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The Role of Immune Defense in *Serratia marcescens* Nosocomial Infections

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***Abstract*—Developing resistance mechanisms leads to various nosocomial infections caused by opportunistic bacteria. S*erratia marcescens* are well known to be opportunistic and are equipped with an armory of virulence factors against host immune response. The study aims to detect the immune defense in patients infected with multidrug-resistant *S. marcescens*. The study includes 132 clinical samples, including burn, wound, otitis media, and urinary tract infection (UTI) at several hospitals in Baghdad, Iraq. All isolates are identified by cultivation on MacConkey agar, nutrient agar, and blood agar, followed by biochemical tests and assessment with the VITEK 2 compact system. The isolates are tested for antibiotic susceptibility tests, interleukin-12 (IL12) levels, neutrophil ability to phagocytosis, and complement C3 and C4 levels. Out of 120 positive cultures, six isolates are identified as *S. marcescens*. The urine samples are the most isolated source and a higher level of antibiotic resistance was noticed in ampicillin and cefotaxime (100%), whereas a lower level is in imipenem. Stimulation (p = 0.005) provided a significant increase in IL-12 production. The infection with the *S. marcescens* stimulated the neutrophil's phagocytosis process compared with the control. The interplay role of virulence factors in *S. marcescens* influences its pathogenesis, antibiotic resistance, and immune response, particularly involving neutrophils and IL-12. Understanding these interactions is crucial for developing effective therapeutic strategies.**

***Index Terms*—Antibiotics susceptibility test, Interleukin-12, Neutrophil, Phagocytosis, *Serratia marcescens*, Virulence factors.**

I. Introduction

Serratia marcescens is a Gram-negative, facultative anaerobe and opportunistic bacteria related to several hospital-acquired infections, such as urinary tract, ocular lens, respiratory tract infections, wounds, septicemia, and osteomyelitis (Hsueh, 2020; Friedrich et al., 2021). Secreting a variety of inflammatory-promoting enzymes, serine protease 56-kDa is the most potent enzyme, known as serralysin, which causes keratitis, cleaves IgG and IgA, lysozyme, and triggers interleukin (IL)-6 and IL-8 (Jupatanakul et al., 2020). Being an Enterobacteriaceae member, this bacterium is widespread and possesses mechanisms for developing antibiotic resistance. It is generally documented that 1–2% of nosocomial infections affect the urinary tract, and 30–35% of asymptomatic patients with a history of instrumentation also get infections of the respiratory tract, surgical wounds, and soft tissues (Moles et al., 2019; Prado et al., 2021). A high fatality rate was associated with *S. marcescens* infections, despite limited epidemiological data, and nosocomial illnesses such as sepsis, meningitis, and endocarditis. The pathogenic isolates of *S. marcescens* often produce proteases, nucleases, lecithin, and hemolysin. A variety of strains exude prodigiosin pigment ranging from dark red color to pink indicating metabolic change over time (Sameer et al., 2023), the capacity to manufacture a beta-lactamase, which is the primary factor in the development of bacteremia and sepsis during hospitalization due to broad-spectrum antibiotic resistance (Tóth et al., 2020). Toll-like receptors (TLRs) and other receptors are used by immune cells (monocytes/macrophages and neutrophils) in response to pathogens such as bacteria, fungi, intracellular parasites, or viruses (IL-12) (Iain et al., 2023). Besides, neutrophils' role in innate immune responses shapes the host's immune defense (Bhor et al., 2021). Through CD41 Th1 cells, phagocytic cells are stimulated to release IL-12 in response to bacterial endotoxin. INF-gamma produced by IL12 controls how much IL-12 neutrophils and macrophages may secrete. IL-12 ties innate and adaptive immunity together. IL-12 and IFN- are produced in response to inflammation in an acute infection, but IL-12 production that is out of control causes septic shock syndrome and autoimmunity in a chronic infection (Ullrich et al., 2020). Therefore, the primary goal of this research is to understand how *S. marcescens* contributes to various clinical infections in Baghdad, Iraq by isolating, identifying,

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and detecting the major virulence factors, followed by the examination of neutrophils' ability to undergo phagocytosis as a marker for innate immunity and their relationship to immune-mediated diseases.

II. Materials and Methods

A. Isolation and Identification

Between June and November 2021, 132 clinical specimens, including otitis media, burn infections, infected wounds, and urinary tract infections (UTIs), were obtained at several hospitals in Baghdad province. The specimen collection followed the Tille (2022) protocol, which involved taking ear swabs by gently rotating the swab in the ear canal and collecting midstream urine and skin scrapes from wounds and burn infections in sterile containers. The specimen swabs were quickly transferred and cultured on the nutrient, blood, and MacConkey agar for biochemical and microbiological characterization. The biochemical test included catalase, oxidase, Voges-Proskauer, citrate utilization, indole production, motility, and sugar fermentation on MacConkey agar. A Gram stain, nutrient agar, and blood agar were used to characterize the isolates. The VITEK 2 compact system (BioMerieux, France) was used to validate the identification (Abhishek and Tanu, 2020).

B. Virulence Factors Detection

S. marcescens isolates' phenotypic virulence characteristics were examined to determine isolates' pathogenicity, biofilm-forming capacity, protease, beta-lactamase, lipase, urease, lecithinase, hemolysin, and motility (Abdul et al., 2019; Abhishek and Tanu, 2020; Tille, 2022).

C. Antibiotics Susceptibility Test

The disk diffusion method was used to determine the antibiotics susceptibility patterns for cefotaxime (CTX 30μg), azithromycin (AZM 30 μg), ampicillin (AMP 10 μg), piperacillin/tazobactam (TZP 110 μg), ceftazidime (CAZ 30 μg), ciprofloxacin (CIP 10 μg), amikacin (AK 30 μg), tobramycin (TOB 5 μg), gentamycin (GEN 10 μg), and imipenem (IPM 10 μg) (bioanalysis/Turkey). The results were interpreted according to the Clinical and Laboratory Standards Institute as described by (Priyanka et al., 2023).

