AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Emergence of *macA* and *macB* Genes in Multi-Drug Resistance *Serratia* Species Isolated from Nosocomial Infections

Rafah Abdul Hadi , Zahraa Yosif Motaweq

Department of biology, Faculty of Science, University of kufa, Kufa, Iraq.

Article history
Received: 18/04/2026
Revised: 13/05/2026
Accepted: 31/05/2026

*Corresponding Author: Rafah Abdul Hadi . *Department of biology, Faculty of Science, University of kufa, Kufa, Iraq.*
Email: rafahabdalhadi@gmail.com

Abstract: *Serratia* species are an opportunistic pathogenic microorganism primarily known as infectious nosocomial agents. **Methods:** 150 diverse specimens were gathered in a sterile manner utilizing appropriately labeled and sterile containers from various regions of Najaf Hospital and private external laboratories, Ethical approval was obtained, and informed consent was secured from all participating patients before specimen collection. Duplicate samples from the same patient were excluded to avoid redundancy in data analysis and strict contamination control procedures were applied to prevent sample contamination during collection and processing, was obtained 21 isolates of *Serratia* 18 (86%) of them *S. marcescens* and 3 (14%) was *S. fonticola*. The specimens were inoculated on culture media (MacConkey and blood agar), using IMVIC testing, Gram stain, culture media and Tests for catalase were conducted, and the colonies were molecularly characterized via use. The isolates were eventually examined against some antibiotics. **Results:** According to the findings, all *Serratia* spp. were results negative for Methyl Red (MR), Voges-Proskauer (VP), Indole Production, and Oxidase. In contrast, all examined isolates had positive results for both catalase and citrate utilization. Additionally, *Serratia*'s antibiotic susceptibility test revealed that all the isolates tested exhibited strong resistance to Ampicillin, Cefuroxime, Ceftazidime, cephalexin 100%, and sensitive for antibiotics Imipenem, Chloramphenicol, Levofloxacin, Ciprofloxacin and Aztreonam. The isolates showed resistance to several drugs 19 (90.5%) of the isolates displayed multidrug resistance MDR and 2 (9.5%) appeared XDR. The results of protease enzyme phenotypically detection test showed that 66.7% out of 21*Serratia* isolates were positive to protease production and gave clear zone of proteolytic around the colonies and 33.3% was protease Negative. **Conclusion:** The most prevalent genes found in *Serratia* spp. were *macA* and *macB* responsible for encoding efflux pump proteins in 100% of isolates and it is the primary cause of bacterial innate and acquired multidrug resistance in clinical settings.

Keywords: *Serratia*, *macA* and *macB*, Efflux pump, Protease.

DOI: 10.36320/ajb/v18.i2.23908

1. Introduction

Contemporary, *Serratia* spp. are responsible for around 2% of all hospital-acquired infections, with a greater proportion seen decisively sick individuals, according to series and surveillance in hospitals surveys. *Serratia* spp., and *S. marcescens* in particular, can cause broad outbreaks in hospitals and healthcare facilities because they can contaminate various kinds of medical equipment, increasing the risk to surgical patients and spreading mainly through direct contact [1]. Due to the presence of R-factors on plasmids, the majority of strains are resistant to many antibiotics [2]. Treating *Serratia* species, especially *S. marcescens* infection, is becoming more challenging since their unique ability to acquire, transmit, and alter the expression of various antibiotic resistance genes [3]. Because preventative antibiotics are used in hospital intensive care units, *S. marcescens* has been changing to acquire antimicrobial resistance (AMR) [4]. Metalloproteases, gelatinase, and alkaline protease are among the exoproteins produced by Serratia species that may contribute to virulence and allow the organism to cause infections, especially ocular illnesses [5].

One of the most prevalent resistance mechanisms in Efflux pumps are transport proteins present in the cytoplasmic membrane of many pathogenic bacteria that actively transfer substances across the membrane. Multiple efflux pumps work together to prevent heavy metal toxicity because periplasmic metal ions can re-enter the cytoplasm in Gram-negative bacteria by lowering both the cytoplasmic and periplasmic concentrations of heavy metal ions [6].
Similar to other Enterobacterales, *Serratia* also picks up resistance genes that code for drug-modifying enzymes, carbapenemases, and extended spectrum beta lactamases [7,8]. Serious challenges, such as frequent treatment failures with unfavorable results, have been brought about by the growth of these multidrug resistant bacteria [9]. The objective of this study is to detect the antibiotic susceptibility of separated species and to isolate, characterize, and phenotypically investigate *Serratia* spp.

