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Short Communication

First report of an *Enterobacter cloacae* ST837 resistant to cefiderocol co-harbouring *bla*$_{IMP-19}$ and *mcr-4.3* resistance genes in Italy

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ABSTRACT

Objective: To describe the molecular characterisation of an *Enterobacter cloacae* strain resistant to cefiderocol and co-harbouring *bla*$_{IMP-19}$ and *mcr-4.3* resistance genes

Methods: The strain was isolated from the rectal swab of a 77-year-old woman during screening for the detection of patients colonised by carbapenemase-producing Enterobacterales (CPE) in Bologna (Italy). The strain was identified at species level by MALDI-TOF mass spectrometry and the presence of active-on-imipenem (IMP) carbapenemase was confirmed both by a phenotypic immunochromatographic method, and by a rapid commercial nucleic acid amplification test. The strain underwent antimicrobial susceptibility testing and its genome was fully characterised to assess (i) the multilocus sequence type, (ii) the presence of antimicrobial resistance (AMR) genes, and (iii) the presence of virulence genes coupled with mobile genetic elements.

Results: Multilocus sequence typing identified the strain as ST837. A plasmid of 60 691 bp, very similar to the MW574937 plasmid of *Escherichia coli*, was identified and carried the following AMR genes: *aac(6′)-II*, bla$_{BEL-1}$, *bla*$_{IMP-19}$, *msr(E), mph(E)*, and *sul1*. This plasmid harboured several *tra* genes (*traB, traC, traD, traG, traI, traJ, traKtraL*, and *traM*) of the transfer operon of the *E. coli* conjugative F plasmid. Other AMR genes were located in the chromosome (*bla*$_{CMH-3}$ and *fosA*) or in other plasmids (*bla*$_{SHV-12}$ and *mcr-4.3*). The strain showed resistance to many β-lactams in vitro, including cefiderocol, whereas, despite the presence of the *mcr-4.3* gene, it was susceptible to colistin.

Conclusions: Further studies are needed to better evaluate the origin of this strain and monitor the presence of Gram-negative bacilli with a similar molecular structure in our area.

1. Introduction

In recent decades, the spread of carbapenemase-producing Enterobacterales (CPE) has been increasingly described worldwide, with epidemics of international proportions in different countries. Invasive infections due to CPE, such as bloodstream and lower respiratory tract infections, have a significant clinical and economic impact, with high morbidity, mortality, and prolonged hospital stays [1].

Plasmids carrying carbapenemase genes play an important role in the spread of CPE, thus underlining the importance of detailed analysis of resistance plasmids in molecular epidemiological studies of CPE [2]. Although complete nucleotide sequences of numerous plasmids carrying carbapenemase genes, such as *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{OXA-48}$-like, have been assessed, detailed information on plasmids carrying *bla*$_{IMP}$ are lacking, partly because CPE-producing active-on-imipenem (IMP) enzymes have been rarely reported [3]. Indeed, IMP-producing Enterobacterales have been mainly identified in the Asia-Pacific region, particularly in *Klebsiella pneumoniae, Escherichia coli*, and *Enterobacter* spp. [4].

IMP enzymes are metallo-β-lactamases (MBLs) potentially able to hydrolyse all β-lactams, including novel β-lactam/β-lactamase inhibitor combinations (e.g. ceftazidime/avibactam and meropenem/vaborbactam), although resistance levels may vary ac-

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cording to different subtypes [3]. Thus, therapeutic options for IMP-producing Enterobacterales are very limited and currently include colistin and the siderophore cephalosporin, cefiderocol, among the possible last-resort sensitive antimicrobials. Here, we describe the genome of an IMP-producing *Enterobacter cloacae* strain resistant to cefiderocol and harbouring the *mcr-4.3* colistin resistance gene isolated from a rectal swab during routine CPE screening in Bologna, Italy.

2. Materials and methods

2.1. Strain isolation

In August 2023, a 77-year-old women was admitted to the Gastroenterology Unit of Maggiore Hospital of Bologna (Italy) for acute pancreatitis of unknown origin. Her medical history comprised a mastectomy for breast cancer, a hysterectomy, and the presence of hypothyroidism and chronic obstructive pulmonary disease. She denied the recent use of antimicrobials as well as travels abroad. During hospitalisation, she underwent rectal sampling for the detection of CPE as part of active screening for the early detection of colonised patients [5].

