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Staphylococcus aureus nasal carriage in centers of Casablanca (Morocco)

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The aim of this study was to determine the prevalence of nasal carriage of *Staphylococcus aureus* among patients and personnel of private centers at Casablanca, and to determine the resistance pattern of isolates. The carriage of virulence toxin genes by the methicillin resistant strains was also investigated. This study was conducted from November 2008 to February 2009. Nasal swabs were taken from 145 and 42 patients and personnel respectively. The susceptibility testing to 16 antibiotics was performed using the agar disc diffusion method. Minimum inhibitory concentrations (MICs) of oxacillin were determined by the agar dilution method for all strains demonstrating resistance to cefoxitin. In addition, resistant isolates were examined for the existence of the *mecA* gene by polymerase chain reaction (PCR). Furthermore, the carriage of 22 virulence toxin genes among strains showing resistance to cefoxitin was investigated by PCR Multiplex. The prevalence of nasal carriage of *S. aureus* was 32.4% (n=47) and 38.1% (n=16) in patients and personnel, respectively. Patients' strains showed 16 resistance patterns against only 4 in personnel strains. No *S. aureus* isolates were found to be resistant to lincomycin, pristinamycin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, rifampicin and vancomycin, while over 90% (n = 59) were resistant to penicillin G. For the other antibiotics, the percentage of resistance varied between 2.63 and 18.75%. One *S. aureus* (1.6%) was methicillin resistant by possession of *mecA* gene. This isolate harboured the staphylococcal enterotoxin genes *sec*, *sed*, *sell*, *selm*, *selo*, *ser* and toxic shock syndrome toxin gene (*tst*). Investigation of *S. aureus* nasal carriage and characterization of isolates among patients undergoing hemodialysis is important to develop infection prevention and to limit the spread of methicillin resistant *S. aureus* (MRSA) strains.

Key words: Hemodialysis centers, patients, personnel, *Staphylococcus aureus*, nasal carriage, antibiotic susceptibility, methicillin resistance, toxin genes.

INTRODUCTION

The anterior are the primary reservoir of *Staphylococcus aureus* in humans and its nasal carriage is recognised as a major risk factor for the development of both community-acquired and nosocomial infections (Boelaert et al., 1995; Koziol-Montewka et al., 2001), particularly in patients who are undergoing long-term hemodialysis

(Watanakunakorn et al., 1992). Infections complications are the main cause of morbidity and the second cause of mortality after cardiovascular diseases in chronic hemodialyzed patients. *S. aureus* is by far the most frequent bacterium implicated in these infections, especially in septicemia (Forestier et al., 2007; Koziol-Montewka et al., 2001; Koziol-Montewka et al., 2006).

Several factors are likely to depress the immune system of these patients, and thus, make them more susceptible to infection, such as old age, concurrent debilitating illnesses, long-term stay in hospital, repeated antibiotic treatment and specific immune defects associated with renal dysfunction (Koziol-Montewka et al., 2001). Therefore, recognition of persons colonized or infected with *S. aureus* is recommended for preventing the spread of the organism within hospitals or in communities. The emergence and dissemination of methicillin-resistant *S. aureus* (MRSA), which is also often multi-drug-resistant renders the treatment of staphylococcal infections more challenging.

Methicillin resistance in *S. aureus* is conferred by carriage of the *mecA* gene that codes for the penicillin binding protein PBP2a, with very low affinity to beta-lactam antibiotics (Beck et al., 1986; Katayama et al., 2000). Many *S. aureus* strains, especially MRSA produces a variety of extracellular toxins and virulence factors (Hu et al., 2008) that contribute to its pathogenic potential, including staphylococcal enterotoxins (SEs), SEs-like, Panton-Valentine leukocidin (PVL), toxic shock syndrome toxin-1 (TSST-1), exfoliative toxin (ET), hemolysins, and coagulase (Hu et al., 2008).

