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PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) AMONG HEALTHY UNIVERSITY STUDENTS

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ABSTRACT
The present study was conducted to determine the prevalence of community acquired Methacillin *Staphylococcus aureus* (CA MRSA) among healthy students of the University of Kabanjaar, Malaysia (UKM) . For this purpose, 340 samples were collected from 85 student volunteers, 55 of whom were highly involved in sport activities. Specimens were collected from skin surfaces, nostrils, upper throat, scars burn and pimples. S*taphylococcus aureus* was isolated only from 85 specimens when grown on manitol salt agar (MSA) and tested by basic bacteriological and biochemical techniques. Out of these isolates, 25 were confirmed to be Oxicillin resistant thus considered as CA MRSA; 17(68%) of these were skin surface and wound specimens. No biochemical or bacteriological differences, using standard procedures was noticed between CAMRSA and *S aureus* ATCC 25923. Further investigation showed large number of CA MRSA isolates that were resistant to Beta - lactam antibiotics such as Penicillin 100%), Ampicillin (23%), as well as non Beta lactam antibiotic such as Fusidic acid (55%), Mupirocin (60%) and Vancomycin (25%). Compared with *S. aureus* ATCC 25923, the latter was found to be more sensitive to all antibiotics other than Penicillin Multiplex PCR was used to detect mecA,mecA1 and PVL genes using 16S gene as internal control to determine their presence in 16S gene in all CA MRSA isolates as well as in *S. aureus* ATCC 25923. The result showed that mecA gene was detected in 8(32%) isolates. mecA1 in 17(68%) isolates and PVL gene in 10(40%) isolates. None of these genes was detected in *S. aureus* ATCC 25923. One of the CAMRSA isolates was shown to have all these 4 genes

KEYWORDS: Methicillin resistant, *Staphylococcus aureus*, manitol salt suger, skin surface, biochemical etc.

INTRODUCTION

Bacterial resistance to antibiotic has become a growing problem affecting control of diseases of bacterial aetiology. *S. aureus*, Gram-positive bacteria is responsible for a variety of serious infections (1). Methicillin resistant *staphylococcus aureus* (MRSA) since it was first reported in Great Britain (2), has increasingly been recognized in association with hospitals out-breaks worldwide as Hospital Acquired MRSA (HA MRSA). (3 & 4) In Malaysia , Norazah *et al* (5) reported 640 MRSA isolates in 9 Malaysian hospitals including UKM Teaching hospital that were resistant to fusidic acid and rifampicin; 72% were identified as ZC PFGE strain of MRSA *S. aureus* that possess the mecA gene. (6 & 3) MecA genes encode for an additional low affinity Penicillin- binding protein (PBP) that produce the methicillin resistance of *S. aureus* (7 & 8). Methods that are usually used to determine MRSA isolates include MRSA screening test such as rapid slide late agglutination test to detect PBP (6), pulse field gel electrophoresis. (PFGE) (9) and multiplex polymerase chain reaction (PCR) (10 & 6). PCR and DNA hybridization of mecA are reliable methods to identify MRSA because, typically, only a few cells within the total population of cells express resistance, which makes detection of MRSA by conventional methods difficult (6). . PCR is an accurate method of diagnosis but not suitable for routine diagnosis because it is expensive and takes long time to perform. MRSA strains can be isolated from blood, sputum, body, fluid, catheter tips, urine, vaginal swabs, abscesses aspirates, respiratory system and skin surfaces (11 & 12). Specimens collected from hospitals patients including those in intensive care unit (13) and from health care workers suggest that transmission of this organism may occur through transit of patients or health workers between hospitals via transiently colonized hands of hospital personal (14). MRSA has emerged as an important cause of community associated staphylococcal infections (15 ,16, 17 &18). Although diversity and variation in their genome and antibiogram background exists (3). The relatively new emerged community associated MRSA (CA MRSA) carry Panton valentine leukocidin (PVL) virulence genes but it possess a novel small mobile staphylococcal cassette chromosome mec (Scc mec) type 1V or V genetic element. This gene is easily transfered to other *S. aureus* stains compared to that of HA MRSA (17,18 &19). PVL virulence gene is a biocomponent leukocidin encoded by LukS-PV and LukF-PV genes responsible for leukocytes destruction and tissue necrosis (20, 21, 22 & Taking into view that, CA MRSA is increasingly becoming an issue of concern. causing global problem since it has emerged as a community associated infection widening its range of prevalence from nosocomial infection, this work is an effort in which we sought to determines whether clone spread of MRSA has occurred among students communities in Malaysia without the students are realizing it.