As suggested by regional guidelines [6], rectal swabs are routinely collected from intensive care units, and medical and surgical wards of hospitals as part of normal CPE screening, both in naïve subjects and during weekly follow-up of colonised patients, in order to prevent CPE transmission and spread.

Briefly, using the WASPLab system (Copan, Brescia, Italy) the rectal swab (eSwab; Copan) was automatically plated onto a selective (i.e. containing a carbapenem agent) chromogenic medium (CHROMagar KPC; Kima Meus, Padua, Italy) and incubated at 35 °C aerobically for 16 h. After the incubation, blue/green bacterial colonies (KES group: *Klebsiella* spp., *Enterobacter*spp., *Serratia* spp.) were noticed and the strain was identified as *E. cloacae* by MALDI-TOF mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany). No colony referable to carbapenem-resistant *Acinetobacter baumannii* or *Pseudomonas aeruginosa* was noticed.

Afterward, the bacterial colonies were tested for carbapenemase production by a multiplex immunochromatographic assay (NG-Test CARBA 5; NG Biotech, Guipry, France) and by a rapid commercial nucleic acid amplification test (XpertCarba-R; Cepheid, Sunnyvale, CA, USA) [7,8]. Additionally, we performed the carbapenem inactivation method to confirm carbapenemase activity, as well as a synergy test (combination disk testing with meropenem ± various inhibitors) to identify the carbapenemase class [9].

The strain underwent antimicrobial susceptibility testing based on microdilution broth assays (MicroScan Walkaway; Beckman Coulter, Atlanta, GA, USA; Sensititre Plate EUMDRXXF; Thermo Fisher Scientific, UK) and obtained MICs were interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints. Susceptibility/resistance to cefiderocol was first determined by a disk diffusion method (30 μg disk), as indicated by EUCAST guidelines [10]. The MIC of cefiderocol was assessed by a broth microdilution panel with iron-depleted cation-adjusted Mueller Hinton broth (ComASP Cefiderocol Test; Liofilchem, Roseto degli Abruzzi, Italy).

2.2. Genomic analysis

Genomic DNA was extracted using a Maxwell HT 96 gDNA Blood Purification Kit (Promega, Madison, WI, USA) and subjected to genomic libraries preparation using an Illumina DNA Prep (M) Tagmentation Kit (Illumina, San Diego, CA, USA). Short-read sequencing was performed using an Illumina MiSeq platform (Illumina) producing paired-end reads (300×2 bp) that were checked and filtered for quality and contamination. Long-read sequencing (Oxford Nanopore Technologies, Oxford, UK) was also performed to close chromosome and plasmids. Short and long reads were assembled using Unicycler v. 0.5.0 [11]. Starting from genome assembly, we detected the following in silico: (i) the multilocus sequence type using the Pasteur BIGSdb [12]; (ii) antimicrobial resistance genes using ResFinder and CARD databases [13,14]; and (iii) virulence genes coupled with mobile genetic elements using MGE Finder v. 1.0.3 (MGEdb v. 1.0.2) [15] and Virulence Factor Database [16]. Comparisons between plasmids were performed and visualised using BRIG Ring Image Generator [17] and Clinker [18].

2.3. Data availability

Raw genomic data are available at European Bioinformatics Institute (EMBL-EBI) under study accession no. PRJEB75786.

3. Results

3.1. Phenotypic analysis

E. cloacae strain, FZ47, tested positive for production of an IMP carbapenemase via the rapid phenotypic immunochromatographic assay. Detection of an IMP enzyme was confirmed via the molecular detection of an IMP-encoding gene by the XpertCarba-R test.

Synergy with ethylenediaminetetraacetic acid confirmed a class B carbapenemase, whereas a positive carbapenem inactivation test highlighted the ability of the carbapenemase to hydrolyse meropenem.

As shown in detail in Table 1, *E. cloacae* strain FZ47 was resistant to ceftazidime/avibactam, ceftolozane/tazobactam, cefepime, ertapenem, and piperacillin/tazobactam. Conversely, susceptibility was noticed to colistin, amikacin, gentamicin, meropenem/vaborbactam, ciprofloxacin, and sulfamethoxazole/trimethoprim. Meropenem was characterised by a MIC value of 4 mg/L (i.e., *I* = susceptible, increased exposure). Finally, the strain was shown to be resistant to cefiderocol (zone diameter <21 mm by the disk diffusion method), showing an MIC of 4 mg/L.

Table 1
Antimicrobial susceptibility testing of the *E. cloacae* FZ47 strain.

