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Article

Serratia marcescens isolated from newborn meningitis in the Iraqi city of Diwaniyah: Molecular characterization

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Abstract
Our study was conducted to detect virulence genes in Serratia marcescens. It has many virulence genes that cause nosocomal infections in immunocompromised persons and neonates. A total of 24/100 (24%) S. marcescens were obtained from neonates suffering from meningitis, and they were identified using culture characteristics biochemical- tests and confirmed by Polymerase chain reaction (PCR) technique, using the 16S rRNA gene. All virulence factors, including the fimA gene that encodes type-1 fimbria, the bsmB gene that encodes exo polysaccharide production, and ampC that encodes ß-lactamase enzymes, were done using the PCR technique. The results revealed that S. marcescens isolates have 16S rRNA gene at the percentage (100%), fimA gene at the percentage (54%), bsmB gene at the percentage (71%) and ampC gene at the percentage (100%). Finally, the DNA sequencing of (fimA, bsmB, and ampC genes) was done using a DNA sequencer technique to determine the sequence of nucleotides. The results revealed the similarities of the genes in local isolates of S. marcescens (98%) with S. marcescens isolates globally registered on the NCBI-Genbank website.

Keywords: fimA gene, bsmB gene, ampC gene, Serratia marcescens, DNA sequences.

Introduction

Meningitis is a major cause of death in neonates and newborn children. It is an infection of the membrane surrounding the spinal cord and brain (the meninges). Viruses, bacteria and fungi can cause meningitis[1]. One of the most common types of bacteria that cause meningitis is S. marcescens, which belongs to the family of Enterobacteriaceae[2]. It is a Gram-negative rod, non-spore-forming, and produces a red pigment on nutrient agar. It is an opportunistic pathogen of human causes (nosocomial infections) and, with outbreaks in immuno-compromised patients and neonates, is multi-resistant antibiotics[3,4]. The colonies of S. marcescens on Macconkey agar appear as dark red colonies, chrom agar as turquoise to metallic blue and on the blood, agar give β-hemolysis[5]. The pathogenicity of S. marcescens belongs to their ability to form biofilm and extracellular polysaccharides, which also produce hemolysin, lipase, nuclease, proteases, chitinase, peroxidase and DNAse [6].

S. marcescens have virulence genes, including the fimA gene that encodes the type I fimbria, the bsmB gene that encodes exopolysaccharide production, the ampC

gene that encodes ß-lactamase enzymes which destroy penicillin antibiotics[7,8,9,10,11].

The aim of our study was the molecular detection of fimA, bsmB, and ampC genes in S. marcescens isolated from neonatal meningitis using PCR technique and DNA sequencing.

Materials and Methods

100 cerebrospinal fluid (CSF) specimens were collected from September 2021 to February 2022 from neonates with meningitis. All specimens were transferred to the Microbiology Lab in the Biology Department.

Isolation of S. marcescens

Isolation of S. marcescens was done by streaking of blood Agar chrom agar and McConkey agar with CSF specimens. The colonies of S. marcescens were observed after incubation at 37 °C for 24 hours as turquoise to metallic blue on chrom agar, McConkey agar as dark-red colonies, and β-hemolysis on blood agar. The results were confirmed using the API-20E system, PCR technique and DNA sequencing 10,12.

Polymerase, chain, reaction

PCR. was done to confirm a diagnosis of S. marcescens using the 16S rRNA gene and some virulence genes (fimA, bsmB and ampC genes). Specific. Primer. as in. table [1]. DNA was extracted from S. marcescens using a Genomic DNA Mini Kit. Nano drop spectrophotometer was used to measure the concentration of DNA. PCR, master. mix. were. applied in 25µl. (total volume) according, to kit. instructions. (AccuPower®. PCR. PreMix. Kit. Bioneer. Korea) by. adding 12.5 µl of PCR- master mix, 5µl. of extracted, DNA ,2.5µl of forward. primer, (F) and 2.5µl of reverse. primer(R) into PCR-premix tube, then complete. then to .25µl. with.deionizer PCR water. The reaction was performed. In a thermo-cycler as in table [2]. The PCR. products. Were obtained by electrophoresis using (2%) agarose gel and UV light 12,13.

primers	Amplicon	Sequence (3'-5')	
16srRNA	522bp	F	CCTGGACAAAGACTGACGCT
		R	CGCTTCTCTTTGTATGCGCC
fimA	435bp	F	GAACAACAACCCGGCCATTC
		R	CTTTTGATAAGGCCGCCACG
bsmB	514bp	F	CCAAACAACAAGCGCAGGAA
		R	TTCCATGATGCCGCTCACAT
ampC	390bp	F	AAGTCCATCCGTTGACGCTT
		R	CAATTTACCGATGGCTGCCG

*F: Forword and R: Reverse

Table 1. DNA primers.

