Nat. Volatiles & Essent. Oils, 2021; 8(4): 12647-12657

NVEO
Natural Volatiles &
Essential Oils

Molecular And Biochemical Study Of Serratia Marcescens Isolated From Sheep In Baghdad Province

***Ro'aa N. Ahmed and * Ikram A. A. Al-Samarraae**

*Microbiology department, Veterinary Medicine College Baghdad University, Baghdad-Iraq.

Abstract

Themolecular and biochemical study for Serratia marcescensisolated from infected sheep in Baghdad province. One hundred and fifty samples from feces of sheep collected from University of Baghdad/College of Veterinary Medicine,Agricultural Research office/ Ruminant Station department, Abu-Ghraib zone, Al-Shu'ala zone, Al-Mahmodia zone, Al-Askan zone in Baghdad province, cultured by pre-enrichment, enrichment, selective and differential media then Gram stain were done;After thatcolonies were cultivated on nutrient agar and incubated at 37°C for 24-48 hours, then examined for form using the naked eye; after purification of cultured bacteria, biochemical tests were done including: Ureas, Oxidase and Catalase, Simmon citrate, and confirmed by Vitek2system and PCR assay16S ribosomal RNA gene (16S rRNA).

Results showed that 7 (4.66%) samples out of 150 samples were positive for Serratia marcescens and it's the first time to isolate from fecal samples of sheep in veterinary medicine field.

Keywords: serratia marcescens, Vitek2, 16S rRNA

Introduction:

Serratia marcescens is an environmental rod-shaped gram-negative bacterium that belongs to the Enterobacteriaceae family (1,2).It's a rod-shaped Gram-negative, facultative anaerobic bacterium (3). They can be found in water, soil, plants, and animals and are normally 1–5 µm long;they do not form spores and can be found in water, soil, plants, and animals. S. marcescens is an opportunistic pathogen of many animals, including humans, and is distinguished from

other members of the order Enterobacterales by their unique production of three enzymes: DNase (nucA), lipase, and gelatinase (serralysin) (1,4, 5).

Serratia are considered to be ubiquitous in the environment and the organism is found in water, soil, plants, insects, animals, mammals including humans and food, particularly those rich in starch, Most Serratia isolates are motile with petrichous flagella (6).There are now 19 Serratia species in existence:S. aquatilis, S. entomophila, S. ficaria, S. fonticola, S. grimesii, S. liquefaciens, S. marcescens, S. microhaemolytica, S. myotis, S. nematodiphila, S. odoriferae, S. oryzae, S. plymuthica, S. proteamaculans, S. quinivorans corrig, S. rubidaea, S. symbiotica, S. ureilytica, S. vespertilionis (7).

Serratia is a virulent organism (8), when it enters the bloodstream, endotoxins are released that cause fever, septic shock, thrombocytopenia and disseminated intravascular coagulation; symptoms depend on the infection site and disease (8).It has been now recognised as a frequent cause of nosocomial extraintestinal infections (9, 10).Due to the importance of Serratia spp. in the domestic animals as a cause of diarrhea and there are less knowledgement of the molecular and biological features of this bacteria, this research was conducted for Isolation and identification of Serratia spp. in sheep of Baghdad Province and detection of Serratia spp. by Vitek2, and PCR 16S ribosomal RNA gene (16S rRNA).

1- Materials and Methods:

a- Bacterial isolation:

One hundred fifty (150) of sheep fecal samples were collected from different regions of Baghdad Province.

b- Bacterial identification:

One gram of each fecal sample was placed in a sterile test tube containing 10 ml of normal saline, mixed well, and left for 1 minute, and then 0.1 ml of the sample suspension was inoculated on the brain heart broth, incubated at 37°C for 24 hours, colonies were evaluated by naked eye for shape after being grown on nutrient agar and incubated at 37°C for 24-48 hours., size and color; After that, they were examined microscopically by Gram-stain and biochemical tests were done including: Ureas, Oxidase and Catalase (11).

c- Vitek2 compact system:

The positive isolates from traditional morphological and biochemical tests were selected to be identified by the Vitek2 system were used for diagnosing the bacterial isolates according to the manufacturer's instructions.

d- Molecular detection of Serratia marcescens:

- **DNA extraction:**

Genomic DNA of Serratia marcescens isolates was extracted by using ZR Fungal/Yeast/Bacterial DNA MiniPrep kit (ZYMO/ USA) according to the manufacturer's instructions:

- **PCR Product Analysis (agarose gel electrophoresis):**

  Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel (12).

- **Determination of DNA Concentration**:

  Table (1): DNA concentration

	Blank	Standard	Sample
Mix	200 µl	200 µl	200 µl
DNA Extraction		2 µl	2 µl

- **The primers:**

  Investigated by IDT (Integrated DNA Technologies Company, Canada).