Increasing resistance to the drugs has recently raised the concerns of both microbiologists and clinicians, especially in the case of MRSA strains. Data on the epidemiology of antibiotic resistance are relevant, as they should provide a basis for the selection of empiric use of antimicrobial agents, either for therapy or for prophylaxis, especially patients receiving hemodialysis are at particular risk for the development of invasive infections caused by staphylococci (Watanakunakorn et al., 1992).
The purpose of this study was, on one hand, to define the frequency of *S. aureus* nasal carriage from patients and personnel of three private hemodialysis centers at Casablanca (Morocco) and to investigate antibiotic resistance rates with the choice of the method suited for the detection of the MRSA; on the other hand, to estimate the frequency and to access the virulence potential of methicillin resistant *S. aureus* isolates.

MATERIALS AND METHODS

Sampled persons

Nasal swabs were collected from patients and personnel (medical staff was not excluded) of three private hemodialysis centres in Casablanca during four months (November 2008 to February 2009). Each person was asked to give informed consent prior to specimen collection. Characteristics of the two study groups are noted (age, sex, treatment period with hemodialysis, history of hospitalisation, antibiotic therapy, and history of underlying diseases such as diabetes).

Nasal swabs

Nasal specimens for culture were taken with sterile, cotton tipped swabs and were obtained by 5 rotations in each anterior nary. All nasal swabs were quickly sent to the bacteriological laboratory in closed boxes and were processed on the day of sampling.

Isolation of *S. aureus* from nasal swabs

The study was conducted at the Laboratory of Molecular Bacteriology of the Pasteur institute, Casablanca, Morocco. Nasal swabs were screened for the presence of *S. aureus*. The swabs were inoculated and streaked on Chapman agar (local production according to its composition), and incubated aerobically at 36°C for 18 to 24 h. Only one isolate from each individual was included. After incubation, media were investigated and presumptive *S. aureus* colonies were identified, based on colony morphology, mannitol fermentation, catalase test and Gram staining, coagulase activity on rabbit plasma (Bio-Mérieux, Marcy l'Etoile, France), and production of clumping factor (Pastorex Plus-Staph, Bio-Rad, Marnes-la-Coquette, France). The homogeneous strains were stored at 4°C in nutrient agar until use, and at -20°C in glycerol stocks using commercial Cryobilles (AES Laboratoire; France).

Methicillin sensitivity

Methicillin susceptible *S. aureus* strains (MSSA) were differentiated from MRSA using agar screen plates on Muller-Hinton (M-H) agar (Bio-Rad, France) containing 30 µg/ml of cefoxitin disc as recommended by the French Microbiological Society Antibiogram Committee (CA-SFM 2009). Minimum inhibitory concentrations (MICs) to oxacillin were determined by the agar dilution procedure according to the CA-SFM guidelines (2009), for all strains showing a growth inhibition zone diameter ≤ 27 mm after incubation at 36°C for 18-24 h. The range of dilution used was 0.004 to 128 mg/L and the breakpoint for the definition of oxacillin resistance was MICs ≥ 2 µg/mL. MRSA reference strain U2A1593 and one methicillin-susceptible *S. aureus* reference strain U2A1594 provided by Pasteur Institute (Paris, France) were used as controls.

Antibiotic susceptibility testing

Antibiotic sensitivity of all *S. aureus* strains was performed on the isolates using the agar disc diffusion method on M-H medium (Bio-Rad, France) according to the recommendations given by the CA-SFM (2009). Disks loaded with the following antimicrobial agents (Bio-Rad, France) were used for susceptibility testing: cefoxitin (30 µg), penicillin G (6 µg), tetracycline (30 µg), erythromycin (15 µg), lincomycin (15 µg), pristinamycin (15 µg), kanamycin (30 µg), tobramycin (30 µg), gentamicin (10 µg), chloramphenicol (30 µg), rifampicin (5 µg), fosfomycin (50 µg), pefloxacin (5 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), fusidic acid (10 µg) and vancomycin (30 µg). In addition, *mecA* negative strains

