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**Original article**

# Circulation and transmission of methicillin-resistant *Staphylococcus aureus* among college students in Malaysia (cell phones as reservoir)

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***Background:*** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen of increasing risk to man.

***Objective:*** We determined the risk of using cell phones as silent and underestimated tools for spreading MRSA in community.

***Methods:*** One hundred swabs of cell phones were collected from college students in Malaysia. A series of identification and differentiating tests were conducted for the precise identification of MRSA bacteria. Moreover, this study compared the efficacy of the different identification tests with gold standard, PCR assay. The tests used were tube coagulase, DNase agar test, antibiogram, several routine biochemical identification tests, and PCR assays. PCR assay used specific primers for resistance or ID -related genes: *mecA, ermA, ermB, ermC, msrA, linA, femA,* and *nuc* genes.

***Results:*** One hundred fifty bacterial isolates were collected from college students' cell phones, non-PCR assays of identification and resistance detection revealed presence and spread of MRSA in cell phones of 14 college students. PCR-amplification of the *nuc* gene was used as a baseline test to detect *Staphylococcus aureus*. Seven isolates (50%) were detected as *Staphylococcus aureus* with the presence of *nuc* gene, and the remaining seven isolates (50%) were negative for *nuc* gene. However, of the seven positive *nuc* gene isolates, six isolates (6/14; 42.9%) were positive for *mecA* gene, making them MRSA. Using PCR as gold standard, the specificity and the sensitivity of antibiogram test in the detection of methicillin resistance was only 55.6% and 40%, respectively. Most of the MRSA carriers were found to study in the field of Science (33.3%) and Education (33.3%).

***Conclusions:*** Cell phones proved to be silent tool for transferring MRSA in the community of college students in South East Asia. Moreover, PCR assay for identification of *S. aureus* and resistance evaluation for MRSA is superior when compared to other conventional methods.

***Keywords:*** Cell phones, MRSA, PCR, resistance, *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a nosocomial pathogen that causes major worldwide infections [1, 2]. MRSA strains are usually resistant to various types of antibiotics especially the β-lactam antibiotics and some of them can be easily transmitted and spread in the community [2-4]. MRSA are readily shed off and easily transfer horizontally by direct physical contact or by contact with formites, cell phones, coins, laptops, etc. Some researchers have reported the colonization of nosocomial pathogens on various objects such as ball pens, keyboards, coins, keys, and cell phones, which can act as a potential

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vector for transmission of these organisms among health-care workers [5-8]. Today, cell phones have become an important communication device in the community, especially among students. The increased use of cell phones has also raised the infection rates as reported by ecological findings [9]. Hence, this potentially pathogenic MRSA may circulate from nasal to hand, hand to cell phones and to other objects, which can cause potential infection in students and transmitted to others.

Since there is no sufficient data on the risk of MRSA contamination of cell phones among college students, this study was undertaken to investigate the potential role of cell phones in the transmission of pathogenic MRSA and its circulation among college students in University Putra Malaysia as a representative well-defined community in the region of South East Asia.

## Materials and methods

### *Sample collection*

The current study was conducted according to the Helsinki congress of ethical standards in biomedical research. Therefore, it was approved by the regional ethical committee. This study was carried out at University Putra Malaysia (UPM), during the period between October and December 2010. One hundred swabs of cell phones were collected from college students (27 males and 73 females) aged between 19 and 34 years. Of them, there are 71 Malay students, 26 Chinese students and 3 Indian students. The study field of each student and his/her history of cold or flu were recorded. Sterile swabs were moistened with sterile phosphate buffered saline, and then rotated over the surface of both sides of cell phones. Collected swabs were cultured in nutrient broth (MERCK, KGaA, Germany) and then incubated at 37°C for 24 hours for pre-enrichment. The cultures were then streaked onto blood agar (MERCK, KGaA, Germany) plates supplemented with 5% defribinated human blood. Plates were incubated aerobically at 37°C for 24 hours. Isolated microorganisms were identified using Gram's staining, morphology, catalase reaction, and glucose oxidation and fermentation reaction according to the standard procedures.

### *Selective/differential plating*

Isolated Gram's positive cultures were cultured on mannitol salt agar (Oxoid, Cambridge, UK) and incubated at 37°C for 24 to 48 hours. The cultures were then characterized and the presumptive *Staphylococcus aureus*, which can ferment mannitol, were isolated and evaluated based on the growth of yellow colonies and yellow zones surrounding the culture [10].