PCR step	Temperature (°C)	Time	Repeat cycle
Initial Denaturation	95°C	3min	1
Denaturation	95°C	30sec.	35 cycle
Annealing	59°C	30sec.	
Extension	72°C	1min	

Final extension	72°C	5min	1
Hold	4°C	Forever	-

Table 2. PCR Thermocycler of 16srRNA, fimA, bsmB and ampC genes.

DNA sequencer technique

The DNA sequencing of fimA, bsmB and ampC genes was performed according to a study of 14. The PCR products with Primer F Primer R were sent to Macrogen company (South Korea), where the AB DNA sequence system was used for DNA sequencing of genes. The results were read using BLAS at the NCBI website.

Results

Isolation and diagnosis

A total of 15//100 (15%) S. marcescens were collected from neonates with meningitis, which was—diagnosed using culture characteristics biochemical tests and confirmed by PCR – technique. The colonies of S. marcescens were observed after incubation at 37 °C for 24 hours as turquoise to metallic blue on chrom agar, dark-red colonies on Macconkey agar, and β-hemolysis on blood agar—. Biochemical assays were summarized. In. Table 3. PCR was done using the 16S rRNA gene and virulence genes (fimA, bsmB, ampC). The results revealed that isolates of S. marcescens have 16S rRNA gene at a percentage of 100%, as in Figure1, fimA gene at a percentage of 54%, as in Figure2, bsmB gene at percentage of 71%, as in Figure3, and ampC gene at percentage 100% as in Figure 4.

Biochemical tests	Results
Catalase test	+
Oxidase test	-
Motility test	+
Hemolysin	**β-hemolysis**
Indole	-
Methyl-red	-
Vogas-Proskaur	+
Citrate utilization	+
DNase test	+
Urease	+
Gelatin hydrolysis	+

Table 3. Biochemical- tests of. S. marcescens.

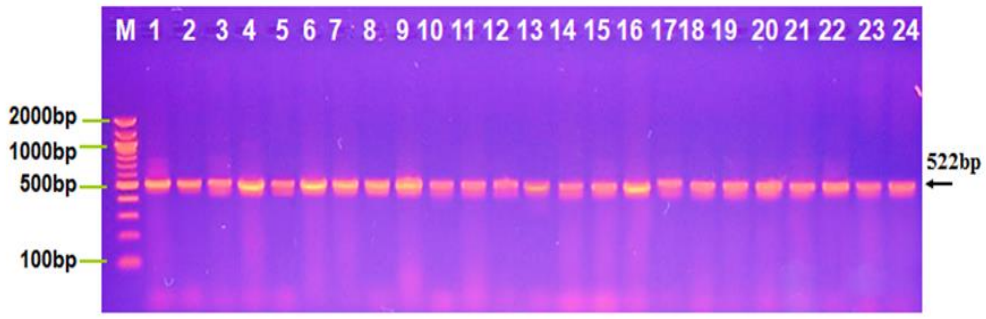

Figure 1. The results of agarose gel electrophoresis of 16S- rRNA gene. In S. marcescens at 522 bp (PCR product size), Lane (1-24) are positive results, M: Marker 100 -2000 bp.

