



Letter to the Editor

Isolation and characterisation of carbapenemase-producing and polymyxin B-resistant *Enterobacter bugandensis* from a vegetable



Editor: Stefania Stefani

To the editor,

The acquisition and rapid dissemination of antibiotic resistance in human pathogens has become a global public-health issue. Carbapenems are the most potent and reliable β -lactam antibiotics for the treatment of infections caused by multidrug-resistant pathogens. Carbapenem resistance is a growing concern and is considered an urgent threat. Food is an important vehicle for transmitting foodborne human pathogens [1]. Vegetables have been involved in numerous foodborne outbreaks [2]. Vegetables can also harbour antibiotic-resistant bacteria. Fresh vegetables that contain carbapenemase-producing microorganisms may serve as a reservoir for the transmission of carbapenem resistance. In this study, we isolated carbapenem-resistant bacteria in retail vegetables and investigated their antimicrobial resistance genes through genomic analyses.

A total of 88 vegetable samples were purchased from seven grocery stores in a 30-mile radius in central Arkansas (USA) from September to December 2020. Vegetable samples (10 g) were mixed with 90 mL of buffered peptone water (BPW) (BD, Franklin Lakes, NJ, USA) and were processed in a stomacher at 300 rpm for 3 min to release bacterial cells. After pre-enrichment in BPW for 3 h and selective enrichment overnight in Enterobacteriaceae enrichment broth (BD), carbapenem-resistant bacteria were screened on CHROMagarTM mSuperCARBATM agar (CHROMagar Microbiology, Paris, France). Bacterial isolates from four organic products (5%) were non-susceptible to carbapenem antibiotics. These isolates included *Enterobacter bugandensis* S68-1, *Aeromonas veronii* S50-1, *Aeromonas hydrophila* S73-1 and *Stenotrophomonas maltophilia* S76-3. Because intrinsic resistance to carbapenem antibiotics often occurs in *Aeromonas* spp. and *Stenotrophomonas* spp., this study only focused on characterisation of *E. bugandensis* S68-1, which was isolated from a vegetable sample containing a mixture of lettuce and other leafy vegetables. *Enterobacter* is a globally important pathogen in the family of Enterobacteriaceae. *Enterobacter bugandensis* is a novel species associated with severe clinical infections.

The antimicrobial susceptibility of *E. bugandensis* S68-1 was determined by the disk diffusion method according to standard Clinical and Laboratory Standards Institute (CLSI) procedures for 11 antibiotics, including ampicillin, amoxicillin/clavulanic acid, cefiderocol, imipenem, meropenem, gentamicin, tetracycline, chloramphenicol, ciprofloxacin, nalidixic acid and trimethoprim/sulfamethoxazole. In addition, susceptibility to polymyxin B was determined by broth microdilution. We found that *E. bugandensis* S68-1 was resistant to ampicillin, amoxicillin/clavulanic

acid and imipenem. Moreover, this isolate showed resistance to polymyxin B with a minimum inhibitory concentration (MIC) of 32 μ g/mL. Carbapenemase production was determined by the modified Hodge test (MHT) and was further confirmed by modified carbapenem inactivation method and EDTA-modified carbapenem inactivation method (mCIM/eCIM) assays, which indicated the types of carbapenemase produced by the tested strain. *Enterobacter bugandensis* S68-1 was positive by the MHT test. Using mCIM/eCIM assays, we found that *E. bugandensis* S68-1 produced a serine carbapenemase.

