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# Characterization of antibiotic resistance of *Staphylococcus aureus* isolated from patients with diabetic foot ulcers in Wasit Province

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## Abstract

Diabetic foot ulcers are one of the most common public health issues worldwide, putting a social strain on those who suffer from them; chronic ulcers that lead to amputation are a severe disease that can reduce diabetic patients' quality of life. A total of seventy-five samples were collected from diabetic foot ulcer with age (30 - 89) years, during the period from 1st October 2020 to15th January 2021, admitted to Al-Karama teaching hospital and private clinics in Wasit province. The results revealed that the frequency among males 50 (67 %) more than female 25 (33 %).The sample distribution according to age it appears high (42.7 %) with group (50-59) years. The results shows that Gram negative bacteria reveals a high rate 57.4% (n=35) which includes *K. pneumoniae* that show a high percentage 24% (n=18), then *E.coli* 13.2% (n=10). followed, *P.aeruginosa* 4.0% (n=3) and *P.mirabilis* 2.7(n=2) and *A.baumannii* 2.7% (n=2). While Gram-positive bacteria recorded 42.6% (n=26) ,which include *S. aureus* was the most isolated bacteria in this study with percentage 33.3 % (n=25) followed by *streptococcus group B* 1.4% (n=1).

Methicillin resistant *Staphylococcus aureus* was recorded with 92.3 % in all isolates and results of MRSA isolates antibiotic susceptibility for *S. aureus* as shown: the maximum resistance level to the Oxacillin (100 %), penicillin (100 %), cefoxitin (92.3 % ), vancomycin (61.5 % ), clindamycin (61.5 % ), followed by gentamicin (53.8 %), erythromycin (53.8 %), while the lowest resistance with ciprofloxacin (38.4 %). The maximal *S. aureus* sensitivity has been to Trimethoprim-Sulfamethoxazole (57.8 %) , vancomycin (38.5 %) , ciprofloxacin (30.7 %) and gentamicin (34.6 %). DNA of twenty-five isolates *S. aureus* were extracted, also purity and concentration were confirmed with Nanodrop, the purity of the nucleic acid in the samples ranged between 1.8-2, while its concentration ranged from (50-360 mg / µl).

Resistance genes possessed by the *S. aureus* isolates were: *mecA* (96%), *ermC* (40%), *msrA* (8.0 %) and *aac(6')-aph(2")* (16 %). while *ermA* and *vanA* genes were absent among all isolates. In conclusion, the findings of the present study revealed that most studied isolates which had multiple antibiotic resistances.

**Keywords:** Diabetic foot ulcer; *S. aureus*; Characterization; Antibiotic resistance genes.

## 1. Introduction

A foot affected by ulceration is associated with neuropathy and peripheral arterial disease of the lower limb in a patient with diabetes. As well as diabetic foot ulcer usually fail to heal and lead to lower extremity amputation (Yazdanpanah et al., 2015). Diabetic foot ulcers are one of the most common public health issues worldwide, putting a social strain on those who suffer from them. Chronic ulcers that lead to amputation are a severe disease that can reduce diabetic patients' quality of life (Rathur and Boulton, 2007). Practically everyone will have one or more *Staph aureus* infections in his or her lifetime ( 20-50%) of the human population are often colonized with *Staph aureus* in addition it is a major

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pathogenic bacteria responsible for community-acquired and nosocomial infections, it is one of the most common human pathogenic bacteria that cause different sequelae of infections in both genders and in all age groups (Suhaili et al., 2018). Methicillin resistant *S. aureus* creates resistance to the beta-lactam antibiotics through the gaining of the *mecA* gene that encodes the penicillin-binding protein 2 a, having an extensively diminished affinity for the β-lactam antibiotics, hence conferring the resistance of β- lactam. Sources have been evaluated by cefoxitin disc diffusion test and the PCR detection of *mecA* gene (Wijesundara et al., 2019).

