ISSN(Online) : 2319-8753
ISSN (Print) : 2347-6710

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 5, Issue 4, April 2016

Isolation and Molecular Identification of a *Serratia* spp. from Suspected Neonatal Sepsis in Intensive Care Unit (ICU) of Basra Province, Iraq

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ABSTRACT: The genus *Serratia* , a member of Enterobacteriaceae family, plays an important role in nosocomial infections. It is responsible for numerous hospital outbreaks occurring in immunodefficient patients,particularly newborns and patients in intensive care unit (ICU). The current study included the collection of one hundred and sixty blood samples from neonatal patients who are suspected of infection by late onset sepsis in the neonatal intensive care unit (ICUs) of Maternity and childhood hospital in Basra province . All samples were tested by using blood cultures to isolation bacteria and molecular identification the positive blood cultures . Result of this study showed 8(5%) samples were found with positive blood cultures while 152(95%) given negative blood cultures. after identification by using the biochemical test,all (8) bacterial isolates were identified as *Serratia* spp. In the second part of this study the 16SrDNA nucleotides sequencing data results for eight alignments of bacterial isolates were showed (5) isolates No1,3,4,7 and 8 similarity the isolates that have accession number in genbank (KT741023.1,KT260868.1,KJ130057.1,KT260868 and KJ877667.1) respectively as *Serratia marcescens* , while (3) isolates No2,5and 6 similarity the isolates that have accession number in genbank (KC191827.1, FJ811864.1and KC191827.1) respectively as *Serratia liquefaciens*.

KEYWORDS: *Serratia* , neonate , sepsis,16SrDNA .

I. INTRODUCTION

Serratia spp. a member of the Enterobacteriaceae are opportunistic Gram-negative , facultative and rod-shaped bacteria [1] . It's wide spreading in the environment and causes disease in vertebrate , invertebrate and plant hosts [2].Itcan recognize species: *Serratia marcescens*, *Serratia ficaria*, *Serratia liquefaciens*, *Serratia rubidaea*, *Serratia fonticola*, *Serratia odorifera*, *Serratia plymuthica*, *Serratia quinivorans*, *Serratia grimesii*, *Serratia proteamaculans*, and *Serratia entomophila* . All species except *S. entomophila* have been isolated from clinical samples [3].
Serratia spp. cause clinically problematic nosocomial infections including peritonitis, pneumonia, sepsis and wound infections because multi-drug resistance is widespread within the species [3,4,5] .The reservoir of *Serratia* spp. are the infected patients and can spread among patients occurs by transient carriage on the hands of nursing or medical staff. Handling of urinary catheters, wound drains, or tracheal tubes of infected[6].The study aims to investigating the *Serratia* spp. that play a role in the neonatal sepsis infection in the neonatal intensive care units (ICUs) by using isolation , biochemical and molecular identification .

II. MATERIALS AND METHODS

2.1.Patients :

A total of (160)blood samples were collected from neonatal patients who are suspected to have infection by late onset sepsis in the neonatal intensive care unit of Maternity and childhood hospital during April/2012 to April /2013 in Basra province .

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2.2.Sampling :

Blood cultures were collected, transported and tested according to Clinical and Laboratory Standards Institute (CLSI) standard [7] , and blood culture bottles were incubated for seven days at 37^{o}C and visually inspected daily to detect positive growth. Positive bottles (turbid) were subculture on nutrient agar and MacConkey agar and incubated overnight at 37^{o}C. The colonies identified by Gram's staining and biochemical test [8,9] .

2.3.Molecular study

The genetic study based on 16SrDNA . The genomic DNA from (8) isolates was extracted from pure cultures by using a Promega genome DNA extraction kit(Wizared® Genomic DNA Extraction Kit ,Promega ,USA) according to the manufactures specifications. The 16SrDNA gene was carried out using (5 µl) of the extracted DNA was amplified with primers F27 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R1492 (5′TACGGYTACCTTGTTACGACTT-3′) [10]. All reactions were carried out in (25 µl) volumes, containing (1 µl) of each primer, (12.5 µl) of GoTaq Thermo master mix (Thermo , England) , completed volume to (25 µl) with (9.5 µl) Nuclease-free water. PCR was performed with the following program: (1) cycles of (5 min.) denaturation at (95°C), followed by (35) cycles of (45 sec.) denaturation at (95°C) , (45 sec.) annealing at (50°C), (90 sec.) extension at (72°C), and a final extension step of (15 min.) at(72°C). The (1 µl) loading (bromophnol blue) was mix with (5 µl) of PCR product and loading in (1%) agarose gel containing (0.2 µl) ethidium bromide with the addition of loading buffer (Tris acetate EDTA buffer) that prepared according to [11] . The (5 µl) of DNA ladder 1kb used as standard marker in electrophoresis and run for (45 min .) in 95V , the products were visualized by using gel documentation system (Dark room gel reader system UVP, MWG,Germany) .

