Research Square

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Comparative Genomic Characterization of Multidrug-resistant Citrobacter Spp. Strains in Fennec Fox Imported to China

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Abstract

Background: To investigate the antimicrobial profiles and genomic characteristics of MDR-*Citrobacter* spp. strains isolated from Fennec fox imported from Sudan to China.

Methods: *Citrobacter* spp. strains were isolated from stool samples. Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS) was used for identification. Antimicrobial susceptibility testing was performed using the broth microdilution method. Whole-genome sequencing was performed on an Illumina Novaseq-6000 platform. Acquired antimicrobial resistance genes and plasmid replicons were detected using ResFinder 4.1 and PlasmidFinder 1.3, respectively. Comparative genomic analysis of 277 *Citrobacter* genomes was also investigated.

Results: Isolate FF141 was identified as *Citrobacter cronae*, isolate FF371, isolate FF414, and isolate FF423 were identified as *Citrobacter braakii.* Of these, three *C. braakii* isolates were further confirmed to be ESBL-producer. All isolates are all MDR with resistance to multiple antimicrobials. Plasmids of incompatibility (Inc) group pKPC-CAV1321. Comparative genomics analysis of *Citrobacter* isolates generated a large core-genome. Genetic diversity was observed in our bacterial collection, which clustered into five main clades. Human, environmental and animal *Citrobacter* isolates were distributed into five clusters.

Conclusions: To our knowledge, this is the first investigation of MDR-*Citrobacter* from Fennec Fox. Our phenotypic and genomic data further underscore the threat of increased ESBL prevalence in wildlife and emphasize that increased effort should be committed to monitoring the potentially rapid dissemination of ESBL-producers with one health perspective.

Background

The worldwide increase and spread of infections caused by multidrug-resistant (MDR) Gram-negative bacteria of human and animal origin is a significant global public health burden in recent decades.[1, 2] Enterobacteriaceae are common bacteria and usually associated with different types of community-, hospital-acquired, and even animal infections,[3–6] so antimicrobial resistance (AMR) in these bacteria has significant potential impacts on control of AMR, with one health perspective.[7, 8] Among these organisms, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae are recognized as the most prevalent group of pathogens due to their mobility.[9, 10] Treatment of infections caused by ESBL, AmpC-, and carbapenemase-producing-producing Enterobacteriaceae strains is challenging, with limited evidence of their efficacy,[11] due to the emergence and spread of carbapenem resistance in ESBL-producing Enterobacteriaceae isolates, which is of particular clinical relevance.[12, 13]

The ESBLs are usually carried by mobile genetic elements, such as a variety of self-transferring plasmids, which can be transferred to other bacteria.[14–16] Thus far, several ESBL types have been identified in Enterobacteriaceae isolates of which the CTX-M, TEM, and SHV β-lactamases are the most prevalent groups.[17, 18] It is noteworthy that ESBL- and carbapenemase-producing Enterobacteriaceae occurring in animals has become a public-health issue in recent years.[4, 18] Zoonotic pathogens contributed to the cross-transmissions of ESBL-producing Enterobacteriaceae.[19] Previously, we also reported the detection of the transmission of AMR across human, animals and environmental compartments in China.[8, 20, 21]

Citrobacter spp. are common Gram-negative bacilli and widely found in water, food, soil, and intestines of animals and humans.[22] AMR encoding genes are frequently reported in *Citrobacter freundii*, and it became a reservoir of antibiotic resistance genes in recent years.[23, 24] In addition, MDR *C. freundii* has been reported in numerous hosts including but are not limited to humans.[25–27] In this work, we identified four MDR *Citrobacter* isolates in Fennec fox imported from Sudan to China. Antimicrobial susceptibility tests, conjugation experiments, whole-genome sequencing, and comparative genomic analysis were performed to study the molecular characteristics of these MDR strains.

Methods And Materials

Bacterial identification and isolation of *Citrobacter* strains

We collected 168 stool samples of wild Fennec fox imported from North Africa to China.[5] Stool samples were cultured by MacConkey agar supplemented with 1mg/L cefotaxime as described previously.[5] Bacterial identification was conducted by MALDI-TOF MS (Bruker, Bremen, Germany) as described.[16] Confirmation of ESBL-producing isolations was further performed by a standard double-disk diffusion method as defined by the Clinical and Laboratory Standards Institute (CLSI) (https://clsi.org/).

