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Isolation and Identification of Methicillin-Resistant *Staphylococcus aureus* from Keys of College Students Using Different Detection Methods

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Research Article

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ABSTRACT

Aims: In this study, the methicillin-resistant *Staphylococcus aureus* (MRSA) were isolated and identified by using biochemical tests, antibiogram and polymerase chain reaction (PCR) to explore the circulation of MRSA among college students.
Study design: Cross-sectional study.
Place and Duration of Study: Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

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between June 2010 and December 2010.
Methodology: A total of 100 samples were collected from keys of college students. There were 39 isolates (39 %) Gram-positive cocci and Catalase positive. 29 (74.36%) were glucose oxidation and fermentation positive. From the 39 isolates, 16 (43.24%) were shown Mannitol Salt Agar (MSA) tests positive. The deoxyribonuclease (DNase) tests and tube coagulase tests with human and rabbit plasma were carried out to improve the efficiency of the MSA test.
Results: 7 (43.75%) DNase positive and 2 (12.5%) tube coagulase positive. Both human and rabbit plasma showed similar sensitivity for the tube coagulase tests in this study. However, both isolates with tube coagulase positive were confirmed as *S. aureus* but not resistant to oxacillin, methicillin, erythromycin and cefoxitin. 2 (66.67%) of 3 (18.75%) isolates which is tube coagulase negative were resistant to erythromycin and 1 (33.33%) of them was resistant to methicillin. Rare strains of *S. aureus* can be coagulase negative. PCR assay was used. 1 (33.33%) of the coagulase negative isolate resistant to erythromycin was found to have *nuc* gene, *mec*A gene, *erm*C gene, *msr*A gene, *lin*A gene, and *fem*A gene. The isolate was confirmed as MRSA.
Conclusion: In conclusion, PCR technique is more sensitive and reliable than tube coagulase test or antibiogram for the detection of MRSA. And keys were shown to be an important source of MRSA and other bacteria circulation in the community.

Keywords: Staphylococcus aureus; MRSA; key; PCR; resistance.

1. INTRODUCTION

Staphylococcus aureus are Gram-positive cocci bacteria (Peacock, 2005). The main reservoirs of *S. aureus* are the mucous membrane and skin of human and animals (Genigeorgis, 1989). These bacteria able to produce enterotoxin that cause harm to human (Tamarapu et al., 2001); therefore *S. aureus* is classified as bacterial pathogen. Under new selective pressure, it has evolved and developed resistance to many antibiotics. The evolution of *S. aureus* is associated with serious community-acquired and nosocomial infections (Day et al., 2001).

Macrolide, lincosamide and streptogramin are the antibiotics that widely used to treat staphylococcal infections (Ardic et al., 2005). However, the resistance ability of *S. aureus* increased due to the widespread use of these antibiotics. The integration of staphylococcus cassette chromosome mec (SCCmec) element into *S. aureus* changed it into methicillin-resistant *Staphyloccocus aureus* (MRSA) (Keiichi et al., 2001). The MRSA contains the ability of resistance to macrolides, aminoglycoside, lincosamide, tetracycline and other antimicrobial drugs (Sabet et al., 2006).

It has been shown that colonization and infection of MRSA in healthy people has increased (Delorme, 2009). MRSA can transmit between person and person, and human and animals (Seguin et al., 1999), air transmission (Shiomori et al., 2001), and contaminated inanimate object to living things (Oie and Kamiya, 1996). MRSA is a pathogen that causes nosocomial and community-acquired infections (Elsayed et al., 2003). The incidence of the infections has increased over the last two decades (Ayliffe, 1997).

MRSA has the ability to be resistant to methicillin due to the acquisition of SCCmec (Hiramatsu, 1995). There are five types of SCCmec elements. Types I, II, and III are found in nosocomial strain of MRSA, whereas types IV and V compose the majority of community-acquired MRSA (Hiramatsu et al., 2001). The SCCmec element carries the mecA genes (Katayama et al., 2000).). It encodes the high molecular weight penicillin binding protein, a 78-kD penicillin-binding protein 2a polypeptide (PBP 2a). The addition of PBP 2a with β-lactams is mainly involved in the mechanism of methicillin resistance of *S. aureus* (Utsui and Yokota, 1985).