2.4.PCR products purification and sequencing

PCR products of the correct size were purified with a QlAquick PCR purification Kit (Qiagen ,USA) according to the manufactures specifications ,the samples prepared by following concentrations and volumes described from WMG operon company , and samples were sent to the address (eurofins MWG operon ,28-32 Brunel Road /Acton / London W3 7XR) .The bacterial 16SrDNA sequences were obtained then aligned with known 16SrDNA sequences in Genbank using the basic local alignment search tool BLAST (www.blast.ncbi.nlm.nih.gov/Blast.cgi.) at the National Center for Biotechnology Information (NCBI) , and percent homology scores were generated to identify bacteria. Bacteria with 16SrDNA sequences ≥99% similarity were depended for the diagnosis [12] . phylogenetic tree were constructed by using Multiple alignment program (MAFFT) program version 7 (http://mafft.cbrc.jp/alignment/server/) [13] .

III. RESULTS AND DISCUSSION

This study showed from (160) blood samples were collected from neonatal suspected late onset sepsis , 8(5%) samples were found with positive blood cultures while 152(95%) given negative blood cultures. after identification by using the biochemical test show in (Table 1,2) ,all (8) bacterial isolates were identified as *Serratia* spp. .The Gram-positive and Gram-negative bacteria cause sepsis related infection .The common Gram-negative bacteria are *Klebsiella* ,*E.coli* , *Pseudomonas*,*Enterobacter*, *Serratia* ,*Acinetobacter*,*Citrobacter* and other Enterobacteriaceae [14]. *Serratia* spp. has been recognized as an opportunistic pathogen in humans[15]. Nevertheless the recent surveillance programme results in the United state and Europe, indicate that *Serratia* spp., average of 6.5% of all Gram-negative infection in intensive care units , and an average of 3.5% in non-intensive care unit (ICU)patients [16]. *Serratia* is the seventh most common cause of pneumonia with percentage 4.1% in the United state, 3.2% in Europe and 2.4% in Latin America [17] . *Serratia* composed tenth most common cause of bloodstream infection with percentage 2.0% amongst hospitalized patients [18,19].

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Table 1: Biochemical test used to identify *Serratia* spp**.**

Number of isolates	Types of test											
	Gra.	Cata.	Oxi.	Ino.	Meth.	Vog.	Citr.	Ure.	Mot.	Orin.	Lys.	Arg.
1	-	+	-	-	-	+	+	-	+	+	+	-
2	-	+	-	-	-	+	+	-	+	+	+	-
3	-	+	-	-	-	+	+	-	+	+	+	-
4	-	+	-	-	-	+	+	-	+	+	+	-
5	-	+	-	-	-	+	+	-	+	+	+	-
6	-	+	-	-	-	+	+	-	+	+	+	-
7	-	+	-	-	-	+	+	-	+	+	+	-
8	-	+	-	-	-	+	+	-	+	+	+	-

6.Vog.: Vogas-proskauer test ,7.Citr.:Citrate test. 8.Ure.:Urease test , 9. Mot.:Motility test , 10.Orin. : Orinthine decarboxylase, 11. Lys.:Lysine decarboxylase, 12.Agr.:Arginine dihydrolase,

Table 2: The additional biochemical test used to identify Serratia spp.

Number of isolates	Types of test								
	Gel.	ONP.	Pig.	DNase.	Gas from glucose	Grow. at $40^{O}C$	Grow in NaCl 7% (W/V)	Grow in NaCl 8.5% (W/V)	Grow. in NaCl 10% (W/V)
1	+	+	-	+	-	+	+	+	-
2	+	+	-	+	+	-	+	-	-
3	+	+	-	+	-	+	+	+	-
4	+	+	-	+	-	+	+	+	-
5	+	+	-	+	+	-	+	-	-
6	+	+	-	+	+	-	+	-	-
7	+	+	-	+	-	+	+	+	-
8	+	+	-	+	-	+	+	+	-

1.Gel.:Gelatin hydrolysis test, 2.ONPG: Ortho-nitrophenyl β.D-galactosidase test , 3.Pig: pigments .

