ACCESS MICROBIOLOGY
an open research platform

SHORT COMMUNICATION
David *et al.*, *Access Microbiology* 2023;5:000565.v3
DOI 10.1099/acmi.0.000565.v3

MICROBIOLOGY SOCIETY
OPEN DATA OPEN ACCESS

Draft genome sequence of *Enterobacter cloacae* ST473 harbouring *bla*CMH-3 isolated from a human patient diagnosed with recurrent bacteriuria in Nigeria

Ebuka Elijah David[1,2,*], Ikechuku Okorie Igwenyi[3], Ifeanyichukwu Romanus Iroha[4], Layla Farage Martins[2], Guillermo Uceda-Campos[2] and Aline Maria da Silva[2]

Abstract

Enterobacter cloacae is among the most frequently isolated species described in clinical infections and is commonly associated with a multidrug resistance (MDR) phenotype. We present the draft genome sequence of a MDR *E. cloacae* isolated in Nigeria from the urine sample of an adult male outpatient diagnosed with symptomatic recurrent bacteriuria. The isolate was found to be resistant to ceftriaxone, cefotaxime, cefepime and levofloxacin. Genome analysis revealed the presence of the beta-lactamase chromosomal gene *bla*CMH-3, which may be responsible for the antibiotic resistance observed in the recurrent *E. cloacae* urinary tract infection.

DATA SUMMARY

The first version of draft genome assembly has been deposited in DDBJ/ENA/GenBank under the accession number JAPOMC000000000 (BioProject accession number PRJNA864081 and BioSample accession number SAMN30073201). The raw sequence reads have been deposited in the Sequence Read Archive (SRA) under the accession number SRR20725478. The 16S rRNA sequence was deposited with GenBank accession number OP932952. Modified double disc synergy tests showing synergy of the zone of inhibition between amoxicillin-clavulanic acid (AMC) and the cephalosporins are shown in the Supplementary Material.

ANNOUNCEMENT

Enterobacter cloacae complex is among the *Enterobacter* species most frequently described in clinical infections, particularly in immune-compromised patients and those hospitalized in intensive care units. *Enterobacter* spp. clinical isolates are commonly associated with a multidrug resistance (MDR) phenotype, which makes their treatment challenging. Isolates from *Enterobacter* species are reported to present intrinsic resistance to ampicillin, amoxicillin-clavulanic acid, first- and second-generation cephalosporins due to the chromosomal encoded AmpC beta-lactamase [1]. Furthermore extended-spectrum beta-lactamases (ESBL) have also been reported in *Enterobacter* species [1, 2]. Analysis of 272 *E. cloacae* genome assemblies belonging to a variety of STs showed that almost all of them encode a β-lactamase gene with *bla*CMH and *bla*ACT being the main *bla*AmpC genes [2]. Among the *bla*CMH genes found in this 272-genome dataset, *bla*CMH-6 (*n*=41), *bla*CMH-4 (*n*=26), *bla*CMH-5 (*n*=21), *bla*CMH-3 (*n*=21) and *bla*CMH-1 (*n*=16) were the most prevalent [2]. *bla*CMH-1 has been identified as plasmid-mediated β-lactamase gene in *E. cloacae* clinical isolates collected in Southern Taiwan in 2015 [3]. *bla*CMH-3 was identified as a chromosome encoded gene in a *E. cloacae* clinical isolate assigned to ST932 harbouring a plasmid-mediated *bla*NDM-1, which was collected in Spainin 2016 from a Ukraine patient [4]. To our knowledge, no other clinical cases of *E. cloacae* harbouring *bla*CMH-3 has been reported

Received 18 January 2023; Accepted 16 June 2023; Published 06 July 2023
Author affiliations: [1]Department of Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Nigeria; [2]Department of Biochemistry, Institute of Chemistry, University of Sao Paulo, Sao Paulo, Brazil; [3]Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria; [4]Department of Microbiology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.
***Correspondence:** Ebuka Elijah David, david.ebuka@funai.edu.ng
Keywords: *Enterobacter cloacae*; extended-spectrum beta-lactamases; urinary tract infection.
Abbreviations: ESBL, extended-spectrum beta-lactamases; MDR, multidrug resistance; MIC, minimum inhibitory concentration; ST, sequence type; ST, multi-locus sequence typing; TSB, trypticase soy broth; UTI, urinary tract infection.
One supplementary figure is available with the online version of this article.

