New Microbes and New Infections 62 (2024) 101546

Contents lists available at ScienceDirect

New Microbes and New Infections

journal homepage: www.journals.elsevier.com/new-microbes-and-new-infections

Letter to the Editor

A novel multidrug-resistant *Enterobacter hormaechei* ST2755 isolated from a wild-caught oyster in Georgia, USA

ARTICLE INFO

Handling Editor: Patricia Schlagenhauf

Keywords:
Antimicrobial resistance
Enterobacter hormaechei
Surveillance
Cephalosporin
Ertapenem
Oysters
One health

Dear Editor,

The *Enterobacter cloacae* complex (ECC) is comprised of seven different species; *Enterobacter asburiae*, *Enterobacter cloacae*, *Enterobacter hormaechei*, *Enterobacter kobei*, *Enterobacter ludwigii*, *Enterobacter mori*, and *Enterobacter xiangfangensis*. *E. hormaechei* is often implicated in nosocomial infections [1]. This is primarily due to its adaptability to antimicrobial treatments and its opportunistic pathogenesis [1].

E. hormaechei has been frequently associated with multidrug resistance (MDR), exhibiting reduced susceptibility to critically important antibiotics. This species naturally produces an inducible chromosomally encoded Ambler class C β-lactamase (cAmpC) characterized by the ability to hydrolyze cephalosporins without being inhibited by clavulanic acid or tazobactam [2]. Additionally, acquired resistance to broad-spectrum cephalosporins can occur through the acquisition of extended-spectrum β-lactamases (ESBL) or by chromosomal mutations, mostly in *ampD* or *ampR* genes that lead to cAmpC overproduction. Notably, the genus *Enterobacter* has been considered a reservoir of *plasmid-borne AmpC β-lactamase-encoding* genes, such as bla_{ACT}, bla_{CMH}, and bla_{MIR}, which can be transferable to other Enterobacterales species. For example, the plasmid-mediated bla_{ACT-1} gene reported in *Klebsiella pneumoniae* has been specifically curated to originate from *E. hormaechei* [3]. Carbapenem resistance can also arise by acquiring carbapenemases or through the alteration or loss of non-specific porins associated with the hyperexpression of the cAmpC gene and/or production of ESBLs.

Bivalve mollusks, such as clams, oysters, mussels, and scallops, are essential components of aquatic ecosystems due to their filter-feeding behavior, which enables them to accumulate particles, including microorganisms, from the surrounding water. The latter can include microbial pathogens and antibiotic-resistant bacteria that might contaminate the environment of the bivalves. Notably, bacterial communities present in bivalves can serve as an indicator of fecal contamination that results from human and animal activities, such as sewage discharge or agricultural runoff, which introduce fecal bacteria into coastal and marine environments [4]. Consequently, public health concerns have been associated with consuming bivalves, especially when eaten raw or lightly cooked, which can expose humans to various enteric pathogens. As importantly, bivalves can also be used to monitor the quality of their water habitat, serving as an indicator for the emergence or spread of different pathogens, including antimicrobial-resistant bacteria. Despite this, studies of MDR bacteria in seafood remain comparatively limited worldwide in comparison to other food-animals such as poultry, beef, and pork. Taken together, comprehensive surveillance of bacteria in bivalves can be essential for assessing human exposure to drug-resistant pathogens via seafood and for monitoring AMR dissemination in the aquatic environment and beyond. Here, we report the isolation and in-depth characterization of an MDR *E. hormaechei* isolated from a wild-caught oyster purchased from a retail store in Georgia, USA.

In August 2024, as part of a project that targeted AMR in seafood in Georgia, a fresh oyster sample was collected, placed on ice, and immediately transported to the laboratory for analysis. The shells were removed using standard sterile procedures, and 20 g were suspended in 1X PBS and homogenized in a stomacher. An aliquot (100 μL) was spread onto RAPID'*E. coli* 2 agar (Bio-Rad, Hercules, CA) supplemented with 36 μg/mL ampicillin (Sigma-Aldrich, St. Louis, MO). After incubation for 24 hours at 37 °C under aerobic conditions, colonies were randomly selected and purified. Antibiotic susceptibility testing using the Kirby-Bauer disk diffusion revealed a notable MDR isolate (11A-DG2), exhibiting an AmpC-overproduction phenotype and resistance to penicillins, extended-spectrum cephalosporins, and ertapenem (Table 1).

