Molecular Detection of Extended-Spectrum $\beta$-Lactamases- Producer *Serratia marcescens* Causing Neonatal Sepsis in Iraq

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**ABSTRACT**

*Serratia marcescens* is an important nosocomial pathogen that causes a variety of infections, especially urinary tract and bloodstream infections. The emergence and spread of multidrug-resistant *Serratia marcescens* producing extended-spectrum beta-lactamases is a threat to public health worldwide at present. Extended-spectrum $\beta$-lactamases (ES$\beta$Ls) including TEM, SHV, and CTX-M are the predominant types that confer resistance to beta-lactam group of antibiotics. Many reports have been investigated ESBL-producing isolates of Enterobacteriaceae in Iraq. However, there are few studies concerned about ES$\beta$L-producing *Serratia marcescens* particularly, detection of ESBLs encoding genes. Therefore this study aimed to identify ES$\beta$Ls encoding genes in *Serratia marcescens* isolates from a neonatal intensive care unit. Fifty isolates were identified phenotypically using the VITEK® 2 compact system. For confirming the identification of bacterial strains, molecular detection of housekeeping LuxS gene was done using species-specific designed primers. Antibiogram was performed using the VITEK® 2 compact system. A phenotypic confirmatory test for ES$\beta$Ls producers was performed using a combination disc method. The ES$\beta$Ls encoding genes, including $bla$TEM, $bla$SHV, and $bla$CTX-M, were amplified using a PCR-based technique; the amplified products of some selected isolates were sequenced. Molecular detection of isolates using PCR-based amplification of the LuxS gene showed that all isolates possessed this gene. The patterns of antimicrobial resistance for isolates under study showed very high resistance to cephalosporins, while they were susceptible to carbapenem drugs and tigecycline. Findings based on the PCR technique showed that the prevalence of ES$\beta$Ls encoding genes of isolates was 13 (26%), 31 (62%), and 46 (92%) for $bla$TEM, $bla$SHV, and $bla$CTX-M respectively. In the present study, it was concluded that $bla$CTX-M gene was the most prevalent ES$\beta$Ls-encoding gene among ES$\beta$Ls producing *Serratia marcescens* isolates.

**INTRODUCTION**

Among *Serratia* species, *Serratia marcescens* is the most common associated with human infections (Merkier et al., 2013). Initially, it is considered as nonpathogenic, but recently it is recognized as an important nosocomial pathogen causing urinary tract infections (Raymann et al., 2019; Mohajerani et al., 2019), bloodstream infections including endocarditis (Marin et al., 2017), and many other types of infections (Mostatabi et al., 2013; Mohajerani et al., 2019).
The emergence and spread of extended-spectrum β-lactamases producing Enterobacteriaceae is a public health threat because infections are caused by these strains associated with an increase in morbidity, mortality, and health-care costs (Liebana et al., 2013). There are various ESβLs encoding genes globally, with the most common members like TEM, OXA, CTX-M, and SHV. Based on Ambler molecular classification, The SHV, TEM and CTX-M enzymes are of class A, while based on Bush-Jacoby classification, CTX-M enzyme is of group 2be, The SHV and TEM enzymes are of group 2b or 2be (Ambler et al., 1991; Bush et al., 1995). In the past decade, Serratia strains causing nosocomial infections have become a growing concern. The horizontal transfer of genes encoding resistance to a broad spectrum of antibiotics in this opportunistic pathogen made effective treatment more difficult (Mahlen, 2011). ESβL-producing enzymes have risen among the Serratia genus and increased multidrug resistance (Młynarczyk et al., 2007). Such β-lactamases are quickly transferable and have become a significant human health concern.

*Serratia marcescens* is a widespread and ubiquitous in the environment; found in water and soil and also associated with plants and other animals (Wamala et al., 2018). It may harbour multidrug-resistant mechanisms, including the production of beta-lactamase enzymes which complicate its treatment (Cristina et al., 2019). *Serratia marcescens* can produce inducible beta-lactamase (IβL) and ESβLs so that they can develop resistance to many beta-lactam antibiotics. This resistance makes the treatment of nosocomial infections caused by *Serratia marcescens* quite tricky. This bacterium can exhibit multidrug resistance to beta-lactam, aminoglycoside, and quinolone group antibiotics as well as natural resistance to many antibiotics (Şimşek, 2019).

