Tripolitana Medical Journal; 2016,Vol. 5, No. 1, pp. 23 - 32

Research Article

ISSN 2077-5628

Prevalence and Clinical Significance of Nasal Colonization with Multi-drug Resistant Bacteria on Libyan Kidney Recipients

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Received 12 March/ 2016/Accepted 10 May 2016

ABSTRACT

We investigated the nasal carriage of multi-drug resistant bacteria and their influences on renal function among Libyan renal transplanted recipients (RTRs). A prospective study of nasal swabs taken from 60 consecutive patients at the time of follow-up clinic has shown 42 (70%) bacterial carriers and 18 (30%) non-carriers. Of 42 positive specimens, 14 specimens contain only one type of bacteria and 28 specimens yielded more than one bacterial isolates. A total of 70 bacterial strains were isolated from 42 nasal swaps where gram positive bacteria represent 60% (42 of 70) and gram negative bacteria represent 40% (28 of 70). The most frequently isolated gram positive organisms were *S. aureous* followed by *S. epidermitis* which both represent the majority of the isolates. Moreover, the most frequently Gram-negative isolated organisms were *Pseudomonas spp, Enterobacter spp,* and *E. coli*. Almost all confirmed isolates exhibited high resistance rates to Augmentin and trimethoprim/sulfamethoxazole and moderate resistance rates to Vancomycin, Rifampicin, and Clindamycin. The only effective antibiotics in this study against the isolates were Imipenem and Ciprofloxacin. The monitoring of renal performance has showed no direct correlation between bacterial nasal carriage and renal performance among RTRs.

Keywords- *Methicillin-Resistant Staphylococcus aureus; Renal Transplanted Recipients.*

INTRODUCTION

Bacterial infections remain the major complications and causes of morbidity and mortality following renal transplantation procedures.[1,2] The anti-rejection therapy and the transplant recipient's epidemiologic exposures rates put renal transplant recipients (RTRs) at high risk of series bacterial infections and/or colonization with highly resistance bacterial strains.[3,4] Generally, Bacterial infections have been reported to occur in 47% of RTRs, which have direct adverse impact on graft and patient's survival rates.[5-7] The range of organisms capable of causing bacterial infection in RTRs is relatively broad and their ability to develop manifested clinical infectious disease depends on the inoculum size, colonization site and organism virulence.[8] Most infections after six months posttransplantation are related to the community exposure.[5,8] During this period the most frequently involving etiological agents are common strains of *Staphylococcus spp*, *Haemophilus influenza, Acinetobacter baumannii, Clostridium difficile, Streptococcus pneumonia*, *Vancomycin-Resistant Enterococci, pseudomembranous colitis, Pseudomonas aeruginosa, E. coli,* and *Enterococcus spp*.[5, 6, 8, 9] A symptomatic nasal colonization with such strains has been reported among individuals and some of them were frequently recovered from the infection site(s).[10] Consequences of environmental selective pressures might turn these strains to acquire new state of pathogenicity and become dramatically associated with moderate to severe infectious disease in immunocompromised patients. Of Particular, *Staphylococcus aureus*[11,12], *Pseudomonas aeruginosa* and *E. coli* have evolved and reached unexpected levels of resistance to antibiotics in immunocompromised patients.[13]

Several comprehensive studies have shown that the nasal vestibular region serves as the major reservoir of *Staphlylococcus aureus* in immunocompetent individuals with nearly 25% of them being persistent nasal carriers. Interestingly, the increased rates of carriage amongst immuno-compromised individuals have been reported.[14] In a recent study, nasal *S. aureus* carriage was found in 10.6% of RTRs where cutaneous bacterial infection was suspected in 42.4% of them.[15] Of great interest, methicillin-resistant *staphylococcus aureus* (MRSA) emerged widely in last decades accounts for the majority of the most serious bacterial infection.[16,17] Based on elsewhere trials, nasal and skin MRSA carriages have higher rates of serious staphylococcal diseases, including skin or cutaneous infections, in both community and hospital settings.[18-20] Unfortunately, MRSA strains have evolved to become more pathogenic and acquire high antibiotic resistant rates against several commercially available antibiotics. Although most MRSA infections were acquired primarily in hospital settings, community-associated MRSA (CA-MRSA) strains have emerged leading to serious morbidity and mortality-associated infections.[21,22] The association between MRSA colonization and all-cause mortality has been reported among patients in intensive care units[23,24], nursing home residence[25]

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and hemodialysis patients.[26] The subsequent risk for skin and soft tissue infections among RTRs colonized with MRSA needs to be addressed clearly. The clinical significance of co-existing of some bacterial strains in the same site has been studied with some respect. This phenomenon is called bacterial interference where a co-existing bacterium of different species affects the survival and/or pathogenicity of an extant species or strain. For example, the interactions between *S. aureus* and the coexisting microbes are either cooperative or competitive.[10,14] However, investigations addressing the clinical effect of MRSA nasal carriage and co-existing of other pathogens on RTRs are of great importance to help in minimizing any chance of graft rejection. Furthermore, accurate periodical and comprehensive screening and profiling of bacterial carriage for all transplant recipients could help physicians assess patients' risks and improve the decisions about treatment and/or hospitalization.

