First case of VIM-1-producing Leclercia adecarboxylata in Oman: A case report and literature review.

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Case Report

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Abstract

*Leclercia adecarboxylata* is a recently acknowledged emerging pathogen. It is a member of the *Enterobacterals* family, formerly thought to be a member of the genus *Escherichia*. Isolation was reported from various animal and environmental specimens. However, it rarely causes infection in humans, and the true frequency is unknown or underestimated. *Leclercia adecarboxylata* showed an ascending resistance grade from pan-sensitive to Carbapenem-resistant due to its ability to produce and harbour hydrolyzing enzymes that challenge daily clinical practices. In our report, the isolate was misidentified as *Citrobacter koseri* by API E and Pantoea species by Vitek 2 but confirmed by MALDI-TOF MS and 16S ribosomal RNA analysis as *Leclercia adecarboxylata*. Conventional PCR revealed the presence of two populations of resistance genes, VIM-1 and OXA-48. Herein, a report of the first emergence of *Leclercia adecarboxylata* producing VIM-1 in a rectal swab of a 63-year-old non-immunocompromised female with acute intracerebral haemorrhage.

Introduction

*Leclercia adecarboxylata* is a motile facultative aerobic, gram-negative bacillus formerly described as *Escherichia adecarboxylata* by Leclerc in 1962 [1]. However, a re-classification of this microorganism as a member of the *Enterobacterals* family was only established following the study of deoxyribonucleic acid homology by Tamura in 1986 [2]. Most commonly, it is isolated from soil and water [3]. In addition, it has been previously isolated from other sources, such as the surface of chicken eggshells and the mouth cavities of sharks [4-5].

Infections by *Leclercia adecarboxylata* are mostly non-lethal due to their excellent antibiotic susceptibility profile and low virulence factors [6]. Opportunistic infections by this pathogen are reported, and associated with predisposing factors, including immunosuppression, the presence of catheters, and wounds [7-10]. Worth mentioning that in the immunocompetent population, *Leclercia adecarboxylata* showed less pathogenicity. And in most cases, it was documented as part of polymicrobial infection [7]. Of significance, it was found as a monomicrobial isolate in blood, peritoneal fluid, urine and sputum [11-14]. *L. adecarboxylata* is usually sensitive to most antibiotics, including tetracycline, tigecycline, amikacin, and carbapenems [15]. However, antibiotic resistance is commonly reported for fosfomycin [16].

*L. adecarboxylata* shares several morphological and microbiological properties that resemble the *Escherichia* genus, leading to the misidentification of these infections when identification methods rely solely on conventional diagnostic techniques [7, 10,17].

Therefore, some researchers have claimed that the incidence of *L. adecarboxylata* infections is more appropriately an underreported figure [15-16]. Consistent with that, a study by Çiçek et al. in 2018 reported that classical culture media such as MacConkey agar, eosine-methylene blue agar, and blood agar exhibit colonies that are morphologically identical to *E.*
*Escherichia coli* may occur in high frequency, particularly in low-resource laboratories, if no further identification is made [18]. Furthermore, with the availability and introduction of advanced biochemical-based systems (e.g., MicroScan, VITEK 2 ID/AST, and BD Crystal), molecular-based technologies (e.g., 16S rRNA sequencing and PCR), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a timely and accurate identification was accomplished, which is essential to achieve an accurate diagnosis, therapy and aid trusted epidemiological studies [19-20].

Herein, we report the first VIM-1-producing *Leclercia adecarboxylata* strain in the literature related to a non-immunocompromised patient. The report aimed to narrate the course of infection, management and follow-ups, outcomes, and the unique morphological and molecular characteristics of the isolate.

**Case Presentation**

A 63-year-old female presented to the emergency department with a sudden onset of screaming, altered sensorium and vomiting 30 minutes prior to arrival at the emergency department. She was found to have elevated blood pressure (270/135 mmHg) and chest crepitations with wheezes.

The patient had a history of type 2 diabetes mellitus (DM2), hypertension, and chronic kidney disease (CKD) not requiring haemodialysis. In addition, she had recent recurrent admissions due to pulmonary oedema, coronary artery disease, and erosive gastropathy.