Antimicrobial	MIC value (mg/L)	EUCAST interpretation
Amikacin	≤2	S
Ampicillin*	>8	R
Amoxicillin-clavulanate*	>32	R
Ceftazidime-avibactam	>16/4	R
Ceftazidime*	>32	R
Ceftolozane-tazobactam	>8/4	R
Ciprofloxacin	0.25	S
Cefotaxime*	>32	R
Ertapenem	>1	R
Cefepime	>16	R
Gentamicin	≤2	S
Meropenem	4	I
Meropenem-vaborbactam	4/8	S
Piperacillin-tazobactam	>32/4	R
Sulfamethoxazole-trimethoprim	≤2/38	S
Colistin	≤0.5	S
Imipenem	2	S
Imipenem-relebactam	2/4	S
Cefiderocol	4	R

* *Enterobacter cloacae* strains have an intrinsic resistance to ampicillin, amoxicillin, amoxicillin-clavulanate, and first-generation cephalosporins owing to the production of constitutive AmpC β-lactamase. The de-repression or hyperproduction of natural AmpCs is due to various genetic changes and confers high-level resistance to cephalosporins (including the third-generation but generally not the fourth-generation compound) and to penicillin-β-lactamase inhibitor combinations.

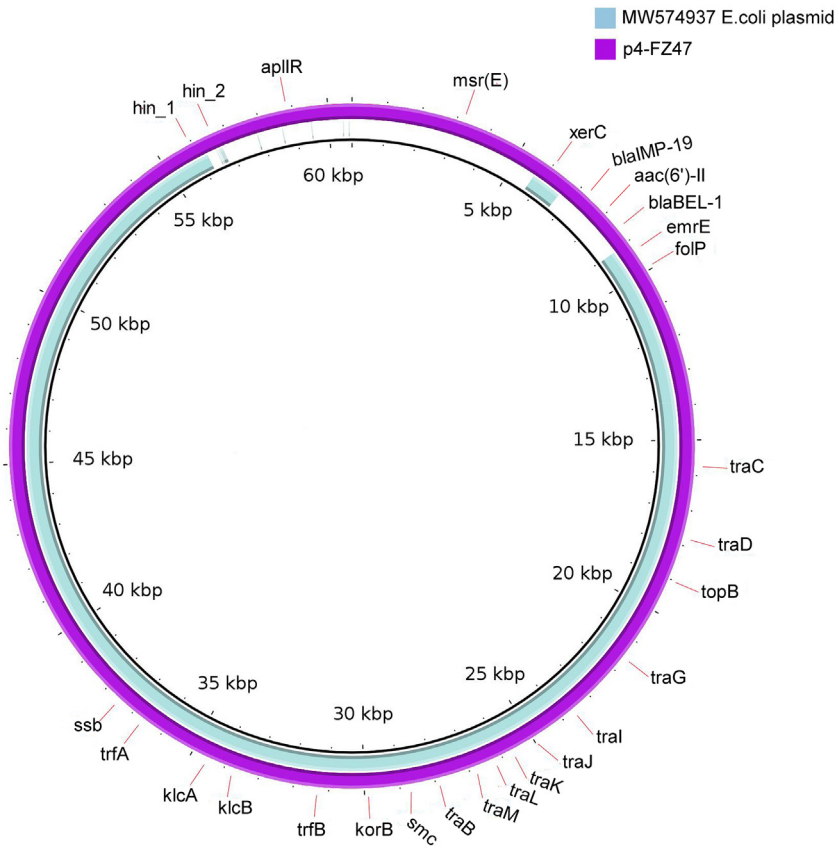

Fig. 1. BRIG graphical representation of the comparison between p4-FZ47 and MW574937 plasmids. Annotation of genes with a known product is shown with labels.

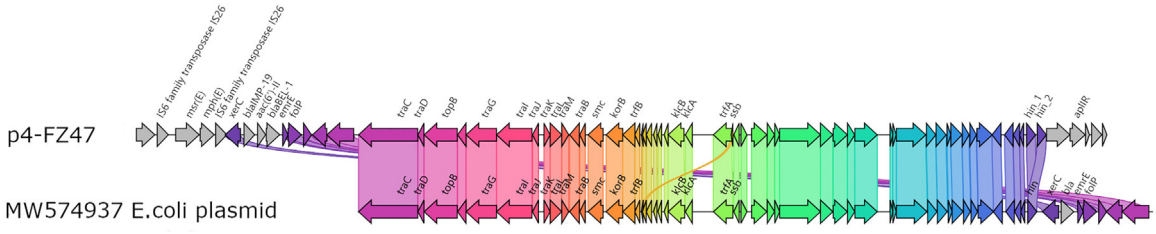

Fig. 2. Gene cluster comparison between p4-FZ47, described in this work, and the MW574937 plasmid of *Escherichia coli*, visualised by using Clinker [13]. Coloured genes are shared between the two plasmids.

