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MOLECULAR DETECTION OF *SERRATIA MARCESCENS* ISOLATED FROM DIFFERENT CLINICAL CASES IN WASIT PROVINCE, IRAQ

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Abstract

Background: *Although previously considered non-pathogenic (harmless saprophyte) Serratia marcescens is now recognized as an important opportunistic pathogen combining a propensity for healthcare-associated infection and antimicrobial resistance. The current study was aimed to detect and identify Serratia marcescens isolated from different clinical cases, using a variety of genotypic and phenotypic criteria*

Materials and Methods*: Totally, 207 different clinical samples collected from Al-Zahra Teaching Hospital, Wasit province, Iraq were included in this study. They were different specimens involving urine (n = 122), skin wound infections (n =59), and, respiratory system infection (n= 26). All isolates wereidentified by traditional culture methods and biochemical tests and analysis profile Vitek® 2 compact system. Then, DNA extractions of all positive 18 isolates were carried out for confirmed with PCR by the amplification of 16S rRNA gene.*

Results: *The culture results were shown an overall 8.69% (18) positive isolates. (15 samples from patients with urinary tract infection,1 sample from patients with respiratory system infection, and 2 samples from patients with wound infection). The highest isolation rate from urine, and the incidence ofSerratia marcescens was higher among females than males.*

Conclusions: *Serratia marcescens played an important role in urinary tract infections. Further studies are needed to determine the prevalence of pathogenic bacteria from the hospital environment and to detect virulence genes in these isolates.*

Keywords: *Serratia marcescens, Urinary tract infections,* DNA extraction ,16S rRNA gene

INTRODUCTION

Serratia marcescens is a rod-shaped Gram-negative bacterium that belongs to the family Enterobacteriaceae (Allen et al., 2022). It is a facultative anaerobic bacterium that can grow in the presence and absence of oxygen at temperatures 30-37°C (Ferreira et al., 2018; Kljakić et al., 2021). *Serratia marcescens* become an opportunist pathogen causing nosocomial infections and is commonly involved inhospital-acquired infections (HAIs); especially urinary tract infections (UTIs), pneumonia, septicemia, meningitis ,and wound infections (Prado et al., 2021). The bacterium *Serratia marcescens* can develop efflux pumps to survive and defend itself. These pumps work to eliminate harmful substances from the bacterial cell, including antibiotics, chemical toxins, dyes, and even small foreign objects like antibiotics used in medical treatment (Poole et al., 2007). Additionally , *S. marcescens* have virulence factors like enzymes such as phospholipases, lipases, nucleases, DNAs, chitinases, and hemolysin, 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 (Abbas and Hegazy, 2020; Barman et al., 2020). Thus, the present study was aim to isolation and identification of *S. marcescens* obtained from different clinical cases to evaluate *S. marcescens* infection in Al-Zahra Teaching Hospital, Wasit province, Iraq.

MATERIALS AND METHODS

Study design and Sample collection

This study was a cross-sectional study carried out in Al-Zahraa hospital in Al- Kut city, Wasit Province, Iraq. A total 207 different clinical samples were collected between November (2022) and February (2023). The clinical different specimens from urinary tract infection patients (n = 122), skin wound infections of suspected patients (n =59), and from patients with respiratory system infection (n = 26). Data on gender age, also were taken. All samples were collected by using sterile swabs ,placed in sterile tubes contain transport medium and placed in cool box for transporting to the laboratory.

Bacterial isolates:

All different clinical cases samples were placed inside 5 ml of nutrient broth and incubated at 37°C overnight to promote growth of bacteria and then subculture were done on nutrient agar, blood agar and Mac Conkey agar (Oxoid), after that incubated aerobically 24-48 h at 37°C. All media were prepared and sterilized according to the company. All growing bacterial colonies were identified The growing bacterial colonies were differentiated according to conventional methods: character of pure bacterial colony and morphology, staining, and pigment because of *S. marcescens* had capability of to produce red non-soluble pigment that is called Prodigiosin. Conventional biochemical tests for the bacterial colonies' isolates were also done to confirm the genus and species of isolated bacteria (Quinn et al 2011), such as: TSI (Triple Sugar Iron), DNase agar, urease, and IMViC test involved (indol, methyl red, Voges proskauer, citrate utilization.