MATERIALS AND METHODS

Collection of specimens and bacterial isolates

Three hundred and forty specimens were collected from 85 student volunteers at UKM, four specimens from each student including skin surfaces, wounds or pimples, upper throat and nostrils. Fifty-five of these students were highly involved in sport activities while thirty of them were not. Each student participated in this study was interviewed using structured questionnaire for data including age, history of hospital treatment, history of delayed wound healing, history of cough, sharing of personal equipments with other students. MRSA isolates were obtained from culturing these specimens.

Microbiological and Biochemical Identification of *Staphylococcus aureus*

S. aureus is catalase +ve, coagulate +ve and oxidase –ve bacteria when these tests performed. Under the microscope it appears as Gram positive cocci in grape clusters, growth on manitol salt agar (MSA) change the red colour of the media to yellow while growth on blood agar produces β- haemolysis zones. All these methods were performed as described elsewhere.

Antibiotic Susceptibility Testing and Minimum Inhibitory Concentration (MIC)

Susceptibility to various antibiotics was performed by the standard disc diffusion methods according to (24).Antibiotics tested were fusidic acid, rifampicin, erythromycin, mupirocin, cefazolin, ceftazidime, clindamycin, topramycin, Cefoxitin, Ceftriaxone, amoxicillin, ciprofloxacin, oxacillin, vancomycin, streptomycin, tetracycine, gentamycin, kanamycin, ampicillin and penicillin using MIC Table (1).

TABLE 1: Results of 85 *S. aureus* isolates resistivity to several antibiotics and concentration used

Antibiotics	Concentration	Number	Percent Resistance
Oxacillin	1 µg	27	32%
vancomycin	30 µg	7	8%
streptomycin	25 µg	2	3%
tetracycline	30 µg	9	10%
gentamycin	10 µg	2	3%
kanamycin	10 µg	2	3%
ampicilin	10 µg	19	22%
penicillin	10 µg	85	100%
Fusidic acid	10 µg	47	55%
Rifampicin	15 µg	9	10%
Erythromycin	15 µg	21	25%
Mupirocin	5 µg	51	60%
Cefazolin	30 µg	4	5%
ceftazidime	30 µg	4	5%
clindamycin	2 µg	21	25%
tobramycin	30 µg	00	0%
Cefoxitin	30 µg	00	0%
ceftriaxone	30 µg	00	0%
Amoxicilin	30 µg.	00	0%
ciprofloxacin	5 µg	00	0%

Simple and Multiplex Polymerase Chain Reaction (PCR)

The presence of the genes 16S, mecA, and PVL were determined by multiplex polymerase chain reaction (multiplex PCR) as described by (6 & 25). Multiplex PCR was used to allow for identification of several genes at the same time for a lot of samples.

Genome Extraction:

For genome extraction bacteria were grown on LB broth media (Difco) and incubated at 37ºC for 18 hour. Extraction of the genome was done using a commercial kit (promega)

RESULTS

Investigation of the specimens by basic microbiological and biochemical methods revealed that 85 isolates were *S. aureus* of which, 62 specimens were from students highly involved in sport activities. Twenty one (24%) were nasal cavity specimens, 26 (31%) were from skin surface, 22 (26%) were from wounds or pimples and 16 (19%) were form the upper throat. Based on sensitivity test to oxacillin, 25 isolates were found to be resistant (thus considered MRSA isolates}. When these isolates were tested for bacterial resistance to several antibiotics, the isolates also showed resistance as presented in (Table 2). .Five of these MRSA isolates obtained from students not involved in sport, three of these were from skin surfaces and 2 from wounds. These isolates were found sensitive to kanamycin but resistant to vancomycin.. The result indicates MRSA is not resistant to beta-lactam antibiotics only but also to aminoglycocides antibiotics which alter bacterial ribosomal protein synthesis. (Table 2). Result of genome analysis using multiplex PCR (Table 3) revealed that all isolate possess 16S gene (~~756bp) including S aureus ATCC 25923,. Nineteen (76%) and 8 (32%) isolates showed the presence of mecA gene using mecA1 (~310bp) and mecA (~533bp) primers respectively. 10(40%) isolates possess PVL gene (~433bp). Only one isolate possess the 4 genes. This isolate is from skin surface specimen collected from a female student aged

