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MOLECULAR CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF MRSA ISOLATED FROM CHRONIC HEMODIALYSIS OUTPATIENTS AND THEIR CORRELATION TO MRSA COLONIZATION AMONG HEALTHCARE WORKERS

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ABSTRACT

The carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among dialysis patients is remarkable not only in terms of the risks of developing infections, but also in playing a principle part in transmission among dialysis unit staff. The aim of this study was to detect the colonization of Methicillin-sensitive *Staphylococcus aureus* and MRSA carriage. Also, our aim was to determine the relatedness of MRSA isolates and the potential routes of transmission using PCR- Restriction Fragment Length Polymorphism (PCR-RFLP) in Hemodialysis Unit of El Zagazig General Hospital, a tertiary medical center in Sharqia, Egypt. This study was conducted on 150 chronic hemodialysis outpatients and 200 non clinical control samples including environmental and healthcare workers (HCWs). Antibiotic susceptibility by VITEK-2 and disc diffusion, PCR amplification of mecA, pvl and *coa* genes and RFLP-PCR were conducted during the study period. In this study 3.3% of the patients and 3.2% of HCWs colonized with pvl positive MRSA. Fifty percent of MRSA isolates showed a single band PCR product amplification of 810bp fragment corresponding to *coa* gene. Ten distinct MRSA RFLP banding patterns designated as H1-H10 were obtained. The majority of strains belonged to RFLP banding pattern H1 (33.33%).The prevalence of MRSA carriage among hemodialysis patients was 14% and 9.7 % among HCWs with similar polymorphism patterns. The presence of one major *coa* gene type confirmed the occurrence of hospital acquired-associated MRSA.

Keywords: Hemodialysis patients; Healthcare workers; MRSA; *coa* gene; pvl gene; RFLP

INTRODUCTION

Colonization and infection with multi-drug resistant (MDR) strains such as MRSA occurs with increasing frequency in hospitals worldwide (**de Kraker *et al*., 2013)**. High mortality and morbidity rates are associated with MRSA infections, also prolonged length of hospitalization and higher healthcare costs are connected to MRSA infections (**Cosgrove *et al*., 2005)**. Plans to control the spread of MRSA in health care setting require standard data of the characteristics and prevalence of circulating MRSA strains and that can be obtained by active surveillance (**Cirkovic *et al*., 2015**). Hemodialysis (HD) patients may be at increased risk for MRSA infections and colonization (**Johnson *et al*., 2009**).
As HD patients have a multitude of risk factors, including repeated exposure to invasive medical devices and regular contact with healthcare workers, they are hundred-times more susceptible to invasive MRSA infections than the general population (**Lu *et al*., 2008**). Actually, MRSA infections are the subsequent leading cause of death among patients with end-stage renal disease (**National Institutes of Health, 2009**). Asymptomatic colonized patients and HCWs are the main sources of MRSA, in the health care settings. Transmission of MRSA among patients is concomitant to HCWs (**Malini *et al*., 2012**). HCWs are at the boundary between communities and hospitals, thus they act as a route of MRSA transmission (**Albrich *et al*., 2008**). Regular periodic testing, of MRSA positive colonization and decolonizing of the patients, is one of the possible policies to prevent MRSA infections among HD patients This is difficult to determine without strain typing whether a newly identified case is the result of health-care associated acquisition.
PCR-based typing methods, such as fragment length polymorphism (PCR-RFLP) analysis of the coagulase (*coa*) gene, play an important role in epidemiological studies and ideal typing method for MRSA analysis during MRSA outbreaks, because PCR-RFLP is rapid, simple, low in cost and applicable (**Godwin and Choyce 2001; Shittu and Lin 2006**). Typing of *coa* gene of *S. aureus* isolated is a simple, specific, discriminatory, and reproducible method, for that reason it has been used to identify and compare *S. aureus* genotypes (**Vimercati *et al*. 2006)**.
Panton Valentine leukocidin (PVL), is encoded by lukF-PV and lukS-PV genes, produced by some *S. aureus* strains, is one of the significant cytotoxins and responsible for dermal and soft tissue infections (**Genestier *et al*., 2005)**. Limited data available about the epidemiology of Egyptian PVL -positive MRSA strains (**Helal *et al*., 2012 and Hefzy *et al*., 2016)**

The present study aimed to assess the relatedness of MRSA isolates and the potential routes of transmission using PCR- RFLP by examining environmental samples and HCWs nasal swabs in relation to MRSA recovered from patients of Hemodialysis Unit of El Zagazig General Hospital, Sharqia, Egypt. In addition, the presence of PVL staphylococcal virulence factor gene was investigated.

