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MOLECULAR DISTRIBUTION, VIRULENCE FACTORS AND ANTIBIOTIC RESISTANCE OF *SERRATIA MARCESCENS*; FROM ENVIRONMENT TO HOSPITAL AND VICE VERSA

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Abstract: The clonal distribution of *S. marcescens* in the environment and hospital sites is of great importance since the virulence and resistance factors are similar in both cases, and these serve the species to colonize diverse niches either at 30°C and/or 37°C. Therefore, the objective was focused on determining the clonality, pathogenicity profile and antibiotic resistance of clinical and environmental *S. marcescens* at 30°C and 37°C. For this purpose, 26 strains were isolated from different environments and causing healthcare-associated infections (HAI), the ability to produce hemolysins, proteases, lipases, prodigiosin and biofilms, resistance to various antibiotics, and their clonal profile were measured, with the aim of describing their distribution and profile. It was detected that both groups of strains share similar profiles of resistance and production of metabolites associated with virulence, only proteases differ with temperature and clone A is widely distributed, and only clone B is found in the environment and the hospital or vice versa, leading to a distribution of these in both environments.
Keywords: *S. marcescens*, antibiotics, temperature, clonal distribution and virulence.

INTRODUCTION

Serratia marcescens is a bacterium with the capacity to adapt to diverse ecological niches (human and animal intestines, water bodies, pipes, handles, rhizosphere, animal and insect skin, air currents and hospital supplies, among others) (Van Houdt *et al*., 2007). The expression of their virulence factors is affected in some cases by temperature, in environmental ones their optimum temperature is 30°C and in clinical ones it is usually 37°C (Petersen, L. M.., & Tisa, L. S. (2012 and 2013)), these abilities serve not only to colonize or infect humans and animals, but to adapt to their ecological niches, and have described the resistance to antibiotics in S. *marcences* and that may hinder their treatment, likewise they are able to acquire resistance genes by selective pressure of being in environments with such antibiotics. The clonal diversity of *S. marcencens* is fundamental to know the molecular epidemiology of the clones in the environment and to help us to know their capacity of adaptation. The objective was to determine the clonality, pathogenicity profile and antibiotic resistance of *S. marcescens* clinical and environmental at 30°C and 37°C.

MATERIALS AND METHODS

29 strains of *S. marcescens* were obtained (environmental strains came from air currents near hospitals, treated water, insect surfaces, tomato root and amphibian skin), and clinical isolates were obtained from Health Care Associated Infections (HAI). Identification and antibiotic resistance was done using the Vytek 2® system, in addition, their ability to produce secondary metabolites as virulence factors at 30°C and 37 °C was determined according to protocols already described, for prodigiocin, hemolysins, proteases, lipases (Harris AKP *et al*., (2004), AL-Ghanem M M., (2018)), biofilm (Peeters., *et al.* (2008), Petersen LM, Tisa LS. (2014)), likewise, DNA extraction was performed by heat shock technique, and bacterial clonality analysis was performed using ERIC-PCR described by Toni G. Patton., *et al.* (2001) and interpreted according to Tenover *et al*., (1995).

RESULTS AND DISCUSSION

The strains of *S. marcences,* 26 are environmental (water, rhizosphere, air, insects, and amphibians) and only 3 were causative of HCAI, all were evaluated for their resistance, virulence and clonality profiles. 100% are resistant to ampicillin, 100% of the environmental strains are sensitive to chloramphenicol and 100% of