## 2. Material and methods

Totally seventy-five samples were collected from diabetic foot ulcer with age (30 - 89) years, during the period from 1st October 2020 to15th January 2021 admitted to Al-Karama teaching hospital and private clinics in Wasit province. Isolation the Swab samples were collected from diabetic foot ulcer with age (30 - 89) years. Culture on Manitol salt agar, Blood agar and aerobically incubated for 24h at 37°C (Bhatia, 2008). The isolated bacteria were identified according to morphological, biochemical tests and Vitek2. Antibiotic Susceptibility testing this test has been conducted by the Kirby-Bauer process on the Muller Hinton agar (Bauer et al., 1966) and the results have been interpreted based on the (CLSI, 2020). Primers were supplied by the manufacturer (Integrated DNA Technologies, USA) as lyophilized powder in eppendorf tubes (1.5 ml) as the procedure mentioned by Dahwash et al., (2021) (Table 1).

**Table 1** Primer's sequence of resistance genes

| Genes | Primer sequences (5′- 3′) | | size (bp) | Source of primer |
|---|---|---|---|---|
| *vanA* | F | GGC AAG TCA GGT GAA GAT G | 713 | (Cabrera *et al*., 2020) |
| | R | ATC AAG CGG TCA ATC AGT TC | | |
| *ermA* | F | TATCTT ATC GTT GAG AAG GGA TT | 138 | |
| | R | CTACAC TTG GCT TAG GAT GAA A | | |
| *ermC* | F | CTT GTTGAT CAC GAT AAT TTC C | 189 | |
| | R | ATC TTTTAG CAA ACC CGT ATT C | | |
| *msrA* | F | TCC AATCAT TGC ACA AAA TC | 162 | |
| | R | AAT TCCCTC TAT TTG GTG GT | | |
| *aac(6′)-aph(2″)* | F | TTG GGAAGA TGA AGT TTT TAG A | 173 | |
| | R | CCT TTACTC CAA TAA TTT GGC T | | |
| *mec A* | F | GGGATC ATAGCG TCA TTATTC | 527 | (Poulsen *et al.,* 2003) |
| | R | AAC GAT TGT GAC ACGATAGCC | | |

### 2.1. Detection of *Staph. aureus* Resistance genes

This was achieved by multiplex PCR. For *ermC, ermA, msrA and aac(6′)-aph(2″), mecA* and *vanA* genes, 1µl (25pmol/µl) of forward primer and 1µl (25pmol/µl) of reverse primer were added to PCR master mix which was prepared as in Table (4) and Monoplex PCR Mixture.

**Table (2)** Thermal cycling program for monoplex*: mecA*

| PCR step | Temp. | Time | No. of cycles |
|---|---|---|---|
| initial Denaturation | 95°C | 5min. | 1 |
| denaturation | 95°C | 1min. | 32 cycle |
| Annealing | 51°C | 30sec. | |
| Extensions | 72°C | 1min. | |
| finale extensions | 72°C | 5min. | 1 |
| Hold | 4°C | ∞ | - |

**Table (3)** Thermal cycling program for monoplex: vanA

| PCR step | Temp. | Time | No. of cycles |
|---|---|---|---|
| initial Denaturation | 94°C | 5min. | 1 |
| denaturation | 94°C | 1min. | 40 cycles |
| Annealing | 55°C | 1min. | |
| Extensions | 72°C | 2min. | |
| finale extensions | 72°C | 5min. | 1 |
| Hold | 4°C | ∞ | - |

**Table 4** Thermal cycling program for multiplex pool. 4: *ermC, ermA, msrA and aac(6′)-aph(2″)*

| PCR step | Temperatures | Time | No. of cycles |
|---|---|---|---|
| initial Denaturation | 94°C | 3min. | 1 |
| denaturation | 94°C | 30sec. | 30 cycle |
| Annealing | 55°C | 30sec. | |
| Extensions | 72°C | 30sec. | |
| finale extensions | 72°C | 4min. | 1 |
| Hold | 4°C | ∞ | - |

Then gel electrophoresis and documentation according to (Sambrook and Russell, 2001). Statistical analysis of data has been done with the use of SAS (Statistical Analysis System - version 9.1). In addition, the percentages have been compared with the use of Chi-square test. $P < 0.05$ is considered to have statistical significance.