Initial laboratory investigations showed normal complete blood count (CBC), electrolytes, liver function tests (LFT), coagulation profile, and c-reactive protein (CRP). Arterial blood gas analysis indicated a slight acidosis with a pH of 7.320. Expectedly for a patient with a history of CKD, renal function tests (RFT) illustrated unfavourable results in urea, creatinine and e-GFR(MDRD). Moreover, a mild elevation was detected in troponin. Detailed laboratory investigations from day1-27 are provided in Table 1, (supplementary material).

During the examination in the emergency department, the patient became agitated with time, experienced several episodes of vomiting, and started to express a gradual loss of consciousness. To secure the airway, she was intubated. Based on brain CT findings and cerebral CTA, the patient was diagnosed with cerebral haemorrhage without tonsillar herniation or midline shift. The patient entered a in a deep coma and was admitted to the intensive care unit (ICU). Due to the worsening of the RFT parameters, she was subjected to haemodialysis. Repeated CT brain two days later, showed a significant growth in the brain hematoma.

She stayed in ICU for 13 days, with two sets of microbiological cultures obtained on the 3rd and 11th days of her stay in ICU. These include blood cultures, endotracheal cultures (ET) and urine cultures. Blood cultures reports were negative in both sets of cultures. Urine cultures revealed *Escherichia coli* in both sets of cultures. ET cultures grew *klebsiella pneumonia* first set of cultures, whereas *Elizabethkingia meningoseptica* in the second set of cultures. During her lengthy stays in ICU, she received amoxiclav injections (D6) for seven days, erythromycin (D10) was administered as a prokinetic to facilitate
gastrointestinal motility, Vancomycin injection (D11) was given for only one day, and Tazocin (D13) for five days.

After 13 days in ICU, she shifted to an isolation room (D14) due to the development of an infected sacral bedsore grade II that measures approximately 5 cm in diameter. The infected sacral bedsore required prompt surgical intervention, followed by a course of Vancomycin and levofloxacin (D16), which was later upgraded to meropenem (D18).

Unfortunately, she experienced rectal bleeding that caused a significant decrease in haemoglobin level, where it reached 6.85 g/dL and triggered an urgent blood transfusion of two units of packed red blood cells.

On a subsequent day, a swab culture was taken from the infected sacral bedsore that grew three types of colonies (Table 1). These strains were referred to Central Public Health Laboratories (CPHL), the national reference laboratories, for confirmation and further testing. On day 24, an injection of Colistin Sulphomethoate was initiated. Unfortunately, the patient passed away on day 27 of hospital stay and was already on day 2 of colistin administration due to the long list of comorbidities and risk factors described above.

**Methodology**

*Strain Isolation*

The strains recovered from our patient's clinical sample were sub-cultured on blood agar and MacConkey's agar (Bio-Oman, United labs, Oman). The cultured plates were subjected to overnight incubation at 35-37°C.

*Isolate Identification*

Identify the isolate at the local hospital laboratory using API E (BIOMERIEUX, USA). Initial identification at CPHL was carried out via Vitek 2 version 8.01 (bioMérieux, France) using a GN card and matrix-assisted laser desorption / ionization time of flight (MALDI-TOF) (Maldi Biotyper MBT Compass 4.1.100, Bruker Daltoniks GmbH, Bremen, Germany).

*Further identification*

*Genomic DNA Extraction*

Bacterial colonies from overnight growth (18 – 24h) on a blood agar plate at 37 degrees Celsius were suspended in 200 μL of distilled H2O, and genomic DNA was extracted by automated nucleic acid
extractor (QIAsymphony SP/AS) using DSP virus/pathogen (Qiagen Valencia, CA) kit as per manufacturer instructions. DNA was eluted in 85 mL and stored at 4-80°C.

**16S rRNA PCR Amplification and Sequencing**

Bacterial universal primers (27F & 519R) were used for 16S rRNA PCR amplification. 16S/23S rRNA sequencing. The same primers were used for the big dye terminating cycle sequencing. For 16S rRNA PCR amplification, the master mix was prepared using 0.4µM final 0.4 M concentration of each primer, 12.5 µl Master Mix (AmpliTaq Gold® 360), 6.7µL of nuclease-free water, and 5 µL of template genomic DNA. A known bacteria and nuclease water were processed along with samples as positive and negative controls, respectively. The primer sequences and thermal cycler run conditions are provided in **Table 2, (supplementary material)**.