2. Methodology

Isolation of Serratia spp. (Samples collection)

Different differential, selective, and enrich media (MacConkey and blood agar) were used to grow the bacteria from urine Specimens, ear, burns and wounds swabs taken from different patients in different ages who were infected with Serratia spp. during 2025 from July to November study periods at AL-Najaf Hospital / Iraq and private external laboratories. Each urine sample was collected, centrifuged at 3000 rpm for 5 minutes, and then the precipitate was cultured on selective and differential medium. Identification of Serratia spp. was done by culture characteristics, biochemical reactions and Vitek-2 compact system.

Antibiotics Sensitivity

Every sample of bacteria was cultivated using the Muller Hinton agar medium. 0.1ml of the bacterial suspension (in comparison to 0.5 from the MacFrland tube) was spread on the agar surface, and each plate received an antibiotic disc. The antibiotic discs were then incubated at 37°C for 24 hours, antibiotics were selected and the inhibition zone was measured according to CLSI, (2024) [10].

phenotypic Efflux Pump Identification Using the EtBr-Agar Cartwheel Method

All bacterial isolates were diluted using sterile physiological salt solution, and the turbidity was measured using the McFarland Standard (0.5) instrument. Bacterial isolates with antibiotic resistance were examined using the cartwheel agar-EtBr technique, which uses trypton soy agar and ethidium bromide dye in different concentrations [11].

Protease Production

Protease production test was performed by inoculating Skim – Milk agar with bacterial isolates, and incubated for 24 to 36 hours at 37 °C. Appearance clear zone of proteolytic for casein protein around colonies indicated the positive result [12].

Genomic DNA Extraction

After 12 hours of incubation at 37°C on BHI media, DNA was extracted from a BHI broth culture using the Wizard® genomic DNA Purification kit (Promega Co., Madison, WI, USA). genomic DNA was successfully recovered from Serratia isolates. The concentration and purity of extracted DNA was evaluated by the RNA/DNA spectrophotometer (Biodrop) instrument directly, extracted DNA purity ranged between 1.8-2, and using PCR technique for amplification double strand DNA then Gel electrophoresis was used to confirm and analyze the extracted DNA [13].

Amplification of Genes

Primer type of macA and macB

According to the manufacturer's instructions the polymerase chain reaction (PCR) of the macA and macB genes were performed using the universal Primer for bacteria macA-F (GCA CAA CAA GCA CCG AAC AT), macA-R (CAT ATC CAG CCG CAG CAA AC) and Primer for macB-F (GCG TGA GCG GCG ATT ATT TT), macB-R (AAA CGC GTG AGT TGC TGT, TC)[14].

The condition of the primers was included: Initial Denaturation 94\5m, followed 35 cycles of (Denaturation 95\30 sec, annealing 58.3\30 sec for macA while 57.2/30 sec for macB and Extension 72 \30 sec), finally the Final Extension was 72\7min. Before being used, the reaction mixture (monoplex) was carried out in a final volume of 25 µl (master mix 12 µl, forward primer 2.5 µl, reverse primer 2.5 µl, DNA template 5 µl, Nuclease free water 3 µl) and was kept at 4°C, and the last extension step was conducted for around ten minutes at 72°C. Each and every PCR amplification was performed using a Verity Thermal Cycler (Agilent, UK). Then, all of the PCR results were dyed with harmless dye and analyzed using 1% agarose gel electrophoresis. The agarose gel was prepared through melting 1% from agarose powder (Himedia, India) in 100 ml of TBE buffer (1X). The mixture was put in a microwave for 1 min at 90˚C, then let to cool to 50˚C, and 3 µl Green safe stain was added the electric current was set on 75 volt for 50 minutes, 5 µl of the amplified PCR products were loaded to the agarose gel wells followed by the DNA marker (ladder) to one of the wells. The gel tray was fixed in an electrophoresis chamber and 1X TBE buffer was added to the chamber until the surface of the gel was covered. Lastly, Gel was visualized with UV transilluminator and photographed by using digital Camera the electrophoresis results were identified using the gel documentation system.

