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

Ehsan A. Hassan\(^1\), Mona Hussein Abdel-Rahim\(^1\), Thanaa Hassan Mohamed Hassan\(^2\), Nashwa Mostafa A. Azoz\(^3\) and Mona Embarek Mohamed\(^1\)*

\(^1\)Medical Microbiology and Immunology Department, Faculty of Medicine, Assiut University, Egypt
\(^2\)Pharmacist at Abnob Hospital, Assiut Governorate, Egypt
\(^3\)Nephrology Unit, Internal Medicine Department, Assiut University Hospitals, Egypt

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...
the wound or surgical incision and causes infection. The organism has the ability to adhere to, and form a biofilm on tissues or medical indwelling devices that conver resistance to antimicrobial agents. 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. Methicillin-resistant Staph. aureus (MRSA) remains among the most frequently identified pathogens associated with nosocomial respiratory tract infections. 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. The intercellular adhesion (ica) locus in Staph. aureus codes for an intercellular adhesion molecules which are crucial for biofilm formation. 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. 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 37°C. Blood culture bottles were incubated aerobically at 37°C 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. 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 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*\(^1\), *mecA*\(^2\), *PVL*\(^3\), *γHL*\(^4\), and *icaA*\(^5\) by conventional PCR.

**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’  | Denaturation at 94°C for 15 min | Variable (180-600) |
|        | R 5’-CAGCAGTAGTGCCGTTTGCTT-3’  | 94°C for 30 sec, 59°C for 1 min, 72°C for 1 min | 40 cycles |
|        |                                 | Final extension at 72°C for 10 min | |
| MecA   | F 5’-GTGTAATGTCGGTGGTTTG-3’    | Denaturation at 92°C for 5 min | 310 |
|        | R 5’-CTTCCACATACCATCTTCTTAAC-3’| 95°C for 30 sec, 56°C for 30 sec, 72°C for 3 min | 35 cycles |
|        |                                 | Final extension at 72°C for 6 min | |
| PVL    | F 5’-ATCATTAGTAAATGTCTGGACATGATCCA-3’ | Denaturation at 94°C for 5 min | 433 |
|        | R 5’-GCATCAAATGGATAGCAAAAGC-3’ | 94°C for 30 sec, 55°C for 30 sec, 72°C for 10 min | 30 cycles |
|        |                                 | Final extension at 72°C for 7 min | |
| γHL    | F 5’-GCCAATCCTGTTAGAAAAATGC-3’ | Denaturation at 94°C for 5 min | 937 |
|        | R 5’-CCATAGACGTAGCAAACGGAT-3’ | 95°C for 30 sec, 55°C for 30 sec, 72°C for 10 min | 30 cycles |
|        |                                 | Final extension at 72°C for 10 min | |
| IcaA   | F 5’-TCTCTTGCAGGAGCAATCAA-3’   | Denaturation at 94°C for 5 min | 188 |
|        | R 5’-TCAGGCATACATCCAGCA-3’     | 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min | 50 cycles |
|        |                                 | Final extension at 72°C for 10 min | |

Genomic DNA was extracted by the boiling method as described previously by Oliver *et al.*\(^6\). 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.
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. Chi² 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 (Chi²: 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)    | 7.3±13.9 | --     |
|                               | Median (range) (to all 90 patients)| 3 (1-96) | --     |
|                               | Infected (86 patients)            | 7.35±14.2 | 0.622  |
|                               | Non-infected (4 patients)         | 6±4.2 | --     |
| Duration of IVA insertion (days) | Mean ± SD (to all 90 patients)    | 23 ± 14.4 | --     |
|                               | Median (range) (to all 90 patients) | 83 (7-90) | --     |
|                               | Infected (86 patients)            | 23.4±14.7 | 0.019  |
|                               | Non-infected (4 patients)         | 16.75±3.5 | --     |
| 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     | 166.6 ± 45 | --     |
|                               | after dialysis                    | 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:                                            |           |                         |                                            |                |              |
| Staph. aureus                                             |           | 9                       | 21                                         | 50             | 50           |
| CoNS                                                     |           | 4                       | 10                                         | 19             |              |
| Klebsiella spp.                                          |           | 1                       | 2                                          | 3              |              |
| Pseudomonas spp.                                         |           | 1                       | 2                                          | 3              |              |
| Polymicrobial:                                            |           |                         |                                            |                |              |
| Staph. aureus + CoNS                                     |           | 2                       | 5                                          | 8              |              |
| Staph. aureus + Pseudomonas spp                          |           | 1                       | 4                                          | 5              |              |
| Staph. aureus + Klebsiella spp.                          |           | 1                       | 6                                          | 7              |              |
| CoNS + Pseudomonas spp.                                  |           | 1                       | 3                                          | 4              |              |
| CoNS + Klebsiella spp.                                   |           | 1                       | 2                                          | 3              |              |
| Klebsiella spp. + Pseudomonas spp.                       |           | 0                       | 2                                          |                |              |
| Staph. aureus + E. coli                                  |           |                         |                                            |                |              |
| No infection                                              |           |                         |                                            |                |              |
| No infection                                              |           | 4 (4.4%)                |                                            | 4 (4.44%)      |              |