The amplicon (~500bp) was visualized on 1% agarose gel stained with Ethidium bromide. The gel was run for 35 minutes at 80 volts.

**16S rRNA sequencing**

16 S rRNA sequencing was performed using the sanger cycle sequencing method using BigDye TM Terminator v3.1 - Cycle Sequencing (Applied Biosystems) chemistry. 16S rRNA PCR amplicon was purified using the EXOSAP kit as per the kit instructions (details of the kit). The cycle sequencing master mix was prepared using 4µl of BigDye Terminator v3.1 ready reaction mix, 2µL of BigDye TerminatorV3.1 buffer, 1µl of primer (3uM) and 12µl of nuclease-free water and 1 µl (5 ng) of the purified amplicon. The thermocycler conditions are provided in appendix A. After cycle sequencing, the product is again purified using AGENCOURT CLEANSEQ. The product was sequenced using a 3500 genetic analyzer (Applied Biosystems).

The forward and reverse sequences were aligned using the GENEDOC program to obtain a consensus sequence. The consensus sequence was blasted against the 16S rRNA gene database library (Blastn, NCBI) and the Greengenes database (16S rRNA specific database) to identify the organism. The quality of the sequences was checked for any contamination or noise.

**Antimicrobial susceptibility testing (AST)**

AST was carried out via Vitek 2 version 8.01 (bioMérieux, France) using AST GN card 215 and E test (predefined gradient concentrations, bioMérieux, France).
Interpretation of results was based on Clinical and Laboratory Standards Institute (CLSI), M100, 2022 breakpoints (reference: Performance Standards for Antimicrobial Susceptibility Testing, M100, 32nd edition. Clinical and Laboratory Standards Institute. 2022 Feb)

### 3.5.1. CPE confirmation

Phenotypic CPE confirmation was carried out through Modified Hodge Test (MHT) using meropenem disc (BioMerieux, France) as described (CLSI 2016 M100, reference: Performance Standards for Antimicrobial Susceptibility Testing, M100, 26th edition. Clinical and Laboratory Standards Institute, 2016 Jan) and Modified Carbapenem Inactivation Method (mCIM) preformed as described (CLSI 2022 M100).

### 3.5.2 CPE multiplex PCR

Molecular characterization of the CPE genes was done by multiplex PCR targeting blaOXA-48, blaNDM, blaKPC, blaIMP & blaVIM as described previously by D Doyle, G Peirano, C Lascols, et al. 2012. Primer sequences, master mix composition and PCR conditions are provided in Table2 appendix A.

### 3.5.3. VIM confirmation by sequencing

VIM-F and VIM-R primers (same as used in VIM simple Lex PCR) were used to sequence the VIM amplicon. Sequencing was done using the sanger cycle sequencing method using BigDyeTM Terminator v3.1 - Cycle Sequencing chemistry using the same method as used for 16S rRNA sequencing.

**Ethical approval**

The case report was conducted in accordance with the 1964 Helsinki declaration, and informed consent was obtained from a rightful next of ken of the deceased patient by the research focal point; available upon request for publication purpose to be submitted only for the editor in chief of the medical journal.

**Results**

*Isolate colonial morphology*

Leclercia adecarboxylata grew as pink lactose fermenting colonies in MacConkey’s and as non-haemolytic colonies in blood agar (Figure 1).
Isolate Identification

API E in the local hospital lab gave the Citrobacter koseri ID with 99.9%. In CPHL, the Vitek 2 system gave the ID of Pantoea species with a probability of 93%, while MALDI-TOF gave L. adecarboxylata with a score of 1.88.

16S rRNA sequencing

The consensus sequence was blasted in BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch) and the Greengeens database (https://rnacentral.org/expert-database/greengenes). Leclercia species were found with the highest query coverage (99%) and percentage identity (99.24%).

Antimicrobial susceptibility testing (AST)

The AST showed a multidrug resistant organism with high MIC to cephalosporins, aminoglycosides, cotrimoxazole, piperacillin-tazobactam, and carbapenems (Table 2).