There are some genes in MRSA responsible for different drugs resistance. The ermA genes, ermB genes, and ermC genes are responsible for erythromycin resistance but ermB genes are formally found in animal strain (Eady et al., 1993). The msrA genes are responsible for macrolide resistance (Ardic et al., 2005). linA genes are responsible for the resistance to lincosamides. The nuc genes are responsible for oxacillin resistance; nuc genes are specific in *S. aureus* (Rushdy et al., 2007). Moreover, femA genes are the factors essential for methicillin resistance in *S. aureus* and staphylococci (Ehlert et al., 1997).

Several biochemical tests were carried out to identify *Staphylococcus aureus*. The suspected isolates were identified by using Mannitol Salt Agar tests, Catalase tests and oxidation and fermentation of glucose. Then, DNase and tube coagulase tests were done to increase the efficiency of detection of *S. aureus*. In the tube coagulase tests, rabbit plasma and human plasma were used. However, human plasma was not recommended due to several factors (Koneman et al., 1997). There was no single test that gave reliable results for the detection of *S. aureus*. Therefore, Mannitol Salt Agar tests, DNase tests and tube coagulase were carried out together to improve the identification of *S. aureus*. After that, antibiogram tests were carried out for those MSA positive isolates (Rohani et al., 2000) to identify MRSA. The susceptibility tests were confirmed by using polymerase chain reaction method (PCR) (Manal et al., 2009). The nuc gene was used to identify *S. aureus* (Rushdy et al., 2007). MRSA was identified by the detection of mecA genes, ermA genes, ermB genes, ermC genes, msrA genes, linA genes, and femA genes.

The infections by MRSA were noticeable among young people. No previous study was done in Malaysia; moreover, College students were not previously covered for exploring the circulation of MRSA in population. Therefore, a study was performed among college students. The objectives of the current study were to isolate and identify *S. aureus* from key samples by using biochemical test and identify MRSA by using antibiogram and PCR method. The reason behind using different modes of detection including PCR is to give high credibility for the MRSA detection, render the current study comparable with other studies in other parts of the world that use some of these detection methods, and at the same time comparing among these different methods of detection to know which one is the best.

2. MATERIALS AND METHODS

2.1 Collection of Samples

The students from University Putra Malaysia were selected for the study. A total of 100 samples were randomly collected from the keys of different college students. The samples were collected by using sterile cotton swabs. The swabs were moistened with nutrient broth and swabbed the keys. Then, the swabs were kept in sterile screwed cap test tubes with 5 ml of nutrient broth. The samples were incubated for 2 to 6 hours at 37°C in the incubator.

MRSA ATCC was obtained from Faculty of Medicine and Health Sciences. The MRSA was examined on the selective media, morphology test and biochemical tests. The results were used as positive control.

Escherichia coli was obtained from Microbiology Laboratory of Faculty Biotechnology and Biomolecular Sciences. It was cultured on the selective media, and subjected to morphology and biochemical tests and the results served as negative control.

2.2 Isolation of Bacteria

A drop of inoculated nutrient broth was dropped on the Blood agar (BBLTM, United State of America) by using the cotton swab. Then, the culture was streaked by used sterile inoculating loop. The plates were incubated at 37ºC for 18 to 24 hours. The colony morphology on blood agar was identified. Pure colony was obtained by subculturing onto nutrient agar (Oxoid, United Kingdom).

2.3 Identification of Bacteria

2.3.1 Gram stain

A smear of bacteria was prepared by placing a drop of distilled water on a clean slide to perform Gram staining.

2.3.2 Mannitol salt phenol red agar

The pure colonies on nutrient agar were picked using a sterile inoculating loop and sub-cultured onto the surface of selective agar, Mannitol Salt Phenol Red agar (Oxoid, United Kingdom). Then, the plates were incubated at 37ºC for 18 to 24 hours. The changes in color of the medium from pink to yellow indicated positive results.

2.3.3 Catalase test

One drop of 3% hydrogen peroxide was placed on a clean slide. The suspected isolate was picked by using sterile inoculating loop and put into 3% hydrogen peroxide. The presence of bubbles was indicative for positive results.

