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# SOME VIRULENCE GENES OF STAPHYLOCOCCUS AUREUS ISOLATED FROM INFECTED VASCULAR ACCESSES IN HEMODIALYSIS PATIENTS AT ASSIUT UNIVERSITY HOSPITALS

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*We aimed in this work to detect bacterial pathogens causing infection of the vascular accesses in hemodialysis patients and demonstration of important genes responsible for virulence and biofilm formation in Staphylococcus aureus isolates namely; spa, mecA, PVL, γ-hemolysin, and icaA genes. The study was conducted from March 2018 to September 2020 and included 90 patients with infected hemodialysis accesses. Blood samples were collected for blood culture. Distal catheter end was cut and placed in brain heart infusion broth. Total bacterial isolates were 234. Samples were identified by conventional bacteriological methods. Staph. aureus was the most common isolated microorganism. Virulent genes in Staph. aureus were detected by PCR. Fifty (96.25%) of the 52 Staph. aureus isolates were mecA gene positive. The most common spa genotype was at S3 followed by S2. Forty of 52 (76.90 %) Staph. aureus isolates were icaA positive. Only 2(3.85%) Staph. aureus isolates were gamma-hemolysin positive. All 52 isolates of Staph. aureus were negative for Panton-Valentine leukocidin gene.*

**Keywords:** *γ-hemolysin; icaA; mecA; spa; Staphylococcus aureus*

## INTRODUCTION

A vascular access is needed in patients under hemodialysis (HD)[1]. Arteriovenous fistula (AVF) is the most preferred among vascular accesses because of its lower mortality and lower infection rate. The use of central venous catheter (CVC) was accompanied with greater infection risk which ultimately resulted in higher mortality [2]. Nevertheless, over half of incident HD patients inevitably start dialysis via CVC[3]. Complications of this hemodialysis access (i.e., thrombosis or infection) are common, that in urgent need for access-related procedures or medical interventions[4]. Infection comes secondly (after cardiovascular events) as a major cause of morbidity in HD patients. In addition, the annual mortality secondary to sepsis is approximately 100-300 folds higher in HD patients than the general population[5]. Predisposition to infection in HD patients is attributed to the changes that occur in primary host defense, with most of patients with are old age, and many of them suffer comorbid conditions such as diabetes mellitus (DM), malnutrition, invasive dialysis procedures, disruption of skin and mucosal barriers, and susceptibility to nosocomial infection[6]. The pathogens which are mainly responsible for infections in HD patients are Staphylococcus, Gram-negative enteric bacilli, *Pseudomonas aeruginosa*, and Candida spp. [7]. *Staph. aureus* is an important pathogen causing infection in HD patients with the organism exists in the anterior nares and skin as normal flora, from where it can penetrate the skin barriers through

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the wound or surgical incision and causes infection[8]. The organism has the ability to adhere to, and form a biofilm on tissues or medical indwelling devices that convers resistance to antimicrobial agents[9]. Nowadays, most of *Staph. aureus* isolates show resistance to methicillin which is attributed to the presence of *mecA* gene located on the staphylococcal chromosomal cassette. *MecA* gene represents the main factor responsible for methicillin resistance in *Staph. aureus*[10]. Methicillin-resistant *Staph. aureus* (MRSA) remains among the most frequently identified pathogens associated with nosocomial respiratory tract infections[11]. Protein A of *Staph. aureus*, binds to the immunoglobulin G (IgG) molecules by their Fc portion and inhibits phagocytosis of bacteria, thus contributes to the development of the disease. Protein A is encoded by the staphylococcal protein A (*spa*) gene which is considered as one of the important virulence factors in *Staph. aureus*[12]. The intercellular adhesion (*ica*) locus in *Staph. aureus* codes for an intercellular adhesion molecules which are crucial for biofilm formation[13]. Panton-Valentine leukocidin (PVL) is a cytotoxin secreted by virulent strains of *Staph. aureus* causes destruction of human leukocytes. Isolates secreting PVL are commonly associated with necrotizing skin lesions and pneumonia [14]. We aimed in this study to detect bacterial pathogens associated with infection of the vascular accesses in end-stage renal disease (ESRD) patients undergoing HD and detection of five important genes in *Staph. aureus* isolates associated with virulence and biofilm formation namely; *spa*, *mecA*, *PVL*, γ-hemolysin (γ- HL), and *icaA* genes.

## MATERIAL AND METHODS

***Study design, patients, and ethical considerations***

This is a hospital-based descriptive study that included 90 ESRD patients undergoing HD admitted to the Renal Unit of Assiut University Hospitals with suspected infected vascular accesses over the period from March, 2018 to September, 2020. Diagnosis of suspected infection of the hemodialysis accesses was manifested by the clinical findings such as fever, chills, redness, pain, swelling and pus discharge at the catheter site. Demographic and clinical data were obtained from participants that included: age, sex, duration of dialysis, duration and frequency of CVC insertion, and associated comorbidities. Approval for this study was obtained from the Institutional Review Board of Faculty of Medicine, Assiut University (IRB number 17101222). All participants received a clear, written consent form indicating the purpose of the study and their freedom to participate or withdraw at any time.

***Samples collection and bacteriological identifications***

Blood samples (each 5-10 ml) were collected from patients using sterile syringes into blood culture bottles. Ninety samples were collected from the catheter tip and peripheral vein termed confirmed (or definitive) infection; a blood sample was obtained from the peripheral vein and the catheter tip (about 5 cm) was cut off by a sterile forceps and placed in tubes containing brain heart infusion (BHI) broth. Another 90 samples were obtained from the external site of the catheter termed suspected infection. Samples were transported to the Infection Control Research Lab. at the Medical Research Center of Assiut University Hospitals. Catheter tips in BHI broth were incubated for 24 hrs at 37ºC. Blood culture bottles were incubated aerobically at 37ºC for 7 days and examined for bacterial growth every other day. Samples were subcultured on blood agar, mannitol salt agar, nutrient agar, MacConkey's agar, and eosin methylene blue (EMB) agar. Identification of the causative microorganisms was confirmed by the Gram staining, colonial morphology, motility test, and biochemical reactions that included catalase, coagulase, DNase, oxidase, indole, urease, citrate, and triple sugar iron tests as described[15]. Samples were transported to the Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, where the laboratory procedures were conducted.