Molecular diagnosis by PCR assay:

Genomic DNA was isolated from pure bacterial grow according to the protocol of Promega genome DNA extraction kit (Wizared® Genomic DNA Extraction Kit, Promega, USA). The purity of bacterial DNA was evaluated by OD260/280. Specific primers were use for molecular identification of the 16S rRNA gene for *S. marcescens* (Table 1).

The PCR-Master mix for all reactions were prepared at a total volume of

25µl, which contain (1 µl) of each primer (12.5 µl) of GoTaq Thermomaster mix, completed volume to (25 µl) with (5.5 µl) Nuclease free water. The tubes of the master mix were mixed well using the vortex and PCR was conducted using thermal cycler with the following conditions; An initial denaturation of (1 cycles) 5 min at 94°C was followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, followed by a final extension at 72°C for 5 min and hold step of 4°C. Five microliters of PCR products (DNA samples) were detected by electrophoresis in 1.5 % agarose gels for 45min at 95 V. and 100 bp DNA ladder as a standard molecular weight marker, after that the gels were stained with ethidium bromide stain . Finally, visualize the DNA using U.V. light for detection of expected DNA bands (Sambrook and Russell, 2001).

Table 1. Primer used for molecular diagnosis of *S. marcescens* in different clinical cases samples

Genes	Primer Sequences (5′-3′)		Product Size (bp)	Reference
16S rRNA	**F**	AGAGTTTGATCCTGGCTCAG	1500 bp	(Embaby et.al.,2014)
	R	GGTTACCTTGTTACGACTT		

DNA Sequencing

After determining the *S. marcescens* DNA amplification by conventional PCR and no control strains were available for the investigated genes, PCR product were sent to Sanger sequencing utilizing ABI3730XL, automated DNA sequences (Macrogen Corporation – Korea) to confirm the DNA sequencing in genes.

Statistical analysis

Data were entered and analyzed using the software program Statistical Package for Social Sciences (SPSS). All numerical variables were represented by mean and median as measures of central tendency. While categorical variables were presented by frequencies and percentages. Considering a*P-value* equal to or less than 0.05 a significant (Gharban, 2022).

RESULTS:

Socio-demographic characteristics of this study:

In this study, was conducted on 207 different clinical specimens were included which 44.0% (n=91) and 56.0% (n=116) of patients' samples were male and female, respectively. The age of the patients ranged from 15 to 90 years. The lowest prevalence was among those aged 76-90 (1.4%), while the greatest incidence was among those aged 90 and higher. The 15-30 age group 40.1% whereas the clinical specimens included urinary tract infection 122 (58.9%), skin wound infections of suspected patients 59 (28.5%) raspiratory system infection patients 26 (12.6%), (Table 2).

Table (2): Demographic characteristics of participants' patients in this study

Gender of total patients	**No. of Patients (%) (n=160)**
Male	(91) 44.0%
Female	(116) 56.0%
Mean ±Standard deviation	**39.24±17.40**
Age Groups (yrs.)	
15-30	(83) 40.1%
31-45	(52) 25.1%
46-60	(44) 21.3%
61-75	(25) 12.1%
76-90	(3) 1.4%
Type of clinical specimens	
urinary tract infection	(122) 58.9%
skin wound infections	(59) 28.5%
respiratory system infection	(26) 12.6%

Serratia marcescens isolates and the type of infection

The results showed that *S. marcescens* was present in 8.69% (18/207) of samples. Among these isolates, 15(83.3%) were obtained from UTIs patient (included: urine samples and urine catheterization), and two swabs 2(11.1%); showed positive bacterial growth culture from various skin infection sites (included wound infections), then only 1 (5.6%) from the respiratory tract infection patients (included: sputum and mouth and nose swabs), (Table 3).