### *Tube coagulase test*

Colonies of 150 µl test isolates (not more than Mc Farland standard 2.0) were suspended in 350 µl of citrated rabbit and human plasma in sterile glass test tubes. Positive control tubes of methicillin-resistant *Staphylococcus aureus* ATCC were included. In addition, negative control tubes were the tubes containing citrated plasma alone (without culture inoculated). The tubes were incubated at 37°C for 4 hours. Where clotting did occur, the tubes were incubated at 37°C for additional 18 hours to examine fibrinolysin reaction. Positive coagulase reactions were confirmed by the clots formation that gels the whole contents of the tubes or loose web of fibrin in the tubes. To confirm the fibrinolysin reaction, disappearance of the clots in the initially positive coagulase tubes after overnight incubation indicated a positive fibrinolysin results.

### *DNase agar test*

The isolates were streaked on DNase agar (Oxoid, Cambridge, UK) and incubated at 37°C for 24 hours. After incubation, the colonies on DNase agar were flooded with an excess (~15 ml) of 1N HCl. Excess acid was removed with a vacuum pipette and the reaction was observed. Clear zones around the bacterial colonies indicated a positive DNase reaction [10].

### *Antibiogram typing*

The presumptive *Staphylococcus aureus* isolates were tested for their resistance to eleven antibiotics, which is methicillin 10 µg, cefoxitin 30 µg, erythromycin 30 µg, oxacillin 1 µg, gentamycin 10 µg, vancomycin 30 µg, tetracyclines 30 µg, chloramphenicol 30 µg, trimethoprin 1.25 µg, ampicilin 10 µg, and penicillin G 1 µg (Oxoid, Basingstoke, Hampshire, England) following a previously reported method [11]. Isolates (McFarland standard 0.5) were spread on Mueller Hinton Agar (MERCK, KGaA, Germany) plates and were allowed for 5 minutes to dry. Antibiogram procedure was conducted according to well-known art [12, 13].

***Polymerase chain reaction (PCR) for identification of mecA, ermA, ermB, ermC, nuc, femA, msrA, and linA genes***

Genomic DNA was extracted from Staphylococcal isolates by using the GeneJET Genomic DNA Purification Kit and the procedure was done according to the manufacturer instructions [Fermentas, #K0721]. The extracted genomic DNA was used as a template for PCR amplification [14, 15]. The primers used in this study were designed from published GenBank sequences that provided specific PCR products (**Table 1**). PCR was carried out on 14 staphylococcal isolates that were resistant to one of the antibiotics (Cefoxitin, Methicillin, Erythromycin, and Oxacillin) as well as the positive control MRSA strains. The PCR assays were preceded with an initial denaturation step (4 minutes at 94°C) and followed with a final extension step (10 minutes at 72°C). The amplification cycle of each gene was done as described in **Table 1**. After amplification, 4 µl of the amplicons were mixed with 1 µl of DNA loading buffer and electrophoresed in a 1% agarose gel in TAE buffer (Tris, acetate and EDTA). After electrophoresis, gels were stained with ethidium bromide for 10 minutes and photographed under UV light (**Figure 1**). The results were reported as positive or negative [12].

## Results

One hundred fifty bacterial isolates were isolated from college students' cell phones. Of them, 97 isolates (64.7%) were characterized as Gram's positive cocci isolates and 53 isolates (53.3%) were non-Gram's positive cocci isolates. All of 97 Gram's positive cocci isolates were subjected to catalase test, glucose oxidation and fermentation test, and solid screening medium on mannitol salt agar. Of them, 96 isolates (99%) gave catalase-positive reactions while only one isolate (1%) gave catalase-negative reaction. Catalase test was coupled with glucose oxidation and fermentation test and selective and differential solid screening medium using mannitol salt agar to isolate *Staphylococcus aureus* isolates. For glucose oxidation and fermentation test, results were compared with the positive control to determine positive and negative reactions and eliminate non-staphylococcal isolates (give negative results in either glucose oxidation or glucose fermentation tubes). Out of the 97 tested isolates, 63 isolates (65%) were shown to be positive in both glucose oxidation and fermentation tests while 34 isolates (35%) were shown to be negative in either glucose oxidation or glucose fermentation tubes. *Staphylococcus aureus* can be identified by using mannitol salt agar in which it is able to tolerate the high salt content and ferment mannitol for growth. It was detected that 38 isolates (39.2%) were able to grow on MSA producing yellow coloration surrounding the culture. Accordingly, these isolates were identified as *Staphylococcus aureus*. On the other hand, 59 isolates (60.8%) gave negative results in mannitol-salt agar; therefore, they were identified as non-*S. aureus* isolates.

