

Detection of Extended-Spectrum β -Lactamases (ESBLs), TEM, SHV and CTX-M genes among *Staphylococcus haemolyticus* isolates from cesarean section infections

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SUMMARY

AUTHORS' CONTRIBUTION: (A) Study Design · (B) Data Collection · (C) Statistical Analysis · (D) Data Interpretation · (E) Manuscript Preparation · (F) Literature Search · (G) No Fund Collection

Background: Cesarean section is regarded as a crucial risk factor for postpartum inflammatory because of uterine skin rupture, bladder catheterization, and endometritis. The *S. haemolyticus* is the second most recurrently pathogenic of clinical nosocomial infections, especially with sepsis, on skin and soft tissue infections mainly existing as abscess, paronychia, and serious infections in different the body systems.

Methods: One hundred and fifty swab samples were collected from women who had caesarean sections at Al-Basrah Teaching Hospital between October 2022 to January 2023. The Vitek®2 system test revealed for identify positive bacterial growth. Then double Disk Approximation Method (DAM) was used to tested *S. haemolyticus* isolates for produce Extended Spectrum β -Lactamase (ESBLs). For the β -lactamase gene amplification, three specific primers were employed: blaTEM, blaSHV and blaCTX-M genes.

Results: From October 2022 to January 2023, 150 swab samples were collected. The samples showed 57 (38%) positive bacterial growth, distributed 51(89. 5%) Gram-positive bacterial isolates, while 6(10. 5%) Gram-negative bacterial isolates, by Using the Vitek® 2 system, various bacterial species were identified, *Staphylococcus haemolyticus* the most predominant. Out of 34 isolates, 31 (91. 2%) were divided *S. haemolyticus* 39 (68. 42%) isolates, *Staphylococcus aureus* 6(10. 53%), *Klebsiella* spp. 4(7. 00%) isolates, *Staphylococcus saprophyticus* 3 (5. 30%) isolates, *Escherichia coli* 2 (3. 50%) isolates, *Staphylococcus sciuri* 1(1. 75%.) isolate, *Staphylococcus hominis* 1(1. 75%) isolate, and *Enterococcus faecium* 1 (1. 75%) isolate. Out of (n=34) isolates that distributed to 31 (91. 2%) isolates were divided into *S. haemolyticus* 25 (74%), and *S. aureus* 6 (18%) isolates were gave positive results for producing extended-spectrum β -lactamases (ESBLs). While, 3(8. 8%), isolates of *S. haemolyticus* were shown negative results for producing ESBLs by using of the double approximation method (DAM) The results of the current study revealed from (n=34) *Staphylococci* spp. were distributed to 28 (82%) *S. haemolyticus* and 6(18%), that 33(97. 1%) *Staphylococcus* spp. isolates gave positive results for the detection of TEM and SHV gene. While the 1(2. 9%) isolate was shown negative result for the detection of TEM, SHV and CTX-M genes. While in the present study the results showed that all 34 (100%) *Staphylococcus* spp. were gave positive results for detection of the CTX-M gene

Conclusion: The most isolates of *S. haemolyticus* and *S. aureus* were multi drug-resistant (MDR) were detected by using Vitek®2 compact system and most isolates of *S. haemolyticus* and *S. aureus* were producing extended-spectrum β - lactamases(ESBLs).

Keywords: Cesarean section infections; *Staphylococcus haemolyticus*; ESBLs; TEM; SHV; CTX-M genes

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INTRODUCTION

Cesarean section is regarded as a crucial risk factor for postpartum inflammatory because of uterine skin rupture, bladder catheterization, and endometritis [1,2]. The inappropriate antimicrobial agent, or prolonged use of the antibiotics may lead to bacterial resistance and increased SSI rates [3,4]. *S. haemolyticus* is considered one of the predominantly Coagulase Negative *staphylococci* (CONS). *S. haemolyticus* is non-motile, non-sporulation, Gram-positive and facultative anaerobic. However, the *S. haemolyticus* can be grown in optimal temperature between 30-40 °C with the presence of O₂ and 10% of NaCl [5]. *S. haemolyticus* is regarded as inhabitants of the human and animal microorganisms, has been many reports as a relationship with nosocomial pathogens because biodiversity of microbial and virulence apparatus including resistance to antibiotics [6-8].