This study aimed to describe the prevalence and antimicrobial profile of bacterial strains carried in the nose of RTRs as well as to address whether the detection of MRSA nasal carriage and other multidrug resistant bacteria in RTRs could predict higher renal function defect. Special attention was paid on the correlation between MRSA nasal carriage rates and serum creatinine level.

MATERIALS AND METHODS

Study Design, Setting, and Population

This investigation was a cross-sectional and prevalence study conducted in December 2012 at Organ Transplant Center, Tripoli Central Hospital, Tripoli, Libya. The study proposal was reviewed and approved by the Department Review Board. Participant informed consent was obtained in all cases. 60 transplant recipients were subjected to the study. The following recipient covariates were assessed in multivariate analysis: age, sex, months post-transplantation, donor source (living versus deceased), hypertension, diabetes mellitus, infectious diseases, and serum creatinine, urea, uric acid level upon follow-up date. Follow-up time was defined as the time span between date of swab collection (study entry) and the time point when the patient definitely left the center. All clinical information was provided by the nephrology clinic staff. Demographic data and information on risk factors of all participants were collected and recorded.

Bacterial isolation and identification

The specimens for culture were obtained from both anterior nares by means of rotating a pre-moistened cotton swab around the sampling site.[27] The swab was immediately inoculated onto nutrient broth tubes which were then incubated for 24 hours at 35°C. After that, a sample from the broth was streaked directly on nutrient agar and a methicillin (5 µg) disk is placed on the surface and incubated at 35°C for 24 hours (Oxoid, Tripoli, Libya). Any discernible growth within the zone of inhibition when seen using transmitted light is treated as methicillin resistance isolates. In addition, the isolates are considered methicillin resistant when they grow in plates with zone diameter of ≤9 mm around the methicillin disk (CLSI2011). Standard microbiology identification and isolation procedures were used to analyse the swab contents for the presence of *S. pneumoniae*, *H. influenzae, M. catarrhalis, S. aureus, S. epidermidis, P. aeruginosa* and *E. coli*. This was performed by using colony morphology, gram stain, capsule stain, manitol salt agar, mackoncy agar, blood agar, triple sugar iron (TSI) test, indol test and oxidase test (Oxoid, Tripoli, Libya) . MRSA chromogenic media was used to confirm MRSA isolates (biomerieux, Tripoli, Libya). Gram negative isolates were subsequently verified by Analytical Profile Index (API20E kit) (biomerieux, Tripoli, Libya). All procedures were undertaken according to methodology described in CLSI2011. All media and reagents were used according to the manufacturer's instructions.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was determined using the Kerby-Bauer disk diffusion method against: vancomycin, rifampin, tri-sulfamethoxazole, imipenem, augamantin, clindamycin, ciprofloxacin, and ceftriaxone. All the procedures and interpretive criteria were according to the latest National Committee for Clinical Laboratory Standards (NCCLS) recommendations [Clinical and Laboratory Standards Institute, Performance Standard for Antimicrobial Disk Susceptibility Tests, 2011]. After 24-h incubation at 37^{0}C, the zone diameter was measured and compared to NCCLS guidelines. Accurate detection of vancomycin-resistant enterococci by the disc diffusion test requires that any zone surrounding the vancomycin disc be examined carefully with transmitted light for evidence of small colonies or a light film growing within the zone. *S. aureus* ATCCC 29213 (sensitive) and 27R MRSA (resistance) were used as control strains. Double-disk diffusion tests (D-tests) were performed for each isolate to evaluate the presence of inducible clindamycin resistance (MLSBi). Isolates with positive D-tests were reported as resistant to clindamycin.[28]

Biochemical and blood evaluation

Renal function tests were measured from a morning blood sample using automated analyser (cobas integra 400 plus, Roche, Basel, Switzerland) to measure serum urea, creatinine, C-RP, and Uric acid. Longitudinal laboratory data (complete blood count, total and differential white blood cells count, CRP, and hemoglobin) was measured by using automated analyzer count (Sysmex, Kobe, Japan). The data were collected from the most recent data in the patients' files and were repeated when necessary. At least three readings must be taken for each factor during the time study. Graft dysfunction was defined as more than 20% rise in serum creatinine.

Statistical analysis

All data was entered into Microsoft Access XP software and exported into statistical package for GraphPad Prism5. Quantitative (continuous) variables were expressed as mean ± standard deviation, while qualitative (categorical) variables were shown as frequency (percentage). Student 't' test was used to compare groups for continuous variables. The Chi square test was used to compare proportions (percentages). A *P* value less than 0.05 was considered as significant and 95% confidence intervals were used for analyses.