***Antibiotic susceptibility patterns of bacterial isolates***

The antimicrobial susceptibility of bacterial isolates was conducted by the Kirby-Bauer disc diffusion method according to the

Clinical & Laboratory Standards Institute guidelines[16] using the following antibiotic discs that purchased from Bioanalyse, Turkey: ampicillin (10 µg), penicillin (10 µg), vancomycin (30 µg), amikacin (30 µg), ceftriaxone (30 µg), tetracycline (10 µg), rifampicin (5 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), erythromycin (15 µg), amoxicillin/clavulanic acid (30 µg), amoxicillin (10 µg), chloramphenicol (30 µg) and trimethoprim/ sulfamethoxazole (25 µg).

***Molecular detection of virulent genes of Staph. aureus by conventional polymerase chain reaction (PCR)***

The identified *Staph. aureus* isolates were further tested for the presence of the following virulent genes: *spa*[17], *mecA*[18], *PVL*[19], γHL[20], and *icaA* [21] by conventional PCR.

Genomic DNA was extracted by the boiling method as described previously by Oliver *et al.*[22]. The quantity of DNA extract was determined by a nanodrop spectrophotometer (EPOCH BioTek Instrument, USA). The oligonucleotides sequences and amplification programs used are shown in tables 1. PCR was performed using the thermal cycler (Bio-Rad T100, USA). The amplification reactions were performed at a defined volume of 25 µl containing 12.5 µl PCR master mix, 6 µl genomic DNA, 1 µl of each primer forward and reverse, and 4.5 µl PCR water. The PCR products were visualized by 1.5% agarose gel electrophoresis after staining with ethidium bromide under ultraviolet (UV) light.

**Table 1 :** Primers and amplification programs used in PCR amplification of resistance genes in *Staphylococcus aureus* isolates

| Primer | Oligonucleotide sequence (5'-3') | PCR protocol | | Expected size of amplicon (bp) |
|---|---|---|---|---|
| *Spa* | F 5′-TAAAGACGATCCTTCGGTGAGC-3′<br>R 5′- CAGCAGTAGTGCCGTTTGCTT-3′ | Denaturation at 94°C for 15 min | | Variable (180-600) |
| | | 94°C for 30 sec, 59°C for 1 min, 72°C for 1 min | 40 cycles | |
| | | Final extension at 72ºC for 10 min | | |
| *MecA* | F 5′-GTTGTAGTTGTCGGGTTTGG-3′<br>R 5′-CTTCCACATACCATCTTCTTTAAC-3′ | Denaturation at 92°C for 5 min | | 310 |
| | | 95°C for 30 sec, 56°C for 30 sec, 72°C for 3 min | 35 cycles | |
| | | Final extension at 72ºC for 6 min | | |
| *PVL* | F 5′-ATCATTAGGTAAATGTCTGGACATGATCCA-3′<br>R 5′-GCATCAASTGTATTGGATAGCAAAAGC-3′ | Denaturation at 94°C for 5 min | | 433 |
| | | 94°C for 30 sec, 55°C for 30 sec, 72°C for 10 min | 30 cycles | |
| | | Final extension at 72ºC for 7 min | | |
| *γHL* | F 5′-GCC AATCCGTTATTAGAAAATGC-3′<br>R 5′-CCATAGACGTAGCAACGGAT-3′ | Denaturation at 94°C for 5 min | | 937 |
| | | 95°C for 30 sec, 55°C for 30 sec, 72°C for 10 min | 30 cycles | |
| | | Final extension at 72ºC for 10 min | | |
| *IcaA* | F 5′-TCTCTTGCAGGAGCAATCAA-3′<br>R 5′-TCAGGCACTAACATCCAGCA-3′ | Denaturation at 94°C for 5 min | | 188 |
| | | 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min | 50 cycles | |
| | | Final extension at 72ºC for 10 min | | |

***Statistical analysis***

Statistical analysis was conducted with the SPSS 20.0 version software. Data were described by number and percentage for categorical variables or mean and standard deviation (Mean ± SD) for numerical variables. $Chi^2$ or Fisher's exact tests were used for testing statistical significance for categorical variables and the student-*t* test used for testing the significance between numerical variables. Pearson's correlation was used to test the association between variables. A *p* value <0.05 was considered statistically significant.

## RESULTS AND DISCUSSION

### Results

**Demographic and laboratory characteristics of patients**

Our patients aged 49 ± 13.6 (range 17-80) years with 53 (59%) males and 37 (41%) females. Patients started dialysis (onset of ESRD) since 7.3 ± 13.9 (ranged 1-96) months. The mean frequency of intravenous access (IVA) insertion was 2.6 ± 1.17 times. Duration of IVA insertion was 23 ± 14.4 (ranged 7-90) days. There was no significant differences in the incidence rate of infection according to the onset of ESRD (*p*=0.622) nor the frequency of IVA insertion (*p*= 0.3), while there was statistically significant difference between infected and non-infected patients regarding the duration of IVA insertion (student-*t* test; *p*=0.019). Nearly half of patients suffered from DM either solely or concomitant with hypertension. A considerable number of patients suffered from hepatitis C virus (HCV) (14; 15.6%). There were no statistically significant differences in the infection rates regarding neither DM, hypertension, nor HCV positivity ($Chi^2$; *p* values were: 0.654, 0.856, and 0.498, respectively). Most patients (85 patients; 94.4%) suffered from anemia where the mean hemoglobin (HB) levels in the studied cohort were 8.96 ± 2.1 (range 5.0-14.0) gm/dL. Other demographic and laboratory data of patients are shown in table 2.