MATERIALS AND METHODS

This study was conducted in the hospital-based outpatient Hemodialysis Unit of El Zagazig General Hospital, a tertiary medical center in Sharqia, Egypt for the period between January 2014 and September 2014. During the study period, 190 clinical specimens were collected from 150chronic hemodialysis outpatients. With regard to the clinical origin, the clinical specimens were isolated from blood(n=63),sputum(n=33),urine (n=30), nasal swabs (n=29), under nail swabs(n=20), purulent discharge(n=12) and skin swabs (n=3).A total of 200 non clinical control samples (Environmental cultures and health care personnel) were collected from the hemodialysis unit as follows; Water samples(n=30) (samples from incoming water tap before reverse osmosis, samples after storage tank), dialysate solution (n=30), effluent tubes of dialysis machines (n=30),beds, bed rails and doors (n=44), health care workers' skin swabs(n=31) , nasal swabs (n=27) and nail swabs (n=8).

Demographic and clinical data

Baseline information and medical data were collected from medical records of patients after signing informed consent form prior to their inclusion in the study. The study protocol was approved from the Ethics Committee of the Faculty of Pharmacy, Al-Azhar University. Descriptive information regarding participants' age, sex, and exposure to antimicrobial treatment and underlying chronic disease were collected.

Identification of isolates

Screening for Gram positive isolates was done using mannitol salt agar (Becton-Dickinson, Sparks, MD) and incubated for 24 h at 35°C and MRSA screen plates (Hardy Diagnostic, NY, USA) used to detect MRSA recovered from environmental samples and HCWs. Methicillin resistance was also detected using a cefoxitin disc (FOX, 30 mcg) on Mueller-Hinton agar plates supplemented with

4% NaCl according to Clinical and Laboratory Standards Institute guidelines (**Patel *et al*., 2014**).
Full identification and susceptibility testing of Gram positive isolates recovered from clinical isolates of patients was done using VITEK 2 (bioMerieux, Marcy l'E´toile, France) automated machine.

Susceptibility testing

Antibiotic susceptibility testing of all isolates was performed by VITEK-2 and modified Kirby Bauer disc diffusion method as recommended by CLSI guidelines (**Patel *et al*., 2014**) using Mueller Hinton agar. The antibiotics used in this study were amikacin (AN, 30 mcg), amoxicillin/clavulanic (AmC, 20/10 mcg), cefepime (FEP, 30 mcg), cotrimoxazole (SXT, 30 mcg),doxycycline (D, 30 mcg), erythromycin (E, 15 mcg), imipenem (IMP, 10 mcg), nitrofurantoin (F, 300 mcg), ofloxacin (OFX, 5 mcg), and rifampin (RA, 5 mcg) (Oxoid ltd., Basin Stoke, Hants, England).