WGS analysis identified strain 11A-DG2 as *E. hormaechei* and showed that the bacterium belonged to ST2755, a novel sequence type that has not been reported in the literature previously. Analysis using ResFinder v4.6 revealed that the strain carried two resistance genes, bla_{ACT-7} and *fosA*, encoding resistance to beta-lactams and fosfomycin, respectively. Since bla_{ACT-7} and *fosA* are located on a large contig of 499,923 bp, these genes are likely chromosomally encoded. A subsequent BLAST search of this contig against the nucleotide database confirmed its alignment with

https://doi.org/10.1016/j.nmni.2024.101546
Received 1 November 2024; Received in revised form 27 November 2024; Accepted 28 November 2024
Available online 29 November 2024

Table 1
Genome analysis and antimicrobial susceptibility patterns of the *Enterobacter hormaechei* 11A-DG2 isolated from a wild-caught live oyster in Georgia, USA.

Isolate ID	GenBank accession number of the assembled genome	Genome size (bp)	No. Of contigs	N50 (bp)	L50	GC content (%)	Non-susceptibility to selected antimicrobials[a,g]	Susceptibility to selected antimicrobials[b,g]	Acquired antimicrobial resistance genes[c]	Quinolone resistance-determining region (QRDR) mutations	Plasmid replicons detected by WGS[d]	Sequence type[e]	Human pathogenicity probability (virulence genes)[f]
11A-DG2	GCF_041877835.1	4659198	81	450100	3	55.5	AMP, AMC, PIP, CFZ, CXM, FOX, CFM, CTX, CRO, CAZ, CPD, ATM, ERT	TZP, FEP, IMP, MEM, DOR, STR, GMN, TMN, KMN, TET, DOX, NOR, CIP, CHL, SXT, FUR, CST	bla_{ACT-7}; *fosA*	–	–	ST2755	0.762 (*iroN*; *nlpI*)

[a] AMP, ampicillin; AMC, amoxicillin-clavulanate; PIP, piperacillin; CFZ, cefazolin; CXM, cefuroxime; FOX, cefoxitin; CFM, cefixime; CTX, cefotaxime; CRO, ceftriaxone; CAZ, ceftazidime; CPD, cefpodoxime; ATM, aztreonam; ERT, ertapenem.
[b] TZP, piperacillin-tazobactam; FEP, cefepime; IMP, imipenem; MEM, meropenem; DOR, doripenem; STR, streptomycin; GMN, gentamicin; TMN, tobramycin; KMN, kanamycin; TET, tetracycline; DOX, doxycycline; NOR, norfloxacin; CIP, ciprofloxacin; CHL, chloramphenicol; SXT, trimethoprim-sulfamethoxazole; FUR, nitrofurantoin; CST, colistin.
[c] Acquired antimicrobial drug resistance genes detected by ResFinder v4.6 (http://genepi.food.dtu.dk/resfinder) using the default thresholds of 90 % minimum identity and 60 % minimum coverage.
[d] No plasmid types were detected by PlasmidFinder v2.0.1 (https://cge.food.dtu.dk/services/PlasmidFinder/) using 80 % identity as cut-off.
[e] Sequence type (ST) was determined by PubMLST (https://pubmlst.org/bigsdb?db=pubmlst_ecloacae_seqdef).
[f] Using PathogenFinder v1.1 (https://cge.food.dtu.dk/services/PathogenFinder/), 11A-DG2 was predicted to be a human pathogen. Virulence genes were detected by VirulenceFinder v2.0 (https://cge.food.dtu.dk/services/VirulenceFinder/) using *Escherichia coli* as the selected species and the default thresholds of 90 % minimum identity and 60 % minimum length.
[g] Resistance to antimicrobials was determined using the Kirby-Bauer disk diffusion and broth microdilution assays according to the Clinical and Laboratory Standards Institute (CLSI-M100) guidelines (https://clsi.org/standards/products/microbiology/companion/using-m100/). The *Escherichia coli* ATCC®25922™ strain was used for quality control. To define MDR, we adopted the standardized international definition of resistance to three or more antimicrobial classes.