**Table 2** : Demographic and laboratory characteristics of the studied patients (No=90)

| Variable | Category | * | *P* ** |
|---|---|---|---|
| Age in years | Mean ± SD | 49 ± 13.6 | -- |
| | Median (Range) | 50 (17 - 80) | |
| Sex | Male | 53 (59%) | -- |
| | Female | 37 (41%) | |
| Infection | Yes: | 86 (95.6%) | -- |
| | No: | 4 (4.4%) | |
| Duration of dialysis (months) | Mean ± SD (to all 90 patients)<br>Median (range) (to all 90 patients) | 7.3±13.9<br>3 (1-96) | -- |
| | ➢ Infected (86 patients)<br>➢ Non-infected (4 patients) | 7.35±14.2<br>6±4.2 | 0.622 |
| Duration of IVA insertion (days) | Mean ± SD (to all 90 patients)<br>Median (range) (to all 90 patients) | 23 ± 14.4<br>83 (7-90) | -- |
| | ➢ Infected (86 patients)<br>➢ Non-infected (4 patients) | 23.4±14.7<br>16.75±3.5 | **0.019** |
| Frequency of IVA insertion (times) | Mean ± SD | 2.6±1.17 | -- |
| | Median (range) | 2 (1-7) | |
| Associated comorbidities | DM | 55 (61%) | -- |
| | Hypertension | 41 (45.6) | -- |
| | HCV positivity | 14 (15.6%) | -- |
| Laboratory characteristics | Creatinine (mg/dL) | 7.5±2.5 | -- |
| | Urea (mg/dL): before dialysis<br>after dialysis | 166.6 ± 45<br>75.6 ± 20 | -- |
| | HB (gm/dL) | 8.96 ± 2.1 | -- |

***Abbreviations*:** DM=diabetes mellitus; ESRD=end stage renal disease; HB=haemoglobin level; HCV=hepatitis C virus; IVA=intravenous access. * Data were expressed as either number (%) or mean ± standard deviation. ** *P* value < 0.05 was considered statistically significant.

**Frequency of different bacterial isolates from the collected samples**

A total number of 234 bacterial strains were isolated from patients either in single or mixed infections. Positive samples at confirmed infection were 86 (95.55%). Bacterial isolates from the catheter tip and peripheral vein (confirmed infection) were 122 isolates (50 isolates in single infection; and 72 isolates in mixed infection). Positive samples from the external site of the catheter (suspected infection) were 72 (80%). Bacterial isolates from suspected infection were 112 isolates (32 isolates in single infection; and 80 isolates in mixed infection (table 3).

**Table 3:** Frequency of isolates at the catheter tip and peripheral vein (confirmed infection) and from the external site of catheter (suspected infection)

| **The catheter tip and peripheral vein (confirmed infection)** | | | | | | |
|---|---|---|---|---|---|---|
| Infection | Catheter tip number (%) | Catheter tip and peripheral vein number (%) | Total Number (%) | | Total isolates | |
| **Monomicrobial:**<br>*Staph. aureus*<br>CoNS<br>*Klebsiella spp.*<br>*Pseudomonas spp.* | 15 (16.66%)<br>9<br>4<br>1<br>1 | 35 (38.88)<br>21<br>10<br>2<br>2 | 50 (55.55 %) | 86 (95.55%) | 50 | 122 |
| **Polymicrobial:**<br>*Staph. aureus* + CoNS<br>*Staph. aureus* + *Pseudomonas spp*<br>*Staph. aureus* + *Klebsiella spp.*<br>CoNS + *Pseudomonas spp.*<br>CoNS + *Klebsiella spp.*<br>*Klebsiella spp.*+ *Pseudomonas spp.*<br>*Staph. aureus* + *E. coli* | 8 (8.8%)<br>2<br>1<br>2<br>1<br>1<br>1<br>0 | 28 (31.11)<br>5<br>4<br>6<br>6<br>3<br>2<br>2 | 36 (40%) | | 72 | |
| No infection | Catheter tip number (%) | Catheter tip and peripheral vein number (%) | | | | |
| No infection | 4 (4.4%) | | 4 (4.44%) | | | |

| **The external site of the catheter (suspected infection/colonization)** | | | | |
|---|---|---|---|---|
| Infection | Total number (%) | | Total isolates | |
| **Monomicrobial:**<br>*Staph. aureus*<br>CoNS<br>*Klebsiella spp.*<br>*Pseudomonas spp*<br>*Candida spp.* | 32 (35.55%)<br>17<br>7<br>3<br>2<br>3 | 72 (80%) | 32 | 112 |
| **Polymicrobial:**<br>*Staph. aureus* + CoNS<br>*Staph. aureus* + *Pseudomonas spp.*<br>*Staph. aureus* + *Klebsiella spp.*<br>CoNS + *Pseudomonas spp.*<br>CoNS + *Klebsiella spp.*<br>*Klebsiella spp.*+ *Pseudomonas spp.*<br>*Staph. aureus* + *E. coli* | 40 (44.44%)<br>6<br>9<br>8<br>6<br>5<br>4<br>2 | | 80 | |
| No infection | 18 (20%) | 18 (20%) | | |

*Abbreviations*: CoNS= Coagulase negative Staphylococci; *E.coli*=*Escherichia coli*

In confirmed infection, Gram-positive (G+ve) bacterial isolates were detected in 84 /122 (69%) strains; while Gram-negative (G-ve) bacteria were detected in 38/122 (31%) strains. In suspected infection, G+ve isolates were detected in 66/112 (59%) strains, while G-ve bacteria were detected in 43/112 (38%) strains. Candida was isolated from 3/112 (2.7%) specimens. *Staph. aureus* was the most common organism isolated from specimens in both confirmed and suspected infections followed by coagulase-negative Staphylococci (CoNS). Then both *Klebsiella spp.* and *Pseudomonas spp.* were isolated consecutively. The least organism detected in the specimens was *Escherichia coli* (*E. coli*) (table 4).