Table 3: Percentage of *S. marcescens* isolates according to the type of infection

Type of infection	***Serratia marcescens* No. (%)**		**Total (n=207)**	**P-value**
	Yes	**No**		
Urinary tract infection	15(83.3%)	107(56.6%)	122(58.9%)	0.088
Skin infection	2(11.1%)	57(30.2%)	59(28.5%)	
Respiratory tract infection	1(5.6%)	25(13.2%)	26(12.6%)	

Phenotypic Characterization of *S. marcescens* isolates

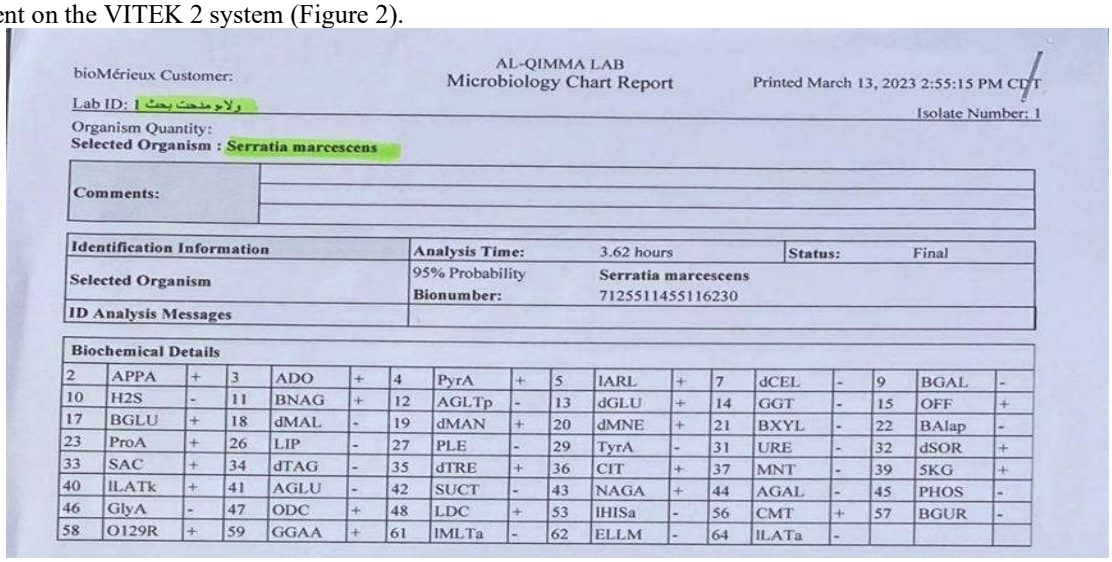
The findings showed according to culture methods and biochemical tests, the bacterial colonies may be culture on MacConkey agar, blood agar and also on Nutrient agar plates under the aerobic conditions. On MacConkey agar, *S. marcescens* colonies were appear small, pale and pink smooth round colonies (non- lactose fermenter). However, in blood agar, the colonies of S. marcescens were appeared large, round, red colonies and showed hemolytic activity were appear as clear and translucent zones on human andsheep blood agar plates in addition to this zone could be larger when bacteria grown at 30°C than at 37°C, and the colonies on Nutrient agar showed red pigmentation at 25°C

Prodigiosin is considered one of the secondary metabolites produced by

S. marcescens isolates (Stella, 2019). Finally, the suspected *S. marcescens* colonies look like as typical species when they were subculture on Chrom agar or when incubated for 24 hours at 37°C, *S. marcescens* shown green colonies on

Chrom agar (Pérez-Viso et al., 2021). The direct microscopic investigation of pure bacterial colonies was stained and carried out for studying size ,shape of bacteria also, Gram staining. according to biochemical tests, *S. marcescens* isolates indicated negative results for indole and oxidase but given positive results for motility test, catalase, Vogesproskier, Simmon citrate. While giving variable results for urease test and methyl red, *S. marcescens* were grown on TSI agar and given alkaline - acid (pink yellow) on the slant but the bottom was not appearing gas bubbles and H_2S production was negative (Abdullah et al, 2017).