RESULTS

Characteristics of participants

Demographic characteristics and risk factors of our study population are outlined (Table 1). A total of 60 nasal swaps

were obtained from 60 kidney transplant patients (35 females; 58% and 25 males; 42%), invited for the study. The patients live in different geographic areas and attend the outpatient clinic at Organ Transplant Centre, Tripoli Central Hospital, Tripoli, Libya, regularly. The specimens were collected at the Organ Transplant Centre and investigated at department of Microbiology and Immunology, Faculty of Pharmacy, University of Tripoli at same day. The age of patients was not normally distributed, ranging from 16-67 years with a mean of 39.8 years (±13.63). It was the first transplant for all patients and all of the patients received a kidney from live donor. Years post-transplantation vary among patients, where it is distributed in the following manner: < 1 year = 20 (33%), 1- 3 years = 15 (25%), and > 3 years = 25 (42%). The averages of total leukocyte count (7.8 $x10^3$±1.78$x10^3$), hemoglobin (12.6±1.8 g/dl), serum creatinine (1.43±0.94 mg/dl), and uric acid (6.7±2.3 mg/dl) were within the normal ranges. In contrast, significantly higher levels of blood urea (42.7±20 mg/dl) and C-RP (9.47±14.14 mg/l) were observed among participants. All the cases were without history of hospitalization in last 30 days. The follow-up time was less than 4 hours/visit for all patients. The data has shown that 20 (36.4%) patients were suffering from bacterial infection. Of these, upper respiratory tract infection was reported in 7 (12%) cases, lower respiratory tract infection in 10(17%) cases, and urinary tract infection in 3 (5%) cases. Out of 60 patients, 8 (15%) kidney transplant recipients were under short antibiotic regimen. In general, data on risk factors and immunosuppressant regimen has been summarized (Table 1).

In the study group, 42 of 60 (70%) patients were identified as carriers for bacteria and a total of 70 multidrug resistant strains were isolated and characterized. Of 42 positive specimens, 14 specimens contain only one type of bacteria, either S. *aureous* or *S. epidermitis,* and 28 specimens contain two bacterial isolates. Notably, the nasal carriage of *S. aureous* and *S. epidermitis* were reported in 27/60 (45%) and 15/60 (25%) patients, respectively (table 2). Of that, MRSA and methicillin-resistant *S epidermidis* (MRSE) strains were isolated from 22 (37%) and 14 (23%) patients, respectively. *P. areuginosa* was isolated from 15/60 (25%) patients, where 10 isolates were isolated from 10 patients colonized with MRSA and 5 isolates were isolated from 5 patients colonized with MRSE. In the same manner, *Enterobacter spp* and *E. coli* were isolated from patients colonized with MRSA and MRSE, as shown in table 2.

The sources and causative organisms were community-acquired multidrug resistant bacteria. Among the etiologic agents, 60% of isolates (42/70) were gram positive bacteria and 40% of isolates (28/70) were gram negative bacteria (Table 3). The most frequently isolated gram positive organisms were *S. aureous* (38.5%; 27 of 70) followed by *S. epidermitis* (21.5%; 15 of 70), which both represent the majority of the isolates. Importantly, 31% (22 of 70) of the isolated *S. aureous* were methicillin resistant strains. Therefore, methicillin resistant *Staphylococcus aureus* (MRSA) are considered as community-acquired methicillin resistant *staphylococcus aureus* (CA-MRSA), since they were isolated from out-patient clinic. On the other hands, 20% (14 of 70) of the identified *S. epidermitis* isolates were methicillin resistant strains.

Table 1: Demographic characteristics and risk factors of the study group.***Sources and causative organisms***

Variable		Study group (%) n= 60
Age, mean years (39.8±13.63)		
	16-25 y	6(10%)
	26-50 y	34(57%)
	>50 y	20(33%)
Gender		
	Females	35(58%)
	Males	25(42%)
Donor source		
	Live	60(100%)
	Deceased	0(0%)
Follow-up time		
	< 4 hours	60 (100%)
	> 4 hours	0 (0%)
Years post-transplantation		
	< 1 year	20(33%)
	1-3 years	15(25%)
	>3 years	25(42%)
Laboratory data		
	Leukocytes (per mm^3)	7.8±1.78
	Hemoglobin (g/dl)	12.6±1.8
	S. creatinine (mg/dl)	1.43±0.94
	Urea (mg/dl)	42.7±20
	Uric acid (mg/dl)	6.7±2.3
	C-RP (mg/l)	9.47±14.14
Antibiotic use		10 (17%)
Hospitalization in last 30 days		0 (0%)
Infection	20(33%)	
	URTI	7(12%)
	LRTI	10(17%)
	UTI	3(5%)
Hypertension		15 (27.3%)
Diabetes mellitus		15 (27.3%)
Smoking		0 (0%)
Immunosuppressant *		
	Cyclosporine	23(42%)
	Cellcept	30(54.5%)
	Prednisolon	20(36.3%)
	Prograf	8(15%)
	Rapamine	2(3.6%)

*All patients were under cocktail of more than one immunosuppressant drug.

