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Original Article

Nasal Carriage of *Staphylococcus aureus* among Paramedical Students in Alexandria and Evaluation of Dry Spot Staphytect Latex Kit as a Rapid Screening Method for *Staphylococcus aureus*

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Abstract

Background: *Staphylococcus aureus* (*S. aureus)* is a common cause of nosocomial and community acquired infections worldwide. Nasal carriage of *S. aureus* acts as an endogenous reservoir for clinical infections in the colonized individual and also as a source of cross-colonization for community spread. Infections caused by *S. aureus* range clinically from minor skin infections to severe life threatening infections; with mortality rates ranging from 6 to 40 %.Treatment of staphylococcal infections has now become more challenging with emergence of methicillin resistant *S. aureus* (MRSA) strains; which are often also multidrug-resistant.

Objectives: The present study was conducted to screen for the prevalence rate of nasal carriage of *S. aureus* among paramedical students studying at the Faculty of Allied Medical Sciences, Pharos University, Alexandria, Egypt. The study also aimed at studying the validity of Dry Spot Staphytect Latex Kit as a rapid screening test for identification of *S. aureus.*

Methods: Nasal swabs were collected from 100 volunteer students over a period of three months (February-April 2015). Swabs were cultured both on blood agar and mannitol salt agar and all isolates that were confirmed microscopically and biochemically as *S. aureus* were tested for antibiotic sensitivity using modified Kirby Bauer technique. Dry Spot Staphytect Latex Kit was evaluated as a rapid method for identification of *S. aureus*, setting the positive result of tube coagulase test as a gold standard.

Results: The nasal carriage rate of *S. aureus* detected was 34%, 18 % of which were MRSA strains. The highest level of sensitivity to antibiotics among *S. aureus* isolates was recorded for vancomycin and mupirocin, (97.1%) each. Regarding MRSA strains, 100% were sensitive to mupirocin and 100% were resistant to oxacillin. Dry Spot Staphytect Latex Kit had a sensitivity of 97.06 %, a specificity of 96.97 %, a positive predictive value of 94.29 %, a negative predictive value of 98.46 % and an accuracy of 97 % in rapid identification of *S. aureus.*

Conclusion: Paramedical university students are a high risk group for nasal carriage of *S. aureus* and MRSA. Dry Spot Staphytect latex test is recommended for use as an efficient rapid, sensitive, specific and accurate screening test for *S. aureus* and MRSA.

Key words: *S. aureus*, MRSA, nasal carriage, paramedical students, Dry Spot Staphytect Latex Kit

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INTRODUCTION

Staphylococcus aureus (S. aureus) is a pathogen of high concern because of its ability to cause a various array of life-threatening nosocomial and community-acquired infections and its capacity to adapt fast to different environmental conditions. The infections caused by *S. aureus* have clinical range from minor skin infections to severe life threatening infections such as endocarditis, osteomyelitis, toxic shock syndrome and septicemia; with mortality rates ranging from 6 to 40%. In the relationship between man and *S. aureus*, overt infection is merely the tip of an epidemiological iceberg. Beneath the surface lurks a silent epidemic of unapparent skin and nasal carriage which

plays a crucial role in persistence and spread of *S. aureus* and in its outstanding success as a nosocomial pathogen.[1]

S. aureus exhibits a propensity to asymptomatically colonize human hosts either at a single or multiple body sites. Common anatomic locations of asymptomatic carriage include anterior nares, throat, groin, perineal region, mammary folds, axilla, umbilicus, and the sites where the skin integrity has been breached.[1,2]

Colonization of the human nose by *S. aureus* represents a commensal relationship, and carriage is inconsequential to the healthy human host in every-day life. However, *S. aureus* nasal carriage translates into a three to four fold higher infection rate in health care settings compared to non-carriers.[1,2] There are three lines of evidence that support the association between *S. aureus* nasal carriage and staphylococcal disease. First, the rates of infection are higher in persistent carriers than others. Second, prospective and retrospective studies using high-resolution molecular typing by pulsed-field gel electrophoresis has shown that more than 80% of nosocomially infected nasal carriers suffer from a *S. aureus* strain that is clonally identical to the commensal strain carried in their noses, thereby strongly implicating an endogenous origin. Finally, eradication of this microorganism by nasal application of an antistaphylococcal drug temporarily decolonizes the nose and other body sites, which prevents infection.[3]