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Table 1. Details of personnel and hemodialyzed patients *Staphylococcus* carriers and non-carriers

Parameter	*S. aureus* carriers	*S. aureus* non-carriers
Number of patient and percentage (n= 145)	47 (32.4%)	98 (67.6%)
Mean age		
Sex	22 men (46.8%) 25 women (53.2%)	
Number of personnel and percentage (n= 42)	16 (38.1%)	26 (61.9%)
Mean age		
Sex	8 men (50%) 8 women (50%)	

with reduced susceptibility to cefoxitin were submitted to the action of the following betalactamins antibiotics: cefalotin, amoxicillin and amoxicillin+clavulanic acid. Any penicillin G susceptible strain (inhibition diameter greater or equal to 29 mm) was submitted to a chromogenic test (cefinase test) for confirmation. The performance of the susceptibility testing was monitored by the quality control strains *S. aureus* ATCC 25923.

All isolates of *S. aureus* with reduced susceptibility to cefoxitin were grown in brain heart infusion media at 36°C overnight. Their genomic DNA used for PCR was extracted by using a standard phenol-chloroform procedure as described by Sambrook et al. (1989). In addition, the *nuc* gene responsible for the production of thermostable nuclease was detected by PCR assay (see *nuc* and *mecA* genes detection) in order to confirm that the isolates were indeed *S. aureus* and not other staphylococcal species.

nuc and *mecA* genes detection

The presence of the *mecA* gene was determined by Polymerase Chain Reaction methods (PCR) in isolates that showed cefoxitin zone sizes smaller or equal to 27 mm in order to confirm the assumption that the methicillin (cefoxitin) resistant strain was a MRSA. Duplex PCR was performed for the simultaneous detection of the *nuc* (encoding for the *S. aureus* specific thermonuclease) and the *mecA* (encoding for the PBP2a) genes using protocols and primers as described by Chesneau et al. (1993) and Vannuffel et al. (1995), respectively. The control organisms included *S. aureus* U2A 1594 (MSSA negative *mecA*) and *S. aureus* U2A 1593 (MRSA, positive *mecA*).

Virulence profile of *S. aureus* strains

To assess the virulence potential of strains demonstrating resistance to cefoxitin (CMI value of oxacillin ≥ 2 µg/mL), several Multipex PCRs for the parallel detection of the presence of genes coding for: the classical staphylococcal enterotoxins A, B, C and D (*sea, seb, sec* and *sed*) SEs, and SE*ls* H, K, L, M, O, P, Q and R (*seh, selk, sell, selm, selo, selp, selq* and *selr*), the toxic shock syndrome toxin-1 (*tst*), the exfoliative toxins A, B and D (*eta, etb* and *etd*), the Panton Valentine leukocidin (PVL) (*luk*S-PV; *luk*F-PV), the Luk-M leukocidin (*luk*M), the epidermal cell differentiation inhibitor A, B and C (*edin* A, B and C) and β- hemolysin (*hlb*) as described previously (Holtfreter et al., 2007; Jarraud et al., 2002; Tristan et al., 2003), were performed. Positivity was verified using reference strains *S. aureus* ATCC19095 (*sec, seh, sell, seg, sei, selm, seln, selo* and *seu*); FRI913 (*sea, sec, see, selk, sell, selq* and *tst*); ATCC14458 (*seb*). However, control chromosomal DNA samples for *sed*, *selr, selp, luk*-PV, *luk*M, *eta*, *etb*, *etd*, *edin* A/B/C and *hlb* genes were obtained from our standard laboratory controls.

RESULTS

Nasal carriage

In this study, we investigated a total of 42 personnel members (20 men (47.6%) and 22 women (52.4%)) and 145 patients (73 men (50.3%) and 72 women (49.7%)), who were between 15 and 94 years of age. The mean time on dialysis treatment was 72 months (range 3-264 months). Among hemodialyzed patients, 30 have stayed in a hospital or clinic at least 3 days during the current year (or year of study). Nasal screening identified 47 (32.4%) and 16 (38.1%) *S. aureus* carriers among patients and personnel respectively with a male to female ratio of 0.8 and 1, respectively (Table 1).

