CASE REPORT

Isolation of *Leclercia adecarboxylata* from a Chronic Pus Sample in a German Shepherd Dog: A Case Report

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Leclercia adecarboxylata is a peritrichous, motile, Gram-negative, facultative anaerobic bacterium belonging to the Enterobacteriaceae family. It was initially classified as *Escherichia adecarboxylata* by Leclerc, H. in 1962 before being reclassified into the *Leclercia* genus based on phenotypic and genotypic analyses (Anuradha, 2014). Originally isolated and identified in water, foods, and environment as also from clinical specimens including blood, stool, sputum, urine, and wound pus, *L. adecarboxylata* is increasingly recognized as a rare opportunistic pathogen in humans and animals primarily affecting immunocompromised patients and has been associated with a variety of chronic infections and inflammations including wound, urinary tract, soft tissue, peritonitis, post-traumatic polymicrobial infection, and bloodstream infections (Choudhary *et al.,* 2018; Keyes *et al.,* 2020; Zayet *et al.,* 2021).

In veterinary medicine, reports of *L. adecarboxylata* are scarce and its pathogenic role remains largely under-recognized. Most documented cases involve co-infection with other bacteria; however, isolated reports emphasize its potential to cause significant diseases such as wound infections, abscesses, and septicaemia in animals (Choudhary *et al.,* 2018). The pathogenicity of *L. adecarboxylata* in animals remains poorly characterized; however, its recurrent association with skin infections across various reports suggests its ability to function both as a primary or secondary opportunistic pathogen, especially in cases where skin integrity is compromised (Jung *et al.,* 2017; Spiegelhauer *et al.,* 2019; Yamada and Takahashi, 2020; Al Shuhoumi *et al.,* 2023). Factors such as immune status, prior antibiotic use, and environmental exposure are likely key contributors or determinants of its pathogenic potential (Hess *et al.,* 2008; Al Shuhoumi *et al.,* 2023). Current reports on isolation of the bacterium stated that multidrug resistant strains are increasing (Alosaimi and Kaaki, 2020; Garza-Gonzalez *et al.,* 2021; Di Gregorio *et al.,* 2023). Despite its clinical relevance, the identification of *L. adecarboxylata* can be challenging due to its phenotypic similarities with other Enterobacteriaceae members (Gimpel and Kamerbeek, 2015; Morris and Patel, 2018), emphasizing the importance of integrating biochemical, molecular, and susceptibility testing in diagnostics. This document reports isolation and characterization of ESBL positive *Leclercia adecarboxylata* from canine clinical sample, perhaps for the first time in India.

Case History and Observations

A three-year-old German Shepherd intact male was presented with a chronic non-healing pustular skin lesion persisting for over 10 months. The lesion was located on the lateral abdomen, characterized by recurrent pustules and intermittent serous discharge. The dog had undergone multiple courses of antibiotics with limited success. Aiming to identify the causative agent, aseptic pus sample collected was inoculated into Brain Heart Infusion (BHI) broth for enrichment. After incubation, subcultures were performed on BHI agar, and Gram negative selective media MacConkey Lactose Agar (MLA) with bromocresol purple indicator (HiMedia Lab, Mumbai, India; Fig. 1, 2). Morphological examination of colonies indicated small, Gram-negative rods. A comprehensive conventional biochemical profiling and ABIS online laboratory tool (ABIS Database Version 3.17.18) confirmed the bacterial isolate as *L. adecarboxylata*.

Antimicrobial susceptibility testing of isolate was performed using the Kirby-Bauer disk diffusion method using 14 different antibiotic disks. For initial screen test and phenotypic confirmation of ESBL producers HiMedia Hexa G-minus 23 and Hexa G-minus 24, respectively, were selected (Table 1; Fig. 3, 4). The isolate exhibited multidrug resistance and tested positive for extended spectrum beta-lactamase (ESBL) production.

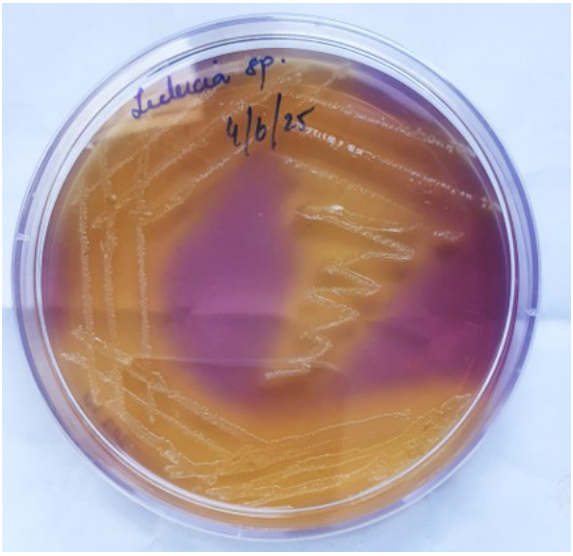

Fig. 1: Yellow color colony *Leclercia adecarboxylata* on MLA purple agar showing fermentation of lactose.