**MATERIALS AND METHODS**

**Bacterial Isolates**

During a period of eight months from April to November 2018, Fifty *Serratia marcescens* isolates were collected from blood specimens of neonatal intensive care unit of Fatima Al-Zahraa hospital – Baghdad city. These isolates were identified phenotypically using VITEK® 2 compact system (*bioMérieux, France*) and genotypically using a genus and species-specific primers Table 1 of the LuxS quorum-sensing gene as a housekeeping gene.

**Antimicrobial susceptibility test**

Antimicrobial susceptibility test for *Serratia marcescens* isolates was done by VITEK® 2 compact system (*bioMérieux, France*) with the using gram-negative antimicrobial susceptibility test cards (AST-GN82) according to the manufacturer’s instructions. The tested antimicrobials were: Ampicillin/Sublactam, Pipercillin/Tazobactam, Cefazolin, Cefazidime, Ceftriaxone, Cefepime, Aztreonam, Imipenem, Ertapenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Levofloxacin, Tigecycline, and Trimethoprim/sulfamethoxazole.

**Phenotypic confirmation test for ESβLs producers**

A combination disc method was used according to the Clinical and Laboratory Standard Institute protocol (CLSI, 2014). The zone of inhibition for the cefepime discs was compared to that of cefepime Plus clavulanic acid combination discs (Mast Co, UK). A difference of ≥ 5 mm in the zone of growth inhibition diameter between cefepime disks and their respective cefepime /clavulanate disk confirmed the ESβLs phenotype among the isolates.

**Molecular detection of ESβLs encoding genes**

Genomic DNA has been isolated using a Genomic DNA purification kit (Geneaid, Thailand) according to the manufacturer’s protocol. Molecular detection of ESβLs-encoding genes (blaCTX-M, blaSHV, blaTEM) was performed using a monoplex PCR-based technique. The primers were used to amplify the ESβLs-encoding genes listed in Table 1.

**Sequencing of PCR products**

The amplified PCR products were sequenced using the Sanger sequencing method (Macrogen, Inc, South Korea). DNA sequences data were analyzed using BLAST (Basic Local Alignment Search Tool) available in the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov /BLAST).

**Genebank accession numbers**

The LuxS, *blaCTX-M*, *blaTEM*, and *blaSHV* genes sequences of some selected isolates were deposited in GenBank database under accession numbers MN928776 to MN928787 (till now not released on the website).

**RESULTS**

Each DNA sample extracted from bacterial isolates was subjected to a monoplex-PCR reaction with a pair of designed primers of the LuxS gene. The findings of PCR-based amplification showed that all isolates gave positive results for amplified products of both designed primers, which are species-specific
Table 1: The Sequences of Primers Used in this Study

| Primer                      | Sequences (5’—–3’)                                                                 | Product size (bp) | Reference                        |
|-----------------------------|------------------------------------------------------------------------------------|-------------------|----------------------------------|
| Genus-specific LuxS         | F-GGGATGATGATAACAAATGCGCG                                                          | 931               | This study                       |
|                             | R-GCACCGCAACGTCGGGAGCTG                                                            |                   |                                  |
| Species-specific LuxS       | F-TTGGAAAAGGTTCGCGGTAGGA                                                          | 690               | This study                       |
|                             | R-GGCCATACCTGGCAGGGTCCAG                                                          |                   |                                  |
| blaCTX-M                    | F-TCGTCTCTCCAGAATAGG                                                              | 1025              | (Schneider et al., 2009)         |
|                             | R-AAGGAGAACAGAACCAGC                                                              |                   |                                  |
| blaCTX-M-1-all              | F-ATGGTTAAAAATTACACTGCG                                                           | 876               | (Shen et al., 2017)              |
|                             | R-TTACAAACCCTGGTGGAGT                                                            |                   |                                  |
| blaTEM                      | F-CGCTCATGAGACAAATAACCTCT                                                      | 1000              | This study                       |
|                             | R-GGATCTTACCTAGATCCTT                                                            |                   |                                  |
| blaSHV                      | F-GCCGGGTATTTATTTGTCGCC                                                       | 977               | (Mulvey et al., 2011)            |
|                             | R-TCTTCCGATGCGCCCGCCAGTCA                                                       |                   |                                  |

for Serratia marcescens (690 bp) and genus-specific for Serratia spp. (931 bp).