despite the detection of this *bla*AmpC variant in several publicly available *E. cloacae* genomes [2]. Here we report the draft genome sequence of an *E. cloacae* isolate collected in March 2021 at the Laboratory unit of Alex Ekwueme Federal Teaching Hospital Abakaliki, Ebonyi State, Nigeria from the urine sample of an adult male outpatient with recurrent urinary tract infection (UTI).

The isolate was collected, stored in nutrient broth with glycerol. Antibiotic susceptibility testing was performed by the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2021 on Muller–Hinton (MH) agar [5]. After 24 h of growth at 37 °C, the isolate was evaluated for its susceptibility to ciprofloxacin (5 µg), levofloxacin (5 µg), cefepime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), amoxicillin-clavulanic acid (20/10 µg), ampicillin (10 µg), imipenem (10 µg) and fosfomycin (200 µg). A modified DDST (modified double disc synergy test) with the use of cefepime was also performed to improve the detection of ESBL-producing isolates, which co-produce AmpC [6] (Fig. S1, available in the online version of this article). The isolate was found to be resistant to the two third generation cephalosporins (ceftriaxone and cefotaxime) and showed intermediate resistance (MIC of 15 µg ml^{-1} with zone of inhibition of 18.4 mm ±0.01) to cefepime, a fourth-generation cephalosporin. The isolate was susceptible to amoxicillin-clavulanic acid and imipenem, and resistant to ampicillin. Regarding the fluoroquinolones, the isolate showed intermediate resistance to ciprofloxacin (MIC of 0.5 µg ml^{-1} with zone of inhibition of 23 mm ±0.00) and resistance to levofloxacin. We also verified that the isolate showed resistance to fosfomycin.

The isolate was cultured in Tryptic Soy Agar (TSB) broth and 0.5 ml was used for genomic DNA extraction with the Wizard Genomic Purification kit (Promega, USA). Purified DNA was used as a template for 16S rRNA gene amplification with universal primers 27F (5′AGAGTTTGATCMTGGCTCAG-3′) and 1525R (5'- AAGGAGGTGWTCCARCCGCA-3′) as previously described [7]. The amplicon was subjected to sequencing reactions using Big Dye terminator v3.1 cycle sequencing kit (ThermoFisher Scientific) with 27F or 1525R as sequencing primers and then injected on an ABI PRISM 3130XL genetic analyser (ThermoFisher Scientific). The obtained sequences were used to assemble a 16S RNA 1546 bp sequence, which was then BLASTn searched against the GenBank/NCBI database allowing taxonomic identification of the clinical isolate as *E. cloacae* (100% identity, 100% query cover).

Whole-genome sequencing of the *E. cloacae* isolate was performed on Illumina MiSeq platform (Illumina, CA, USA) at the Center for Advanced Technologies in Genomics (CATG), Institute of Chemistry, University of Sao Paulo, using the genomic DNA purified with Wizard Genomic Purification kit (Promega, USA) DNA. The shotgun genomic library was prepared using the Nextera DNA Library Prep (Illumina) with a total DNA input of ~20 ng and subjected to a run using an Illumina MiSeq Reagent Kit v3 (2×300 cycles). The reads were checked for quality phred=30 and filtered using Fastp v0.12.4 [8]. Filtered rawR1 reads were used for *de novo* assembly using SPAdes v3.15.4 [9]. Assembly completeness and contamination were verified with CheckM v. v1.2.0 [10] and the assembly quality was evaluated with QUAST v5.0.2 [11]. The assembled genome was annotated using PROKKA v.1.14.6 [12]. General features of the genome assembly and annotation are presented in Table 1.