4.5.1. CPE confirmation

Phenotypic tests: both MHT and mCIM were positive

4.5.2. CPE molecular characterization

The VIM sequencing data were blasted in NCBI and it was confirmed that the amplified product (amplicon) was VIM-1 and not any random amplicon. The CPE multiplex PCR identified the presence of blaVIM and blaOXA-48. Co-presence of VIM and OXA in the isolate was also confirmed by single plex PCRs targeting VIM and OXA-48 (Figure 2).

Table 1. Summary table of isolated pathogens. The table demonstrates the polymicrobial existence of the pathogen on the rectal swab, their antibiotic susceptibility testing, identification methodologies and additional remarks.
<table>
<thead>
<tr>
<th>Microorganism type</th>
<th>Susceptibility</th>
<th>Identification method</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>VRE-Enterococcus faecium</td>
<td><strong>Resistant:</strong> VA, TEC</td>
<td>MALDI-TOF &amp; AST by VITEK2</td>
<td>E-test VA: &gt;256 ug/ml, Disk diffusion for VA, TEC, LZD, 6mm, 7mm, 32mm respectively.</td>
</tr>
<tr>
<td></td>
<td><strong>Sensitive:</strong> LZD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDR-Ab</td>
<td><strong>Resistant:</strong> CTX, CRO, CIP, CN, TAZ, TE, SXT, AK, CAZ, DO, MEM</td>
<td>API E (99.6% score) &amp; AST by disk diffusion</td>
<td>- Testing carried out using API E due to Previous MDR-Ab was grown in a wound swab collected one day prior to rectal swab that was diagnosed via MALDI-TOF MS and AST by VITEK2.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Colistin E-Test: no CLSI interpretative guidelines</td>
</tr>
<tr>
<td>CPE-Citrobacter koseri*</td>
<td><strong>Resistant:</strong> CTX, CRO, CIP, CN, TAZ, TE, SXT, AK, CAZ, DO, MEM</td>
<td>API E &amp; AST by disk diffusion</td>
<td>Misidentified with Leclercia adecarboxylyata which was confirmed by MALDI-TOF MS and 16S ribosomal analysis</td>
</tr>
</tbody>
</table>

*; Reflects the results of L. adecarboxylyata because Citrobacter koseri was misidentified as Leclercia adecarboxylyata, VRE; vancomycin resistant enterococci, MDR-Ab; Multidrug-resistant Acinetobacter baumannii, CPE; Carbapenemase-producing Enterobacterals, MIC; minimum inhibitory concentration, API E; Analytical Profile Index for Enterobacterals, AST; antibiotic susceptibility testing, MALDI-TOF MS; matrix-assisted laser desorption / ionization time of flight mass spectrometry, CTX; Cefotaxime, CAZ; Ceftazidime, CRO; Ceftriaxone, CIP; Ciprofloxacin, CN; Gentamycin, TE; Tetracycline, TZP; Piperacillin+Tazobactam, MEM; Meropenem, SXT; Trimethoprim + Sulfamethoxazole, DOXY; Doxycycline, LZD; linezolid, TEC; teicoplanin, VA; vancomycin.

**Table 2. Leclercia adecarboxylyata antibiogram**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>16</td>
<td>S</td>
</tr>
<tr>
<td>Amoxicillin/Clavulanic Acid</td>
<td>16</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>16</td>
<td>R</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>32</td>
<td>R</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>32</td>
<td>R</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>R</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8</td>
<td>R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>2</td>
<td>I</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2</td>
<td>I</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>4</td>
<td>R</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>64</td>
<td>R</td>
</tr>
<tr>
<td>Trimethprim/sulfamethoxazole</td>
<td>160</td>
<td>R</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.75</td>
<td>-</td>
</tr>
<tr>
<td>Colistin</td>
<td>≤0.25</td>
<td>-</td>
</tr>
</tbody>
</table>

MIC; Minimal inhibitory concentration, S; Sensitive, R; Resistant, I; Intermediate, SXT; Trimethprim + Sulfamethoxazole, DOXY; Doxycycline, LZD; linezolid, TEC; teicoplanin, VA; vancomycin, IPM; imipenem, F; nitrofurantoin, KZ; cefazolin. ‘-’: No CLSI interpretative breakpoint.
Discussion