Antimicrobial susceptibility testing (AST)

Susceptibility to 16 antibiotics (amikacin, aztreonam, cefpirome, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, florfenicol, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, polymyxin E, tigecycline, tobramycin, and trimethoprim-sulfamethoxazole) for four MDR *Citrobacter* isolates were evaluated. The MICs were determined via an agar dilution method for all antibiotics except for colistin and tigecycline, for which a broth microdilution method was used according to the CLSI standards. *E. coli* ATCC 25922 was used as control.

Whole-genome sequencing (WGS) and bioinformatics analysis

Genomic DNA was extracted from four MDR *Citrobacter* isolates using the Qiagen Blood/Tissue kit (Qiagen, Hilden, Germany).[6] The sequencing library was prepared by using Illumina Nextera XT kit (Illumina, San Diego, CA, USA) and sequenced using the Illumina NovaSeq 6000-PE150 platform (Illumina). Paired reads were then assembled into scaffolds using Velvet version 1.2.10.[43] Acquired antimicrobial resistance genes and plasmid replicons were performed using the CGE server (http://www.genomicepidemiology.org). Antibiotics Resistance Genes (ARGs) were identified using the ResFinder 4.1 database.[44] Genotyping was performed to query the seven domesticated genes (*aspC, clpX, fadD, mdh, arcA, dnaG, and lysP*) via the MLST database (https://pubmlst.org/organisms/citrobacter-spp). We further created a core genome-based phylogenetic tree using 4 *Citrobacter* genomes sequenced in this study and 272 randomly selected publicly available *Citrobacter* genomes (**Table S1**). The isolate collection includes strains from clinical (n = 159) and the environment (n = 133) sources that were widely distributed over time and geographical locations. *Citrobacter* genomes were annotated using Prokka[45] and RAST tool.[46] The core genes were identified using Prokka[45] and maximum likelihood-based phylogenetic reconstruction was performed with Roary.[47] Phylogenetic tree visualizations were generated by using iTOL (https://itol.embl.de/).

Plasmid characterization

The transferability of plasmids carrying MDR encoding genes was determined by filter mating as described previously.[20] Animal isolates and *Escherichia coli* J53 were used as donors and acceptors, respectively. The Animal isolates and J53 strains were mixed in (LB) broth at a ratio of 1:3 and incubated at 37°C for 18h. Transduction and binding were selected on MacConkey agar plates containing cefotaxime (2 µg/ml) and sodium azide (150µg/ml) for 12h. Susceptibility test was performed to determine the horizontal transferability of drug resistance, and the corresponding transduction conjugate was confirmed by PCR amplification and pulsed field gel electrophoresis (PFGE).

Data availability

The whole-genome sequences of four *Citrobacter* spp. isolates have been deposited in the GenBank under the BioProject number PRJNA656097.

Results

Isolation and identification

In this work, four *Citrobacter* spp. isolates resistant to cephalosporins were cultivated by selective medium plates. Among these strains, isolate FF141 was identified as *Citrobacter cronae*, isolate FF371, isolate FF414, and isolate FF423 were identified as *Citrobacter braakii.* Of these, three *C. braakii* isolates were further confirmed to be ESBL-producer (Table 1).

Table 1

Isolate	TZP	CTX	CAZ	CPO	IPM	MEM	AMK	ATM	CIP	CHL	FLR	GEN	TOB	FOS	TGC	COL
FF141	64	32	64	1	0.25	0.0075	8	64	0.25	64	16	4	1	0.25	0.25	2
FF371	128	> 128	64	128	0.25	0.003	32	128	16	128	128	> 128	64	256	0.25	2
FF414	2	> 128	64	128	0.25	0.003	16	128	16	128	128	> 128	64	256	0.25	2
FF423	2	> 128	64	> 128	0.25	0.003	16	128	16	128	> 128	> 128	64	256	0.25	2

TZP: Piperacillin/Tazobactam; CTX: Cefotaxime; CAZ: Ceftazidime; CPO: Cefpirome; IMP: Imipenem; MEM: Meropenem; AMK: Amikacin; ATM: Aztreonam; CIP: Ciprofloxacin; CHL: Chloramphenicol; FLR: Florfenicol; GEN: Gentamicin; TOB: Tobramycin; FOS: Fosfomycin; TGC: Tigecycline; COL: Colistin

Assessment of antibiotic susceptibility and MLST analysis

Four *Citrobacter* isolates are all MDR with resistance to multiple antimicrobials (Table 1). The total resistance rate was observed for cefotaxime, ceftazidime, aztreonam, and chloramphenicol (100%). All the isolates were susceptibility to. Imipenem, meropenem, amikacin, tigecycline, and colistin. Interestingly, only FF371 was resistant to piperacillin-tazobactam. Among these *Citrobacter* isolates, we found two sequence types (STs), which were ST350 (FF371, FF414, and FF423) and ST370 (FF141).