These phenotypic characterization results of *S. marcescens* isolates were confirmed by identification of isolates was dependent on the VITEK 2 system (Figure 2).



bioMérieux Customer: AL-QIMMA LAB Microbiology Chart Report Printed March 13, 2023 2:55:15 PM CDT

Lab ID: 1 ولاد مدحت بحث Isolate Number: 1

Organism Quantity:
Selected Organism : Serratia marcescens

Comments:	

Identification Information	Analysis Time:	3.62 hours	Status:	Final
Selected Organism	95% Probability	Serratia marcescens		
	Bionumber:	7125511455116230		
ID Analysis Messages				

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 system Biomerieux for *S. marcescens* isolates .

Molecular identification

All *S. marcescens*isolates were DNA extracted and purified with a genomic DNA purification kit. Identification of *S. marcescens* isolates were confirmed by PCR technique and using 16S RNA gene amplified product size of about 1500bp as shown in Fig 2. To confirm the validity of the results in this study, forward primers were sent to Macrogen Company in South Korea for DNA sequences. The obtained sequences of *S. marcescens* isolates' samples were aligened using NCBI software.

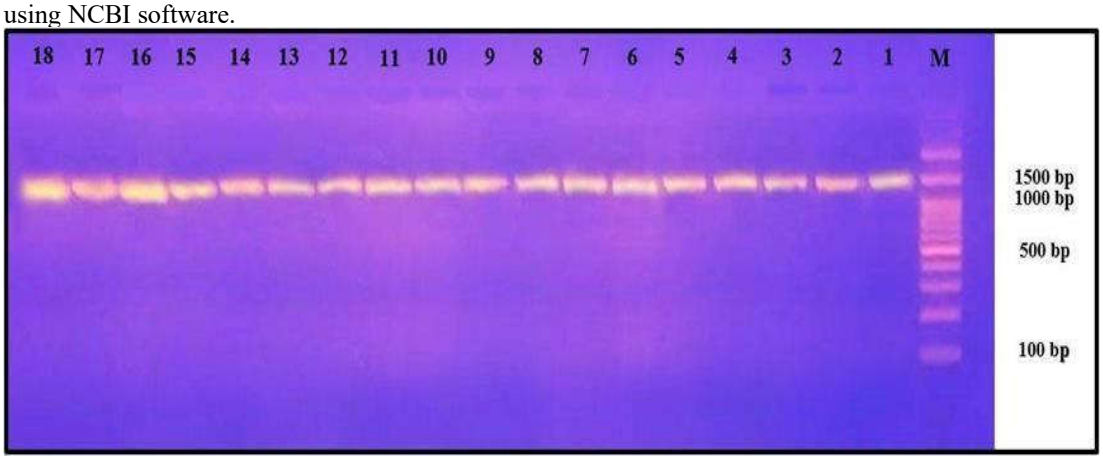


FIGURE 2: Agarose gel electrophoresis for PCR products targeting 16S rRNA gene in *S. marcescens* at size 1500bp in 1.5% agarose gel for 90min. M: Marker ladder (100 -1500 bp).

Discussion

Serratia marcescens was considering of the more important an opportunistic nosocomial bacterial pathogen and It was playing essential role and responsible for several types of illnesses in worldwide such as meningitis, wound infections, lung infections and urinary tract infections (UTIs), (Abbas and Hegazy, 2020).