Sequence type was defined using MLST v 2.0.9 and multi locus sequence typing (MLST) allele sequences and profile data obtained from PubMLST.org [13, 14]. ResFinderFG v 2.0 was used to identify antibiotic resistance determinants [15]. MobileElementFinder v 1.0.3 and ISfinder were used to identify insertion sequences and mobile genetic elements and their relation to antimicrobial resistance genes and virulence factors [16, 17]. Table 2 lists the antibiotics resistance genes (ARGs) detected in the *E. cloacae* draft genome. It is worth noting a chromosomal *blaCMH-3* gene with 100% identity and 100% coverage to the reference gene. Hence, this is the first time multidrug-resistant *E. cloacae* with an ESBL phenotype harbouring a single narrow spectrum *blaCMH-3* beta-lactamase is reported in Nigeria. A fosfomycin resistance gene *fosA* was also detected. Genes of the RND (resistance nodulation division) drug efflux system, *OqxAB*, related to fluoroquinolone resistance was also observed. The *E. cloacae* isolate seems to carry a plasmid without antibiotic resistance genes, which is similar to plasmid ColRNAI originally found in *Klebsiella pneumoniae*

Table 1. General features of the *E. cloacae*draft genome

Assembly							Annotation		
Contigs number	**Estimated chromosome length (bp)**	**N50 (bp)**	**GC (%)**	**Completeness**	**Contamination**	**Sequencing coverage**	**CDSs**	**tRNAs**	**rRNA operons**
15	5012585	857935	55.12	99.97	0.33	125x	4657	72	8

Table 2. Antibiotic resistance genes detected in *E. cloacae* genome assembly

Gene	Description	Protein identity %	Query coverage %	Reference accession no.
*bla*CMH-3	Cephalosporin-hydrolysing class C beta-lactamase CMH-3	100	100	WP_045295573.1
*fos*A	fosfomycin resistance glutathione transferaseFosA	100	100	AFM58300.1
OqxAB	multidrug efflux RND permease	100	100	WP_262761476.1

strains where it encodes carbapenems resistance genes [18]. The genomic features of *E. cloacae* clinical isolate described here suggest that a single AmpC beta-lactamase may be responsible for a broader extended spectrum beta-lactamase drug resistance. Despite the cost associated to whole-genome sequencing of clinical isolates, which might be prohibitive in regions with limited resources, elucidating the genetic basis of resistance is highly valuable to enable initial antibiotic selection to minimize use of ineffective antibiotics [19]. Based solely on the susceptibility testing phenotypic assays, a successful treatment of the recurrent UTI reported in this study could be achieved with amoxicillin-clavulanic acid. However, in vitro susceptibility does not necessarily translate to in vivo susceptibility. This may be related to the current clinical practice of high amoxicillin-to-clavulanate ratios, which has been found to result in the most rapid adaptation to antibiotics via gene dosing responses [20].

Funding information
E.E. David was the recipient of the Arturo Falaschi ICGEB Fellowship and Grant (S_NGA20-01-1) from the International Centre for Genetic Engineering and Molecular Biology (ICGEB). The funder had no role in study design, data collection, and analysis; in the decision to publish; or in the preparation of the manuscript.

Author contributions
Conceptualization, E.E.D, I.O.I and I.R.I.; methodology, E.E.D and L.F.M and A.M.d.S.; formal analysis, E.E.D and G.U.; investigation: E.E.D and L.F.M.; writing – original draft preparation, E.E.D.; writing – review and editing, E.E.D and A.M.d.S.; supervision, A.M.d.S, I.O.I and I.R.I.; funding acquisition, E.E.D. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
As this study involved urine samples collected in the routine of the hospital clinic it was exempted from ethical approval according to the section B, item 'c' of the guidelines of National Health Research Ethics Committee of Nigeria (NHREC) available at http://nhrec.net/nhrec/NCHRE_10.pdf.