2.3.4 Oxidation-fermentation test

Bromcresol purple glucose fermentation broth was used. Two tubes were needed for each sample. The suspected isolates were picked and inoculated into the medium by using sterile inoculating loop. One of the tubes was incubated aerobically and another tube was incubated anaerobically by sealing the surface with 2 to 3 ml of sterile mineral oil. The tubes were incubated for 18 to 24 hours at 37°C in the incubator. The positive results were shown yellow in color and the negative results were purple in color.

2.3.5 DNase test

The pure colonies on nutrient agar were picked by using a sterile inoculating loop and subcultured onto the surface of DNase agar (Oxoid, United Kingdom). Then, the plates were

incubated at 37ºC for 18 to 24 hours. After that, the plates were flooded with 1 N HCl. The clear zone around the colonies were observed and recorded.

2.3.6 Coagulase test (tube tethod)

The tests were carried out by using human plasma and rabbit plasma. The suspected isolates were picked and inoculated into a sterile test tube with 5 ml of Phosphate-Buffered Saline. The O.D. of the bacterial suspension was measured by using spectrophotometer at 600 nm. The O.D. of the bacterial suspension should not exceed 0.3. After that, 150 µl of bacterial suspension and 350µl of human blood plasma were pipetted into sterile hemolysis test tube and mixed well. The test tubes were incubated at 37ºC for 4 hours and the clot formation was observed for every 30 minutes. For the negative samples, the test tubes were further incubated for 24 hours and observed for clot formation. For the positive results, the test tubes were re-incubated for overnight to observe the clot lyses. This indicated that fibrinolysin enzyme was produced by the bacteria.

2.3.7 Antibiogram test

Antimicrobial susceptibility was determined by the disk diffusion method, referred to the Clinical and Laboratory Standard Institute (CLSI, 2007). The suspected isolates were picked by using sterile inoculating loop and inoculated into a sterile test tube with 5 ml of Phosphate-Buffered Saline. The O.D. of the suspension was measured by spectrophotometer. The O.D. should not exceed 0.5 McFarland (0.08 to 0.1) at 600nm wavelength. Then, sterile cotton swabs were used to spread the growth evenly on the surface of Mueller-Hilton agar (Oxoid, United Kingdom). The antibiotic discs such as Methicillin (10 µg), Erythromycin (30 µg), Gentamycin (10 µg), Penicillin G (1 µg), Tetracycline (30 µg), Vancomycin (30 µg), Ampicillin (2 µg), Oxacillin (1 µg), Chloramphenicol (30 µg), Cefoxitin (30 µg), and Trimethoprim (5 µg) were placed on the surface of MH agar plates. The MH agar plates were incubated for 18 to 24 hours at 37ºC. The antibiogram was read and recorded the diameter of zone of the inhibition.

2.3.8 DNA extraction (gene all DNA purification kits)

The pure single colony of suspected isolates was picked and subcultured on 5 ml of Luria-Bertani broth (non-selective media) using sterile inoculating loop. The bacterial suspension was incubated in shaker incubator at 37ºC for 16 to 20 hours. Then, bacterial suspension was pippetted in a 1.5 ml microcentrifuge tube. The bacterial suspension was centrifuged at 10, 000 rpm for 10 minutes. The supernatant was discarded and the pellet was resuspended in 200 µl of Gram- Positive bacteria lysis buffer. The suspension was incubated for 30 minutes at 37℃. After that, 20 µl of Proteinase K and 200 µl of Buffer BLL were added and mixed well by vortexing. The suspension was incubated at 56℃ for 30 minutes in water bath shaker, followed by further incubation at 70℃ for 30 minutes. After cooling down, 200 µl of absolute ethanol were added and mixed well.

All the lysate was transferred into Gene All DNA purification SV Column with inserted collection tube. The column was centrifuged for 1 minute at 9800 rpm. The collection tube with flow-through solution was discarded and replaced a new collection tube. 600 µl of Buffer BW were added and centrifuged for 1 minute at 9800 rpm. The collection tube with flow-through solution was discarded and replaced a new collection tube. 700 µl of Buffer TW were added and centrifuged for 1 minute at 9800 rpm. The flow-through solution was discarded and the collection tube was inserted back to the SV column. Then, the column

was centrifuged at full speed (> 14,500 rpm) for 1 minute to discard the flow-through solution completely. The SV column was placed into a sterile 1.5 ml microcentrifuge tube. 100 µl of Buffer AE were added and incubated for 1 minute in room temperature. Then, the column was centrifuged at full speed (> 14,500 rpm) for 1 minute. The SV column was discarded. The purified DNA in microcentrifuge tube was kept in -20℃ for temporary storage.