3.2. Genome analysis

The entire genome sequence was assembled into a single chromosome (total length 5 106 263 bp) and seven closed plasmids ranging from 2018 to 230 638 bp. Multilocus sequence typing, using Pasteur BIGSdb, identified the strain as ST837.

A plasmid of 60 691 bp (here named p4-FZ47), very similar to the MW574937 plasmid of *E. coli* [19], was identified (79% coverage, 99.98% identity) and carried the following AMR genes *aac(6′)-II*, bla_{BEL-1}, bla_{IMP-19}, *msr(E)*, *mph(E)*, and *sul1* (Fig. 1) conferring resistance to aminoglycosides, penicillins and cephalosporins, macrolides, and sulphonamides, as reported in detail in Table S1. In Fig. 2, the gene cluster comparison between MW574937 and p4-FZ47 showed that all the coding sequences of MW574937 were conserved in p4-FZ47 with the exception of the bla_{OXA} gene, which was only present in the *E. coli* plasmid. This gene was replaced in p4-FZ47 by a cluster of AMR genes, namely *aac(6′)-II*, bla_{BEL-1}, bla_{IMP-19}, and *emrE* (Fig. 2). Interestingly, these AMR cassettes were surrounded, in both MW574937 and p4-FZ47 plasmids, by *xerC* coding for a site-specific recombinase similar to the integron integrase family protein possibly involved in gene cassette integration/substitution [20]. Ten genes were unique to p4-FZ47 and therefore missing in MW574937. Among these latter genes, two additional resistance genes were found: *mph(E)* and *msr(E)*, coding for a macrolide phosphotransferase and an ABC-F subfamily protein, respectively. Significantly, two IS26 insertion sequences surrounding the *mph(E)* and *msr(E)* resistance genes formed a composite transposon that could possibly move this gene cassette. p4-FZ47 as well as MW574937 harboured several *tra* genes (*traB, traC, traD, traG, traI, traJ, traK, traL*, and *traM*) of the transfer operon of the conjugative *E. coli* F plasmid [21]. Other AMR genes were located within the chromosome (such as bla_{CMH-3} and *fosA*) or in other plasmids (bla_{SHV-12} and *mcr-4.3* in the p1-FZ47 and p3-FZ47 plasmids, respectively) (Table S1). Virulence gene analysis was performed using Virulence Factor Database that revealed the presence of several virulence genes on the chromosome (Table S2).

4. Discussion

To the best of our knowledge, this is the first report of an *E. cloacae* strain isolated in Italy that is resistant to cefiderocol and co-harbouring $bla_{\text{IMP-19}}$ and *mcr-4.3* colistin resistance genes.

At first, we noticed that the strain was characterised by the presence of numerous acquired antimicrobial resistance genes, both located within the chromosome as well as the plasmids (including *fosA*, $bla_{\text{CMH-3}}$, $bla_{\text{SHV-12}}$, *mcr-4.3, qacE,* $bla_{\text{IMP-19}}$, *sul1, aac(6′)-II*, and $bla_{\text{BEL-1}}$), theoretically conferring resistance to many antimicrobials such as β-lactams, aminoglycosides, sulfamethoxazole, and colistin.

Presence of the *emr(E)* gene could have contributed to the multidrug-resistant phenotype. Emr(E) is a small multidrug transporter seen in *E. coli* and *P. aeruginosa* that functions as a homodimer and that couples the efflux of small polyaromatic cations from the cell with the import of protons down an electrochemical gradient. This transporter is a key player in the defence of multidrug-resistant pathogens to toxins and other homeostasis-perturbing compounds, including antimicrobials [22].

Nevertheless, the presence of AMR genes was not always associated with reduced susceptibility in vitro. For example, despite the presence of *mcr-4.3* and *sul1* genes, the *E. cloacae* FZ47 strain appeared susceptible to both colistin and sulfamethoxazole-trimethoprim. As previously reported, even in presence of *mcr* genes, Enterobacterales are able to retain susceptibility to colistin, probably due to the absence of compatible regulatory genes within the genome [23].