Detection of MRSA

All 63 *S. aureus* isolates were tested for cefoxitin resistance using a disk diffusion method and most (82%) were found to be susceptible to cefoxitin. For the remaining 11 cefoxitin nonsusceptible patient's isolates, MICs to oxacillin were determined (Table 2) and detection of the *mecA* gene was done. Four of the 11 strains had MIC to oxacillin higher than 2 µg/mL but only one strain (D11) was *mecA* carrier (Figure 1). The remaining strains were *mecA* negative and expressed resistance to cefalotin and amoxicillin and susceptibility to amoxicillin-clavulanic acid.

Antibiotic susceptibility

On the whole, 18 various resistance phenotypes are found for the 63 *S. aureus* strains (Table 3). The patient's isolates showed the greatest number (16 against only 4 in personnel isolates). Wild-type phenotype (*S. aureus* susceptible to all antimicrobial agents tested) represented a small fraction, that is, two personnel strains (3.4%) of the all 63 isolates tested. Multiresistance to more than three antibiotic classes was not observed in personnel isolates while 2 (4.3%) and 3 (6.4%) *S. aureus* patient's isolates showed multiresistance to 4 and 5 antibiotics respectively (Table 3).

Table 2. Range of methicillin (oxacillin) MICs for 11 isolates of MRSA (Diameter zone to cefoxitin ≤ 27 mm) from hemodialyzed patients by agar dilution method.

Strain code	Inhibition diameter of cefoxitin (in mm)	oxacillin MICs values (µg/mL)
D4	24	1.0
D6	26	1.0
D11	**15**	**16**
D20	**24**	**8.0**
D21	25	0.5
D46	26	0.5
D52	25	0.5
D59	**24**	**8.0**
D80	24	0.5
D88	**24**	**2.0**
D93	**24**	**0.5**

MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*.

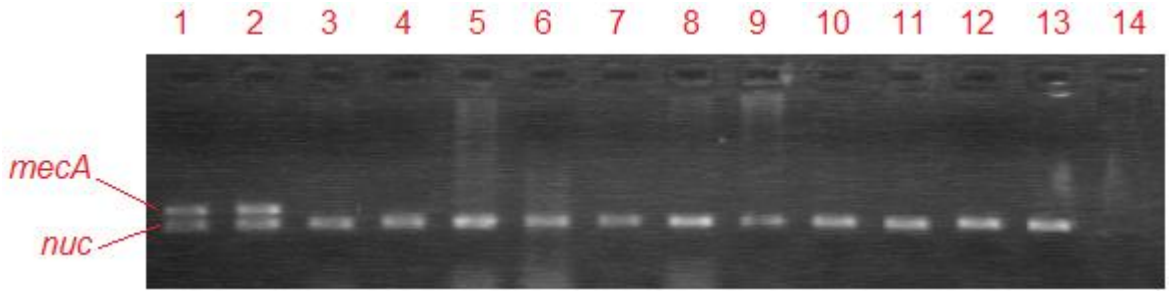

Figure 1. Detection of *nuc* and *mecA* genes by PCR duplex. Lanes 1 and 13, positive control *mecA* (MRSA strain U2A1593) and negative control *mecA* (MSSA strain 1594), respectively; lane 2: *mecA* positive strain (D11); lines 3 to 5: *S. aureus* BORSA (D20, D59, D88); lines 6 to 12: the others *S. aureus* isolates (D4, D6, D21, D46, D52, D80 and D93); line 14: control negative.

Table 3. Resistance profile of *S. aureus* strains isolated from personnel (n=16) and hemodialyzed patients (n=47) to 16 tested antibiotics.