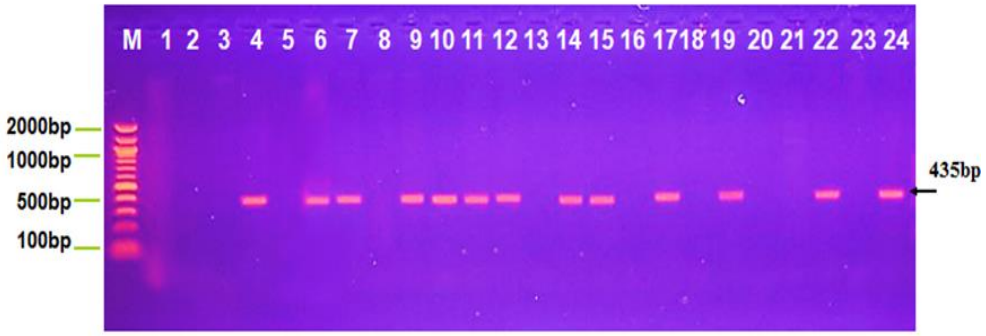

Figure 2. The results of agarose gel electrophoresis of the fimA- gene in S. marcescens at 435 bp (PCR product size), Lane (4-6-7-9-10-11-12-14-15-17-19-22-24) are positive results, Lane (1-2-3-5-8-13-16-18-20-21-23) are negative results M: Marker 100 -2000 bp.

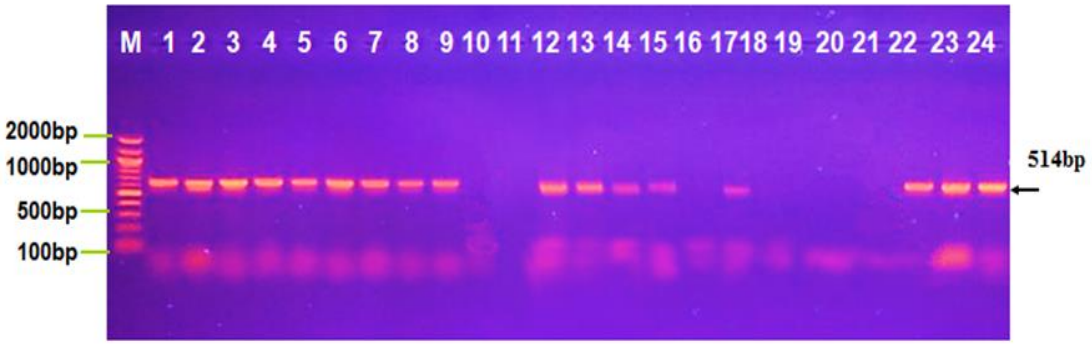

Figure 3. The results of agarose gel electrophoresis of bsmB- gene in S. marcescens at 514 bp (PCR product size), Lane (1-2-3-4-5-6-7-8-9-12-13-14-15-17-22-23-24) are positive results, Lane (10-11-16-18-19-20-21) are negative results M: Marker 100 -2000 bp.

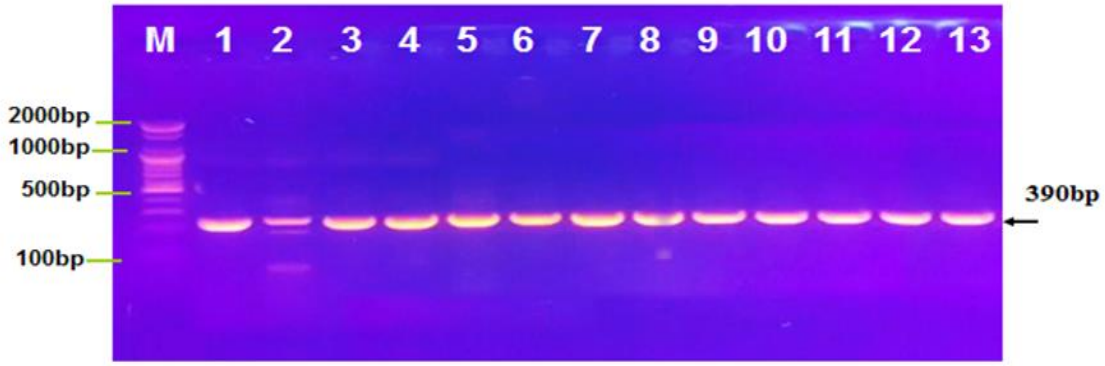

Figure 4. The results of agarose gel electrophoresis of ampC- gene in S. marcescens at 390 bp (PCR product size), Lane (1-13) are positive results, M: Marker 100 -2000 bp.