## 3. Results and discussion

The percentage of bacterial growth, which was isolated as shown in figure (1) , this results are consistent with findings from other Iraqi studies (Raheema, 2016a; and Al-Saeedi and Raheema, 2019). Also similar result was recorded by Raheema, (2016b) and Raheema, and Abed, (2019).

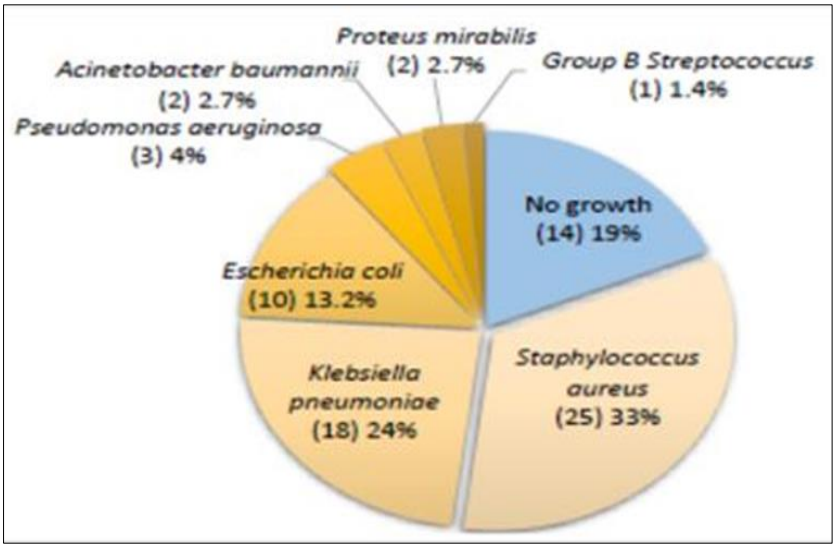


**Figure 1** Distribution of bacterial species

The results of this study, MRSA showed the highest resistance for cefoxitin with 92.3% *Staphylococcus aureus* espealis MRSA, has spread to all parts of the word and is become of significant concern in public health as one the most common causes of nosocomial infections (Raheema, and Qaddoori, 2020).The results of this study have been similar to results obtained by Al-Dahbi, and Al-Mathkhury, (2013) and Idbeis, (2019) who found all the isolates revealed complete resistant (100%) to oxacillin and cefoxitin.

The results of the antibiotic susceptibility for *S. aureus* showed that the maximum resistance level to the Oxacillin (100 %), penicillin (100 %) , cefoxitin (92.3 % ), vancomycin (61.5 % ), clindamycin (61.5 % ), followed by gentamicin (53.8 %), erythromycin (53.8 %), while the lowest resistance with ciprofloxacin (38.4 %) and Trimethoprim-Sulfamethoxazole (38.4 %). The maximal *S. aureus* sensitivity has been to Trimethoprim-Sulfamethoxazole (57.8 %), vancomycin (38.5 %), ciprofloxacin (30.7 %) and gentamicin (34.6 %) as shown in figure (2).