***Antibiotic sensitivity testing for bacterial isolates at the definitive infections***

All 52 (100%) *Staph. aureus* isolates were resistant to ceftriaxone and ampicillin. Resistance was high against penicillin, amoxicillin/clavulanic acid, and amoxicillin. On the other hand, the highest susceptibility was to vancomycin. All 32 (100%) CoNS isolates were resistant to ceftriaxone and amoxicillin. High resistance rates were detected against penicillin, ampicillin, and trimethoprim/sulfamethoxazole, while the highest susceptibility was to chloramphenicol, amikacin, and vancomycin.

Isolated *Klebsiella spp*. were all resistant to ofloxacin, rifampicin, ciprofloxacin, penicillin, ampicillin, vancomycin, and amoxicillin. All *Pseudomonas spp.* isolates were resistant to rifampicin, erythromycin, penicillin, ampicillin, amoxicillin/clavulanic acid, and ceftriaxone. The highest susceptibility was to ofloxacin, ciprofloxacin, amikacin, and tetracycline. The two *E. coli* isolates showed susceptibility for amikacin and chloramphenicol only and were resistant to other antibiotics (table 5).

**Table 4:** Frequency of different microorganisms isolated from patients

| **Bacterial isolates at the catheter tip anBacterial isolates at the catheter tip and peripheral vein** | | | |
|---|---|---|---|
| *Isolated microorganisms* | Total number of confirmed CR-BSI | | Total (122) |
| | colonization | definitive CR-BSI | |
| *Staph. aureus* | 14 (11.5%) | 38 (31.14%) | 52 (43%) |
| Coagulase negative Staphylococci (CoNS) | 8 (6.55%) | 24 (19.67%) | 32 (26%) |
| *Klebsiella spp*. | 5 (4%) | 13 (10.6%) | 18 (15%) |
| *Pseudomonas spp.* | 4 (3.3%) | 14 (11.4%) | 18 (15%) |
| *Escherichia coli* | 0 | 2 (1.64%) | 2 (1.6%) |
| **Bacterial isolates at the external catheter (suspected infection)** | | | |
| *Isolated microorganisms* | Total suspected isolates (112) | | |
| | **No**. | **%** | |
| *Staph. aureus* | 42 | 37.5% | |
| Coagulase negative Staphylococci (CoNS) | 24 | 21.4% | |
| *Pseudomonas Spp***.** | 21 | 18.75% | |
| *Klebsiella Spp***.** | 20 | 17.85% | |
| Candida | 3 | 2.7% | |
| *Escherichia coli* | 2 | 1.7% | |

*N.B. Colonization = strains isolated from catheter tip only with no growth at the peripheral vein. CR-BSI = catheter-related blood stream infection (strains isolated from catheter tip and peripheral vein)*

**Table 5:** Number (percentage) of resistant strains of tested bacterial isolates

| Antibiotics | Bacterial isolates | | | | |
|---|---|---|---|---|---|
| | *Staph. aureus* (52) | CoNS (32) | *Klebsiella spp.* (18) | *Pseudomonas spp.* (18) | *E. coli* (2) |
| **Ofloxacin** | 16 (30.8%) | 13 (40.6%) | 18 (100%) | 0 (0.0%) | 2 (100%) |
| **Tetracycline** | 10 (19.2%) | 11(34.4 %) | 12 (66.7%) | 0 (0.0%) | 2 (100%) |
| **Rifampicin** | 10 (19.2%) | 12 (37.5%) | 18 (100%) | 18 (100%) | 2 (100%) |
| **Ciprofloxacin** | 36 (69.2%) | 18 (56.2%) | 18 (100%) | 0 (0.0%) | 2 (100%) |
| **Penicillin** | 49 (94.2%) | 31 (97%) | 18 (100%) | 18 (100%) | 2 (100%) |
| **Chloramphenicol** | 3 (5.8%) | 4 (12.5%) | 10 (55.5%) | 17 (94.4%) | 0 (0.0%) |
| **Ampicillin** | 52 (100%) | 28 (87.5%) | 18 (100%) | 18 (100%) | 2 (100%) |
| **Erythromycin** | 9 (17.3%) | 19 (59.4%) | 16 (89%) | 18 (100%) | 2 (100%) |
| **Trimethoprim/ sulfamethoxazole** | 28 (53.8%) | 28 (87.5%) | 16 (89%) | 10 (55.5%) | 2 (100%) |
| **Vancomycin** | 2 (3.85%) | 4 (12.5%) | 18 (100%) | 16 (88.9%) | 2 (100%) |
| **Amoxicillin/ clavulanic acid** | 49 (94.2%) | 25 (78%) | 16 (89%) | 18 (100%) | 2 (100%) |
| **Ceftriaxone** | 52 (100%) | 32 (100%) | 17 (94.4%) | 18 (100%) | 2 (100%) |
| **Amikacin** | 22(42.3 % ) | 5 (15.63%) | 10 (55.55%) | 0 (0.0%) | 0 (0.0%) |
| **Amoxicillin** | 48 (92.3%) | 32 (100%) | 18 (100%) | 18 (100.0%) | 2 (100%) |

*Abbreviations*: CoNS=coagulase-negative Staphylococcus; *E. coli*= *Escherichia coli*; *Staph. aureus* = *Staphylococcus aureus*.

## Molecular detection of virulent genes in *Staph. aureus* isolates by PCR

- ***Molecular detection of the spa gene***

The *spa* gene was amplified in 52 (100%) different *Staph. aureus* isolates. These products showed 3 different types of band patterns. The most common *spa* genotype detected among our *Staph. aureus* isolates was at S3 (350 bp) band that was detected in 34/52 (65.4%) of the isolates, followed by S2 which appeared at (240, 350 bp) that was identified in 10/52 (19.2%) of the isolates. Only 8/52 (15.4 %) of the isolates showed S1 genotype at 600 bp (figure 1).

