Molecular detection of MRSA isolated from female Patients in Al-Diwaniya city

Nagham Taleb Maki Al-Ibraheemi*[1], Mohammed Abdulwahab Ati Al-Askeri[2]
[1]Iraqi ministry of Health, Al-Diwaniyah health directory, Women's and Children General Hospital in Al-Diwaniyha/Iraq
[2] University of Al-Qadsiyah, College of Biotechnology/Iraq
*Corresponding author nagham.talib@gmail.com

Abstract
Staphylococcus aureus may be a gram-positive infective agent that is main reason of loads of diseases like toxic shock syndrome syndrome, pneumonia, food poisoning, and coccus scaled-skin syndrome in Iraq. Staphylococcus aureus strains are showing proof against many generations of antibiotics, significantly methicillin-resistant strains (MRSA). yet, no enough previous studies were conducted to research the biological science aspects of enetrotoxins in clinical isolates of Staphylococcus aurus in patients in Iraq.the study was conducted in AD Diwaniyah teaching hospital . Patients diagnosed cancer who had cellulitis, abscesses in addition as soft tissue infections were enclosed within the study. Staphylococcus aureus were cultivated and diagnosed . Polymerase chain reaction was used to identify the antibiotic susceptibility test and enterotoxins genes. Our findings revealed that 30 S.aureus isolates were obtained from 50 specimens, including 8 (26.6%) from cellulitis, 10 (33.3%) from abscesses, and 12 (40%) from other soft tissue infections. Antibiotic resistance was confirmed in S.aureus isolates. Seb genes were found in 21 (48%) of 25 S.aureus isolates. We concluded that isolated *Staphylococcus aureus* from patients had the ability to produce enterotoxins and antibiotic resistance.
Keywords: Toxins , Antibiotics, PCR, Iraq and Seb genes

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that can cause a lot of diseases like toxic shock syndrome, pneumonia, food poisoning, and staphylococcal scaled-skin syndrome[1] . *Staphylococcus aureus* strains resistant to many classes of antibiotics, particularly methicillin-resistant strains (MRSA), are prevalent in nosocomial infections and are associated with high morbidity and mortality rates[2, 3].
The pathogenicity of *S. aures* mainly arises from the virulence factors: proteases, enterotoxins, cytolytic toxins, protein A, clumping factor, and others . Antibiotic resistance is typically caused by antibiotic resistance proteins, such as penicillin binding protein 2a produced by the mecA gene (MRSA)[4-6]. S. aureus produces a variety of exo-proteins, including staphylococcal enterotoxins (SEA; SEB; SECn; SED; SEE; SEG, SHE; and SEI)[7].
Exfoliative pollution which include ETA, ETB, leukocidin toxic (surprise syndrome toxinTSST-1) and staphylococcal enterotoxins (pyrogenic toxin superantigens PTSAgs) were discovered to have an essential position in proliferation no matter antigenic specificity[8, 9].
The majority of S. aureus isolates from diabetic foot ulcers produced a excessive range of Sags, at the same time as SAg exotoxins have been discovered to make a contribution extensively to different principal illnesses[10, 11]. A better range of S. aureus traces remoted from wound grades "2-4" primarily based totally on (Wagner Classification System) have been discovered to have genes encoding enterotoxins SEA and SEI than traces from grade "1" ulcer, making them effective markers to distinguish colonization inside infection. -toxin is a major pathogenicity component of S. aureus in pores and skin infections[12]. This (pore-forming toxin) is composed mostly of beta sheets that are released as a water-soluble monomer via the majority of S. aureus lines and preferentially target purple blood cells[13]. The (Panton-Valentine leukocidin) is a very efficient cytotoxic component composed of distinct protein components, specifically (LukS-PV slow) (LukF-PV fast)[14]. The energetic toxin causes neutrophil lyses by the creation of holes in its membrane, which is linked to dermonecrosis, ongoing SSTI, and recurrent mucocutaneous infection[15].
The motive of this observe changed into to investigate the virulence elements as entrotoxin genes in S.aureus isolated from patients in AD Diwanyah hospital so that you can increase a singular method for contamination control.

2. Materials and Methods

Isolation of *S. aureus* from clinical specimens

A total of forty specimens (cellulitis, abscesses, and sore tissue infection) were collected from patients at AD Diwaniyah Teaching Hospital in Al-Qadsiyah

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governorate in order to isolate S. aureus. All collected samples were immediately cultivated in nutrient broth medium and incubated at 37 0C for twenty-four hours. Following incubation, serial dilutions for each pattern were performed, and 1 was spread on blood agar and mannitol agar media and incubated at 37 0C for 24 hours. According to, the isolates were microscopically and biochemically characterized.