To determine the genetic determinants responsible for resistance to imipenem, the genome of *E. bugandensis* S68-1 was sequenced and analysed. Genomic DNA was extracted using a ZymoBIOMICSTM Kit (Zymo Research, Irvine, CA, USA). A sequencing library was prepared using an NEBNext[®] UltraTM II DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA). DNA sequencing was performed on an Illumina NextSeq 500 System using a mid-output kit (2 \times 150 bp) (Illumina Inc., San Diego, CA, USA). The quality of paired-end reads was assessed before and after the trimming step using FastQC v.0.11.9, and adapters were removed using fastp v.0.20.1 with 'detect_adapter_for_pe' option. Genome assembly from raw reads was performed using SPAdes v.3.14.1 with 'error-correction' and 'careful' options. Assembled contigs of <400 bp were removed, obtaining a final number of 32 scaffolds with an N_{50} of 315 733 and >200 \times coverage. Final assembly was submitted to the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) [3] successfully passing contamination screening. Bacterial species identification was determined using a k-mer-based approach with Kraken2 using the standard database. Antimicrobial resistance genes in the assembled genome were identified using Resistance Gene Identifier (RGI) v.5.1.1 against the CARD database v.3.1.1. Multilocus sequence typing (MLST) was carried out using the PubMLST database (<https://pubmlst.org/>).

Enterobacter bugandensis S68-1 was assigned to sequence type 921 (ST921). Genomic analyses indicated that strain S68-1 possesses a gene encoding an Ambler class A carbapenemase, imipenemase IMI-1 (accession no. **MBO0402124.1**), in a chromosomally integrated 29-kb putative Xer-dependent integrative mobile element (IMEX) (Fig. 1), which is presumably responsible for the mobility of *bla*_{IMI-1} in *Enterobacter* [4]. *Enterobacter* strains carrying the resistance gene *bla*_{IMI-1} have been isolated in seafoods imported from Southeast Asia to Europe and North America [5,6]. Most previously described IMI-producing *Enterobacter cloacae* complex isolates were derived from human clinical samples. IMI-1-producing *Enterobacter* clinical isolates with concomitant polymyxin E (colistin) resistance were reported previously [7]. In addition, the fosfomycin resistance gene (*fosA2*) and genes encoding efflux pumps, such as *arcA*, *arcB*, *tolC*, *oqxA*, *oqxB*, *emrA*, *emrB*, *mdtB* and *mdtC*, were found in the genome of *E. bugandensis* S68-1. No sequences with significant similarity to the mobilised colistin

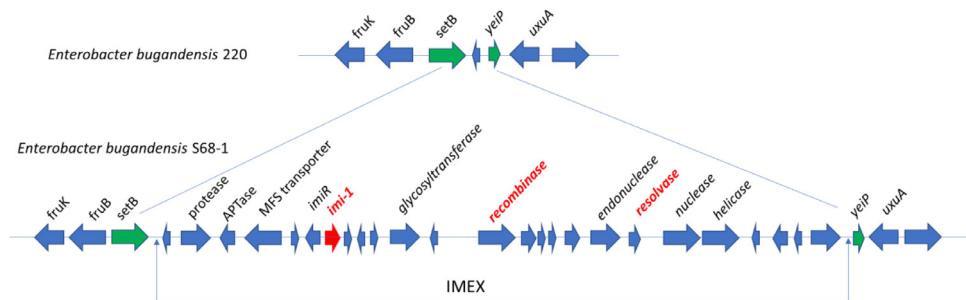


Fig. 1. Comparison of the genomic region (GenBank accession no. [JAFLWR010000003.1](#)) of *Enterobacter bugandensis* S68-1 carrying the *bla_{IMI-1}* gene and the corresponding chromosomal region of a clinical isolate, *E. bugandensis* 220, (GenBank accession no. [NZ_CP039453.1](#)), revealing the chromosomal insertion of the putative Xer-dependent integrative mobile element (IMEX) in *E. bugandensis* S68-1.

resistance gene *mcr* were identified in the genome of *E. bugandensis* S68-1. The underlying mechanisms of polymyxin B resistance in *E. bugandensis* S68-1 remain unknown. Other research suggested that the two-component system PhoPQ regulates 4-amino-4-deoxy-L-arabinose (L-Ara4N) modification of lipid A, contributing to polymyxin resistance in *E. cloacae* ATCC 13047 [8].