2.3.9 Polymerase chain reaction (PCR)

Multiplex PCR was carried out by using mecA gene, ermA gene, ermB gene, ermC gene, msrA gene, linA gene, nuc gene and femA gene primers. The forward and reverse primers for mecA gene were 5'-TCCAGATTACAACTTCACCAGG-3' and 5'-CCACTTCATATCTTGTAACG-3' (Oliver and Lencastre, 2002). The forward and reverse primers for ermA gene were 5'-GTTCAAGAAC AATCAATACA GAG-3' and 5'-GGATCAGGAA AAGGACATTT TAC-3' (Lina et al., 1999). The forward and reverse primers for ermB gene were 5'-CCGTTTACGA AATTGGAACA GGTAAAGGGC-3' and 5'-GAATCGAGAC TTGAGTGTGC-3' (Lina et al., 1999). The forward and reverse primers for ermC were 5'-GCTAATATTG TTTAAATCGT CAATTCC-3' and 5'-GGATCAGGAA AAGGACATT TAC -3' (Lina et al., 1999). The forward and reverse primers for msrA were 5'-GGCACAATAA GAGTGTTTAA AGG-3' and 5'-AAGTTATATC ATGAATAGAT TGTCCTGTT -3' (Lina et al., 1999). The forward and reverse primers for linA were 5'-GGTGGCTGGG GGGTAGATGT ATTAACTGG-3' and 5'-GCTTCTTTTGAAATACATGGTATTTTCGATC-3' (Lina et al., 1999). The forward and reverse primers for nuc were 5'-GCGATTGATGGTGATACGGTT-3' and 5'AGCCAAGCCTTGACGAACTAAAGC-3' (Rushdy et al., 2007).). The forward and reverse primers for femA were 5'-CTTACTTACTGCTGTACCTG-3' and 5'-ATCTCGCTTGTTATGTGC-3' (Ardic et al., 2005). The PCR mixture was prepared as shown in Table 1.

Table 1: PCR mixture

Reagent	Amount per reaction (µl)
DNA template	1.25
Forward primer	0.25
Reverse primer	0.25
Enzyme	6.25
$MgCl_2$	0.25
dH_2O	4.25
Total	12.5

The predenaturation for PCR conditions was at 94℃ for 4 minutes and the final extension was at 72℃ for 10 minutes. The PCR conditions for 6 primers were shown in Table 2.

For gel electrophoresis, our (4) µl of PCR product with 1 µl of loading dye were added into the well of 1% agarose gel. Then, 6 µl of 100bp DNA ladder with loading dye were transferred into the first well of the agarose gel to serve as a marker. The electrophoresis was performed at 80 Volts for approximately 45 minutes. The gel was removed and was stained with ethidium bromide for 10 minutes. The gel was destained with distilled water. Then, the bands on the gel were visualized under UV transilluminator. The image of the bands was captured by using a gel documentation system.

Table 2: PCR conditions

Primer	PCR conditions	Size (bp)
*mec*A	32 cycles of 94°C for 30 s, 53°C for 30 s, 72°C for 50 s	162
*erm*A	32 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 60 s	421
*erm*B	32 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 60 s	359
*erm*C	32 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 60 s	572
*msr*A	30 cycles of 94°C for 60s , 50°C for 60 s, 72°C for 90 s	940
*lin*A	32 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 60 s	323
nuc	32 cycles of 94°C for 35 s, 52°C for 35 s, 72°C for 50 s	276
*fem*A	32 cycles of 94°C for 40s, 48°C for 40 s, 72°C for 50s	684

3. RESULTS AND DISCUSSION

There were 73 female and 27 male students selected for the study. The students were from different races. Among the 71 Malay students, 52 (73.24%) female Malay students and 19 (26.76%) male Malay students were involved. The total of 26 Chinese students composed of 18 (69.23%) female and 8 (30.77%) male. There were only 3 female Indian students participated in the study. In this study, several age ranges were concerned. Students below or equal 20 years old contributed 20%. The percentage for age 21, 22, 23 and 24 were 24%, 24%, 15%, and 9%, respectively. The students who 25 years old or above were 8 % of the total students. The field of study of the students played important role in the study. Education (18%), Microbiology (18%), Biotechnology (9%), Agriculture (9%), Human Development (8%), Biochemistry (8%), Science (7%), Biology (5%), Social Science (4%), Computer Science (4%), Consumer Studies (3%), Business and Economic (3%), Food Science (2%), Chemistry (1%) and Music (1%). The history of flu in a month before conducting the study was taken into account. 37 % of them had history of flu in the previous month and 63 % of them did not have.