D. Clinical Sample Collection

Blood samples were obtained from six patients infected with *S. marcescens*, and healthy individuals who served as the control group. Two tubes were prepared from each blood sample. The first tubes were used to test the neutrophils' phagocytosis capacity, and the second part of the blood samples yielded serum stored in a sterile container for laboratory testing.

E. Enzyme-Linked Immunosorbent (ELISA)

The assessment of plasma IL-12 levels using commercial ELISA kits is crucial for understanding the cytokine's role in various therapeutic contexts, commercial ELISA kits (IBL international IBL GMBH, Germany) following the manufacturer's instructions were applied to the plasma level of IL-12 patients. The kits' standard curves tested the cytokines' concentration, and the results detected were done in ng/ml. The data were examined in the median value, and each sample was run twice.

F. Evaluation of Neutrophil Activity

The nitro-blue tetrazolium (NBT) test is a widely utilized method for assessing the phagocytic capacity of neutrophils, particularly in various clinical contexts. This assay measures the ability of neutrophils to reduce NBT, indicating their functional status in phagocytosis and oxidative burst activity (Nurasyikin et al., 2022). Venous blood from healthy (control) and patients infected with *S. marcescens* was incubated with a solution of nitro blue-tetrazolium (NBT).

G. Complement Level Assessment

Single radial immunodiffusion (SRID) is a reliable technique for quantifying serum proteins, including complement components, using a small volume of serum. The method typically employs 5 μL of patient serum, as recommended by manufacturers, to ensure accurate results while optimizing sample usage. SRID accurately measures components C3 and C4, particularly at elevated concentrations. Endplates were used to create C3, and C4, the plate's lids were tightly closed after being slightly opened for 5 min to eliminate moisture droplets and left on the bench at room temperature for 48–72 h. The diameter of the immunological precipitation ring was measured to an adjacent 0.1 mm using a definite ruler (Ayano and Horiuchi, 2023).

H. Statistical Methods

Statistical analysis ANOVA was performed using the PROC MIXED model (SAS 8.2; SAS Institute, Cary, NC, USA). T-test was used to compare means of tests and controls. P-values indicate statistical significance if $p < 0.05$. To adjust the alpha (α) level, Bonferroni Correction was applied using an online calculator: https://www.statology.org/bonferroni-correction-calculator

III. Results and Discussion

In this study, *S. marcescens* was isolated from 6 (out of 132) clinical specimens. The organism was identified as a Gram-negative bacillus, catalase-positive, oxidase-negative, reacts positively in the Voges–Proskauer test, and utilizes citrate as a sole carbon source. *S. marcescens* colonies on nutrient and blood agar appeared circular with pink or red pigmentation, attributed to prodigiosin production. They did not ferment lactose on MacConkey agar and most of the isolates are pigment producers (66.7%) (Fig. 1).

Virulence factor secretion contributes to bacterial pathogenicity. Consequently, these bacteria produce various metabolites during infection. Table I and Fig. 2 show the virulence factors of isolates.

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TABLE I
VIRULENCE FACTORS OF *S. MARCESCENS* ISOLATES IN THIS STUDY

Isolated number of *S. marcescens*	Virulence factor							
	Motility	Hemolysis	Beta lactamase	DNase	Urease	Lipase	Lecithinase	Prodigiosin
1	+	+	+	+	+	+	-	+
2	+	+	+	-	+	-	-	+
3	+	+	+	+	-	-	-	+
4	+	+	+	+	+	-	+	-
5	+	+	+	+	-	-	-	-
6	+	+	-	-	-	+	-	+
%	100	100	83.3	66.7	50	33.3	16.63	66.7

S. marcescens: Serratia marcescens

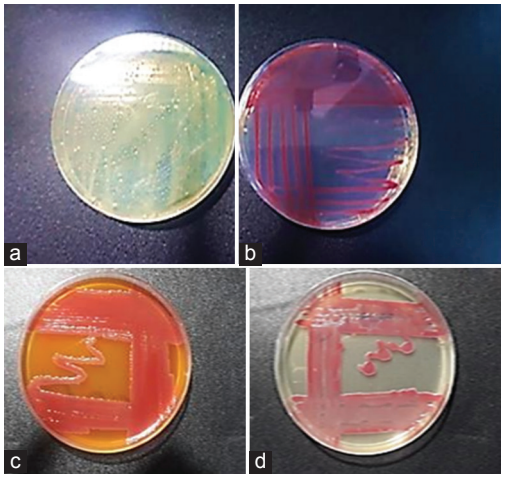

Fig. 1. S*erratia marcescens* colonies. (a) Non-producing a pigment on nutrient agar. (c) produces a red colony on blood agar. (b and d) The colonies are red because of a pigment (prodigiosin) produced by this organism on nutrient agar.

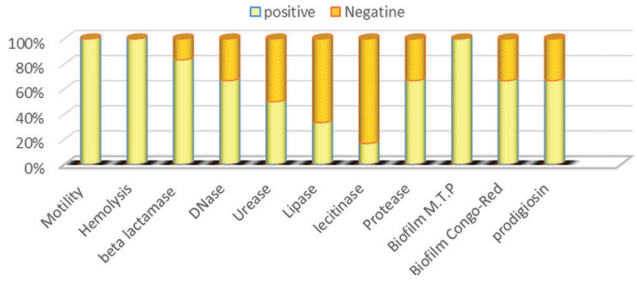

Fig. 2. Virulence factors percentage of *Serratia marcescens* isolates.

Notably, the strain that synthesized prodigiosin did not secrete lecithinase. The production of prodigiosin in *S. marcescens* is intricately regulated by various systems, notably the EnvZ/OmpR system, which responds to environmental stimuli. This multifaceted regulatory landscape encompasses metabolic pathways and biosynthetic processes, influencing pigment synthesis under specific conditions (Jia et al., 2022).