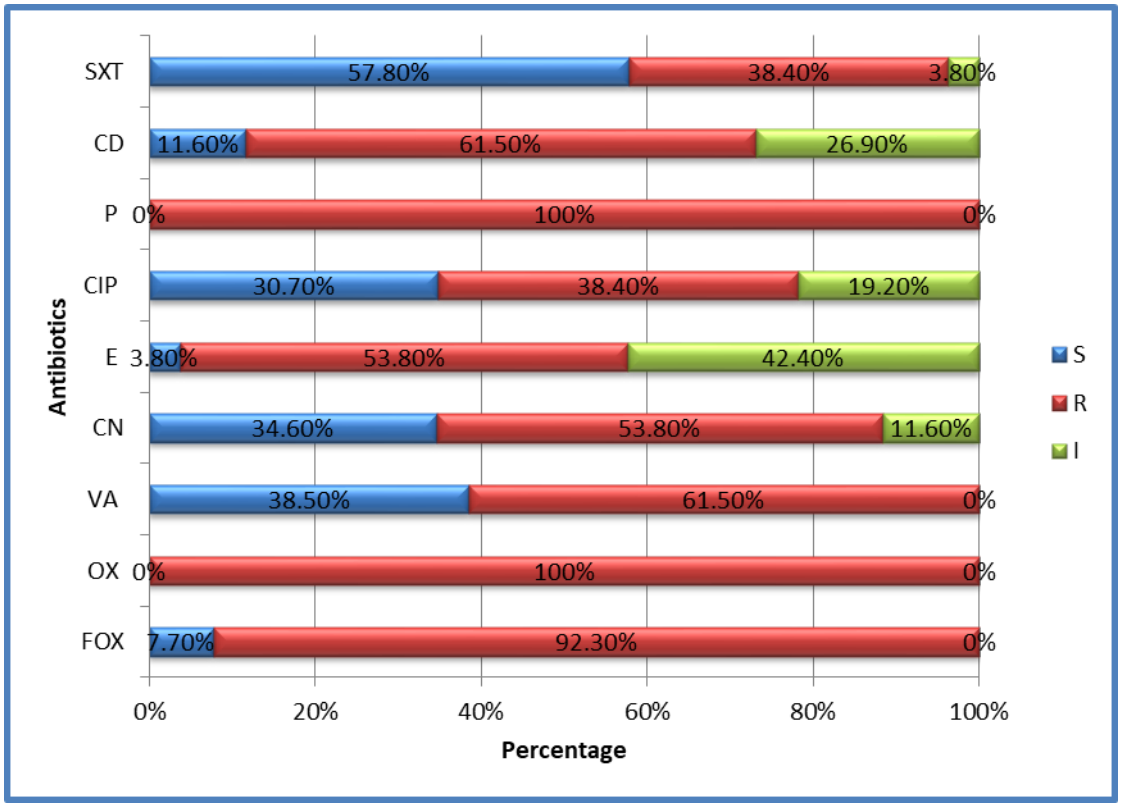


**Figure 2** The percentage of resistance for *S. aureus* isolates against antibiotics

SXT = Trimethoprime - sulfomethoxazole , CD = Clindamycin , P = Penicillin , CIP = Ciprofloxacin , E = Erythromycin , CN = Gentamicin , VA = Vancomycin , OX = Oxacillin , FOX = Cefoxitin .

The results showed that (100 %) of *S. aureus* isolates were resistant for penicillin, oxacillin this result agreed with the results that had obtained in other local studies done by each of Dogramachy, (2018); Idbeis, (2019) and Khudher and Jabur (2020). The isolates appeared high levels of resistance to penicillin (100%), also showed that 61.5% of *S.aureus* isolates were resistant to vancomycin; this result disagreed with results obtained by Saber et al., (2018) who found that the rate of resistance for vancomycin was 8%, such resistance possibly mediated via increasing thickness the cell wall of the bacteria, making it difficult for the vancomycin to enter the cell. The mutations or mechanisms that are needed for such change in cell walls haven't been specified yet (Howden et al., 2020). Vancomycin resistance has been mediated through acquiring *vanA* gene from the closely related enterococci. The gene's expression permits the modifications in peptidoglycan precursors that decrease the vancomycin binding affinity (Zhu et al., 2010).

The susceptibility rate of the isolates toward the Cefoxitin was 7.7%, this result somewhat near to the study of Al-Khafaji, (2013) who found that *S. aureus* isolates were highly resistant to methicillin in 92.3 %. Methicillin-resistant may harbor the genes of the resistance in other genome sites and on plasmids, in addition to carrying the genes of resistance in SCCmec (Deurenberg and Stobberingh, (2008) which is a possible explanation of the reason why the MRSA could resist more than 1 antibiotic besides the β-lactam (Multidrug Resistant). Reducing the permeability of the cell wall, production of chromosomal and plasmid mediated beta - lactamase are considered to be the main mechanism of resistance to methicillin (Katzif et al., 2005). In the current study, multi resistant isolates were divided into MDR, XDR and possible PDR according to the criterion proposed by Magiorakos et al., (2012), which were MDR 52%, XDR 32 % and PDR 4 %.

