Original Article

Occurrence and Bacterial Performance of *Leclercia Adecarboxylata* Isolated from Lung Infections

Abbas Atiya Hammoudi[1]*, Mohammed Sh. Jebur[2]

[1]College of Health and Medical Technologies, Al-Bayan University, Baghdad, Iraq.
Email: abbas.at@albayan.edu.iq
[2]Al-Rafidain University College, Baghdad, Iraq.
Email: mohammed.shamkhi@rur.edu.iq

Abstract

Background: This current study designed for isolation and identification of *Leclercia adecarboxylata* from patients infected with different types of lung infections in Baghdad Teaching Hospital of Medical City by Department of Medical Laboratory Techniques of Al-Rafidian College University during the period from December 2022 to March 2023. **Materials and Methods:** Specimen of sputum and bronchial fluid were collected from 200 patients including both sexes with different ages, who suffered from lung infections that clinically diagnosed by the consultants. These 200 specimen were assessed directly after inoculation on the proper media for isolation and identification. Correspondingly, the identification was confirmed by API 20 system (Biomeriux Company, France), Vitek 2 system and 16S rRNA gene sequencing. Antimicrobial susceptibility profile of *Leclercia adecarboxylata* was patterned against many types of antibiotics that used for treatment of lung infections such as Amoxicillin, Fluoroquinolones, Cephalosporin, Piperacillin, Clindamycin, Erythromycin, Levofloxacin, Linezolid, Imipenem, Trimethoprim/ Sulfamethoxazole (SMX-TMP), Vancomycin, Fosfomycin. **Results:** Results showed polymicrobial distribution of 8 types of pathogenic bacterial isolates in 163 out of 200 specimens these were *Klebsilla pneumoniae* 27 (16.56%), *Acinetobacter baumannii* 25 (15.33%), *Staphylococcus aureus* 23 (14.11%), *Streptococcus pneumoniae* and *Escherichia coli* 20 (12.26%) for each one, 19 (11.65%), isolates for *Streptococcus pyogenes* and *Haemophilus influenzae* and 10 (6.13%) *Leclercia adecarboxylata* isolates. **Conclusion:** Antimicrobial susceptibility pattern of *L. adecarboxylata* isolates presented multidrug resistance through high susceptibility (100%) for Amoxicillin, Fluoroquinolones, Cephalosporin, Piperacillin, Erythromycin and Imipenem. Also it was susceptible to Levofloxacin (90%), Clindamycin and Trimethoprim/Sulfamethoxazole (SMX-TMP) (80%), Linezolid (50%) and Vancomycin (10%).

Keywords: *Leclercia Adecarboxylata*, Lung Infections, Multidrug Resistance, Rare Pathogens.

Introduction

Lung infections are the inflammation of various parts of lung tissue, which perform as acute lung infections or chronic[1] Lung infections occurs due to disease-causing microorganism such as bacteria, virus, fungi, parasite and or any pathogenic agent that causes damage and inflammation to human lungs.[1] Unfortunately, lung infections might happened as co-infection or secondary infection.[2]

There are no greatly differences of lung infections between people of any age or gender to develop these infections but some forms are more common in people of certain ages.[3] Lung infections can be a serious sickness via itself or also by leading to many health problems.[4]

Asthma, bacterial pneumonia, COVID-19, Influenza, Whooping Cough (Pertussis), Tuberculosis, cystic fibrosis, pulmonary disease are the frequent lung infections that affect lung tissues and surrounding extents.[5]

Bacterial infections which typically occasionally diagnosed as secondary pneumonia or lung infections at some point during Covid-19 hospitalization has been reported in about 6-15% of patients where bacterial infection was measured.[6-8] So that Hassan *et al.*[9] showed that the most widespread separated organisms were the Gram negative bacteria mainly *K. pneumoniae*, *P.*

Access This Article Online

Quick Response Code:

Website: www.jnsbm.org

DOI: https://doi.org/10.5281/zenodo.17335771

Address for Correspondence: College of Health and Medical Technologies, Al-Bayan University, Baghdad, Iraq
Email: abbas.at@albayan.edu.iq

Submitted: 12th November, 2025 **Received:** 24th December, 2025
Accepted: 03rd January, 2026 **Published:** 05th January, 2026

How to Cite This Article: Hammoudi A A, Jebur M S J. Occurrence and Bacterial Performance of Leclercia Adecarboxylata Isolated from Lung Infections. J Nat Sc Biol Med 2026;17(1):1-5

aeruginosa, *A. baumannii*, and *E. coli*, while the Gram-positive bacteria mainly *S. epidermidis* and *S. aureus*.[9] *Leclercia adecarboxylata* is a infrequent opportunistic microorganism, which belongs to *Enterobacteriaceae* and usually lead to soft tissue infections through that, its Gram negative bacillus bacterium which is motile and growth on blood and chocolate agar. Also it is aerobic or facultative anaerobic rod and contributed oxidase-negative.[10] The study aimed to investigate the multidrug resistance of *Leclercia adecarboxylata* bacterium which isolated from different lung infection cases.

