

Original Article

Leclercia adecarboxylata as an emerging pathogen in human infections: a 13-year retrospective analysis in Southern HungaryMárió Gajdács¹, Marianna Ábrók², Andrea Lázár², Gabriella Terhes², Katalin Burián^{2,3}¹ Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Szeged, Hungary² Institute of Clinical Microbiology, Faculty of Medicine, University of Szeged, Szeged, Hungary³ Department of Medical Microbiology and Immunobiology, Faculty of Medicine, University of Szeged, Szeged, Hungary**Abstract**

Introduction: The clinical role of *Leclercia adecarboxylata* as an opportunistic pathogen in the context of human infections have been highlighted by multiple published case reports, describing these bacteria as novel or emerging pathogens.

Methodology: The study included *L. adecarboxylata* isolates and laboratory data collected, corresponding to a 13-year time period (between 1 January 2005 and 31 December 2017). Presumptively identified *L. adecarboxylata* isolates were re-identified using VITEK 2 Compact ID/AST and MALDI-TOF MS analysis.

Results: n = 34 isolates were verified by VITEK 2 system and MALDI-TOF. The fosfomycin-agar and CPS Elite agar were effective in the phenotypic differentiation of the isolates. N = 18 (52.9%) of *L. adecarboxylata* was considered as clinically significant pathogens (based on the clinical signs and symptoms), while n = 16 (47.1%) were considered as contaminants. These pathogens were isolated from wound/abscess samples (n = 9), urine samples (n = 6) and blood cultures (n = 3). 31 out of 34 isolates (91.2%) were pan-sensitive (i.e. wild type) to the tested antibiotics. The median age of affected patients was 57 years (range: 12-80 years), 11 out of 18 patients (61.1%) presented with underlying immunosuppression at the time of isolation.

Conclusions: Based on the finding of this study, the actual (published) frequency of *L. adecarboxylata* infections needs to be re-evaluated as the risk of misidentification (and reporting the isolate as a pan-sensitive *Escherichia coli*) is high. Additional reporting of cases, both from a microbiological and clinical standpoint, could help clinicians develop a better understanding of the potential of this organism as a pathogen.

Key words: *Leclercia adecarboxylata*; identification; biochemistry; MALDI-TOF; Enterobacterales; epidemiology; retrospective; immunocompromised.

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Introduction

Leclercia adecarboxylata is a Gram-negative, oxidase-negative, motile (with peritrichous flagella) facultative anaerobic rod, belonging to the Enterobacterales order (based on Adeolu *et al.* 2016) [1,2]. *L. adecarboxylata* was first isolated from drinking water by Leclerc in 1962 as *Escherichia adecarboxylata* (or 'Enteric group 41', however, based on the protein electrophoretic and nucleic acid-based analyses (G+C content of these bacteria is between 52-54%) of Tamura, this species was reassigned and renamed in 1986 [3,4]. Since then, this species has been detected from various natural environments (natural surface waters, soil, from the surface of plants), animal sources and food [5]. The clinical relevance of *L. adecarboxylata* in human infections has not been well established [6]. Before the 1990s, this species was

mostly considered as a contaminant or a bystander, if isolated together with a significant pathogen [7]. The clinical role of *L. adecarboxylata* as an opportunistic pathogen in the context of human infections have been highlighted by multiple published case reports, describing these bacteria as novel or "emerging" pathogens [8]. *L. adecarboxylata* was principally implicated in infections of immunocompromised, severely debilitated patients (the majority of case reports/series attest to this), however, in the last several years, reports of *L. adecarboxylata* infections in immunocompetent patients, associated with trauma or cuts, in addition to the overuse of non-steroidal anti-inflammatory drugs (NSAIDs) and proton pump-inhibitors (PPIs) [9-11]. The isolation frequency of these microorganisms is very low (the true epidemiology of infections is unknown), and it is most

frequently detected as a member of a polymicrobial flora; this pathogen has been isolated from blood, urine, faeces, sputum, wound secretions, pus, abscesses, synovial fluid, cerebrospinal fluid and peritoneal fluid [6,12,13].