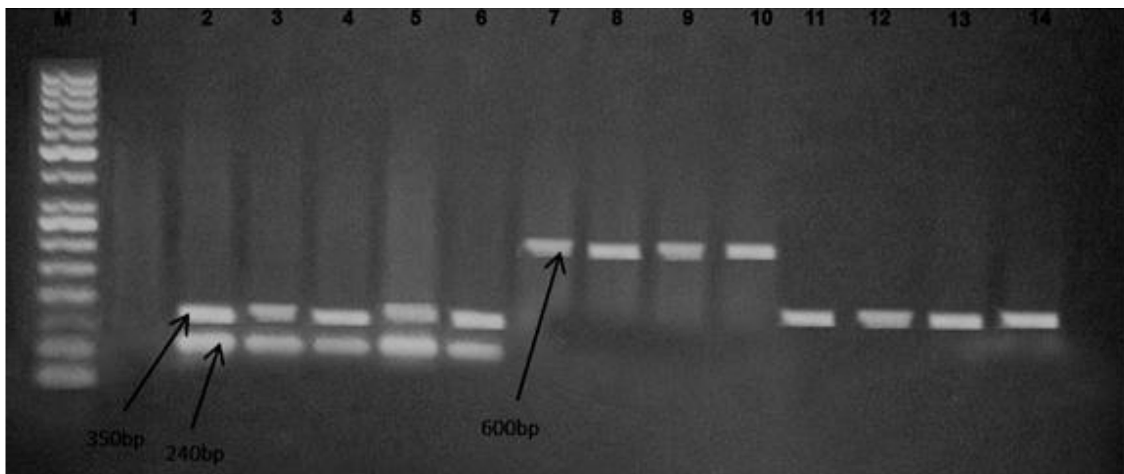


**Fig. 1:** PCR amplification of the *spa* gene among *Staphylococcus aureus* isolates on 1.5 % agarose gel

Fragments of 240 and 350 bp (positive S2 isolates) were detected in lanes 2-6, fragments of 600 bp (positive S1 isolates) were detected in lanes 7-10, and fragments of 350 bp (positive S3 isolates) were detected in lanes 11-14; lane 1 (NC): negative control (distilled water), and M: 100bp DNA ladder. bp= base pair

- ***Molecular detection of the mecA* gene**

Fifty of 52 (96%) *Staph. aureus* isolates were *mecA* positive showing band at 310 bp (figure 2)

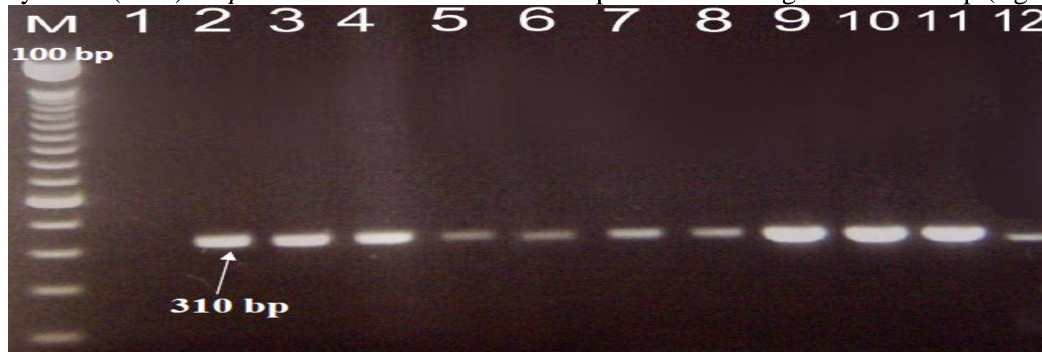


**Fig. 2:** PCR amplification of the *mecA* gene among *Staphylococcus aureus* isolates on 1.5 % agarose gel A fragment of 310 bp was detected. Lanes 2-12: positive isolates; lane 1 (NC): negative control (distilled water), and M: 100bp DNA ladder. bp= base pair

- ***Molecular detection of the icaA gene***

Forty of 52 (77%) *Staph. aureus* isolates were *icaA* positive showing bands at 188 bp (figure 3)

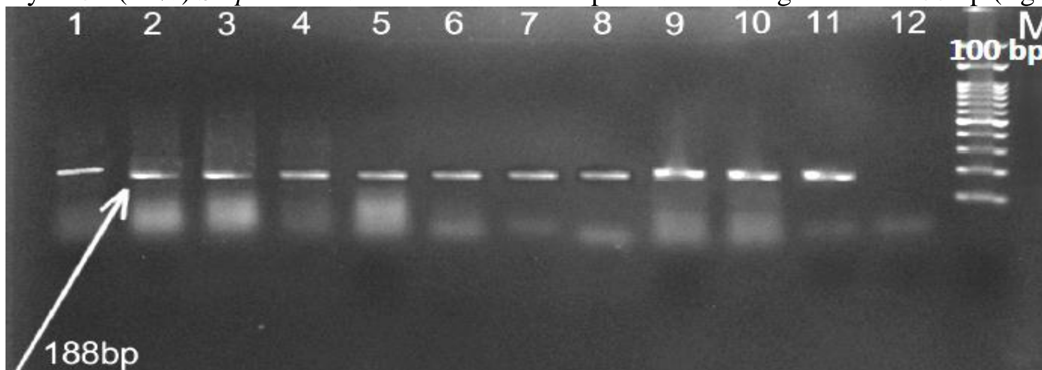


**Fig. 3:** PCR amplification of the *icaA* gene among *Staphylococcus aureus* isolates on 1.5 % agarose gel A fragment of 188 bp was detected. Lanes 1-11: positive isolates; lane 12 (NC): negative control (distilled water), and M: 100bp DNA ladder. bp= base pair

- **Molecular detection of γ-*haemolysin* gene**

Only two (4%) *Staph. aureus* isolates were γ-*haemolysin* positive showing bands at 937 bp (figure 4).