Materials and Methods

Study Design

A cross-sectional research was done for detection of *L. adecarboxylata* isolates from patients infected with different types of lung infections from Baghdad Teaching Hospital in Medical City by Department of Medical Laboratory Techniques of Al-Rafidian College University during December 2022 to March 2023.

Patient's Study

Patient's specimens were collected from lung infection cases. These patients had different demographic features such as age and sex, as well as qualities of the current lung infections, outcome from patients' medical records about duration of infections, clinical feature (that diagnosed by specialist physician), bacteremia joined or not and antimicrobial drugs which used for treatment or prophylaxis. Patients were individually asked and verbally explained about the research aims and objective and the fate of samples collected from them with signed approval consent forms. The study was approved by College of Health and Medical Technologies, Al-Bayan University (Iraq), approval letter code 502 on 16 Sep 2024.

Specimen Collection

Specimens of sputum and bronchial fluid were collected from 200 patients including both sexes with different ages, who suffered from lung infections that clinically diagnosed by the consultants. These patients presenting to Hospitals in Baghdad Teaching Hospital in Medical City. The demographic features of collected cases and history for each case was recorded in a questionnaire paper. The study included some criteria for patients like that all cases were newly diagnosed as lung infections cases, that appeared early severe respiratory symptoms and yet all patients were not taken any antimicrobial agents before. While the exclusion criteria of the study were that patients with COVID-19 patients. COVID-19 patients were excluded because the disease causes typical immune responses and coagulopathy that might confound primary outcomes.

Identification and Diagnosis

Registered specimens were replicate number identified, mono or polymicrobial bacterial lung infections, these 200 sputum and bronchial fluid were identified directly after inoculation on the appropriate media for isolation and identification.[10] Blood agar plates, MacConky agar and Chocolate agar were used for bacterial culture isolation and identification.[11] Isolates were detected based on the morphological characteristics on culture medium and biochemical tests according to the bacterial classification that described by MacFaddin[12].

Biochemical tests also were done for final identification to all isolates by bacterial commercial systematic biochemical tests that carried out then confirmed by API 20 system (Biomeriux Company, France). Also all positive bacterial isolates were then confirmed again through Vitek 2 system.[12]

Antimicrobial Susceptibility Profile

Antimicrobial resistance pattern was confirmed by the disc diffusion assay via MHA. Preparation of bacterial inoculum were done by suspended in 5 ml BHI-B, then the inoculated broth incubated (37˚C for 18-24 hrs). There as the actively growing broth culture was accomodated to gain turbidity optically comparable to the 0.5 McFarland standard tubes (growth equivalent to 1.5 $X10^8$ cell/ml).[13] Antimicrobial susceptibility profile of *Leclercia adecarboxylata* was patterned against many types of antibiotics that used for treatment of lung infections such as Amoxicillin, Fluoroquinolones, Cephalosporin, Piperacillin, Clindamycin, Erythromycin, Levofloxacin, Linezolid, Imipenem, Trimethoprim/ Sulfamethoxazole (SMX-TMP), Vancomycin, Fosfomycin. The zone of inhibition observed and measured. Minimum inhibitory concentration (MIC) of antibiotics on bacterial growth also detected by Vitek 2 system and the concentrations were monitored according to instruction of the biomeriux company.

Molecular Study

Identification of *Leclercia adecarboxylata* was confirmed by 16S rRNA gene sequencing. So that, DNA extraction of bacterial colonies from overnight growth (18–24h) on a blood agar plate at 37 °C were suspended in 200 µL of distilled H_2O, and genomic DNA was extracted according to manufacturer instructions. PCR amplification of bacterial 16S rRNA universal primers were used, furthermore the master mix was prepared using 0.4 µM final 0.4 M concentration of the primer.[14] While was achieved via using the Sanger cycle sequencing method using Big Dye TM Terminator V3.1-Cycle Sequencing chemistry.[14]

Statistical Analysis

Descriptive statistical analysis was evaluated, nonparametric data were outlined as frequencies and percentages. Parametric data were presented as mean ± standard deviation. All statistical analyses were performed using Statistical Package for Social Science (SPSS) software (IBM SPSS 24, USA).