| The external site of the catheter (suspected infection/colonization) | Infection | Total number (%) | Total isolates |
|---------------------------------------------------------------------|-----------|----------------|---------------|
| Monomicrobial:                                                       |           |                |               |
| Staph. aureus                                                        | 32 (35.55%) |               | 32            |
| CoNS                                                                | 17        |               |               |
| Klebsiella spp.                                                      | 3         |               |               |
| Pseudomonas spp.                                                     | 2         |               |               |
| Candida spp.                                                         | 3         |               |               |
| Polymicrobial:                                                       |           |                |               |
| Staph. aureus + CoNS                                                 | 6         | 72 (80%)       | 112           |
| Staph. aureus + Pseudomonas spp.                                     | 9         | 80             |               |
| Staph. aureus + Klebsiella spp.                                      | 8         |                |               |
| CoNS + Pseudomonas spp.                                              | 6         |                |               |
| CoNS + Klebsiella spp.                                               | 5         |                |               |
| Klebsiella spp. + Pseudomonas spp.                                   | 4         |                |               |
| Staph. aureus + E. coli                                              | 2         |                |               |
| 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 | Total number of confirmed CR-BSI | Total (122) |
|---|---|---|
| Isolated microorganisms | 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) | Total suspected isolates (112) |
|---|---|
| Isolated microorganisms | 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 (55.5%)          | 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](image)