Another notable point was the strains producing urease with strong biofilm producers (see isolate numbers 1, 2, and 4 in Tables I and II). Clinical *S. marcescens* isolates demonstrate significant adaptive plasticity through urea-induced acyl-homoserine lactonase, particularly in response to environmental signals including urea and population density (quorum sensing). This adaptability allows the bacteria to regulate gene expression based on external cues (Tuttobene et al., 2024).

Protease activity is also linked to these cells' invasion and demise (Ferreira et al., 2020). All isolates produce hemolysin and are known to induce cytotoxicity and inflammatory mediator cell secretion. These substances can invade and kill HeLa cells and fibroblasts, degrade the complements (C1 and C5a) system, and increase vascular permeability (Ferreira et al., 2020). *S. marcescens* nuclease has both intracellular and extracellular degradation properties and is resistant to high temperatures (Cai et al., 2024). Lipase contributes to degrading host lipids by hydrolysis of triglycerides, providing fatty acids that can be utilized for energy, and growth, and may also disrupt cellular membranes, facilitating tissue invasion and immune evasion. Thus, supporting bacterial proliferation in host environments (Nwachukwu et al., 2017).

Despite the bacteria isolation rate was 4.6%, UTI samples occupied the first position in isolation (50%), followed by burns, wounds, and otitis media (16.63%) in each sample (Table III). The present results agreed with the local studies (Suhad et al., 2023; Sadeq and Neamah 2024) which noticed a higher incidence of urine and burns.

Most *S. marcescens* were isolated from female patients 4 (66.7%) compared with males 2 (33.3%) (Table IV). Females are exposed to more infections, such as UTIs, than males. This result contrasts with the finding by Ferreira et al. (2020), who found isolation incidences were more recurrent in males. That means the bacteria invade individuals that have the prevalent disease.

The prevalence of *S. marcescens* increased with age peaking at 50% in the age range of (36–55) since, the majority in this age range are workers, who may experience an accident at work. Some of them may also be immunocompromised, such as individuals with diabetes mellitus, and 30% of people with *S. marcescens* infection carry the bacterium in their intestines, which is the main reservoir for the bacteria (Ferreira's et al., 2020; Drummond et al., 2023). Regarding the third age group (36–55), most bacteria were isolated from their UTI specimen, wound, and burn swab (33.3% for each).

TABLE II
CAPACITY BIOFILM FORMATION AND PROTEASE PRODUCTION BY *S. MARCESCENS*

Biofilm Congo-Red	Biofilm (M.T.P)	Protease by (mm) lysis diameter	Isolation source	Isolated number of *S. marcescens*
1	Wounds	-	S/0.395	+
2	UTI	16	S/0.273	+
3	burns	11	M/0.189	−
4	UTI	21	S/0.454	+
5	UTI	10	M/0.238	+
6	Otitis media	12	M/0.126	−
Control			0.114	
%		66.7	100	66.7

S=Strong biofilm forming,
M=Moderate biofilm forming
S. marcescens: Serratia marcescens

TABLE III
NUMBER AND PERCENTAGE OF S. MARCESCENS ISOLATES ACCORDING TO SPECIMENS' TYPE

Study groups	Total number of specimens	No. of *S. marcescens* isolated	(%)
UTI	55	3	50
burns	34	1	16.63
Wounds	25	1	16.63
Otitis media	18	1	16.63
Total	132	6	100

S. marcescens: Serratia marcescens

A. Virulence Factors

Evaluating the diameter of the lysis area on skim milk agar media, determined that five isolates were protease producers (or wells method). The lowest protease activity measured 10 mm in diameter, while the maximum activity measured 21 mm in diameter, was found in four isolates.

In the Microtitre Plate (M.T.P.), all *S. marcescens* isolates formed biofilm. In the quantitative assay results, three isolates exhibited moderate biofilm formation, whereas three isolates demonstrated significant biofilm formation. Out of all isolates tested, only 4 (66.7%) formed biofilms on Congo-red agar. The results are listed in Table II and Fig. 2.

The opportunistic traits such as colonization and antibiotic resistance are influenced by biofilm formation (Wu et al., 2024). Biofilm development allows the bacteria to thrive in challenging conditions and confer resistance to a wide antimicrobial treatment (Haidar et al., 2024). *S. marcescens* can produce biofilm associated with biotic or abiotic surfaces (Srinivasan et al., 2021). This ability has been linked to *Serratia*'s capacity to form colonies and endure in medical devices such as catheters and prostheses. In addition, the bacteria are able to attach to the host's epithelial cells through the production of biofilms (Weber et al., 2023).

Antibiotics susceptibility

All isolate susceptibility tests were conducted on 10 antibiotics: Ampicillin, cefotaxime, azithromycin, ceftazidime, piperacillin/tazobactam, ciprofloxacin, amikacin, tobramycin, gentamicin, and imipenem.

The data in Fig. 3 display a high-level resistance of *S. marcescens* medical isolates to the greatest number of antibiotics beneath the test. All isolates were resistant to ampicillin and cefotaxime, with notable resistance rates to

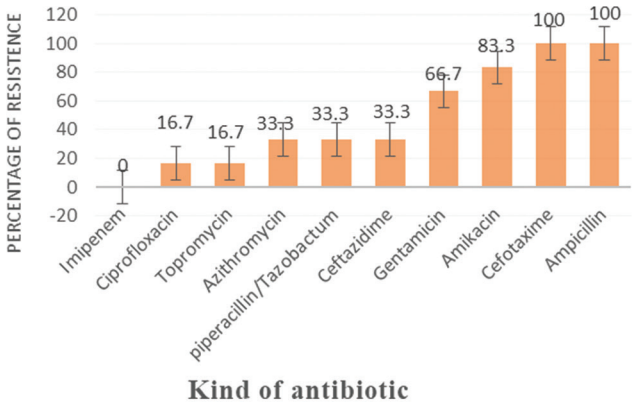

Fig. 3. Antibiotic resistance to *Serratia marcescens* isolates.

amikacin (83.3%) and gentamicin (66.7%). The bacteria can acquire resistance genes encoded to aminoglycoside-modifying enzymes which inactivate amikacin and gentamicin (Tavares-Carreon et al., 2023). However, our results contrast with a systematic review meta-analysis, which shows no resistant strains against amikacin, while three strains out of 26 isolates were resistant (Zivkovic Zaric et al., 2023).