Regarding *mecA* gene in the present study, its prevalence was 96 % among *S. aureus* clinical isolates (Figure 3). Polymerase chain reaction amplification of mecA gene has been applied as the " gold standard" for identification of MRSA (Jonas et al., 2002) .The prevalence of *mec*A gene was in agreement with previously published studies that showed *mec*A prevalence to be 94.33% among *S. aureus* isolates (Raheema, 2019) and disagree with Kareem et al., (2015) who reported 75.5%.

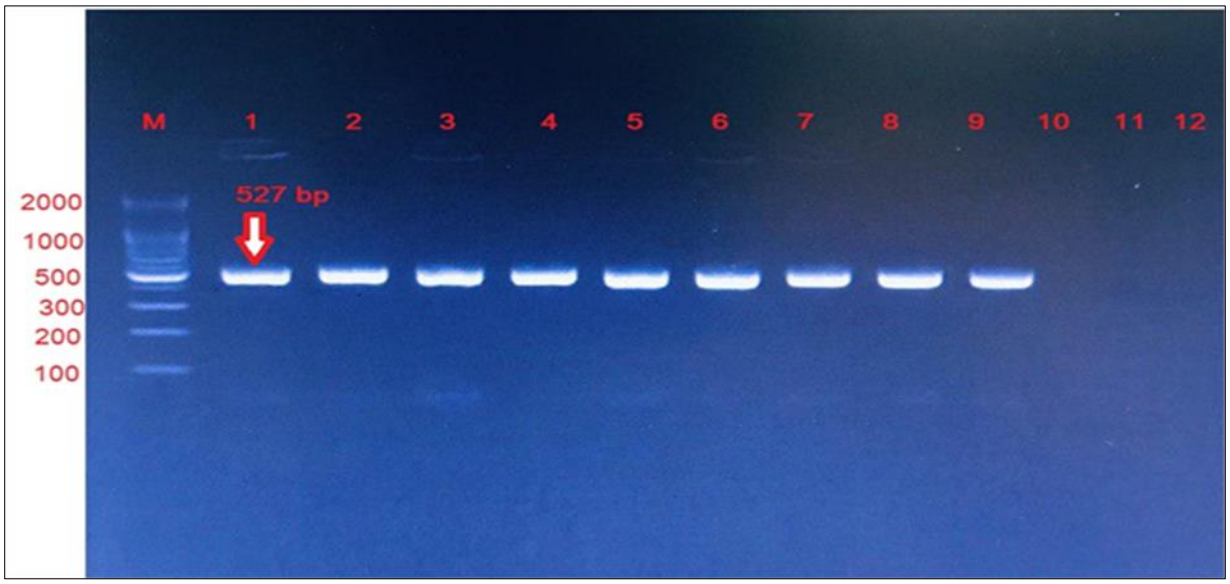


**Figure 3** Gel electrophoresis of amplified mecA gene from *S. aureus* traditional PCR. Agarose 2%, 70V/cm for 40 min, which has been stained by the ethidium bromide dye and observed on an ultraviolet trans-illuminator. Lane (M): 100bp DNA ladder. Lane (1-12 ): Amplicons mecA (527) bp gene

In the present study, erythromycin was in high frequencies (40%) with *ermC, msr*A (8.0 %) whereas, no PCR-amplification products with *erm*A Figure (4). *S. aureus* clinical isolates. This study was in accordance with different studies that had shown the *erm*A gene (7.35%) prevalence, of *erm* C gene (5.88%) and no *erm*B gene that has been recovered from the isolates of the *S. aureus* (Al-Hasnawy, 2020) in Iraq also the most prevalent resistance gene determinates was ermC (88.4%) among MRSA isolates. While the frequency of ; ermA and msrA were (76.9%) and (80.7%), respectively (Cabrera et al., 2020) and disagree with Adwan et al., (2014) in Spain who found *ermC* (61.5%) and *msrA* genes (23.1%).