Analysis of Statistics

All data analyzed by used SPSS version 27. The categorical variables showed as percentages and frequencies. The Chi-square test analysed the relationship between antimicrobial susceptibility patterns (S, R, I). The Fisher–Freeman–Halton exact test was employed when the expected cell count fell below 5. A p-value less than 0.05 was important in statistics.

4. Results and discussion

Phenotypic Characterization of Serratia spp.

Of the 150 specimens collected for this investigation about 21 Serratia spp. isolates had been isolated from patients of ages different and based on Phenotypic characterization of isolates of Serratia spp. revealed that the bacterial colonies were cultured on MacConkey agar and blood agar under aerobic conditions based on biochemical assays and culture techniques. On MacConkey agar, Serratia spp. colonies were appear small, pale because non lactose fermentation and smooth round colonies. On blood agar, however, Serratia spp. colonies showed as big, spherical colonies, the biochemical tests, Gram staining method diagnosed as Serratia spp. that including Serratia marcescens (18 isolates) and Serratia fonticola (3 isolate) were confirmed through vitek compact system. The results of this study revealed that all 21 isolates were Gram-negative under the microscope. Gram staining was used to examine the size and shape of pure bacterial colonies under a microscope. Based on biochemical tests, isolates of Serratia spp. showed negative results for oxidase and indole but positive results for motility test, catalase, Voges-Proskauer (VP), Simmon citrate, methyl red and urease tests. When Serratia spp. were cultivated on TSI agar and exposed to alkaline-acid (pink yellow) on the slant, gas bubbles did not emerge at the bottom, and H2S generation was negative. The VITEK 2 system was used

to identify the isolates, confirming the phenotypic characterization results of S. marcescens isolates.

During this study 150 specimens of urine, sputum, ear, wound and burn swabs were collected and tested in order to diagnosis the pathogenic Serratia spp. which can cause some types of diseases to human after swelled such as diarrhea, endocarditis, urinary tract infections, endotoxic shock, Septicemia, wound infections, and occasionally pneumonia have been reported. The findings found that 21 isolates were diagnosed as Serratia spp. depending on the biochemical tests, and vitek compact system. Among these isolates were 85.7% S. marcescens (18 isolates) and 14.3% S. fonticola (3 isolates). It is worthy to mention that, some Serratia spp. can transport from patients during direct hand touch through wounds in skin [15].

Antibiotics susceptibility

The antibacterial susceptibility test was conducted for 21 Serratia isolates utilizing the disk diffusion technique against 13 widely used antibacterial drugs, according the Clinical and Laboratory Standards Institute (CLSI, 2024) [10]. The results of this test showed that Serratia spp. isolates has been high resistance to many commonly antibiotics which used infection, the highest rate of resistance the seen with Ampicillin, Cefuroxime, Ceftazidime and cephalexin in 21(100%) class cephem, and sensitive for antibiotics carbapenem 20(95.24%), Chloramphenicol 18(85.71%), Levofloxacin 17(80.95%), Ciprofloxacin 14(66.67%) and Aztreonam 11(52.38%) class imipenem, quinolones ,monobactam and other, Multidrug resistance MDR was defined as isolates that were resistant to at least 3 classes of the tested antimicrobial agents, making infections difficult to treat. Figure (1) and Table (1). The most widely accepted reference standard MDR is the international definition proposed by: European Center for Disease Prevention and control and Centers for Disease Control and Prevention. According to reference by Magiorakos et al., (2012) [16]. Clinical and Laboratory Standard institute establishes standard for antimicrobial susceptibility testing and interpretive breakpoints, but it is not the organization that defined MDR itself. The classification of MDR bacteria requires standardized criteria based on: Standardized antimicrobial susceptibility testing methods Internationally accepted breakpoints Classification of antibiotics into antimicrobial categories Interpretation of results according to guidelines from Clinical and Laboratory Standards Institute or European Committee on Antimicrobial Susceptibility Testing. The present study results incompatible with previous study by Liang and colleagues [17] discovered that S. marcescens is sensitive to cefazolin and resistant to Cefepime, Ceftazidime and Cefotaxime can be easily transferred to human during tap water such as S. marcescens. Serratia's inherent resistance to ampicillin, amoxicillin clavulanic acid, first-generation cephalosporins, macrolides, and colistin is really a crucial characteristic.[18].