Resistant and virulence determinants of Citrobacter isolates

The acquired resistance genes detected in four *Citrobacter* isolates are summarized in Fig. 1A. The following genes were identified in three *C. braakii* isolates: the phenicol resistance gene *floR*; the trimethoprim resistance gene *dfrA17*; the tetracycline resistance gene *tet(A)*; the disinfectant resistance gene *qaeE*; the quinolone resistance gene *qnrS1*; the fluoroquinolone and aminoglycoside resistance gene *aac(6')-Ib-cr*, the aminoglycoside resistance genes *aph(6)-Id*, *aac(6')-Ib3*, *aph(3")-Ib*, *aac(3)-IId*, and *aadA5*; the macrolide resistance gene *mph(A)*; the β-lactam resistance genes $bla_{CTX-M-55}$, bla_{CMY-82}, and bla_{OXA-1}; the rifampicin resistance gene *ARR-3*; sulphonamide resistance genes *sul1*, and *sul2*. Of note, *C. braakii* FF414 and FF423 encoded the fosfomycin resistance gene *fosA3*, while *C. cronae* FF141 and *C. braakii* FF371 were negative. Among four isolates, FF141 encoded fewer resistance genes than other isolates, which carried bla_{CMY-98}, *qnrB34, tet(A)*, *dfrA12*, and *aadA2.* Plasmids of incompatibility (Inc) group pKPC-CAV1321, was identified in FF141, and IncR was detected in other *Citrobacter braakii* isolates. The antimicrobial genes *dfrA12*, *tet(A)*, and *aadA2* genes were co-harbored by IncR plasmid in three *C. braakii* isolates.

Virulence gene analysis showed that the presence of genes encoding efflux pump protein (*arcB*), the transport of siderophores (*fepC*), enterobactin (*entB* and *entE*), outer membrane protein A (*ompA*), extracellular nucleation factors (*csgB*, *csgD*, *csgE*, and *csgF*), and type VI secretion system-related proteins (*hcp/tssD*) in all isolates (Fig. 1B). Moreover, three *C. braakii* isolates also carried genes that involved polymer synthesis (*tviB-tviE*), and cell surface localization of the CPS (*vexA-vexE*).

Comparative genomics analysis of Citrobacter isolates

Roary matrix-based gene sequence analysis generated 60,923 total genes and a large core-genome of 1,578 gene clusters of 277 whole genomes. The whole-genome phylogeny (Fig. 2) revealed a population structure. Genetic diversity was observed in our bacterial collection, which clustered into five main clades. Interestingly, *C. braakii* isolates have close relatedness with a clinical isolate from Viet Nam and an environmental strain, while *C. cronae* FF141showed a high similarity with a clinical isolate from Nigeria and a clinical isolate from India (Fig. 2 **& Table S1**).

Discussion

The wide dissemination of MDR Enterobacteriaceae is a global concern with one health perspective.[28] In clinical settings, *Citrobacter* spp. isolates represent up to 6 % of all isolated Enterobacteriaceae from clinical specimens.[29] Members of the genus *Citrobacter* are reported to associate with nosocomial infections for a high mortality rate.[30] The *Citrobacter* genus is most closely related to *Escherichia coli* and *Salmonella*, and is divided into 11 different genomospecies.[31] There is some information indicating a high prevalence of MDR Enterobacteriaceae in wild animals.[32, 33] Although previous studies suggest that fur animals are potential reservoirs of AMR,[5, 34] little

is known about the antimicrobial patterns, and genomic characteristics of MDR *Citrobacter* isolates from wildlife. In the present study, we first described the isolation of MDR-*Citrobacter* strains from Fennec Fox. We subsequently obtained the antimicrobial resistance profiles and genomic information by AST and WGS. We also identified antimicrobial resistance and virulence-associated genes.