  Table (2): The primers for detection of Serratia marcescens

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'- AGAGTTTGATCCTGGCTCAG- 3'	54.3	50.0	1250 base pair
Reverse	5'- GGTTACCTTGTTACGACTT- 3'	49.4	42.1	

- **Diagnosis of Gene**

Table (3): Mixture of the specific interaction for diagnosis gene.

Components	Concentration
Taq PCR PreMix	5µl
Forward primer	10 picomols/µl (1 µl)
Reverse primer	10 picomols/µl (1 µl)
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table (4): The optimum condition of detection:

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	94°C	3 min.	1 cycle
2-	Denaturation -2	94°C	45sec	35 cycle
3-	Annealing	56°C	45sec	
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	7 min.	1 cycle

Results and Discussion:

A total One hundred and fifty (150) fecal samples of infected sheep from different regions of Baghdad province were taken for detection of Serratia marcescens; there were (7) samples were positive.

The samples cultured on enrichment, selective and differential media and produce red pigment on nutrient agar represent prodigiosin pigment that produced by Serratia spp.

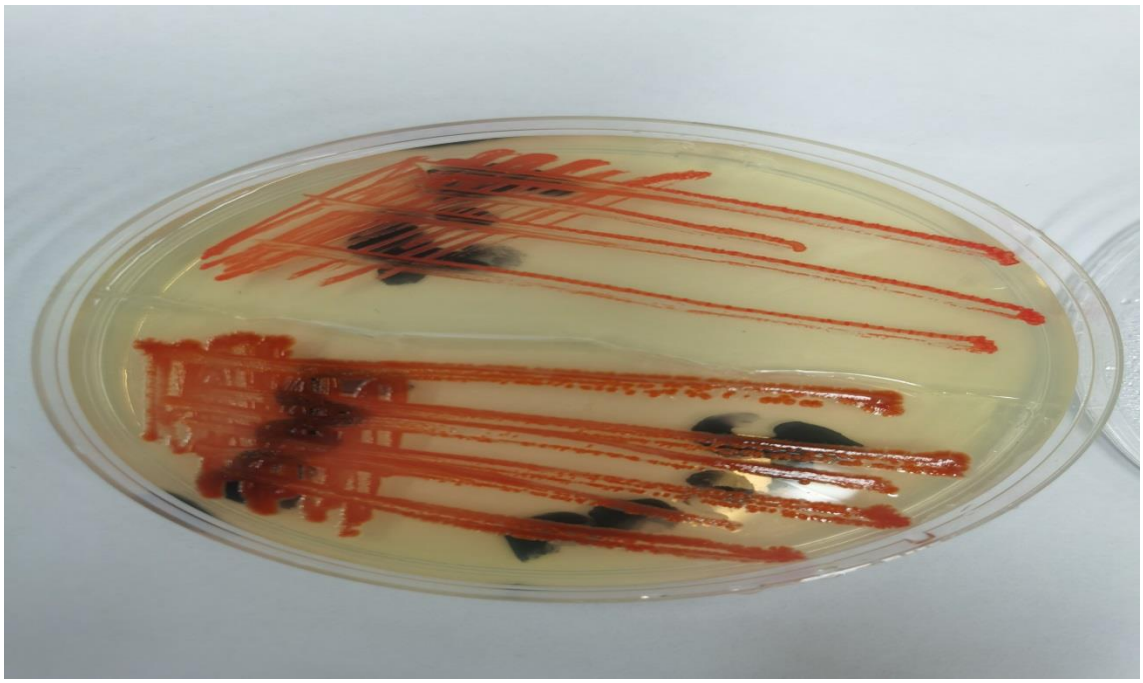

Figure (1): Serratia marcescens on nutrient agar produced red pigment

This result is in agreement according to Darshan & Manonmani (13); they approved that Serratia marcescens produce Prodigiosin pigment; were subjected to biochemical tests such as Urease, Oxidase and Catalase.

The result of Vitek2 compact system showed that the isolatedbacteria in this research was Serratia marcescens (99%) as shown in table (5).

Table (5): Vitek 2 compact system test for Serratia marcescens isolates.