The most frequently Gram-negative isolated organisms are distributed in the following manner; *Pseudomonas* spp (21.5%; 15 of 70), *Enterobacter* spp (10%; 7 of 70), *E. coli* (6%; 4 of 70), and others (2.5%; 2 of 70) (Table 4).

Antibiotic susceptibility profiles

Antibiotic susceptibility tests were performed during the time of sample collection on all strains. Almost all confirmed isolates exhibited remarkably high resistance rates to Augmentin and Trimethoprim/sulfamethoxazole. Imipenem followed by ceftriaxone and Ciprofloxacin were the most effective antobiotics. *S. aureus* exhibited high resistance rates to Augmentin (88%), Vancomycin (75%), Rifampicin (64%), and Trimethoprim/sulfamethoxazole (70%) and were highly susceptible to the other antibiotics. *S. epidermidis* were highly resistant to Augmentin (80%), Vancomycin (70%), Trimethoprim/sulfamethoxazole (87%), and Clindamycin (60%) (Table 5).

On the other hand, *Enterobacter spp* was characterized with intermediate susceptibility to most antibiotics except Augmentin and vancomycin, where it showed resistant rates of about 80% and 60%, respectively. Particularly, the spectrum of antibiotic resistance of *Pseudomonas spp* was high, as it was resistance to Augmentin (87%), Vancomycin (60%), Trimethoprim/sulfamethoxazole (86%), Rifampicin (72%), and Clindamycin (86%), and moderate resistance to Ceftriaxone (43%). *E. coli* showed high resistance rates to Augmentin (75%) and Trimethoprim/sulfamethoxazole (75%) and intermediate resistance rate to Rifampicin and Clindamycin (50% for each) (Table 5).

Double-disk diffusion tests (D-tests) were performed for each isolate to evaluate the presence of inducible Clindamycin resistance (MLSBi). The isolates did not show any inducible clindamycin resistance (MLSBi) activity.

Table 2: Distribution of bacterial strains among the RTRs

	G+ve/patients	Strain(s)/patients	Number of patients	Frequency (%)
Total number of positive samples (patients) (42 samples; 70%)	*S. aureus** (27 samples; 45%)	*S. aureus* alone	11	26%
		+ *P. areuginosa*	10	24%
		+ *Enterobacter spp*	5	12%
		+ *E. coli*	1	2%
	*S. epidermidis*** (15 samples; 25%)	*S. epidermidis* alone	3	7%
		+ *P. areuginosa*	5	12%
		+ *Enterobacter spp*	2	5%
		+ *E. coli*	3	7%
		+ *others*	2	5%

*MRSA represents 22 specimens

**MRSE represents 14 specimens

Table 3: The percentages of gram positive bacteria among the isolates

Bacteria	*Strains*	*Percentage*	*Methicillin resistant strains (%)*
Gram positive bacteria 60% (42/70)	*S. aureus*	38.5% (27/ 70)	31% (22/70)
	S. epidermidis	21.5% (15/ 70)	20% (14/70)

Table 4: The percentages of gram negative bacteria among the isolates

Bacteria	Strains	Percentage
Gram negative bacteria 40% (28/70)	*Pseudomonas spp*	21.5% (15 /70)
	Enterobacter spp	10% (7/70)
	E. coli	6% (4 /70)
	Others	2.5% (2 /70)

Association between bacteria nasal carriage and renal function

The blood levels of parameters such serum creatinine, total WBCs, urea, and uric acid used to assess the renal function were measured during the time study to address the impact of bacterial nasal colonization on renal function in RTRs. The statistical analysis was done on the available data for 14 nasal noncarriers and 33 nasal carriers. Noncarrier patients (*n=14*) had marginally higher serum creatinine (1.53±0.257 mg/dl vs. 1.37±0.139 mg/dl: *P=0.6*; Figure. 4a) and marginally higher blood urea (46.14±7.696 mg/dl vs. 41.36±2.621 mg/dl: *P=0.454;* Figure 4c) than carrier patients (*n=33*). Noncarriers (*n=14*) and carriers (*n=33*) patients appeared to have similar total WBCs values (7462±392 vs. 7891±352: *P=0.44*; Figure 4b). Carrier patients (*n=33*) had significantly higher blood uric acid (7.52±0.313 mg/dl vs. 5.62±0.335 mg/dl: *P*=0.043; Figure 4d) than noncarriers patients (*n=14*).