PCR detection of themecA, pvl and*coa* genes

Genomic DNA was extracted from identified MRSA isolates, *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 using QiaAmp Mini DNA kit (Qiagen, Hilden, Germany).PCR was performed to detect mecA and pvl genes. The following specific primers were used; 5′-AAAATCGATGGTAAAGGTTGGC-3′, and 5′-AGTTCTGGAGTACCGGATTTGC-3′ (**Murakami *et al*., 1991)** for amplification of 533bp of mecA gene, whereas 5′-ACACACTATGGCAATAGTTATTT-3′ and 5′-AAAGCAATGCAATTGAT-3′ (**McDonald *et al*., 2005**) primers with amplicon size of 176bp were used for pvl genes detection. The amplification reaction consisted of an initial denaturation step at 94 ˚C for 5 min followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 50˚C for 30 sec, extension at 72˚C for 30 min, followed by final extension at 72˚C for 7 min. PCR was performed for *coa* gene detection, using the following primers; 5'-CGAGACCAAGATTCAACAAG-3' and 5'-AAAGAAAACCACTCACATCA-3', **(Himabindu *et al*. 2009)** which were designed to amplify the 3'end hypervariable region containing 81bp tandem repeats of *coa* gene. The amplification reaction consisted of an initial denaturation step at 94 ˚C for 5min, followed by 30cycles of denaturation at 95˚C for 30 sec, annealing at 55˚C for 45sec, extension at 72˚C for 2 min, followed by final extension at 72˚C for 7 min.

PCR-RFLP of the coagulase gene

The 3' end region of the coagulase gene was amplified by PCR and RFLPs of the amplicons were determined by digestion with *HhaI* (New England Biolabs, MA, USA) as described by manufacturer.

Statistical analysis

Data are presented using absolute frequencies and percentages for categorical variables, and mean ± standard deviation for continuous variables. Chi-square test was performed. A p-value of <0.05 was considered indicative of a statistically significant difference.

RESULTS

Among 284 chronic hemodialysis outpatients admitted to the hemodialysis unit at El Zagazig General Hospital from January to September 2014, only 150 patients agreed to participate in the study. Out of 150 chronic hemodialysis patients 139 clinical isolates (60 Gram positive and 79 Gram Negative) were recovered from different 190 clinical specimens. Patients' ages ranged from 23–80 years old with mean of 52.4 ± 11.8 SD with male predominance 81/150 (54%). The patient's clinical history characteristics are summarized in table 1. A total of 60 (31.5.4%) *Staphylococcus* species were isolated from different patient samples of which 21 (35 %) were identified as *S. aureus* whereas 39 (65 %) isolates were *S. epidermidis*. All *S. aureus* isolates were shown to be MRSA by cefoxitin disc diffusion test, and by mecA PCR (Figure 1A). Thirty one Staphylococcus species were recovered from the nails and nasal swabs of HCWs (3 *S. aureus* and 28*S.epidermidis*). MRSA colonization was detected in 14% of the patients (n=150). Whereas, only 3(9.7%) MRSA isolates were identified among HCWs, recovered from nasal swabs (n=27) of HCWs (n=31). MRSA was not detected in any of environmental samples (n=134; water samples, dialysate solution, effluent tubes of dialysis machines, beds, bed rails and doors). The current study revealed that, species of bacteria (n=78) isolated from environmental samples were confined to four types, *Bacillus* species (68 %), S. epidermidis (17.9 %), K. pneumonia (7.7%), and K. oxytoca (6.4 %).
Twenty one MRSA isolates recovered from hemodialysis patients. The highest percentage of MRSA isolates fell in the age group from 40 to 60 years (42.8%) followed by (28.6%) in age group 20–40 years and (28.6%) in 60–80 years. Eleven (52.4%) of MRSA isolates were recovered from male patients (Table 1). The three health care workers age were between 40-60 years and all of them were females.

Table 1 Demographic characteristics and clinical data of 150 chronic hemodialysis patients