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:e00002-19.
2. Hu J, Li J, Liu C, Zhang Y, Xie H, *et al*. Molecular characteristics of global β-lactamase-producing Enterobacter cloacae by genomic analysis. *BMC Microbiol* 2022;22:255.
3. Ku Y-H, Lee M-F, Chuang Y-C, Yu W-L. Detection of plasmid-mediated β-Lactamase genes and Emergence of a novel AmpC (CMH-1) in *Enterobacter cloacae* at a medical center in Southern Taiwan. *J Clin Med* 2018;8:8.
4. López-Hernández I, García Barrionuevo A, Díaz de Alba P, Clavijo E, Pascual A. Characterization of NDM-1- and CMH-3-producing *Enterobacter cloacae* complex ST932 in a patient transferred from Ukraine to Spain. *Enferm Infecc Microbiol Clin* 2020;38:327–330.
5. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100, 31st edition. *J Clin Microbiol* 2021;59:e0021321.
6. Kaur J, Chopra S, Mahajan G, Sheevani. Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. *J Clin Diagn Res* 2013;7:229–233.
7. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, *et al*. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013;41:e1.
8. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 2018;34:i884–i890.
9. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
10. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;25:1043–1055.
11. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 2013;29:1072–1075.
12. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
13. Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018;3:124.
14. Wee SK, Yap EPH. GALAXY workflow for bacterial next-generation sequencing De Novo assembly and annotation. *Curr Protoc* 2021;1:e242.
15. Gschwind R, Perovic SU, Weiss M, Petitjean M, Lao J, *et al*. ResFinderFG v2.0: a database of antibiotic resistance genes obtained by functional metagenomics. *bioRxiv* 2022.
16. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res* 2006;34:D32–D36.
17. Johansson MHK, Bortolaia V, Tansirichaiya S, Aarestrup FM, Roberts AP, *et al*. Detection of mobile genetic elements associated with antibiotic resistance in *Salmonella enterica* using a newly developed web tool: MobileElementFinder. *J Antimicrob Chemother* 2021;76:101–109.
18. Yu X, Zhang W, Zhao Z, Ye C, Zhou S, *et al*. Molecular characterization of carbapenem-resistant *Klebsiella pneumoniae* isolates with focus on antimicrobial resistance. *BMC Genomics* 2019;20:822.
19. Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, *et al*. Molecular mechanisms of antibiotic resistance revisited. *Nat Rev Microbiol* 2023;21:280–295.
20. Allen RC, Brown SP. Modified antibiotic adjuvant ratios can slow and steer the evolution of resistance: co-amoxiclav as a case study. *mBio* 2019;10:e01831-19.

Peer review history

VERSION 2

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000565.v2.5

Nihal Bandara; University of Bristol, Bristol Dental School, Lower Maudlin Street, UNITED KINGDOM, Bristol

Date report received: 16 June 2023
Recommendation: Accept

Comments: This is a study that would be of interest to the field and community.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000565.v2.4

Ángel Rodríguez-Villodres; Hospital Universitario Virgen del Rocío, Microbiology, Sevilla, SPAIN
https://orcid.org/0000-0001-6373-9724

Date report received: 14 June 2023
Recommendation: Accept

Comments: Thank you for considering my recommendations.

Please rate the manuscript for methodological rigour
Good

Please rate the quality of the presentation and structure of the manuscript
Good

To what extent are the conclusions supported by the data?
Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000565.v2.3

Shobha Gunathilaka; Rajarata University of Sri Lanka Faculty of Medicine and Allied Sciences, Department of Microbiology, Anuradhapura, SRI LANKA
https://orcid.org/0000-0002-0367-302X

Date report received: 12 June 2023
Recommendation: Accept

Comments: The authors have effectively addressed the feedback provided during the initial review. Given that this article presents a concise communication announcing the genome of a clinically significant bacterium, I highly recommend its publication in the journal. However, I do suggest further refinement of the language to enhance the clarity of the message through a thorough language review.

Please rate the manuscript for methodological rigour
Good

Please rate the quality of the presentation and structure of the manuscript
Satisfactory

To what extent are the conclusions supported by the data?
Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

SciScore report

https://doi.org/10.1099/acmi.0.000565.v2.1

iThenticate report

https://doi.org/10.1099/acmi.0.000565.v2.2

Author response to reviewers to Version 1

May 10, 2023

Dear Dr. Dr Nihal Bandara

Editor, Access Microbiology

Microbiology Society

Thank you for your email of Apr 14, 2023. We thank you and the Reviewers for the time taken to read our manuscript and for the insightful comments and valuable suggestions that were provided which were helpful for improving the manuscript.