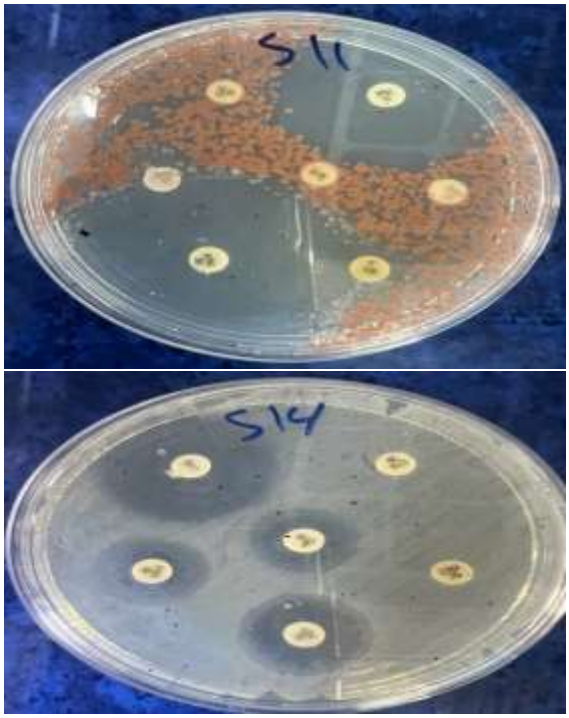

Fig 1: Antibiotics Resistance of *Serratia spp.* Isolates by disk diffusion method.

Differences in antimicrobial resistance patterns arise due to variations in the mechanisms of action of antibiotics, as well as the diverse bacterial strategies used to counteract them, including enzyme production, target site modification, efflux pumps, and reduced membrane permeability. In addition, selective pressure resulting from the overuse or misuse of specific antibiotic classes leads to the emergence and spread of distinct resistance profiles in different geographic regions and healthcare settings. Horizontal gene transfer also plays a major role

DOI: 10.36320/ajb/v18.i2.23908

in disseminating resistance determinants among bacterial populations, contributing to variability across species and environments [19].

The presence of resistance genes on the chromosome causes intrinsic resistance. Indirectly reflecting patterns of antimicrobial usage, research from throughout the world show that isolates have varied rates of resistance to these medications [20] DNA gyrase mutations, modified porin channels, and an elevated efflux pump are responsible for resistance in S. marcescens. Study by Ferriera and colleagues [7] discovered that only 18.5% of Serratia strains were resistant to ciprofloxacin, 14.8% to amikacin, and 18.5% to gentamicin. Additionally, 96.3% of bacteria were resistant to colistin, and all strains demonstrated resistance to beta lactam antibiotics.

Table 1: Antibiotics Resistance of *Serratia spp.*

No (%)			
Antibiotics	**S**	**I**	**R**
FOX	4 (19.5)	2 (9.52)	15 (71.43)
CTX	2 (9.52)	3 (14.2)	16 (76.1)
CIP	14 (66.67)	2 (9.52)	5 (23.81)
C	18 (85.71)	1 (4.76)	2 (9.52)
LEV	17 (80.95)	0 (0.00)	4 (19.05)
CXM	0 (0.00)	0 (0.00)	21 (100.00)
CLM	1 (4.76)	4 (19.05)	16 (76.19)
F	4 (19.05)	5 (23.81)	12 (57.14)
CAZ	0 (0.00)	0 (0.00)	21 (100.00)
KF	0 (0.00)	0 (0.00)	21 (100.00)
ATM	11 (52.38)	1 (4.76)	9 (42.86)
AMP	0 (0.00)	0 (0.00)	21 (100.00)
IPM	20 (95.24)	1 (4.76)	0 (0,00)
Chi-square (P value)		X^2=294.01 (P<0.001*)	