Nasal carriage of *S. aureus* not only acts as endogenous reservoir for clinical infections in the colonized individual but also as a source of cross-colonization for community spread. There are four prerequisites to becoming a nasal carrier of *S. aureus.* First, the nose has to come in contact with *S. aureus*. Second, *S. aureus* needs to adhere to certain receptors in the nasal niche. Third, *S. aureus* needs to overcome the host defenses. Finally, *S. aureus* should be able to propagate in the nose. Hands are the main vector for transmitting *S. aureus* from surfaces to the nasal niche (nose picking). *S. aureus* nasal carriage and hand carriage are strongly correlated. *S. aureus* may also reach the nose directly through the air[4,5] Followed longitudinally, approximately 10–35% of persons are colonized persistently with *S. aureus*, 20-75% are intermittently colonized, and 57% never, or rarely, are colonized. The mean number of CFUs of *S. aureus* in the nose is significantly higher in persistent carriers than in intermittent ones, resulting in an increased risk of infection in the first category of individuals. Persistent carriers are often colonized by only one single strain over extended periods up to 10 years, while intermittent carriers carry many different strains over time. Patients with persistent carriage have been shown to exhibit a higher risk of *S. aureus* infection than patients with other statuses, at least in part by inducing a higher dispersion of *S. aureus* in the environment.[6]

Risk factors for nasal colonization with *S. aureus* include young age, male sex, crowding and large family size, underlying comorbidities, sharing a carrier's household, smoking, having a history of hospitalization, and recent contact with animals. Increased nasal colonization rates have been noted in insulin dependent diabetes, individuals on hemodialysis, those on ambulatory peritoneal dialysis, intravenous drug users and patients receiving routine allergy injections. It has also been suggested that patients with symptomatic human immunodeficiency virus (HIV) infection have an increased colonization risk. Activities involving close physical contact and the risk of minor injuries, such as sports, are also positively correlated with *S. aureus* spread and acquisition.[7]

The development of antimicrobial resistance among nosocomial pathogens creates a serious threat to public health. Methicillin resistant *S. aureus* (MRSA) is one among the potent nosocomial pathogens worldwide. MRSA are those *Staphylococcal* isolates, which are resistant to penicillinase stable penicillins like methicillin, oxacillin, dicloxacillin and flucloxacillin. Patients once colonized with MRSA, are at increased risk (11-25%) to develop acute infections and 3-15% to develop chronic infections in their subsequent one year when compared to non-colonized healthy individuals.[8] Based on Centers for Disease Control and Prevention (CDC) reports, 1 % of all staphylococcal infections and 50% of healthcare-associated staphylococcal infections are caused by MRSA. In 2007, CDC reported that MRSA causes 19000 deaths every year in United States of America (USA), which is more than HIV/AIDS cases.[9] Almost 20% of people who contract MRSA are reported to die from it, and an increasing number of its victims are young. Methicillin resistance rates of *S. aureus* vary considerably between countries. In the USA the figure was reported as 0.8-1.2% in the general population.[10] The figure in long-term facilities in France was 38%.[11]In the United Kingdom (UK), a sharp drop of healthcare-associated MRSA bacteremia from 1.8% in 2006 to <0.1% in 2011 was reported.[12] The prevalence of MRSA colonization among healthcare workers (HCWs) was assessed to be around 5%.[13,14] Almost 25% of the HCWs are stable nasal carriers, and 30 to 50% of them also possess the bacteria on their hands. HCWs; including paramedical personnel are important in the transmission of MRSA, but more frequently act as vectors, rather than being the main sources of MRSA transmission. Colonized HCWs are most often transiently colonized, but they may become persistent carriers if they have chronic dermatitis or sinusitis.[14]

Today, the gold standard for *S. aureus* identification is positive result in the tube coagulase test. However, confirmation of *S. aureus* by this method may take as long as 24 hours. Commercially available agglutination tests became an attractive alternative for *S. aureus* identification in clinical routine laboratory. Tests can be performed directly from the primary culture plate and results are available within a few seconds. In these test systems, particles precipitate with one or multiple *S. aureus* surface antigens, and allow *S. aureus* and coagulase negative staphylococci (CNS) isolates to be distinguished within a few seconds. Unfortunately, the accuracy of these test systems is limited. In particular, MRSA may give false-negative results in agglutination tests, which might be due to changes in various surface components, such as capsular polysaccharides, the clumping factor or protein A.[15]

The present study was conducted to screen for the prevalence rate of nasal carriage of *S. aureus* among paramedical students studying at the Faculty of Allied Medical Sciences, Pharos University, Alexandria, Egypt. The study also aimed at studying the validity of Dry Spot Staphytect Latex Kit as a rapid screening test for identification of *S. aureus.*

METHODS

This cross sectional study was carried out over a 3- month period

(February-April 2015).One hundred volunteer paramedical students (aged 18-21 years) studying at Faculty of Allied Medical Sciences, Pharos University, Alexandria, Egypt were randomly selected. Students filled in a questionnaire covering some demographic and medical data and nasal swabs were collected from them simultaneously. Samples were collected using sterile cotton swabs that had been moistened with sterile saline (to prevent nasal cavity irritation) by rotating one cotton swab tip in both nares of each participant for about five seconds each.

1- Isolation and identification of *S. aureus* strains:[16]
All swabs were cultured on blood and mannitol salt agar (MSA) plates, and incubated at 37°C aerobically for 24 hours. The strains were identified as *S.aureus* in accordance to standard laboratory protocols, including Gram staining, typical colonial morphology, and biochemical reactions. Isolates that were Gram positive cocci arranged in clusters, with yellow to golden mannitol fermenting colonies on MSA plates and/ or yellow to golden beta hemolytic colonies on blood agar plates were suspected of being *S. aureus*. On the other hand, red colonies on MSA were suspected as CNS. Suspected colonies were further tested for catalase, bound coagulase and free coagulase production using catalase, slide coagulase and tube coagulase tests, respectively. Strains that were positive for: catalase, bound coagulase, and free coagulase production, were considered as *S.aureus* .Others which were positive for catalase but negative for coagulase tests were considered as CNS.

2- Antimicrobial susceptibility testing: Identified *S. aureus* strains were screened for their antimicrobial susceptibility patterns using the single disc diffusion method described by Bauer et al., [17] The test was done on Mueller Hinton agar plates, using the selected antibiotic discs with various concentrations including Vancomycin (VA30), Cefazolin (KF30), Ciprofloxacin (CIP5), Cefuroxime (CXM30), Cephotaxime (CTX30),Gentamicin (CN10), Clindamycin (DA2), Amoxicillin (AML20), Amikacin (AK30), Oxacillin (Ox1), Tetracycline (TE30),Trimethoprim (TMP5), Rifampin (RA30), Erythromycin (E15)and Mupirocin (MUP200) [Oxoid]. Inhibition zones were measured and susceptibility was interpreted as susceptible (S), intermediate (I) or resistant (R) according to standard tables published by Clinical and Laboratory Standards Institute (CLSI).[18]

3- Latex agglutination test: (Dryspot staphytect plus: Oxoid):[19] Dry Spot Staphytect Latex Kit was evaluated as a rapid method for identification of *S .aureus;* setting the positive result of tube coagulase test as a gold standard.

Principle: Approximately 97% of human strains of *S.aureus* possess both bound coagulase and extracellular staphylocoagulase.Protein A is found on the cell surface of about 95% of human strains of *S.aureus* and has the ability to bind the Fc portion of immunoglobulin G (IgG).MRSA may express undetectable levels of clumping factor and protein A. However, these strains all possess capsular polysaccharide that can mask both protein A and the clumping factor thereby preventing agglutination. Dryspot staphytect plus uses blue latex particles coated with both porcine fibrinogen and rabbit IgG including specific polyclonal antibodies raised against capsular polysaccharide of *S.aureus.* Blue latex particles sensitised with non reactive globulin are used in control reaction areas.The reagent is dried onto the reaction card. When the reagent is mixed on the card with colonies of *S.aureus* emulsified in saline ,rapid agglutination occurs through the reaction between i) fibrinogen and clumping factor, ii) Fc portion of IgG and capsular polysaccharide. Agglutination may also occur with other species which possess clumping factor or protein A such as *S. hyicus* and *S. intermedius*. If neither clumping factor, Protein A nor specific capsular polysaccharides are present, agglutination will not occur and the result will be regarded as negative .

Standard test method:[16]

- One drop of saline (0.85%) was added to the small rings at the bottom of each oval in both the test and control reaction areas ensuring that the liquid did not mix with the dried latex reagent.
- A sterile loop was used to pick –up the equivalent of 5 average –size suspected staphylococcal colonies from a MSA plate and carefully emulsified in the saline drop ensuring that the resulting suspension was smooth.
- The suspension was mixed into the dry control latex spots until completely suspended and spread to cover the reaction area using the loop.They were watched for autoagglutination.
- A separate loop was used to proceed in the same way with the test latex.
- The card was picked up and rocked for up to 20 seconds and observed for agglutination under normal lighting conditions.

Interpretation: Positive results of *S. aureus* identification were recorded if agglutination was clearly seen after rocking the cards within a time of 20 seconds. Strains which showed autoagglutination reactions with control latex were excluded.

4- Statistical analysis:[20].

The results of the present study were tabulated and statistical analyses were conducted using PC with the software: Statistical Package for the Social Sciences (SPSS) version 20and Excel. Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. Comparison between the studied groups was also done using independent t-test, while for abnormally distributed data, comparison between them was done using Mann Whitney (Z-test). Statistical significance was set at 5% ($P < 0.05$).

RESULTS

In a 3- month period, a total of 34 *S. aureus* strains were isolated from 100 nasal swabs (34%),collected from paramedical students (60 females and 40 males), ages ranging from (18-21 years), studying at Faculty of Allied Medical Sciences, Pharos University ,Alexandria, Egypt. Out of those strains 16strains (47.1%) were MRSA strains, while 18 (52.9%) of which were methicillin sensitive *S. aureus* (MSSA) (Table 1). Out of the 34 *S. aureus* isolates, only 16 (47.1%) were detected among males versus 18 (52.9%) among females. The difference between both sexes was not statistically significant ($p>0.05$). As regards MRSA; 12 out of 16 (75%) were isolated from females

versus 4 (25%) from males. There was a statistically significant difference between both sexes ($p \leq 0.05$). Raising pet animals at home, sharing tools like razors or towels with others, recent hospitalization in the year prior to sampling and smoking were not proved to be risk factors for nasal carriage of *S. aureus* or MRSA. Differences between studied groups were not statistically significant ($p>0.05$). In fact, it is worth mentioning that 61.8 % and 75 % of *S. aureus* and MRSA strains, respectively, were isolated from passive smokers. Also all the isolated strains (100%) of *S. aureus* generally and MRSA specifically were detected among city dwellers compared to none among dwellers of the countryside.

Table (1): Distribution of the 100 students according to nasal carriage of *S. aureus* and MRSA, (Alexandria 2015)

Isolated strain	No.	%
Coagulase positive staphylococci (*S. aureus*)	34	34.0
– MRSA	16	16.0
– MSSA	18	18.0
Coagulase negative staphylococci (CNS)	66	66.0

Nasal allergy and recent intake of antibiotics in the three months prior to sampling were proved to be risk factors for nasal carriage of *S. aureus* ($p \leq 0.05$); where 79.4 % of the isolates were detected among students who complained of nasal allergy and 70.6 % of which were detected among students who were on antibiotic regimens in the past three months (Tables 2 & 3). The highest level of sensitivity to antibiotics among isolated strains of *S. aureus* was recorded for vancomycin and mupirocin (97.1% each) while the least level was recorded for erythromycin (29.4%). All the isolated MRSA strains (100%) were sensitive to mupirocin and were resistant to oxacillin. Only one MRSA strain was also a vancomycin resistant *S. aureus* (VRSA) strain (Figures 1 & 2).

Table (2): Relationship between nasal carriage of *S. aureus* and some related personal, familial and environmental factors

	S. aureus				**Test of Sig.**	***P***
	-ve (n=66)		**+ve (n=34)**			
	No.	**%**	**No.**	**%**		
Sex						
Male	24	36.4	16	47.1	χ^2=1.070	0.301
Female	42	63.6	18	52.9		
Residence						
Country side	12	18.2	0	0.0	χ^2=7.025*	^{FE}p = 0.007*
City	54	81.8	34	100.0		
Nasal allergy	18	27.3	27	79.4	χ^2=24.648*	<0.001*
Recent hospitalization	8	12.1	8	23.5	χ^2=2.173	0.140
Raising pet animals	23	34.8	8	23.5	χ^2=1.344	0.246
Sharing tools with others	49	74.2	23	67.6	χ^2=0.484	0.487
Smoking						
No	14	21.2	8	23.5	χ^2=0.392	0.822
Active	13	19.7	5	14.7		

Table (3): Relationship between methicillin resistance of the isolated *S. aureus* strains and some related personal, familial and environmental factors. Alexandria 2015

	Methicillin resistance among the 34 *S. aureus* isolates				**Test of Sig.**	**P**
	-ve (n=18)		**+ve (n=16)**			
	No.	**%**	**No.**	**%**		
Sex						
Male	12	66.7	4	25.0	χ^2=5.903*	0.015*
Female	6	33.3	12	75.0		
Residence						
Country side	0	0.0	0	0.0	-	-
City	18	100.0	16	100.0		
Nasal allergy	14	77.8	13	81.3	χ^2=0.062	^{FE}p= 1.000
Recent hospitalization	4	22.2	4	25.0	χ^2=0.036	^{FE}p= 1.000
Raising pet animals	3	16.7	5	31.3	χ^2=1.001	^{FE}p= 0.429
Sharing tools with others	13	72.2	10	62.5	χ^2=0.366	0.545
Smoking						
No	5	27.8	3	18.8		
Active	4	22.2	1	6.3	χ^2=2.472	^{MC}p= 0.338
Passive	9	50.0	12	75.0		
Recent antibiotic treatment	12	66.7	12	75.0	χ^2=0.283	^{FE}p= 0.715

N.B Methicillin resistance was checked for the 34 S. aureus isolates (n=34)

χ^2: Value for Chi square FE: Fisher Exact test MC: Monte Carlo test

Z: Z for Mann Whitney test *: Statistically significant at $p \leq 0.05$

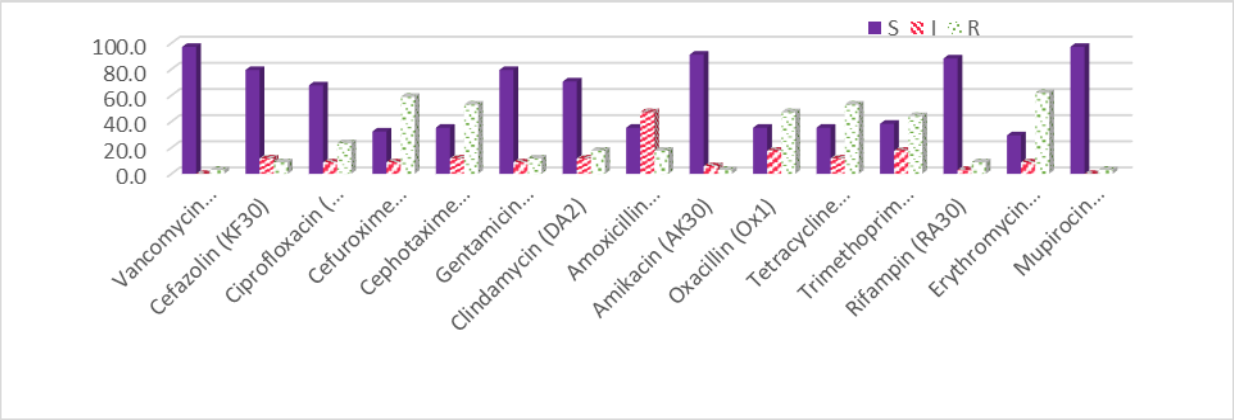

Figure (1): Distribution of the thirty four isolated strains of *S. aureus* according to the results of antibiotic sensitivity test
*S= sensitive I= intermediate R= resistant

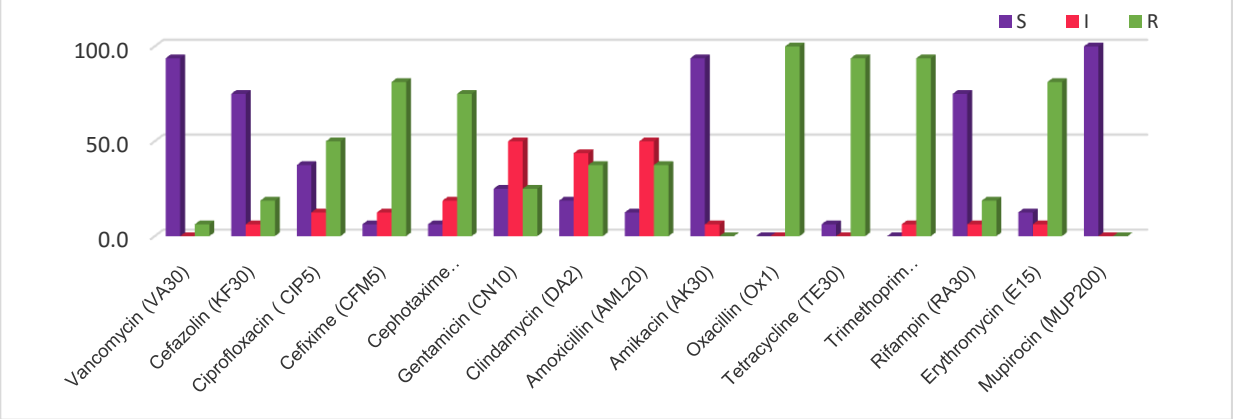

Figure (2): Distribution of the sixteen isolated strains of MRSA according to the results of antibiotic sensitivity test

On evaluation of the results of the Dry Spot Staphytect Latex Kit versus the results of the tube coagulase test, it showed a sensitivity of 97.06 %, a specificity of 96.97 %, a positive predictive value of 94.29 %, a negative predictive value of 98.46 % and an accuracy of 97% in rapid identification of *S. aureus*. All (100%) of MRSA strains were confirmed as *S. aureus* by this kit (Table 4).

Table (4): Comparative evaluation of the results of Dry Spot Staphytect latex test kit versus the results of tube coagulase test

Tube coagulase test / Dry spot staphytect latex	Negative (n=66)		Positive (n=34)		χ^2	p	Sensitivity	Specificity	PPV	NPV	Accuracy
	No.	%	No.	%							
Negative	64	98.5	1	2.9	87.209*	<0.001*	98.06	96.97	94.29	98.46	97.0
Positive	2	1.5	33	97.1							

χ^2: Chi square test *: Statistically significant at $p \leq 0.05$ PPV: positive predictive value NPV: negative predictive value

DISCUSSION

S. aureus is a major cause of serious hospital and community-acquired infections associated with morbidity and mortality rates with rapid development of resistance. The infections are commonly endogenous, i.e. caused by the strain that has already been colonizing the patient. The most common site of colonization by *S. aureus* is the nasal

mucosa. MRSA is one of the highest ranking pathogens worldwide and represents a real challenge to the clinical practice with significant public health concern.[21] Within hospital, MRSA spreads rapidly by hands of medical personnel. Colonized employees of hospital such as asymptomatic nasal and hand carriers acting as reservoirs are important sources for spreading this organism.[22] HCWs are at the interface between hospitals and communities. Various studies showed that reducing the carriage rate of *S. aureus* in HCWs reduces the chance of epidemics in hospitals.[23]

Nasal carriage rates of *S. aureus* among young adults worldwide ranges from 8 to 40%. This variation is attributed to the characteristics of the quality and size of samples, population under study, use of different culture techniques and different interpretation guidelines. [24] For Western Europe, carriage rates were found to be 24– 25.2% in the Netherlands, 27.3–27.6% in Norway and 36.4% in Switzerland.[25]In USA the prevalence was reported to be 30.4% in the period from 2001 to 2004. Similar rates were found among healthy Japanese volunteers showing a nasal carriage of 35.7%.[25] For South and Southeast Asia quite variable carriage rates have been found: 9.1% in Indonesia ,14.8% in Pakistan ,23.4% in Malaysia, (24.1%) in Taiwan and 29.4% in India. Recent reports from Africa showed carriage rates of 33.3% in Nigeria, 13% in Tunisia, 29% in Gabon and 18.3% in Kenya. In Mexico carriage rate was reported to be 37.1%.[25] In France rates ranging from 6 to 24.5% [26] and in Nigeria carriage rates ranging from 10.7 to 43% were reported.[7, 27]

The prevalence of MRSA nasal carriage varies considerably among various countries ranging from (<1%) in Sweden, Norway and Netherlands, UK (2%), Ireland (5%) Austria (8.2%), Switzerland (11.5%), Germany (19.5%), France (24.5%) and Italy (33.5%).[26] In India, the reported prevalence rate of MRSA was (3.1 %)[8] compared to >50% in Portugal [26] and to 83% in Pakistan.[28]

Nasal carriage of *S. aureus* is a common finding among HCWs; including paramedical students who attend many practical sessions in hospitals and laboratories and come in contact with patients and medical samples.

In the current study, 34% of the participant students were found to be *S. aureus* nasal carriers. Higher rates were reported in Brazil (40.8%)[29], Nepal (43.8%)[30], Pakistan (48%)[31] and India (49.9%)[23]. Lower rates were also reported elsewhere: Nigeria (14%)[32], Egypt (18.5%)[33], Palestine (19.4%)[34], KSA (25.4%) [35] and Ethiopia (28.8%).[36]

Reports of studies carried out in the same country may widely vary according to the standard of the health care facility investigated; regards the infection control measures adopted and the degree of contact between the studied HCWs and the patients or medical samples. For example in Australia, *S. aureus* nasal carriage rates ranged from (29 to 35.2%) [37], Malaysia (29 to 76%) [38], China (15.4 to 23.1%) [24, 3], Iran (31-38.46%) [39] and India (13.33 to 37.3%). [40]
In the present work, a nasal carriage rate of 16% was reported for MRSA among the participants. This suggests that the studied population is at risk of carriage of this drug-resistant strain. This is not surprising as life-threatening MRSA infections typically occur more in healthcare settings, while < 2% of carriers are found in the community. The current report is much higher than the pooled prevalence reported in two reviews conducted in 2014 and 2008 in Europe and USA: (4.3 and 4.6%), respectively.[41, 42] A significant increase of MRSA in Egyptian hospitals has been reported.[43]The current report is higher than others reported in Egypt, like that of Rashwan *et al.*, (5.2%)[44], coincides with that reported by Shehab *et al.,* (16%)[45]and lower than that reported by El Behedy *et al.,* (25%)[46], Girguis *et al*., (34.8%)[33]and Rushdy *et al.,* (61.45%).[47] The present report is comparatively higher than others reported worldwide: India (0% up to 6.66%)[8,40], Palestine (2.6%)[34], Pakistan (13.95%)[31], Iran (5.3% up to 31%)[48], Turkey (9.1%)[49], Taiwan (11.3%)[50] and Australia (3.4%) .[51] On the other hand, the current result is lower than the rates reported in Camiron (27.2 and 41.3%)[52,21], Japan (17.6 and 44%) [53] and KSA (18.3%).[35]

In this study, 47.1% of the *S. aureus* isolates were detected among male students, while 52.9% were detected among females. No statistically significant difference between both sexes was detected (*p>0.05).* This finding coincides with findings reported by Khorvash *et al*., (2012) and Ghasemian *et al.,* (2010). [54,55] On the other hand, one of the recent population based studies conducted in Denmark (2013) reported that men had a higher risk of *S. aureus* nasal carriage indicating gender specific risk factors, in line with observations from Norway, Denmark, Australia, New Zealand and USA. This finding was attributed to the females, behavior regarding cleaning their faces regularly.[56]

Currently, 75% of MRSA carriers were females while only 25% were males. This was statistically significant (p<0.001); which highlights a gender difference in prevalence of MRSA in favor of females. More female carriers were also observed in the National Health and Nutrition Examination Survey(2001) in the USA[55] and higher rates of MRSA carriage among female HCWs were also reported by Gonsu *et al.*, in Camiron (2013) [21]. These findings revive discussion on hormonal disposition to *S. aureus* carriage.

Results of this study showed that the prevalence of *S. aureus* nasal carriage tends to be significantly higher among city residents (88%) than the country side residents (12%). The 16 isolated MRSA strains (100%) were reported among the city residents and no MRSA strains were reported among the country side residents (p<0.005). In contrast to such findings Chatterjee *et al*., reported nearly similar prevalence of nasal carriage of *S. aureus* in participants from urban (48.4%) and from rural areas (56.1%) [22]. This can be explained by that inhabitants of urban areas, specially university students living in groups, live in overcrowded settlements or housings compared to those living at home in rural areas.

Although in the current work, sharing tools with others, raising pet animals at home, recent hospitalization and smoking didn't show any significant association with nasal carriage of *S. aureus* in general or MRSA in specific, yet higher rates of colonization were detected among those who didn't share tools with others, who didn't raise pets and among passive smokers.

The relationship with close contacts such as sharing bath towels with MRSA nasal carriage was ruled out in other studies[22] which appears incompatible with our findings. Regards recent hospitalization, worldwide studies supported the finding that visiting health-care facilities in the past year was an independent risk factor for *S. aureus* colonization.[40]

In agreement with the current findings, there is

evidence from large population-based studies for an inverse association between current smoking and *S. aureus* carriage.[57] Possible explanations for the suggested protective effect of smoking include the bactericidal activity of cigarette smoke, the increased immune activity associated with smoking-induced hypoxia and the increased biofilm formation and enhancement of fibronectin binding after cigarette smoking exposure. On the other hand, several reports found that smoking, particularly among ex-smokers, was associated with *S. aureus* colonization. Smoking is known to alter the respiratory mucosal surface, facilitating the binding of potential pathogens, particularly *Streptococcus pneumoniae* and *Haemophilus influenzae*, and to a lesser extent *S. aureus*. This leads to an increased risk of airway colonization.[31,58]

In this study, 79.4% of *S aureus* isolates were detected among participants who suffered from nasal allergy with a statistical significance compared to carriers with no nasal allergy ($p<0.001$). At the same time, 81.3% of MRSA carriers had history of nasal allergy. In agreement with the current results, other researchers also emphasized that nasal allergy and rhinitis affect *S. aureus* colonization in the nasal cavity and its super antigens production. [59]The current work revealed that *S. aureus* nasal carriage among students who were on recent antibiotic treatment represented about 80 % of the recorded *S. aureus* nasal carriers. It was also noted that 75% of MRSA isolates were related to individuals who were on antibiotic treatment regimens recently. This highlights the role of antibiotic treatment specially abused regimens in decreasing the general immune response of the different body systems to infectious agents, mainly due to its passive effect on the normal flora.

The treatment of staphylococcal infections is generally carried out with a group of antibiotics called beta-lactams which include methicillin, oxacillin, penicillin, and amoxicillin; MRSA is however generally resistant to these antibiotics. The highest level of sensitivity to antibiotics among *S. aureus* isolates in the present work was recorded for vancomycin and mupirocin; (97.1%) each, while the least level was recorded for erythromycin (29.4%).Regarding MRSA strains; they were (100%) sensitive to mupirocin and (100%) resistant to oxacillin. There was only one MRSA strain resistant to vancomycin (VRSA).

In the current work, sensitivity to vancomycin was recorded for 93.75% of MRSA strains while other researchers reported a 100% sensitivity.[29,44,54,58,60] The high susceptibility to vancomycin may be due to the fact that it is a relatively expensive and newer antimicrobial drug, therefore less available for abuse. This indicates that vancomycin could be reliably effective whenever there is an outbreak of MRSA in hospitals.

In the current work, 2.9% of *S. aureus* and none (0%) of MRSA isolates were resistant to mupirocin. This report is comparable to other studies carried out in Australia (2009) and in Brazil (2010) which reported 2.5% and 5.8% resistance to mupirocin, respectively.[29,58] This suggests that local use of mupirocin nasal ointment treatment of carriers would be an effective measure.

The current findings denoted a (17.65%) and (37.5%) resistance to amoxicillin among *S. aureus* and MRSA isolates, respectively. This is inconsistent with previous findings; where resistance ranged from (75 to 100 %).[23, 61] Resistance to penicillin results from B lactamase production by *S. aureus*. The increasing resistance to penicillin is attributed to the uncontrolled availability of the agent in every drug vendor, which leads to its frequent use. Misuse exerts greater selection pressure for the resistant strains, thereby makes such agents almost useless in the treatment of staphylococcal infections.[62]

The resistance of *S. aureus* isolates in the current work was tested for different generations of cephalosporins. Resistance to cefuroxime was 81.25%; which is near to that reported by Vinodkumar *et al.*, (89%)[63] and which is higher than that reported by Ahmed *et al.*, and Sasiskala *et al.*, (53%) and (33%), respectively.[61,64] Regards resistance to cefotaxime; (75%) of the current isolates showed resistance, which is consistent to Shinde et al.'s report: (81.8%)[23] but higher than reports by Ahmed *et al.*, and Sasiskala *et al.*, [(25%) and (27%), respectively].[61,64]

As regards sensitivity of our isolates to fluoroquinolones as ciprofloxacin, it was recorded as (67.6%), which is in line with reports of similar studies carried out in Africa[7,27] and in India.[40] Higher levels of sensitivity were reported in Basrah and Egypt: (100%)[44,60] and in Brazil (91.2%).[29] Wide use of ciprofloxacin has resulted in a steady increase in incidence of fluoroquinolone resistant staphylococci.[31] When quinolones are used to treat infections caused by other bacterial pathogens, subjects colonized with *S. aureus* are likely to be exposed to sub therapeutic antibiotic doses and are therefore at risk of becoming colonized with resistant strains. These resident, resistant strains then become the reservoir for future infections.

In the current work, *S. aureus* sensitivity to aminoglycosides: (gentamicin) was (79.4%) and (amikacin): (91.2%). This compares favorably with reports published previously.[7, 27] On the other hand, it is lower than that reported in Basrah, Egypt and Brazil: (100%) sensitivity to aminoglycosides).[29,44,60] Resistance against aminoglycosides, results through mutations, decrease uptake of the antibiotic and modification of aminoglycosides by aminoglycoside-modifying enzymes.[31]

The higher resistance of the isolates against these commonly used antibiotics might be due to the mutation or gene transfer, misuse and/or overuse of antibiotics, and a lack of standardized antimicrobial susceptibility testing before the prescription of drugs. Therefore, the drugs, which are more commonly used, which are generally inexpensive lead to development of bacterial resistance in developing countries.[31,36]Also the presence of residues of antibiotics used in agriculture and in livestock, as well as the bacterial selection due to such use could be responsible for development of resistant strains in environments. Patients and HCWs are indirectly exposed to antimicrobials that act on endogenous microbiota, selecting resistant bacteria that are dispersed through individual contact.[31]Thus HCWs have great importance in the increasing resistance of contaminants, serving as a source of transmission.

Dry Spot Staphtect Latex kit was evaluated in the current work as a confirmatory test for identification of *S. aureus* comparing its results versus results of the tube coagulase test; used as a gold standard for comparison. It showed a sensitivity of 97.06%, a specificity of 96.97%, a positive predictive value of 94.29 %, a negative predictive value of 98.46% and an accuracy of 97%. It is also worth

mentioning that 100% of MRSA isolates were identified as *S. aureus* using Dry Spot Staphtect Latex kit. This highlights the efficacy of the kit as a rapid diagnostic test that would be highly specific, sensitive and accurate in screening for nasal carriage of *S. aureus.*

Cunny *et al.*, compared the results of three latex kits designed only for detection of the clumping factor of *S. aureus* to the results of Dry Spot Staphtect Latex kit. They reported that the later had a sensitivity of 99.5% and a specificity of 97%; which was higher compared to the other three kits.[65]They also stated that tests designed only for the detection of clumping factor of *S. aureus* are highly specific but not sensitive enough for correct detection of *S.aureus* and MRSA, in contrast to the increased sensitivity of test kits containing additional components such as IgG and antibodies against the capsular polysaccharides. They also noted that the specificity of Dry Spot Staphtect Latex kit is only slightly reduced with regard to a positive reaction of *S. schleiferi* that probably possesses another surface component as well as the clumping factor that reacts with plasma constituents other than fibrinogen.[65]

The sensitivity reported for Dry Spot Staphtect Latex kit in the current work (97.06%) is lower than that reported by Weist *et al.*, (100%)[15] and by Wichelhans *et al.*, (99.4%).[66]

Comparing the present findings to those of Wichelhens *et al.*, they reported a lower specificity (91.3%) but higher positive and negative predictive values (97.9% and 100%), respectively.[66]The false negative results which result in decreased specificity and positive predictive values of the tested kit could be explained by presence of other antigens on some isolates, that cross react with any particular antibody utilized in this assay. Protein A for example was demonstrated to be present in up to 2% of CNS in previous studies.[15,16]

CONCLUSIONS AND RECOMMENDATIONS

– Paramedical university students are at the interface between health care facilities and communities and they are a high risk group for nasal carriage of *S. aureus* and MRSA

– Cross sectional studies on wider scales are needed to screen out for nasal carriers among HCWs and there is an arising need to develop and implement strategies for elimination of nasal carriage of *S. aureus* to prevent severe infections in our environments.

– Rational antibiotic prescribing by physicians; based on local guidelines to prevent the development of bacterial resistance.

– Vancomycin remains the main stay of treatment of MRSA, so it is recommended to be used rationally. Alternative antibiotic regimens can be used to treat MRSA infections, in particular rifampin and gentamycin.

– There is a need for a screening program for VRSA in hospitals especially at locations where vancomycin is being heavily used to avoid spread of VRSA in hospitals and communities.

– Dry Spot Staphytect latex test can be recommended for use as an efficient rapid, sensitive, specific and accurate screening test for *S. aureus* and MRSA.

Conflict of Interest: None to declare

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