In summary, carbapenem-resistant *E. bugandensis* S68-1 was isolated from a retail vegetable and was characterised. Production of IMI-1 serine carbapenemase might be responsible for the resistance to imipenem. To the best of our knowledge, this is the first report describing the isolation and characterisation of a carbapenem- and polymyxin B-resistant bacterium in a vegetable product in the USA. A limitation of the study is the relatively small sampling number. Our findings highlight the need for antimicrobial resistance surveillance in fresh produce products.

GenBank nucleotide sequence

The whole-genome shotgun project has been deposited in GenBank under the accession no. [PRJNA705736](#).

Acknowledgment

The authors thank the Genomics Core Laboratory at the University of Arkansas for Medical Sciences for sequencing the bacterial genome.

Funding

This work was supported in part by awards to EH by Arkansas Biosciences Institute, the major research component of the Arkansas Tobacco Settlement Proceeds Act of 2000. The work was supported in part by awards to SRJ through the Translational Research Institute [grant UL1 TR003107] through the National Institutes of Health (NIH) National Center for Advancing Translational Sciences. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This research was supported in part by awards to SRJ through the National Science Foundation [Award No. OIA-1946391]. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation.

Competing interests

None declared.

Ethical approval

Not required.

References

- [1] Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;17:7–15. doi:10.3201/eid1701.P11101.
- [2] Berger CN, Soda SV, Shaw RK, Griffin PM, Pink D, Hand P, et al. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ Microbiol* 2010;12:2385–97. doi:10.1111/j.1462-2920.2010.02297.x.
- [3] Tatusova T, DiCuccio M, Badretdin A, et al. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 2016;44:6614–24. doi:10.1093/nar/gkw569.
- [4] Das B, Martínez E, Midonet C, Barre FX. Integrative mobile elements exploiting Xer recombination. *Trends Microbiol* 2013;21:23–30. doi:10.1016/j.tim.2012.10.003.
- [5] Brouwer MSM, Rapallini M, Geurts Y, Harders F, Bossers A, Mevius DJ, et al. *Enterobacter cloacae* complex isolated from shrimps from Vietnam carrying *bla_{IMI-1}* resistant to carbapenems but not cephalosporins. *Antimicrob Agents Chemother* 2018;62:e00398 -18. doi:10.1128/AAC.00398-18.
- [6] Janecko N, Martz SL, Avery BP, Daignault D, Desrusseau A, Boyd D, et al. Carbapenem-resistant *Enterobacter* spp. in retail seafood imported from Southeast Asia to Canada. *Emerg Infect Dis* 2016;22:1675–7. doi:10.3201/eid2209.160305.
- [7] Norgan AP, Freese JM, Tuin PM, Cunningham SA, Jeraldo PR, Patel R. Carbapenem- and colistin-resistant *Enterobacter cloacae* from Delta, Colorado. 2015. *Antimicrob Agents Chemother* 2016;60:3141–4. doi:10.1128/AAC.03055-15.
- [8] Kang KN, Klein DR, Kazi MI, Guérin F, Cattoir V, Brodbelt JS, et al. Colistin heteroresistance in *Enterobacter cloacae* is regulated by PhoPQ-dependent 4-amino-4-deoxy-L-arabinose addition to lipid A. *Mol Microbiol* 2019;111:1604–16. doi:10.1111/mmi.14240.

Sun Hee Moon

Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AR, 72205, USA

Zulema Udaondo

Department of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA

Xinhui Li

Department of Microbiology, University of Wisconsin-La Crosse, La Crosse, WI, 54601, USA

Xu Yang

Department of Nutrition and Food Science, California State Polytechnic University, Pomona, CA, 91768, USA

Se-Ran Jun

Department of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR, 72205, USA

En Huang*

Department of Environmental and Occupational Health, University of Arkansas for Medical Sciences, 4301 West Markham Street, Little Rock, AR, 72205, USA

*Corresponding author. Tel.: +1 501 526 6627.
E-mail address: ehuang@uams.edu (E. Huang)

Revised 13 July 2021