Testing for antimicrobial susceptibility

The patterns of antimicrobial resistance for the isolates showed high resistance to cephalosporins [cafazolin (100%), ceftazidime (80%), and ceftriaxone (70%)] and aztreonam (76%). However, the isolates showed lower rates of resistance for aminoglycosides [amikacin (26%), gentamycin (50%), tobramycin (6%)], fluoroquinolones [ciproflaxacin (10%) levofloxacin (4%)] and Trimethoprim/sulfamethoxazole (8%). Furthermore, some isolates showed significant intermediate resistance for tobramycin in 21(42%), while they were susceptible to carbapenem drugs (meropenem and ertapenem) and tigecycline.

Phenotypic confirmation test for ESBLs producers

Findings obtained from combination disc method showed that 34 (68%) of isolates were extended-spectrum beta-lactamase producers according to the inhibition zone of cefepime discs compared to cefepime plus clavulanic acid.

Genotypic detection of ESβLs encoding genes

We found the prevalence of blaTEM entire gene (1000bp) was 13 (26%) of the total number of isolates. Besides gene as the whole of blaSHV (977bp) was found in 31 (62%) of isolates under study. First genotypic detection of the black X-M gene was conducted using PCR-based technique to amplify (876bp) as partial coding sequence of the blaCTX-M-1 group which includes (blaCTX-M-1, 3, 10, 12, 15, 18, 30 and FEC-1) then the amplification of entire blaCTX-M gene was performed using the designed primers to confirm the primary detection mentioned above and to investigate if these were other groups of blaCTX-M among the isolates. blaCTX-M entire gene (1025bp) was detected in almost all of the isolates. The current rate of the black X-M-1 group and entire blaCTX-M gene among Serratia marcescens isolates were 14 (28%) and 46 (92%) respectively Table 3.

DISCUSSION

ESβL-producing bacteria are a significant issue in the management of specific bacterial diseases, and they are a real concern in hospitals where there is a regular use of antibiotics and patients in critical conditions (Yadav and Chauhan, 2016). The most common antimicrobial resistance mechanism is an expression of β-lactamase enzymes, which work by hydrolyzing β-lactam ring of β-lactam antibiotics leading to inactivate them (Wilke et al., 2005).

Findings of MICs using the VITEK® 2 compact system revealed that most isolates of Serratia marcescens were highly resistant to β-lactam antibiotics, especially oxyimino-cephalosporin and aztreonam. This pattern of resistance gives an indicator...
Table 2: Antibiograms pattern of isolates towards antimicrobials used in this study

| Antimicrobial     | Resistance percentage | Intermediate percentage | Sensitive percentage |
|-------------------|-----------------------|-------------------------|----------------------|
| Cefazolin         | 50(100%)              | 0(0%)                   | 0(0%)                |
| Ceftazidime       | 40(80%)               | 2(4%)                   | 8(16%)               |
| Ceftriaxone       | 35(70%)               | 0(0%)                   | 15(30%)              |
| Aztreonam         | 38(76%)               | 0(0%)                   | 12(24%)              |
| Ertapenem         | 0(0%)                 | 0(0%)                   | 50(100%)             |
| Meropenem         | 0(0%)                 | 0(0%)                   | 50(100%)             |
| Amikacin          | 13(26%)               | 0(0%)                   | 37(74%)              |
| Gentamicin        | 25(50%)               | 1(2%)                   | 24(48%)              |
| Tobramycin        | 3(6%)                 | 21(42%)                 | 27(54%)              |
| Ciprofloxacil     | 5(10%)                | 4(8%)                   | 41(82%)              |
| Levofloxacin      | 2(4%)                 | 9(18%)                  | 39(78%)              |
| Tigecycline       | 0(0%)                 | 0(0%)                   | 50(100%)             |
| Trimethoprim/Sulfamethoxazole | 4(8%) | 0(0%) | 46(92%) |