We believe we have responded and clarified all the points raised by the reviewers. The changes we have made are highlighted in blue in the manuscript file as well as duly addressed in our point-by-point responses to the reviewers. As a result, this version of the manuscript is an improvement with respect to the previous one.

We hope the changes and corrections we have made are satisfactory. We look forward to hearing from you as to the suitability of our manuscript for publication.

Sincerely,

Ebuka Elijah David

Point-by-point responses to the Reviewer´s comments on the manuscript #ACMI-D-22-00218 entitled "Draft genome sequence of *Enterobacter cloacae*ST473 harbouring blaCMH-3 isolated from a human patient diagnosed with recurrent bacteriuria in Nigeria"

In the point-by-point responses, the line numbers refer to the revised manuscript (word file). The changes in the manuscript text are highlighted in blue. Below we provide our responses to the comments made by the Reviewers. The comments by the Reviewers are in normal fontand our responses are in italics.

Reviewer 1

Comments to Author: I have some recommendations:

Abstract:

The authors say "asimptomatic bacteriuria" in the abstract, and "urinary tract infection" (line 60) in the main text. This is not the same. Which is the correct one?

R:*We thank the reviewer for bringing this error to our attention. The abstract has been corrected to "symptomatic bacteriuria" (please see line 34) which was as a result of UTI.*

Announcement:

Line 40: Enterobacter cloacae complex (italiziced)

R:*Corrected as suggested(please see line 41)*

Line 44: amoxicillin (with clavulanic acid?)

R:*We apologize for the mistake. We have corrected to amoxicillin-clavulanic acid and added a reference for this information (please see lines 46-47).*

Line 45: Please, remove the sentence "as well as to the acquisition of numerous genetic mobile...."

R:*Thesentence "as well as to the acquisition of numerous genetic mobile elements containing resistance genes" was removed as suggested (please see line 47).*

Line 65: What year are the CLSI guides used? Please, add it.

R:*The year (2021) has been added in the text (please see line 65).*

Line 69: I don't see the result in the text of the modified DDST. Please, could you add it?

R:*As requested, the result*of the modified DDST*has been added as supplementary file (Supplementary Figure S1)(please see line71). The modified DDST is a phenotypic evidence of an ESBL-producing isolate which co-produces blaAmpC. This observation was later confirmed through the draft genome sequencing and found to be blaCMH-3.*

Line 72: In the "Intermediate resistance to cefepime", please, add the MIC to cefepime.

R:*The MICto cefepime (15µg/ml with zone of inhibition of 18.4mm±0.01) has been added(please see lines72-73).*

Line 73: The isolate was susceptible to amoxicillin-clavulanic acid?? E. cloacae has intrinsic resistance to this antibiotic. Please, could you explain it?

R:*The reviewer is correct in pointing out thatE. cloacaes hows intrinsic resistance to amoxicillin-clavulanic acid. According to Davin-Regli et al 2019 (doi:10.1128/CMR.00002-19),E.cloacaeisolates are reported to present intrinsic resistance to ampicillin, amoxicillin-clavulanic acid and to first and second generation cephalosporins dueto the chromosomal encoded AmpC beta-lactamase. We have revised the sentence in lines 45-46 to clarify this information. Nevertheless, the E.cloacaeisolate described in this work is susceptible amoxicillin-clavulanic acid and imipenem, and resistant to ampicillin mentioned as mentioned in lines 74-75. See also the result of the modified DDSTpresented in Supplementary Figure S1.*

Line 75: It is unusual resistance to levofloxacin and intermediate resistance to ciprofloxacin. Please, confirm it and add the MIC to both antibiotics.