Ceftazidime, piperacillin/tazobactam, and azithromycin were recorded to be moderate resistance (33.3%) followed by tobramycin and ciprofloxacin (16.63%). The most effective drug from the carbapenem group was imipenem 100%. This finding is consistent with a local study (Abdul et al., 2022). However, the effectiveness of imipenem, particularly in the context of *S. marcescens* infections has been a topic of research and investigation. While imipenem is a potent carbapenem antibiotic, its efficacy can vary based on the resistance mechanisms present in the bacterial strains (Mughrabi et al., 2023).

Ampicillin, a member of the penicillin family, appeared to be highly resistant (100%); as a result, researchers found that *S. marcescens* possesses intrinsic ampicillin resistance (Gravrand et al., 2022). Increasing the production of AmpC beta-lactamase by Enterobacteriaceae is linked to treating infections with third-generation broad-spectrum cephalosporins (cefotaxime and ceftazidime). *S. marcescens* expresses extended-spectrum-lactamases (ESBLs) through a plasmid and is chromosomally encoded for the AmpC beta-lactamase (Hayashi et al., 2021): Third-generation cephalosporins

and aztreonam hydrolyzed by ESBLs to treat the variety of infections, including UTIs. *S. marcescens* isolates showed a 49.4% resistance rate to third-generation cephalosporins, with 32.2% producing ESBLs (Radeva et al., 2022). Resistance levels in *S. marcescens* have been significantly enhanced in various clinical isolates, particularly within hospital environments (Xu et al., 2024). Mutant strains have emerged due to the combination of antibiotics used to treat severe Gram-negative infections, particularly the combination of cefotaxime and amikacin (Sharma et al., 2022).

B. Immunological Study

IL-12

The amount of IL-12 increases when a bacterial infection occurs in a room. In the course of our research, the IL-12 in wounds and UTIs with *S. marcescens* was examined. As presented in Fig. 4, IL-12 level was increased significantly in wounds and UTI patients compared to the normal control patients ([217.32 ± 48.9 pg/mL], and [81.65 ± 13.64 pg/mL; $p < 0.001$]). Certain patients who expected no treatment had a great level of IL-12. Nevertheless, based on the extensive deviation in IL-12 level, the individuals were divided into two collections: patients with a high IL-12 level, and healthy patients with an ordinary level. As shown in Fig. 4, levels of IL-12 in patients were significantly higher compared to the control with normal IL-12 levels ($p < 0.05$). Although there was a significant correlation between IL-12 levels, the levels in patients with wounds and UTIs contrasted with those in the control group. P-value (one-tailed) = 0.003765, p-value (two-tailed) = 0.007531, The *post hoc* test revealed significant results in each comparison, a $p < 0.02500$.

This interaction activates several signals and upregulates the expression of molecules involved in the presentation of Ag, and cytokines inflammatory mediators such as IL-12, TNF-gamma, and CCL2. Then, IL-12 promotes innate and adaptive immune responses by activating T and B cells and controlling the growth and differentiation of the Th1-type immune response (Ullrich et al., 2020). IL12 secretion fluctuates and is consistently produced at the beginning of an infection, where it then controls the production of other cytokines and upholds acquired immunity (Jiang et al., 2020). Cytokines are produced and matured during the immune system's control of infections. IL12 is suppressed or activated by pathogen contacts with immune cells' cytokine release, which regulates immunological responses and affects the development of infectious illnesses (Ullrich et al., 2020). Therefore, an increase in IL12 stimulates endosomes to destroy Gram-positive bacteria. This process is essential for effectively removing pathogens such as *Staphylococcus aureus* and *Mycobacterium tuberculosis* (Peignier et al., 2024).

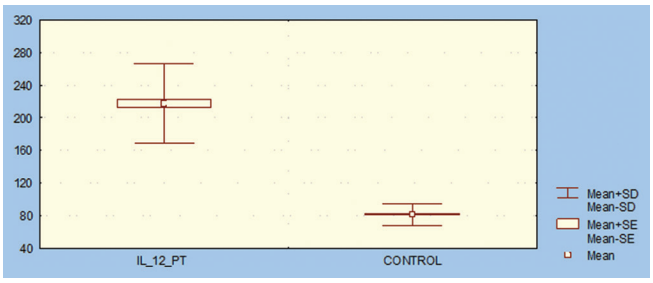

Fig. 4. Levels of interleukin-12 in patients compared with control

The bacterium hindered the innate immune response by employing a broad strategy of immune evasion. For instance, it secretes serralysin metalloprotease, which degrades adhesion molecules on immune cells, thereby inhibiting their ability to adhere and clear bacteria effectively and accelerating immune cell death through lipopolysaccharide and flagella. (Ishii et al., 2014; Vale de Macedo et al., 2021). This is due to IL12 secretion by *S. marcescens.*

Neutrophils phagocytic activity

In comparison to control groups, the findings revealed a statistically significant rise ($p < 0.05$) in the phagocytic action of polymorphonuclear neutrophils in *S. marcescens* infections. In burns infection, the average patient with positive cells was 9.72±*25.17 compared to the control 4.76±10.17, and the largest proportion of reduction in NBT was seen. The rate was lower in patients with otitis media infection 16.33±4.51 compared to control 5.03±10.67, as shown in Table V and Fig. 5.

This demonstrated that bacterial infections boosted the phagocytic action of polymorphonuclear cells in all groups. Neutrophils are known to take part in phagocytic activity, one of the immunological reactions that are non-specific and are triggered by the presence of foreign agents. The NBT dye reduction test is a cytochemical immunoassay revealing macrophages' involvement in phagocytosis (Nurasyikin et al., 2022).