Resistance phenotype profile	Frequency in personnel isolates (n = 16)	Frequency in hemodialyzed patients isolates (n=47)
Wild-type	0	2 (4.2%)
PG	9 (56.2%)	22 (46.8%)
RF	-	1 (2.1%)
Total	**9 (56.2%)**	**23 (49%)**
PG-Fox	3 (18.7%)	6 (12.8%)
PG-TE	-	3 (6.4%)
PG-E	3 (18.7%)	-
PG-Pef	-	1 (2.1%)
PG-FA	-	1 (2.1%)
E-RF	-	1 (2.1%)
Total	**6 (37.5%)**	**12 (25.5%)**

Table 3. Contd.

PG-TE-RF	1 (6.25%)	2 (4.2%)
PG-RF-FA	-	1 (2.1%)
PG-E-K	-	1 (2.1%)
PG-K-FA	-	1 (2.1%)
Total	**1 (6.25%)**	**5 (10.6%)**
PG-E-K-Fox	-	1 (2.1%)
PG-K-TE-FA	-	1 (2.1%)
Total	**0**	**2 (4.2%)**
PG-K-TM-Fox-FA*	-	1 (2.1%)
PG-K-TE-Fox-RF	-	1 (2.1%)
PG-TE-RF-Pef-FA	-	1 (2.1%)
Total	**0**	**3 (6.4%)**

PG: Penicillin G, GM: Gentamicin, K: Kanamycin, TM: Tobramycin, TE: Tetracycline, L: Lincomycin, E: Erythromycin, PT: Pristinamycin, C: Chloramphenicol, Pef: Pefloxacin, Fos: Fosfomycin, Fox: Cefoxitin, FA: Fusidic acid, RF: Rifampicin, VA: Vancomycine and SXT: Trimethoprim-sulfamethoxazole. * *mecA*(+).

Table 4. *S. aureus* strains antibiotic susceptibility pattern in hemodialyzed patients and personnel

Antimicrobial	Antimicrobial resistance rate (%)				
	Patients strains				Personnel strain
	MSSA (n=43)	BORSA (n=3)	MRSA (n=1)	Total (n = 47)	MSSA (n = 16)
Penicillin G	43 (100%)	3 (100%)	1	47 (100%)	14 (87.5%)
Lincomycin	-	-	-	0	0
Erythromycin	1 (2.3%)	1 (33.3%)	1	3 (6.4%)	3 (18.75%)
Pristinamycin	-	-	-	0	0
Kanamycin	3 (6.9%)	2 (66.6%)	1	6 (12.8%)	0
Tobramycin	0	-	1	0	0
Gentamicine	-	-	-	0	0
Chloramphenicol	-	-	-	0	0
Tetracycline	7 (16.2%)	1 (33.3%)	-	8 (17%)	1 (6.25%)
Cotrimoxazole	0	0		0	0
Cefoxitin	-	3[a] (100%)	1[b]	4 (8.5%)	0
Rifampicin	6 (14%)	1 (33.3%)	-	7 (14.9%)	1 (6.25%)
Fosfomycin	-	-	-	0	0
Pefloxacin	2 (4.6%)	-	-	2 (4.25%)	0
Vancomycin	-	-	-	0	0
Fusidic Acid	5 (11.6%)	-	1	6 (12.8%)	0

a: Borderline *S. aureus* isolate (BORSA). b: *S. aureus* isolate harbouring the *mec*A gene.

In vitro, antibiotic susceptibilities of *S. aureus* isolates (MSSA, Borderline *S. aureus* (BORSA) and MRSA) are shown in Table 4. For all 63 isolates, no *S. aureus* isolates were found to be resistant to lincomycin, pristinamycin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, fosfomycin and vancomycin, while over 90% were resistant to penicillin G. For the other antibiotics, in all, the percentage of resistance is relatively low and varied between 4.25 and 18.75%. The greatest percentage in resistance was observed against erythro-

mycin (18.75%) and tetracycline (16.2%) for the personnel and patients isolates respectively.