**Table 1.** Sequences, primers, and PCR conditions used in amplification of *mecA* gene, *ermA* gene, *ermB* gene, *ermC* gene, *msrA* gene, *linA* gene, *femA* gene, and *nuc* gene

| Target gene | Primer sequences | PCR condition | Size (bp) | Reference |
|---|---|---|---|---|
| *mecA* | 5'-TCCAGATTACAACTTCACCAGG-3'<br>5'-CCACTTCATATCTTGTAACG-3' | 32 cycles of 94°C for 30s, 53°C for 30s, and 72°C for 50s | 162 | 15 |
| *ermA* | 5'-GTTCAAGAAC AATCAATACA GAG-3'<br>5'-GGATCAGGAA AAGGACATTT TAC-3' | 32 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 60s | 421 | 14 |
| *ermB* | 5'-CCGTTTACGAAATTGGAACAGGTAAAGGGC-3'<br>5'-GAATCGAGAC TTGAGTGTGC-3' | 32 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 60s | 359 | 14 |
| *ermC* | 5'GCTAATATTG TTTAAATCGT CAATTCC-3'<br>5'-GGATCAGGAA AAGGACATTT TAC-3' | 32 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 60s | 572 | 14 |
| *msrA* | 5'-GGCACAATAA GAGTGTTTAA AGG-3'<br>5'-AAGTTATATC ATGAATAGAT TGTCCTGTT-3' | 30 cycles of 94°C for 60s, 50°C for 60s, 72°C for 90s | 940 | 14 |
| *linA* | 5'-GGTGGCTGGG GGGTAGATGT ATTAACTGG-3'<br>5'-GCTTCTTTTG AAATACATGG TATTTTCGATC-3' | 32 cycles of 94°C for 30s, 57°C for 30s, and 72°C for 60s | 323 | 14 |
| *femA* | 5'-CTTACTTACTGCTGTACCTG-3'<br>5'-ATCTCGCTTGTTATGTGC-3' | 32 cycles of 94°C for 40s, 48°C for 40s, and 72°C for 50s | 684 | 16 |
| *nuc* | 5'-GCGATTGATGGTGATACGGTT-3'<br>5'-AGCCAAGCCTTGACGAACTAAAGC-3' | 32 cycles of 94°C for 35s, 52°C for 35s, and 72°C for 50s | 276 | 17 |

To confirm the isolates as *Staphylococcus aureus*, DNase agar test and tube coagulase test (TCT) were performed. Out of 38 presumptive *Staphylococcus aureus* isolates, it was found that 16 isolates (42.1%) were DNase-positive while the rest, 22 isolates (57.9%) were DNase-negative. For TCT, citrated rabbit plasma and human plasma were used to test the coagulase activity in the presumptive *S. aureus* isolates. When rabbit plasma was used in TCT, it was found that 11 isolates (28.9%) were identified as coagulase-positive *S. aureus* and 27 isolates (71.1%) were identified as coagulase-negative *S. aureus*. There was a difference when human plasma was used in which only 10 isolates (26.3%) gave coagulase-positive reaction and the rest, 28 isolates (73.7%) gave coagulase-negative reaction as shown in **Table 2**.

***Antibiogram typing***

The percentage of each antibiotic resistance among all presumptive *Staphylococcus aureus* isolates was shown in **Table 3**. The resistance for methicillin percentage was 13.2% (5 isolates), and the resistance percentages for other antibiotics were: Cefoxitin 10.5% (4 isolates), Erythromycin 13.2% (5 isolates), Oxacillin 15.8% (6 isolates), Vancomycin 13.2% (5 isolates), Tetracyclines 2.6% (1 isolate), Trimethoprim 7.9% (3 isolates), Ampicillin 78.9% (30 isolates) and Penicillin G 73.7% (28). Of the twenty-seven coagulase negative isolates, four isolates (14.8%) were resistant to methicillin and the other 23 isolates (85.2%) were susceptible. For coagulase positive isolates, it was shown that only one isolate (9.1%) was resistant to methicillin, making it MRSA and the other 10 isolates (90.9%) were susceptible. All 38 tested isolates were susceptible to gentamycin and chloramphenicol (**Table 3**).