Demographic and clinical data	**Patients (n=150)**	**MRSA carrier (n=21)**	**Non MRSA carrier (n=129)**	**Chi^2/P value**
Female, no, (%)	69(46)	10(47.6)	59 (45.7)	0.026/0.872
Male, no, (%)	81(54)	11(42.4)	70 (54.3)	
Age group, no, (%)				
>20-40	36(24)	6 (28.6)	30 (23.3)	1.09 / 0.58
40-60	80(53.3)	9 (42.8)	71(55)	
≥61	34(22.7)	6 (28.6)	28 (21.7)	
Underlying diseases, no, (%)				
Hypertension	113(75.4)	19 (90.5)	104(80.6)	0.309/0.578
Hepatitis C	81(54)	11(42.4)	70 (54)	0.258/0.872
Heart diseases	27(18)	8 (38)	19(14.7)	6.68 /0.009
Diabetes mellitus	40(26.7)	6 (28.6)	34 (26.4)	0.045 /0.831
Respiratory tract infections*	60(40)	12 (57.1)	48 (37.2)	2.99/0.0837
Urinary tract infections	30(20)	0 (0)	30(23.3)	-
Artery and Vein shunt	70(46.7)	8 (38)	62 (48)	0.721/0.396
Underlying Surgery	150(100%)	21(100)	129(100)	-
Smokers	46(30.7)	4 (19)	42 (32.6)	1.55/0.213071
Non-smokers	104(69.3)	17(80.9)	87 (67.4)	1.550 /0.213
Antibiotic exposure*	150(100)	21(100)	129(100)	-
≥3 years undergoing hemodialysis	150(100)	21(100)	129(129)	-

Legend: * ≤3 months before sampling; P-value of <0.05 was defined as statistically significant.

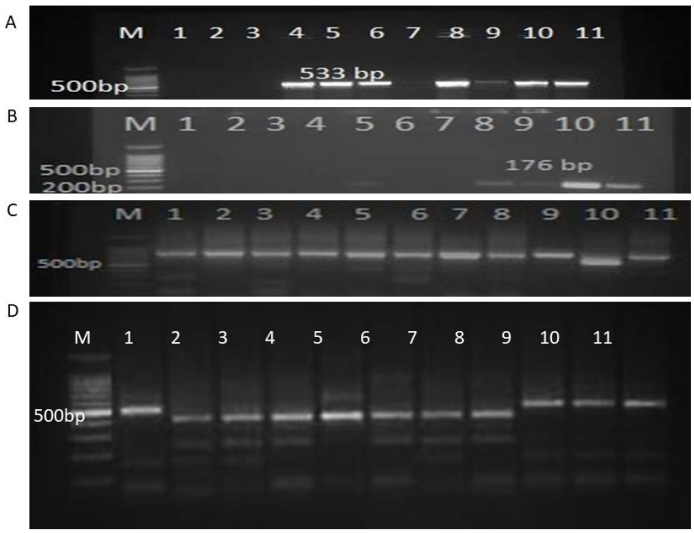

Figure 1 Gel electrophoresis of the PCR amplified products of mecA, PVL and coa genes and RFLP patterns of the coa amplicons after digestion**.** (A) Gel electrophoresis of the PCR amplified products of *mecA* gene with 533 bp amplification fragment. M: 100 bp DNA ladder; lane 1: *S. aureus* ATCC 25923; lane 2: *S. epidermidis* ATCC 12228; lane 3: negative control; lanes 4 to 11: different identified MRSA isolates. (B) Gel electrophoresis of the PCR amplified products of pvl gene with 176 bp amplification fragment. M: 100 bp DNA ladder; lane 1: *S. aureus* ATCC 25923; lane 2: *S. epidermidis* ATCC 12228; lane 3: negative control; lanes 4 to 11: different identified MRSA isolates. (C) Gel electrophoresis of the PCR amplified products of *coa* gene. M: 100 bp DNA ladder; lane 1: *S. aureus* ATCC 25923; lanes 2 to 11: different identified MRSA isolates. (D) *HhaI* restriction enzyme digests of the PCR *coa* gene products. M: 100bp DNA ladder; lanes 1 to 11: different identified MRSA isolates.

Antimicrobial susceptibility

MRSA isolates susceptibility to different antibiotics was tested. The results of antibiotic susceptibility are shown in Table 2. Susceptibility testing on one isolate from each MRSA carrier revealed that twenty (95%) out of 21 Staphylococcus isolates were resistant to both cefepime and erythromycin. Only one (4.8%) isolate showed resistance toward nitrofurantoin. The resistance rates to amoxicllin/clavulanic, doxycycline, amikacin, imipenem, ofloxacin, cotrimoxazole, and rifamycin were 80.8%, 61.9%, 47.6%, 28.6%, 28.6%, 14.3% and 9.5%, respectively. All the MRSA recovered from HCWs were resistant to amoxicillin/clavulanic acid, cefepime, and co- trimoxazole, while, all of them were sensitive to rifampin.