Results and Discussion

Results listed in table (1) showed polymicrobial distribution of 8 types of pathogenic bacterial isolates in 163 out of 200 specimens these were *Klebsilla pneumoniae* 27 (16.56%), *Acinetobacter baumannii* 25 (15.33%), *Staphylococcus aureus* 23 (14.11%), *Streptococcus pneumonia* and *Escherichia coli* 20 (12.26%) for each one, 19 (11.65%), isolates for *Streptococcus pyogenes* and *Haemophilus influenzae* and 10 (6.13%) *Leclercia adecarboxylata* isolates.

Table 1: Types of Pathogenic Bacterial Isolates in Current Study.

	Type of Bacteria	No.	%	Chi-Square Test (P – value)
1	*K. pneumoniae*	27	16.56	
2	*A. baumannii*	25	15.33	
3	*S. aureus*	23	14.11	
4	*S. pneumoniae*	20	12.26	
5	*E. coli*	20	12.26	5.473 (0.0018) **
6	*S. pyogenes*	19	11.65	
7	*H. Influenza*	19	11.65	
8	*Leclercia adecarboxylata*	10	6.13	
	Total	163	99.95	

** Highly significant (P≤0.01), Confidence interval 95%

Zhang *et al.*[15] detected that 612 (57.89%) patients developed secondary bacterial infections after lung pneumonia and *K. pneumoniae* isolates were the major pathogen of lung infections like Covid-19, so that findings of this study were similar to current results of *K. pneumoniae* isolates which was the predominant pathogen.[16] While Calcagno *et al.*[17] exhibited that *Strep. pneumoniae* isolates gave high level of secondary bacterial infections isolated from lung infection cases followed by *K. pneumoniae and H. influenzae*. In addition, study of Said *et al.*[18] showed many patients developed secondary bacterial infections after severe Covid-19 cases.

Also the present results were agreement with results obtained by Karataş *et al.*[19] who recognised that the bacterial pathogens detected in cases of viral lung infections were largely Gram-negative bacteria, particularly *K. pneumoniae*.

Results of *Acinebacter baumannii* showed that it was found with 25 (15.33%), so that same results were confirmed by Karataş *et al.*[19] showed that there were (13.3%) *A. baumannii* isolates had positive diagnosis by Vitek-2 in lung infection cases. Also Khurana *et al.*[20], Lavrinenko *et al.*[21] and Calcagno *et al.*[17] showed that the predominant pathogen isolate was *A. baumannii* from viral lung infections with frequencies; 20 (74%), 1 (32.3%) and 7 (2.8%), respectively.

Isolation and identification of *Staphylococcus aureus* and *Escherichia coli* isolates from lung infection cases were 23 (14.11), 20 (12.26) respectively as main pathogenic agents or as secondary or/and co-infections. This fact of isolation was agreement to bacterial isolation findings of Sharifipour *et al.*[22], Al Shuhoumi *et al.*[23], and Calcagno *et al.*[17] studies. *Leclercia adecarboxylata* was isolated with lowest number, it is beneficial to point out, that its isolation from lung infection cases or pulmonary pneumonia be determined by results of microscopic appearance which presented that *L. adecarboxylata* was Gram negative bacillus, a motile grow well on blood and chocolate agar (Figure 1), it was motile facultative aerobic, gram- negative bacillus and contributed oxidase-negative,[24] so its grew as pink lactose fermenting colonies on MacConkey agar and equally non-haemolytic colonies on blood agar. Also results of API 20 test confirmed its isolation and identification. Through that Vitek2 biochemical test for diagnosis of *L. adecarboxylata* isolates were documented positive isolation with 10 (6.13%) from positive bacterial specimens.