the clinical strains are resistant, 100% are sensitive to meropenem, ciprofloxacin, and gentamicin, in the case of tobramycin 80% of the environmental strains are resistant and 100% of the clinical strains are sensitive. The capacity to produce secondary metabolites is shown in Table 1. The virulence factors at 30 and 37°C are also shown, 100% produce biofilms, 88% (4/26) produce lipases and proteases with the exception of those isolated from frog skin and IAAS, there are changes in the detection of proteases based on temperature, where it is observed that at 30°C the strains are negative and at 37°C positive, the other metabolites remain the same at both temperatures as shown in Table 1, indicating that their detection does not depend on temperature and that both environmental and clinical strains have the same possibilities of adapting to different ecological niches. On the other hand, it is demonstrated with the clonal distribution of all strains, clone A, is widely distributed in 90% of the ecological niches, as for clone B present in the IAAS, some are found in the legs of flies, air outside hospitals and the rhizosphere of tomato, and as for clone C we found it in a sample of water and air (Figure 1). The clonal distribution of strains isolated from the environment and associated with HAIs, demonstrates the great capacity of movement that bacteria have, and the expression of virulence factors and resistance to both temperatures make them successful in colonizing diverse environments (Gupta N., *et al* (2014)), whether clinical or environmental, this leads us to propose the distribution of *S. marcescens*, being strains SH2, M and T457 with better expression of the metabolites analyzed at both 30°C and 37°C, and strains SH2, A2, M, SH1, T4-14 despite being isolated in different niches (air, root, amphibians, insects and hospitals) come from the same lineage (Table 1).

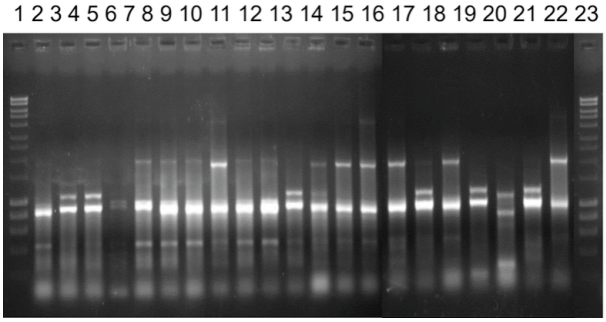
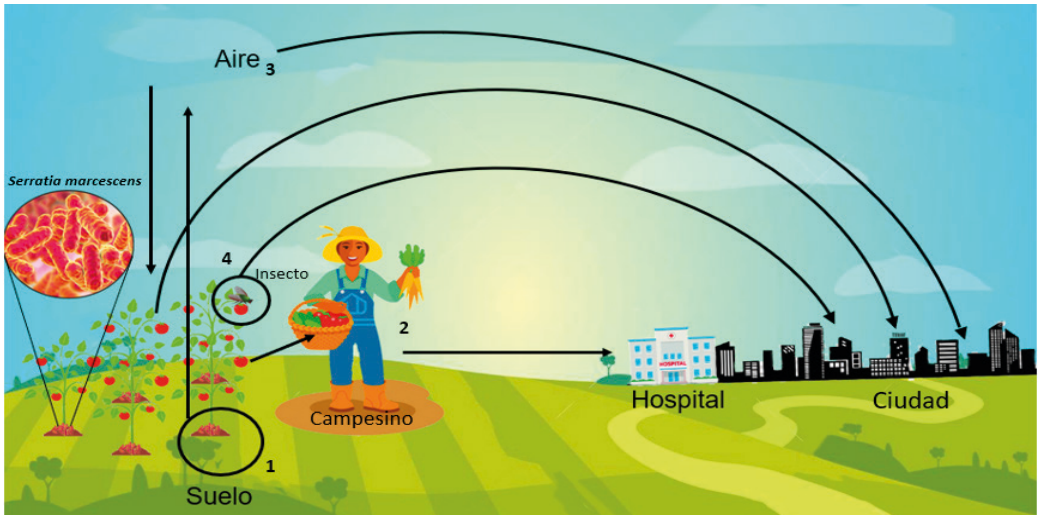


Figure 1. Detection of the clonal origin of the *S. marcescens* isolated from clinical and environmental settings by ERIC-PCR Lanes 1 and 23 molecular weight marker 1Kb, Lane 2 T2-2, lane 3 SH2, lane 4 A2, lane 5 AMO, lane 6 A5, lane 7 T4-28, lane 8 C6, lane 9 T4-52, lane 10 T2-6, lane 11 T2-32, lane 12 M, lane 13 SA4, lane 14 T3-33, lane 15 T3-23, lane 16 T2-21, lane 17 T4-11, lane 18 T3-38, lane 19 SH1, lane 20 SA1, lane 21 T4-14, lane 22 T4-57.