The study of Gupta *et al.*(2014) referred to that *Serratia* infection is responsible for about 2% of nosocomial infections of the bloodstream, lower respiratory tract, urinary tract, surgical wounds, and skin and soft tissues in adult patients [20]. The second part in this study showed that individual band of 16SrDNA was characterized in 1500 bp by comparison with the standard molecular DNA ladder (1kb) on agarose gel 1% (figures 1).

The 16SrDNA nucleotides sequencing data results for eight alignments of bacterial isolates were identified from the suspected neonatal late onset sepsis .All isolates in this study were compared with (6) reference strains from genbank ,that identified (5) isolates (No1,3,4,7 and 8) similarity the isolates that have accession number in genbank(KT741023.1,KT260868.1,KJ130057.1,KT260868 and KJ877667.1) respectively *as Serratia marcescens* (100%) , while (3) isolates (No2,5and 6) similarity the isolates that have accession number in genbank (KC191827.1, FJ811864 and KC191827.1) respectively as *Serratia liquefaciens* (100%).

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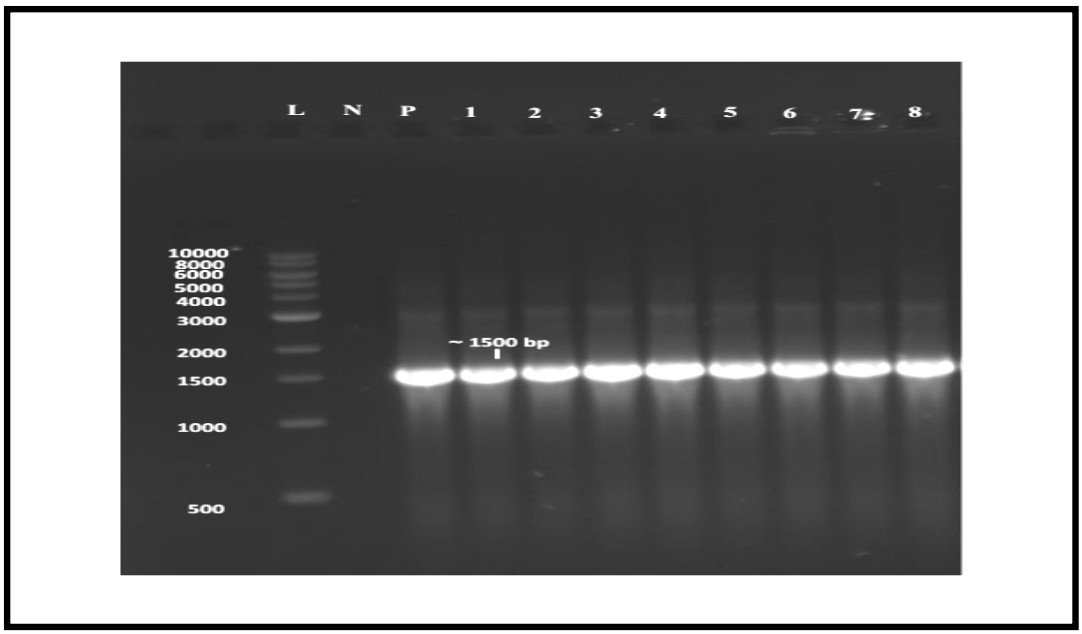

Fig. 1. Agarose electrophorsis patterns show PCR amplified products of 16S rDNA .Lane L: (1kb DNA ladder) ,Lane N:(negative control), Lane P: (*E.coli* positive control) , Lane:(no. 1-8) 16SrDNA band of bacterial isolates .

The present study focused on the use of the 16SrDNA to detect the rate of accurate biochemical test identification of the *Serratia* spp.The 16SrDNA result gives a clear picture about the significance of biochemical tests that were used for the identification of *Serratia* spp from clinical samples .The 16SrDNA sequencing has played a very important role in the accurate identification of bacterial isolates and in the discovery of noval bacteria in clinical microbiology laboratories [21] .

The result of nucleotide sequence data for the (8) isolates in this study were concatenated producing a sequence length (702) base depend on the shorter one among the sequences .The root phylogenetic tree was constructed and displayed in (figure 2) .This tree show the distribution of phylogenetic relationships among the studied bacteria and their identical reference strains .The phylogenetic tree presents the distribution of the genetic relationship between the isolates in two clades A and B.