The biochemical profile of *L. adecarboxylata* is very similar to a prevalent member of the Enterobacterales order, *E. coli* [14]. Therefore, some studies postulate that the incidence of *L. adecarboxylata* infections is most probably underestimated and underreported due to misidentification of these bacteria by clinical microbiology laboratories [6,14]. On classically-used culture media relevant in the differentiation of Gram-negative bacteria (e.g. blood agar, eosine-methylene blue [EMB] agar, MacConkey agar), *L. adecarboxylata* colonies resemble those of *E. coli*, which may frequently occur (especially in low-resource settings) if the colonies are not investigated further [15]. The following biochemical characteristics are used for the differentiation of *L. adecarboxylata* from other members of the order: citrate-, lysine-decarboxylase-, ornithine-decarboxylase-, H₂S-, myo-inositol-, D-sorbitol-, while lactose+, L-rhamnose+, raffinose+, esculin+, indole+, Voges-Proskauer-test+, adonitol+, D-melibiose+ and sucrose+ [16]. However, some reports highlight that some isolates of *L. adecarboxylata* are adonitol-negative, while sorbitol-positive, which is again, characteristic for *E. coli* [15]. The accurate identification of *L. adecarboxylata* from clinical specimens is of utmost importance for diagnostic, therapeutic and epidemiological purposes. Nevertheless, with the advances in automated biochemical-based systems (e.g., BD Crystal, VITEK 2 ID/AST, MicroScan, molecular biological methods (e.g. PCR, 16S rRNA sequencing) and matrix-assisted laser desorption/ionization time-of flight mass spectrometry (MALDI-TOF MS), the accurate and timely identification of various bacterial pathogens is more ensured than previously [17,18].

The aim of our present study is to assess the biochemistry, epidemiology and clinical relevance of the isolation of *L. adecarboxylata* from clinical samples over a long surveillance period, in addition, to assess the antibiotic susceptibilities of these isolates.

Methodology

Study design and population

The present retrospective cohort study was conducted at the Albert Szent-Györgyi Clinical Center, a 1,820-bed primary and tertiary-care teaching hospital serving over 600,000 citizens in the Southern Region of

Hungary [19]. The Institute of Clinical Microbiology serves as the primary diagnostic microbiology laboratory of the Clinical Center, working 8 hours 7 days a week, in addition to an on-call system. The study included *L. adecarboxylata* microbiological isolates and laboratory data collected, corresponding a 13-year time frame (between 1 January 2005 and 31 December 2017). The utilization of hospital beds was between 62-71% and the number of nursing days per year was ranging between 408,000-477,000 days during the study period [19]. Data collection was carried out electronically, in the anonymized records of the laboratory information system (LIS), corresponding to samples positive for *L. adecarboxylata* isolates, which were identified using differential media and phenotypic (classical) biochemical methods. All isolates were re-identified using novel methods (see the *Bacterial identification* section).

To evaluate the demographic characteristics of these infections, patient data was also collected, which was limited to sex, age at the sample submission, indicators/disease corresponding to sample submission, presence/absence of immunosuppression and inpatient/outpatient status. Both clinically-significant *L. adecarboxylata* isolates (this was evaluated based on consultation with the treating physicians) and contaminants were included in the analysis, while isolates (and corresponding data) that turned out to be different species after re-identification were excluded from the study. Only the first isolate per patient was included in the study; however, isolates with different antibiotic-susceptibility patterns from the same patient were considered as different individual isolates [20].