multiple *E. hormaechei* chromosomes available in GenBank. Furthermore, no plasmid types were identified by PlasmidFinder v2.0.1. The presence of chromosomally encoded resistance genes suggests a stable and heritable reservoir of AMR determinants in *E. hormaechei* populations. This increases the potential for long-term persistence and dissemination of resistance traits in the human-animal-environment interface. Detecting these genes in wild-caught oysters further highlights the critical role of environmental reservoirs, particularly aquatic ecosystems, in the spread of MDR bacteria. These findings raise concerns about the risk of human exposure to MDR pathogens through contaminated water sources and seafood, emphasizing the need for enhanced surveillance and mitigation strategies. Additionally, the discovery of ST2755, a novel ST, associated with an MDR profile is notable, because it might signal the emergence of new resistant clones in the environment.

Using PathogenFinder v1.1 and Virulence Finder v2.0, 11A-DG2 was predicted to be a human pathogen (76.2 %). *E. hormaechei* 11A-DG2 harbored two virulence genes, an enterobactin siderophore receptor protein (encoded by *iroN*) and new lipoprotein I (*nlpI*), which are important in facilitating the bacterium's ability to survive in human hosts. Enterobactin, a potent siderophore, allows the bacterium to sequester iron from the host environment, while NlpI, an outer membrane-anchored lipoprotein, contributes to the maintenance of the bacterial cell envelope, promoting its ability to resist environmental stress and evade host immune responses [5].

In conclusion, this study reports the first isolation of a novel MDR *E. hormaechei* from wild-caught live oysters in the USA. Our findings highlight the role of bivalves as bio-accumulators, which can be exploited as an effective tool for surveillance of AMR bacteria/pathogens in contaminated aquatic environments and beyond. Taken together, our findings emphasize the need for robust and innovative One Health strategies to monitor and control the dissemination of AMR in humans, animals, and environment.

CRediT authorship contribution statement

Nivin A. Nasser: Writing – original draft, Investigation. **Marwan Osman:** Writing – original draft, Investigation. **Jouman Hassan:** Investigation, Formal analysis. **Tongzhu Xu:** Software, Formal analysis. **David Mann:** Software, Formal analysis. **Xiangyu Deng:** Software, Formal analysis. **Issmat I. Kassem:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The WGS sequence of *Enterobacter hormaechei* strain 11A-DG2 has been deposited in the GenBank database under the accession number JBHDUA000000000.

Funding information

None to declare.

Declaration of competing interest

The authors declare that they have no known competing interests.

Acknowledgments

We thank the Center for Food Safety (UGA) and UGA's Presidential Interdisciplinary Seed Grants for partially funding of this work.

References

[1] Davin-Regli A, Lavigne J-P, Pagès J-M. *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin Microbiol Rev 2019;32. https://doi.org/10.1128/cmr.00002-19.

[2] Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother 2010;54:969–76.

[3] Roh KH, Song W, Chung HS, Lee YS, Yum JH, Yi HN, et al. Chromosomal cephalosporinase in *Enterobacter hormaechei* as an ancestor of ACT-1 plasmid-mediated AmpC β-lactamase. J Med Microbiol 2012;61:94–100.

[4] Grevskott DH, Svanevik CS, Sunde M, Wester AL, Lunestad BT. Marine bivalve mollusks as possible indicators of multidrug-resistant *Escherichia coli* and other species of the enterobacteriaceae family. Front Microbiol 2017;8:24.

[5] Chopjitt P, Boueroy P, Jenjaroenpun P, Wongsurawat T, Hatrongjit R, Kerdsin A, et al. Genomic characterization of vancomycin-resistant *Enterococcus faecium* clonal complex 17 isolated from urine in tertiary hospitals in Northeastern Thailand. Front Microbiol 2024;14.

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