2023 لسنة 9 العدد
No. 9 – May 2023
المجلة العراقية للبحوث الانسانية والاجتماعية والعلمية
Iraqi Journal of Humanitarian, Social and Scientific Research
Print ISSN 2710-0952 -Electronic ISSN 2790-1254

Molecular Identification Of Some Virulence Factors genes of *Serratia marcescens* Isolated From Clinical samples.

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Abstract

Serratia marcescens bacteria have emerged as main nosocomial pathogens. Virulence factor is important in enhancing ability to cause disease. Some of which are very significant in the pathogenesis of diseases caused via them. Resistance genes only does not indicate pathogenicity of bacteria, presence them with virulence factors it can cause the strain to be dangerous. This study aims to specify the virulence genes of *Serratia marcescens* isolated from different clinical samples in Al-Qadisiyah province, Iraq. Twenty *Serratia marcescens* isolates recovered from different clinical samples. All isolates identified by MacConkey agar, biochemical tests and, Vitek® 2 compact system and the virulence factors were detection by conventional PCR. It was found that Prodigiosin gene, Adhesion gene, and Proteases gene observed in all *Serratia marcescens* isolates that isolated from different clinical samples with 100%.

Keywords : *S. marcescens*, virulence factors genes, Proteases, Prodigiosin, Adhesion.

التعرف الجزيئي لبعض جينات عوامل الضراوة في سيراتيا مارسيسنس المعزولة من العينات السريرية

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خلاصة

ظهرت بكتيريا Serratia marcescens كمسببات أمراض المستشفيات الرئيسية. عامل الفوعة مهم في تعزيز القدرة على التسبب في المرض. بعضها مهم جدًا في التسبب في الأمراض التي تسببها. جينات المقاومة فقط لا تشير إلى إمراضية البكتيريا ، ووجودها مع عوامل الضراوة يمكن أن يسبب السلالة لتكون خطيرة. تهدف هذه الدراسة إلى تحديد جينات الفوعة لـ Serratia marcescens المعزولة من عينات سريرية مختلفة في محافظة القادسية ، العراق. تم استرجاع عشرين عزلة من Serratia marcescensمن عينات سريرية مختلفة. تم الكشف عن جميع العزلات التي تم تحديدها بواسطة أجار MacConkeyوالاختبارات البيوكيميائية ونظام Vitek® 2 المضغوط وعوامل الضراوة بواسطة PCRالتقليدي. وجد أن جين البروديجيوسين وجين الالتصاق وجين البروتياز لوحظ في جميع عزلات سيراتيا مارسيسنس المعزولة من عينات سريرية مختلفة بنسبة 100٪.

الكلمات المفتاحية S. marcescens :، جينات عوامل الضراوة ، البروتياز ، البروديجيوسين ، الالتصاق.

1. Introduction

Previously, *Serratia marcescens* was thought to be a benign saprophytic organism that grew on decaying organic matter, including plants, animals, food, water, and soil. It is now recognized as an opportunistic pathogen that can cause nosocomial infections, particularly in intensive care units of pediatric hospitals. It is linked to a variety of hospital-acquired infections, including pneumonia,

otitis media, meningitis, endocarditis, conjunctivitis, bacteremia, septicemia, and osteomyelitis [1,2]. *S. marcescens* produces virulence factors like enzymes such as proteases, lipases, chitinases, nucleases, and phospholipases, as well as the ability to form biofilms on abiotic or biotic surfaces, which aid bacteria in colonizing and persisting in medical devices such as prostheses and catheters, as well as increasing antibiotic resistance [3]. *S. marcescens* produce a particular class of proteolytic enzyme known as protease is necessary for both the development and differentiation of cells as well as the creation of fully developed organisms. Extracellular proteases are employed in many different fields and have a wide range of applications. A family of enzymes known as "protease" catalyzes the breakdown of proteins [4]. Protease, nuclease, lipase, hemolysin, and other virulence factors that *S. marcescens* possesses, as well as its capacity to swarm, swim, and build biofilm, are thought to contribute to its pathogenicity [5]. Prodigiosin, a red pigment made by *S. marcescens*, has various antifungal, antibacterial, and antiprotozoal effects [3]. Prodigiosin also has immunosuppressive properties [6]. Studies have shown that *S. marcescens* can adhere to the host surface and that it can do so to hydrocarbons and polystyrene that are present there. It also has the ability to adhere to non-living material surfaces due to the presence of the Pilli-like O antigen [7]. This study aims to molecularly identify virulence genes of *S. marcescens* isolated from different clinical samples.