Bacterial identification, ancillary biochemical testing

Sample processing in our Institute was carried out according to guidelines for routine clinical bacteriology, which have been previously described [21]. At the time of the sample submission, relevant samples were cultured on blood agar, chocolate agar, EMB agar, MacConkey agar (bioMérieux, Marcy-l'Étoile, Lyon, France) and UriSelect agar (in case of urine samples; Bio-Rad, Berkeley, CA, USA) plates and incubated at 37 °C for 24–48 hours, aerobically. Isolates that were identified as “*L. adecarboxylata*” (n = 42) based on presumptive biochemical (tube-based) methods and API 20E strips (bioMérieux, Marcy-l'Étoile, Lyon, France) were stored at -80°C until further analysis. After the surveillance period (2005-2017) had ended, all isolates were inoculated onto blood agar, EMB agar, MacConkey agar, CHROMID CPS Elite agar (bioMérieux, Marcy-l'Étoile, Lyon, France) and

fosfomycin-agar (containing of 32 mg/L fosfomycin and 50 mg/mL of glucose-6-phosphate; as most of the *L. adecarboxylata* isolates are fosfomycin-resistant) and incubated at 37 °C for 24 hours [14,15]. After the incubation period, the phenotypic characteristics of Bacterial strains were re-identified using the VITEK 2 Compact ID/AST automated system (bioMérieux, Marcy-l'Étoile, Lyon, France; according to manufacturers' instructions) and MALDI-TOF MS analysis. MALDI-TOF measurements were performed with direct spotting with HCCA matrix using the microFlex LT Biotyper (Bruker Daltonik GmbH, Bremen, Germany) mass spectrometer. The sample preparation, methodology, and the technical details of the MALDI-TOF MS measurements were described elsewhere [22]. The generated protein profile was analysed using the MALDI Biotyper RTC 3.1 software (Bruker Daltonik GmbH, Bremen, Germany) and compared to the MALDI Biotyper Library 3.1.

Isolates identified as any species other than *L. adecarboxylata* (n = 8), based on the results of VITEK 2 and mass spectrometry were excluded from the study and further analysis. For verification purposes, *L. adecarboxylata* isolates were subjected to manual tube tests for the fermentation of adonitol, D-melibiose, sucrose, raffinose, L-rhamnose, myo-inositol and D-sorbitol [16]. For all tests, colonies after 24 h of incubation were used and tests were read after 48 h. *E. coli* ATCC 25922 and *L. adecarboxylata* ATCC 23375 were used as quality control strains.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method and when appropriate, E-tests (Liofilchem, Abruzzo, Italy) on Mueller–Hinton agar plates for ampicillin, amoxicillin-clavulanic acid, piperacillin, cefoxitin, cefuroxime, ceftriaxone, cefepime, meropenem, norfloxacin, ciprofloxacin, gentamicin, nitrofurantoin and trimethoprim-sulfamethoxazole based on the European Committee of Antimicrobial Susceptibility Testing (EUCAST) standard methods [23]. In addition, for the verification of discrepant results, VITEK 2 Compact ID/AST (bioMérieux, Marcy-l'Étoile, France) was also used. Verification of fosfomycin-resistance was performed using the fosfomycin-agar, as described previously [14,15]. The interpretation of the results was based on EUCAST Clinical Breakpoints v.9 for Enterobacterales. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 35659, *E. coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 and *Pseudomonas*

aeruginosa ATCC 27853 were used as quality control strains.

Statistical analysis

Due to low number of relevant isolates (n = 34), only descriptive statistic analysis was performed, categorical variables were summarized by frequencies and percentages. Continuous data were presented as mean ± standard deviation and counts or percentages (%) [24]. All statistical analyses were performed using Statistical Package for Social Science (SPSS) software (IBM SPSS Statistics for Windows 24.0, IBM Corp., Armonk, NY, USA).

Ethical considerations

The study was deemed exempt from ethics review by the Institutional Review Board of the University of Szeged, and informed consent was not required as data anonymity was maintained throughout the study.

