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PREVALENCE OF *CLFA* AND *CLFB* GENES IN *STAPHYLOCOCCUS AUREUS* ISOLATED FROM PATIENT WITH URINARY TRACT INFECTION IN AL-DIWANIYA, IRAQ

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ABSTRACT

Background: *Staphylococcus aureus is a major nosocomial pathogen responsible for a vast array of infections, including urinary tract infections (UTIs). Biofilm formation, mediated by factors like ClfA and ClfB proteins, contributes to S. aureus pathogenesis. This study aimed to investigate the occurrence of ClfA and ClfB genes alongside the antimicrobial susceptibility testing of S. aureus isolates from patients with UTIs.* ***Methods:*** *A total of 42 S. aureus isolates were recovered from patients diagnosed with UTIs attended to private clinics in Al-Diwaniya city/ Iraq during January to May 2023. Identification was confirmed using the VITEK-2 system. Antimicrobial susceptibility testing was done by the Kirby-Bauer method against a panel of commonly used antibiotics. Conventional PCR was employed to detect the presence of ClfA and ClfB genes.* ***Results:*** *The study revealed high resistance rates were observed for Ampicillin (100%), Ampicillin/Cloxacillin (83.3%), Cefoxitin (78.6%), and Amikacin (73.8%) and Methicillin (71.4%). Conversely, resistance rates were lower for Meropenem (19.0%), Ciprofloxacin (21.4%), and Vancomycin (35.7%), indicating their potential continued use for UTI treatment. PCR analysis demonstrated a high occurrence of ClfA and ClfB genes within the S. aureus population. A significant majority (92.85%) of isolates harbored the ClfA gene while, 76.2% of isolates possessed the ClfB gene, highlighting its potential contribution to S. aureus pathogenesis in UTIs.* ***Conclusion:*** *ClfA and ClfB is highly distributed in S. aureusa, studies needed to evaluate molecular pattern of these genes and relationship with other virulence factors. Results of the present study highlight the need for continued invastigation of antibiotic resistance profile in S. aureus across in the country.*

INTRODUCTION

Staphylococcus aureus (S. aureus) is a ubiquitous pathogen responsible for a wide spectrum of infections, from minor skin ailments to life-threatening pneumonia and sepsis. This versatility stems from its potent virulence factors and concerning ability to resist a broad range of antibiotics (1). ClfA and ClfB proteins, anchored within the S. aureus cell wall, play a critical role in pathogenesis (2). Notably, ClfA binds to various host proteins, including fibrinogen, to enhance bacterial adherence and biofilm formation, a crucial step in establishing chronic infections (2). Researches revealed *ClfA* may also bind to host cells directly, enhancing immune evasion and tissue invasion (3). Similarly, *ClfB* participates bacteria to adhere to host epithelium, especially in the nasal canals, promoting colonization - a key factor in establishing infection (4). The possible relation between *ClfA/ClfB* and resistance to antibiotics is an interesting to investigate. However, some studies showed that there is no direct correlation (5), recent studies suggest a greater complexity interaction. For example, a study in 2023 showed that *ClfA* expression could trigger susceptibility to some β-lactam antibiotics through regulation.

METHODOLOGY

Bacterial Isolation and Identification

Urine samples were collected from patient suffering of urinary tract infection who attended to private clinics in Al-Diwaniya city/ Iraq during January to May 2023. samples collected by a sterile container and was streaked onto blood agar using standard loop method and incubated at 37˚C for 24 hr. isolated colonies in positive cultures that had morphological characteristics of staphylococcus were sub cultured onto mannitol salt agar (Accumix /India). Bacterial isolates that showed positive culture in mannitol salt agar tested for identification using VITEK 2-compact system Biomerieux (France) according manufacture instructions: First, a small amount of a bacterial colony is mixed with saline solution. This mixture is then adjusted to McFarland standard solution (1.5×108 cell/ml). The adjusted sample is loaded into the testing cassette, which is then placed into the VITEK 2. The machine reads barcode information from the cassette and the sample to begin the test.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was done based on Kirby-Bauer disks diffusions methods (11) using Muller Hinton agar and antibiotic disks (Himedia / India): Amikacin(30µg), Ampicillin(25µg), Ampicillin\Cloxacillin (30µg), Azithromycin (15µg), Cefoxitin(30µg), Ceftriaxone (30µg), Ciprofloxacin (5 µg), Erythromycin(10µg), Meropenem(10µg), Methicillin(10µg) and Vancomycin(30µg). Bacterial growth inhibition zone around the discs were measured by caliper and compared with National Community for Clinical Laboratory Standard (CLSI, 2020) (13).

