Journal of Global Antimicrobial Resistance 29 (2022) 113–115

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Genome Note

Phylogenomic analysis of CTX-M-15–producing *Enterobacter hormaechei* belonging to the high-risk ST78 from animal infection: another successful One Health clone?

Brenda Cardoso[a,b,1], Fábio P. Sellera[a,c,d,1], Elder Sano[a,b], Fernanda Esposito[a,e], Lourdes A.V. Seabra[d], Milton R. Azedo[d], Fabio C. Pogliani[c], Nilton Lincopan[a,b,e,*]

[a] One Health Brazilian Resistance Project (OneBR), Brazil
[b] Department of Microbiology, Instituto de Ciências Biomédicas, University of São Paulo, São Paulo, Brazil
[c] Department of Internal Medicine, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil
[d] School of Veterinary Medicine, Metropolitan University of Santos, Santos, Brazil
[e] Department of Clinical Analysis, School of Pharmacy, University of São Paulo, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 21 December 2021
Revised 4 February 2022
Accepted 12 February 2022
Available online 18 February 2022

Edited by: Prof Marco R Oggioni

Keywords:
Enterobacterales
ESBL
Dairy cattle
Antimicrobial resistance
Genomic surveillance
Brazil

ABSTRACT

Objectives: Extended-spectrum β-lactamase (ESBL)–producing *Enterobacter cloacae* complex (ECC) members have been a leading cause of severe infections in hospital setting and have lately been recognized as important pathogens for animals. In this article, we report phylogenomic data of a multidrug-resistant and CTX-M-15–positive *E. hormaechei* belonging to ST78 isolated from a calf with omphalitis.
Methods: Genomic DNA was extracted and sequenced using the Illumina NextSeq platform. *De novo* assembly was performed by Unicycler and *in silico* prediction accomplished by curated bioinformatics tools. Single nucleotide polymorphism (SNP)-based comparative phylogenomic analysis was conducted by using publicly available ECC genomes belonging to ST78.
Results: The genome size was calculated at 3 8465 40 bp, comprising 4717 total genes, 3 rRNAs, 43 tRNAs, 7 ncRNAs, and 74 pseudogenes. The animal-associated *E. hormaechei* (ECBEZ strain) ST78 harboured the $bla_{CTX\text{-}M\text{-}15}$ ESBL gene in addition to other critically important resistance genes conferring resistance to β-lactams, aminoglycosides, fosfomycin, phenicol, quinolones, sulphonamides, tetracyclines, and trimethoprim. Phylogenetic analysis revealed that ECBEZ is closely related to human-isolated strains from Asian and African countries.
Conclusion: Phylogenomic analysis of CTX-M-15-producing *E. hormaechei* from animal infection reveals that ST78 is a successful One Health clone among ECC members. Furthermore, data presented in this study reinforce the urgent need to monitor ESBL-producing ECC members in veterinary settings.

1. Introduction

Enterobacter cloacae complex (ECC) members are common nosocomial pathogens that cause a broad spectrum of life-threatening infections, being frequently associated with resistance to clinically important antibiotics, including β-lactams [1]. The ECC has shown genomic heterogeneity and currently comprises seven species: *Enterobacter asburiae, Enterobacter carcinogenus, Enterobacter cloacae, Enterobacter hormaechei, Enterobacter kobei, Enterobacter nimipressuralis,* and *Enterobacter mori* [2]. The World Health Organization has recently classified carbapenemase- and/or extended-spectrum β-lactamase (ESBL)-producing ECC as critical priority pathogens [3]. Particularly, ECC strains belonging to sequence types (STs) ST78 and ST171 are considered emergent and high-risk clones responsible for the spread of clinically important antimicrobial resistance genes [4].

Omphalitis is a generic term to encompass all inflammatory disorders that affect the extra-abdominal portion of the umbilicus. In dairy farms, it frequently affects calves on the first 30 days of life,

* Corresponding author at: Instituto de Ciências Biomedicas, Universidade de São Paulo. Av. Prof. Lineu Prestes 1374, Butantã, São Paulo, 05508-000, Brazil
E-mail address: lincopan@usp.br (N. Lincopan).
[1] These authors have contributed equally to this work.

https://doi.org/10.1016/j.jgar.2022.02.010

as a result of contamination by bacteria found in the environment with further infection via the umbilical cord. Herein, we present genomic and phylogenomic data of a CTX-M-15-producing *E. hormaechei* strain belonging to the global ST78 isolated from a calf with omphalitis in Brazil.