2. Methodology

2.1. Sampling, Isolation, and Identification of Serratia marcescens.

This cross-sectional study gathered approximately 200 clinical samples from hospitals Al-Qadisiyah province, Iraq. from September to January 2023. To isolate the *Serratia marcescens*, All clinical samples were cultured on MacConkey agar and blood agar under sterile conditions and incubated for 24 hr at 37°C. Used Gram stain, catalase test, oxidase test, and IMVIC tests were performed for the early identification of *Serratia marcescens*, then was confirm identify of *S. marcescens* isolates through used Vitek® 2 compact system [8].

2.2. DNA extraction and PCR testing

To extract genomic DNAs from *S. marcescens* isolates, a DNA extraction kit (Scientific Research Company, Iraq) was used in accordance with the manufacturer's instructions. Using the technique described by Sambrook and Russell [9].

2.3. Identifying the virulence genes by PCR method

PCR was applied using specific primes to identify the virulence factors genes including the Prodigiosin gene, Protease gene, and Adhesion gene, Tables 1 and 2. PCR was carried out using a DNA thermal cycler (Master Cycler Gradient, Eppendorf, Germany). The amplicons were stained with ethidium bromide before being electrophoresed in 1.5% agarose gel at 80 V for 30 min. PCR results were examined and captured using UV doc gel documentation devices

(Uvitec, UK). The PCR results were contrasted against a 100 bp DNA marker (Fermentas, Germany).

Table 1: The oligonucleotide primers used for amplification of virulence genes.

Genes		Primer Sequences (5′-3′)	Product Size (bp)	References
Prodigiosin gene	F	TCAATACATCCGCTTCAACGAA	456bp	Primer design depend on
	R	AAAATGCTTTGCCAGCTGC		
Protease gene	F	GCTTCGTGGCGGGTGATAAA	311bp	Primer design depend on
	R	GACCTTGCAGTTTTGGTGGC		
Adhesion gene	F	TCTCTACTGCTTGGCTTGGC	268bp	Primer design depend on
	R	AATTTGCGGATAACGCGTCG		

Table 2. The PCR programs

Genes	Step	Temperature	Time	Cycle
Prodigiosin Protease Adhesion genes	Initial denaturation	95.0 C°	5 min	1
	Denaturation	95.0 C°	1 min	35
	Annealing	58,56,55.0 C°	1 min	
	Extension	72.0 C°	1 min	
	Final Extension	72.0 C°	10 min	1
	Hold	4.0 C°	forever	1

3.Results

Out of 200 clinical samples, *S. marcescens* was detected in 20 samples (10%). All samples tested positive microbiological were tested positive in a phenotyping study conducted using a culture media and biochemical tests. These results are confirmed that these isolates belong to S. marcescens by Identification of isolates was dependent on the VITEK2 compact system the result of this study was 99% probability S. marcescens as shown in Fig. 1.