Origin	Strains	Enzyme expression at 30 °C							Enzyme expression at 37°C					Antibiogram								ERIC-PCR
		HEM	PRO	LIP	BF	CBF	BSF	PRD	HEM	PRO	LIP	BF	CBF	AM	CL	MEM	TE	CN	STX	CIP	TOB	CLONA
Water	AMO	+	-	+	-	**F**	+	+	+	+	+	+	M	R	S	S	R	S	S	S	R	C
Fly	M	+	-	+	+	F	+	+	+	+	+	+	F	R	S	S	R	S	S	S	R	B
Air	A1	+	-	-	-	-	-	+	+	+	-	+	-	R	S	S	R	S	S	S	R	A
	A2	+	-	+	-	**F**	-	+	+	+	+	+	D	R	S	S	R	S	S	S	R	B
	A3	+	-	-	-	-	-	+	+	+	-	+	-	R	S	S	R	S	S	S	R	ND
	A4	+	-	-	-	-	-	+	+	+	-	+	-	R	S	S	R	S	S	S	R	ND
	A5	+	-	-	+	**F**	-	+	+	-	+	+	M	R	S	S	R	S	S	S	R	A
	A6	+	-	+	+	-	-	+	+	-	+	+	-	R	S	S	R	S	S	S	R	ND
	A7	-	-	-	+	D	-	-	+	-	-	+	D	R	R	S	S	S	S	S	R	C
	A8	-	-	-	+	-	-	+	+	-	-	+	-	R	R	S	S	S	S	S	R	ND
	A9	-	-	-	+	**F**	-	-	+	-	-	+	M	R	S	S	R	R	R	S	R	A3
Tomato root	T2-2	-	+	+	+	**D**	+	+	-	-	+	+	M	R	R	S	R	S	R	S	R	A2
	T2-6	-	+	-	+	**F**	+	+	-	-	-	+	M	R	S	S	S	S	R	S	S	A
	T2-21	-	+	+	+	**F**	+	+	+	-	+	-	D	R	R	S	R	R	R	S	R	A
	T2-22	-	+	-	+	M	+	+	-	-	+	+	F	R	S	S	R	S	S	S	S	A
	T3-23	-	+	-	+	F	-	+	-	-	+	+	F	R	S	R	R	S	S	S	S	A1
	T3-33	+	-	-	+	**F**	-	+	+	+	-	+	F	R	R	R	S	S	S	S	S	A
	T3-38	-	+	-	+	**F**	-	+	-	+	+	+	M	R	S	R	S	S	S	S	S	A
	T4-11	-	+	-	+	**F**	+	+	-	-	+	+	D	R	S	S	R	S	S	S	S	B1
	T4-14	-	+	-	+	**F**	-	+	+	-	+	+	D	R	S	S	S	S	S	S	S	B
	T4-28	-	+	+	+	**F**	-	+	+	+	+	+	D	R	S	R	R	S	S	S	S	A
	T4-52	-	+	-	+	F	+	+	+	-	+	+	F	R	S	S	R	S	S	S	R	A1
	T4-57	+	+	-	+	D	+	+	+	+	+	+	F	R	S	S	R	S	S	S	S	A1
	C6	-	-	-	+	**D**	-	+	-	-	+	+	M	R	S	S	S	S	S	S	S	A
Rana	E1	-	-	-	+	-	-	+	-	-	-	+	-	R	S	S	S	S	S	S	S	ND
	E2	+	-	-	+	-	+	+	+	-	-	+	-	R	S	S	S	S	S	S	S	ND
IAAS	UI1	+	-	-	+	-	-	+	-	-	-	+	-	R	S	S	S	S	S	S	S	B
	SH1	+	-	-	+	**F**	-	-	+	+	-	+	M	R	S	S	S	S	S	S	S	B
	SH2	+	-	+	+	**F**	+	+	+	+	+	+	D	R	S	S	S	S	S	S	R	B

Table 1. Detection of virulence factors (enzymes) at 30 and 37°C, antibiogram and clonality of environmental and clinical strains of *S. marcenscens*.