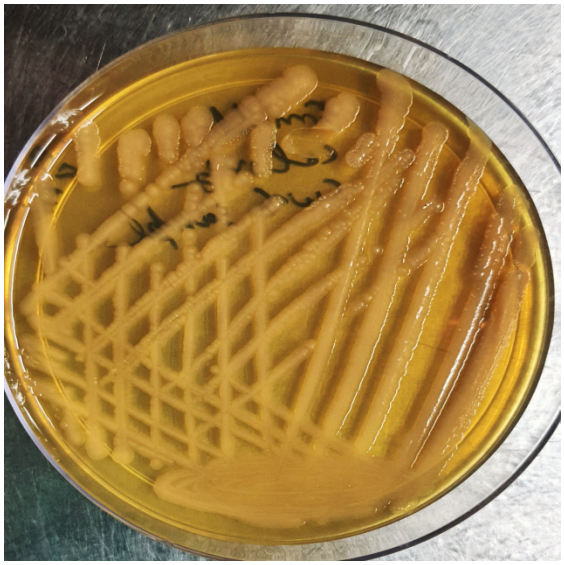

Fig. 2: *Leclercia adecarboxylata* on BHI agar (HiMedia Lab, Mumbai, India)

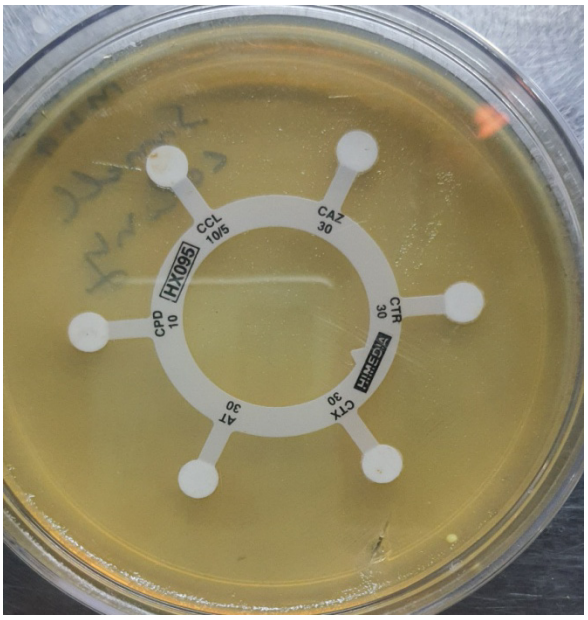

Fig. 3: Screening of ESBL producer with HiMedia Hexa G-minus 23

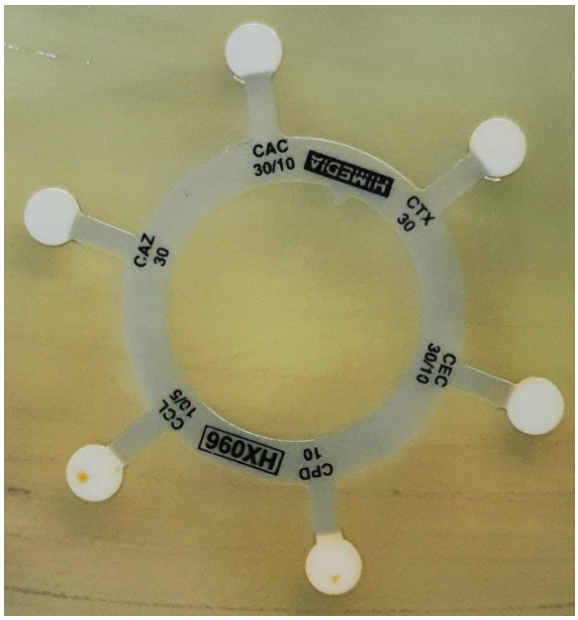

Fig. 4: ESBL phenotypic confirmation with HiMedia Hexa G-minus 24

Laboratoey Findings and Discussion

L. adecarboxylata was isolated from clinical sample as part of a polymicrobial infection with *E. coli* pathogen. Subculture on MacConkey Purple Agar and Gram staining revealed the presence of typical *Enterobacteriaceae* Gram-negative rods, positive for catalase activity but negative for oxidase test. The organism demonstrated positive indole and methyl red tests, urease activity, esculin hydrolysis, nitrate reduction, and fermentation tests showed acid and gas production on triple sugar iron (TSI) agar, acid production from dulcitol and melibiose. The positive indole and MR tests, urease activity and fermentation pattern aligned with known profiles of this species indicating the production of indole and presence of active fermentation pathways. Voges-proskauer (VP), citrate utilization tests, lysine decarboxylase tests, and hydrogen sulphide were negative. Biochemical test results showed 90.1% similarity with *Leclercia adecarboxylata* using ABIS database system.