As expected, the resistance rates among isolates from patients were higher compared with the rates among *S. aureus* isolates from personnel. All personnel isolates were found to be susceptible to tobramycin, kanamycin, pefloxacin and fusidic acid while resistance to these antibiotics was noted among patients' isolates. Resistance profiles also differ between MSSA, BORSA and MRSA isolates with regard to some antibiotics and to resistance frequency (Table 4).

Virulence profile

The MRSA strain D11 harboured the enterotoxin genes *sec*, *sed*, *sell*, *selm*, *selo*, *ser* in combination with *tst*. D59 BORSA strain had none of the toxin genes investigated while enterotoxin gene *selo* was detected in D20 and D88 BORSA isolates in combination with *selm* and *tst* respectively.

DISCUSSION

A major part of dialysis-related infection is endogenous and related to high frequency nasal and skin carriage of *S. aureus*. Dialysis patients generally tend to have a higher carrier rate of *S. aureus* (32 to 82%) than other hospital patients and the personnel working in dialysis (Herwaldt, 2003; Herwaldt et al., 2003).

We revealed that 32.4 and 38.1% of hemodialyzed patients and personnel respectively, were *S. aureus* carriers. This percentage is close to that reported by Saxena and Panhotra (2003) among hemodialyzed patients in Saudi Arabia (38%), but was lower than that found in the studies of Edoh et al. (2003) in Ivory Coast where it reaches 85.7 and 87.5% in hemodialyzed patients and personnel respectively. In a more recent study, Oumokhtar and colleagues (2012) have found, in Fez city (Morocco), the rate prevalence of 42.9%.

As with ordinary strains of *S. aureus*, some patients harbour MRSA on their skin or nose and are at increased risk of developing infection. The emergence and dissemination of MRSA that often demonstrate multi-drug resistance is of great global concern, particularly in healthcare settings. Increased surveillance, including screening of high-risk patients, has been recognized as an important component of effective infection control programs to limit the spread of MRSA in hospitals and in the community. So, the choice of the suitable method for the detection of MRSA is crucial. The study conducted by Gueudet and Lemble (2004) on the comparison of five usual techniques for detection of MRSA, has demonstrated that the disk diffusion method using cefoxitin 30 µg, on M-H agar plate has a specificity of 100%. In our study, among the 11 *S. aureus* isolates that demonstrating resistance to methicillin by the disc diffusion test with cefoxitin, only one isolate harboured *mecA* gene and three were BORSA isolates, whereas seven strains were susceptible to oxacillin. We confirm that the gold standard for determining if a strain of *S. aureus* is MRSA, MSSA or BORSA is to test the isolate for the minimum inhibitory concentration to oxacilin (or cefoxitin) and to define the presence of the *mecA* gene using PCR methods. So, any prevalence of MRSA, determined only by disk diffusion tests, should be undertaken with extreme caution since they can be erroneous.

The surveys of the frequency of methicillin resistance in *S. aureus* were particularly determined among hospital strains. The epidemiology of MRSA varies considerably on a global basis and even shows remarkable differences at regional level (Grundmann et al., 2006). In Europe, a north to south gradient has been reported, with the highest proportions of resistant isolates found in the Mediterranean countries (Stefani and Varaldo, 2003). Greece, Spain, Italy, Israel and Croatia have all reported prevalence of 25% or more for methicillin resistance within *S. aureus* blood culture isolates (Tiemersma et al., 2004).

The lowest overall MRSA proportion was found in Tunisia (18%) and Morocco (at Ibn Rochd hospital, Casablanca) (19%) (Borg et al., 2007). In recent study, conducted by Elhamzaoui et al. (2009), the rate of MRSA was 13.5% (by using agar diffusion method with cefoxitin 30) among *S. aureus* strains isolated from various samples collected in several care units in two Moroccan teaching hospitals. Lowest values are found among community *S. aureus* strains isolated from different specimens collected in several private laboratories, 1.9 and 1.4% rates are reported by studies of Belabbes et al. (2001) and Elazhari et al. (2011), respectively.