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

| Antibiotics | Susceptibility patterns | No (%) | Spa (350 pb, 600 pb, and 240,350 pb in order) | MeCA | IcaA | γ-haemolysin |
|-------------|------------------------|--------|-----------------------------------------------|------|------|--------------|
| Ofloxacin   | S                      | 23     | 12, 4, 7                                      | 21   | 19   | 1            |
|             | R                      | 16     | 12, 3, 1                                      | 16   | 11   | 0            |
|             | I                      | 13     | 10, 1, 2                                      | 13   | 10   | 1            |
|             | **P value**            |        | **.32**                                       | **.4** | **.65** | **.72**     |
| Tetracycline| S                      | 30     | 20, 4, 6                                      | 30   | 24   | 1            |
|             | R                      | 10     | 5, 2, 3                                       | 8    | 9    | 0            |
|             | I                      | 12     | 9, 2, 1                                       | 12   | 7    | 1            |
|             | **P value**            |        | **.75**                                       | **.034** | **.2** | **.67**     |
| Rifampicin  | S                      | 39     | 24, 6, 9                                      | 37   | 31   | 1            |
|             | R                      | 10     | 7, 2, 1                                       | 10   | 8    | 0            |
|             | I                      | 3      | 3, 0, 0                                       | 3    | 1    | 1            |
|             | **P value**            |        | **.64**                                       | **.9** | **.21** | **.15**     |
| Ciprofloxacin| S                    | 16     | 9, 2, 5                                      | 14   | 15   | 0            |
|             | R                      | 36     | 25, 6, 5                                      | 36   | 25   | 2            |
|             | **P value**            |        | **.37**                                       | **.09** | **.08** | **.566**    |
| Penicillin  | S                      | 3      | 2, 0, 1                                      | 2    | 3    | 0            |
|             | R                      | 49     | 32, 8, 9                                      | 48   | 37   | 2            |
|             | **P value**            |        | **.73**                                       | **.11** | **.58** | **1.0**     |
| Chloramphenicol| S                 | 40     | 25, 7, 8                                      | 38   | 35   | 0            |
|             | R                      | 5      | 3, 1, 1                                       | 5    | 2    | 1            |
|             | I                      | 7      | 6, 0, 1                                       | 7    | 3    | 1            |
|             | **P value**            |        | **.816**                                      | **.97** | **.007** | **.05**     |
| Ampicillin  | R                      | 52     | 3, 8, 10                                     | 50   | 0    | 2            |
| Erythromycin| S                      | 37     | 23, 7, 7                                      | 35   | 28   | 2            |
|             | R                      | 6      | 4, 0, 2                                      | 6    | 4    | 0            |
|             | I                      | 9      | 7, 1, 1                                       | 9    | 8    | 0            |
|             | **P value**            |        | **.69**                                       | **.89** | **.67** | **1.0**     |
| SXT         | S                      | 16     | 9, 3, 4                                      | 15   | 13   | 1            |
|             | R                      | 28     | 18, 5, 5                                      | 27   | 21   | 1            |
|             | I                      | 8      | 7, 0, 1                                      | 8    | 6    | 0            |
|             | **P value**            |        | **.63**                                       | **1.0** | **.91** | **1.0**     |
| Vancomycin  | S                      | 50     | 32, 8, 10                                    | 8    | 39   | 2            |
|             | R                      | 2      | 2, 0, 0                                      | 2    | 1    | 0            |
|             | **P value**            |        | **.75**                                       | **1.0** | **.412** | **1.0**     |
| AMC         | S                      | 3      | 2, 0, 1                                      | 2    | 3    | 0            |
|             | R                      | 49     | 32, 8, 9                                      | 48   | 37   | 2            |
|             | **P value**            |        | **.729**                                      | **.11** | **.576** | **1.0**     |
| Ceftriaxone | R                      | 52     | 34, 8, 10                                    | 50   | 40   | 2            |
| Amikacin    | S                      | 30     | 21, 3, 6                                      | 29   | 2    | 1            |
|             | I                      | 22     | 13, 5, 4                                      | 21   | 16   | 1            |
|             | **P value**            |        | **.5**                                       | **1.0** | **.74** | **1.0**     |
| Amoxicillin | S                      | 4      | 2, 1, 1                                      | 3    | 4    | 0            |
|             | R                      | 48     | 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. Nearly all patients in this study suffered catheter-related bloodstream 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\textsuperscript{23,24}. Researchers documented DM and longer duration of IVA insertion to carry the greatest risk for catheter-related infections in HD patients\textsuperscript{25,26}. In this work, \textit{Staph. aureus} was the most common isolated bacteria. This is consistent with enormous previous reports\textsuperscript{27,30}. HD patients are more vulnerable to \textit{Staph. aureus} infection due to their exposure to the nasal carriage of \textit{Staph. aureus} among health care provided medical staff\textsuperscript{30}. Other bacterial isolates in this study were CoNS and various strains of Gram-negative bacteria. This is in agreement with other previous findings\textsuperscript{31,32}. In this work, high resistance rates to most antibiotics were detected that was supported by others\textsuperscript{33}. All \textit{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\textsuperscript{34}. The transmission of resistance (R-factor), a plasmid-mediated genetic determinant, may be credited with the development of MDR among these isolates\textsuperscript{34}. Studies have shown an upward pattern in the incidence of \textit{Staph. aureus} isolates with multiple antibiotic resistance\textsuperscript{35}. Most of the \textit{Staph. aureus} isolates were susceptible to vancomycin. This is in accordance with the findings of\textsuperscript{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.\textsuperscript{37} who reported the same resistance rate to ampicillin. The results of this study showed that above half of \textit{Staph. aureus} isolates were resistant to trimethoprim/sulfamethoxazole. On the other hand, some previous Egyptian studies reported lower sensitivity rates to trimethoprim/sulfamethoxazole\textsuperscript{38,39}. The results of the present study showed high susceptibility rates for chloramphenicol, tetracycline, erythromycin, and rifampin, as reported previously\textsuperscript{32} that susceptibility patterns of \textit{Staph. aureus} strains ranging from 57-83. In the current study, the distribution of the virulence genes of \textit{Staph. aureus} isolates and the molecular typing data were determined. Forty (76.9\%) \textit{Staph. aureus} show prevalence of icaA gene that was agree with Omidi et al.\textsuperscript{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.\textsuperscript{41} reported icaA in 10.3\% of \textit{Staph. aureus} isolates. Mirzaee et al.\textsuperscript{42} demonstrate that the prevalence of icaA, were 95.8\%. Another report detected high prevalence of ica genes among \textit{Staph. aureus} where all isolates were reported to possess icaA and icaD genes\textsuperscript{43}. The PVL genes, which encode a pore-forming cytotoxin and cause tissue necrosis and leukocyte destruction, are frequently present in community associated-MRSA\textsuperscript{44}. The prevalence of PVL genes in \textit{Staph. aureus} from various samples is diverse, with 79.5\% of \textit{Staph. aureus} from recurrent furunculosis and 2.63\% from lower respiratory tract infections harboring PVL genes\textsuperscript{45,46}. Reports from various countries show the increasing prevalence of PVL among MRSA isolates\textsuperscript{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\%)\textsuperscript{49}. Similar study by D'Souza et al.\textsuperscript{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\textsuperscript{52-55}. The surveillance of pvl in \textit{Staph. aureus} showed low occurrence which was consistent with results from previous reports\textsuperscript{56-59}. In the current study all 52 isolates of \textit{Staph. aureus} were PVL negative. The obtained results are in agreement with Johler et al.\textsuperscript{60} who reported that no Panton-Valentine leucocidin (PVL) or methicillin resistant genes were detected. Also, the results obtained are in the same line with previous findings by Gheorghe et al.\textsuperscript{61} that reported the isolates did not carry genes associated with typical virulence factors for \textit{Staph. aureus} such as the PVL. The gene that encodes for protein A (spa) in \textit{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 \textit{Staph. aureus} strain variability\textsuperscript{62,63}. In the present study, 3 types of spa gene were
detected; comparable results were reported by Rezaee et al., who detected 4 types. However, Hosseini et al., 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. who detected spa bands ranging from 192 to 1392 bp. Meanwhile, larger band sizes were detected using the same primer in previous reports as 1152 to 1491 bp, 1000 to 1450 bp, and 1000 to 1500 bp. In this study 2 Staph. aureus isolates were γ-haemolysin positive. These results are in agreement with previous findings by Ben Nejma et al. 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. 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.