The distributions of virulence-determinant genes among *S. marcescens*. It was found that the Prodigiosin gene was observed in 20 (100%) (Fig. 2A). These result is agree with other studies [10],[11]. and disagree with the result of [12]. The increased prevalence of the Prodigiosin gene in *S. marcescens* may shed light on the function of this gene in the prevention of cancer by inducing apoptosis in primary human cancer cells as well as antifungal, antibacterial, antiprotozoal/antimalarial, and immunosuppressive actions [6]. The biosynthesis of prodigiosin in the genus *S. marcescens* is dependent on the *pig* gene cluster

consisting of *pigA-N* or *pigA-O* [13]. Both pigmented and non-pigmented *S. marcescens* strains are pathogenic for humans. However, there is concern that non-pigmented strains are more virulent due to cytotoxin production and antibiotic resistance [14]. Prodigiosin may also play a physiological function in interspecies competition, which occurs when it prevents the growth of a variety of Gram-positive and Gram-negative bacteria that thrive in comparable settings. [15,16].

PCR results showed Protease gene was found in 20 (100%) of *Serratia marcescens* (Fig. 2B). The result of this study agrees with Shanks *et al.*, [17]. identify SlpB, a cytotoxic protease from *S. marcescens* and this result is in agreement with the result previous studies. Al Murjani [18]. reported that all their *S. marcescens* isolates were positive for this, in the same manner Pinaa *et al.* [19]. in his study revealed 100% positive isolates for protease production. *S. marcescens'* protease activity has also been connected to host cell invasion and devastation. Extracellular products known as proteases are linked to virulence, colonization, and invasiveness [20]. According to researchs, proteases control the creation and activation of proteins to keep track of physiological processes [21]. Proteases have a role in a range of physiological processes, including as growth, aging, birth, and death. proteases are primarily vital in the reproduction and spread of infectious pathogens in addition to being incredibly important for medication development [22]. Proteases indeed play a crucial part in a number of disorders, including arthritis, malignancies, and even treatments for metastatic cancer. Additionally, the development of neurological diseases and infection have both been linked to the presence of one or more proteases [23].

The results of PCR test shows that 20 (100%) of *S. marcescens* isolates in this study produces Adhesion gene as indicated in (Fig. 2C)., this result is in agreement with the result obtained by previous studies. Ramanathan *et al.*, [24], [3]. While disagree with the results [25].

Pathogenic microorganisms normally express several virulence determinants facilitating their invasion and evasion of the host defenses and causing disease [26]. Fimbria or pili are used by bacteria to attach to tissues, causing infection [27]. The *S. marcescens* fimbriae expressed by the genes *fimA*, *fimC*, and *fimH* are crucial for surface adhesion and colonization [28]. Additionally, *flhD* controls flagella-controlled swarming motility, which aids in cell surface attachment [29].

bioMérieux Customer:
System #:
Patient Name:
Isolate: 21231a-1 (Qualified)
Card Type: GN Bar Code: 2411993403177442 Testing Instrument: 000014EEB97D (VITEK2C)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: Labadmin
Patient ID:

Bionumber: 6521711455104230
Organism Quantity: Selected Organism: Serratia marcescens

Comments:	

Identification Information	Card: GN	Lot Number: 2411993403	Expires: May 12, 2023 13:00 CDT
	Status: Final	Analysis Time: 3.87 hours	Completed: Jan 2, 2023 22:28 CST
Organism Origin	VITEK 2		
Selected Organism	99% Probability Serratia marcescens Bionumber: 6521711455104230 Confidence: Excellent identification		
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	+	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	-	15	OFF	-
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	+
40	lLATk	+	41	AGLU	-	42	SUCT	-	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	+	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Figure. 1 : Results sheet of VITEK-2 compact system for *S. marcescens.*