DNA Extraction

S. aureus DNA extracted and purified using Genomic DNA Mini Kit (Geneaied/ Taiwan) according to manufactures' instructions.

PCR assay

DNA templates were set to PCR using sets of primers (F and R) listed in (table 1). All PCR components were mixed on ice under sterile condition in laminar flow by using PCR reaction mixtures conducted in 0.2 ml Eppendorf tube with 25 µl reaction volumes, which contain: 12.5µl Go Taq® Green Master Mix X2, 2.5 µl(10 pico/ µl) forward primer, 2.5 µl(10 pico/ µl) reveres primer, 5 µl DNA template(250 ng), and2.5 µl nuclease-free water.

Table (1) sequence of primers of the present study

gene	Sequence	Source	Product Size	Reference
clfA	F-GCATTTAATAACGGATCAGG	Macrogen/ Korea	957 bp	12
clfA	R-TGAATTAGGCGGAACTACA			
clfB	F-ATGGTGATTCAGCAGTAAATCC		828 bp	12
clfB	R-CATTATTTGGTGGTGTAACTCT			

PCR tubes were placed in thermal cycler (BioRad, USA) for amplification of genes and programmed as mentioned in table (2).

Table (2) PCR thermocycling conditions of primers of the present study

Gene	Temperature (C °)/ Time					hold	Cycle number
	Initial denaturation	Cycling condition			Final extension		
		denaturation	annealing	extension			

clfA	105/5 min	94/30 sec	61.5/30sec	72/ 2min	72 / 5	4 C	30
clfB	105/5 min	94/30 sec	60/1min	72/2min	72/10min	4 C	30

After thermocycling, gel electrophoresis in agarose gel 1.5% (g/v) prepared in electrophoresis buffer TBE IX and visualized by staining with ethidium bromide. DNA bands size measured, under UV light, in comparison with 100 bp ladder. IX TBE buffer loaded to the electrophoresis chamber to cover surface of the gel. The electric current was conducted at70 volt for 1.5 hour. Finally results visualized using UV light.

RESULTS AND DISCUSSION

A total of 42 *S. aureus* recovered from patients suffering of urinary tract infections who attended to private clinics in Al-Diwaniya city/ Iraq during January to May 2023 regardless of gender and age, VITEK 2-compact system used in present study which is a colorimetric based system that has a high accuracy in species identification (15,16)., However, VITEK 2-compact system technology is still being used in Iraq for routine work in the healthcare laboratories.

Antimicrobial susceptibility testing of the present study to 42 *S. aureus* revealed that all isolates were multidrug resistant (MDR), they are resistant to at minimum three classes of antibiotics (14). Results showed that (100%) isolates were Ampicillin resistant, in addition, *S. aureus* showed high resistance rate to Ampicillin\Cloxacillin (83.3%), Cefoxitin (78.6 %), Amikacin (73.8 %) and Methicillin (71.4 %), table (3). The results emphasizing the emergence of high resistance in *S. aureus* strains, this finding agrees with a previous finding in Iraq (17, 18) which reported a high rate of MDR *S. aureus*. On the other hand, results of the present study demonstrated that isolates showed a low resistance rate to Meropenem, Ciprofloxacin and Vancomycin in percentage (19.0 %), (21.4%) and (35.7%) respectively, table (3). However, there is a positive aspect, the study shows an encouraging susceptibility to these antibiotics which still effective treatment choice to *S. aureus* infections in the country, this result is similar to other results reported a continued susceptibility to these antibiotics in some *S. aureus* strains. (12, 19).\

Table (3): antimicrobial susceptibility testing of 42 *S. aureus*

Antibiotic	Resistant	Sensitive	Total
Amikacin	31(73.8 %)	11(26.2%)	42
Ampicillin	42(100.0%)	0(0%)	42
Ampicillin\Cloxacillin	35(83.3%)	7(16.7)	42
Azithromycin	19(45.2 %)	23(54.8%)	42
Cefoxitin	33(78.6 %)	9(21.4%)	42
Ceftriaxone	17(40.5 %)	25(59.5%)	42
Ciprofloxacin	9(21.4%)	33(78.6%)	42
Erythromycin	24(57.1 %)	18(42.9%)	42
Meropenem	8(19.0 %)	34(81.0%)	42
Methicillin	30(71.4 %)	12(28.6%)	42
Vancomycin	15(35.7%)	27(64.3%)	42