Funding
This work was not funded by any funding resources.

REFERENCES
1. Y.H. Tseng, M.Y. Wong, T.Y. Huang, B.S. Lin, C.W. Tung and Y.K. Huang, "Molecular characterization of clinical isolates from vascular access infection: A single- institution study", MicrobiologyOpen, 9(11), e1126 (2020).
2. H. Lawson, L.E. Niklason and P. Roy-Chaudhury, "Challenges and novel therapies for vascular access in haemodialysis", Nature Reviews Nephrology, 16, 586-602 (2020).
3. D.H. Kim, J.I. Park, J.P. Lee, Y.-L. Kim, S.-W. Kang, C.W. Yang, N.-H. Kim, Y.S. Kim and C.S. Lim, "The effects of vascular access types on the survival and quality of life and depression in the incident hemodialysis patients", Renal failure, 42(1), 30-39 (2020).
4. S.M. Gage and H. Reichert, "Determining the incidence of needle-related complications in hemodialysis access: We need a better system", The Journal of Vascular Access, 22(4), 521-532 (2021).
5. J. Sarnak and B.L. Jaber, "Mortality caused by sepsis in patients with end-stage renal disease compared with the general population", Kidney international, 58(4), 1758-1764 (2000).
6. S.H. Abbasi, R.A. Aftab and S.S. Chua, "Risk factors associated with nosocomial infections among end stage renal disease patients undergoing hemodialysis: A systematic review", PloS one, 15(6), e0234376 (2020).
7. T. Gauna, E. Oshiro, Y.C. Luzio, A.M.M. Paniago, E.R.J.C. Pontes and M.R. Ching, "Bloodstream infection in patients with end-stage renal disease in a teaching hospital in central-western Brazil", Revista da Sociedade Brasileira de
8- S.Y. Tong, J.S. Davis, E. Eichenberger, T.L. Holland and V.G. Fowler Jr, "Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management", Clinical microbiology reviews, 28(3), 603-661 (2015).

9- B.E. Nuradeen, S.A. Omer, D.A. Sharif and T.S. Othman, "Catheter-related blood stream infection among hemodialysis patients: incidence and microbiological profile.", Journal of Sulaimani Medical College, 8(4), 223-235 (2018).

10- M. Rahbar, S. Karami and J. Vand Yousefi, "Evaluation of five phenotypic methods for detection of methicillin resistant Staphylococcus aureus (MRSA)". Iranian Journal of Pathology, 6, 27-31 (2011).

11- M. Tadros, V. Williams, B.L. Coleman, A.J. McGeer, S. Haider, C. Lee, H. Iacovides, E. Rubinstein, M. John and L. Johnston, "Epidemiology and outcome of pneumonia caused by methicillin-resistant Staphylococcus aureus (MRSA) in Canadian hospitals", PloS one, 8(9), e75171 (2013).

12- N.K. Sharma, C.E. Rees and C.E. Dodd, "Development of a single-reaction multiplex PCR toxin typing assay for Staphylococcus aureus strains". Applied and Environmental Microbiology, 66(4), 1347-1353 (2000).

13- M. Omidi, F. Firoozeh, M. Saffari, H. Sedaghat, M. Zibaei and A. Khaledi, "Ability of biofilm production and molecular analysis of spa and ica genes among clinical isolates of methicillin-resistant Staphylococcus aureus", BMC Research Notes, 13(1), 19 (2020).

14- A. Adler, V. Temper, C.S. Block, N. Abramson and A.E. Moses, "Panton-Valentine leukocidin-producing Staphylococcus aureus", Emerging infectious disease., 12(11), 1789-1790 (2006).

15- D. Bergey and J. Holt, "Bergey’s Manual of Determinative Bacteriology, 9th Edn Philadelphia", in, PA: Lippincott Williams & Wilkins, volume (2000).