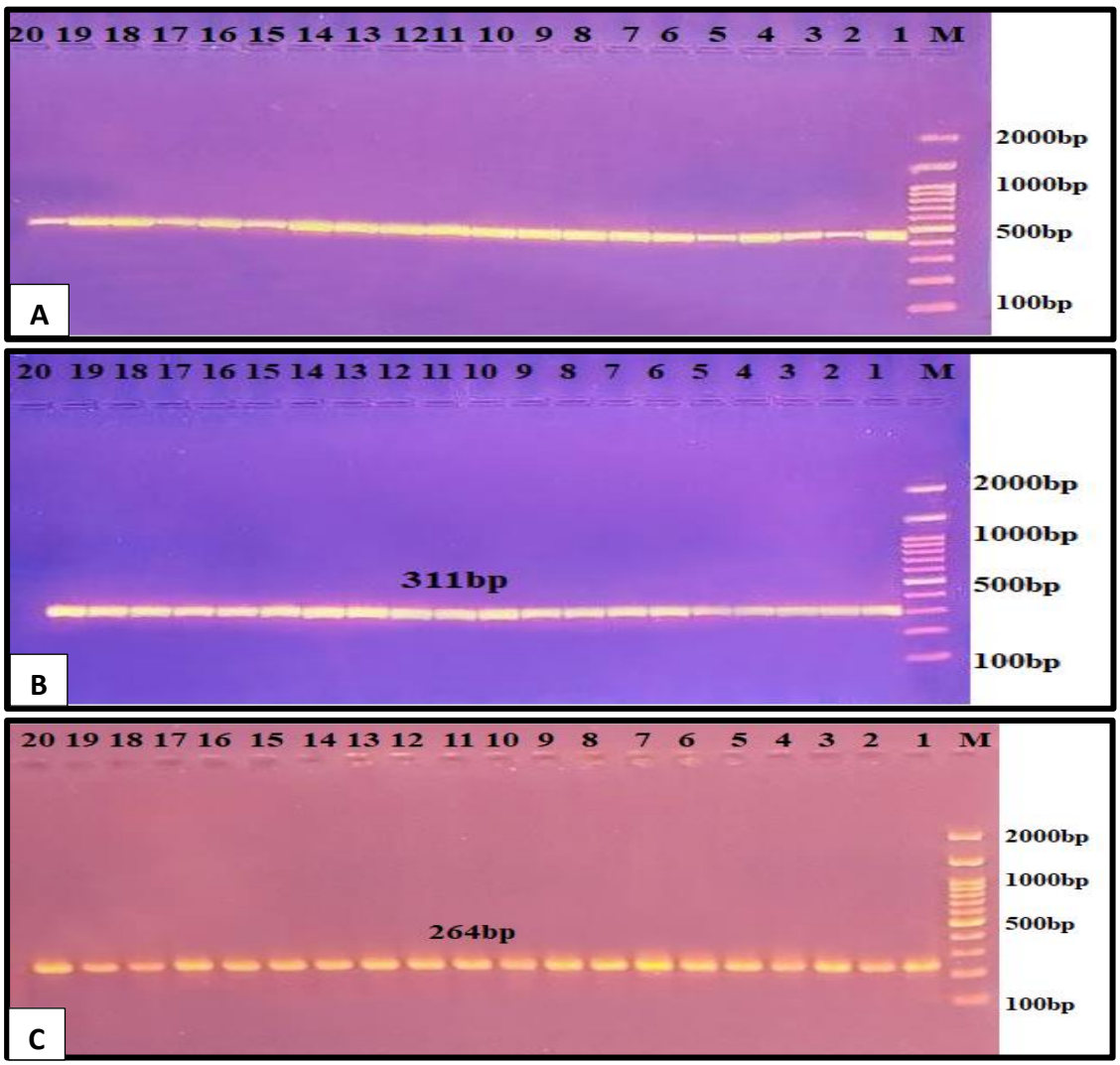

Fig. 2. Gel electrophoresis (1.5%) of amplified products of (A): Prodigiosin gene (468bp). (B): Protease gene (311bp). And (C): Adhesion gene (264bp) Size Marker 1000bp.

4.Conclusions

Virulence factors commonly prevalent in *Serratia marcescens* isolated from different clinical samples in this study, Thus *Serratia marcescens* has become a concern in hospitals.

Ethics statement

The study was approved by the scholarliness committee of the Faculty of Education/ Al-Qadisiyah University.

Acknowledgements

The authors thank the staff at the hospitals Al-Qadisiyah province, Iraq.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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