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RESEARCH ARTICLE

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[1,2]. 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[3].

There is a high incidence of infections caused by *S. aureus* among the patients with renal disease; specifically, those undergoing hemodialysis or kidney transplantation[4,5]. 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*)[6-8]. 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[9-11]. 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[12]. However, such colonization of *S. aureus* can cause wound and tissue infections; septicemia; and chronic infections[13-16].

Infections cause significant morbidity and mortality among dialysis patients and HD patients are a high-risk population for blood-stream infection[17,18]. 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%[19]. 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

Table 1. Whole genome sequencing results showing MLST type 1

Sequence Type: ST1 (MLST Scheme: *Staphylococcus aureus*)[2]					
Gene	% identity	Alignment Lenght[3]	DB Allele Lenght	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[20].

Polymerase Chain Reaction for mec genes

PCR was used to determine the type of mec gene in MRSA isolate[21]. The primers used for detection of types of mec gene were F: 52 -GTAGAAATGACTGAACGTCCGATGA-32 and R: 52 -CCAATTCCACATTGTTTCGGTCTAA-32 .

Table 2. Whole genome sequencing results showing MLST type 80

Sequence Type: ST80 (MLST Scheme: *Staphylococcus aureus*)[2]					
Gene	% identity	Alignment Lenght[3]	DB Allele Lenght	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

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[22]. 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.*

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

Reistance Gene	% Identity	DB Allele/ Alignment Lenght	Contig ID	Poistion in Contig	Phenotype	Accession No.
ant(6)-Ia	100	909/909	NODE_11_lenght_6968 _cov_40.318600	2019..2927	Aminoglycoside reistance	AF3300699
aph(3')-III	100	795/795	NODE_11_lenght_6968 _cov_40.318600	3559..4353	Aminoglycoside resistance	M26832
msr(A)	99.73	1467/1467	NODE_10_lenght_6376 _cov_88.807556	4297..5763	Macrolide, Lincosamide and Streptogramin B reistance	X52085
norA	91.59	1167/1142	NODE_15_lenght_25906 _cov_27.324635	5508..6649	Fluroquinotone reistance	M97169
mecA	100	2007/2007	NODE_84_lenght_8411 _cov_ 30.187254	1736..3742	Beta-lactam reistance	AB 033763
blaZ	99.55	846/441	NODE_480_length_434 _cov_166.806458	1/1/0441 12:00:00 AM	Beta-lactam reistance	AJ302698
fusB	100	642/642	NODE_30_lenght_2646 _cov_44.031746	1364..2005	Fusidic acid reistance	AM292600

aureus)[6-8]. 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[9-11]. 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[17]. 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)-III, 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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