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Research Article

Isolation and Identification of *Serratia marcescens* from Suspected Late Neonatal Sepsis in Intensive Care Unit

Jabbar S Hassan[1], Ahmed E Salman[2], Ahmed S Obeid[3], Thana R Abdul Rhman[1]

[1]*Medical Microbiology department/ College of Medicine, Al-Nahrain University*
[2]*Clinical Laboratory Science department/College of pharmacy, Mustansiriyah University*
[3]*Al-Rasheed University College/ Department of Laboratory Analyses*

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ABSTRACT

Background: Neonatal sepsis stays one of the main sources of morbidity and mortality both among infant in ICU, in light of the planning of the disease neonatal sepsis has been categorized into early and late-onset sepsis, where the latter occurs after one week of life and is often more insidious in onset than the former. Objective: To detect the rate of *Serratia marcescens* infection in neonatal sepsis in ICU by molecular technique. Methods: A total of 50 neonates with the age group 8 days to 30 days who were admitted to AL-Kadhumiya Teaching Hospital/ Baghdad during the period January to March ,2017 were recruited for this cross-sectional study. Approximately 3 ml of venous blood were obtained from each patients. These samples were examined for septicemia by blood culturing followed by API20 for quick identification of relevant bacteria. Furthermore, bacteria DNA was isolated directly from blood samples, and conventional PCR based on luxS gene, highly specific to *S. marcescens*, was achieved. Results: Blood culture were positive in 36(72 %) out of 50 samples; the most common bacterial causes were *Staphylococcus aureus* (18.7%), *Pseudomonas aeruginosa* (13.8%) and *Serratia marcescens* (11.1%), Molecular method revealed specific amplification of luxS gene in 12 samples (24%). Conclusion: *Serratia marcescens* has risen as a most widely recognized causative agent in late onset sepsis.

Keywords: neonatal sepsis, intensive care unit, *Serratia marcescens*, *luxS* gene.

INTRODUCTION

Newborns are particularly at risk for some diseases because their immune systems are not developed enough to fight pathogens. Neonatal sepsis is considered as one of the major causes of morbidity and mortality in newborns. According to the time of onset, the neonatal sepsis is categorized into two types: Early-onset neonatal sepsis (EOS) occurring within 72 hours in infants and pathogens transmitted to infants vertically, and Late-onset sepsis (LOS) occurring after 72 hours of life in term infants where pathogens may be acquired both vertically or horizontally[1]. Many studies have pointed out the risk of cross infection in neonatal intensive care unit (NICU) as an important source in neonatal sepsis[2,3].

The bacterial etiological agents for neonatal sepsis is fluctuated. Epidemiological data do not only differ greatly between developed and developing countries, but also within same country. bacterial organisms with expanded resistance to antibiotic have additionally developed further complicated the management of neonatal sepsis[4]. *Serratia marcescens* is an important opportunistic nosocomial pathogen, rod-shaped gram-negative bacteria, late lactose fermenter, and can cause potentially life-threatening infections such as meningitis, blood sepsis and pneumonia[5]. Outbreaks of *S. marcescens* in NICU were frequently reported during last years (References). Unfortunately, sepsis caused by this microorganism is difficult to treat because most S. *marcescens* strains are resistant to many antibiotics[6].

The current study aimed to detect the rate of *S. marcescens* as a cause of neonatal sepsis in in NICU Kadhumiya Teaching Hospital/ Baghdad by molecular identification.

SUBJECTS AND METHODS

The Study Population

Fifty neonates with age from 7 days to 30 days who were admitted to NICU at AL-Kadhumiya Teaching Hospital/ Baghdad hospital during the period January to March 2017 were recruited for this cross-sectional study**.** Clinical manifestations were determined by consultation of a pediatric specialist and verification of the information in the medical record. Each child's parent has signed a consent letter, and the Research Ethical Committee at College of Medicine of Al-Nahrain University approved the study.

Sample Collection and Processing

About 3ml of venous blood were obtained from each participate. Each sample was separated into two parts, the first one about (1.5-2 ml) underwent blood culture by using brain heart infusion broth and incubated aerobically at 37ºC for 5 days. Aliquots from the culture bottles were sub cultured on blood agar and MacConkey agar, incubated at 37ºC for 24 hours under aerobic condition and Chocolate agar under 5% Co2 conditions[7]. The cultivated bacteria

**Author for Correspondence: jabbarhassan@trientedu.edu*

Table 1: Primer sequences and gene targets for *S. marcescens* with *β-globin* housekeeping gene.

Genes	Nucleotide sequences (5'to3')	Reference	Products (bp)
luxS	F:GCTGGAACACCTGTTCGC R:ATGTAGAAACCGGTGCGG	(8)	102
B-globin	F:ACACAACTGTGTTCACTAGC R:GAAAATAGACCAATAGGCAG `	(9)	408

Table 2: Demographic character of studied neonates.