Results

Bacterial identification, ancillary biochemical testing

Out of the n = 42 isolates identified as “*L. adecarboxylata*” by presumptive biochemical methods, n = 34 isolates were verified by VITEK 2 system and MALDI-TOF analysis. In case of n = 8 isolates, misidentification occurred during the primary isolation and identification of the isolates: n = 6 isolates were *Pantoea agglomerans*, while n = 2 were *E. coli*. The agreement between the identification results of VITEK 2 and the MALDI-TOF were 100% (42/42). The fosfomycin-agar and CPS Elite agar were effective in the phenotypic differentiation of the isolates: the former detected 33 out of the 34 *L. adecarboxylata* isolates, while the chromogenic agar detected all relevant isolates (*L. adecarboxylata* presented with turquoise green colonies, while the other isolates produced pink colonies). On EMB and MacConkey agar, 29/34 *L. adecarboxylata* isolates showed lactose-fermentation, while 3/34 isolates showed hemolysis on blood agar. The results of the biochemical tests were the following: adonitol-positivity: 33/34, D-melibiose-positivity: 34/34, sucrose-positivity: 34/34, raffinose-positivity: 32/34, L-rhamnose-positivity: 33/34, myo-inositol-negative: 34/34, D-sorbitol-negative: 32/34.

Epidemiology of L. adecarboxylata infections, patients

The complete epidemiology and the patient characteristics associated with the isolation of *L. adecarboxylata* as a true pathogen or a contaminant is summarized in Table 1. Based on the collected data corresponding to the affected patients, in addition to the

consultation with the physicians at the time of primary isolation, n = 18 (52.9%) of *L. adedecarboxylata* was considered as clinically-significant pathogens (based on the clinical signs and symptoms), while n=16 (47.1%)

as contaminants. These pathogens were isolated from wound/abscess samples (n=9), urine samples (n = 6) and blood cultures (n = 3). Out of these 18 patients, in n = 6, *L. adedecarboxylata* was the only isolated

Table 1. Epidemiology and the patient characteristics associated with the isolation of *L. adedecarboxylata* (2005-2017)

Isolate	Study year	Age	Gender	Culture source	Pathogen	Contaminant	Medical condition at isolation	Immuno-suppressed	Co-pathogen*
1	2005	26	M	Midstream urine	-	+	Pneumonia	-	
2	2005	46	F	Wound secretion	-	+	Erysipelas	-	
3	2005	33	M	Stool	-	+	Diarrhoea	-	
4	2006	50	M	Midstream urine	+	-	Urinary tract infection	+	None
5	2006	29	F	Cervical swab	-	+	Pregnancy (screening)	+/-	
6	2006	9	M	Throat swab	-	+	Asthma bronchiale	-	
7	2007	63	F	Sputum	-	+	Pneumonia	-	
8	2008	41	M	Aerobic blood culture	+	-	Bacteremia	+	<i>S. epidermidis</i> , <i>S. haemolyticus</i>
9	2008	56	F	Wound secretion	+	-	Decubitus ulcers	+	<i>C. striatum</i> , <i>S. agalactiae</i> , <i>P. aeruginosa</i>
10	2009	17	M	Wound secretion	+	-	Fracture of tarsal bones	-	None
11	2009	68	F	Wound secretion	+	-	Ulcerated lower limb	+	<i>P. vulgaris</i> , <i>B. fragilis</i> , <i>Clostridium spp.</i>
12	2009	60	M	Wound secretion	+	-	Deep cutting injury	+	<i>K. pneumoniae</i>
13	2009	30	F	Stool	-	+	Sine morbo (screening)	-	
14	2010	64	F	Aerobic blood culture	+	-	Chronic pancreatitis	+	<i>K. pneumoniae</i>
15	2010	16	M	Wound secretion	+	-	Deep cutting injury	-	<i>E. coli</i> , <i>S. agalactiae</i> , <i>C. acnes</i>
16	2011	23	F	Midstream urine	-	+	Cystitis acuta	-	
17	2011	46	M	Aerobic blood culture	+	-	Septicaemia	+	None
18	2012	67	M	Wound secretion	+	-	Soft tissue infection	-	None
19	2012	28	M	Aerobic blood culture	-	+	Pneumonia	-	
20	2012	12	M	Abscess	+	-	Deep cutting injury	-	<i>E. coli</i> , <i>K. oxytoca</i> , <i>C. freundii</i>
21	2013	17	M	Throat swab	-	+	Pharyngitis	-	
22	2013	44	F	Midstream urine	+	-	End-stage kidney disease	-	None
23	2013	55	F	Cervical swab	-	+	Aerobic vaginitis	-	
24	2014	58	F	Midstream urine	+	-	Kidney cyst	-	<i>K. pneumoniae</i> , <i>E. cloacae</i>
25	2014	80	F	Wound secretion	+	-	Ulcerated lower limb	+	<i>S. aureus</i> , <i>S. haemolyticus</i> , <i>C. tertium</i> , <i>P. aeruginosa</i>
26	2014	77	F	Catheter-specimen urine	+	-	End-stage kidney disease	+	None
27	2015	62	M	Catheter-specimen urine	+	-	End-stage kidney disease	+	<i>S. aureus</i>
28	2015	35	M	Sputum	-	+	Persistent cough	-	
29	2015	37	F	Cervical swab	-	+	High-risk pregnancy (screening)	+/-	
30	2016	59	M	Wound secretion	+	-	Ulcerated lower limb	-	<i>S. putrefaciens</i> , <i>E. cloacae</i> , <i>S. agalactiae</i>
31	2016	52	F	Urine (non-specified)	+	-	Pyelonephritis	+	<i>K. pneumoniae</i>
32	2017	35	F	Stool	-	+	Sine morbo (screening)	-	
33	2017	24	F	Cervical swab	-	+	High-risk pregnancy (screening)	+/-	
34	2017	31	F	Midstream urine	-	+	Headache	-	