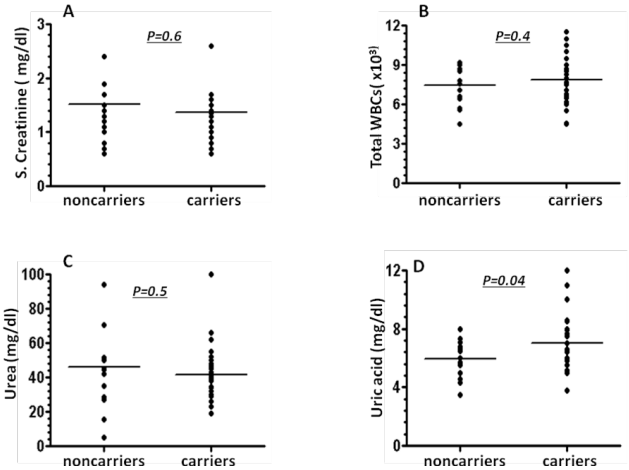

Figure 4: Renal function tests and monitoring of graft rejection episodes in carriers and noncarriers patients.

Serum creatinine (*P*=0.6; A), total WBCs (*P*=0.4; B) and blood urea (*P*=0.5; C) were not significantly different in noncarriers (n= 14) and carriers (n=33). Uric acid (*P*=0.04) was significantly higher in carriers (n=33) than in noncarriers (n=14). The average of four readings during the time study (one reading/month) was taken for each patient. Mean values are depicted by horizontal bars.

Association between MRSA/ P. aeruginosa nasal bacteria and renal function

Serum creatinine concentration is the most used biomarker for assessing renal function. Therefore, serum creatinine levels of seven *P. aeruginosa* and nine MRSA nasal carriages were compared to eight MRSA/*P. aeruginosa* nasal carriages to address the influence of bacterial strain (s) on graft function. MRSA/*P. aeruginosa* nasal carriages (n=8) had significantly higher serum creatinine (1.54±0.171 mg/dl vs. 1.03±0.0781 mg/dl: *P*=0.0139; Fig. 5) than MRSA nasal carriages (*n=9*). MRSA/*P. aeruginosa* nasal carriages (n=8) had marginally higher serum creatinine (1.54±0.171 mg/dl vs. 1.2±0.139 mg/dl: *P*=0.157; Figure. 5) than *P. aeruginosa* nasal carriages (*n=7*). *Pseudomonas aeruginosa* nasal carriages (n=7) had slightly higher serum creatinine (1.2±0.139 mg/dl vs. 1.033±0.0781 mg/dl: *p*=0.288; Figure 5) than MRSA nasal carriages (n=9). Of note, none of the patients had ever been screened for nasal swaps and received decolonization therapy for MRSA. All MRSA/*P. aeruginosa* nasal carriages (n=8) suffered from respiratory complications and received ciprofloxacin 250 mg.

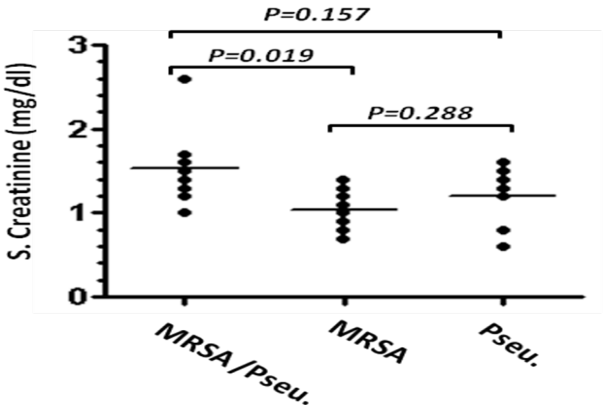

Figure 5: Serum creatinine levels in MRSA and *Pseudomonas aeruginosa* nasal carriages.

Serum creatinine (*P*=0.019) was significantly higher in

Table 5: Antibiotic resistance profile

Confirmed resistant isolates	Antimicrobial resistance of confirmed isolates (%)							
	AMC	VAN	IPM	SXT	RD	CRO	CIP	CD
S. aureus	88%	75%	4%	70%	64%	41%	56%	37%
S. epidermidis	80%	70%	0%	87%	40%	40%	53%	60%
Enterobacter spp	80%	60%	0%	50%	50%	30%	40%	50%
Pseudomonas spp	87%	60%	0%	86%	72%	43%	14%	86%
E. coli	75%	40%	0%	75%	50%	0%	25%	50%

MRSA/*Pseudomonas areuginosa* nasal carriages (n=9) than in MRSA nasal carriages (n=8). Serum creatinine (P=0.156) was not significantly different in MRSA/Pseudomonas areuginosa nasal carriages (n=9) and in *Pseudomonas areuginosa* nasal carriages (n=7). The average of four readings during the time study (one reading/month) was taken for each patient. Mean values are depicted by HORIZONTAL bars.