Production of Protease

Serratia spp. isolates were cultured by streaking on skim milk agar. Results showed that 14 (66.67%) out of 21 Serratia isolate was positive result gave clear zone around the colonies (Figure 2) while Serratia isolates were Negative to protease production 7 (33.33%) non producer protease, Microbial proteases contribute to pathogenicity as result of the disintegration of defense proteins of the host by breaks down peptide bonds between the amino acids that make up a protein thus increasing the ability of bacteria to spread thus its ferocity. These enzymes are known to harm the host organism's defensive proteins [21]. A difference was observed in the ability of the isolates to produce protease. Femi-Ola [22] discuss that it was discovered that 40°C was the ideal temperature and a pH of 7 was ideal for the test isolate to produce protease. At pH 6, 8, and 9, a high protease yield was also attained. Research by Nguyen and Quyen [23] who said that using casein as a substrate resulted in the best protease production. It was discovered that the development of the bacterial culture was linked to the synthesis of enzymes. The association between protease production and MDR phenotypic in Serratia isolates may be attributed to the coexistence of virulence and resistance determinants, enhanced biofilm formation, and selective pressure in hospital environments. Possible aspects of relationships include joint genetic regulation, Biofilm, hospitalization and protease as virulence factor.

Fig 2: Production of Protease of *Serratia* spp. incubation on Skim Milk Agar on 37°C for 24 hours.

Detection of Efflux Pumps

The present study showed that some isolates have efflux pumps (no fluorescence), through variable degree at different concentration of Ethidium bromide (EtBr) dye (25, 50, 100, 150, 200) using tryptic-soya agar media. The result shows that 2 isolates have efflux pumps at a concentration of 25µg/ml of (EtBr) dye, one isolate have efflux pumps at a 50 µg/ml concentration 2 isolates have efflux pumps at a concentration of 100 µg/ml, 3 isolates have efflux pumps at concentration of 150 µg/ml and 1 isolate have efflux pumps at 200 µg/ml of concentration. The percentage of isolates with efflux

DOI: 10.36320/ajb/v18.i2.23908

pumps was 4(19.05%) positive while the percentage of isolates with efflux pumps was 17(80.95) negative as show in Figure (3).

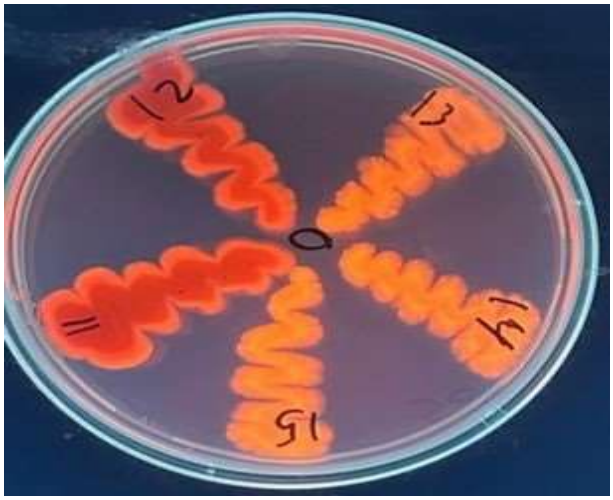

Fig 3: Efflux pumps are detected phenotypically using the Cart Wheel technique. Fluorescent isolates under the ultraviolet light with negative.

The percentage of isolates with efflux pumps was 4(19.05%) positive, while the percentage of isolates without efflux pumps was 17(80.95%) Negative. Research by Khadim and Hussein, (2025) [24] who explain that out of the 20 S. marescens clinical isolates tested were 8(40%) demonstrated positive using the ethidium bromide-agar cartwheel technique (EtBr-CW) to measure efflux activity. Fluorescence intensity under ultraviolet light was used to differentiate efflux-positive (weak or no fluorescence) from efflux-negative (strong fluorescence) isolates. Suresh et al., (2023) [25] evaluated the resistant isolates' efflux pump performance from various freshwater fish farms was assessed. Following incubation, variation in fluorescence of bacterial mass were noticed, depending on their ability to efflux EtBr. Several laboratory methods are used to detect or evaluate and inhibition efflux pump activity in bacteria. These include fluorescent dye accumulation assays such as ethidium bromide, where increased intracellular fluorescence indicates reduced efflux activity. Efflux pump inhibitors (EPIs), such as CCCP and PAβN, are also used by comparing minimum inhibitory concentrations (MICs) in the presence and absence of inhibitors to demonstrate the contribution of efflux mechanisms to antimicrobial resistance. In addition, fluorometric assays and molecular techniques like real-time PCR can be used to assess efflux pump activity or gene expression at the functional and genetic levels. These approaches are mainly applied to investigate the role of efflux pumps in antimicrobial resistance mechanisms [19]. One of the most prevalent resistance mechanisms across a variety of dangerous bacteria is antibiotic efflux. The bacterial cytoplasmic membrane contains transport proteins called efflux pumps, which actively transfer the chemical across the membrane. According to earlier research, S. marcescens used plasmids and efflux pumps to increase its infectivity and innate resistance to antibiotics. This led to the formation of several drug-resistant variants and ultimately made treatment more difficult [26].