Variables	No,%
Age (mean±SD)	12.3 ±7.76
Gender	
Male	29 (58%)
Female	21 (42%)
Gestational age	
Preterm	24 (48%)
Full term	26 (52%)
Mode of delivery	
cesarean section	30 (60%)
Vaginal	20 (40%)
Place of birth	
Hospital	37 (74%)
Outside hospital	13 (26%)
Birth weight	
≥ 2.5 Kg	23(46%)
< 2.5 Kg	27 (54%)

Table 3: Organisms isolated from blood culture from neonate with sepsis by API system.

API 20 of bacteria	Total No (36)
Pseudomonas aeruginosa	5(13.8 %)
Acinetobacter baumannii	3(8.3%)
Citrobacter freundii	3(8.3%)
Serratia marcescens	4 (11. 1%)
Klebsiella pneumoniae	2 (5.5%)
E.coli	3(8.3%)
(Burkholderia cepacian)	1(2.7%)
Staphylococcus aureus	6(18.7 %)
Staphylococcus hemolytica	2(5.5%)
Staphylococcus xylosus	2(5.5%)
Staphylococcus lentus	1(2.7%)
Streptococcus agalactia	3(8.3%)
viridans streptococci	1(2.7%)

were diagnosed primarily according to morphological characteristics of the colonies. Diagnosis was confirmed by using Api20E system for Enterobacteriaceae, Api Staph System, Api 20 Strep System according to instructions of manufacturing company (bio-Merieux). The second part of blood was used in extraction of bacterial DNA using ready kit (Geneaid Total DNA Extraction/ Taiwan) following manufacturer's instructions.

Molecular detection of Serratia marcescens

Specific oligonucleotide primer sequences were used in conventional PCR to detect *luxS* gene which is highly specific for *S. marcescens* to produce a DNA fragment of 102 base pair[8]. Primers for *β-globin* housekeeping were used to examine the integrity of the extracted DNA with 480 base pairs amplicon[9]. The sequences of these primers are illustrated in table 1. PCR mixture without DNA template (non-template negative control) were used as negative control. Cyclic conditions were: 94°C for 3 min followed by 40 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 60 seconds, terminating in 72°C for 7 min, 10 µl of each PCR product was subjected to 1% (wt/vol) agarose gel electrophoresis with ethidium bromide (0.5 µg /ml). PCR products electrophoresis amplicon visualization was performed using an UV light transilluminator.

Statistical analysis

Statistical Analysis system (SAS) software was used for all statistical analysis continuous variables were expressed in mean ± standard deviation (SD). The Pearson's Chi-square test or Fisher exact test was used for comparing the categorical variable. A two-sided significant level of 0.05 was considered to indicate a statistically significant difference.

RESULTS

The demographic baseline of the neonates is shown in table 2. In current study, males were slightly more affected than females. Interestingly, it seems that different gestational ages or birth weight do not influence the rate of neonatal sepsis. On the other hand, neonates born in hospital represent about three-quarter of cases, while those born outside the hospital represent only one-quarter. Furthermore, cesarean section seems to increase the chance of neonatal sepsis compared to vaginal delivery. Out of 50 samples cultivated, 36 (72 %) were successfully grown, among which 21(58.3%) gram negative and 15(41.6%) gram positive. Growing colonies were examined by the different strips API system which was tailored to certain groups of microbes to identify the bacterial isolate species. It was found that the most prominent bacterial causes were *Staphylococcus aureus* (18.7%), *Pseudomonas aeruginosa* (13.8%) and *Serratia marcescens* (11.1%) table (3).

Molecular detection

Conventional PCR was done for the amplification of *luxS* gene, which is highly specific to *S. marcescens*, by using specific set of primers sequences. The results showed that, amplification products were obtained for this gene in 12 (24%) out of 50 blood samples, PCR product of this gene was 102 bp figure (1).

DISCUSSION

In the current study male are affected more than female (58%, 42% respectively). Such finding is incompatible with those obtained in a local study conducted by Albahadle, and Abdul Abass[10], who found that male are less likely to get the neonatal sepsis than females. However, in another study, males were found to be more infected than females[11]. This heterogeneity in results may