DISCUSSION

This study provides new insight regarding the prevalence of multi-drug resistant bacteria nasal carriages among Libyan RTRs. Furthermore, this study was designed to addresses the influence of MRSA and other MDR nasal colonization on kidney function among RTRs. However, most of the available studies addressing the clinical significance of MRSA were carried out on immunocompetent individuals.[26,29]

RTRs invited in this study have shown to have a high incidence of *S. aureus* and *S. epidermidis* nasal carriage. Similarly, liver transplant recipients have been shown to have a high incidence (up to 46%) of *Staphylococcal* nasal carriage.[30,31] Importantly, colonization of anterior nares with MRSA and MRSE was observed in 37% and in 23% of RTRs, respectively. Our findings are in contrary to other observations that reported not more than 10% of participants were MRSA carriers.[32-35] In this context, while Gualdoni *et al*., (2012) has indicated low prevalence rates of nasal colonization by MRSA in Central Europe, the Mediterranean region indeed constitutes a high prevalence region for MRSA.[36] A supporting study from Libya by Abouzeed, *et al*., (2010) has confirmed 51% of the isolates he worked on were MRSA strains.[37] Collectively, the occurrence of community-associated *S. aureus* (CA-MRSA) infection varies by geographic area.[38-40] However, the incidence and prevalence of MRSA nasal carriages worldwide is increasing among individuals outside hospital settings.

Nasal carriage of MRSA is a recognized risk factor for subsequent infection of endogenous origin and a useful predictor of *S. aureus* infections in susceptible patients such as RTRs. Community-based studies by Ellis *et al.,* (2004) and Steven *et al.,* (2010) have concluded that skin infections were more likely to develop in MRSA carriers compared with MSSA carriers and non–*S. aureus* carriers.[41,42] However, healthy *S. aureus* nasal carriages are more likely to develop immunity to MRSA over period of time and minimize the risk of MRSA infection by producing significant levels of IgG and IgA against staphylococcal toxic shock syndrome toxin-I (TSST-1)[43] as compared to immunocompromised individuals. This might explain to some extent that RTRs who are under restricted immunosuppressant regimen are more vulnerable to carry broad range of bacterial strains and develop serious bacterial infections than healthy individuals.[2,44] Therefore, eradication and elimination of *S. aureus* nasal carriage has been proven to reduce infection rates and the severe consequences of infection in patients at risk, such as RTRs.[45] The high prevalence of MRSA in the community has important implication for the clinical management and for hospital infection control programmers. When the incidence of MRSA increases in the community among RTRs, CA-MRSA have a propensity to replace HA-MRSA in health-care settings**,** making infection control measures less effective for reducing the prevalence of MRSA.[46]

Based on our results, the presence of *S. epidermidis* in the nasal cavities was correlated with the absence of *S. aureus*. In agreement with our results, several studies indicate that *S. epidermidis* uses multiple strategies to antagonistic and inhibit *S. aureus* colonization.[10] *S. epidermidis* is well known as nosocomial pathogen, frequently isolated from the normal skin flora of patients and healthy individuals.[47,48] Similar results from prospective study of skin swabs suggest that 25% of patients have MRSE on their skin and this strain was highly associated with serious bacterial infection.[49] The incidence of MRSE on the skin is more likely to increase when patients spend some time in health-care settings for investigation or follow-up purposes. In fact, the importance of these isolates is due to their association with sever chronic infections, such as *S. epidermidis* bacteremia[50], postoperative wound infections[51] pediatric bloodstream infections.[52]

Considerable number of multi-drug resistant (MDR) Gram negative bacteria, such as *P. areuginosa*, *enterobacter spp*, and *E. coli* was characterized as well. These strains are not considered to be part of normal nasal flora but were found to multiply easily in moist environments outside the human body. *P. aeruginosa*, an opportunistic human pathogen is commonly associated with respiratory infections, particularly nosocomial, ventilator-associated infections, community-acquired pneumonia and *Pseudomonal pneumonia* in immunocompromised patients. Based on our results, nasal swaps indicated 25% of patients were positive for *P. areuginosa.* In contrast, elsewhere study by Clarke *et al* has shown low *P. areuginosa* nasal carriage rates among healthy individuals (less than 3%).[53] Furthermore, a comparative population-based prevalence study conducted to measure bacterial organisms in nasal swabs of the Eritrean and German pediatric population has revealed that the less prevalent organism was *P. areuginosa* (1% in each group).[54] The remarkable difference between the results of the studies cited previously and the results of our study is most likely because of the difference between the study groups, i.e immunocompetent patients and the immunocompromised patients. In our results, all of *P. aeruginosa* isolates appeared as mucoid strains on MacConkey agar selective for gram-negative bacteria. It is well known that mucoid strains of *P. aeruginosa* grow as biofilms in airways of patients and are significantly more resistant to several antibiotics.[55] With regard to our results, 15 patients were positive for *P. aeruginosa* in their nasal cavities, and 10 of them was co-colonized with MRSA. Interestingly, all MRSA/*P. aeruginosa* nasal carriages (n=10) suffered from respiratory tract infection complications and received ciprofloxacin 250 mg. Detailed analyses of several *in vitro* and *in vivo* observations reviewed by Nair *et al.,* (2014) has demonstrated that the majority of the interactions between *S. aureus* and *P. aeruginosa* are competitive in nature, and only a few interactions are cooperative. Polymicrobial