The *S. haemolyticus* is the second most recurrently pathogenic of clinical nosocomial infections, especially blood cultures of patients with sepsis, on skin and soft tissue infections mainly existing as abscess, paronychia, and serious infections in different the body systems involving endocarditis, meningitis, joint prosthetic infections, peritonitis, otitis media, urinary tract infections, septicemia and it is widespread in the hospital situation also on the hands of workers in the health care [9,10]. Furthermore, the increased of infectious associated with medical implanted and devices were caused by the pathogen interpreting Multi-Drug Resistant (MDR) Profiles [11,12]. Detect of ESBLs-producing organisms is critical for infection control and nosocomial outbreak prevention. The purpose of this study was detect Extended-Spectrum β -Lactamases (ESBLs) genes TEM, SHV and CTX-M genes in *S. haemolyticus* isolates from cesarean section infections from Al-Basrah governorate, Iraq.

MATERIALS AND METHODS

Collection of specimens

One hundred and fifty swab samples were collected from women underwent cesarean section for both emergency cesarean delivery, and elective cesarean delivery during period from Oct. 2022 to Jan. 2023 in Al-Basrah Teaching Hospital were selected depending on their medical history.

Isolation and identification

The samples were collected from patients underwent cesarean section. The swab samples were cultivated, the samples that gave positive results for bacterial growth identified by Vitek² system.

Detection of Extended Spectrum β -Lactamase (ESBLs)

Double Disk Approximation Method (DAM)

The *S. haemolyticus* isolates was tested for produce Extended Spectrum β -Lactamase (ESBLs) by cultured on Mueller-Hinton agar plates, with disks of 20 μ g amoxicillin / 10 μ g clavulonic acid was placed in the center of the agar surface in center, (30 μ g) cefotaxime, (30 μ g) ceftriaxone, (30 μ g) ceftazidime, and (30 μ g) aztreonam arranged around it were approximately (20-30 mm center to center). The test was positive after an overnight incubation, with an enhanced zone of inhibition between disks [13].

Detection of ESBLs genes

Three specific primers that were used for the amplification of β -lactamase genes include: *TEM*, *SHV* and *CTX-M* genes **Tab. 1**.

RESULTS

From Oct. 2022 to Jan. 2023, 150 swab samples was collected from cesarean section patients at Al-Basrah Teaching Hospital were gave 57(38%) positive bacterial growth, 93(62%) negative for bacterial growth, and 51(89.5%) Gram -positive bacterial isolates, while 6(10.5%) Gram-negative bacterial isolates. As shown in **Tab. 2**.

Identification of bacterial growth by using Vitek² system have been emerged various bacterial species, the most predominant were *S. haemolyticus*, out of 57 bacterial isolates the 39(68. 42%) isolates, *S. aureus* 6(10.53%), *Klebsiella* spp 4(7.00%) isolates, *Staphylococcus saprophyticus* 3(5. 30%) isolates, *E. coli* 2 (3.50%) isolates, *S. sciuri* 1(1.75%) isolate, *S. hominis* 1(1.75%) isolate, and *E. faecium* 1(1.75%) isolate, as shown in **Fig. 1**.

In present study emerged that out of (n=39) isolates

were distributed to 28 (82%) *S. haemolyticus* isolates and 6(18%) *S. aureus* isolates was choice. The 25 (74%) *S. haemolyticus* isolates and *S. aureus* 6 (18%) isolates were gave positive results for producing extended-spectrum β -lactamases (ESBLs). While, 3(8. 8%) *S. haemolyticus* isolates were shown negative results for producing ESBLs by using of the double approximation method (DAM), as shown in **Fig. 2**.

Also the results of the current study revealed out of 28(82%) *S. haemolyticus* isolates and 6(18%) *S. aureus* isolates the 33(97.1%) isolates gave positive results for the detection of *TEM* and *SHV* gene. While the 1(2.9%) isolate was shown negative result for the detection of *TEM* and *SHV* gene as shown in **Fig. 3**. and **Fig. 4**. While the results in present study were showed that all 34(100%) *Staphylococcus* spp. were gave positive results for detection of the *CTX-M* gene as shown in **Fig. 5**.

DISCUSSION

The study found that Gram-positive bacteria isolates (89.5%) had a higher prevalence than Gram-negative bacteria (10.5%), contradicting previous studies that found a higher prevalence of Gram-negative bacteria (63.6%) and Gram-positive bacteria (36.4%) [14-16], these results are not compatible with this study. Whereas, another study results showed that (68. 4%) isolates Gram-negative bacteria and (31.6%) Gram-positive [17].

This variation could be attributed to environmental factors, geographical location, education level, and patients who took antibiotics before samples [18-20]. Factors such as hormonal changes, depression, menopause, hospital-acquired infections, immunocompromised patients, and long hospital stays can also contribute to the increased risk of infections [21-23]. Antibiotic resistance genes are also a significant factor [24-26].