*L. adecarboxylata* is an aero-anaerobic, pigmented, peritrichous-flagellated, mesophilic bacilli that belong to the *Enterobacterials* family. The term enteric group 41 or *Escherichia adecarboxylata* was initially described by Leclerc in 1962 as referring to the bacteria. However, various electrophoretic and nucleic acid methods separated it from the genus *Escherichia* and renamed it *L. adecarboxylata* [1-2]. *L. adecarboxylata* is commonly distributed in nature in ubiquity and has been isolated from various environmental sources such as water, soil, oil reservoirs, milk, industrial fat, and eggshells. It has been a normal flora of the gastrointestinal tract of normal humans and part of the gut flora in animals [16, 21]. Interestingly, it has been found in various clinical samples, such as urine, blood, wound, bone, bronchial wash, gall bladder, and cardiac valve [16, 22-25].

In the current report, *L. adecarboxylata* was isolated from a rectal swab to screen for the presence of multi-drug resistant organisms (MDROs). Less than 48 hours prior to the rectal swab collection, the patient suffered from an infected sacral bedsore where she was subjected to prompt surgical intervention and wound swab culture. The wound swab revealed significant growth of *multidrug-resistant Acinetobacter baumannii* (MDR-Ab) that exhibited a symmetrical antibiotic susceptibility testing to the detected MDR-Ab in rectal swab, Table 1.

Two studies have suggested a low pathogenic potential by *L. adecarboxylata* [7,14]. These findings are consistent with that of other studies, which asserted that the immunological status of infected patients by *L. adecarboxylata* tends to demand other germs as a co-infecting agent to establish an infection in immuno-competent patients. In contrast, it emerged as a monomicrobial infection in immunocompromised subjects [15]. In the current case, both aerobic and anaerobic blood cultures were negative in all collection intervals, which supports previous reports suggesting that this bacterium is less invasive. Additionally, considering that the patient is non-immunocompromised and found as a polymicrobial infection, it seems likely that the strain is dependent on other co-infecting germs to establish an infection.

These results corroborate the findings of a previous study on the cytotoxic effect of *L. adecarboxylata* in human HeP-2 epithelial cells in an in-vitro study where the bacteria failed to invade or adhere to the cells [28]. However, several cases presented as monomicrobial infections in subjects without pre-existing conditions (non-immunocompromised) [21,27, 29-32]. The contradicting scientific evidences highlight nothing but one notion that there are many gaps and unexplored spots in the analysis of the pathogenicity of *L. adecarboxylata*.

The source of infection by *L. adecarboxylata* is exceptionally blurry. Moreover, there is limited comprehensive knowledge about the route of transmission. However, there are several former hypotheses of infectious transmission that different authors have described: (I) bacterial translocation via the genitourinary tract, (II) host invasion through catheter or wounds access, and (III) bacterial translocation
in the gastrointestinal tract across the mucosal barrier [15,24,26,27,34]. The second and third are possible routes of infection in our patient. Undeniably, catheter-induced infections are reported in the literature, with apparent evidence of the role of catheter and urinary tract infections [35-36]. A bacterial translocation through the genitourinary tract and catheter-induced implantation is less likely, as urine cultures were negative for *L. adecarboxylata* at all intervals. Keeping in mind that there are no reported cases of *L. adecarboxylata* in the same healthcare setting, positively favouring the argument to exclude transmission through healthcare workers' hands.

It is essential to note that our patient has a history of a long list of underlying conditions which lead to fluctuations in her immune system but with no diagnostic evidence of low numerical metrics in the white blood cell count in the general or absolute cell-cell count. In line with that, according to a study by Simpson [37], opportunistic infections were found in association with leukopenia in a context of bacterial infection caused by immunodeficiency in incidences other than AIDS. Other evidence has been reported demonstrating leukopenia, low CD4 count, and a significantly high vulnerability to opportunistic infections in a context other than AIDS as a causative agent for various opportunistic infections [38].