Additionally, even though the modifying enzyme aminoglycoside acetyltransferase *aac(6′)-II* is able to inactivate tobramycin and gentamicin [24], the *E. cloacae* FZ47 strain was fully susceptible to gentamicin, possibly indicating a failure in gene expression.

The lack of resistance to imipenem (MIC = 2) and meropenem (MIC = 4) is not surprising. Indeed, considering that carbapenemases are expressed at various levels, the carbapenem MICs for CPE may be below clinical breakpoints but above epidemiological cut-off values, as defined by EUCAST [9].

This aspect also explains the retained susceptibility to meropenem-vaborbactam (MIC = 4) and imipenem-relebactam (MIC = 2), even though metallo-β-lactamases are not inhibited by β-lactamase inhibitors, such as vaborbactam and relebactam.

Interestingly, the *E. cloacae* FZ47 strain was resistant to cefiderocol, one of the last-resort drugs used in the treatment of MBL-producing Enterobacterales. As several different mechanisms could contribute to cefiderocol resistance, we primarily confirmed the presence/integrity of *ompC* and *ompF* genes, coding for porins, and the *cirA* gene coding for a siderophore receptor. Other than the possibility of efflux pump overexpression, the presence of $bla_{\text{SHV-12}}$ and bla_{BEL-1} genes could explain the resistant phenotype: indeed, SHV- and BEL-type β-lactamases have the potential to contribute to cefiderocol resistance as they were associated with increased cefiderocol MICs following their introduction to isogenic mutants [25].

Regarding the bla_{IMP} gene, the most common species associated with IMPs among the *Enterobacteriaceae* include *K. pneumoniae, E. coli*, and *Enterobacter* spp. IMP genes are often situated within class 1 integrons harboured on broad host-range plasmids, with the exception of some bla_{IMP}-encoding class 2 and 3 integrons. These mobile genetic elements play an important role in the interspecies distribution of IMP types of carbapenemases [3]. Few studies have reported the presence of $bla_{\text{IMP-19}}$, which is predominantly founded in *Acinetobacter* species. Interestingly, a recent report on the chromosomal integration of carbapenemase gene $bla_{\text{IMP-19}}$ in *Acinetobacter baumannii* suggests the importance of monitoring the spread of mobile elements, such as integrons carrying carbapenemase genes, which could integrate within the chromosome and therefore be maintained in the population [20].

Genomic analysis of the FZ47 strain genome revealed the presence of seven plasmids, three of which carried resistance genes (*mcr-4.3,* $bla_{\text{SHV-12}}$, $bla_{\text{IMP-19}}$, *sul1, aac(6′)-II*, and $bla_{\text{BEL-1}}$). In particular, we focused on plasmid p4-FZ47, which harboured $bla_{\text{IMP-19}}$ on a mobile AMR cassette including *aac(6′)-II* and $bla_{\text{BEL-1}}$; flanking gene distribution and the presence of an integrase/recombinase suggested the presence of an integron-like mobile element. Nearby, another mobile cassette carrying *mph(E)* and *msr(E)* resistance genes was mobilised by an IS26 array. Moreover, P4-FZ47 was equipped with several *tra* genes involved in the transfer of an *E. coli* conjugative F plasmid, raising the possibility of transfer to other strains. This feature, combined with possible mobilisation of the single AMR cassette, suggests the importance of monitoring the spread of these mobile elements over time.

In conclusion, here we reported the molecular characterisation of a *E. cloacae* strain co-harbouring $bla_{\text{IMP-19}}$ and *mcr-4.3* resistance genes showing resistance to cefiderocol. These genes were isolated during active rectal screening for the detection of colonised patients in northern Italy.

Further studies are needed to better evaluate the origin of this strain and monitor the presence of Enterobacterales and non-fermenting Gram-negative bacilli with a similar molecular structure in our geographic area.

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Declaration of competing interests: None declared.

Ethical approval: This study was carried out in accordance with the declaration of Helsinki, under the terms of relevant local legislation, and was cleared by the institutional review Board. The requirement for informed consent was waived due to the observational nature of this study.

Author contributions: ES, TL, SP, CF, and SA conceived and designed the study. IM, ES, BS, AD, CF, and SA performed the experiments and analysed the data. IM, ES, CF, and SA wrote the paper. All authors read, reviewed and approved the final version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2025.01.023.

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