between 21-23 years, not actively involved in sport, she did not share personal equipments with other students.nor did she experience wounds that take long period to recover; she had never received treatment from a hospital.

TABLE 2: Result of sensitivity test of 25 CA MRSA isolates to several antibiotics according to the specimen source

Isolate Number	Specimen Source	Antibiotic Resistance
1	skin	OX, PEN, CAZ,CLIN
2	skin	OX, PEN, AMP, FUS
3	skin	OX,FUS,MUP,AMP,TET, PEN
4	skin	OX, PEN, AMP, FUS, CZ, MUP
5	skin	OX, PEN, MUP, FUS, AMP
6	skin	OX, PEN, MUP,CLIN
7	wound	OX, FUS, AMP, PEN, MUP
8	nasal cavity	OX, PEN, MUP, FUS
9	wound	OX, RIF, AMP, PEN, VAN, FUS
10	wound	OX, PEN, VAN, RIF, FUS
11	wound	OX, FUS, PEN,CLIN
12	Mouth	OX, VAN, AMP, PEN
13	Nasal cavity	OX, AMP, PEN, MUP
14	wuond	OX, AMP, PEN, MUP,CLIN
15	Nasal cavity	OX, PEN, MUP, ERYT, FUS
16	Mouth	OX, AMP, TET, PEN, ERYT
17	wound	OX, AMP, PEN, VAN, FUS,CLIN
18	mouth	OX, AMP, PEN
19	Nasal cavity	OX, AMP, PEN, MUP
20	Nasal cavity	OX, TET, PEN, MUP
21	Skin	OX,AMP,STP,PEN
22	Skin	OX, ,TET,VAN,PEN
23	Skin	OX,AMP,STP,PEN
24	wound	OX,,AMP,STP,PEN
25	wound	OX,VAN,PEN, AMP
S. aureus ATCC 25923	-	PEN

OX; oxacilin, FUS; fusidi,, MUP; mupurocin, PEN; penicilin, RIF; rifampicin, AMP; ampicilin, TET; tetracyline, ERYT; erytromycin, VAN; vancomycin, CAZ; ceftazidime, CZ; cefazolin. STP; streptomycin, CLIN; clindamycin.

TABLE 3: Results of genome analysis of 25 CA-MRSA Isolates

Isolate Number	16S (~756 bp)	*mecA*(~533 bp)	*mecA1* (~310bp)	PVL (~433 bp)
1	+		+	+
2	+	+		+
3	+	+		+
4	+		+	
5	+		+	
6	+		+	
7	+		+	+
8	+		+	
9	+		+	+
10	+		+	+
11	+		+	+
12	+	+		
13	+		+	-
14	+	+		+
15	+		+	
16	+		+	
17	+		+	+
18	+	+		
19	+		+	
20	+		+	

