prokaryote genome comparisons, *BMC Genomics* 12 (2011) 402, <https://doi.org/10.1186/1471-2164-12-402>.
- [24] M.J. Sullivan, N.K. Petty, S.A. Beatson, Easyfig: a genome comparison visualizer, *Bioinformatics* 27 (2011) 1009–1010, <https://doi.org/10.1093/bioinformatics/btr039>.
- [25] N.J. Croucher, A.J. Page, T.R. Connor, A.J. Delaney, J.A. Keane, S.D. Bentley, J. Parkhill, S.R. Harris, Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins, *Nucleic Acids Res.* 43 (2015) e15, <https://doi.org/10.1093/nar/gku1196>.
- [26] M.N. Price, P.S. Dehal, A.P. Arkin, FastTree: computing large minimum evolution trees with profiles instead of a distance matrix, *Mol. Biol. Evol.* 26 (2009) 1641–1650, <https://doi.org/10.1093/molbev/msp077>.
- [27] R. Memesh, M. Yasir, R.G. Ledger, H. Zowawi, A.J. McBain, E.I. Azhar, An update on the prevalence of colistin and carbapenem-resistant gram-negative bacteria in aquaculture: an emerging threat to public health, *J. Appl. Microbiol.* 135 (2024), <https://doi.org/10.1093/jambio/txad288>.
- [28] H.Y. Pekkle Lam, M.J. Lai, W.J. Wu, Y.H. Chin, H.J. Chao, L.K. Chen, S.Y. Peng, K. C. Chang, Isolation and characterization of bacteriophages with activities against multi-drug-resistant Acinetobacter nosocomialis causing bloodstream infection in vivo, *J. Microbiol. Immunol. Infect.* 56 (2023) 1026–1035, <https://doi.org/10.1016/j.jmii.2023.07.012>.
- [29] L. Huang, T.L. Chen, Y.T. Lee, M.H. Lee, S.C. Kuo, K.W. Yu, H.Y. Dou, C.P. Fung, Risk factors for imipenem-nonsusceptible Acinetobacter nosocomialis bloodstream infection, *J. Microbiol. Immunol. Infect.* 47 (2014) 311–317, <https://doi.org/10.1016/j.jmii.2013.02.002>.
- [30] K.L. Chew, K.X. Tan, N.A.B. Abu Bakar, R. Lin, J.W.P. Teo, Genotypic diversity and antimicrobial resistance phenotype of carbapenem-resistant and carbapenem-susceptible Acinetobacter species isolates, *Pathology* 57 (2025) 637–642, <https://doi.org/10.1016/j.pathol.2024.12.647>.
- [31] M. Aman, I. Altaf, B.A. Fomda, S. Roohi, A. Mir, S. Tariq, U. Qadri, Uncommon non-fermentative gram-negative superbugs - a surge in bacteraemia patients at an apex super speciality hospital, *Indian J. Med. Microbiol.* 60 (2026) 101046, <https://doi.org/10.1016/j.ijmm.2026.101046>.
- [32] S. Lenz, S. Biswas, I. Terry, L. Tran-Basha, A.K. Lyons, T. Mazumdar, F. Whitehill, A.L. Halpin, L. Franco, M. Karlsson, M. Williams, A. Coulliette-Salmond, Detection of Carbapenem-resistant Bacteria in skilled nursing facility wastewater, *J. Infect. Dis.* 233 (2026) 59–69, <https://doi.org/10.1093/infdis/jiaf534>.
- [33] N. Rakovitsky, M. Lurie-Weinberger, A. Keren-Paz, Y. Carmeli, Genomic and phenotypic characterization of six multidrug-resistant Acinetobacter pittii isolates, *Sci. Rep.* 15 (2025) 29577, <https://doi.org/10.1038/s41598-025-15848-2>.