Another potential route of infection is the bacterial translocation in the gastrointestinal tract across the mucosal barrier. It is a multifaceted route of transmission, some have been investigated, and others are yet to be explained. It has been evidenced that bacteraemia could occur on such a transmission route. To date, only five cases reported in the literature will be used to evaluate the certainty of the previously stated route of transmission. A recent study reported bacteraemia by translocation of *L. adecarboxylata* into the gastrointestinal tract in a competent patient with Hirschsprung disease [39]. Three other cases reported bacteraemia with no history or current record of undergoing invasive medical interventions [24, 40-41]. Furthermore, a 5th case reported bacteraemia due to an invasive intervention on the gastrointestinal tract, which led to severe alterations in the mucosal membranes.

In our patient, blood culture bottles were negative during her lengthy hospital stay. These relationships can be hypothetically partly explained by another possible route of infection that has not yet been described: the possibility of gut flora borne by rectal bleeding (we describe it as rectal bleeding-borne gut flora). As it is known that *L. adecarboxylata* is part of the normal gastrointestinal flora [16,21], bearing in mind the chronic history of the patient having erosive gastropathy and heavy rectal bleeding before rectal swab collection, Hence, it could possibly be hypothesized that intestinal bleeding has been carried out by various sections of the intestinal microbiota in the rectal area. In reviewing the literature, no data was found on the association between rectal bleeding and *L. adecarboxylata*. However, dysbiosis in the gastrointestinal microbiota ecosystems could cause significant changes [43].

The frequency of *L. adecarboxylata* in human samples is unknown in Oman and it is the case in most other countries. However, a relatively recent 13-year retrospective single institutional study on *Leclercia adecarboxylata* infections in southern Hungary revealed 2-3 isolate frequency per year with a range of 1-4
[44]. The same study [44] asserted a median age of 57 years old (age range: 12-80) with more frequency in males (1.25 male-female ratio: 10 males: 8 females). Indeed, a variation in demographic characteristics could exist in other studies. The study also reported that 61.1% of the study population has underlying immunosuppression. In another study, the frequency of *L. adecarboxylata* in clinical specimens is estimated to be 0 to 5. However, the absolute frequency of infections caused by *L. adecarboxylata* is still to be determined, as it has been underreported for many decades since its first emergence [39].

The underestimation of *L. adecarboxylata* infections stemmed principally from the misidentification with other strains of the same family, *Enterobacterals*, such as *Escherichia coli*, as both strains share resemblance metabolic and morphological characteristics features [16, 45-56]. It should be noted that the two strains exhibited close and indistinguishable yields by automated systems, and it is misidentified if no further investigations have been carried out [15-16]. Thus, it indicates that the risk of misidentification is extremely eminent in the case where biochemical approaches are followed, which is the typical case in low setting laboratory. Despite that, the introduction of advanced automated systems, molecular sequencing, and MALDI-TOF MS revolutionized identification methods [47-48]. A similar situation was phased in our patient, *L. adecarboxylata* was misidentified as *Citrobacter koseri* using API and as *Pantoea* species using the Vitek2 system, which has not been reported in the literature, triggering an urgent comparative study in the same manner as conducted with *E. coli* [16,18]. The strain was correctly identified using MALDI-TOF, although a score of <2 triggered further identification through 16S rRNA.

It has been postulated that, in laboratories with low resources, the utility of chromogenic media such as CPS Elite agar and fosfomycin agar is promoted to differentiate between *E. coli* and *L. adecarboxylata* [16,18].

There are no guidelines for treating *L. adecarboxylata* infections, yet antibiotic regimens have shown efficient resolution of infections, including fluoroquinolones and beta-lactams [15,49]. Another study revealed more than 90% pan sensitivity in 31 of 34 *L. adecarboxylata* isolates [44]. However, resistance has been reported commonly with fosfomycin [16]. In the Spiegelhauer et al. study [15], 9 of 30 isolates showed resistance to ampicillin, and 8 of 10 isolates were resistant to fosfomycin. It can thus be suggested that these antibiotics are not recommended to treat *L. adecarboxylata infections*.