Antimicrobial susceptibility test

A modified Kirby Bauer's Disk diffusion technique and commercially available antibiotic discs were used to test antibiotic susceptibility. According to the manufacturer's instructions and WHO, the resistance test was carried out based only on the diameter of the inhibitory zone. The antibiotics used were, Oxacillin(OX,1 µg), Azithromycin (AZM, 15 µg), Ciprofloxacin(CIP,5 µg), Tetracycline(T,30 µg), Gentamicin (G, 10µg), Penicillin(P,10 µg) , doxycycline (DO,30 µg), Cefoxitin (CX,30 µg) , Vancomycin (VA,30 µg), Ofloxacin(OFX,5 µg), Chloramphenicol (C,30 µg) and Ampicillin(A, 10 µg).

Molecular characterization of Enterotoxin genes

Genomic DNA extraction

The genomic DNA of S.aureus was extracted using the promega kit (USA) in accordance with the manufacturer's instructions, and the extracted genomic DNA was kept at -20°C for amplification with the use of polymerase chain reaction.

Amplification of Enterotoxin genes

Using ahead and opposing oligonucleotide primers, conventional PCR became applied to increase enterotoxin genes[16-18]. These primers had been furnished in lyophilized shape via way of means of (bioneer DNA Company, Korea) and dissolved in unfastened nuclease distilled water to offer a very last awareness of 100 picomole /l in line with the manufacturer company's recommendations, then 10 picomole /l of every primer became prepared via way of means of mixing 10 ul of primer stock solution with 90 ul of unfastened nuclease distilled water and aliquoted and stored at -20C as showed in Table 1 and 2 .

PCR became finished on a cycler machine (LABNET) the use of amplification settings of 40 cycles of 94°C/4 minutes, 94°C/30 seconds, 59°C/forty five seconds, and 72°C/one minute, observed via way of means of a very last extension step of 72°C/5 minutes.

PCR products were analyzed on an Agarose gel (2%) using a horizontal electrophoresis device, after which the gel was immersed in 1X TBE buffer and samples were placed into the gel's wells. After one hour, the electrophoresis was completed. The gel was stained with 10ul of GelGreen stock solution after electrophoresis. Using a UV transilluminator and DNA bands were visible[19, 20].

Table (1) : Sequence of oligo nucleotide used for amplification of Enterotoxin gene

Gene	Sequences	Product size
SEB	F 5- CAACCAGACCCTATGCCAGA-3 R 5- AGTTTTACCACCTGTAACTTTACCT-3	321 bp

Table (2): Preparation of PCR reaction mixture

Volume for single tube (µl)	Content	No.
12.5	Green master mix	1
1	Forward Primer	2
1	Reverse Primer	3
2	DNA template	4
8.5	Nuclease free water	5
25	Total volume	

3. Results and Discussion

Our findings examined that 30 S.aureus isolates had been recovered from 50 samples, at the side of twelve (30%) from cellulitis, twenty (50%) from abscesses, and 8 (20%) from mild tissue infections. All isolates capable of fermenting mannitol and generating catalase. Catalase production is a virulence factor in S. aureus, allowing bacteria to withstand intra- and extracellular lack of existence via hydrogen peroxide (H2O2). Antibiotic responses of S.aureus isolates severa based mostly on antimicrobial resistance percentages. Penicillin (90%) and Ampicillin (90%) had the very first-rate resistance, followed via Chloramphenicol (85%), Vancomycin (74%), Cefoxitin (68%), and Ofloxacin (50%), at the same time as the isolates had been more sensitive to the following antibiotics: Gentamicin (34%), Ciprofloxacin (29 %), Doxycycline (34%), Azithromycin (30%), and Tetracycline (30%), as demonstrated in figure (1)