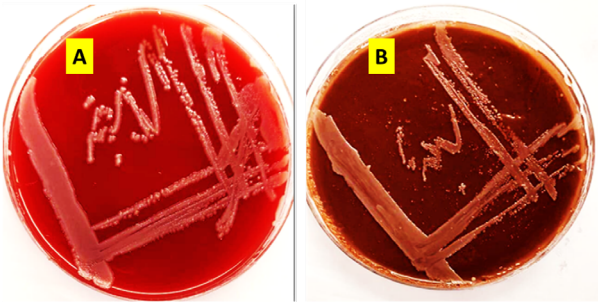

Figure 1: (A) *L. adecarboxylata* on Blood agar (B) *L. adecarboxylata* on Chocolate agar**.** Colony Morphology shown Representative Color and Pigmentation Related to Hemolysis on Blood Agar with Growth Characteristics on Chocolate Agar and Colony showed Characteristic Texture and Opacity.

Clark *et al.*[25], Aarab *et al.*[24] and Al Shuhoumi *et al.*[23], isolated *L. adecarboxylata* from different specimen with various percentages. Antimicrobial susceptibility pattern of *L. adecarboxylata* isolates presented MDR based on recent drug classification guidelines, an almost equal number of drugs were found highly effective against the isolates of current study.

For instance, *L. adecarboxylata* showed high susceptibility (100%) for Amoxicillin, Fluoroquinolones, Cephalosporin, Piperacillin, Erythromycin and Imipenem. Also it was susceptible to Levofloxacin (90%), Clindamycin and Trimethoprim/Sulfamethoxazole (SMX-TMP) (80%), Linezolid (50%) and Vancomycin (10%). Consequently, all results of bacterial susceptibility of present study were matched the results obtained by Zayet *et al.*[26]. Rawson *et al.*[7] showed that Piperacillin and Vancomycin were a scientific treatment for *L. adecarboxylata* cases that approved the results of study.[7] While, Clark *et al.*[25], found that *L. adecarboxylata* isolates were resistance to amoxicillin/clavulanate, ceftriaxone, imipenem, and gentamicin with low percentage (34%) in Covid-19 patients that consider as lung infections and these results justified consequences of present study (Table 2).

On other hands, *L. adecarboxylata* isolates displayed high resistance for Fosfomycin (100%), Vancomycin (90%) and with less levels for Clindamycin, Levofloxacin, Linezolid, Trimethoprim/ Sulfamethoxazole (SMX-TMP) and (20%, 10%, 50%, and 20%) respectively. Thus, results of resistance

approved by other many studies detected antimicrobial susceptibility profile of lung bacterial causes.[27-29]

Table 2: Antibiotic Susceptibility Profile of *Leclercia Adecarboxylata*.

Resistance (%)	Sensitivity (%)	Antibiotic
-	100	Amoxicillin
-	100	Fluoroquinolones
-	100	Cephalosporin
-	100	Piperacillin
20	80	Clindamycin
-	100	Erythromycin
10	90	Levofloxacin
50	50	Linezolid
-	100	Imipenem
20	80	Trimethoprim/ Sulfamethoxazole
90	10	Vancomycin
100	-	Fosfomycin

Genomic DNA was obtained from the bacterial culture by using protocol of ABIO pure extraction to *L. adecarboxylata* bacterium, which cultured on artificial media overnight. Concentration of DNA was determined by using Quintus Fluorimeters. The presented DNA concentration range (10-25ng/µl), and the DNA integrity was checked through agarose gel electrophoresis. DNA must be displayed as a single sharp be and when visualized under UV light following ethidium bromide staining. Through that Shabir *et al.*[28] showed that 16S rRNA gene is used for pathogenic bacteria isolation and identification as used in current study (Figure 2).

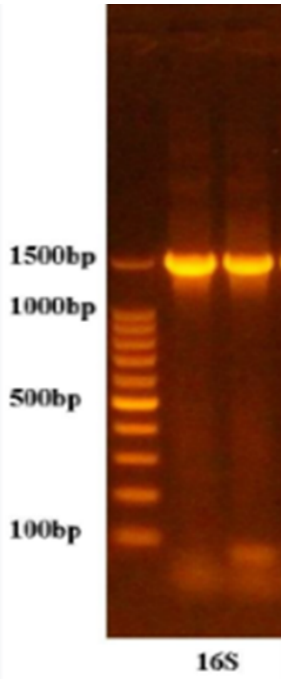

Figure 2: Results of the Amplification of 16S rRNA Gene of *L. adecarboxylata* Bacterium (2 Lanes resemble 1500bp PCR products).

Conclusion

Antimicrobial susceptibility pattern of *L. adecarboxylata* isolates presented multidrug resistance through high susceptibility for Amoxicillin, Fluoroquinolones, Cephalosporin, Piperacillin, Erythromycin and Imipenem. Also it was susceptible to Levofloxacin, Clindamycin and Trimethoprim/Sulfamethoxazole, Linezolid and Vancomycin.