R:*We have confirmed the resistance of theE.cloacaeisolate to levofloxacin and intermediate resistance to ciprofloxacin. The respective MIC was added (please see line 76). It should be noted that both levofloxacin and ciprofloxacin are recommended for clinical application in UTIs, despite evidence that uropathogens are more sensitive to ciprofloxacin(Afriyie et al., 2018;Humphries et al., 2019; Cao et al., 2021).*

Afriyie D. K., Adu L. B., Dzradosi M., Amponsah S. K., Ohene-Manu P., Manu-Ofei F. (2018). Comparative in vitro activity of ciprofloxacin and levofloxacin against isolated uropathogens in Ghana: a pilot study. Pan Afr. Med. J. 30, 194. Doi: 10.11604/pamj.2018.30.194.15457.

Humphries R. M., Hindler J. A., Shaffer K., Campeau S. A. (2019). Evaluation of ciprofloxacin and levofloxacin disk diffusion and etest using the 2019 Enterobacteriaceae CLSI breakpoints. J. Clin. Microbiol. 57 (3). Doi: 10.1128/JCM.01797-18.

Cao D, Shen Y, Huang Y, Chen B, Chen Z, Ai J, Liu L, Yang L, Wei Q. Levofloxacin Versus Ciprofloxacin in the Treatment of Urinary Tract Infections: Evidence-Based Analysis. Front Pharmacol. 2021; 12:658095. Doi:10.3389/fphar.2021.658095.

Line 109: ESBL phenothype? Is this correct? blaCMH-3 is a class C betalactamase that confers a AmpC phenotype.

R: *This is correct. The organism showed phenotypic evidence of ESBL productionbecause of resistance to the beta-lactams up to the third generation (see lines 72-73) and an additional phenotype for AmpC-production as in a synergy between the third generation beta-lactams and cefepime, the fourth-generation. The presence of AmpC-lactamase was also phenotypically seen in the unusual intermediate resistance to cefepime and confirmed by whole genome sequencing as chromosomally encoded blaCMH-3 (lines 72-73 and 110).*

Reviewer 2

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?

Reviewer 2: No: Although a human biological sample is used no identification details mentioned. It has been mentioned that Nigerian guidelines states ethical approval is not necessary, anyway, its better if the authors have given reference ti the said guidelines.

R:*As requested we have added reference to the guidelines of National Health Research Ethics Committee of Nigeria (NHREC)that inform the research activities in which the involvement of human participants are exempt from health research ethics committee oversight, such as those involving the collection or study of diagnostic specimens if the information is recorded by the investigator in such a manner that participants cannot be identified, directly or through identifiers linked to the participants (please see the revised text in lines 158-159).*

Reviewer 2 Comments to Author: The article is a genome announcement. The use of genome analysis to identify the resistance mechanism is a commendable approach, as it provides a detailed understanding of the genetic basis of resistance.The presence of the beta-lactamase chromosomal gene blaCMH-3 is an important finding, as it sheds light on the mechanism of resistance to beta-lactam antibiotics in this isolate.

The findings of this study could have implications for the management of patients with recurrent urinary tract infections caused by MDR E. cloacae, particularly in regions with limited resources for microbiological testing and antibiotic susceptibility testing.

The article does not discuss any potential limitations of the study, such as selection bias, potential contamination of the sample, or limitations of the genome analysis.Such discussion would is of importance to evaluate the robustness of the findings and their implications for clinical practice.

R:*We thank the reviewer for the positive evaluation of our work, particularly the contribution of this study for the management of patients with recurrent urinary tract infections caused by MDR E. cloacaein regions with limited resources for microbiological testing and antibiotic susceptibility testing.As suggested,we have added a brief discussion about potential limitations of the study (please see lines 119-127).*

Further I have noted some typographical errors

Eg;- Enterobacter cloacae is written in different ways eg : 'cloacae' in line 31 and 'cloaca' in line 58, please strict to one correct way.

R:*The typographicalerror has been corrected (please see line 59). The text was further checked for typos.*

In summary, the article comprised of a concise genome announcement which is of a an organism with some clinical implications. I would suggest to expand the article including the potential limitations and to correct the grammar and spelling errors.

R:As mentioned above we have added a brief discussion about potential limitations of the study (please see lines *119-127*). The text was further checked for grammar and spelling errors.