16- CLSI, "CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-ninth Informational Supplement M100-S29 USA. Wayne, PA, USA: CLSI", in, Wayne, PA: Clinical and Laboratory Standards Institute; USA, volume (2019).

17- A. Larsen, M. Stegger and M. Sørum, "spa typing directly from a mecA, spa and pvl multiplex PCR assay—a cost-effective improvement for methicillin-resistant Staphylococcus aureus surveillance", Clinical Microbiology and Infection, 14(6), 611-614 (2008).

18- C. Wielders, A. Fluit, S. Brisse, J. Verhoef and F. Schmitz, "mecA gene is widely disseminated in Staphylococcus aureus population", Journal of clinical microbiology, 40(11), 3970-3975 (2002).

19- D.R. Bhatta, L.M. Cavaco, G. Nath, K. Kumar, A. Gaur, S. Gokhale and D.R. Bhatta, "Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant Staphylococcus aureus (MRSA) in Western Nepal: a matter of concern for community infections (a hospital based prospective study)", BMC infectious diseases, 16, 1-6 (2016).

20- G. Lina, Y. Piémont, F. Godail-Gamot, M. Bes, M.-O. Peter, V. Gauduchon, F. Vandenesch and J. Etienne, "Involvement of Panton-Valentine leukocidin—producing Staphylococcus aureus in primary skin infections and pneumonia", Clinical infectious diseases, 29(5), 1128-1132 (1999).

21- C.R. Arciola, L. Baldassarri and L. Montanaro, "Presence of icaA and icaD genes and slime production in a collection of staphylococcal strains from catheter-associated infections", Journal of clinical microbiology, 39(6), 2151-2156 (2001).

22- A. Oliver, A. Pascual, J. Rodriguez-Bano, G. Ruiz-Carrascos, P. Ruiz-Garbajosa, L. Zamorano, V. Bautista, M. Perez-Vazquez, J. Campos J.T. Wang, U.I. Wu, T.L. Lauderdale, M.C. Chen, S.Y. Li, L.Y. Hsu and S.C. Chang, "Carbapenem-nonsusceptible Enterobacteriaceae in Taiwan", Antimicrobial agents and chemotherapy, 10, e0121668 (2015).
23- F. Sahli, R. Feidjel and R. Laalaoui, "Hemodialysis catheter-related infection: rates, risk factors and pathogens", Journal of infection and public health, 10(4), 403-408 (2017).

24- X. Lemaire, M. Morena, H. Leray-Moragués, D. Henriet-Viprey, L. Chenine, C. Defez-Fougeron and B. Canaud, "Analysis of risk factors for catheter-related bacteremia in 2000 permanent dual catheters for hemodialysis", Blood purification, 28(1), 21-28 (2009).

25- J.-J. Parienti, M. Thirion, B. Mégarbane, B. Souweine, A. Ouchikhe, A. Polito, J.-M. Forel, S. Marquè, B. Misset and N. Airapetian, "Femoral vs jugular venous catheterization and risk of nosocomial events in adults requiring acute renal replacement therapy: a randomized controlled trial", Jama, 299(20), 2413-2422 (2008).

26- K. Wang, P. Wang, X. Liang and X. Lu, Z. Liu, "Epidemiology of haemodialysis catheter complications: a survey of 865 dialysis patients from 14 haemodialysis centres in Henan province in China", BMJ open, 5(11), e007136 (2015).

27- Z. Nabi, S. Anwar, M. Barhamein, H. Al Mukdad and A. El Nassri, "Catheter related infection in hemodialysis patients", Saudi Journal of Kidney Diseases and Transplantation, 20(9), 1091-1095 (2009).

28- M. Mattous, K. Djiguiba, N. Ouzeddoun, F. Ezaitouni, R. Bayahia and L. Benamar, "Infections liées aux cathéters centraux d’hémodialyse: facteurs de risque et écologie bactérienne", Néphrologie & Thérapeutique, 5, 332-333 (2011).

29- S. Sanavi, A. Ghods and R. Afshar, "Catheter associated infections in hemodialysis patients", Saudi Journal of Kidney Diseases and Transplantation, 18(1), 43-46 (2007).

30- R.A.S. Isidre, F.R. Acosta, C.R.C. Vargas, G.A.V. Romero, J.F.P. Perrota and R.M.G. Fretes, "Molecular Characterization of Staphylococcus aureus Isolates Obtained from Hemodialyzed Patients at the Hospital de Clínicas of Paraguay: A pilot study", International journal of medical students, 5, 14-19 (2017).