M: Male; F: Female; *Co-pathogens were only interpreted in case of clinically-relevant isolates.

pathogen, while for the other patients, this pathogen was isolated with other co-pathogens, most frequently with other members of the gut flora, (e.g. *E. coli*, *K. pneumoniae*, *C. freundii*, *P. vulgaris*) and *Staphylococcus* spp. (Table 1.). The median age of patients with clinically-relevant *L. adecarboxylata* infections was 57 years (range: 12-80 years), including 10 male and 8 female patients (male-to-female ratio: 1.25). 11 out of 18 patients (61.1%) presented with underlying immunosuppression at the time of isolation.

Antibiotic susceptibility of L. adecarboxylata isolates

Antibiotic susceptibility of the thirty-four *L. adecarboxylata* isolates to the tested antibiotics were the following: ampicillin: 32 out of 34, amoxicillin-clavulanic acid: 34 out of 34, piperacillin: 34 out of 34, cefoxitin: 34 out of 34, cefuroxime: 34 out of 34, ceftriaxone: 34 out of 34, cefepime: 34 out of 34, meropenem: 34 out of 34, norfloxacin: 32 out of 34, ciprofloxacin: 34 out of 34, gentamicin: 34 out of 34, nitrofurantoin: 32 out of 34 and trimethoprim-sulfamethoxazole: 31 out of 34. Thus, 31 out of 34 isolates (91.2%) were pan-sensitive to the tested antibiotics. In addition, no differences in susceptibility were shown between the clinically-relevant isolates and contaminants.

Discussion

L. adecarboxylata is an uncommon Gram-negative bacterium of the Enterobacterales order, sharing many biochemical and phenotypical characteristics with other members of the order present in the gut flora. In this study, cases of isolation (both clinically significant and contamination) of *L. adecarboxylata* were collected over a 13-year period in a single institution. The isolation frequency of this microorganism was around 2-3 isolates/year (range: 1-4), thus, it should be considered a species with low isolation-frequency. Most of the patients were middle-aged and in almost two-thirds of the patients, an immunocompromised state was verified. More than 90% of species were sensitive to all tested antibiotics, significant resistance-levels were not shown in our Institution.