DNA sequencer technique was used to determine the sequence of nucleotides in fimA, bsmB, and ampC genes. The blast program was used to analyze the nucleotide sequence of (fimA, bsmB, ampC genes) and compare it with the wild-type sequences of standard isolates. Results revealed that fimA and bsmB gene sequences were 98% similar to the standard isolate sequence, while the ampC gene was 96% similar to the standard isolate sequence. In other words, substitution mutations occur in the nucleotide sequences of the genes in our study.

The substitution mutations at the fimA gene were five mutations as in Figure 5, including adenine replaced with thymine (A-T), adenine replaced with guanine (A-G), thymine replaced with cytosine(T-C), cytosine replaced with thymine (C-T) and adenine replace with guanine (A-G). The number of substitution mutations at the bsmB gene was five mutations as in Figure 6, including guanine replaced with adenine (G- A), adenine replaced with thymine (A-T), cytosine replaced with thymine(C-T), thymine replaced with cytosine(T-C) and cytosine replace with thymine(C-T). The substitution mutations at the ampC gene were four mutations, as in Figure 7, including cytosine replacement with thymine(C-T), adenine replaced with cytosine (A-C), guanine replaced with adenine (G-A), and adenine replace with guanine (A-G).

Finally, compared the results of the amino acid translation of fimA, bsmB, and ampC genes with the amino acid translation of standard isolate, the results revealed a change in protein translation; thus, histidine was converted to methionine and glutamine to asparagine.

Score	Expect	Identities	Gaps	Strand
650 bits(353)	0.0	395/402(98%)	1/402 (0%)	Plus/ Minus

```
Query  345  ACAGGCAAACAGTGGCGGGGTATCAGCCTGCTGCACTTAGCCACCTATACAGCGGGTGGC  404
Sbjct  1    ACAGGCAAACAGTGGCGGGGTATCAGCCTGCTGCACTTAGCCACCTTTACAGCGGGTGGC  60

Query  405  CTGCCGCTGCAGATCCCCGATAACGTTACGGATAAAGCCGCATTACTGCGCTTTTATCAA  464
Sbjct  61   CTGCCGCTGCAGATCCCCGATGACGTTACGGATAAAGCCGCATTACTGCGCTTTTATCAA  120

Query  465  AACTGGCAACCACAATGGACTCCGGGCGCTAAGCGTCTTTACGCTAACTCCAGCATTGGT  524
Sbjct  121  AACTGGCAACCACAATGGGCTCCGGGCGCTAAGCGTCTTTACGCTAACTCCAGCATTGGT  180

Query  525  CTGTTTGGTGCGCTGGCGGTGAAACCTTCAGGTATGAGCTACGAAGAGGCAATGACCAGA  584
Sbjct  181  CTGTTTGGTGCGCTGGCGGTGAAACCTTCAGGTATGAGCTACGAAGAGGCAATGACCAGA  240

Query  585  CGCGTCCTGCAACCATTAAAACTGGCGCATACCTGGATTACGGTTCCGCAAAGCGAACAA  644
Sbjct  241  CGCGTCCTGCAACCACTAAAACTGGCGCATACCTGGATTACGGTTCCGCAAAGCGAACAA  300

Query  645  GATGAGAAGATCGGTGAACTTGCAGGCATAACCCGTAATGCTGATCGCAGTCAGAGTGGT  704
Sbjct  301  GATGAGAAGATCGGTGAACTTGCAGGTATAACCCGTAATGCTGATCGCAGTCGGAGTGGT  360

Query  705  GACGCCGAAGCCTATGGCGTGAAATCCAGCGTTATCGATATG  747
Sbjct  361  GACGCCGAAGCCTATGGCGTGAAATCCAGCGTTATCGGTATG  402
```

Figure 5. Nucleotide- sequence. of fimA gene, in S. marcescens compared. To sequence of standard isolate.