Acknowledgement

Authors would like to thank the staff of Baghdad Medical hospital with their cooperation for the work could be done successfully.

References

1. Biagi C, Rocca A, Poletti G, Fabi M, Lanari M. Rhinovirus Infection in Children with Acute Bronchiolitis and Its Impact on Recurrent Wheezing and Asthma Development. Microorganisms. 2020; 8(10): 1620. doi: https://doi.org/10.3390/microorganisms8101620.
2. Darweesh O, Kurdi A, Merkhan M, et al. Knowledge, Attitudes, and Practices of Iraqi Parents Regarding Antibiotic Use in Children and the Implications. Antibiotics (Basel). 2025; 14(4): 376. doi: https://doi.org/10.3390/antibiotics14040376.
3. Abuelgasim H, Albury C, Lee J. Effectiveness of honey for symptomatic relief in upper respiratory tract infections: a systematic review and meta-analysis. BMJ Evid Based Med. 2021; 26(2): 57-64. doi: https://doi.org/10.1136/bmjebm-2020-111336.
4. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens. Jama. 2020; 323(20): 2085-86. doi: https://doi.org/10.1001/jama.2020.6266.
5. Linden D, Guo-Parke H, Coyle PV, et al. Respiratory viral infection: a potential "missing link" in the pathogenesis of COPD. Eur Respir Rev. 2019; 28(151): 180063. doi: https://doi.org/10.1183/16000617.0063-2018.
6. Langford BJ, So M, Raybardhan S, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect. 2020; 26(12): 1622-29. doi: https://doi.org/10.1016/j.cmi.2020.07.016.
7. Rawson TM, Moore LSP, Zhu N, et al. Bacterial and Fungal Coinfection in Individuals With Coronavirus: A Rapid Review To Support COVID-19 Antimicrobial Prescribing. Clin Infect Dis. 2020; 71(9): 2459-68. doi: https://doi.org/10.1093/cid/ciaa530.
8. Huang Z, Kłodzińska SN, Wan F, Nielsen HM. Nanoparticle-mediated pulmonary drug delivery: state of the art towards efficient treatment of recalcitrant respiratory tract bacterial infections. Drug Deliv Transl Res. 2021; 11(4): 1634-54. doi: https://doi.org/10.1007/s13346-021-00954-1.
9. Hassan I, Gupta P, Ray P, Tiewsoh K. Leclercia adecarboxylata Causing Spontaneous Bacterial Peritonitis in a Child with Nephrotic Syndrome: A Case Report and Review of Literature. J Lab Physicians. 2020; 12(3): 222-24. doi: https://doi.org/10.1055/s-0040-1721162.