Although the number of studies on the clinical role of *L. adecarboxylata* as an opportunistic pathogen is increasing, the frequency of reports is currently still too small to estimate the route of transmission and significance of this pathogen properly [25]. It has been suggested that this microorganism has a low pathogenic potential and the virulence factors of this species have not been adequately characterized [26]. Thus, to cause infection, there has to be some kind of breach in the

anatomical continuity of the tissue, an underlying disease or immunosuppression: e.g. compression ulcers, penetrative injuries, burns, chronic alcoholism, diabetes, liver cirrhosis, total parenteral nutrition, extensive use of corticosteroids or monoclonal antibodies, malignant diseases and anticancer agents, kidney failure and/or hemodialysis and presence of central venous catheters [6,27]. Of interest, reports of diarrhoea and gall bladder infections have also been described, which is similar to the role other members of gut commensals may play as opportunistic pathogens [28-30]. Nonetheless, community-acquired infections in immunocompetent individuals and outpatients have also been reported (predominantly presenting as UTIs) [6]. Recently, a systematic review article summarized the published case reports on *L. adecarboxylata* corresponding to the time period between 1991 and 2017: from 61 publications, 74 patients were affected by this pathogen, out of which, only four cases (5.4%) were fatal [6]. Most cases (91%) were published from North America (n = 26), Europe (n = 22) and Asia (n = 21), with males being affected twice as much as females. Similarly to this study, isolates were the most frequently recovered from the blood (25%), urine and wound samples (in addition to peritoneal fluid); around 30% of isolations were monobacterial [6].

Most of the published reports were in agreement and have shown very low levels of resistance in *L. adecarboxylata* isolates for all relevant anti-Gram-negative antibiotics (apart from benzyl-penicillin), which was further verified by our study [6]. The two largest laboratory-based studies on the susceptibility of these pathogens have been published by Tamura *et al.* in 1986 [4] and by Stock *et al.* in 2004 [14]. Similarly to other published reports, the overwhelming majority of isolates were susceptible to tested antibiotics in both mentioned studies. Nevertheless, ESBL or AmpC-producing isolates, resistant isolates to cefotaxime, ceftazidime and cefepime were described, in addition to an *bla*_{NDM-1}-producing strain isolated from a male patient, suffering an open ankle fracture and crush injury to his left foot [31,32]. Comparably to other Gram-negative gut bacteria, the emergence and spread of multidrug-resistant strains is to expected [33].

Due to similar biochemical properties, the risk of misidentification between *L. adecarboxylata* and *E. coli* is significant if only classical biochemical methods are used (which is common resource-scarce settings), however, the introduction of automated identification systems and MALDI-TOF MS play a crucial role in the identification of uncommon bacteria and in establishing their clinical relevance [34,35]. In low-resource

settings, the use of fosfomycin-agar and the strategic selection of chromogenic media (e.g. CPS Elite agar) that are capable of differentiating *L. adecarboxylata* and *E. coli* is warranted for their successful isolation and identification [14,15].

Conclusion

Long-term epidemiological and clinical studies (similar to the present report) are required and encouraged to ascertain the true prevalence of *Leclercia* infections. Isolation of this pathogen is usually monobacterial in immunocompromised patients, while in immunocompetent patients, the isolation predominantly occurs in a part of a polymicrobial culture. To the best of our knowledge, this is the first and longest study describing the epidemiology of this bacterial pathogen in Hungary. Based on the findings of this study, the actual (published) frequency of *L. adecarboxylata* infections needs to be re-evaluated as the risk of misidentification (and reporting the isolate as a pan-sensitive *E. coli*) is high. Additional reporting of cases, both from a microbiological and clinical standpoint, could help clinicians develop a better understanding of the potential of this organism as a pathogen.

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