31- M. Sedlacek, J.M. Gemery, A.L. Cheung, A.S. Bayer and B.D. Remillard, "Aspirin treatment is associated with a significantly decreased risk of Staphylococcus aureus bacteremia in hemodialysis patients with tunneled catheters", American Journal of Kidney Diseases, 49(3), 401-408(2007).

32- H.A.H. Maamoun, A.R. Soliman and R. El Sherif, "Carriage of S taphylococcus aureus in the nose of patients on regular dialysis treatment using hemodialysis catheters", Hemodialysis International, 15(4), 563-567(2011).

33- J. Tanwar, S. Das, Z. Fatima and S. Hameed, "Multidrug resistance: an emerging crisis", Interdisciplinary perspectives on infectious diseases, 2014, 541340 (2014).

34- O.E. Akanbi, H.A. Njom, J. Fri, A.C. Otigbu and A.M. Clarke, "Antimicrobial susceptibility of Staphylococcus aureus isolated from recreational waters and beach sand in Eastern Cape Province of South Africa", International journal of environmental research and public health, 14(9), 1001 (2017).

35- P. Skórczewski, Z.J. Mudryk, J. Miranowicz, P. Perlin ski and M. Zdanowicz, "Antibiotic resistance of Staphylococcus-like organisms isolated from a recreational sea beach on the southern coast of the Baltic Sea as one of the consequences of anthropogenic pressure", Oceanological and Hydrobiological Studies, 43, 41-48 (2014).

36- M. Ahangarzadeh Rezaee, S.F. Mirkarimi, A. Hasani, V. Sheikhalizadeh, M.H. Soroush and B. Abdinia, "Molecular typing of Staphylococcus aureus isolated from clinical specimens during an eight-year period (2005-2012) in Tabriz, Iran", Archives of Pediatric Infectious Diseases, 4(2) (2016).

37- H. M. Abdulghany, R.M. Khairy, "The frequency of methicillin-resistant Staphylococcus aureus and coagulase gene polymorphism in Egypt", International journal of bacteriology, 2014,680983 (2014).
38- N.Y. Omar, H.A.S. Ali, R.A.H. Harfoush and E.H. El Khayat, "Molecular typing of methicillin resistant Staphylococcus aureus clinical isolates on the basis of protein A and coagulase gene polymorphisms", International journal of microbiology, 2014, (2014).

39- S.M. Hosseini - P. Karami, H. Kazemian, Z. Karimitabar, A.M. Bardbari, A. Khaledi and M.R. Arabestani, "Relationship between antibiotic resistance with spa gene polymorphism coding protein A and its typing with PCR-RFLP technique in S. aureus isolated from foodstuffs", Avicenna Journal of Clinical Microbiology and Infection, 4(3), 12105 (2017).

40- M. Omidi, F. Firoozeh, M. Saffari, H. Sedaghat, M. Zibaei and A. Khaledi, "Ability of biofilm production and molecular analysis of spa and ica genes among clinical isolates of methicillin-resistant Staphylococcus aureus", BMC research notes, 13, 1-7 (2020).

41- B. Diemand-Hernández, F. Solórzano-Santos, B. Leaños-Miranda, L. Peregrino-Bejarano and G. Miranda-Novales, "Production of icaADBC-encoded polysaccharide intercellular adhesin and therapeutic failure in pediatric patients with staphylococcal device-related infections", BMC infectious diseases, 10, 1-6 (2010).

42- M. Mirzaee, S. Najar-Peerayeh, M. Behmanesh, M. Forozan-deh-Moghadam and A.-M. Ghasemian, "Detection of intracellular adhesion (ica) gene and biofilm formation Staphylococcus aureus isolates from clinical blood cultures", Journal of Medical Bacteriology, 3(1), 1-7 (2014).

43- E. Torlak, E. Korkut, A.T. Uncu, Y. Şener, "Biofilm formation by Staphylococcus aureus isolates from a dental clinic in Konya, Turkey", Journal of infection and public health, 10(6), 809-813 (2017).

44- C.K. Hesje, C.M. Sanfilippo, W. Haas and T.W. Morris, "Molecular epidemiology of methicillin-resistant and methicillin-susceptible Staphylococcus aureus isolated from the eye", Current eye research, 36(2), 94-102 (2011).

45- L. De-Zhi, C. Yu-sheng, Y. Jing-ping, Z. Wei, H. Cheng-Ping, L. Jia-Shu, M. Lan, H. Ying-hui, G. Rong and H. Ke, "Preliminary molecular epidemiology of the Staphylococcus aureus in lower respiratory tract infections: a multicenter study in China", Chinese medical journal, 124(5), 687-692 (2011).

46- K. Garbacz, L. Piechowicz, W. Baranska-Rybak and M. Dabrowska-Szponar, "Staphylococcus aureus isolated from patients with recurrent furunculosis carrying Panton-Valentine leukocidin genes represent agr specificity group IV", European Journal of Dermatology, 21(1), 43-46 (2011).