The metabolic alterations happen in neutrophils and monocytes after phagocytosis and are related to the decrease in NBT *in vitro* inside the phagocytic vacuole (Britt et al., 2022). It has been demonstrated that the neutrophil NBT-reduction test is a useful indicator of bacterial infection (Nurasyikin et al., 2022). Although neutrophil phagocytosis's role removes pathogens, the initial anti-inflammatory cytokines secreting through neutrophil cells develop an immune paralysis response and hyperinflammatory response which lead to the insufficient removal of pathogens and subsequently cause septicemia (Kwok et al., 2023). Neutrophils account for

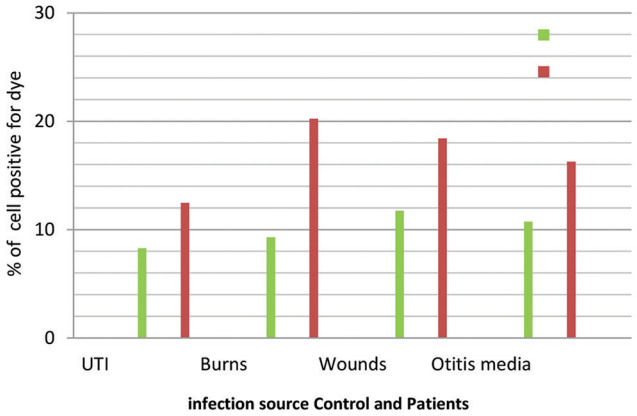

Fig. 5. Percentage of polymorphonuclear neutrophils in patients and control.

60%–80% of leukocyte cells rise tenfold during infections. Neutrophil membrane adhesion is reduced in response to bacteria endotoxin and hyperinflammatory cytokines secreted leading to inflammation of tissues and initial sepsis (Hortová-Kohoutková et al., 2020). The pro-inflammatory cytokines TNFα, IL-1β, IL-6, and IL-12 are secreted in response to Gram-negative bacteria infections through the outer membrane and LPS which TLR4 recognizes expressed on the neutrophil cell surface and trigger pro-inflammation signals (Ernst et al., 2021). Then the complement activates and recruits the neutrophils (Maqsood et al., 2024).

Patients' serum complements level (infected by S. marcescens)

As shown in Table VI the complements C3 and C4 levels in the serum of patients were 102.67±6.43, and 51.67±3.06, whereas the control levels of C3 and C4 were 85.33±5.03, 38.33±11.93, respectively. An increased level of complements in patients compared to control. Statistical analysis showed no significant differences; however, the *post hoc* test revealed that the means of the C3 and C4 tests were equal and significant. Complement levels are often significantly elevated after infection or injury (Rognes et al., 2021). The serum complements C3 and C4 are critical biomarkers for assessing complement pathways, diagnosing infectious diseases, and managing immune disorders. Their measurement provides insights into the functionality of the complement system, which plays a vital role in innate immunity and the pathogenesis of various diseases (Wang and Liu, 2021).

In addition, the classical pathway of complement activation plays a crucial role in enhancing the opsonization of bacteria, particularly through the binding of immunoglobulin IgM to bacterial surface molecules or extracellular material in biofilms. This process initiates complement activation, leading to the formation of C3 convertase, which cleaves C3 protein and facilitates opsonization (Pinto et al., 2024) (Table VI). These levels also revealed the state of the individual's innate immunity, especially C3 is the main element of the complement pathway, whereas C4 levels were increased. A drop in C3 is a reliable sign that a close, non-intrusive bacterial infection is forcing the complement system to use an alternative pathway (Tzoumas et al., 2021). Even though few studies have concentrated on immune responses to bacterial biofilm, the development of the biofilm plays a significant role in evading the host immune defense because it shields bacteria from antimicrobial peptides, neutrophil phagocytosis, complement deposition, and antibodies (Dong et al., 2022). The complement C3 controls the inflammatory response and macrophage phagocytosis (Bai et al., 2022). Moreover, the complement C4 expression is raised by lipopolysaccharide, IFNγ, and IL6 (Wang and Liu, 2021). The pathogens use different mechanisms to inhibit the innate immunity of the complement system, and the ability to do that reflects the bacteria's pathogenicity (Syed et al., 2020).

S. marcescens is an opportunistic pathogen that presents significant challenges in clinical environments due to its capability to evade host immune responses. The mechanisms

TABLE IV
Distribution of Serratia marcescens isolates according to patient's gender and age with specimen types

Ag group (Years)	Patient No	Gender		Study group			
		Male No.	Female No.	UTI No.	Wound No.	Burns No	Otitis media No.
≤20	1	-	1	1	-	-	-
21–35	1	-	1	1		-	-
36–55	3	1	2	1	1	1	-
>55	1	1	-	-	-	-	1
Total no. (%)	6 (100)	2 (33.3)	4 (66.7)	3 (50)	1 (16.63)	1 (16.63)	1 (16.636.7)

TABLE V
Percentage of Polymorphonuclear Neutrophils that Tested Positive for the Dye Nitro Blue Tetrazolium

Clinical source	Group	% cells positive for dye (mean±SD)	P-value	**Post hoc test*
UTI	Control	8.33±1.35	0.046	Non-significant
	Patients	*15.33±5.13		
Burns	Control	4.76±10.17	0.008	Significant
	Patients	9.72±*25.17		
Wounds	Control	1.15±10.67	0.011	Significant
	Patients	2.65±*18.00		
Otitis media	Control	5.03±10.67	0.220	Non-significant
	Patients	16.33±4.51		

*significance ($p<0.05$) comparison to the healthy
**Post hoc* P=0.0125

TABLE VI
Complements Level in *Serratia marcescens*-Infected Patient Sera

Complements	Patients' sera (M±standard deviation)	Control	P-value	**Post-hoc test*
C3 (mg/dL)	102.67±6.43	85.33±5.03	0.021 nonsignificant	Significant
C4 (mg/dL)	51.67±3.06	38.33±11.93	0.134 nonsignificant	Significant

**Post hoc* P=0.025

by which *S. marcescens* escapes immune detection are multifaceted, primarily involving its capsule polysaccharides, which play a crucial role in its virulence and survival during infections (Anderson et al., 2024).