Table 2 Antibiotic susceptibility pattern of MRSA recovered from patients

Antibiotic	**(n=21) Susceptible, no (%)**	**Intermediate, no (%)**	**Resistant, no (%)**
Cefepime	1 (4.8%)	0 (0%)	20 (95.2%)
Co-trimoxazole	3 (14.3%)	15 (71.4%)	3 (14.3%)
Erythromycin	0 (0%)	1 (4.8%)	20 (95.2%)
Nitrofurantoin	16 (76.2%)	4 (19%)	1(4.8%)
Rifampin	16 (76.2%)	3 (14.3%)	2 (9.5%)
Imipenem	12 (57.1%)	3 (14.3%)	6 (28.6%)
Amoxicillin/Clavulanic acid	3 (14.3%)	1 (4.8%)	17 (80.8%)
Ofloxacin	12 (57.1%)	3 (14.3%)	6 (28.6%)
Doxycycline	6 (28.6%)	2 (9.5%)	13 (61.9%)
Amikacin	6 (28.6%)	5 (23.8%)	10 (47.6%)

Presence of PVL gene

Out of 21 MRSA isolates from patients, 5 (32.8%) were pvl gene positive, and out of the three MRSA isolates from HCWs one (33.33%) was pvl gene positive (Figure 1B).

Coagulase gene typing

The *coa* gene amplified products (figure 1 C) ranged from nearly 81bp to 810 bp, and these products showed seven different types of band patterns as shown in Table 3.PCR products of *coa* gene revealed that, most MRSA (11/21; 52.4%) isolated from patients produced a single band of 810 bp fragment followed by double amplicons of 810bp and 648bp (4/21; 19%) and a single pattern of 729 bp (2/21; 9.5%).On the other hand all MRSA recovered from health care worker produced three different banding patterns of *coa* gene that range approximately from 567bp – 810bp.

Table 3 PCR product of mecA, PVL and coa genes and RFLP patterns

Isolate number	Source	MecA gene	PVL gene	*Coa* PCR products (approximate bp)	RFLP pattern
1	Patients	+	-	810	H1
2	Patients	+	-	810	H1
3	Patients	+	-	729	H4
4	Patients	+	+	810	H2
5	Patients	+	-	405, 810	H5
6	Patients	+	+	810	H2
7	Patients	+	-	810	H1
8	Patients	+	-	810	H2
9	Patients	+	-	810	H1
10	Patients	+	-	810	H1
11	Patients	+	-	810	H1
12	Patients	+	-	810 , 81	H6
13	Patients	+	+	648, 810	H3
14	Patients	+	-	648, 810	H3
15	Patients	+	+	648, 810	H3
16	Patients	+	+	729	H4
17	Patients	+	-	810 , 567, 81	H7
18	Patients	+	-	810	H2
19	Patients	+	-	810	H1
20	Patients	+	-	648, 810	H8
21	Patients	+	-	810 ,324, 81	H9
22	HCWs	+	+	648,810	H3
23	HCWs	+	-	810	H1
24	HCWs	+	-	567, 810,	H10

Legend: There was not a significant correlation between PVL carriage MRSA isolated from 21 patients and 3HCWs at $p < 0.05$ (P-value is 0.72158).

***Coa*-RFLP Typing using *HhaI* Restriction Enzyme**

RFLP patterns and genotype frequency are shown in Table 4.Restrictiondigestionwasperformedonthe amplified coagulase PCR products with *HhaI*. Ten distinct RFLP banding patterns (designatedasH1, H2, H3, H4, H5, H6, H7, H8, H9 and H10) were obtained among the 24 MRSA isolates. The produced fragments of amplicon products of *coa* gene generated different quantities of bands, varying from 2 to 5, and their sizes were approximately 81 - 648 bp (figure 1 D). Eight out of 24 (33.3%) isolates studied belongedtoRFLPbandingpatternH1 (one of them isolated from HCWs and the remaining 7 were isolated from patients). Four MRSA isolates belonged to pattern H3; 3 of them were recovered from patients' samples and 1 from HCWs. Only one MRSA isolate belonged to pattern H10 which was recovered from HCWs. All MRSA isolates that belonged to patterns H2, H4, H5, H6, H7, H8 and H9 were isolated from patients.