The Vitek² system is an automated instrument designed for rapid and accurate identification of most staphylococci in clinical specimens [27-29]. It has been proven effective in detecting Gram-positive cocci and Gram-negative rods, with 99% accuracy and reproducibility confirmed by multiple independent studies [29]. The Colorimetric Vitek² GP card is suitable for clinical samples, and has been praised for its performance in detecting Gram-positive and Gram-negative rods [30].

Tab. 1. Specific primers used for detection of extended spectrum β -lactamase genes.

Primers	DNA Sequences	Length	Product Size bp	Reference
<i>bla_{TEM}</i>	F: 5'-CATTTCGGTGTCCCTTATTC- 3'	22	800 bp	Perez, et al. [14]
	R: 5'-CGTTCATCCATAGTTGCCTGAC- 3'	22		
<i>bla_{SHV}</i>	F: 5'- AGCCGCTTGAGCAAATTAAC- 3'	21	713 bp	Perez, et al. [14]
	R: 5'- ATCCCGCAGATAATCACCAC- 3'	21		
<i>bla_{CTX-M}</i>	F: 5'- CGCTGTTGTTAGGAAGTGTG- 3'	20	754 bp	Ramachandran, et al. [15]
	R: 5'- GGCTGGGTGAAGTAAGTGAC- 3'	20		

Tab. 2. Percentage of bacterial species isolated from cesarean section infections.

Total Number of Isolates	Gram +ve	Gram -ve	Percentage Total
57	51(89.5%)*	6(10.5%)	(100%)

Fig. 1. Frequency of bacterial species isolated from cesarean section infections.

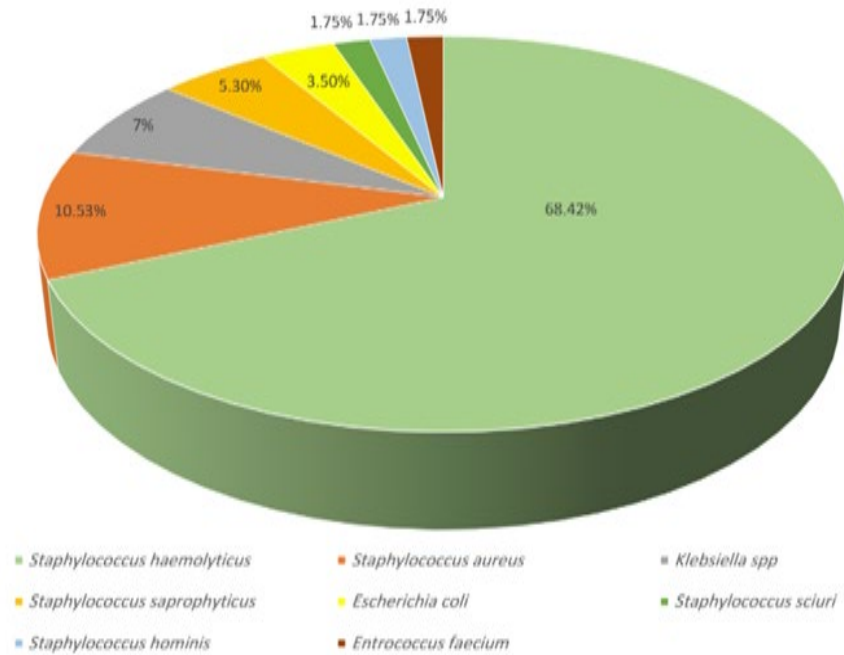


Fig. 2. Double-disc approximation (DAM) test to detect ESBLs produced isolates. **A)** Positive result for detect ESBLs and **B)** Negative result for detect ESBLs.

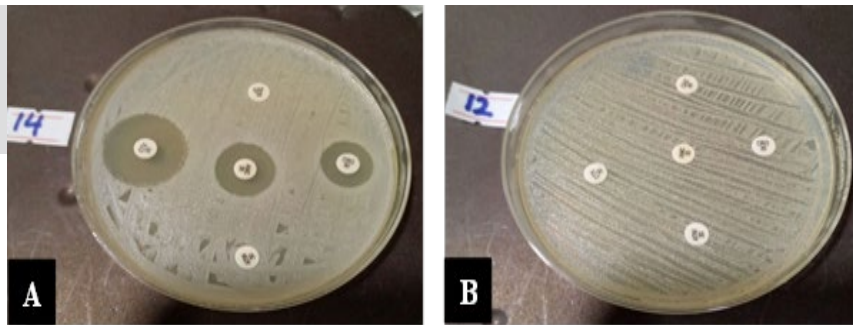


Fig. 3. Agarose electrophoresis patterns of TEM gene.