S. marcescens was the fifth most commonly recovered organism of the *Enterobacteriaceae* family, representing 4% of all *Enterobacteriaceae* clinical isolates (Empel, *et al*., 2008). In other hand, itwas the eighth most commonly reported Gram-negative rod from Pierce County hospitals, USA (Mahlen, 2011). Recent methods to identify *S. marcescens* from

specimens needs a multistep procedure for traditional culture, for bacterial determination, and then genotyping methodologies via detection it by used conventional PCR assay for 16SrRNA gene of *S. marcescens* (Sutherland et al., 2010). Our work was identification *S. marcescens* to be isolate from in a large-hospital in Al- Kut city (Al-Zahraa hospital, Wasit Province, Iraq). The determined these pathogenic bacteria were mainly depend on phenotyping and genotyping methods. In this investigation, a total number of 207 samples were bacteriological examination for isolating *S. marcescens*. A number of positive *Serratia* isolates among the examined samples were 18 positive isolates (9.3%). This result corresponded to many studies that focused on *Serratia* spp. among clinical samples.

The percentage of infection with *Serratia* spp. in the present study was lower than that reported by some researchers in Iraq, Ali (2007) who reported that 25 *Serratia* spp. isolates (16.66%) were obtained from 150 clinical specimens 12 *S. marcescens* was collected from patients with nosocomial infections in Mosul City, Iraq, but agreement with another recent local study by Ali (2012), 200 blood sample collected from neonates with clinical sepsis from pediatric hospital and Baghdad hospital neonatal intensive care unit, 11(12.24%) cases of *Serratia* spp. bacteremia were obtained.
Identification of *Serratia* to the species levels by the conventional identification tests remains difficult in the clinical microbiology laboratory (Tariq and Prabakaran, 2011). Therefore, in this study the Vitek 2 system and PCR assay were used to confirm the identification and to avoid the variability in findings of biochemical tests. The results indicate that *16S rRNA* gene are highly specific (100%) and suitable for development of PCR assays for identification and detection of *Serratia* isolates. Urine samples were collected from 122 patients with UTI during a study period. However, among 18 *Serratia* spp. isolates, 15 (83.3%) isolates were recovered from patients with urinary tract infections (UTI) patients in this study. This finding is similar to that of Ali (2007)who obtained 12 *Serratia* spp. isolates from 50 urine samples of patients with UTI in Mosul, Iraq, also it was not agreement (Shaimaa *et.al*. 2018) shown (3 isolates (10.7%)) of *Sarretia spp.* from UTI samples from catheterized patients from four Iraqi hospitals .

As well, this result was disagreement with Ahmed Abbas Hamza (2023) were obtain (12.5%) from UTIs from hospitals Al-Qadisiyah province, Iraq Detection of *Serratia* spp. as the pathogen of community and hospital associated UTI has been extensively reported in many studies (Yang *et al*., 2012).

In the present study, the incidence of Ur pathogenic*Serratia* spp. was found higher in females 9 (4.66%) as compared to males 5 (2.50%), and the frequency of *Serratia* spp. was also found higher among old-aged patients (15-30) (40.1%) than other ages. The incidence increases with advancing ages making them a high-risk group, in which case, 92% of the isolates were *S. marcescens* (Laupland *et al.,* 2008).

Eventually, the detection of *Serratia spp.* in wound samples 2 (11.1%) isolates could be due to the ability ofthis pathogen to colonize sterile wounds. However, the damaged infected tissue may cause suppression of the local immunity that increases the chance of infection. Al-Daoodi (2002) isolated *S. marcescens* from wound infections of patients who were subjected to invasive instrumentation in the surgery units in Mosul, Iraq.

The results indicate that only 1 isolate were sputum of the infected patient. However, the possible virulence factors found in *Serratia* isolates are the formation of fimbriae, potent siderophores, presence of cell wall antigens, ability to resist the bactericidal action of serum, production of proteases, the ability of *Serratia* cells to attach human buccal epithelial cells and human urinary bladder surfaces (Mahlen, 2011; Yang *et al*., 2012).

CONCLUSION

In the current study, it was found that *S. marcescens* is a source of nosocomial infections in hospitals with the highest isolation rate from urine, and the incidence was higher among females than males. Further studies are needed to determine the prevalence of pathogenic bacteria from the hospital environment and to detect virulence genes in these isolates.

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