Table 4 Restricted fragment size patterns of *coa* gene of the MRSA isolates

RFLP pattern (10)	**Size of *HhaI* fragments (approximate bp)**	**24 MRSA isolates (%)**		
		Patient isolates	HCWs isolates	
H1 (3 bands)	162-243-405	7	1	(12.5%)
H2 (3 bands)	81, 243, 486	4	0	(29%)
H3 (4 bands)	81,162-567-648	3	1	(4.2%)
H4 (3 bands)	81, 162, 486	2	0	(20.8%)
H5 (3 bands)	81,405 , 648	1	0	(4.2%)
H6 (4 bands)	81, 162, 243, 405	1	0	(4.2%)
H7 (5 bands)	81, 162, 243, 405, 567	1	0	(4.2%)
H8 (5 bands)	81, 162,243, 324, 648	1	0	(4.2%)
H9 (5 bands)	81, 162, 243,324, 405	1	0	(4. 2%)
H10 (4 bands)	162, 243,405, 567	0	1	(12.5%)

DISCUSSION

The distribution of circulating MRSA among hemodialysis patients admitted to outpatient hemodialysis unit (El Zagazig General Hospital, Sharqia, Egypt) as well as among healthcare workers were studied here. MRSA represented 100% of total *S. aureus* isolates in our study. Nasal swabs were the most common source of recovered MRSA (61.9%). In other Egyptian study by Omar *et al*., MRSA represented 75% of total *S. aureus* isolates and 35 (46.7%) were isolated from respiratory system**(Omar *et al*., 2014)**.

The most common causative agents of peritonitis concomitant with peritoneal dialysis are *S. aureus* and coagulase-negative Staphylococcus, worldwide **(Piraino *et al*., 2005)**. Although, *S. aureus* is coupled with increased chances of hospitalization, the most severe episodes, catheter removal and mortality, the main etiological agent of peritonitis is coagulase-negative Staphylococcus in the world **(Barrett*et al*., 2007; Grothe*et al.*2010)**.

The present study showed that 21 (14%) *S. aureus* and 39(26%) *S. epidermidis* was isolated from the hemodialysis patients (n=150). The antimicrobial resistance rates in the case of *S. epidermidis* isolates are higher than that of MRSA except for Amoxicillin/Clavulanic acid (Table 2).

Close contact between patients and HCWs carrying MRSA (on their skin or in nasal cavity), is the reason of the dissemination of infection in hospitals. Screening of MRSA among HCWs in the hospital is mandatory. HCWs act as the source of infection to their immune-compromised patients, leading to prolog hospitalization **(Malini et al., 2014)**, since HCWs are at the border between communities and hospitals. In our study, nasal and skin swabs were collected from 31 health care personnel who are working at the hemodialysis unit in El Zagazig General Hospital. The estimated prevalence of MRSA (11.1%) recovered from HCWs nasal swabs (n=27) in our study was much lower than that reported in other countries; 12.4% in Libya **(Al-haddad *et al*., 2014)**, 17.5% in India **(Radhakrishna *et al*., 2013)**, 20.8% in West Bank of Palestine **(Kaibni *et al*., 2009)** and 21% in Kuwait **(Dimitrov *et al*., 2003)**. Lower rates (3.4%) were detected in Nepal **(Khanal *et al*., 2015)**.these differences in MRSA carriage could be explained by differences in adherence to infection control measures in each hospital.