Citrobacter spp. isolates can have chromosomal AmpC β-lactamases, as well as plasmid encoded carbapenemases, which results in ineffective of many antimicrobial agents for treatment.[21, 23, 35, 36] *C. cronae* was identified as a new *Citrobacter* species from human stool samples very recently,[29] the antimicrobial profiles of *C. cronae* are largely unknow. In this study, we first identified an AmpC β-lactamase encoding gene (bla_{CMY-98}) in *C. cronae*, which provides a glimpse of antimicrobial insight into this species. Occurrence of ESBL-producing *C. braakii* isolated from animals and food product are occasionally reported.[37] Our detection of three ESBL-producing *C. braakii* isolates from Fennec Fox further suggests the risk of zoonotic potential MDR *C. braakii* from animals and animal products deservedly garners considerable attention.

Previous investigations found that CTX-M-55 became one of the prevalent ESBL type detected among clinical, animals, and environment in some countries.[14, 15, 38, 39] Very recently, the occurrences of CTX-M-55-producing *Escherichia coli* were also increasing reported in environment and diverse animal species in Europe [40, 41]. Our previous work confirmed that CTX-M-55-producing *Escherichia coli* was the most prevalent ESBL-producer from Fennec Fox.[5] These findings further strengthened that wildlife may act as potential reservoirs and vectors of CTX-M-55, although some of these isolates carried $bla_{CTX-M-55}$ genes on the chromosome.

Interestingly, the diverging clonality of the human, environmental and animal *Citrobacter* isolates was confirmed by the fact that strains originating in these three sources distributed into five clusters. It is still not sure whether the ecological and animal strains are highly related to the human strains in terms of genetic phylogeny. The previous investigation highlighted the challenges associated with species designation of *Citrobacter* by core genome analysis, particularly in regards to *Citrobacter freundii*, which did not constitute a discrete phylogenetic group.[42] As we noted in our data, *C. cronae* and *C. braakii* strains were clustered into the same clade, which suggests further accurate taxonomic inquiry is needed to clarify the lineage of *Citrobacter* members.

Conclusion

In summary, this investigation involved the first survey of MDR *Citrobacter* isolates in Fennec Fox. We characterized the phenotypic characteristics and genomic basis of MDR *Citrobacter* strains. Fennec Fox may serve as a common vector for the rapid dissemination of ESBL-encoding genes via animal contact and thereby threaten public health. Our findings further underscore the threat of increased ESBL prevalence in Enterobacteriaceae, and improved multisectoral surveillance for ESBL-producing *Citrobacter* is warranted.

Declarations

Ethical approval：Not required.

Consent for publication：Yes.

Competing interests：None declared.

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References

1. Kern WV, Rieg S. Burden of bacterial bloodstream infection-a brief update on epidemiology and significance of multidrug-resistant pathogens. Clin Microbiol Infect. 2020;26(2):151–7.

