Molecular Characterization of *Staphylococcus aureus* Isolated from Renal Hemodialysis (HD) Patients from Saudi Arabia

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Abstract

*Staphylococcus aureus*, including methicillin resistant *S. aureus* (MRSA) is the most commonly isolated pathogen in hospitals worldwide. The aim of present study was molecular characterization of *Staphylococcus aureus* isolated from renal hemodialysis (HD) patients from Ha’il region of Saudi Arabia. A total of 392 samples were screened from 204 HD patients for colonization of *S. aureus*. The isolated bacteria were identified by MALDI-TOF-MS. Antibiotic susceptibility testing was performed using Microscan. Among these isolates, 72 *S. aureus* (43% MRSA and 57% MSSA) were identified. The isolates were considerably resistant with varied profile to the antibiotics tested except being 100% susceptible to vancomycin, linezolid and teicoplanin. Of the isolates, 22.2% were positive for biofilm assay. Four representative MRSA isolates were selected and whole genome sequence analysis was performed using MiSeq. Two out of the 4 MRSA were found to be ST-1 and 2 were found to be ST-32. Among MRSA isolates, 25.8% were negative for mecA and all of them were negative for mecC gene. A high prevalence of MRSA in HD patients as well as high percentage of biofilm production in MRSA isolates highlights the vital role for standardized surveillance along with validated molecular typing methods to evaluate the incidence of MRSA and accordingly to control its spread.

Keywords: Hemodialysis, *Staphylococcus aureus*, Whole genome sequencing Pathogenic bacteria, MALDI-TOF-MS.

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INTRODUCTION

*Staphylococcus aureus* is an opportunistic bacterial pathogen responsible for a large number of human and animal infections. *Staphylococcus aureus* is associated with asymptomatic colonization of the skin and mucosal surfaces of about 30% of normal humans. The Staphylococcal infections have been found regularly among the patients with compromised immune system and when the skin or mucosal barriers are breached, following insertion of a foreign body. There is a high incidence of infections caused by *S. aureus* among the patients with renal disease; specifically, those undergoing hemodialysis or kidney transplantation. Because of frequent use of antimicrobials for a prolonged time and use of catheters, the hemodialysis (HD) patients are at a higher risk of colonization and infection by multi-drug resistant *S. aureus* including MRSA (methicillin resistant *S. aureus*). The bacterial infections are the major cause of morbidity and mortality during receiving hemodialysis and *S. aureus*, particularly MRSA, is one of the most common pathogen. Mortality from all causes in patients on dialysis treatment is 6.5–7.9 times higher than that of the general population. *S. aureus* can colonize almost half the dialysis population without any indication of disease. However, such colonization of *S. aureus* can cause wound and tissue infections; septicemia; and chronic infections. Infections cause significant morbidity and mortality among dialysis patients and HD patients are a high-risk population for bloodstream infection. Renal disease, especially hemodialysis is a complex health care issue globally, including Saudi Arabia. Saudi Center for Organ Transplantation (SCOT) estimated a total of 19,659 dialysis patients, 18,270 of them are treated by hemodialysis (HD) and the remaining 1,389 by peritoneal dialysis (PD) with the mortality of about 9%. There is a lack of data regarding the prevalence of *S. aureus* among hemodialysis patients from Saudi Arabia; therefore, the aim of present study was molecular characterization of *Staphylococcus aureus* isolated from renal hemodialysis (HD) patients from Ha’il region of Saudi Arabia.

MATERIALS AND METHODS

**Bacterial isolates**

In this study, a total of 392 samples were screened from 204 HD patients from King Khalid Hospital, Ha’il, Saudi Arabia. The samples were collected from catheter tips, catheter site swabs and nose swabs.

The identification of bacterial isolates was performed on MALDI-TOF-MS (Bruker Daltonics Germany) according to the manufacturer’s guidelines. Briefly, a fresh bacterial colony from overnight culture was smeared on target plate overlaid with 1 µl of a saturated a-cyano-4-hydroxy-cinnamic acid (HCCA) matrix solution in 50% acetonitrile-2.5% trifluoroacetic acid (Bruker Daltonics) with the help of a sterile toothpick and air dried at room temperature. The plate was loaded in to the machine and the operation was run. The identification and analysis of mass spectra were performed the MALDI Biotyper software package (version 3.0).

**Identification and Antibiotic susceptibility by Microscan**

Microscan walkaway (Siemens Healthcare Diagnostics, Sacramento, CA, USA); an automated system used for bacterial identification and antibiotic susceptibility test was used for confirmation of identification and antimicrobial susceptibility of the bacterial strains. In this method, a small portion of a well isolated colony was taken and added to a Gram-positive Microscan combo panel. The panel was loaded into the Microscan walkaway machine according to the manufacturer's guidelines.

**Table 1. Whole genome sequencing results showing MLST type 1**

| Gene | % identity | Alignment Length | DB Allele Length | Gaps | Best Match |
|------|------------|------------------|------------------|------|------------|
| arcc | 100        | 456              | 456              | 0    | arcc_1     |
| aroe | 100        | 456              | 456              | 0    | aroe_1     |
| glpf | 100        | 465              | 465              | 0    | glpf_1     |
| gmk  | 100        | 417              | 417              | 0    | gmk_1      |
| pta  | 100        | 474              | 474              | 0    | pta_1      |
| tpi  | 100        | 402              | 402              | 0    | tpi_1      |
| yqit | 100        | 516              | 516              | 0    | yqit_1     |
manufacturer’s protocol. Results were available after 24-48 hrs.

**Biofilm assay**

Biofilm assay was performed according to a previously published method\(^2\).