*L. adecarboxylata* resistant species producing ESBL were reported in the literature [39,45,50], which complicates therapeutic decisions leading to the use of broad-spectrum antibiotics such as carbapenems. More terrifying are the cases of Carbapnemase-Producing strains (CPE). In a study reported by Shin et al. [51], *L. adecarboxylata* was isolated from a blood culture of an immunocompromised patient with malignancy (breast cancer) in chemotherapy via a central catheter inserted peripherally. PCR confirmed the isolate for the presence of blaCTX-M-3 (resistant to aminoglycosides), blaTEM-1 (resistant to trimethoprim-sulfamethoxazole) and intl1 with aadA2 and dfrA12 genes. Sun et al. [52] reported isolation of *L. adecarboxylata* from a 43-year-old male sputum
sample harboured the genes blaNDM-1, blaTEM, blaOXA-1, and blaCTX-M-1 that illustrated resistance to all antibiotics tested. Another study by Geffen et al. [53] revealed the blaKPC-2 gene in a rectal swab confirmed by 16S of rDNA sequencing. Only one reference in the literature has reported *L. adecarboxylata* with the resistant blaVIM-1 gene in a non-clinical sample [54]. The study carried out by Papagiannitsis et al. [54] aimed to study the compliance to hand hygiene in Na Homolce Hospital staff, Prague, Czech Republic, that identified the strains with MALDI TOF MS confirmed it via 16s rRNA gene sequence. To our knowledge and through searching the literature, the current report is the first evidence to highlight *L. adecarboxylata*-resistant species producing blaVIM-1 in a rectal swab in the scientific literature.

Several limitations must be conveyed. The patient's death renders further examination to verify or falsify the previously described propositus concerning the possible route of infection. Not to mention the inability to pursue an accurate evaluation of the course of antibiotic therapy, although it was widely reported in the literature. There is ample room for further progress in determining the association of biofilms and bacterial resistance [55], because it has been neglected in discussing the possible path of infections of *L. adecarboxylata*.

**Conclusion**

In conclusion, *L. adecarboxylata* is an under-reported pathogen that could carry a threat to health care settings considering its potential to acquire multidrug resistance genes limiting therapeutic options. The isolate exhibited the resistant gene blaVIM-1 and blaOXA, making it the first reported case in the literature. These findings contribute to our understanding of the transmission route of *L. adecarboxylata* and provide a basis for future studies. The patient sadly passed away before the confirmation test was released, and keeping the long list of comorbidities, it is unquestionably challenging and inaccurate to attribute the death as a result of *L. adecarboxylata* infection. Further studies are urgent to determine the real risk factors and pathogenesis. Continued efforts are needed to make advanced diagnostic tools more accessible to ensure appropriate systems, services, accurate diagnoses, and strict infection control measures are essential to control its spread.

**Declarations**

**Ethics approval**

This is a case report in which Ethical clearance is waived due to the type of the study design.

**Consent to participate**

Written informed consent was obtained by the next-ken of our diseased and beloved patient.
Consent to publish

Written informed consent was obtained by the next-ken of our diseased and beloved patient.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mohammed Abdullah Al Shuhoumi, Abdulrahman Al Mhrooqi, Azza Al Rashdi, Rajesh Kumar, Ahood Al-Jabri, Amal Al-Kalbani, Abdulrahman Al Mhrooqi, Amina Al-Jardani. The first draft of the manuscript was written by Mohammed Abdullah Al Shuhoumi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Data availability

All data are available in the manuscript, however, additional supplementary files are will be submitted as a supportive evidences. If the supplementary files caannot be published; The datasets generated during and/or analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Figures
Figure 1

Colony morphology of Leclercia adecarboxylata in culture media. (a) Exhibits the morphological characteristics of Leclercia adecarboxylata in blood agar; (b) shows the morphological characteristics of Leclercia adecarboxylata in MacConkey's agar.

Figure 2

CPE Multiplex PCR. The above gel image shows OXA-48 singlex PCR results (extreme left), VIM singleplex results in the middle and CPE multiplex PCR results (extreme right) for isolate (Lab Id 222). The isolate was run in duplicate for all three PCR reactions. The molecular marker (100 bp) was run in the first well, and negative and positive controls were run in each PCR reaction. In Multiplex PCR, positive controls were run for all 5 types of targets (blaIMP, blaKPC, blaNDM, bla OXA-48 & blaVIM).