2. Giacobbe DR, Ciacco E, Girmenia C, Pea F, Rossolini GM, Sotgiu G, Tascini C, Tumbarello M, Viale P, Bassetti M, et al. Evaluating Cefiderocol in the Treatment of Multidrug-Resistant Gram-Negative Bacilli: A Review of the Emerging Data. Infect Drug Resist. 2020;13:4697–711.
3. Ouchar Mahamat O, Kempf M, Lounnas M, Tidjani A, Hide M, Benavides JA, Carriere C, Banuls AL, Jean-Pierre H, Ouedraogo AS, et al. Epidemiology and prevalence of extended-spectrum beta-lactamase- and carbapenemase-producing Enterobacteriaceae in humans, animals and the environment in West and Central Africa. Int J Antimicrob Agents. 2021;57(1):106203.
4. Madec JY, Haenni M, Nordmann P, Poirel L. Extended-spectrum beta-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: a threat for humans? Clin Microbiol Infect. 2017;23(11):826–33.
5. Feng C, Wen P, Xu H, Chi X, Li S, Yu X, Lin X, Wu S, Zheng B: **Emergence and Comparative Genomics Analysis of Extended-Spectrum-beta-Lactamase-Producing Escherichia coli Carrying mcr-1 in Fennec Fox Imported from Sudan to China**. *mSphere* 2019, **4**(6).
6. Zheng B, Xu H, Lv T, Guo L, Xiao Y, Huang C, Zhang S, Chen Y, Han H, Shen P, et al: **Stool Samples of Acute Diarrhea Inpatients as a Reservoir of ST11 Hypervirulent KPC-2-Producing Klebsiella pneumoniae**. *mSystems* 2020, 5(3).
7. Cars O, Xiao Y, Stalsby Lundborg C, Nilsson LE, Shen J, Sun Q, Bi Z, Borjesson S, Greko C, Wang Y, et al. Building bridges to operationalise one health - A Sino-Swedish collaboration to tackle antibiotic resistance. One Health. 2016;2:139–43.
8. Zheng B, Xu H, Huang C, Yu X, Guo L, Han H, Zhang J, Jiang X, Chen C, Xiao Y: **Occurrence and Genomic Characterization of Two MCR-1-Producing Escherichia coli Isolates from the Same Mink Farmer**. *mSphere* 2019, **4**(6).
9. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. Clin Microbiol Rev. 2012;25(4):682–707.
10. Rojas LJ, Salim M, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC, Watkins RR, Marshall S, Rudin SD, et al. Colistin Resistance in Carbapenem-Resistant Klebsiella pneumoniae: Laboratory Detection and Impact on Mortality. Clin Infect Dis. 2017;64(6):711–8.
11. Rodriguez-Bano J, Gutierrez-Gutierrez B, Machuca I, Pascual A. **Treatment of Infections Caused by Extended-Spectrum-Beta-Lactamase-, AmpC-, and Carbapenemase-Producing Enterobacteriaceae**. *Clin Microbiol Rev* 2018, 31(2).
12. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9(4):228–36.
13. Grundmann H, Glasner C, Albiger B, Aanensen DM, Tomlinson CT, Andrasevic AT, Canton R, Carmeli Y, Friedrich AW, Giske CG, et al. Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. Lancet Infect Dis. 2017;17(2):153–63.
14. Zhang J, Zhou K, Zheng B, Zhao L, Shen P, Ji J, Wei Z, Li L, Zhou J, Xiao Y. High Prevalence of ESBL-Producing Klebsiella pneumoniae Causing Community-Onset Infections in China. Front Microbiol. 2016;7:1830.
15. Zhang J, Zheng B, Zhao L, Wei Z, Ji J, Li L, Xiao Y. Nationwide high prevalence of CTX-M and an increase of CTX-M-55 in Escherichia coli isolated from patients with community-onset infections in Chinese county hospitals. BMC Infect Dis. 2014;14:659.
16. Zheng B, Feng C, Xu H, Yu X, Guo L, Jiang X, Song X. Detection and characterization of ESBL-producing Escherichia coli expressing mcr-1 from dairy cows in China. J Antimicrob Chemother. 2019;74(2):321–5.
17. Bush K, Bradford PA. **Epidemiology of beta-Lactamase-Producing Pathogens**. *Clin Microbiol Rev* 2020, 33(2).
18. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M beta-lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother. 2017;72(8):2145–55.
19. Katale BZ, Misinzo G, Mshana SE, Chiyangi H, Campino S, Clark TG, Good L, Rweyemamu MM, Matee MI. Genetic diversity and risk factors for the transmission of antimicrobial resistance across human, animals and environmental compartments in East Africa: a review. Antimicrob Resist Infect Control. 2020;9(1):127.
20. Zheng B, Huang C, Xu H, Guo L, Zhang J, Wang X, Jiang X, Yu X, Jin L, Li X, et al. Occurrence and Genomic Characterization of ESBL-Producing, MCR-1-Harboring Escherichia coli in Farming Soil. Front Microbiol. 2017;8:2510.
21. Xu H, Wang X, Yu X, Zhang J, Guo L, Huang C, Jiang X, Li X, Feng Y, Zheng B. First detection and genomics analysis of KPC-2-producing Citrobacter isolates from river sediments. Environ Pollut. 2018;235:931–7.
22. Liu LH, Wang NY, Wu AY, Lin CC, Lee CM, Liu CP. Citrobacter freundii bacteremia: Risk factors of mortality and prevalence of resistance genes. J Microbiol Immunol Infect. 2018;51(4):565–72.
23. Chi X, Guo J, Zhou Y, Xiao T, Xu H, Lv T, Chen C, Chen J, Zheng B. Complete-Genome Sequencing and Comparative Genomic Characterization of an IMP-4 Producing Citrobacter freundii Isolate from Patient with Diarrhea. Infect Drug Resist. 2020;13:1057–65.
24. Jiang X, Cui X, Liu W, Xu H, Zheng B. Genetic characterization of a novel sequence type of multidrug-resistant Citrobacter freundii strain recovered from wastewater treatment plant. Infect Drug Resist. 2019;12:2775–9.