Score	Expect	Identities	Gaps	Strand
650 bits(353)	0.0	360/365(98%)	0/365(0%)	Plus/ Plus

```
Query  5    GGAGTTAGTGCAGCCTCCAGTGCATCCCTCATAGGGGCCCCGATAAGCATGCTGGTGAGT  64
Sbjct  1    GGAGTTAGTGCAGCCTCCAGTGCATCCCTCATAAGGGCCCCGATAAGCATGCTGGTGAGT  60

Query  65   GCATTAACCGGTACGATATCTGGCATTCTGGAAGCATCAAAACAGGCTATGTTTGAGCAC  124
Sbjct  61   GCATTAACCGGTACGATATCTGGCATTCTGGAAGCATCAAAACTGGCTATGTTTGAGCAC  120

Query  125  GTTGCAGACAAATTCGCTGCTCGGATCAATGAATGGGAAAAGGAGCATGGCAAAAATTAT  184
Sbjct  121  GTTGCAGATAAATTCGCTGCTCGGATCAATGAATGGGAAAAGGAGCATGGCAAAAATTAT  180

Query  185  TTTGAGAATGGCTATGACGCAAGACATGCTGCGTTTTTAGAAGACTCTCTGTCTTTGCTT  244
Sbjct  181  TTTGAGAATGGCTATGACGCAAGACATGCTGCGTTTTTAGAAGACTCTCTGTCTTTGCTT  240

Query  245  GCTGATTTTTCTCGTCAGCATGCAGTAGAAAGAGCTGTCGCAATAACCCAGCAACATTGG  304
Sbjct  241  GCTGATTTTTCTCGTCAGCATGCAGTAGAAAGAGCCGTCGCAATAACCCAGCAACATTGG  300

Query  305  GATGAGAAGATCGGTGAACTTGCAGGCATAACCCGTAATGCTGATCGCAGTCAGAGTGGT  364
Sbjct  301  GATGAGAAGATCGGTGAACTTGCAGGTATAACCCGTAATGCTGATCGCAGTCAGAGTGGT  360

Query  365  AATAA  370
Sbjct  361  AATAA  365
```

Figure 6. Nucleotide- sequence of bsmB gene in S. marcescens compared to sequence of standard isolate.

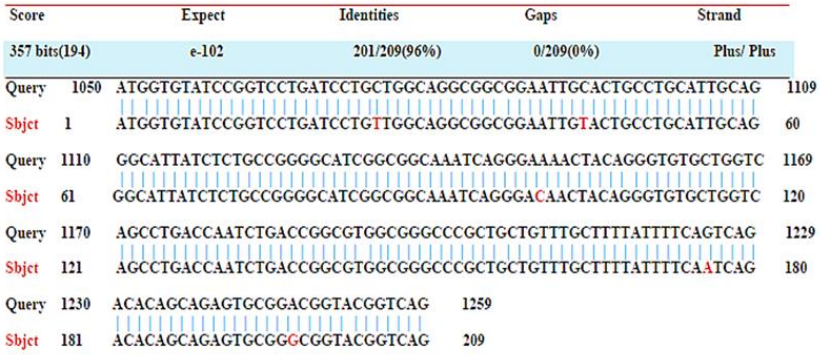

Figure 7. Nucleotide- sequence of ampC gene. In S. marcescens compared .to sequence of standard isolate.

Discussion

The high percentage of S. marcescens in patients with meningitis is due to immature defense mechanisms in patients, and the long period of patients in the hospital may lead to the transfer of S. marcescens from person to patient (nosocomial- infection). PCR and DNA sequencer techniques are most important in biological research, and they also enter medical diagnostics, virology and biotechnology [15]. Mutations in a genome or gene cause an alteration in the function and structure of the genes, thus changing gene expression. Sometimes, the replacement of amino acids in protein does not affect its function because the genetic code (codon) consists of three nitrogen bases, and the change may occur in one base [16]. The genetic variation between local and standard isolates may be due to point mutations or substitution mutations within the sequence of the nucleotides of the genes, causing alteration of a single nitrogen base in the DNA sequence. During replication, these changes were replicated, thus causing a permanent change in the genome [17,18,19]. In point mutations, if purines were replaced with purines, it is called transition mutation. If Purines were replaced with pyrimidines or vice versa, it is called transversion mutation [14]. The genetic analysis of local isolates showed that the highest ratio of point mutations converted the cytosine into thymine and adenine into guanine. High ratio of mutations due to the ease of replacing the nitrogen bases with one chemical category rather than replacing nitrogen bases that have a different chemical class [21,22].

Conclusions

The present study concluded the prevalence of S. marcescens in meningitis, especially in neonates. S. marcescens is resistant to ß-lactam antibiotics and dominance of ampC gene in all isolates. Also, local isolates of S. marcescens were identical to the standard isolate at a percentage of 98%.

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