47- T. Conceicao, M. Aires-de-Sousa, M. Füzi, A. Toth, J. Paszti, E. Ungvári, W. Van Leeuwen, A. van Belkum, H. Grundmann and H. De Lencastre, "Replacement of methicillin-resistant Staphylococcus aureus clones in Hungary over time: a 10-year surveillance study", Clinical Microbiology and Infection, 13(10), 971-979 (2007).

48- A. Budimir, R. Deurenberg, Z. Bošnjak, E. Stobberingh, H. Cetkovic and S. Kalenic, "A variant of the Southern German clone of methicillin-resistant Staphylococcus aureus is predominant in Croatia", Clinical microbiology and infection, 16(8), 1077-1083 (2010).

49- K.S. Ko, J.-Y. Lee, J.Y. Suh, W.S. Oh, K.R. Peck, N.Y. Lee and J.-H. Song, "Distribution of major genotypes among methicillin-resistant Staphylococcus aureus clones in Asian countries", Journal of clinical microbiology, 43(1), 421-426 (2005).

50- N. D'Souza, C. Rodrigues and A. Mehta, "Molecular characterization of methicillin-resistant Staphylococcus aureus with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India", Journal of clinical microbiology, 48(4), 1806-1811 (2010).

51- F. Rahimi, "Characterization of resistance to aminoglycosides in methicillin-resistant Staphylococcus aureus strains isolated from a tertiary care hospital in Tehran, Iran", Jundishapur journal of microbiology, 9(5), e32388 (2016).
T. Ida, R. Okamoto, C. Shimauchi, T. Okubo, A. Kuga and M. Inoue, "Identification of aminoglycoside-modifying enzymes by susceptibility testing: epidemiology of methicillin-resistant Staphylococcus aureus in Japan", *Journal of clinical microbiology*, 39(9), 3115-3121 (2001).

T. Hauschild, P. Sacha, P. Wieczorek, M. Zalewska, K. Kaczyńska and E. Tryniszewska, "Aminoglycosides resistance in clinical isolates of Staphylococcus aureus from a University Hospital in Bialystok, Poland", *Folia Histochemica et Cytobiologica*, 46(2), 225-228 (2008).

A. Azimian, S.A. Havaei, H. Fazeli, M. Naderi, K. Ghazvini, S.M. Samiee, M. Soleimani and S.N. Peerayeh, "Genetic characterization of a vancomycin-resistant Staphylococcus aureus isolate from the respiratory tract of a patient in a university hospital in northeastern Iran", *Journal of clinical microbiology*, 50(11), 3581-3585 (2012).

F. Ghanbari, H. Ghajavand, R. Havaei, M. S. Jami, F. Khademi, L. Heydari, M. Shahin and S.A. Havaei, "Distribution of erm genes among Staphylococcus aureus isolates with inducible resistance to clindamycin in Isfahan, Iran", *Advanced biomedical research*, 5,62 (2016).

L. Shallcross, K. Williams, S. Hopkins, R. Aldridge, A. Johnson and A. Hayward, "Panton-Valentine leukocidin associated staphylococcal disease: a cross-sectional study at a London hospital, England", *Clinical Microbiology and Infection*, 16(11), 1644-1648 (2010).

E. Năstase, O. Dorneanu, T. Vremeră, C. Logigan, E. Mițode and C.M. Dorobăț, "MecA and pvl genes detection in Staphylococcus aureus strains isolated from lower respiratory tract infections", *Revista medico-chirurgicala a Societății de Medici si Naturalisti din Iasi*, 114(4), 1162-1168 (2010).

Q. Li, Y. Zhu, K. Dong, C. Liu, Y. Zhou, Y. Ni and X. Guo, "A novel sequence-based coa genotyping method to discriminate nosocomial methicillin-resistant Staphylococcus aureus isolates", *Irish journal of medical science*, 180(2), 463-468 (2011).

C.-M. Ho, M.-W. Ho, C.-Y. Lee, N. Tien and J.-J. Lu, "Clonal spreading of methicillin-resistant SCC mec Staphylococcus aureus with specific spa and dru types in central Taiwan", *European journal of clinical microbiology & infectious diseases*, 31(4), 499-504 (2012).

S. Johler, G. Macori, A. Bellío, P. Acutis, S. Gallina and L. Decastelli, "Characterization of Staphylococcus aureus isolates of bovine mastitis", *Journal of dairy science*, 101(4), 2915-2920 (2018).