A PCR assay was conducted in this study to detect *ClfA* and *ClfB* genes in *Staphylococcus aureus* isolates. The results revealed a high prevalence of both genes, with 39 isolates (92.85%) amplifying the *ClfA* gene, resulting in a PCR product size of 957bp (Figure 1). Similarly, 32 isolates (76.2%) yielded amplification products of 828bp using *ClfB*-specific primers (Figure 2). These findings are in partial agreement with Eftekhar *et al*. (20), who reported a detection rate of 71.4% for the ClfB gene but showed a lower prevalence (78.6%) for ClfA compared to this study. Conversely, the results align more closely with Al Ani et al. (21) who detected *ClfA* and *ClfB* genes in 100% and 81.3% of *S. aureus* isolates from Baghdad, respectively. Interestingly, this study's findings significantly diverge from those of Mohammadi et al. (22), who observed *ClfA*

and *ClfB* in only 50.6% and 54.2% of *S. aureus* isolates, respectively. This observed variability in gene prevalence across different studies highlights the potential for geographical or strain-specific variations in the distribution of these virulence factors among *S. aureus* populations.

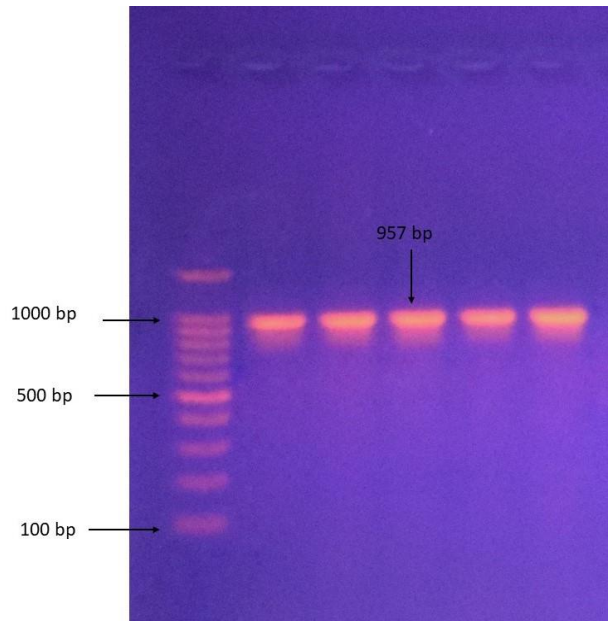

Figure (1): positive results of agarose stained with Ethidium bromide of PCR products amplified with *ClfA* primers of S. aureus. extracted DNA (957bp).

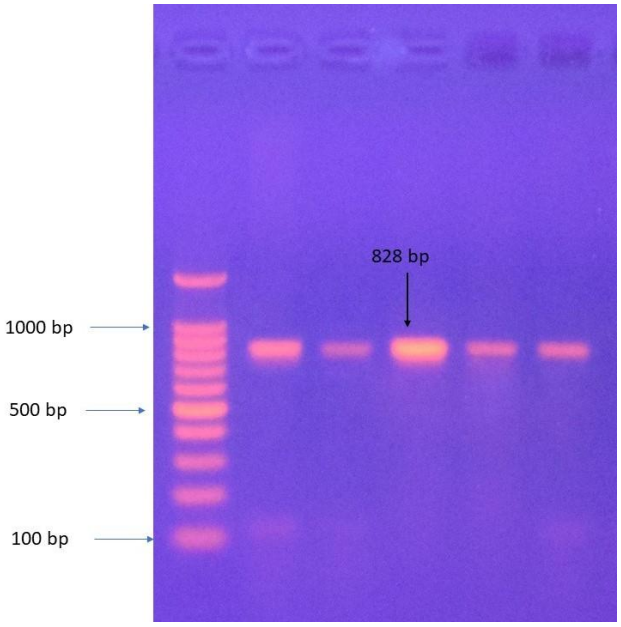

Figure (2): positive results of agarose stained with Ethidium bromide of PCR products amplified *ClfB* primers of S. aureus. extracted DNA (828bp).

CONCLUSION

In conclusion, *ClfA* and *ClfB* is highly distributed in S. *aureus*, however, studies needed to investigate other aspects in these genes regarding with molecular pattern and relationship with other virulence factors. Results of the present study highlight the need for continued researches of antibiotic resistance profile in *S. aureus* across in the country. on the other hand, it is a crucial to explore of alternative treatment choices and researching of a new effective antibiotic directed to resistant strains.

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