10. Larsson C, Bergström S. A novel and simple method for laboratory diagnosis of relapsing Fever borreliosis. Open Microbiol J. 2008; 2: 10-2. doi: https://doi.org/10.2174/1874285800802010010.
11. Dacarro C, Picco AM, Grisoli P, Rodolfi M. Determination of aerial microbiological contamination in scholastic sports environments. J Appl Microbiol. 2003; 95(5): 904-12. doi: https://doi.org/10.1046/j.1365-2672.2003.02044.x.
12. MacFaddin JF. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia; 2000.
13. Ali AJM, Alkhudhairy MK, Tobal DD. Immunological Study of Klebsiella Pneumoniae Isolated from Pneumoniae Infection Patients. Biomedicine and Chemical Sciences. 2022; 1(1): 11-19. doi: https://doi.org/10.48112/bcs.v1i1.80.
14. Kim HM, Chon CY, Ahn SH, et al. Fatal spontaneous bacterial peritonitis by Leclercia adecarboxylata in a patient with hepatocellular carcinoma. Int J Clin Pract. 2008; 62(8): 1296-8. doi: https://doi.org/10.1111/j.1742-1241.2005.00780.x.
15. Zhang H, Zhang Y, Wu J, et al. Risks and features of secondary infections in severe and critical ill COVID-19 patients. Emerg Microbes Infect. 2020; 9(1): 1958-64. doi: https://doi.org/10.1080/22221751.2020.1812437.
16. Wu T, Ge Y, Zhao K, et al. A reverse-transcription recombinase-aided amplification assay for the rapid detection of N gene of severe acute respiratory syndrome coronavirus 2(SARS-CoV-2). Virology. 2020; 549: 1-4. doi: https://doi.org/10.1016/j.virol.2020.07.006.
17. Calcagno A, Ghisetti V, Burdino E, et al. Co-infection with other respiratory pathogens in COVID-19 patients. Clin Microbiol Infect. 2021; 27(2): 297-98. doi: https://doi.org/10.1016/j.cmi.2020.08.012.
18. Said KB, Alsolami A, Moussa S, et al. COVID-19 Clinical Profiles and Fatality Rates in Hospitalized Patients Reveal Case Aggravation and Selective Co-Infection by Limited Gram-Negative Bacteria. Int J Environ Res Public Health. 2022; 19(9): 5270. doi: https://doi.org/10.3390/ijerph19095270.
19. Karataş M, Yaşar-Duman M, Tünger A, Çilli F, Aydemir Ş, Özenci V. Secondary bacterial infections and antimicrobial resistance in COVID-19: comparative evaluation of pre-pandemic and pandemic-era, a retrospective single center study. Ann Clin Microbiol Antimicrob. 2021; 20(1): 51. doi: https://doi.org/10.1186/s12941-021-00454-7.
20. Khurana S, Singh P, Sharad N, et al. Profile of co-infections & secondary infections in COVID-19 patients at a dedicated COVID-19 facility of a tertiary care Indian hospital: Implication on antimicrobial resistance. Indian J Med Microbiol. 2021; 39(2): 147-53. doi: https://doi.org/10.1016/j.ijmmb.2020.10.014.
21. Lavrinenko A, Kolesnichenko S, Kadyrova I, Turmukhambetova A, Akhmaltdinova L, Klyuyev D. Bacterial Co-Infections and Antimicrobial Resistance in Patients Hospitalized with Suspected or Confirmed COVID-19 Pneumonia in Kazakhstan. Pathogens. 2023; 12(3): 370. doi: https://doi.org/10.3390/pathogens12030370.
22. Sharifipour E, Shams S, Esmkhani M, et al. Evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients admitted to ICU. BMC Infect Dis. 2020; 20(1): 646. doi: https://doi.org/10.1186/s12879-020-05374-z.
23. Al Shuhoumi MA, Al Mhrooqi A, Al Rashdi A, et al. First clinical case of VIM-1-producing Leclercia adecarboxylata: A case report and literature review. Med Microecol. 2023; 15: 100075. doi: https://doi.org/10.1016/j.medmic.2022.100075.
24. Aarab A, Saddari A, Noussaiba B, et al. Leclercia adecarboxylata invasive infection in a patient with Hirschsprung disease: A case report. Ann Med Surg (Lond). 2021; 71: 102927. doi: https://doi.org/10.1016/j.amsu.2021.102927.
25. Clark A, Jit M, Warren-Gash C, et al. Global, regional, and national estimates of the population at increased risk of severe COVID-19 due to underlying health conditions in 2020: a modelling study. Lancet Glob Health. 2020; 8(8): e1003-e17. doi: https://doi.org/10.1016/s2214-109x(20)30264-3.
26. Zayet S, Lang S, Garnier P, et al. Leclercia adecarboxylata as Emerging Pathogen in Human Infections: Clinical Features and Antimicrobial Susceptibility Testing. Pathogens. 2021; 10(11): 1399. doi: https://doi.org/10.3390/pathogens10111399.
27. Church DL, Cerutti L, Gürtler A, Griener T, Zelazny A, Emler S. Performance and Application of 16S rRNA Gene Cycle Sequencing for Routine Identification of Bacteria in the Clinical Microbiology Laboratory. Clin Microbiol Rev. 2020; 33(4): e00053-19. doi: https://doi.org/10.1128/cmr.00053-19.
28. Shabir U, Dar JS, Bhat AH, Ganai BA, Mahmoud MH, Batiha GE. Uncovering the antimicrobial activity of G-type lysozyme 2 derived from Cyprinus carpio mucus against bacterial and fungal pathogens. Dev Comp Immunol. 2024; 153: 105135. doi: https://doi.org/10.1016/j.dci.2024.105135.
29. Barrios-Villa E, Pacheco-Flores B, Lozano-Zaraín P, et al. Genomic insights of Leclercia adecarboxylata strains linked to an outbreak in public hospitals in Mexico. Genes Genomics. 2023; 45(5): 569-79. doi: https://doi.org/10.1007/s13258-022-01348-4.