2. Methods

In January 2019, a 15-day-old female Holstein calf was diagnosed with omphalitis at a dairy farm in Pirassununga city, southeast Brazil. Clinically, the calf presented with weight loss and mild dehydration, but umbilical inflammation, swelling, purulent and malodorous discharge, and pain on palpation of the umbilical region were also observed. Veterinary medical records revealed that the animal did not receive any antibiotic treatment. A swab sample was collected from the umbilicus and submitted to microbiological culture. A Gram-negative bacilli (ECBEZ strain) was recovered and initially identified as ECC by using MALDI-TOF/MS. The ECBEZ strain displayed a multidrug-resistant (MDR) profile to amoxicillin/clavulanic acid (MIC ≥32 μg/mL), ampicillin/sulbactam (MIC ≥32 μg/mL), piperacillin/tazobactam (MIC ≥128 μg/mL), cefoxitin, ceftiofur, ceftriaxone (MIC ≥64 μg/mL), cefotaxime (MIC ≥32 μg/mL), ceftazidime (MIC ≥64 μg/mL), cefuroxime (MIC ≥64 μg/mL), cefepime (MIC ≥64 μg/mL), aztreonam, amikacin (MIC ≥64 μg/mL), gentamicin (MIC ≥16 μg/mL), nalidixic acid (MIC ≥32 μg/mL), ciprofloxacin (MIC ≥4 μg/mL), enrofloxacin, norfloxacin (MIC ≥16 μg/mL), levofloxacin, trimethoprim/sulfamethoxazole (MIC ≥320 μg/mL), tetracycline, and ampicillin (MIC ≥32 μg/mL), remaining susceptible to fosfomycin, ertapenem (MIC ≤0.5 μg/mL), imipenem (MIC ≤0.25 μg/mL), and meropenem (MIC ≤0.25 μg/mL). The antimicrobial susceptibility tests were determined by the Kirby–Bauer disk diffusion and Vitek 2 system (bioMérieux, France), according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2020).

Genomic DNA of the ECBEZ strain was extracted using PureLink™ Quick Gel Extraction kit (Life Technologies, Carlsbad, CA, USA), and Qubit 2.0 fluorometer (Life Technologies, Carlsbad, CA, USA) was used to measure DNA concentration. Library preparation was performed using the Nextera DNA Flex kit (Illumina, San Diego, CA, USA) and sequenced by using NextSeq550 platform (Illumina) paired-end reads (2 × 75 bp) (Illumina, San Diego, CA, USA). Reads with a PHRED quality score below 20 were discarded, and adapters were trimmed using Trim Galore v. 0.6.5 (https://github.com/FelixKrueger/TrimGalore). *De novo* genome assembly was performed with Unicycler v. 0.4.8 (https://github.com/rrwick/Unicycler). Sequences were annotated using NCBI Prokaryotic Genome Annotation Pipeline version 4.10 (http://www.ncbi.nlm.nih.gov/genome/annotation_prok/).

The genome size was calculated at 3 846 540 bp, comprising 4717 total genes, 3 rRNAs, 43 tRNAs, 7 ncRNAs, and 74 pseudogenes. Multilocus sequence typing analysis by MLST 2.0 (https://cge.cbs.dtu.dk/services/MLST/) revealed that the *E. hormaechei* ECBEZ strain belonged to ST78, which has been recognized as a high-risk clone of global interest, being increasingly reported in nosocomial infections in Europe and Asia, associated with ESBL (CTX-M-15) and/or carbapenemase production [4,5].

To assess the phylogenetic relationship, we downloaded 1468 *E. hormaechei* genome assemblies from NCBI datasets with data for country, source, and collection date. MLST v2.11 (https://github.com/tseemann/mlst) was used to identify the ST of each isolate, and all ST78 assemblies (n = 50) were used for comparative analysis. A maximum-likelihood tree based on single nucleotide polymorphism (SNP) alignment was generated using default settings of CSI Phylogeny (https://cge.cbs.dtu.dk/services/CSIPhylogeny). The chromosome sequence of *E. hormaechei* ST78 RIVM_C010068 strain