3.1 Isolation of Bacteria on Blood Agar and Gram Stain

Among the 100 key samples, 97% were grown on blood agar. There were 10 samples that contained 2 types of bacteria. Hence, 109 bacterial isolates were detected and grown on blood agar. 80 (73.39%) of the bacterial colonies were grey color, 24 (22.02%) were white in color and 5 (4.59%) were yellow in color. 36.70% of the bacterial colonies did not show hemolysis on blood agar. And 53.21% of the bacterial colonies showed β-hemolysis and 10.09% showed α-hemolysis. In the total of 109 bacterial isolates, 63 (57.80%) isolates were Gram-positive bacilli while 39 (35.78%) were Gram-positve cocci and 7 (6.42%) were Gram-negative rods.

3.2 Biochemical Tests for Identification

Several biochemical tests were carried out to identify the *S. aureus*. In the Mannitol Salt Agar (MSA) tests, 16 (41.03%) of the bacterial isolates showed positive results and 23 (58.97%) showed negative results. All Gram-positive isolates gave positive results in the Catalase tests. Bromcresol purple glucose fermentation broth was used for the oxidation and fermentation test of the bacteria isolates. It was found that 29 (74.36%) of the bacterial isolates showed positive results for both oxidation and fermentation of glucose; 6 (15.38%) showed positive results in the oxidation of glucose but negative results in the fermentation of glucose; 4(10.26%) showed negative results in both oxidation and fermentation of glucose.

In order to support the previous biochemical tests, DNase tests and tube coagulase tests were carried out. The combination of all the biochemical tests increased the sensitivity to identify the *S. aureus* among the bacterial isolates. DNase and tube coagulase tests were carried out among the 16 MSA positive bacterial isolates. In DNase tests, 7(43.75%) of the 16 bacterial isolates showed positive results and 9 (56.25%) showed negative results. In tube coagulase tests, human plasma and rabbit plasma were used. However, both plasma gave similar results. Only 2 (12.50%) of the 16 MSA positive bacterial isolates showed tube coagulase positive results and 14 (87.50%) showed negative results. Both tube coagulase positive samples showed fibrinolysin tests negative due to inability to lyse the clot after 24 hours incubation.

3.3 Antibiogram

The 16 MSA positive bacteria isolates underwent the antiobiogram tests. The oxacillin 1µg, methicillin 10µg, erythromycin 30µg and cefoxitin 30µg were applied and tested to indicate the presence of MRSA. There were 3 (18.75%) suspected bacterial isolates. Isolates number 7i and 11 were resistant to erythromycin and isolate number 57 was resistant to oxacillin and methicillin (Table 3). However, these bacterial isolates were tube coagulase negative. In order to confirm the identity of the bacteria, molecular methods were carried out.

Table 3. Antibiogram for the 3 tube coagulase negative bacteria

Antibiotic discs	Isolate number 7i	Isolate number 11	Isolate number 57
Oxacillin 1µg	S	S	R
Methicillin 10µg	S	S	R
Chloramphenicol 30µg	S	S	S
Gentamycin 10µg	S	S	S
Vancomycin 30µg	S	S	R
Tetracycline 30µg	S	S	S
Ampicillin 2µg	R	R	R
Trimethoprim 5µg	S	S	R
Penicillin G 1µg	R	R	R
Erythromycin 30µg	R	I	S
Cefoxitin 30µg	S	S	S

3.4 Polymerase Chain Reaction (PCR)

Eight types of primers were used in the PCR test to identify the presence of MRSA for the 3 coagulase negative bacteria. The *nuc* gene which is specifically used to detect *S. aureus* was present in isolate number 7. Besides, mecA gene, ermC gene, *msr*A gene, *lin*A gene, and *fem*A gene were detected in isolate number 7. For isolate number 11, only *mec*A gene, *msr*A gene and *lin*A gene were detected. There were 4 genes detected in isolate number 57, which were *mec*A gene, *msr*A gene, *lin*A gene and *fem*A gene (Table 4).