21	+	+	+		
22	+		+		
23	+	+			
24	+		+		
25	+	+	+	+	
S. aureus ATCC 25923	+	-	-		

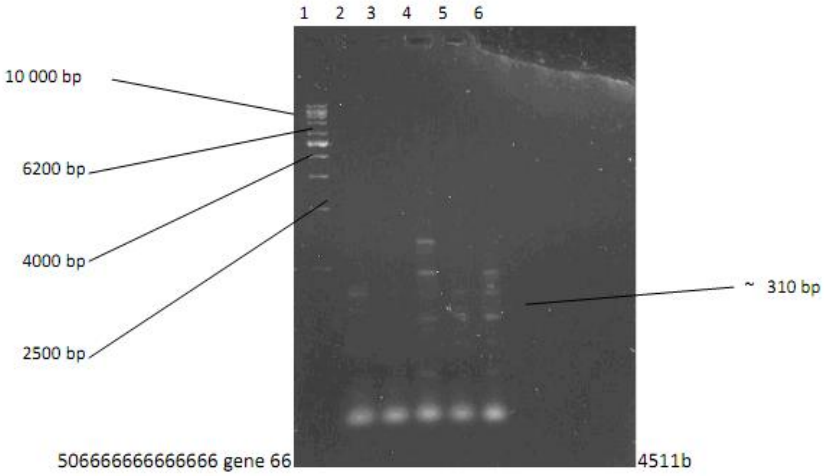

Fig. 1 :The presence of mec A1 gene in MRSA

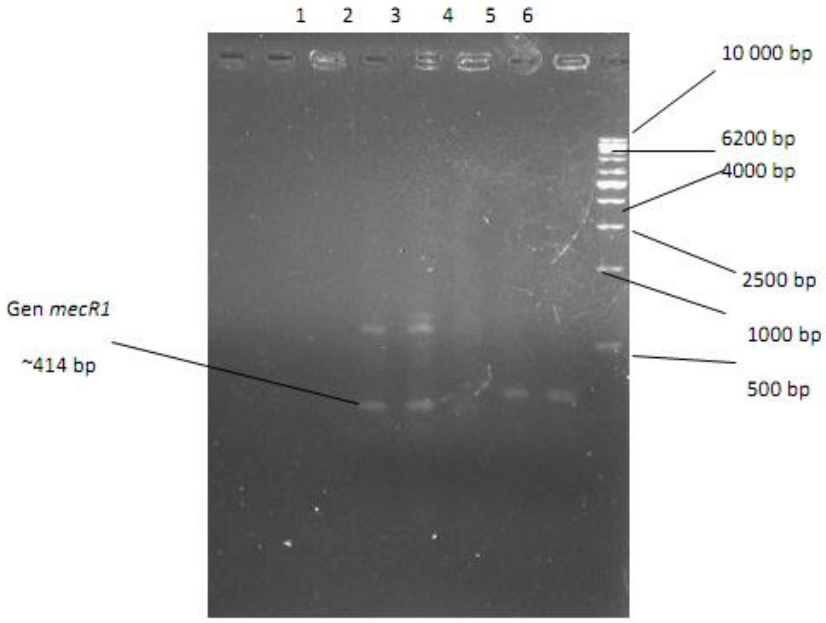

Fig 2. Presence of mge mec1 in MRSA

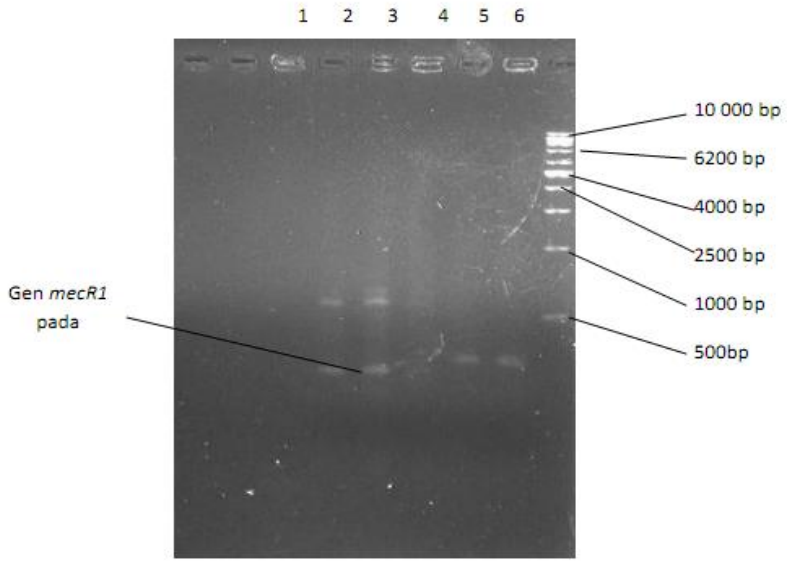

Fig. 3: Presence of mecR1 gene in MRSA

DISCUSSION

The present finding that most of the positive CA MRSA isolates were recovered from skin surface and wound sources (68%) agrees with the findings of Huang *et al*(12) who reported 85% and McClure *et al* (3) who reported that most of the MRSA isolates were from skin source. CA MRSA is more sensitive to aminoglycocides antibiotics group such as kanamycin, streptomycin and erythromycin and usually resistant to Beta-lactam group like methicillin and oxacillin. The present study revealed that CA MRSA is sensitive to five antibiotics; namely ciprofloxacin, topramycin, cefoxaline, cifriaxoneand amoxicillin. The results also revealed significant increase in the resistance of CA MRSA to fusidic acid, mupirocin and rifampicin, Eighty percent of the CA MRSA isolates, that found resistant to mupirocin, were of nasal passage origin. This result agrees with Karim *et al* (26) and provides support for the fact that mupirocin has been frequently used for the treatment of MRSA nasal passage infections. Apart from mupirocin, resistance, fusidic acid is also investigated in this study. Results obtained showed that 55% of CA MRSA isolates were resistance to fusidic acid. In contrast, Norazah *et al.* (5) reported only 32 out of 640 (5%) MRSA isolates were resistant to fusidic acid in Malaysia. This result clearly indicates an increase in the prevalence of the resistance to fusidic acid that has been formerly reported (27) . Reports of such resistance to fucidic acid appear to be in an increase as it became not very effective when used alone. Aatherefore, it is preferable if Fusidic acid is used in a combination with other antibiotic such as vancomycin or rifampicin.. It was found that 67% of MRSA infections can be be treated efficiently with fusidic acid when it is combined with aminoglycosides such as kanamycin or fluoroquanilone such as ciprofloxacin.(28). Ten percent resistance to rifampicin that was found in this study is almost close to the 5% and the 6.3% reported by other workers (5 & 29) Rifampicin can also be used in combination with vancomycin, if vancomycin alone fails to treat MRSA infection (30).