R: reference strain

Fig.2. Rooted Neighbour Joining phylogenetic tree constructed from concatenated sequences of (702 bp) for each strain derived from an alignment of 16S rRNA gene sequences then produced from a MAFFT alignment by using the Average linkage (UPGMA) , and visualized using forester version1035. This N-J tree showing the distribution and phylogenetic relationships between 8 *Serratia spp.* isolated in this study , 6 reference strains (R) .

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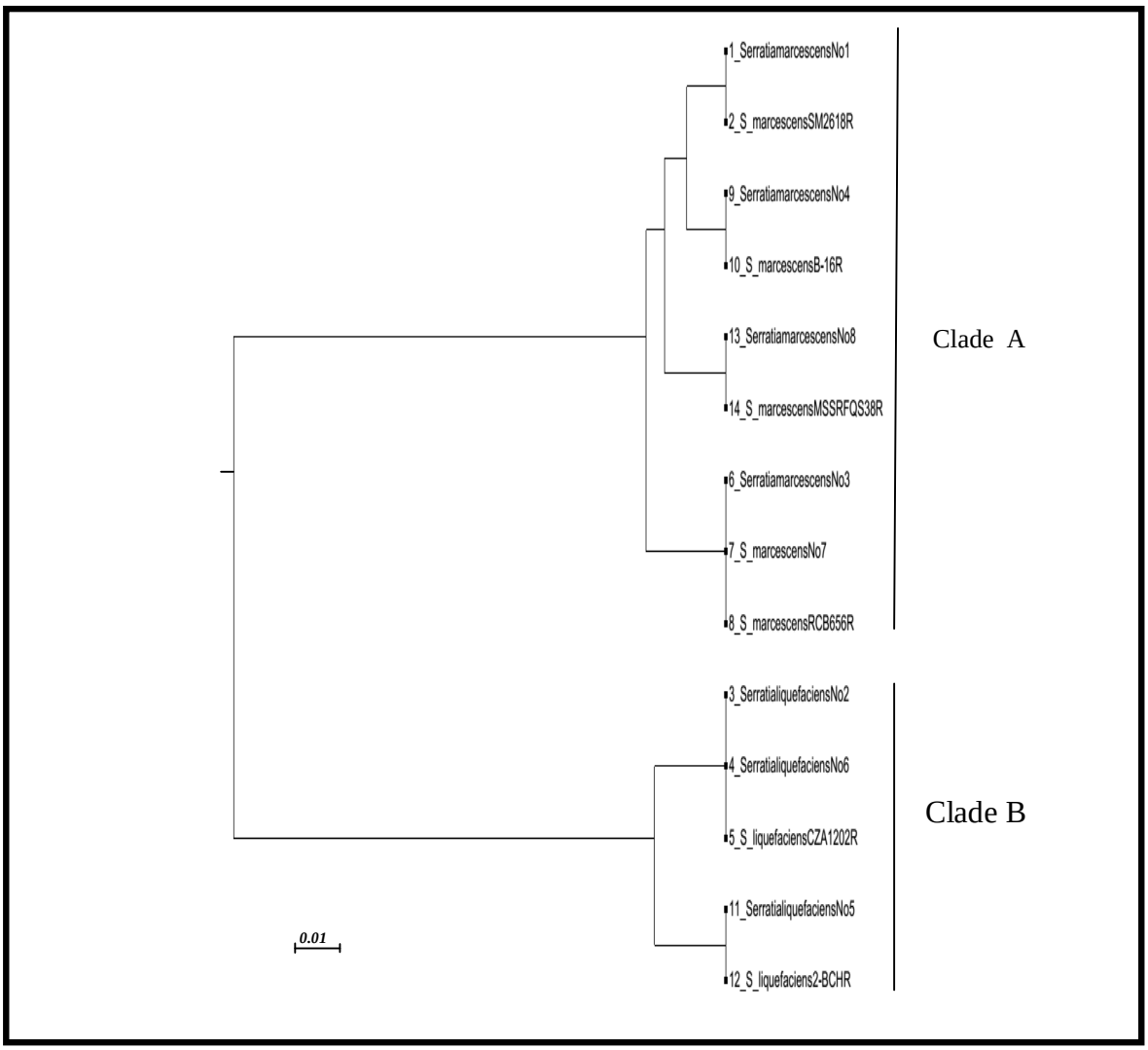

All vertical branch lengths were drawn to scale Bootstrap values after 1000 repetitions are indicated.

IV. CONCLUSION

Serratia spp. has emerged as an important healthcare-associated pathogen and play an important role in the neonatal sepsis infection in the neonatal intensive care units and needed more studies in Iraq .

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