Table 4. Resistant genes detected in the 3 tube coagulase negative bacteria

Gene	Isolate number 7	Isolate number 11	Isolate number 57
*mec*A	Yes	Yes	Yes
*erm*A	-	-	-
*erm*B	-	-	-
*erm*C	Yes	-	-
*msr*A	Yes	Yes	Yes
*lin*A	Yes	Yes	Yes
nuc	Yes	-	-
*fem*A	Yes	-	Yes

Based on the results, there were 35.78% of Gram-positive cocci and 6.42% of Gram-negative cocci on the key samples. This data showed that the cleanliness of the college students were low. The presence of these bacteria was often related to improper handling (Hatakka, 2000). There were 39 bacterial isolates from 37 key samples which represented 37 of college students. There were 24 (64.87%) of Malay students and 13 (35.13%) of Chinese students. This data showed that the personal hygiene of Malay students was lower than of other races. Among the 37 of college students, female students contributed 26 (70.27%) and the male students contributed 11 (29.73%). This data showed that the inanimate objects of female students were highly contaminated by bacteria compared to the male students. The percentage of students with positive bacterial isolates in relation to different age ranges was 7 (18.92%) at or below 20 years old, 15 (40.54%) at 21 years old, 8(21.62%) at 22 years old, 4(10.81%) at 23 years old and 3(8.11%) at or above 24 years old. The students from field of Microbiology were highly colonized by bacteria; up to 32.43% of the 37 students. Students with and without the history of flu were covered 48.65% and 51.35, respectively.

Stapbylococcus aureus is Gram-positive bacteria (Peacock, 2005). Therefore, the 39 Gram-positive bacteria isolates were selected. On Blood agar, *S. aureus* colonies were golden yellow in colour (Sasidharan et al., 2011). Besides, *S. aureus* may show hemolysis on Blood agar around the area of the colonies. Mannitol Salt Agar (MSA) is the selective media usually used to detect the *S. aureus*. *S. aureus* isolates produce yellow colonies on MSA (Zadik et al., 2001). Among the 39 bacterial isolates, 16 of them showed yellow colonies on the MSA. They were suspected as *S. aureus*. In order to support the results of MSA, all the 39 isolates underwent Catalase tests and oxidation and fermentation of glucose. *S. aureus* isolates would produce bubbles when 3% hydrogen peroxide added because Staphylococci are able to produce enzyme catalase that breakdown hydrogen peroxide to water and oxygen. All the 39 bacterial isolates produced bubbles. The Staphylococci are able to use up glucose by oxidation or fermentation. From the study, 74.36% of the isolates showed oxidation and fermentation of glucose positive. The color of the bromcresol purple glucose broth changed from purple to yellow. All the 16 MSA positive isolates showed Catalase positive and oxidation and fermentation of glucose positive.

The sensitivity of MSA increased by performing DNase tests and coagulase tests. DNase tests were used to detect the DNase activity of Staphylococci. Among the 16 MSA positive isolates, only 7 of the isolates showed clear zone around the colonies. For the tube colagulase tests, human plasma and rabbit plasma were used. Two types of plasma were used because different choice of plasma can influence the efficiency of the test. Both plasma were used in order to compare the results and increase the sensitivity of the detection of *S. aureus*. However, human plasma was not recommended because human blood containes

variable amounts of Coagulase-Reacting Factors and anti-staphylococcal antibodies (Koneman et al., 1997). In this study, both plasma gave similar results. Only 2 of the samples showed clot in the test tubes. The positive results were let to be further incubated for 24 hours. The clots were still remained. This indicated that the bacterial isolates are unable to produce fibrinolysin enzyme to lyse the clot. With the combination of all the biochemical tests, there were 2 bacteria isolates *S. aureus*, isolate number 33 and 85.