The rate of MRSA (by possession of *mecA* gene) in hemodialyzed patients revealed by our study was 2.1% (1/47). If we take into account the whole strains including personnel isolates, this percentage is only 1.6%. The strain was cultured from a 61 year-old female not suffering from diabetes. The patient had not a history of previous hospitalisation. This MRSA strain was susceptible to 9/16 tested antibiotics including vancomycin. Decreasing susceptibility of MRSA isolates to vancomycin, which is currently the drug of choice for MRSA infections are, reported (Hiramatsu, 1998), what is very worrying. MRSA strain D11 (Table 4) has the following phenotype of resistance: PG-K-TM-Fox-FA (resistance to penicillin G, kanamycin, tobramycin, cefoxitin and fusidic acid).

The presence of multi-drug resistance, which has been considered a characteristic feature of nosocomial MRSA (Diep et al., 2004; Fey et al., 2003; Okuma et al., 2002), suggested that the strain might be a member of Hospital-Acquired MRSA (HA-MRSA) since this group of staphylococci is commonly resistant to the majority of other non beta-lactam antibiotics (Fey et al., 2003). The

hypothesis was subsequently corroborated by the fact that the MRSA strain was negative for the PVL leukocidin gene by PCR. Indeed, the expression of PVL has been strongly associated with Community-Acquired MRSA (CA-MRSA) (Boyle-Vavra and Daum, 2007; Holmes et al., 2005). Nevertheless, the elucidation of the origin of the MRSA was not possible with certainty. The prevalence of MRSA (*mec*A +) strains (2.1 or 1.6%) in our study was inferior to those reported by other authors: Ternois et al. (1993), Kresken et al. (2004) and Edoh et al. (2003) found 13, 20.7 and 22.7%, respectively. Although our rate was slightly inferior to result (5.3%) published by Askarian et al. (2009). In more recent work, Omokhtar and co-workers (2012) found 3.3% MRSA among hemodialyzed nasal carriage *S. aureus* in Morocco.

All the others *S. aureus* isolates were found to be susceptible to lincomycin, pristinamycin, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, rifampicin and vancomycin (Table 4). The rate of nasal colonization of BORSA in hemodialyzed patients sampled revealed to be 6.4% (n=3). The mechanism of resistance exhibited by BORSA includes excessive penicillinase production or plasmid mediated inducible methicillinase (Chambers, 1997; Santhosh et al., 2008). The low level of oxacillin resistance in these strains is thought to be due to their hyperproduction of extracellular β-lactamase since clavulanic acid restore their susceptibility to amoxicillin.

The clinical impact of carriage of these strains by patients undergoing hemodialysis should be considered. Possible risk factors for patients harbouring BORSA in a case control study by (Balslev et al., 2005) showed that, in comparison to the controls, BORSA infected patients were more prone to severe skin infections, were more often hospitalized, and had more bed-days.

The MRSA D11 has accumulated enterotoxins genes (*sec*, *sed*, *sell*, *selm*, *selo* and *ser*) and *tst* gene that would increase its pathogenic potential. D20 and D88 isolates harboured the enterotoxin genes *selo* in combination either with *ser* or *tst* respectively.

Enterotoxins and toxic shock syndrome toxin (TSST-1) are important virulence factors, and as pyrogenic toxin superantigens, they have profound effects on their host. Thus, the circulation of TSST-1 producing *S. aureus* among patients prone to staphylococcal infections is a worrying issue. Furthermore, these bacterial populations with variable virulence represent a new challenge in terms of pathogenicity, treatment, and prevention of transmission.

Although colonized patients have no signs or symptoms of infection, they can still serve as a source from which transmission may occur. Colonized personnel can also serve as reservoirs. Hence, adequate establishment of staphylococcal nasal carrier status accompanied by characterization of cultured isolates along with anti-biotic susceptibility testing is crucial in patients particularly prone to infections caused by these bacteria, such as those receiving hemodialysis in order to develop infection prevention measures and treatment strategies.

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