IV. Conclusion

S. marcescent is one of the important causes of infections in hospitals, with the highest isolation rate depicting the pathogenicity of the isolates. The incidence was high among females, and the isolates were multi-resistant to most antibiotics and induced an inflammatory immune response, IL-12. The study showed that infection with a virulent bacterium stimulates the process of phagocytosis in neutrophil cells compared to the control.

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References

Abdul, F.R., Abbas, M.K., Rasool, K.H., and Raheem, I.A., 2022. The contribution of interleukin (17) in Iraqi patients with type I diabetes and positive agglutination sera with *Morganella morganii* antigens. *International Journal of Design and Nature and Ecodynamics*, 17(5), pp.767-772.

Abdul, F.R., Subhi, H.T., Taher, N.A., and Raheem, I., 2019. Activity of Iron oxide nanoparticles on bacterial biofilm formation. *International Journal of Pharmaceutical Sciences and Research*, 11(3), pp.1126-1130.

Abhishek, C., and Tanu, J., 2020. *Biochemical and Molecular Methods for Bacterial Identification*. Springer, Germany, pp.425-468.

Ayano, M., and Horiuchi, T., 2023. Complement as a biomarker for systemic lupus erythematosus. *Biomolecules*, 13(2), pp.367-367.

Bai, H., Mu, L., Qiu, L., Chen, N., Li, J., Zeng, Q., Yin, X., and Ye, J., 2022. Complement C3 regulates inflammatory response and monocyte/macrophage phagocytosis of *Streptococcus agalactiae* in a teleost fish. *International Journal of Molecular Sciences*, 23(24), p.15586.

Bhor, R., Rafati, S., and Pai, K., 2021. Cytokine saga in visceral leishmaniasis. *Cytokine*, 147, p.155322.

Britt, E.C., Lika, J., Giese, M.A., Schoen, T.J., Seim, G.L., Huang, Z., Lee, P.Y., Huttenlocher, A., and Fan, J., 2022. Switching to the cyclic pentose phosphate pathway powers the oxidative burst in activated neutrophils. *Nature Metabolism*, 4(3), pp.389-403.

Cai, J., Zhang, Y., Jiang, X., Hu, Y., and Chen, W., 2024. Thermally resistant nuclease in *Serratia marcescens* hinders PCR reactions and degrades PCR products. *Cell Biochemistry and Function*, 42(4), p.e4032.

Dong, J., Wang, W., Zhou, W., Zhang, S., Li, M., Li, N., Pan, G., Zhang, X., Bai, J., and Zhu, C., 2022. Immunomodulatory biomaterials for implant-associated infections: From conventional to advanced therapeutic strategies. *Biomaterials Research*, 26(1), p.72.

Drummond, S.E., Maliampuraka A., Jamdar A., Melly L., and Holmes S., 2023. *Serratia marcescens* causing recurrent superficial skin infections in an immunosuppressed patient. *Skin Health and Disease,* 3, p.e283.

Ernst, O., Khan, M.M., Oyler, B.L., Yoon, S.H., Sun, J., Lin, F.Y., Manes, N.P., MacKerell, A.D., Jr., Fraser, I.D.C., Ernst, R.K., Goodlett, D.R., and Nita-Lazar, A., 2021. Species-specific endotoxin stimulus determines toll-like receptor 4-and caspase 11-mediated pathway activation characteristics. *mSystems*, 6(4), p.e0030621.

Ferreira, R.L., Rezende, G.S., Damas, M.S.F., Oliveira-Silva, M., Pitondo-Silva, A., Brito, M.C.A., Leonardecz, E., De Góes, F.R., Campanini, E.B., Malavazi, I., Da Cunha, A.F., and Pranchevicius, M.D.S., 2020. Characterization of KPC-producing *Serratia marcescens* in an intensive care unit of a Brazilian tertiary hospital. *Frontiers in Microbiology*, 11, p.956.

Friedrich, I., Bodenberger, B., Neubauer, H., Hertel, R., and Daniel, R., 2021. Down in the pond: Isolation and characterization of a new *Serratia marcescens* strain (LVF3) from the surface water near frog's lettuce (*Groenlandia densa*). *PLoS One*, 16(11), p.e0259673.

Gravrand, V., Ariey, F., Terracol, L., Poupet, H., Doloy, A., Poyart, C., and Mammeri, H., 2022. Frameshift mutation (690delG) in cpxA contributes to the extensive drug resistance of a *Serratia marcescens* clinical isolate. *The Journal of Antimicrobial Chemotherapy*, 77(6), pp.1611-1616.

Haidar, A., Muazzam, A., Nadeem, A., Atique, R., Naveed, A., Sharif, J., Perveen, A., Fatima, H. R., and Samad, A., 2024. Biofilm formation and antibiotic resistance in *Pseudomonas aeruginosa*. *The Microbe*, 3, p.100078.

Hayashi, W., Yoshida, S., Izumi, K., Koide, S., Soga, E., Takizawa, S., Arakawa, Y., Nagano, Y., and Nagano, N., 2021. Genomic characterisation and epidemiology of nosocomial *Serratia marcescens* isolates resistant to ceftazidime and their plasmids mediating rare blaTEM-61. *Journal of Global Antimicrobial Resistance*, 25, pp.124-131.

Hortová-Kohoutková, M., Tidu, F., De Zuani, M., Šrámek, V., Helán, M., and Frič, J., 2020. Phagocytosis-inflammation crosstalk in sepsis: New avenues for therapeutic intervention. *Shock*, 54(5), pp.606-614.