Detection of macA and macB genes

Conventional PCR amplification was performed for Serratia spp. using a particular primer, a fragment of DNA that is amplified by PCR is detected by gel electrophoresis, as seen in Figure (4), to verify the presence of macA and Figure (5) to verify the presence of macB. The results indicated that 21/21 (100%) of Serratia spp. isolates have macA and macB genes. The difference between phenotypic detection of efflux pump activity (~80%) and the genotypic detection of macA and macB genes in 100% of Serratia marcescens isolates can be explained by several molecular and regulatory factors, gene regulation, including global stress-response regulators, may also influence expression levels. The presence of resistance genes does not necessarily correlate with their expression, as macA/macB may remain transcriptionally silent or weakly expressed under standard laboratory conditions. Phenotypic assays, in contrast, measure functional activity of efflux systems, which can vary depending on environmental conditions, growth phase, and regulatory mechanisms. Additionally, efflux activity in bacteria is often mediated by multiple transporter systems, and macAB alone may not fully contribute to detectable phenotypic resistance. Variations in Therefore, genotypic positivity does not always translate into phenotypic expression, leading to the observed discrepancy [19].

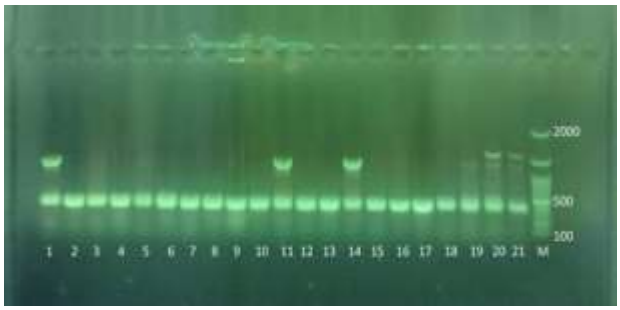

Figure (4): Safe dye- PCR products from extracted total DNA were electrophoresed on a dyed agarose gel of *Serratia* spp. using primer *macB* with product 364bp show all result positive

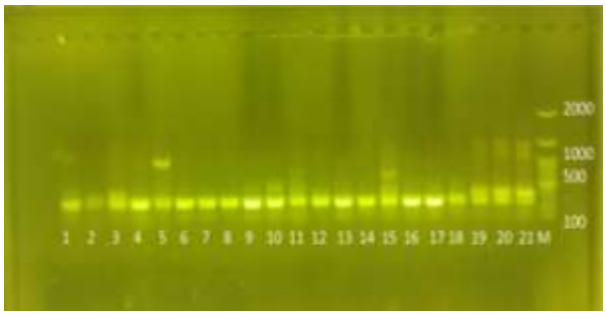

Fig 5: Safe dye- PCR products from extracted total DNA were electrophoresed on a dyed agarose gel of *Serratia* spp. using primer *macB* with product 364bp show all result positive

In the molecular study *macB* gene can be characterized as an ABC-type macrolide efflux transporter that collaborates with TolC, the multifunctional outer membrane channel, and macA, the MFP and the ABC-Type Efflux pump *macAB* is involved in protection of *S. marcescens* against aminoglycoside antibiotics, macrolides, polymyxins, and oxidative Stress and motility and biofilm formation [27]. The primary cause of clinical innate and acquired multidrug resistance in bacteria is the efflux pump, which aids in the excretion of drugs by bacteria [28].