Out of 134 environmental samples there were 56 negative bacterial cultures and 87 positive bacterial cultures. Out of 78 positive bacterial cultures, 53 (68%) Gram positive bacterial rods, 14 (17.9 %) Gram positive cocci and 11(14.1 %) Gram negative bacterial rods were isolated Four types of bacterial species were isolated from environmental samples which including *Bacillus* species, *S. epidermidis*, *K. pneumonia*, and K. *oxytoca*. In the current study MRSA was not detected in any of environmental samples. Variants in the detection percentage of MRSA in hospital environment have been described in different studies maybe due to differences in hospital's cleaning/disinfection procedures, patient colonization load, laboratory techniques used and study strategy (**Boye et al., 1997; Boyce et al., 2007; Freeman et al., 2014; Russotto et al., 2015)**.

MRSA typing is required to outline epidemiological trends and infection control policies. The *coa* gene is one of the principal criterion for the characterization of *S. aureus* isolates **(Montesinos *et al*., 2002)**. The 3′ end of *coa* gene contains an 81 bp tandem short sequence repeat series, the number of repeats varies between strains (**Shopsin *et al*., 2000**). The *coa* gene was identified in all MRSA isolates in this study, indicating 100% type ability. In the present study, amplified *coa* gene presented seven PCR types and a single amplified product of 810bp fragment was corresponding to *coa* gene in most of MRSA isolates. Similarly Himabindu and his colleges reported that the majority of isolates belonged to the band class of 810bp **(Himabindu *et al*. 2009)**.Whereas; they showed that the sizes of *coa* gene amplified products were classified into 3 band classes which was different from our findings. A 2014 Egyptian study reported9 different amplicons of the *coa* gene which was quite similar to our findings **(Omar *et al*., 2014)**.There was variability in *coa* gene PCR sizes and bands detected in this study. This may be attributed to the presence of various allelic arrangements of *coa* gene in MRSA, permitting a single strain to induce multiple amplicons **(Goh *et al*., 1992)**.

Our findings showed that after digesting of PCR products with *HhaI*, 2 to 5 bands for each isolate were observed. The size of the bands produced by enzyme digestion was divided into 8 different band classes (81, 162, 243, 324, 405, 486, 567, 648bp) each containing multiples of 81bp tandem repeat units. Ten distinct RFLP patterns were observed based on the number and size of the produced bands (Table 4). The majority of isolates (8/24=33.33%) belonged to RFLP banding pattern H1. Although MRSA colonization was detected in 3 HCWs only, the transmission among HCWs and patients was high. This is evident in our findings as in case of patterns H1 and H3.

PVL is the major virulence factor of *S. aureus*, responsible for necrosis, apoptosis and destruction of white blood cells. .Currently, we reported pvl gene carriage rate of 25% (6/24) among MRSA isolates. The prevalence of pvl gene positive MRSA varies between countries ; 2.7% in Algiers, 4.9 % in UK, 5 % in France, 7% in Taiwan, 8.1 % in Saudi Arabia, 14.3 % in Bangladesh, 17% in Egypt; 64% in India and 100% in Tunisia (**Lina *et al*., 1999; Souza *et al*., 2010;Holmes *et al*., 2005; Moussa *et al*., 2008; Afroz *et al*., 2008; Antri *et al*., 2011; Helal *et al*., 2012; Ben Nejma *et al.*, 2013)**, indicating that the prevalence of PVL genes differs greatly between strains in different geographic areas and inhabitants.

In this study, pvl gene-positive MRSA was clustered under three restriction types (H2, H3 and H4). Only the H3 restriction pvl gene-positive MRSA type was identified between patients and HCWS.

CONCLUSION

There was a low prevalence of MRSA colonization among hemodialysis out patients (14%) and HCWs (9.7%). We found a correlation between MRSA isolated from the out patients and HCWs. We observed heterogeneity among patients and HCWs associated MRSA isolates using coa gene polymorphisms, but only two types were dominant between both patients and HCWs.The molecular typing of MRSA from patients, hospital atmosphere and HCWs, as a routine practice, will help to detect nosocomial spread in a hemodialysis centers, and opens the possibility of a rapid response. The presence of the PVL carriage MRSA possibly reflected the existence of community acquired MRSA which serve as a reservoir for MRSA transmission in hospitals. It is important to stick to infection control measures in order to reduce the spread of infection by MRSA among susceptible individuals.

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