Table 3: Distribution of ESβLs encoding genes among isolates

| Gene             | percentage |
|------------------|------------|
| blaCTX-M-1 group | 14(28%)    |
| blaCTX-M         | 46(92%)    |
| blaTEM           | 13(26%)    |
| blaSHV           | 31(62%)    |

that these isolates may be ESβLs producers. Detection of ESβLs production Serratia marcescens using the combination disc method was confirmed that most isolates were ESβLs producers.

Detection of ESβL-producing Serratia marcescens in laboratories has significant importance for the proper treatment of patients, efforts of infection prevention and control, as well, for tracking these pathogens in surveillance systems. The cost-cutting practices and unawareness of the relevant CLSI guideline are the most difficulties facing detecting ESβL-mediated resistance in many laboratories, particularly in developing countries like our country. So the implementation of the measures and protocols related to CDC and CLSI guidelines are significant to control the spread of antimicrobials-resistant isolates, especially ESβLs producers.

The present study found that CTX-M is the most prevalent ESβL among Serratia marcescens isolates, notwithstanding the small number of amplified products of a blaCTX-M gene have been sequenced of selected blaCTX-M-harboring isolates; we found that the CTX-M-15 was dominant among these isolates.

Although the prevalence of blaCTX-M gene among ESβLs-harboring isolates in many studies, other studies have obtained mixed results. Rezende (2019) study in the intensive care unit of a tertiary hospital in Tocantins, Brazil, reported that the ratio of ESBLs encoding genes among ESBLs-harboring isolates of Serratia marcescens was 100%, 0%, and 12.96% for blaTEM, blaSHV, and blaCTX-M-15 respectively (Rezende, 2019). An outbreak occurred in the neonatal intensive care units (NICU) by the dissemination of a blaSHV-5-harboring cephalosporin-resistant Serratia marcescens strains that resulted in 26% lethality in a tertiary pediatric hospital Mexico City (Monteros et al., 2008).

blaCTX-M gene is prevalent in many bacterial species especially in the members of the family Enterobacteriacea, and for it is plasmid-mediated, it can be easily transferred and distributed among bacteria (Sun et al., 2017).

The recognition of CTX-M β-lactamases as the predominant type of ESβL among Gram-negative pathogens raise the concern in many countries (Hussein and Hamed, 2017; Piri et al., 2018). There are shreds of evidence that the epidemiology of organisms producing CTX-M enzymes is very different from those that provide TEM- and SHV...
derived ESβLs. CTX-M enzymes are not limited to nosocomial infections caused by Enterobacteriaceae, and their potential for spread beyond the hospital environment serves to exacerbate public health concerns. Also, the existence of mobile elements such as ISEcp1 in the upstream region of CTX-M β-lactamase-encoding genes leads to an increase in spreading among bacteria in the community and health-care settings.

**CONCLUSION**

Nosocomial infections related to *Serratia marcescens* are associated with a high rate of morbidity and mortality in the neonatal intensive care units (NICU). The proper investigation of outbreaks-causing pathogens, early recognition of the precise mechanism of antimicrobials resistance, particularly beta-lactamases production, and adopt the necessary measures of prevention and control diseases including determining appropriate antimicrobial therapy are clinically significant. Despite the small size of the study samples, the prevalence of CTX-M among ESβLs-producing isolates was investigated in this study. Consequently, this result may give an inference that these genes disseminated among our local isolates, so many studies need to be conducted extensively on the epidemiology of *Serratia marcescens* isolates especially in NICU and detection of different types of beta-lactamases including AmpC and carbapenemase, as well as an investigation of the origin of these nosocomial-causing strains should be carried out.

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**Conflict of Interest**

The authors declare that they have no conflict of interest for this study.

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