Identification Information				**Analysis Time: 3.65 hours**							
Selected Organism				99% Probability **Serratia marcescens**							
Analysis Messages				Bionumber: 6125511455056230 Confidence: Excellent identification							
Biochemical Details											
APPA	-	ADO	+	PyrA	+	lARL	+	dCEL	-	BGAL	-
H2S	-	BNAG	+	AGLTp	-	dGLU	+	GGT	-	OFF	+

BGLU	+	dMAL	-	dMAN	+	dMNE	+	BXYL	-	BAlap	-
ProA	+	LIP	-	PLE	-	TyrA	-	URE	-	dSOR	+
SAC	+	dTAG	-	dTRE	+	CIT	+	MNT	-	5KG	+
lLATk	-	AGLU	-	SUCT	-	NAGA	+	AGAL	-	PHOS	+
GlyA	-	ODC	+	LDC	+	lHISa	-	CMT	+	BGUR	-
O129R	+	GGAA	+	lMLTa	-	ELLM	-	lLATa	-		

The results are in agreement with Rafii(1999) (14) who proved the reliability of this system for the identification of Serratia marcescens, result showed that theisolated bacterium in this research was Serratia and the speciesmarcescens.

Confirm identification of Serratiaisolate by PCR analysis, the isolate was tested to present 16S rRNA. Hence, the isolate was positive for 16SrRNA gene amplification ˜1250 pb as showed in figure (2):

Figure (2) PCR product the band size 1250 bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. N: DNA ladder (1000 plus).

The concentration and purity of DNA was (6.1 ng/ml and 1.780 ng/ml) respectively as shown in table (6):

No.	Isolate	Nucleic acid conc. (ng/ml)	260/280 purity
1	SMR (1) / N.A	6.1	1.780

Table (7): PCR result by 16S rRNA gene:

X	DNA Extraction	PCR Result16srRNA 1250 bp
Serratia marcescens	+	+

Sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov). The expectation value is defined to give an estimate of the number of times expected to get the same similarity coincidental and the lower the value of E. This indicates that the degree of similarity was high between sequences which give greater confidence. The value of a very close to zero means that these sequences are identical and the bit Score: statistical measure of the moral similarity and the higher value indicates that the high degree of similarity, and if dropped from the class of 50 points, the sense that there is no similarity mention as shown in appendix.

The application of PCR-based assays for detection and identification of Serratia marcescens has been increased due to their accuracy, sensitivity, speed and ability to work with DNA rather than the highly infectious live cultures (15). This finding can be ascribed to the fact that there are new causes of gastrointestinal infections in animals other than the traditional infections known, and this is attributed to environmental pollution and microbial resistance to antibiotics in addition to the mutations that result from it (16).

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Appendices:

bioMérieux Customer:
System #:
Patient Name:
Isolate: 1-1 (Qualified)
Card Type: GN Bar Code: 2411441403306230 Testing Instrument: 0000144FB5E1 (Vitek Compact)
Setup Technologist: Laboratory Administrator(Labadmin)

Laboratory Report

Printed by: Labadmin
Patient ID:

Bionumber: 6125511455056230
Organism Quantity:

Selected Organism: Serratia marcescens

Comments:	

Identification Information	**Card:**	GN	**Lot Number:**	2411441403	**Expires:**	Nov 6, 2021 13:00 CDT
	Status:	Final	**Analysis Time:**	3.65 hours	**Completed:**	Mar 29, 2021 04:53 CDT
Organism Origin	VITEK 2					
Selected Organism	99% Probability		**Serratia marcescens**			
	Bionumber: 6125511455056230			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	-			

Installed VITEK 2 Systems Version: 9.02
MIC Interpretation Guideline:
AES Parameter Set Name:

Therapeutic Interpretation Guideline:
AES Parameter Last Modified:

Page 1 of 2

Gene: 16S ribosomal RNA gene						
No.	Type of substitution	Location	Nucleotide	Sequence ID with compare	Source	Identities
1	Transition	217	G\A	ID: MN691630.1	Serratia marcescens	99%
	Transvertion	357	G\C			

1

Serratia marcescens strain NPK2_1_20 16S ribosomal RNA gene, partial sequence

Sequence ID: MN691630.1Length: 1139Number of Matches: 1

Range 1: 9 to 419GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
733 bits(812)	0.0	409/411(99%)	0/411(0%)	Plus/Plus

Query 1 GCAGCTACACATGCAGTCGAGCGGTAGCACAGGGGAGCTTGCTCCCTGGGTGACGAGCGG 60
Sbjct 9 68

Query 61 CGGACGGGTGAGTAATGTCTGGGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGG 120
Sbjct 69 128

Query 121 TAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATC 180
Sbjct 129 188

Query 181 AGATGTGCCCAGATGGGATTAGCTAGTAAGTGGGGTAATGGCTCACCTAGGCGACGATCC 240
Sbjct 189 .....G..... 248

Query 241 CTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC 300
Sbjct 249 308

Query 301 GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCACCCATGCCGCGT 360
Sbjct 309 .....G..... 368

Query 361 GTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTCAGCGAGGAGGAAGGTG 411
Sbjct 369 419