Hsueh, K., 2020. Fevers and sepsis. In: Spec, A., Escota, G.V., Chrisler, C., and Davies, B., Eds. *Comprehensive Review of Infectious Diseases*. 1st ed. Elsevier, USA, p.181.

Iain, L., Hirschfeld, J., Kantarci, A., Wilensky, A., and Shapira, L., 2023. The role of the host-neutrophil biology. *Periodontology 2000*, 10.1111/prd.12490, pp.1-47.

Ishii, K., Adachi, T., Hamamoto, H., and Sekimizu, K., 2014. *Serratia marcescens* suppresses host cellular immunity via the production of an adhesion-inhibitory factor against immunosurveillance cells. *The Journal of Biological Chemistry*, 289(9), pp.5876-5888.

Jia, X., Zhao, K., Liu, F., Lin, J., Lin, C., and Chen, J., 2022. Transcriptional factor OmpR positively regulates prodigiosin biosynthesis in *Serratia marcescens* FZSF02 by binding with the promoter of the prodigiosin cluster. *Frontiers in Microbiology*, 13, p.1041146.

Jiang, H., Zhai, T., Yu, Y., Li, X., Gong, P., Zhang, X., Li, G., and Li, J., 2020. Delayed IL-12 Production by macrophages during *Toxoplasma gondii* infection is regulated by miR-187. *Parasitology Research*, 119(3), pp.1023-1033.

Jupatanakul, N., Pengon, J., Selisana, S.M.G., Choksawangkarn, W., Jaito, N., Saeung, A., Bunyong, R., Posayapisit, N., Thammatinna, K., Kalpongnukul, N., Aupalee, K., Pisitkun, T., and Kamchonwongpaisan, S., 2020. Serratia marcescens secretes proteases and chitinases with larvicidal activity against *Anopheles dirus*. *Acta Tropica*, 212, p.105686.

Kwok, A.J., Allcock, A., Ferreira, R.C., Cano-Gamez, E., Smee, M., Mentzer, A.J., Todd, J.A., and Knight, J.C., 2023. Neutrophils and emergency granulopoiesis drive immune suppression and an extreme response endotype during sepsis. *Nature Immunology*, 24(5), pp.767-779.

Maqsood, M., Suntharalingham, S., Khan, M., Ortiz-Sandoval, C.G., Feitz, W.J.C., Palaniyar, N., and Licht, C., 2024. Complement-mediated two-step NETosis: Serum-induced complement activation and calcium influx generate NADPH oxidase-dependent NETs in serum-free conditions. *International Journal of Molecular Sciences*, 25(17), p.9625.

Anderson, M.T., Himpsl, S.D., Kingsley, L.G., Smith, S.N., Bachman, M.A., and Mobley, H.L.T., 2024. *Infection characteristics among Serratia marcescens capsule lineages*. bioRxiv: The Preprint Server for Biology. https://doi.org/10.1101/2024.08.23.609398

Moles, L., Gómez, M., Moroder, E., Jiménez, E., Escuder, D., Bustos, G., Melgar, A., Villa, J., Del Campo, R., Chaves, F., and Rodríguez, J.M., 2019. *Serratia marcescens* colonization in preterm neonates during their neonatal intensive care unit stay. *Antimicrobial Resistance and Infection Control*, 8, p.135.

Mughrabi, A., Maamari, J., Philips, T., Alabbasi, A., Brooks, A., Nuriev, R., Zenkin, L., Jaber, B., and Nader, C., 2023. 2787. Carbapenem versus non-carbapenem based therapy for blood stream infection caused by *Serratia marcescens*: A multicenter, retrospective cohort study. *Open Forum Infectious Diseases*, 10(Suppl 2), p.S1232

Nurasyikin, Y., Yasin, N.M., Yousuf, R., Wahab, A.A., and Aziz, S.A., 2022. Comparison of neutrophil respiratory oxidative burst activity between flow cytometry using dihydrorhodamine (DHR) 123 and conventional nitroblue tetrazolium test (NBT). *Bangladesh Journal of Medical Science*, 21(3), pp.626-633.

Nwachukwu, E., Ejike, E.N., Ejike, B.U., Onyeanula, E.O., Chikezie-Abba, R.O., Okorocha, N.A., and Onukaogu, U.E., 2017. Characterization and optimization of lipase production from soil microorganism (*Serratia marcescens*). *International Journal of Current Microbiology and Applied Sciences*, 6(12), pp.1215-1231.

Peignier, A., Kim, J., Lemenze, A., and Parker, D., 2024. Monocyte-regulated interleukin 12 production drives clearance of *Staphylococcus aureus*. *PLOS Pathogens*, 20(10), p.e1012648.

Pinto, A.J., Chouquet, A., Bally, I., Thielens, N.M., Dumestre-Perard, C., Kunert, R., Gaboriaud, C., Ling, W.L.W., and Reiser, J.B., 2024. The Fc Fragment of Soluble IgMs Binds C1q to Activate the Classical Complement Pathway, While Inhibiting Complement-Dependent Cytotoxicity. bioRxiv: the preprint server for biology, 2024.07.10.602503.

Prado, L.C.D.S., Giacchetto Felice, A., Rodrigues, T.C.V., Tiwari, S., Andrade, B.S., Kato, R.B.,… & Soares, S.D.C., 2021. New putative therapeutic targets against Serratia marcescens using reverse vaccinology and subtractive genomics. *Journal of Biomolecular Structure and Dynamics*, 30, pp.1-16.

Priyanka, G., Vivek, H., Rath, R.S., Mohanty, A., Singh, P., and Rukadikar, A., 2023. Interpretation of antimicrobial susceptibility testing using European committee on antimicrobial susceptibility testing (EUCAST) and clinical and laboratory standards institute (CLSI) breakpoints: Analysis of agreement. *Cureus*, 15, p.e36977.