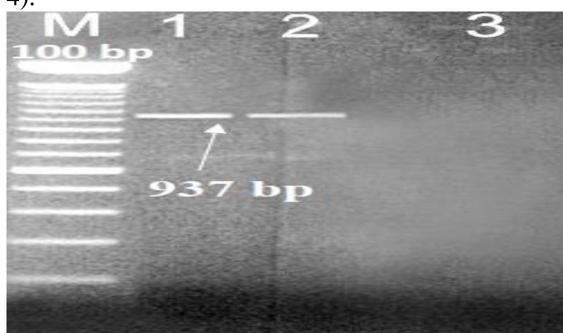


**Fig. 4:** PCR amplification of the γ-*haemolysin* gene among *Staphylococcus aureus* isolates on 1.5 % agarose gel

A fragment of 937 bp was detected. Lanes 1 and 2: positive isolates, and lane 3 (NC): negative control (distilled water), and M: 100bp DNA ladder. bp = base pair.

- ***Molecular detection of pvl genes***

All the 52 isolates of *Staph. aureus* were *pvl* gene negative.

**Relation of the detected virulence genes in *Staph. aureus* isolates and antibiotic susceptibility patterns**

Table 6 showed the different susceptibility patterns and the detected virulence genes in *Staph. aureus* isolates. For most of the detected *Staph. aureus* isolates, there were no significant associations found between the detected virulence genes and the patterns of antibiotic sensitivities for most of the antibiotics used. There was a statistical significant difference in antibiotic susceptibility pattern to tetracycline and chloramphenicol antibiotics among *Staph. aureus* isolates that are *mecA* and *icaA* genes producers and non-producers ($p$=.034, and .007, respectively). *Staph. aureus* isolates positive for the γ-HL gene, showed higher resistance to the chloramphenicol antibiotic ($p$=.05).

**Table 6:** Antibiogram and the detected virulence genes in *Staph. aureus* isolates

| | | | | Detected genes in *Staph. aureus* isolates | | | |
|---|---|---|---|---|---|---|---|
| **Antibiotics** | **Susceptibility patterns** | **No** | **(%)** | ***Spa*** **(350 pb, 600 pb, and 240,350 pb in order)** | ***MecA*** | ***IcaA*** | **γ-*haemolysin*** |
| **Ofloxacin** | S | 23 | 44 | 12, 4, 7 | 21 | 19 | 1 |
| | R | 16 | 31 | 12, 3, 1 | 16 | 11 | 0 |
| | I | 13 | 25 | 10, 1, 2 | 13 | 10 | 1 |
| | ***P* value** | | | .32 | .4 | .65 | .72 |
| **Tetracycline** | S | 30 | 58 | 20, 4. 6 | 30 | 24 | 1 |
| | R | 10 | 19 | 5, 2, 3 | 8 | 9 | 0 |
| | I | 12 | 23 | 9, 2, 1 | 12 | 7 | 1 |
| | ***P* value** | | | .75 | **.034** | .2 | .67 |
| **Rifampicin** | S | 39 | 75 | 24, 6, 9 | 37 | 31 | 1 |
| | R | 10 | 19 | 7, 2, 1 | 10 | 8 | 0 |
| | I | 3 | 6 | 3, 0, 0 | 3 | 1 | 1 |
| | ***P value*** | | | .64 | .9 | .21 | .15 |
| **Ciprofloxacin** | S | 16 | 31 | 9, 2, 5 | 14 | 15 | 0 |
| | R | 36 | 69 | 25, 6, 5 | 36 | 25 | 2 |
| | ***P* value** | | | .37 | .09 | .08 | .566 |
| **Penicillin** | S | 3 | 6 | 2, 0, 1 | 2 | 3 | 0 |
| | R | 49 | 94 | 32, 8, 9 | 48 | 37 | 2 |
| | ***P* value** | | | .73 | .11 | .58 | 1.0 |
| **Chloramphenicol** | S | 40 | 77 | 25, 7, 8 | 38 | 35 | 0 |
| | R | 5 | 9.6 | 3, 1, 1 | 5 | 2 | 1 |
| | I | 7 | 13.5 | 6. 0, 1 | 7 | 3 | 1 |
| | ***P value*** | | | .816 | .97 | **.007** | **.05** |
| **Ampicillin** | R | 52 | 100 | 3, 8, 10 | 50 | 0 | 2 |
| **Erythromycin** | S | 37 | 71 | 23, 7, 7 | 35 | 28 | 2 |
| | R | 6 | 11.5 | 4, 0, 2 | 6 | 4 | 0 |
| | I | 9 | 17 | 7, 1, 1 | 9 | 8 | 0 |
| | ***P value*** | | | .69 | .89 | .67 | 1.0 |
| **SXT** | S | 16 | 31 | 9, 3, 4 | 15 | 13 | 1 |
| | R | 28 | 54 | 18, 5, 5 | 27 | 21 | 1 |
| | I | 8 | 15 | 7, 0, 1 | 8 | 6 | 0 |
| | ***P value*** | | | .63 | 1.0 | .91 | 1.0 |
| **Vancomycin** | S | 50 | 96 | 32, 8, 10 | 8 | 39 | 2 |
| | R | 2 | 4 | 2, 0, 0 | 2 | 1 | 0 |
| | ***P value*** | | | .75 | 1.0 | .412 | 1.0 |
| **AMC** | S | 3 | 6 | 2, 0, 1 | 2 | 3 | 0 |
| | R | 49 | 94 | 32, 8, 9 | 48 | 37 | 2 |
| | ***P value*** | | | .729 | .11 | .576 | 1.0 |
| **Ceftriaxone** | R | 52 | 100 | 34, 8, 10 | 50 | 40 | 2 |
| **Amikacin** | S | 30 | 58 | 21, 3, 6 | 29 | 2 | 1 |
| | I | 22 | 42 | 13, 5, 4 | 21 | 16 | 1 |
| | ***P value*** | | | .5 | 1.0 | .74 | 1.0 |
| **Amoxicillin** | S | 4 | 8 | 2, 1, 1 | 3 | 4 | 0 |
| | R | 48 | 92 | 32, 7, 9 | 47 | 36 | 2 |
| | ***P value*** | | | 1.0 | .15 | .37 | 1.0 |