Although historically regarded as opportunistic bacterium associated with human immunocompromised hosts, recent reports are increasingly implicating *L. adecarboxylata* in various infections across species. Choudhary *et al.* (2018) reported isolation of the bacterium from respiratory infection

in cattle in Chhatisgarh, India. The current isolation of this bacterium from a chronic wound exudate in a three-year-old German Shepherd dog, which had undergone multidrug treatment over a ten-month period, underscores its potential pathogenic role in companion animals. This prolonged persistence of the lesion in the patient and resistance to previous antibiotic regimens suggested that *Leclercia adecarboxylata* may possess virulence factors enabling sustained survival within host tissues, which is consistent with findings of Di Gregorio *et al.* (2023), who highlighted the bacterium as an emerging pathogen with potential virulence attributes facilitating persistence in the environments. Notably, to our knowledge, there are no published reports of *L. adecarboxylata* isolation from canine hosts in India, highlighting the need for further investigation into its clinical significance and zoonotic implications.

Antimicrobial susceptibility testing profile indicated that the bacterial isolates were sensitive to aminoglycosides such as amikacin, gentamicin, and to β-lactam antibiotics like ticarcillin/ clavulanic acid and cefoperazone (Table 1). These antibiotics are likely to be effective choices for empirical or targeted therapy. Conversely, significant resistance was observed against commonly used antibiotics such as ampicillin, cefixime, and cotrimoxazole, suggesting that they may not be suitable for treating infections caused by these isolates. The intermediate susceptibility to certain cephalosporins and fluoroquinolones warrants cautious use, with close clinical follow-up for treatment success. The detection of resistant colonies within inhibition zones indicates the presence of heterogeneous resistance mechanisms, underscoring the need for molecular investigations to elucidate specific resistance genes.

Table 1: Antibiotic susceptibility profile of the isolate *Leclercia adecarboxylata*

Antibiotic	Zol (mm)	Interpretation
Amikacin (30 µg)	23.5	Sensitive
Ampicillin (10 µg)	<10.0	Resistant
Ampicillin/Sulbactam (10/10 µg)	20.0	Intermediate
Amoxicillin/Sulbactam (30/15 µg)	13.0	Intermediate
Ceftazidime (30 µg)	17.5	Intermediate
Cefixime (5 µg)	12.0	Resistant
Cefoperazone (30 µg)	21.5	Sensitive
Cefamandole (30 µg)	13.0*	Resistant
Cotrimoxazole (25 µg)	<10.0	Resistant
Enrofloxacin (5 µg)	20.0	Intermediate
Gentamicin (10 µg)	25.5	Sensitive
Moxalactam (30 µg)	21.0*	Intermediate
Ticarcillin/Clavulanic acid (75/10 µg)	20.0*	Sensitive
Tobramycin (10 µg)	24.5	Sensitive

*Resistant bacterial colonies observed within the zone of inhibition

The isolation of *Leclercia adecarboxylata* from a canine clinical sample is noteworthy due to its rarity in veterinary settings, highlighting the emerging presence of this bacterium in animal health. Significantly, the isolate exhibited multidrug resistance and tested positive for extended spectrum beta-lactamase (ESBL) production, indicating the heightened ability to withstand multiple classes of antibiotics. Although such isolations are uncommon in veterinary practice, the human clinical reports documented multiple cases of the bacterium developing resistance to antimicrobials. The presence of MDR and ESBL positive strain in both human and animal populations raises concerns regarding the potential zoonotic transmission and the role of veterinary environments to the broader context of antimicrobial resistance. This highlights the critical need for vigilant surveillance, antimicrobial stewardship, and molecular characterization of such isolates to better understand their epidemiology and resistance mechanisms.

This report documents the first isolation of *Leclercia adecarboxylata* from canine clinical case in India, associated with chronic polymicrobial wound infection. The isolate demonstrated multidrug resistance including ESBL production and given its emerging pathogenic potential in both human and veterinary medicine, this finding highlights the need for increased surveillance, accurate identification, and antimicrobial stewardship to address its zoonotic and clinical significance.

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