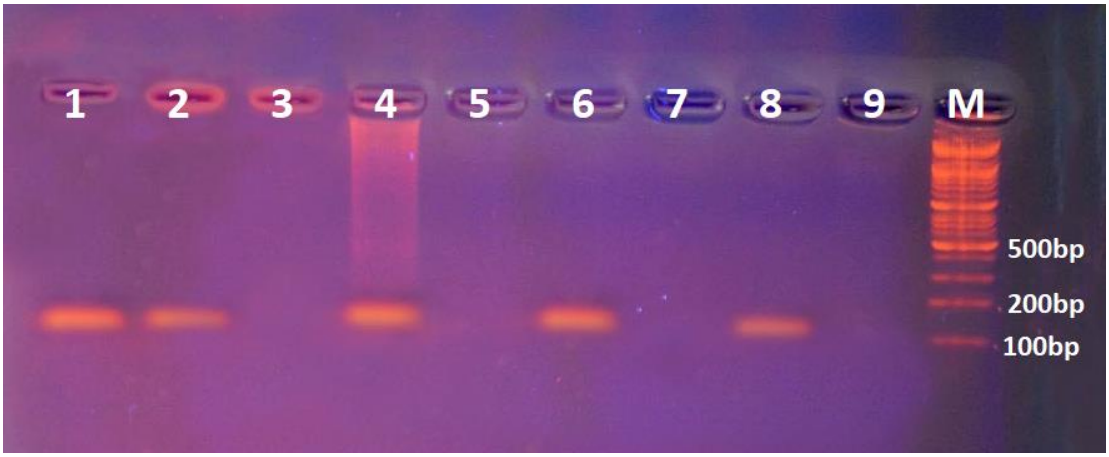

Figure 1: Gel electrophoresis of PCR of *S. marcescens* for luxS gene. Lanes 1, 2, 4, 6, 8: positive results (102 pb), lanes 3, 5, 7 negative results, lane 10: negative control.

be attributed to the society's view regarding gender. In most families, attention is more to males than females, or might be conceivable by the higher number of males than females in children that was born during the study period. In this study, the ratio of full term patients was (52%) and 30 (60%) were born by cesarean section delivery. The possible explanation for such results is that the majority of children were born by caesarean sections, which is often performed when the pregnant woman completes her pregnancy and there were no signs of normal vaginal delivery. The majority of neonates in the current series were hospital inborn. Many factors influence infection in neonatal period including, nosocomial infection, obstetrical procedures and use of intrapartum instrumentation which is considered as a risk factors for neonatal sepsis. Regarding birth weight, 27(54%) with low birth weight (<2.5 kg). These findings are compatible with prior study in Iraq by Abdul-Kareem *et al.*[12], and with international studies by Birju and James[13] and Mukhopadhyay[14] in that neonates with low birth weight are more susceptible for neonatal sepsis may be due to repeating handling of the baby by the health professionals and his/her families which increase the contamination rate. The rate of neonatal sepsis is fluctuating with time from one hospital to another and from one area to another. In this investigation, blood culture positivity rate was 72%. This rate is higher than many previous studies in Iraq, such as the study by Zuhair[15] who reported that 70.9% blood culture positive and a study by Abdul-Karem and Areege[16], who found that 66.9% of neonates in NICU were blood culture positive. The difference in these results can be attributed to a number of reasons, including the volume of blood taken from the patients which greatly affects the yield of blood culture. Furthermore, conditions of blood culture incubation and the treatment given to the patients before blood collection have a significant impact on the outcome of blood culture.

In order for fast identification of the different types of bacteria that have been isolated from blood culture, three types of API system were used including API- Staph. API-Strep and API-20E. According to this system, the most predominant bacteria was *Staphylococcus aureus* (18.7%), *Pseudomonas aeruginosa* (13.8%) and *Serratia marcescens* (11.1%). The present study is incompatible with many studies in this field[17,18]. It is remarkable that there is a difference in the types of bacteria causing the LOS between this study and the others, which can be explained by acquisition of bacteria either from the community or from previous admission to the NICU.

S. marcescens is one of the most opportunistic pathogen that emerged as nosocomial infection in ICU and non-intensive care unit patients[19,20]. A critical feature of *S. marcescens* is its capability to produce a beta-lactamase that confers resistance to extensive-spectrum beta-lactam antibiotics[21]. 2.0% of hospitalized patients with bacteremia are due to *S. marcescens*[22]. In a study in USA, Neil G *et al* reported that *S. marcescens* as one of the leaded causes of blood stream infection in patients who received parenteral nutrition (PN)[23]. Sepsis caused by multidrug resistant *S. marcescens* have been reported, and many strains carry both chromosomally encoded and plasmid-mediated resistance determinants for several different types of antibiotics. An interesting point is that infection with MDR *S. marcescens* makes neonatal infection even more devastating and associated with significant morbidity and mortality[24].

In our study 12 (24%) out of 50 patients were positive *luxS* gene *S. marcescens*. This result was found to be higher than that recorded by Saad which was 5%[19] and Zuhair, 2.3%[15]. The variation in these results may be related to the diagnostic methods. In current study the diagnosis process was based on the extraction of bacterial DNA directly from the blood samples of patients then used highly specific gene for *S. marcescens*, while in the prior studies the diagnostic method based only on blood culture. However, the result in this study is close to another global study by Ostrowsky *et al*[25]. Interestingly, PCR-dependent method revealed three-time greater positive samples compared to traditional method from the same samples. This indicates the highly sensitivity of molecular method in the detection of bacterial infection.

From the aforementioned data, it can be concluded that *Serratia marcescens* has risen as most widely recognized causative agent in late onset sepsis and physicians particularly in intensive care unit must emphasize it.

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CONTRIBUTION DETAILS
Samples selections, design, acquisition of data and molecular methods diagnosis. Drafting the article and revising it critically for important intellectual content. Analysis and interpretation of results Conventional methods diagnosis and material supplementation. Statistical analysis.

CONFLICT OF INTEREST
The author declares that they have no competing interests.

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