*Abbreviations*: AMC = Amoxicillin/clavulanic acid; *Staph. aureus*=*Staphylococcus aureus*; SXT = Trimethoprim/sulfamethoxazole

## Discussion

HD patients are often predisposed to infection due to diminished host immunity, old age, and presence of associated medical conditions [6]. Nearly all patients in this study suffered catheter related-blood stream infection (CR-BSI) at the catheter tip and peripheral vein. Multiple comorbidities were detected in

the patients group. Moreover, the patients experienced significantly longer duration for intravenous access insertion which coincides with previous findings[23&24]. Researchers documented DM and longer duration of IVA insertion to carry the greatest risk for catheter-related infections in HD patients[25&26]. In this work, *Staph. aureus* was the most common isolated bacteria. This is consistent with enormous previous reports[27-30]. HD patients are more vulnerable to *Staph. aureus* infection due to their exposure to the nasal carriage of *Staph. aureus* among health care provided medical staff[30]. Other bacterial isolates in this study were CoNS and various strains of Gram-negative bacteria. This is in agreement with other previous findings[31&32]. In this work, high resistance rates to most antibiotics were detected that was supported by others[23]. All *Staph. aureus* isolates were multidrug resistant (MDR). This point is worth noting, as it potentially could lead to failure in treatment therapy, prolonged illnesses, increased expenses for health care, and in serious cases, risk of death if patients are infected with such strains[33]. The transmission of resistance (R-factor), a plasmid-mediated genetic determinant, may be credited with the development of MDR among these isolates[34]. Studies have shown an upward pattern in the incidence of *Staph. aureus* isolates with multiple antibiotic resistance[35]. Most of the *Staph. aureus* isolates were susceptible to vancomycin. This is in accordance with the findings of [36]. In the current work, the highest antibiotic resistance was observed to ampicillin and ceftriaxone (100%). This coincides with the findings obtained by Abdulghany *et al.*[37] who reported the same resistance rate to ampicillin. The results of this study showed that above half of *Staph. aureus* isolates were resistant to trimethoprim/ sulfamethoxazole. On the other hand, some previous Egyptian studies reported lower sensitivity rates to trimethoprim/sulfamethoxazole[38&39]. The results of the present study showed high susceptibility rates for chloramphenicol, tetracycline, erythromycin, and rifampin, as reported previously[34] that susceptibility patterns of *Staph. aureus* strains ranging from 57-83. In the current study, the distribution of the virulence genes of *Staph. aureus* isolates and the molecular typing data were determined. Forty (76.9%) *Staph. aureus* show prevalence of *icaA* gene that was agree with Omidi *et al*.[40] who demonstrated that molecular study of *icaA* and *icaD* genes among 24 MRSA strains revealed that 18 (75%) isolates carried *icaA* gene while *icaD* gene was not detected in all MRSA strains. Diemond-Hernández *et al.* [41] reported *icaA* in 10.3% of *Staph. aureus* isolates. Mirzaee *et al*.[42] demonstrate that the prevalence of *icaA*, were 95.8%. Another report detected high prevalence of *ica* genes among *Staph. aureus* where all isolates were reported to possess *icaA* and *icaD* genes [43]. The *PVL* genes, which encode a pore-forming cytotoxin and cause tissue necrosis and leukocyte destruction, are frequently present in community associated-MRSA[44]. The prevalence of PVL genes in *Staph. aureus* from various samples is diverse, with 79.5% of *Staph. aureus* from recurrent furunculosis and 2.63% from lower respiratory tract infections harboring PVL genes[45&46]. Reports from various countries show the increasing prevalence of PVL among MRSA isolates[47, 48]. In a previous report from India, they found 62.85 % of PVL prevalence among MRSA and MSSA (MRSA: 85.1 % and MSSA: 48.8 %)[49]. Similar study by D'Souza *et al*.[50&51] from Mumbai, India, reported prevalence of 64 % PVL positive isolates among MRSA. A lower prevalence of PVL has been reported from other parts of world (5 % in France, 4.9 % in UK, 8.1 % in Saudi Arabia and 14.3 % in Bangladesh)[52-55]. The surveillance of *pvl* in *Staph. aureus* showed low occurrence which was consistent with results from previous reports[56-59]. In the current study all 52 isolates of *Staph. aureus* were *PVL* negative**.** The obtained results are in agreement with Johler *et al.* [60] who reported that no Panton-Valentine leucocidin (PVL) or methicillin resistance genes were detected. Also, the results obtained are in the same line with previous findings by Gheorghe *et al.* [61] that reported the isolates did not carry genes associated with typical virulence factors for *Staph. aureus* such as the PVL. The gene that encodes for protein A (spa) in *Staph. aureus* is the most widely used marker for molecular typing because it contains polymorphic units. *Spa* genes are also a good choice to be able to identify and distinguish *Staph. aureus* strain variability[62&63]. In the present study, 3 types of *spa* gene were

detected; comparable results were reported by Rezaee *et al*.[36] who detected 4 types. However, Hosseini *et al*.[39] detected 6 types. The band sizes of the *spa* PCR products ranged from 240 to 350 bps which were comparable to the band sizes reported by *Omar et al*.[38] who detected *spa* bands ranged from 192 to 1392 bp. Meanwhile, larger band sizes were detected using the same primer in previous reports as 1152 to 1491 bp[63]**,** 1000 to 1450 bp[36], and 1000 to 1500 bp[39]. In this study 2 *Staph. aureus* isolates were γ-*haemolysin* positive. These results are in agreement with previous findings by Ben Nejma *et al*.[64] who reported that the amplification of γ-*haemolysin* gene revealed that this gene was detected only in 2 strains among all isolates. On contrast study by Kreausukon *et al*.[65] showed that γ-hemolysin genes were detected in all isolates.