In the present study, 8% of CA MRSA was found resistant to vancomycin. Resistance to vancomycin was also reported by other workers (18, 29 & 31). This should be an issue of concern as vancomycin at present is highly used for treatment of CA 149MRSA infections in Malaysia. In contrast, some workers found all MRSA isolates were found to be sensitive to vancomycin (1). In a study conducted by the same author to investigate the difference between CA MRSA and HA MRSA he found that 24% of CA MRSA isolates were resistant to clindamycin; the same result was reported in the present study as well. Testing the resistance of MRSA to methicillin is not only requires susceptibility testing but also throough looking for the genes carried by bacterium that determine the presence of mecA genes(32). These genes can detected by simple and multiplex PCR.

Ten (40%) of CA MRSA isolated in this study posses PVL gene is, that is nearly similar to the 30.5% reported in Spain in 2002 (33) and was, then, considered high when compared to only one isolate reported by the same authosr in Spain in 2006 and 1.6% recorded by other workers (34).Two genes unique to CA MRSA that is not present in HA MRSA were regnized (18) ; these genes are PVL and SCC mec1. These genes were reported in CA MRSA isolates from all over Europe. Studies carried by some workers revealed that resistance to Methicillin is carried by genes closely related to mecA gene that act on ribosomal subunit 30S and 50S.(35); mec chromosome said to contain genetic elements such as Tn 164554 encoding the resistance to clindamycin and PT that encodes the resistance to tetracycline. mecA can be detected by using two different primers such as mecA and mecA1. Detection of mecA genes using mecA and mecA1 primers in part of CA MRSA isolates and not in the others could be due to mutation occurred and mecA1 in the genome sequence of the starter plate to thwart the ability

of specific site (36), mecA genes is important genes that ensures the resistance of MRSA to antibiotic by penicillin binding protein (PBP). Production of this protein lowers the ability of beta lactam ring binding to molecular target (37). This is evident when all CA MRSA isolates were resistant to beta lactam antibiotics. mecA gene produce protein signal to detect beta lactam (38).

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