VERSION 1

Editor recommendation and comments

https://doi.org/10.1099/acmi.0.000565.v1.5

Nihal Bandara; University of Bristol, Bristol Dental School, Lower Maudlin Street, UNITED KINGDOM, Bristol

Date report received: 13 April 2023
Recommendation: Major Revision

Comments: The reviewers have highlighted major concerns with the work presented. Please ensure that you address their comments.

Reviewer 2 recommendation and comments

https://doi.org/10.1099/acmi.0.000565.v1.3

Shobha Gunathilaka; Rajarata University of Sri Lanka Faculty of Medicine and Allied Sciences, Department of Microbiology, Anuradhapura, SRI LANKA
https://orcid.org/0000-0002-0367-302X

Date report received: 13 April 2023
Recommendation: Minor Amendment

Comments: The article is a genome announcement. The use of genome analysis to identify the resistance mechanism is a commendable approach, as it provides a detailed understanding of the genetic basis of resistance.The presence of the beta-lactamase chromosomal gene blaCMH-3 is an important finding, as it sheds light on the mechanism of resistance to beta-lactam antibiotics in this isolate. The findings of this study could have implications for the management of patients with recurrent urinary tract infections caused by MDR E. cloacae, particularly in regions with limited resources for microbiological testing and antibiotic susceptibility testing. The article does not discuss any potential limitations of the study, such as selection bias, potential contamination of the sample, or limitations of the genome analysis.Such discussion would is of importance to evaluate the robustness of the findings and their implications for clinical practice. Further I have noted some typographical errors Eg; - Enterobacter cloacae is written in different ways eg : 'cloacae' in line 31 and 'cloaca' in line 58, please strict to one correct way In summery, the article comprised of a concise genome announcement which is of a an organism with some clinical implications. I would suggest to expand the article including the potential limitations and to correct the grammar and spelling errors.

Please rate the manuscript for methodological rigour
Good

Please rate the quality of the presentation and structure of the manuscript
Satisfactory

To what extent are the conclusions supported by the data?
Strongly support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
No: Although a human biological sample is used no identification details mentioned. It has been mentioned that Nigerian guidelines states ethical approval is not necessary, anyway, its better if the authors have given reference ti the said guidelines

Reviewer 1 recommendation and comments

https://doi.org/10.1099/acmi.0.000565.v1.4

Ángel Rodríguez-Villodres; Hospital Universitario Virgen del Rocío, Microbiology, Sevilla, SPAIN
https://orcid.org/0000-0001-6373-9724

Date report received: 06 March 2023
Recommendation: Minor Amendment

Comments: I have some recommendations: Abstract: The authors say "asimptomatic bacteriuria" in the abstract, and "urinary tract infection" (line 60) in the main text. This is not the same. Which is the correct one? Announcement: Line 40: Enterobacter cloacae complex (italiziced) Line 44: amoxicillin (with clavulanic acid?) Line 45: Please, remove the sentence "as well as to the acquisition of numerous genetic mobile...." Line 65: What year are the CLSI guides used? Please, add it. Line 69: I don't see the result in the text of the modified DDST. Please, could you add it? Line 72: In the "Intermediate resistance to cefepime", please, add the MIC to cefepime. Line 73: The isolate was susceptible to amoxicillin-clavulanic acid?? E. cloacae has intrinsic resistance to this antibiotic. Please, could you explain it? Line 75: It is unusual resistance to levofloxacin and intermediate resistance to ciprofloxacin. Please, confirm it and add the MIC to both antibiotics. Line 109: ESBL phenothype? Is this correct? blaCMH-3 is a class C betalactamase that confers a AmpC phenotype.

Please rate the manuscript for methodological rigour
Satisfactory

Please rate the quality of the presentation and structure of the manuscript
Good

To what extent are the conclusions supported by the data?
Partially support

Do you have any concerns of possible image manipulation, plagiarism or any other unethical practices?
No

Is there a potential financial or other conflict of interest between yourself and the author(s)?
No

If this manuscript involves human and/or animal work, have the subjects been treated in an ethical manner and the authors complied with the appropriate guidelines?
Yes

SciScore report

https://doi.org/10.1099/acmi.0.000565.v1.1

iThenticate report

https://doi.org/10.1099/acmi.0.000565.v1.2