Radeva, S.D., Niyazi, D., Bojkova, M., Savova, D., and Stoeva, T., 2022. Resistance rates to 3rd generation cephalosporins and carbapenems in *Serratia marcescens* isolates obtained from various clinical samples from two Bulgarian hospitals. *Medical Sciences Forum,* 12(1), p.32.

Rognes, I.N., Pischke, S.E., Ottestad, W., Røislien, J., Berg, J.P., Johnson, C., Eken, T., and Mollnes, T.E., 2021. Increased complement activation 3 to 6 h after trauma is a predictor of prolonged mechanical ventilation and multiple organ dysfunction syndrome: A prospective observational study. *Molecular Medicine*, 27(1), p.35.

Sadeq, J.N., and Neamah, A.J., 2024. Comparative phenotypic and genotypic properties of *Serratia marcescens* isolates from clinical cases from human in AL-Diwaniyah Province, Iraq. *South Eastern European Journal of Public Health*, Volume XXV S1 pp.580-592.

Sameer, R.S., and Arun, P., 2023. Prodigiosin: An in-depth exploration of a bioactive compound from *Serratia* sp. *Current Bioactive Compounds,* 19, p.13.

Sharma, M.K., Rizvan, M., Ahmad, N., Jain, P., and Kumar, M., 2022. Treatment of MDR Gram-negative bacteria infections: Ongoing and prospective. *Journal of Pure and Applied Microbiology*, 16(3), pp.1575-1589.

Srinivasan, R., Santhakumari, S., Poonguzhali, P., Geetha, M., Dyavaiah, M., and Xiangmin, L., 2021. Bacterial biofilm inhibition: A focused review on recent therapeutic strategies for combating the biofilm mediated infections. *Frontiers in Microbiology*, 12, p.676458.

Suhad, A.A., Al-Kadmy, M.S.I., Sarah, N.A., and Hamzah, I.H., 2023. The predominance of virulence genes associated with multidrug-resistant *Serratia marcescens* isolated from urinary tract infections. *Reviews and Research in Medical Microbiology*, 35(3), pp.160-166.

Syed, S., Viazmina, L., Mager, R., Meri, S., and Haapasalo, K., 2020. Streptococci and the complement system: Interplay during infection, inflammation and autoimmunity. *FEBS Letters*, 594(16), pp.2570-2585.

Tavares-Carreon, F., De Anda-Mora, K., Rojas-Barrera, I.C., and Andrade, A., 2023. *Serratia marcescens* antibiotic resistance mechanisms of an opportunistic pathogen: A literature review. *PeerJ*, 11, p.e14399.

Tille, P.M., 2022. *Bailey and Scott's Diagnostic Microbiology*. 15th ed. Elsevier, Netherlands, pp.334-339.

Tóth, Á., Makai, A.L., Jánvári, L., Damjanova, I., Gajdács, M., and Urbán, E., 2020. Characterization of a rare bla_{VIM-4} metallo-β-lactamase-producing *Serratia marcescens* clinical isolate in hungary. *Heliyon*, 6, p.e04231.

Tuttobene, M.R., Arango Gil, B.S., Di Venanzio, G., Mariscotti, J.F., Sieira, R., Feldman, M.F., Ramirez, M.S., and García Véscovi, E., 2024. Unraveling the role of UilS, a urea-induced acyl-homoserine lactonase that enhances *Serratia marcescens* fitness, interbacterial competition, and urinary tract infection. *mBio*, 15, p.e0250524.

Tzoumas, N., Hallam, D., Harris, C.L., Lako, M., Kavanagh, D., and Steel, D.H.W., 2021. Revisiting the role of factor H in age-related macular degeneration: Insights from complement-mediated renal disease and rare genetic variants. *Survey of Ophthalmology*, 66(2), pp.378-401.

Ullrich, K.A., Schulze, L.L., Paap, E.M., Müller, T.M., Neurath, M.F., and Zundler, S., 2020. Immunology of IL-12: An update on functional activities and implications for disease. *EXCLI Journal*, 19, pp.1563-1589.

Vale de Macedo, G.H.R., Costa, G.D.E., Oliveira, E.R., Damasceno, G.V., Mendonça, J.S.P., Silva, L.D.S., Chagas, V.L., Bazán, J.M.N., Aliança, A.S.D.S., Miranda, R.D.C.M.D., Zagmignan, A., Monteiro, A.D.S., and Nascimento da Silva, L.C., 2021. Interplay between ESKAPE pathogens and immunity in skin infections: An overview of the major determinants of virulence and antibiotic resistance. *Pathogens*, 10(2), p.148.

Wang, H., and Liu, M., 2021. Complement C4, infections, and autoimmune diseases. *Frontiers in Immunology*, 12, p.694928.

Weber, D.J., Rutala, W.A., Anderson, D.J., and Sickbert-Bennett, E.E., 2023. Biofilms on medical instruments and surfaces: Do they interfere with instrument reprocessing and surface disinfection. *American Journal of Infection Control*, 51(11), pp.A114-A119.

Wu, X., Wu, D., Cui, G., Lee, K.H., Yang, T., Zhang, Z., Liu, Q., Zhang, J., Chua, E.G., and Chen, Z., 2024. Association between biofilm formation and structure and antibiotic resistance in *H. pylori*. *Infection and Drug Resistance*, 17, pp.2501-2512.

Xu, Q., Zheng, B., Li, K., Shen, P., and Xiao, Y., 2024. A preliminary exploration on the mechanism of the carbapenem-resistance transformation of *Serratia marcescens in vivo*. *BMC Genomics*, 25(1), p.2.

Zivkovic Zaric, R., Zaric, M., Sekulic, M., Zornic, N., Nesic, J., Rosic, V., Vulovic, T., Spasic, M., Vuleta, M., Jovanovic, J., Jovanovic, D., Jakovljevic, S., and Canovic, P., 2023. Antimicrobial treatment of *Serratia marcescens* invasive infections: Systematic review. *Antibiotics* (*Basel)*, 12(2), p.367.