I. Gheorghe, A.L. Tatu, I. Lupu, O. Thamer, A.I. Cotar, G.G. Pircalabioru, M. Popa, V.C. Cristea, V. Lazar and M.C. Chifiriuc, "Molecular characterization of virulence and resistance features in Staphylococcus aureus clinical strains isolated from cutaneous lesions in patients with drug adverse reactions", *Rom Biotechnol Lett*, 22(1), 12321-12327 (2017).

K. Kuzma, E. Malinowski, H. Lassa and A. Klossowska, "Analysis of protein A gene polymorphism in Staphylococcus aureus aureus isolates from bovine mastitis", *Bull Vet Inst Pulawy*, 49(1), 41-44 (2005).

F. Shakeri, A. Shojaei, M. Golalipour, S. Rahimi Alang, H. Vaez and E.A. Ghaemi, "Spa Diversity among MRSA and MSSA Strains of Staphylococcus aureus in North of Iran", *International journal of microbiology*, 2010, 351397 (2010).

M.B. Nejma, M. Mastouri, S. Frih, N. Sakly, Y.B. Salem and M. Nour, "Molecular characterization of methicillin-resistant Staphylococcus aureus isolated in Tunisia", *Diagnostic Microbiology and infectious disease*, 55(1), 21-26 (2006).

K. Kreausukon, A. Fetsch, B. Kraushaar, K. Alt, K. Müller, V. Krömker, K.-H. Zessin, A. Käsbohrer and B.-A. Tenhagen, "Prevalence, antimicrobial resistance, and molecular characterization of methicillin-resistant Staphylococcus aureus from bulk tank milk of dairy herds", *Journal of dairy science*, 95(8), 4382-4388 (2012).
توصيف لبعض جينات الضراءة لميكروب المكور العنقودي الذهبي المعزول من المداخل الوعائية لمرضى الغسيل الكلوي بمستشفى جامعة أسيوط

أحسان عبد الصبور حسن 1، منى حسن عبد الرحيم 1، ثناء حسن محمد حسن 1، نشوى مصطفى عبد المنعم عزوز 3، منى سلام أبادامك محمد 1

1 قسم الميكروبيولوجيا الطبية والمناعة، كلية الطب، جامعة أسيوط، 21515، أسيوط، جمهورية مصر العربية

2 مستشفى ابنوب العام، محافظة أسيوط، جمهورية مصر العربية

3 وحدة أمراض الكلى، قسم الباطنة العامة، مستشفيات جامعة أسيوط، 21515، أسيوط، جمهورية مصر العربية

أجريت هذه الدراسة من يناير 2016 إلى أكتوبر 2021 على مرضى الغسيل الكلوي في مراكز مراكز الوعاء وذلك عبر تحليل البكتيريا من المراكز الوعائية. تمت جمع البكتيريا من المراكز الوعائية إلى أن تم تحديد البكتيريا المستخدمة في العزلة. البكتيريا كانت البكتيريا الأكثر شيوعًا في الإصابة المشتبه بها بنسبة 37.5٪ (21/52) من عدد البكتيريا المستخدمة، البكتيريا كانت أيضًا أكثر الكائنات الجيني شبيهة في الإصابة المشتبه بها بنسبة 21.43٪ (11/52). خصوصًا (99.2٪ من MRSA، البكتيريا المستخدمة في عزلة المرض). تم التعرف على عزلة المرض عبر معالجة البكتيريا بالبكتيريا المستخدمة في عزلة المرض مع معالجة البكتيريا المستخدمة في عزلة المرض. استخدمت الطرق المستخدمة في تحليل البكتيريا المستخدمة في عزلة المرض. أظهرت النتائج المرضية عالة معالجة حساسية mecA للفلافوناميس (96.1٪) كما تم الكشف عنها بطريقة نشر قرص كيبي باور. تم تشخيص جين bp في عزلة مختلفة من المكورات العنقودية الذهبي. تراوحت أحجام المنتجات من 240 إلى 350 bp، وظهرت هذه المنتجات 3 أنواع مختلفة من جين آل، كان النطاق الجيني للمتبقي الصحي الأكثر شيوعًا الذي تم اكتشافه بين عزلات المكورات العنقودية الذهبي عند 350 bp (240-350 bp) من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. ومن العزلات بينما ظهرت عند (420-430 bp) وعبر عزلات البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدمة في عزلة المرض. من العزلات، وله S2 الذي ظهر عند (420-430 bp) والذي تم تحديده في البكتيريا المستخدم
من 52 (76.90%) المكورات العنقودية الذهبية كانت موجبة لجين الايكا والذي ظهر عند 188bp فقط 2 (3.85%) من المكورات العنقودية الذهبية كانت موجبة لعزلات جاما-الهيوموليسين والتي ظهرت عند 937bp. جميع العزلات الب - 52 من المكورات العنقودية الذهبية كانت سالبة لجين ال pvl.