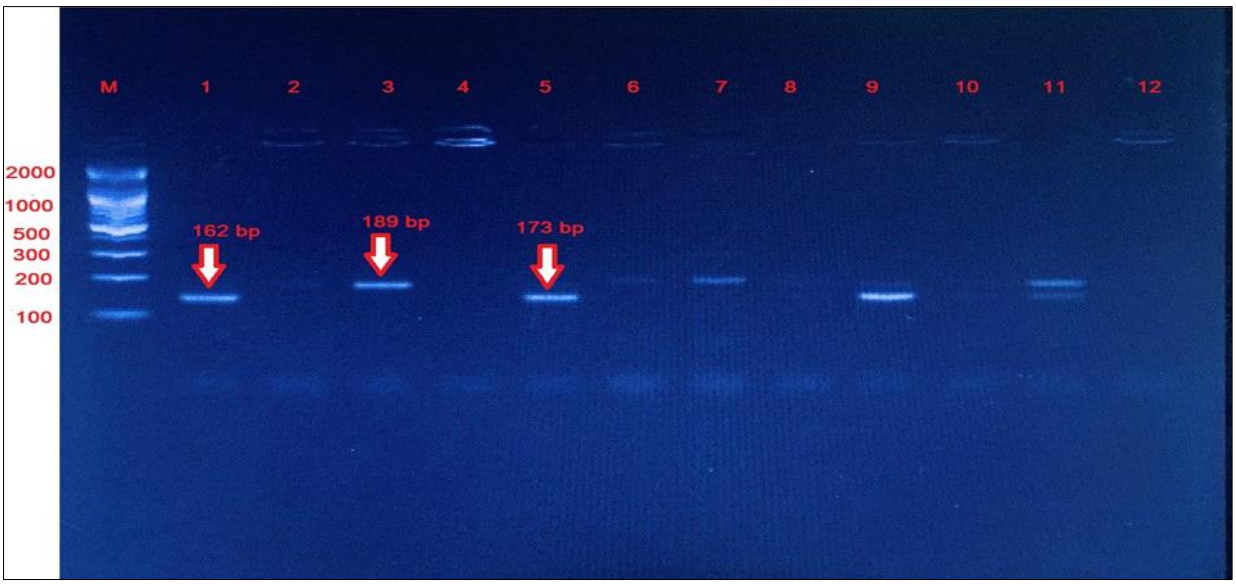


**Figure 4** Gel electrophoresis of amplified ermC , msrA , aac(6)aph(2) gene from *S. aureus* traditional PCR. Agarose 2%, 70V/cm for 40 min, which has been stained by the ethidium bromide dye and observed on an ultraviolet trans-illuminator. Lane (M): 100bp DNA ladder. Lane (1-12 ): Amplicons ermC (189) bp, msrA (162) bp , aac(6)aph(2)( 173) bp gene

Regarding *vanA* gene in the present study, no specific band detected for *vanA* genes *S. aureus* which disagreed with Zhang et al., (2004) who found ( 1.8%) also disagreed with the absence of Vancomycin resistance is due to the lack of vanA and vanB genes (Al-sherees, 2019). Regarding aac(6′)-aph(2″) gene in the present study, its prevalence was (16

% ) among *S. aureus* clinical isolates (Figure 4). This result was in accordance with different researches conducted by Ardic et al., (2006) 34%, Goudarzi et al., (2019) in Japan.

## 4. Conclusion

In conclusion, in studied the *S. aureus* is the findings of the present study revealed that most studied isolates which had multiple antibiotic resistances. Phenotypic and genotypic indicated the wide spread prevalence of Methicillin resistant *Staphylococcus aureus* among all *S. aureus* isolates, also molecular approaches are more reliable than traditional methods for detection.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The authors declared no conflict of interest.

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