25. Sadek M, Ortiz de la Rosa JM, Abdelfattah Maky M, Korashe Dandrawy M, Nordmann P, Poirel L. **Genomic Features of MCR-1 and Extended-Spectrum beta-Lactamase-Producing Enterobacterales from Retail Raw Chicken in Egypt**. *Microorganisms* 2021, 9(1).
26. Shnaiderman-Torban A, Steinman A, Meidan G, Paitan Y, Abu Ahmad W, Navon-Venezia S. Petting Zoo Animals as an Emerging Reservoir of Extended-Spectrum beta-Lactamase and AmpC-Producing Enterobacteriaceae. Front Microbiol. 2019;10:2488.
27. Goldberg DW, Fernandes MR, Sellera FP, Costa DGC, Loureiro Bracarense AP, Lincopan N. Genetic background of CTX-M-15-producing Enterobacter hormaechei ST114 and Citrobacter freundii ST265 co-infecting a free-living green turtle (Chelonia mydas). Zoonoses Public Health. 2019;66(5):540–5.
28. Booton RD, Meeyai A, Alhusein N, Buller H, Feil E, Lambert H, Mongkolsuk S, Pitchforth E, Reyher KK, Sakcamduang W, et al. One Health drivers of antibacterial resistance: Quantifying the relative impacts of human, animal and environmental use and transmission. One Health. 2021;12:100220.
29. Oberhettinger P, Schule L, Marschal M, Bezdan D, Ossowski S, Dorfel D, Vogel W, Rossen JW, Willmann M, Peter S. Description of Citrobacter cronae sp. nov., isolated from human rectal swabs and stool samples. Int J Syst Evol Microbiol. 2020;70(5):2998–3003.
30. Qian C, Du Y, Li H, Wu P, Wang L, Wei Y, Cao H, Yin Z, Zhang Y, Zhu Y, et al. Development of rapid and simple experimental and in silico serotyping systems for Citrobacter. Future Microbiol. 2018;13:1511–22.
31. Petty NK, Bulgin R, Crepin VF, Cerdeno-Tarraga AM, Schroeder GN, Quail MA, Lennard N, Corton C, Barron A, Clark L, et al. The Citrobacter rodentium genome sequence reveals convergent evolution with human pathogenic Escherichia coli. J Bacteriol. 2010;192(2):525–38.
32. Fashae K, Engelmann I, Monecke S, Braun SD, Ehricht R. Molecular characterisation of extended-spectrum ss-lactamase producing Escherichia coli in wild birds and cattle, Ibadan, Nigeria. BMC Vet Res. 2021;17(1):33.
33. Wang X, Kang Q, Zhao J, Liu Z, Ji F, Li J, Yang J, Zhang C, Jia T, Dong G, et al. Characteristics and Epidemiology of Extended-Spectrum beta-Lactamase-Producing Multidrug-Resistant Klebsiella pneumoniae From Red Kangaroo, China. Front Microbiol. 2020;11:560474.
34. O'Hagan MJH, Pascual-Linaza AV, Couzens C, Holmes C, Bell C, Spence N, Huey RJ, Murphy JA, Devaney R, Lahuerta-Marin A. Estimation of the Prevalence of Antimicrobial Resistance in Badgers (Meles meles) and Foxes (Vulpes vulpes) in Northern Ireland. Front Microbiol. 2021;12:596891.
35. Peter S, Bosio M, Gross C, Bezdan D, Gutierrez J, Oberhettinger P, Liese J, Vogel W, Dorfel D, Berger L, et al: **Tracking of Antibiotic Resistance Transfer and Rapid Plasmid Evolution in a Hospital Setting by Nanopore Sequencing**. *mSphere* 2020, 5(4).
36. Hu X, Xu H, Shang Y, Guo L, Song L, Zhang H, Yue F, Jiang H, Zheng B. First genome sequence of a blaKPC-2-carrying Citrobacter koseri isolate collected from a patient with diarrhoea. J Glob Antimicrob Resist. 2018;15:166–8.
37. Gelbicova T, Barakova A, Florianova M, Jamborova I, Zelendova M, Pospisilova L, Kolackova I, Karpiskova R. Dissemination and Comparison of Genetic Determinants of mcr-Mediated Colistin Resistance in Enterobacteriaceae via Retailed Raw Meat Products. Front Microbiol. 2019;10:2824.
38. Nadimpalli M, Fabre L, Yith V, Sem N, Gouali M, Delarocque-Astagneau E, Sreng N, Le Hello S. group Bs: **CTX-M-55-type ESBL-producing Salmonella enterica are emerging among retail meats in Phnom Penh, Cambodia**. J Antimicrob Chemother. 2019;74(2):342–8.
39. Seenama C, Thamlikitkul V, Ratthawongjirakul P. Multilocus sequence typing and bla ESBL characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolated from healthy humans and swine in Northern Thailand. Infect Drug Resist. 2019;12:2201–14.
40. Lupo A, Saras E, Madec JY, Haenni M. Emergence of blaCTX-M-55 associated with fosA, rmtB and mcr gene variants in Escherichia coli from various animal species in France. J Antimicrob Chemother. 2018;73(4):867–72.
41. Ovejero CM, Delgado-Blas JF, Calero-Caceres W, Muniesa M, Gonzalez-Zorn B. Spread of mcr-1-carrying Enterobacteriaceae in sewage water from Spain. J Antimicrob Chemother. 2017;72(4):1050–3.
42. Pilar AVC, Petronella N, Dussault FM, Verster AJ, Bekal S, Levesque RC, Goodridge L, Tamber S. Similar yet different: phylogenomic analysis to delineate Salmonella and Citrobacter species boundaries. BMC Genom. 2020;21(1):377.
43. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res. 2008;18(5):821–9.
44. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67(11):2640–4.
45. Seemann T. Prokka: rapid prokaryotic genome annotation. Bioinformatics. 2014;30(14):2068–9.

46. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, et al. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic Acids Res. 2014;42(Database issue):D206–14.
47. Sitto F, Battistuzzi FU. Estimating Pangenomes with Roary. Mol Biol Evol. 2020;37(3):933–9.

Figures

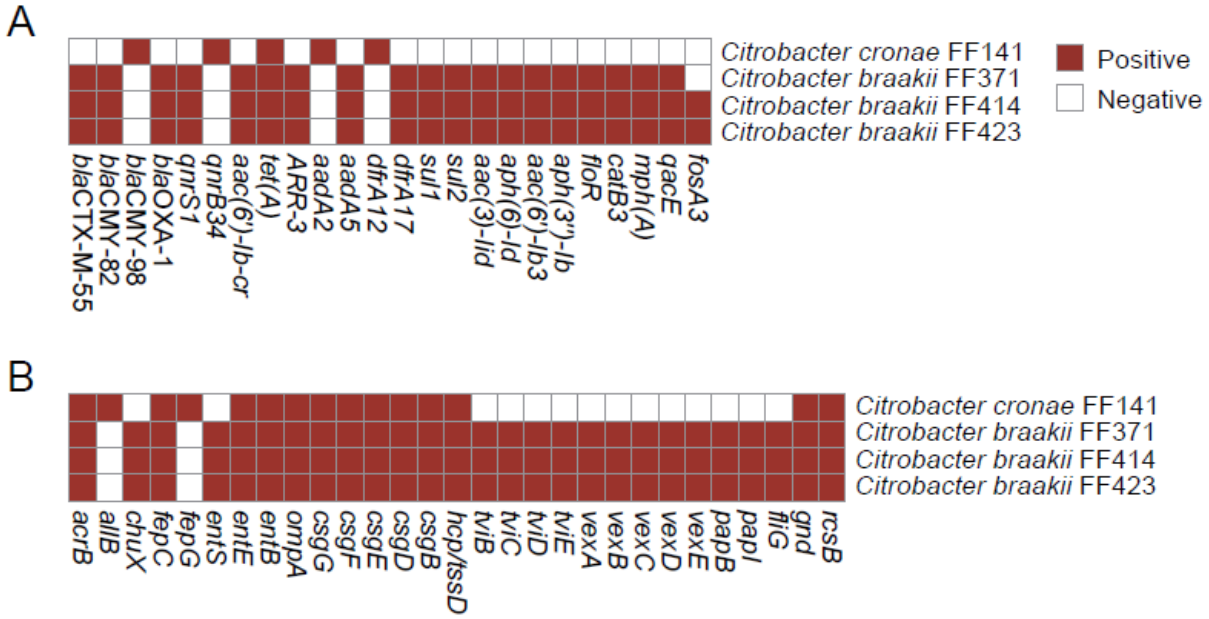

Figure 1

A). Antimicrobial resistance gene profiles of 4 MDR-Citrobacter isolates. The heatmap is used to display the types of acquired AMR genes. Brown indicates the presence of AMR genes, whereas colorless correspond to the absence of the AMR genes. B). The heatmap is used to display the types of virulence-associated genes. Brown indicates the presence of virulence genes, whereas colorless correspond to the absence of the virulence genes.