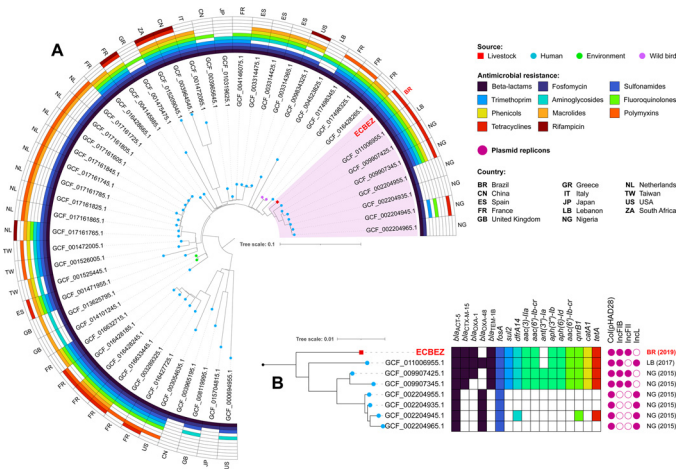

Fig. 1. (A) Single nucleotide polymorphism (SNP)-based phylogenetic tree of 51 *Enterobacter hormaechei* ST78 strains globally identified from human, environment, and wild bird hosts and their predicted phenotypes. The ECBEZ strain clustered (56–68 SNP differences) with seven *Enterobacter hormaechei* ST78 strains isolated from human samples, in Lebanon and Nigeria. (B) Heatmap, including resistome and plasmidome of the *Enterobacter hormaechei* cluster highlighted in (A). The ECBEZ livestock strain analysed in this study (accession number: JAINUR000000000) is represented by red colour. ISO 3166-1 Alpha-2 country codes were used.

(RefSeq assembly accession number: NZ_CP071025.1) was used as a reference. iTOL v. 6 (https://itol.embl.de) was used to annotate the phylogenetic tree with data from ABRicate and NCBI BioSamples. A phylogenetic tree revealed that ECBEZ is genomically related (56-68 SNP differences) to human strains from Lebanon and Nigeria (Fig. 1, Supplementary Table S1).

ECBEZ resistome analysis identified several resistance determinants, encoding resistance to β-lactams [$bla_{\text{ACT-5}}$, $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, and $bla_{\text{TEM-1B}}$], aminoglycosides [*aac(3)-IIa, aph(6)-Id*, and *aph(3′)-Ia*], fosfomycin [*fosA*], phenicol [*catA1*], quinolones [*qnrB1, aac(6')Ib-cr, oqxA* and *oqxB*], sulphonamides [*sul2*], tetracyclines [*tetA*], and trimethoprim [*dfrA14*], which were identified by ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder/), with a 90% threshold for gene identity and coverage. Plasmid incompatibility (Inc) types IncFII, IncFIB, and Col440I were detected using PlasmidFinder 2.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder/) (Fig. 1).

In summary, we report genomic data of a MDR CTX-M-15–producing *E. hormaechei* ST78 isolated from an infected calf in Brazil. The global spread of *E. hormaechei* ST78 carrying broad resistome at the One Health interface might be considered a serious epidemiological issue that requires close surveillance [5]. Our data could be helpful for comparative genomic analyses of *E. hormaechei* ST78 strains that have been emerging globally, highlighting that surveillance of WHO critical priority bacteria isolated from food-producing animals is an urgent demand.

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAINUR000000000. The version described in this article is JAINUR010000000.

Funding

This work was supported, in whole or in part, by the Bill and Melinda Gates Foundation (Grand Challenges Explorations Brazil OPP1193112). Under the grant conditions of the Foundation, a CC BY or equivalent license is applied to the Author Accepted Manuscript version arising from this submission. Additionally, this study was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (2020/08224-9, 2019/15778-4), Con-

selho Nacional de Desenvolvimento Científico e Tecnológico (AMR 443819/2018-1, 312249/2017-9, 422984/2021-3 and 314336/2021-4), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (88882.333054/2019-01).

Competing Interests

None declared.

Ethical approval

Not required.

Acknowledgements

Bill and Melinda Gates Foundation, FAPESP, CAPES, and CNPq research grants are gratefully acknowledged. N.L. is a research fellow of CNPq (314336/2021-4). We thank Silvia R. Santos and Angélica J. Balabakis for MIC assay and Cefar Diagnóstica Ltda. (São Paulo, Brazil) and CEFAP-GENIAL facility for kindly supplying antibiotic discs for susceptibility testing and Illumina sequencing, respectively.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.02.010.

References

[1] Annavajhala MK, Gomez-Simmonds A, Uhlemann AC. Multidrug-resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. Front Microbiol 2019;10:44. doi:10.3389/fmicb.2019.00044.
[2] Davin-Regli A, Lavigne JP, Pagès JM. *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin Microbiol Rev 2019;32:e00002–19. doi:10.1128/CMR.00002-19.
[3] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. WHO Pathogens Priority List Working Group. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis 2018;18:318–27. doi:10.1016/S1473-3099(17)30753-3.
[4] Gomez-Simmonds A, Annavajhala MK, Wang Z, Macesic N, Hu Y, Giddins MJ, et al. Genomic and geographic context for the evolution of high-risk carbapenem-resistant *Enterobacter cloacae* complex clones ST171 and ST78. mBio 2018;9 e00542-18. doi:10.1128/mBio.00542-18.
[5] Villa J, Carretero O, Viedma E, Lora-Tamayo J, Mingorance J, Chaves F. Emergence of NDM-7-producing multi-drug-resistant *Enterobacter hormaechei* sequence type ST-78 in Spain: a high-risk international clone. Int J Antimicrob Agents 2019;53:533–4. doi:10.1016/j.ijantimicag.2018.11.009.