**Polymerase Chain Reaction for mec genes**

PCR was used to determine the type of mec gene in MRSA isolate\(^2\). The primers used for detection of types of mec gene were F: 5'-GTAGAAATGACTGAACGTCGATGA-3' and R: 5'-CCAATTCCACATTGTTCGGTCTAA-3'.

A PCR amplicon of 310 base pairs was analyzed using Sanger sequencer.

**Whole Genome Sequencing**

The sequencing of the bacterial genome for detection of antibiotic resistant genes, virulence factors, plasmids and MLST types was performed by using Illumina methodology using NextEra kit for library preparation\(^2\). The presence of known acquired resistance genes was determined by mapping the data from the isolate to an online database. The ResFinder web server (www.genomicepidemiology.org) and Basespace from Illumina was used to identify acquired antimicrobial resistance genes, MLST types and the presence of different virulent genes in the WGS data, using a threshold of 98% identity.

**RESULTS AND DISCUSSION**

There is a high incidence of colonization followed by infections caused by *S. aureus* among the HD patients\(^5\). The main reason behind this high infection rate among HD patients is because of frequent use of antimicrobials for a prolonged time and use of catheters during the dialysis procedure. Thus HD patients are at a higher risk of colonization and infection by multi-drug resistant *S. aureus* including MRSA (methicillin resistant *S. aureus*).

| Gene | % Alignment Length | DB Allele Length | Gaps | Best Match |
|-----|--------------------|------------------|------|------------|
| arcc | 100 | 456 | 456 | 0 | arcc_1 |
| aroe | 100 | 456 | 456 | 0 | aroe_3 |
| glpf | 100 | 465 | 465 | 0 | glpf_1 |
| gmk | 100 | 417 | 417 | 0 | gmk_14 |
| pta | 100 | 474 | 474 | 0 | pta_11 |
| tpi | 100 | 402 | 402 | 0 | tpi_51 |
| yqit | 100 | 516 | 516 | 0 | yqit_10 |

**Table 2.** Whole genome sequencing results showing MLST type 80

**Table 3.** Whole genome sequencing results showing different antibiotic resistance genes and virulence factors

| Resistance Gene | % Identity | DB Allele/ Alignment Length | Contig ID | Position in Contig | Phenotype | Accession No. |
|-----------------|------------|-----------------------------|-----------|-------------------|-----------|---------------|
| ant(6)-Ia       | 100        | 909/909                     | NODE_11_length_6968 | 2019..2927 | Aminoglycoside resistance | AF3300699 |
| aph(3')-III     | 100        | 795/795                     | NODE_11_length_6968 | 3559..4353 | Aminoglycoside resistance | M26832 |
| msr(A)          | 99.73      | 1467/1467                   | NODE_10_length_6376 | 4297..5763 | Macrolide, Lincosamide and Streptogramin B resistance | X52085 |
| norA            | 91.59      | 1167/1142                   | NODE_15_length_25906 | 5508..6649 | Fluroquinotone resistance | M97169 |
| mecA            | 100        | 2007/2007                   | NODE_84_length_8411 | 1736..3742 | Beta-lactam resistance | A8 033763 |
| blaZ            | 99.55      | 846/441                     | NODE_480_length_434 | 1/1/0441 | Beta-lactam resistance | AJ302698 |
| fusB            | 100        | 642/642                     | NODE_30_length_2646 | 12:00:00 AM | Fusidic acid resistance | AM292600 |
The bacterial infections are the major cause of morbidity and mortality during receiving hemodialysis and *S. aureus*, particularly MRSA, is one of the most common pathogens\(^9\). The current study was aimed at characterization of *S. aureus* isolated from renal hemodialysis (HD) patients from Ha’il region of Saudi Arabia.

A total of 72 *S. aureus* isolates were cultured from patients undergoing HD, and among these, 43.1% were MRSA and 56.9% were methicillin sensitive *S. aureus* (MSSA). Previous studies have highlighted a high percentage of *S. aureus* colonization among HD patients\(^17,23,24\). The percentage of MRSA and MSSA in our study was found similar to that of a study published from Japan\(^7\). The antibiotic profiling of *S. aureus* is very critical in management of the serious infections among the hospitalised patients. The *S. aureus* isolates from our study showed a varied profile to the antibiotics tested with 100% susceptibility to vancomycin, linezolid and teicoplanin. In addition, the capability of biofilm production among *S. aureus* helps it to remain in the hospital environment for prolonged time period leading to colonization of more patients\(^25\). In our study, 22.2% *S. aureus* were positive for biofilm assay.

By using the most advanced technique in Microbiology laboratory, whole genome sequencing can provide a broad analysis of the bacterial strains from all the sources. With the development of bench-top sequencers and rapid analytical softwares, WGS has become a useful tool to guide treatment of the infections caused by bacterial strains. The whole genome sequencing results of MRSA from our study revealed that genome sizes ranging from 2879711 bp to 3012628 bp with 720 to 3408 contigs were successfully sequenced. The MLST data revealed that the most common MLST type of the MRSA from our study were ST1 and ST80 (Table 1 and Table 2). The res finder showed that the MRSA in our study contained the genes which exhibited the resistance to aminoglycosides, macrolides, fluoroquinolones, β-lactams and the most common genes detected were *msr(A)*, *nora*, *blaZ*, *ant(6)Ia*, *aph(3)-II* and *mecA* and *fusB* (Table 3). The presence of these genes by WGS was found to be associated with that of phenotypic antibiotic profiles.

## CONCLUSIONS

This is the first report of molecular characterization of *S. aureus* collected from HD patients in Ha’il region of Saudi Arabia. A high prevalence of MRSA in HD patients as well as high percentage of biofilm production in MRSA isolates were observed in this study. This study emphasizes on the vital role for standardized surveillance along with validated molecular characterization methods to evaluate the incidence of MRSA and accordingly to control its spread among HD patients.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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