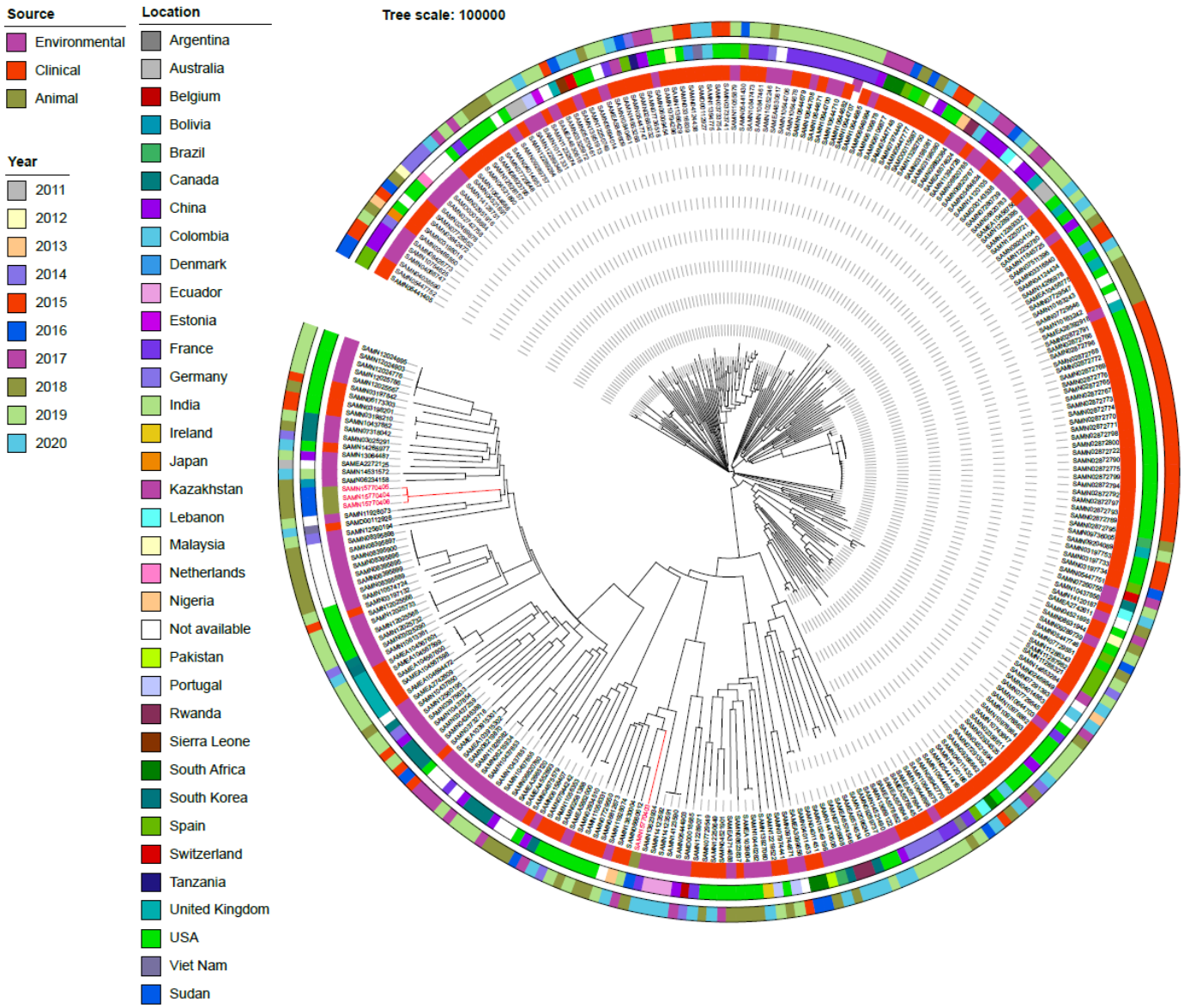

Figure 2

A core-genome analysis of 277 Citrobacter strains, including 4 isolates from this study and 273 strains downloaded from NCBI genome database. The year of the isolation is labelled in the outer ring. The source of the strains is presented in the middle ring. The location of the isolates is colored in the inner ring. Isolates identified in this study were colored in red. NA, details regarding the region of the strains is not available. The bar shows 100000 nucleotide substitution per position.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx