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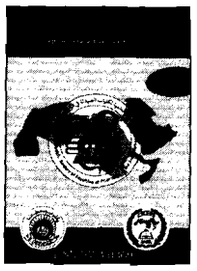

Mec A and *PVL* Genes in Methicillin Resistant Staphylococcus aureus from Nasal Carrier Individuals Exposed and Not Exposed to Hospital Environments

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Abstract

Exposure to hospital environments of limited duration did not cause an increase in the nasal carriage of Staphylococcus aureus whether they are methicillin resistant or sensitive. 56% of MRSA isolated were resistant to erythromycin and lincomycin and more than 50% of those are resistant to gentamicin as well. No vancomycin resistance was encountered. Resistance to other anti-microbials was uncommon among MRSA. One MRSA strain possessed the *pvl* but not *mec* A gene. All other *mec* A positive strains did not have the *pvl* gene.

Key words: nasal carriage, methicillin resistant Staphylococcus aureus (MRSA), methicillin sensitive staphyloccus aureus (MSSA), *mec* A gene, *pvl* gene.

Introduction

Most strains of Staphylococcus aureus, even those acquired in the community are penicillin resistant. In most cases the resistance is attributable to *B*-lactamase production due to a plasmid or chromosomal gene. Some staphylococci that are penicillin resistant are also resistant to the newer *B*-lactamase resistant semi-synthetic penicillins such as methicillin,oxacillin and nafcillin. This resistance is due primarily to the presence of an unusual penicillin binding protein in the cell wall of resistant strains (1).

Clinically significant methicillin resistant Staphylococcus aureus (MRSA) is being isolated with greater frequency in many countries,often pausing problems as causes of nosocomial infections. Infections by these strains significantly affect patient,s morbidity. Infection with MRSA is likely to be more severe a and requires longer hospitalization (2) Since nasal carriage of MRSA plays a key role in the epidemiology and pathogenesis of community associated disease(3, 4), this study was conducted to investigate this phenomenon in two groups of healthy young adults. Risk factors for nasal carriage of MRSA are poorly understood. The possibility that exposure to hospital environments may be one of these was explored. MRSA strains were tested for both *mec* A and *pvl* genes.

Materials and Methods

Subjects:

One hundred and seventy three third and fourth year students of the College of Nursing, Philadelphia University, Amman, Jordan were included in the study. All students have had several six weeks sessions in various hospital departments. They represented the group exposed to hospital environments. Similarly, 178 age matched third and fourth year students of the College of Pharmacy of the same university were studied. Those represented the group not exposed to hospital environments. Both sexes were represented in the two groups. None of the students had a medical history, an infection or treatment with an antibiotic in the last six months. All students consented to participation in the study.

Nasal Swabs:

A swab from both anterior nares was obtained from each student. Swabs were carefully inserted into each nostril so that the tip is entirely at the nasal osteum level (about 2.5

Cm. from the edge of the nare) and gently rolled 5 times.

Culture and Identification:
Swabs were immediately plated on mannitol-salt agar and cultured on brain heart infusion broth for enrichment. Subcultures were made from the broth to mannitol salt agar after 18-24 hours. Inoculated mannitol salt agar plates were incubated at 35^{0}C. for 72 hrs. Mannitol fermenting colonies were examined by Gram stain and tested for catalase production. Subcultures on nutrient agar slants were made and tested further for DNA-ase production and production of Coagulase and possession of protein A using Staph-Latex kit (Plasmatec Laboratory Products Limited, Dorset, U.K.). Strains positive by these tests were labeled as Staphylococcus aureus and slants were kept for further study.

Screen test for MRSA:
A suspension equivalent to Mac Farland 0.5 was prepared from each strain. A swab was dipped and streaked over an area of approximately 2x2.5 Cm. on the surface of a Mueller-Hinton agar supplemented with 4% NaCl and 6 mcg/ml Oxacillin (Sigma-Aldrich). Plates were incubated at 30^{0}C. for 3 days. A growth indicates that the strain is oxacillin resistant.

Sensitivity to other antibiotics:
A Mueller-Hinton agar plate was inoculated with a swab dipped in a suspension of each strain equivalent to Mac Farland 0.5. Sensitivity discs of penicillin, tetracycline, erythromycin, lincomycin, gentamicin, vancomycin, ciprofloxacin and trimethoprim-sulfamethaxasole were used. The disc contents and zones of inhibition were as recommended by the Clinical Laboratory Standards Institute (CLSI),formerly National Committee for Clinical Laboratory Standards.

Molecular Analysis:
Genomic DNA extraction:
Strains were grown on brain heart infusion broth at 37^{0}C. overnight.
DNA was extracted by DNA purification kit (Promega, USA) according to the manufacturer's instructions. The concentration of DNA was estimated spectrophotometrically.

PCR Detection of the *mec* A Gene
The *mec* A gene was detected by PCR as described by Garnier et. al (5) using 5 pmol/ul of each *mec* A p4 and *mec* A p7 primers (6,7 and 8) purchased from invitrogen (U.S.A.). DNA of *mec* A positive S. aureus strain (ATCC 4300) and a negative PCR blank control with nuclease free water instead of DNA were included with each set of 5 reactions to identify false positive results. DNA was amplified in a MJ Mini personal thermal cycler (Bio Rad, U.S.A.) with Hot START Master Mix as recommended by the manufacturer (GENEXPLORER). The following profiling cycle was used: predenatureation at 94^{0}C. for 5 min. followed by 35 cycles of 30sec. at 94^{0}C.,30 sec. at 53^{0}C.,60 sec. at 72^{0}C. and a final extension for 5 min. at 72^{0}C. The 162 bp PCR products were detected in 1.5% agarose gel (Promega.LE, U.S.A.). Band size was assessed by direct comparison with a 100-bp PCR DNA marker (Promega, U.S.A.).

PCR detection of leukocidin gene (*pvl* gene)
The *pvl* (lukS-PV-lukF-pV) gene was detected as previously described (9 and 10) using 5 pmol/ul of each *pvl*-1 and *pvl*-2 primers (Invitrogen, U.S.A.). DNA of positive *pvl* 1 strain (ATCC 49775) and a negative PCR blank control with nuclease free water instead of DNA were included with each set of 5 reactions to identify false positive PCR results. PCR condition was predenaturing at 94°C for 5 minutes, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 57°C, 30 seconds at 72°C, and a final extension of 5 munutes at 72°C. The 433 PCR products were detected in 1.5% agarose gel.

Results
The nasal carrier rate of both groups, the incidence of methicillin resistance and the possession of *mec* A gene is shown in table I.
The table shows that nursing students do not show a higher carrier rate of Staphylococcus aureus but although the number is small, the rate

Table I: Nasal carrier rate, incidence of methicillin resistance and possession of *mec* A gene.

Group	Total Number	Number Positive (%)	Number Resistant (%)	*mec* A gene Positive
Nursing Students	173	16 (9%)	6 (38%)	6
Pharmacy Students	178	30 (11%)	10 (33%)	9

Table-II: Resistance among MRSA and MSSA to antibiotics.

	Total Number Resistant to							
Organism	P	T	Eryth.	Linco.	Gent.	V	SXT	Cipro.
MRSA (16)	16	2	9	9	5	0	3	3
MSSA (30)	29	0	4	3	1	0	1	1

(P=penicillin, T=tetracycline, E=Erythromycin, Linc=Lincomycin, Gent=Gentamycin, V=Vancomycin, Cip= ciprofloxacin, SXT=Trimethoprim-sulfamethaxosole)

of methicillin resistance is slightly higher in this group. All strains labeled as methicillin resistant by the method used had *mec* A gene except one. It was this strain which had the *pvl* gene.

Resistance to other antibiotics among MRSA and methicillin sensitive Staphylococcus aureus (MSSA) is shown in table II.

The table shows that 56% of MRSA are resistant to both erythromycin and lincomycin and more than 50% of those are resistant to Gentamicin. Resistance to Ciprofloxacin and Septrin was uncommon. No resistance to vancoycin was encountered. Resistance among MSSA to antibiotics was less common than MRSA. Almost 97%of MSSA are resistant to penicillin. All *mec* A positive strains were negative to *pvl* gene. The only strain which was *pvl* positive was the strain labeled as MRSA but was *mec* A negative.

Discussion

Nasal carriage of MRSA or MSSA varies in different geographical areas (2, 10, 11, 12, and13). While the prevalence of carriage of methicillin resistance is high and increasing in hospital environments (11) it is rather low among strains colonizing young and healthy members of the community (13).

The present study shows that the carriage rate of both MRSA and MSSA in young healthy adults whether exposed to hospital environments or not is lower than that reported by Al-Zu'bi *et al.* (14). Exposure to hospital environments may not have been long enough to show an increase in the carrier rate since it is known that exposure to hospital environment causes an increase (11). Nevertheless, the carrier rate of MRSA in nursing students (38%) is a little higher than that of pharmacy students (33%). The prevalence of MRSA in some countries is still low. In the Netherlands for example, it is as low as 1% (21).

One strain which was Oxacillin resistant by the method used did not possess the *mec* A gene. The resistance mechanism in this strain may either be due to hyperproduction of β-lactamase or the presence of penicillin binding proteins not related to 2a or 2' (16). It is interesting that this was the only strain which was *pvl* gene positive. The *pvl* gene is a stable marker of most community associated MRSA (18). Such strains are associated with skin infections like furuncles and with necrotizing pneumonia (19).

No Vancomycin resistance was encountered. This agrees with a previous study (15) which did not find vancomycin resistance in clinical isolates or nasal carriers of MRSA. Resistance to other antibiotics (Erythromycin, Lincomycin and Gentamicin) was more common in MRSA than MSSA. Cephalosporins were not included in sensitivity tests since β-lactam antibiotics are clinically ineffective for treatment of MRSA even if they show in vitro sensitivity.

Study of nasal carriage of MRSA is important to the community since it plays a key role in epidemiology and pathogenesis of community associated disease (3 and 4) Risk factors for CA-MRSA carriage are not understood. Some studies have suggested that recent anti-microbial drug use plays a role in CA-MRSA colonization (20, 21). This study does not confirm this hypothesis since none of the students has had recent anti-microbial therapy. Study of CA-MRSA continues to be important. Transmission of infections caused by these strains is readily established by close contact (22). Furthermore, transmission from humans to animals (23) or from animals to man (24) may further complicate the epidemiology of these organisms.

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