No significant associations were found between the antibiotic susceptibility patterns of most *Staph. aureus* isolates in this study and specific virulence genes detected. With exception of an accidental association of the *mecA*, icaA, and γ-HL genes-producers and non-producers with tetracycline and chloramphenicol antibiotics. The reason for this could be explained for the *mecA* gene as nearly all (50 out of 52) *Staph. aureus* isolates in this study were *mecA* gene-producers leaving only 2 isolates were non-producers with the statistical analysis in this case makes no sense. Our study revealed that, these virulence genes had no effect on antibiotic susceptibility patterns of *Staph. aureus* isolates to different antibiotics

## Conclusion

We concluded that *Staph. aureus* is the most common cause of vascular access infection followed by coagulase negative staphylococci (CoNS). There is high resistance rate against different antimicrobial agents among *Staph. aureus* isolates that decrease treatment modalities. There are 3 *spa* genotypes among *Staph. aureus* isolates with band sizes ranged from 240 to 600 bp. The most common *spa* genotype detected among *Staph. aureus* isolates was at S3 (350 bp) band. Biofilm formation is an important cause of antibiotic resistance in Staphylococci isolated from infected vascular access and the presence of *icaA* gene is associated with biofilm formation.

The regular surveillance of biofilm formation by Staphylococci and their antimicrobial resistance profile leads to early treatment of vascular accesses infection.


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نشرة العلوم الصيدليـــة
جامعة أسيوط

# توصيف لبعض جينات الضراوة لميكروب المكور العنقودى الذهبى المعزول من المداخل الوعائية لمرضى الغسيل الكلوى بمستشفيات جامعة أسيوط

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أجريت هذه الدراسة من يناير ٢٠١٨ إلى أبريل ٢٠٢١ على مرضى الغسيل الكلوى والذين ظهرت عليهم اعراض مثل الحمى والرعشة والاحمرار والتورم وتصريف القيح والشعور بالألم فى مكان الجرح في قسم أمرض الكلى بمستشفى جامعة أسيوط. في هذه الدراسة كان العدد الإجمالي للعزلات البكتيرية ٢٣٤ معزولة من ١٨٠ عينة مقسمة إلى نوعين من العدوى. عدوى مفردة ومختلطة: تم تحديد عامل معدي واحد في ٨٢ عينة بينما تم تحديد الإصابات المختلطة في ٧٦ عينة (١٥٢ عزلة بكتيرية). كانت العزلات البكتيرية من الموقع الداخلي للقسطرة (إصابة مؤكده) ١٢٢ عزلة (٥٠ عزلة في إصابة واحدة ، و ٧٢ عزلة في عدوى مختلطة). تم عزل ١١٢ عزلة بكتيرية من الموقع الخارجي للقسطرة (مشتبه في إصابتها بالعدوى) (٣٢ عزلة في إصابة واحدة و ٨٠ عزلة في عدوى مختلطة). المكورات العنقودية الذهبية كانت أكثر الكائنات الحية شيوعا بنسبة ٤٢.٦٢٪ (١٢٢/٥٢) ، في العدوى المؤكده تليها المكورات العنقودية السلبية المخثرة بنسبة ٢٦.٢٣٪ (١٢٢/٣٢). المكورات العنقودية الذهبية كانت أيضًا أكثر الكائنات الحية شيوعًا في الإصابة المشتبه بها بنسبة ٣٧.٥٠ ٪ (١١٢/٤٢) تليها المكورات العنقودية السلبية المخثرة بنسبة ٢١.٤٣ ٪ (112/٢٤). خمسون (٩٦.٢٥٪) من ٥٢ العنقوديات. تم التعرف على عزلات المذهبة من طرف القسطرة والوريد المحيطي على أنها MRSA عن طريق الكشف الجزيئي لجين mecA. أظهرت العزلات المدروسة أعلى معدلات حساسية للفانكومايسين (٩٦.١٥٪) كما تم الكشف عنها بطريقة نشر قرص كيربي باور. تم تضخيم جين *spa* في ٥٢ عزلة مختلفة من المكورات العنقودية الذهبية. تراوحت أحجام المنتجات من ٢٤٠ إلى ٦٠٠ bp ، وأظهرت هذه المنتجات ٣ أنواع مختلفة من جين ال *spa*. كان النمط الجيني للمنتجع الصحي الأكثر شيوعًا الذي تم اكتشافه بين عزلات المكورات العنقودية الذهبية عند 350 bp الذي تم اكتشافه في ٥٢/٣٤ (٦٥.٤ ٪) من العزلات ، يليه S2 الذي ظهر عند (٢٤٠ ، ٣٥٠) bp والذي تم تحديده في ٥٢/١٠ (١٩.٢٪) من العزلات بينما ٥٢/٨ (١٥.٤٪) من العزلات ظهرت عند ٦٠٠ bp و أربعون

من ٥٢ (٧٦.٩٠ ٪) المكورات العنقودية الذهبية كانت موجبه لجين الايكا والذى ظهر عنـــد ١٨٨ bp فقط ٢ (٣.٨٥٪) من المكورات العنقودية الذهبية كانت موجبة لعزلات جاما–الهيموليسين والتى ظهرت عند ٩٣٧ bp . جميع العزلات الـــ ٥٢ من المكورات العنقودية الذهبية كانت سالبة لجين الpvl.