HEM: Hemolysins, PRO: Proteases, LIP: Lipases, BF: Biofilm, CSF: Biofilm quantification, BSF: Biosurfactant, PRD: Prodigiosin, AM: Ampicillin, CL: Chloramphenicol, MEM: Meropenem, TE: Tetracycline, CN: Gentamicin, STX: Sulfamethoxazole and trimethoprim, CIP: Ciprofloxacin, TOB: Tobramycin. (+) Positive, (-) Negative, (.) No result, F: Strong, M: Moderate, D: Weak, R: Resistant, S: Susceptible, I Intermediate

Proposed clonal distribution of *S. marcescens* clone B, ranging from the environment to the hospital and vice versa; 1) *S. marcescens* in the rhizosphere of tomato. 2) contact with farmers. 3) transfer of strains to the air or vice versa and 4) contact with insects.

CONCLUSION

S. marcescens has a great capacity to adapt to different environments and its virulence factors help it to adapt and clone A is the most widely distributed, followed by B which was detected in both environments, which leads to understand the traffic and movements of this species, which stimulates further research on how to block the dissemination to reduce the effect of causing HAIs in various hospital centers.

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CONFLICTS OF INTEREST

The authors declare no conflicts of **interest.**

REFERENCES

AL-Ghanem M M. (2018). Serratia A Novel Source of Secondary Metabolites. Adv Biotech & Micro. 11(3): 555814. DOI: 10.19080/ AIBM.2018.11.555814

Harris AKP, Williamson NR, Slater H, Cox A, Abbasi S, Foulds I, Simonsen HT, Leeper FJ, Salmond GPC. (2004). The Serratia gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species- and strain-dependent genome context variation. Microbiology (Reading) 150:3547–3560.

Gupta N, Hocevar SN, Moulton-Meissner HA, Stevens KM, McIntyre MG, Jensen B, Kuhar DT, Noble-Wang JA, Schnatz RG, Becker SC, Kastango ES, Shehab N, Kallen AJ. (2014). Outbreak of *Serratia marcescens* bloodstream infections in patients receiving parenteral nutrition prepared by a compounding pharmacy. Clin Infect Dis.59:1–8.

Peeters Elke, Hans J. N. (2008). Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. Journal of microbiological methods. pp 157.

Petersen, L. M., & Tisa, L. S. (2012). Influence of temperature on the physiology and virulence of the insect pathogen *Serratia sp*. Strain SCBI. Applied and environmental microbiology, 78(24), pp 8840–8844. https://doi.org/10.1128/AEM.02580-12

Petersen, L. M. & Tisa, L. S. (2013). Friend or foe? A review of the mechanisms that drive Serratia towards diverse lifestyles. Can J Microbiol. 59, pp 627–40.

Petersen LM, Tisa LS. (2014). Molecular characterization of protease activity in *Serratia sp*. strain SCBI and its importance in cytotoxicity and virulence. J Bacteriol. 196:3923–3936.

Van Houdt R. Givkov M. y Michiels C.W (2007). Sensación de quórum en Serratia" FEMS Microbiology Reviews. 31(4). pp 407 – 408

Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol. 33(9). Pp. 2233-2239. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC228385/

Toni G. Patton, Sue Katz, Rodney J. Sobieski, Scott S. (2001). Crupper, Genotyping of clinical. Serratia marcescens isolates: a comparison of PCR-based methods, FEMS Microbiology Letters. 194(1), pp 19–25, https://doi.org/10.1111/j.1574-6968.2001.tb09440.x