infections involving *S. aureus* and *P. aeruginosa* exhibit enhanced disease severity and morbidity. The interactions of both types influence changes in both strains that alter their characteristics and lead to the development of more-persistent strains with altered colony morphology, antibiotic resistance, and increased pathogenicity and level of virulence.[10] It has been shown, Multi-resistant gram-negative aerobes such as *P. aeruginosa* along with MRSA are potential pathogens associated with lung disease in the immunocompromised patients.[55] Based on above mentioned reasons, we expect that these strains were the most expected pathogens caused chest infection in this group of patients. Because it cannot be ruled out that MRSA nasal carriage may activat other pathogenic bacteria of unknown sources to increase the probability of serious complication in RTRs. Sever bacterial pneumonia caused by *P. aeruginosa* are frequently associated with a high rate of mortality particularly in immunocompromised patients, such as RTRs.[56-58] In supporting to our conclusion, a single-centre study by Hoyo *et al* has reported that *P. aeruginosa* was the most common isolated microorganism in RTRs with nosocomial pneumonia.[59] In addition to others, our results suggest that multidrug-resistant gram negative bacteria should be considered in RTRs with severe chest infection. If colonisation of the upper airways, including nasal cavity, precedes pulmonary invasion, appropriate antibiotic treatment at this stage is of great importance to prevent or delay pulmonary disease.

In the current study *Enterobacter spp* has been isolated from 7 (10%) patients and *E. coli* from 4 (6%) patients. It has been shown that *Enterobacter spp* was among the organisms recovered more often from nares. *Enterobacter spp* accounted for 3.5% of the nares isolates, and *E. coli* represented 1.7 of the total nasal isolates. Moreover, the organisms causing persistent nasal colonization were *E. coli* and *Enterobacter spp*.[60] However, a detailed analysis of nasal specimens by Frank *et al.,* (2010) has shown that the prevalence of *Enterobacter spp* in *S. aureus*-colonized inpatients was significantly higher as compared to healthy adults (5.3% vs. 1%, respectively). Moreover, *S. aureus* non-colonized inpatients and healthy individuals have shown similar level of nasal *Enterobacter spp* (1% for each group).[61] *Enterobacter* strains are highly transmissible and clonal expansion and spread among individuals can occur throughout a hospital in very short time.[62] Therefore, patients invited to our study might have acquired *Enterobacter* strains during their regular visit to outpatient clinic. Even though the incidence of *Enterobacter* colonization seems to be low compared to *S. aurus, S. epidermidis* and *P. areuginosa*, it may be a warning of massive cross-transmission in clinical settings, with possibly enhanced morbidity and clinical difficulties.

In the resent study we utilized the disc diffusion susceptibility test which is a valuable method for the accurate, reliable detection of MRSA and for monitoring antibiotic resistance trends among bacterial strains.[63-65] Our observation regarding the antibiotic resistance profile was relatively unexpected in that the vast majority of the isolates were multidrug resistant strains. Importantly, MRSA and MRSE were the most prevalent bacterial isolates in the above mentioned results. The structural gene mecA which encodes PBP 2a' that has a decreased binding affinity for beta-lactam antibiotics has been found to be distributed among different species of staphylococci including *S. aureus* and *S. epidermitis*. Both strains have shown high resistance rates to Augamentin, Vancomycin and Trimethoprim /sulfamethoxazole and were highly susceptible to imipenem. The results extend the findings of earlier studies shown that methicillin resistance was associated with remarkable resistance rates to other antibiotics.[66] Of particular importance is the isolation of methicillin/ vancomycin resistant *S. aureous and S. epidermidis*. In consistent to our observations, a dramatic increase in vancomycin-resistant enterococcal and methicillin- resistant *S. aureus* (MRSA) infections have also been documented among liver transplant recipients.[31,67] MRSA isolates with reduced susceptibility to vancomycin, known as vancomycin-intermediate *S. aureus* (VISA), was first isolated in Japan in 1997.[68] A vancomycn-resistant *S. aureus* was isolated in the USA that had obtained the *vanA* gene from *Enterococcus* spp.[69] Several studies have noted that the wide use of vancomycin for the treatment of severe infections caused by MRSA and methicillin-resistant coagulase-negative *Staphylococcus* spp (MRCNS) and its therapeutic failures have led to an increase in microbial resistance, relapse of infection incidence and worsening of patients' clinical conditions.[70,71] Similar to other study[72], resistance rate of *S. aureous* including MRSA isolates to clindamycin was as high as 37%. Clindamycin has been suggested to inhibit production of Panton-Valentine Leukocidin (PVL), a potent exotoxin commonly harbored by CA-MRSA, which may propose some unique advantages against this strain.[73] The high percentage of isolates that were clindamycin susceptible in the current study (63%) suggests that the MRSA isolates from RTRs were most likely community-acquired strains. Similar moderate resistance rate to ciprofloxacin and ceftriaxone was reported for both *S. aureous and S. epidermidis* isolates, and only 4% of the *S. aureous* isolates exhibited a high level of imipenem resistance. IPM is a carbapenem belonging to the β-lactams class that has excellent activity on Gram-negative bacteria, but has reduced effect on *Staphylococci* resistant to methicillin. The high resistant profile of these isolates may be due to biofilm formation where biofilm-producing isolates presented higher rates of resistance to some conventional antibiotics used in therapy compared to the non producing biofilm isolates