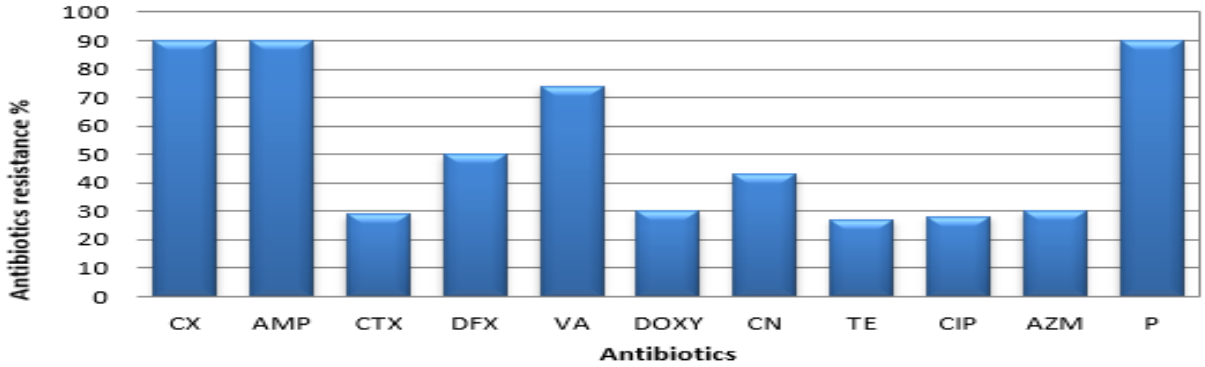

Figure 1: Antibiotics Resistance Percentage

Oxacillin (OX), Azithromycin (AZM), Ciprofloxacin(CIP), Tetracycline(T), Gentamicin (G), Penicillin(P , doxycycline (DO), Cefoxitin (CX) , Vancomycin (VA), Ofloxacin(OFX), Chloramphenicol(C)and Ampicillin(A)

The DNA was extracted with with the PrestoTM Mini gDNA Bacteria Kit ranged among 10 and 87 ng/ml, and horizontal gel electrophoresis become used to decide the purity of the extracted DNA as showed in figure 2.

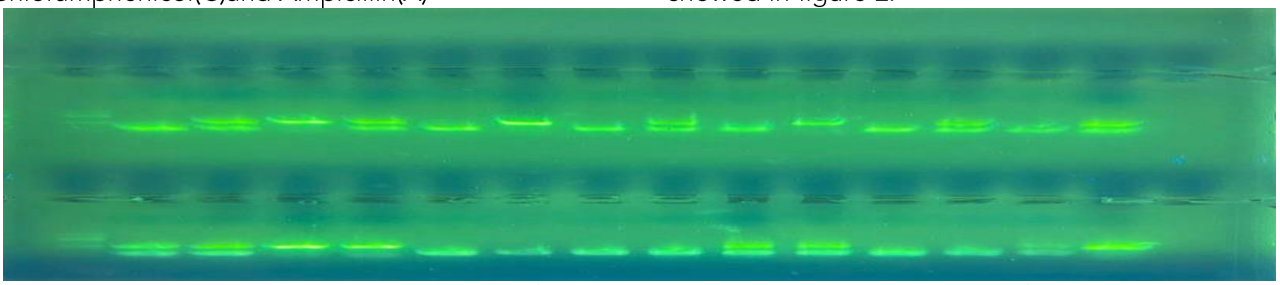

Figure (2): 2% Agarose gel electrophoresis of PCR products obtained by using SEB-specific primer. Seb gene products with 325bp, 100bp DNA ladder , Agarose stained with GelGreen dye.

The current research determined that 21 (48%) of the 25 S.aureus isolates carried *seb* genes, which agreed with preceding findings that 30 of the sixty S. aureus strains (48.3%) harbored genes coding for pollution which includes saw (12.9%), eta (12.9%), and seb (9.6%).

The risky human pathogen *Staphylococcus aureus* creates a mess of pollution that make contributions to its pathogenicity potential. Staphylococcal enterotoxins (SEs) are a subgroup of the staphylococcal terrific antigen family, which additionally includes poisonous surprise syndrome toxin 1 (TSST-1) and enterotoxin-like serotypes[21]. All massive antigen pollution are potent nonspecific stimulators of T lymphocytes. They pass the typical antigen-specific restrictions of immune cell activation by establishing a bridge between the major histocompatibility class (MHC) II receptors on antigen-delivering cells and V chains on T-cell receptors[18, 22, 23].

Our findings confirmed the ability of remoted *Staphylococcus aureus* to produce enterotoxins, which is consistent with the findings of it.

4. Conclusion

The risky human pathogen *Staphylococcus aureus* is a manufacturer of many pollutants that together make a contribution to its virulence cappotential the staphylococcal enterotoxins (SEs) are a subset of the staphylococcal tremendous antigen family, which additionally consists of poisonous surprise syndrome (TSS) toxin 1 (TSST-1) and enterotoxin like serotypes. All major antigen contaminants are potent nonspecific stimulators of T lymphocytes. They bypass the conventional antigen-specific controls of immune cell activation by establishing a bridge between major histocompatibility class (MHC) II receptors on antigen-turning in cells and V chains on T-cell receptors.

Our findings demonstrated the ability of isolated *Staphylococcus auras* to produce enterotoxins, which is consistent with previous research.

5. Acknowledgements

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Ethical Statement

The ethical statement was approved by University Ethics Committee for Human Research

Conflict of Interest

The author has declared no potential conflicts of interest.

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