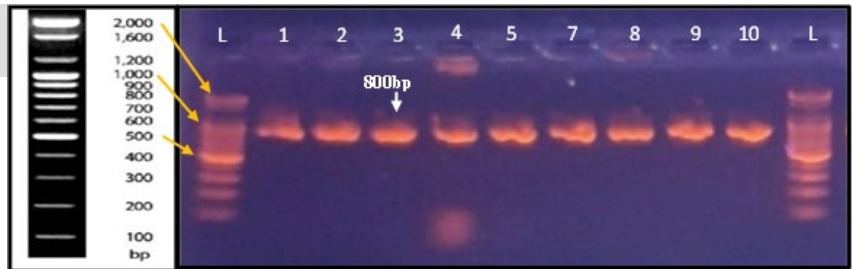
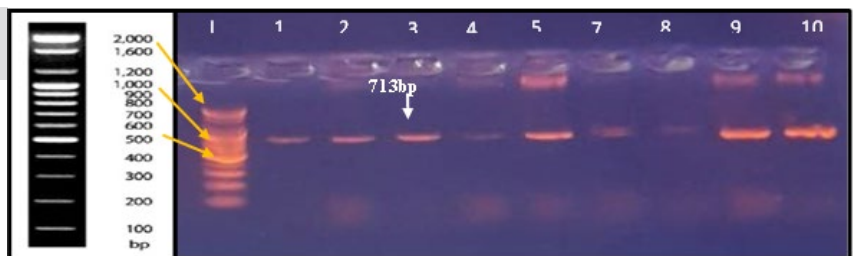


Fig. 4. The PCR amplifying products of SHV gene.

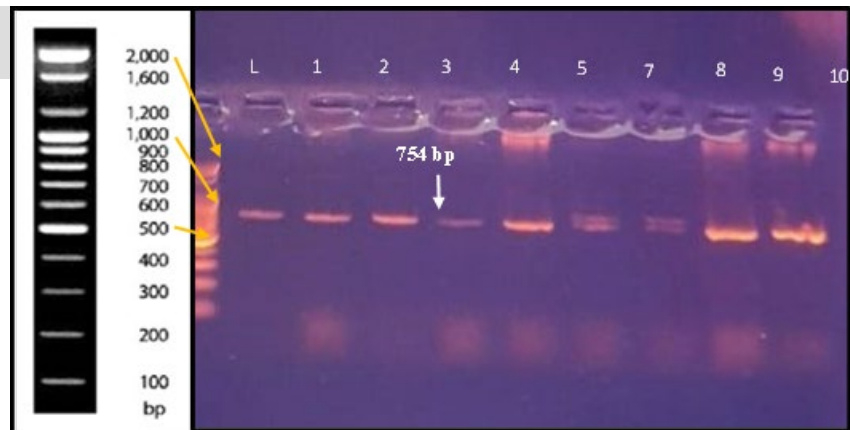


The results of the present study showed that (92%) isolates were gave positive result for producing extended-spectrum β -lactamases, which agree with study by Hassuna, et al. [31] in Egypt that found (59. 7%) of their *E. coli* isolates were have ability to produce ESBLs. While the present study that disagreed with the study by Pandit, et al. [32] in Nepal which found that ESBLs production was at

(40. 3%). Moreover, the results of other study revealed that (90%) isolates were produced ESBLs [33]. These results resembled with results.

The results of the present study showed that (97. 1%) isolates were gave positive result for TEM and SHV genes, this results which not agreement with study of Abdi, et al. [34] who reported (15%) of SHV gene. The results of

Fig. 5. PCR amplified products of CTX-M gene.



the current study emerged that (97.1%) isolates were gave positive results for *SHV* gene, this results was compatible with study of Karimi, et al. [35] who reported that (82%) of isolates have this gene. This result was harmonious with study of Al-Ezee, et al. [36], were found (100%), but the study of Shahid, et al. [37] who were found (20%), and the study of Mahmoud, et al. [19] were found (17%), and the study of Alipour, et al. [38] who reported that absence of *SHV* gene.

The current study showed that (100%) isolates were gave positive results for *CTX-M* gene. While the results of other studies revealed that (77.4%) isolates were *CTX-M* positive results [39]. On the other hand the results of other studies revealed that (43.8%) isolates were gave positive result for *CTX-M* gene [40]. This result harmonious with study of Park, et al. [41]. Also bla *CTX-M* was found in another study of Maleki, et al. [42] who were found (92%)

and [43] (100%). These results were compatible with present results.

CONCLUSION

The most isolates of *S. haemolyticus* and *S. aureus* were multi drug-resistant (MDR) were detected by using Vitek² compact system and most isolates of *S. haemolyticus* and *S. aureus* were producing extended-spectrum β -lactamases (ESBLs).

DISCLOSURE

None.

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