P. aeruginosa is highly resistant to antibiotics and, as with many bacterial pathogens, resistance increases with repeated use and misuse of antibiotics.

In the current study, a high proportion of the confirmed *P. aeruginosa* isolates exhibited high resistance rates to Augamentin, Trimethoprim/sulfamethoxazole, Vancomycin, Clindamycin, and Rifampin. Gram-negative bacteria of the *Enterobacteriaceae* family, such as *Enterobacter spp*, and *E. coli*, as well as *P. aeruginosa* that are resistant to the third generation of Cephalosporins are now the main problem in clinical practice. These strains have been proven to produce the so-called extended spectrum beta-lactamases (ESBL).[74] Additionally, our results show that carriage of ciprofloxacin-

resistant *E. coli* is associated with colonization by MDR bacteria. In agreement to our results, resistance to quinolones (such as ciprofloxacin) has been reported in a variety of important bacterial pathogens, including *E. coli, K. pneumoniae* and other enteric organisms; *P. aeruginosa*; *Chlamydia trachomatis*, *Mycoplasma pneumoniae*; *Campylobacter jejuni*, *B. cepacia*; *S. maltophilia*, *N. gonorrhoeae*, *S. aureus* (especially oxacillin-resistant strains), *Enterococcus faecium* and *S. pneumoniae*. Overall, imipenem seems to be the most effective antibiotic with only 4% of *S. aureus* cultures showing remarkable resistance, although published reports indicate some resistance in a variety of clinical Gram-negative organisms, including *Pseudomonas aeruginosa* and *Enterobacter spp* isolates. The high resistant rates to most of antibiotics used in this study is probably because of prolonged and widespread use of these antibiotics without culture and sensitivity tests which has led to emergence of more resistant strains.

Principally, the elevation in serum creatinine and urea nitrogen levels after kidney transplant is significantly associated with kidney allograft disfunction. The normal lab serum creatinine readings in men are 0.7 to 1.3 mg/dL and for women are 0.6 to 1.1 mg/dL of blood. The increase in serum creatinine (>0.3mg/dl from the baseline value) is one of the main findings that must make us suspect renal toxicity. In addition to serum creatinine and urea nitrogen, we evaluated the total WBCs and uric acid serum levels to assess the effect of microbial nasal carriage on graft function. Our results did not show any significant differences in serum creatinine, urea nitrogen and total WBCs levels between nasal carriers and noncarriers and most of the cases seemed to be within the normal levels. We opted to focus on a more homogeneous patient population (i.e. RTRs with only bacterial pneumonia) in our study, as the elevation in serum creatinine level under opportunistic infections might have been a significant confounding factor. Our results indicated that all MRSA/*P. aeruginosa* nasal carriages during the time of study suffered from respiratory complications. This group had slightly elevated serum creatinine level as compared to those positive for *P. aeruginosa* and MRSA alone. The results of this study are consistent with those of other studies in that *P. aeruginosa and* MRSA were the most common isolated microorganism in RTRs with nosocomial pneumonia.[58,59,75] Due to the lack of more published data, the association between the microbial nasal carriages and graft disfunction needs more understandings. In routine practice, immediate identification of the pathogen responsible for pneumonia in RTRs may not be possible. Therefore, it is very complicated to attribute this slight elevation of serum creatinine to a co-infection with MRSA/*P. aeruginosa.* Although many studies have focused on nasal *S. aureus* colonization in different age groups, the relationship between nasal carriage of staphylococci and Gram-negative bacteria such as *P. aeruginosa* in RTRs is not well understood. It has been shown that patients with chronic kidney disease may develop more severe pneumonia, although only obscure symptoms and signs may be noted at presentation.[76] The results of this study will help us to improve long-term kidney allograft survival by controlling bacterial nasal colonization

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