Conclusion

Lastly, the MDR Serratia species dissemination results obtained in our study proposed a great challenge which may transmit resistance to other pathogens, so permanent monitoring of the MDR Serratia spp. emergency could provide the appropriate treatment methods. MacB 364 bp.

Acknowledgement

The authors thank the staff at the hospitals Al-Najaf province.

Funding Information

There was no outside support for this study.

Ethics

The study was accepted by the University of Kufa's College of Science Ethics Committee, with reference H K/1082, as well as in December 2025. Ethical approval was obtained, and informed consent was secured from all participating patients before specimen collection. Duplicate samples from the same patient were excluded to avoid redundancy in data analysis.

References

1. Liu, D. (Ed.). (2011). *Molecular detection of human bacterial pathogens*. CRC Press. https://doi.org/10.1201/b10818
2. Carrascosa, F. M., Jose, R. S., Caviedes, R., & Carmen, F. (2013). *Serratia marcescens* rhabdomyolysis. *Journal of Advances in Infectious Diseases*, *3*(1), 63–64. https://doi.org/10.4236/aid.2013.31008
3. Moradigaravand, D., Boinett, C. J., Martin, V., Peacock, S. J., & Parkhill, J. (2016). Recent independent emergence of multiple multidrug-resistant *Serratia marcescens* clones within the United Kingdom and Ireland. *Genome Research*, *26*(8), 1101–1109. https://doi.org/10.1101/gr.205245.116
4. Aracil-Gisbert, S., Fernández-de-Bobadilla, M. D., Guerra-Pinto, N., Delgado-Valverde, M., Ruiz-Garbajosa, P., Cantón, R., & Baquero, F. (2024). The ICU environment contributes to the endemicity of the "*Serratia marcescens* complex" in the hospital setting. *mBio*, *15*(4), Article e03054-23. https://doi.org/10.1128/mbio.03054-23

5. Mahlen, S. D. (2011). *Serratia* infections: From military experiments to current practice. *Clinical Microbiology Reviews*, *24*(4), 755–791. https://doi.org/10.1128/CMR.00017-11
6. Gaurav, A., Bakht, P., Saini, M., Pandey, S., & Pathania, R. (2023). Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology*, *169*(5), Article 001333. https://doi.org/10.1099/mic.0.001333
7. Ferreira, R. L., Rezende, G. S., Damas, M. S. F., Oliveira-Silva, M., Pitondo-Silva, A., Brito, M. C. A., ... & Pranchevicius, M. C. S. (2020). Characterization of KPC-producing *Serratia marcescens* in an intensive care unit of a Brazilian tertiary hospital. *Frontiers in Microbiology*, *11*, Article 956. https://doi.org/10.3389/fmicb.2020.00956
8. Omololu-Aso, J., & Awoderu, B. O. (2021). Extended spectrum β-lactamase mobile gene encoded variants CTX-M, TEM, SHV, AmpC and FOX among *Serratia marcescens*, Ile-Ife South Western Nigeria. *International Journal of Immunology and Immunotherapy*, *7*(1), Article 058. https://doi.org/10.23937/2378-3672/1410058
9. Kim, S. B., Jeon, Y. D., Kim, J. H., Kim, J. K., Ann, H. W., Choi, H., ... & Song, Y. G. (2015). Risk factors for mortality in patients with *Serratia marcescens* bacteremia. *Yonsei Medical Journal*, *56*(2), 348–354. https://doi.org/10.3349/ymj.2015.56.2.348
10. Clinical and Laboratory Standards Institute. (2024). *Performance standards for antimicrobial susceptibility testing* (34th ed., CLSI supplement M100).
11. Martins, M., McCusker, M. P., Viveiros, M., Couto, I., Fanning, S., Pagès, J. M., & Amaral, L. (2013). A simple method for assessment of MDR bacteria for over-expressed efflux pumps. *The Open Microbiology Journal*, *7*, 72–82. https://doi.org/10.2174/1874285801307010072
12. Pratika, M., M, A., & Suwastika, I. N. (2021). Protease activity from bacterial isolates of *Nepenthes maxima* Reinw. ex Nees. *Journal of Physics: Conference Series*, *1763*(1), Article 012092. https://doi.org/10.1088/1742-6596/1763/1/012092
13. Jawade, H. A., Motaweq, Z. Y., Rasool, H. D., & Hussain, F. H. (2024). Study of antibiotic resistance in ESKAPE bacteria using β-lactamase and ESBL genes. *Journal of Angiotherapy*, *8*(3), Article 9618.
14. Ali, S. A., & Al-Dahmoshi, H. O. M. (2022). Detection of efflux pumps gene and relation with antibiotics resistance in uropathogenic *Escherichia coli* (UPEC) isolated from patients with cystitis. *Iraqi Journal of Science*, *63*(6), 2388–2397. https://doi.org/10.24996/ijs.2022.63.6.7
15. Ahmed, M. S., & Shareef, H. A. (2024). Investigating the prevalence of some Gram-negative bacteria in patients with lower respiratory tract infections in the city of Mosul and studying their sensitivity to antibiotics. *Samarra Journal of Pure and Applied Sciences*, *6*(3), 145–159. https://doi.org/10.54153/sjpas.2024.v6i3.633
16. Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012). Multidrug-resistant, extensively

drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection*, *18*(3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

17. Liang, Z., Shen, J., Liu, J., Sun, X., Zhang, Y., Wang, Y., ... & Luo, J. (2023). Prevalence and characterization of *Serratia marcescens* isolated from clinical bovine mastitis cases in Ningxia Hui Autonomous Region of China. *Infection and Drug Resistance*, *16*, 2727–2735. https://doi.org/10.2147/IDR.S406325
18. Cristina, M. L., Sartini, M., & Spagnolo, A. M. (2019). *Serratia marcescens* infections in neonatal intensive care units (NICUs). *International Journal of Environmental Research and Public Health*, *16*(4), Article 610. https://doi.org/10.3390/ijerph16040610
19. World Health Organization. (2017). *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*. World Health Organization.
20. Ali, F., Wu, J., & K.C., D. A. (2023). A sixty-nine-year-old female with *Serratia marcescens* infection. *Cureus*, *15*(11), Article e49985. https://doi.org/10.7759/cureus.49985
21. Vachher, M., Sen, A., Kapila, R., & Nigam, A. (2021). Microbial therapeutic enzymes: A promising area of biopharmaceuticals. *Current Research in Biotechnology*, *3*, 195–208. https://doi.org/10.1016/j.crbiot.2021.05.006
22. Femi-Ola, T. O., Akinsanmi, O. P., & Bamidele, O. S. (2014). Production and characterization of protease from *Serratia marcescens*. *Assumption University Journal of Technology*, *18*(1), 1–10.
23. Nguyen, T. T., & Quyen, D. T. (2011). Overproduction of an extracellular protease from *Serratia* sp. DT3 just using soybean powder. *World Journal of Agricultural Sciences*, *7*(1), 29–36.
24. Khadim, M. M., & Hussien, H. A. (2025). Phenotypic and molecular characterisation of efflux pump genes in clinical isolates of *Serratia marcescens*. *Medical Journal*, *8*(2), 208–216.
25. Suresh, K., Pillai, D., Sindhuja, P., & Preethi, A. (2023). Efflux pump and biofilm forming capabilities of antibiotic resistant *Serratia marcescens* from freshwater fish farms in Andhra Pradesh, India. *Fishery Technology*, *60*(3), 196–205.
26. Liou, B. H., Duh, R. W., Lin, Y. T., & Fung, C. P. (2014). A multicenter surveillance of antimicrobial resistance in *Serratia marcescens* in Taiwan. *Journal of Microbiology, Immunology and Infection*, *47*(5), 387–393. https://doi.org/10.1016/j.jmii.2013.04.003
27. Tanabe, M., Szakonyi, G., Brown, K. A., Henderson, P. J., Nield, J., & Byrne, B. (2009). The multidrug resistance efflux complex, EmrAB from *Escherichia coli* forms a dimer in vitro. *Biochemical and Biophysical Research Communications*, *380*(2), 338–342. https://doi.org/10.1016/j.bbrc.2009.01.081
28. Olivares Pacheco, J., Alvarez-Ortega, C., & Martínez, J. L. (2013). RND multidrug efflux pumps: What are they good for? *Frontiers in Microbiology*, *4*, Article 7. https://doi.org/10.3389/fmicb.2013.00007