Table 5. Combination of biochemical test for 16 MSA positive bacteria

Number isolate	MSA	Catalase tests	Oxidation of glucose	Fermentation of glucose	DNase tests	Tube coagulase tests (human plasma	Tube coagulase tests (rabbit plasma
1i	+	+	+	+	+	-	-
7i	+	+	+	+	+	-	-
9	+	+	+	+	-	-	-
11	+	+	+	+	-	-	-
17	+	+	+	+	-	-	-
26i	+	+	+	+	-	-	-
33	+	+	+	+	+	+	+
35ii	+	+	+	+	+	-	-
42	+	+	+	+	-	-	-
57	+	+	+	+	-	-	-
69i	+	+	+	+	-	-	-
69ii	+	+	+	+	-	-	-
70	+	+	+	+	+	-	-
74	+	+	+	+	-	-	-
85	+	+	+	+	+	+	+
93	+	+	+	+	+	-	-

Although only 2 bacterial isolates were tube coagulase positive, all the 16 MSA positive isolates were subjected to antibiogram tests. This action was to avoid any possibility of misdetection of the MRSA. Another reason was that some of the rare strains of *S. aureus* showed DNase tests and tube coagulase tests negative. 11 types of antibiotics were used which were Methicillin (10 µg), Erythromycin (30 µg), Gentamycin (10 µg), Penicillin G (1 µg), Tetracycline (30 µg), Vancomycin (30 µg), Ampicillin (2 µg), Oxacillin (1 µg), Chloramphenicol (30 µg), Cefoxitin (30 µg), and Trimethoprim (5 µg). The zone diameter was referred to the NCCLS guidelines. There were four types of antibiotics that more concerned, which were oxacillin, methicillin, erythromycin and cefoxitin. These four types of antibiotic discs were the major antibiotics that used to detect MRSA. Among the 16 MSA positive bacteria isolates, isolate number 7i and 11 were resistant to erythromycin. Isolate number 57 resistant to both oxacillin and methicillin. The other bacterial isolates were susceptible to most of the antibiotics, including the 2 tube coagulase positive bacteria. Therefore, isolates number 7i, 11 and 57 had undergone molecular methods, which were PCR.

The antibiogram tests were not supportive enough to detect the presence of MRSA. PCR was needed to increase the sensitivity and specificity of detection of MRSA. Although PCR was time consuming and of higher cost, the specificity of PCR was higher than the conventional ways of detection such as antibiogram. In the PCR, nuc gene was used

specifically to detect the presence of *S. aureus*. The other genes such as mecA gene, ermA gene, ermB gene, ermC gene, msrA gene, linA gene, and femA gene were used to detect the MRSA which are resistant to methicllin, erythromycin, oxacillin, cefoxitin, vancomycin and other macrolide drugs. After running PCR, gel electrophoresis was carried out. 100 bp DNA ladder was used as a marker. The 1% agarose gel was performed at 80 volts for 45 minutes. The bands were observed and recorded. The nuc gene was only detected on isolate number 7. This indicated that isolates number 11 and 57 were not *S. aureus*. Isolate number 7 also contained mecA gene, ermC gene, msrA gene, linA gene, and femA gene. Therefore, isolate number 7 was the MRSA which is tube coagulase negative. This showed that the identification of MRSA is not guaranteed by using the common laboratory tests, but also requires highly advanced molecular methods to increase the reliability and sensitivity in the detection of MRSA.

4. CONCLUSION

There was no single way that can guarantee the results of the identification of *S. aureus*. The combination of several tests and molecular methods were needed. In this study, there were 2 *S. aureus* detected in isolates number 33 and 85. Both were isolated from key samples from a female Malay student and a male Malay student. Both students were 21 years old. The female student was in the field of Microbiology and had flu in the previous month. The male student was in the field of Computer Science and did not have the history of flu. Moreover, MRSA was found on the key samples of a 20 years old Chinese female student who studied on Science. This data showed that *S. aurues* is able to be colonized on the surface of keys. Thus, keys can be considered as risk tools for the transmission and circulation of MRSA and other bacteria. The presence of MRSA on keys is very dangerous. The MRSA might circulate throughout the body, and even transmitted to another innocent students or lecturers. Accordingly, hygiene of hands and handholds is essential to prevent or lower the spread of MDR bacteria such as MRSA in all sectors of population even these of high social class. It is recommended, therefore, that educative programs must be introduced to all sectors of community to attract their attention on the importance of hand and handhold hygiene in lowering or avoiding spread of MRSA.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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