**Table 2.** Isolation and identification of S. aureus with common tests

| Test | Gram's staining no. (%) | Catalase test no. (%) | Glucose OF test no. (%) | Mannitol salt agar no. (%) | DNase agar no. (%) | Tube coagulase test no. (%) | |
|---|---|---|---|---|---|---|---|
| | | | | | | Rabbit plasma | Human plasma |
| Positive results | 97 (64.7) | 96 (99.0) | 63 (65.0) | 38 (39.2) | 16 (42.1) | 11 (28.9) | 10 (26.3) |
| Negative results | 53 (53.3) | 1 (1.0) | 34 (35.0) | 59 (60.8) | 22 (57.9) | 27 (71.1) | 28 (73.7) |
| Total isolates | 150 | 97 | 97 | 97 | 38 | 38 | 38 |

**Table 3.** Antimicrobial susceptibility patterns among staphylococcal isolates as measured by disk diffusion method

| Antibiotics | Coagulase positive | | | Coagulase negative | | | General all isolates | | |
|---|---|---|---|---|---|---|---|---|---|
| | S no. (%) | I no. (%) | R no. (%) | S no. (%) | I no. (%) | R no. (%) | S no. (%) | I no. (%) | R no. (%) |
| Methicillin | 10 (90.9) | - | 1 (9.1) | 23 (85.2) | - | 4 (14.8) | 33 (86.8) | - | 5 (13.2) |
| Cefoxitin | 10 (90.9) | - | 1 (9.1) | 24 (88.9) | - | 3 (11.1) | 34 (89.5) | - | 4 (10.5) |
| Erythromycin | 8 (72.7) | 2 (18.2) | 1 (9.1) | 18 (66.7) | 5 (18.5) | 4 (14.8) | 26 (68.4) | 7 (18.4) | 5 (13.2) |
| Oxacillin | 10 (90.9) | - | 1 (9.1) | 22 (81.5) | - | 5 (18.5) | 32 (84.2) | - | 6 (15.8) |
| Gentamycin | 11 (100) | - | - | 27 (100) | - | - | 38 (100) | - | - |
| Vancomycin | 9 (81.8) | - | 2 (18.2) | 24 (88.9) | - | 3 (11.1) | 33 (86.8) | - | 5 (13.2) |
| Tetracyclines | 10 (90.9) | - | 1 (9.1) | 26 (96.3) | 1 (3.7) | - | 36 (94.8) | 1 (2.6) | 1 (2.6) |
| Chloramphenicol | 11 (100) | - | - | 27 (100) | - | - | 38 (100) | - | - |
| Trimethoprim | 10 (90.9) | 1 (9.1) | - | 20 (74.1) | 4 (14.8) | 3 (11.1) | 30 (78.9) | 5 (13.2) | 3 (7.9) |
| Ampicillin | 2 (18.2) | - | 9 (81.8) | 6 (22.2) | - | 21 (77.8) | 8 (21.1) | - | 30 (78.9) |
| Penicillin G | 3 (27.3) | - | 8 (72.7) | 7 (25.9) | - | 20 (74.1) | 10 (26.3) | - | 28 (73.7) |

S = sensitive, I = intermediate, R = resistant

***PCR method***

Fourteen isolates that were resistant to methicillin, cefoxitin, erythromycin and oxacillin in disk diffusion test were subjected to amplification of specific genes by using polymerase chain reaction method. PCR-amplification of the *nuc* gene was used as a baseline test to detect *Staphylococcus aureus*. Seven isolates (50%) were detected as *Staphylococcus aureus* with the presence of *nuc* gene, and the remaining seven isolates (50%) were negative for *nuc* gene. However, of the seven positive *nuc* gene isolates, six isolates (6/14; 42.9%) were positive for *mecA* gene, making them MRSA, and another two isolates (2/14; 14.2%) were positive for *mecA* gene but negative for *nuc* gene, making them methicilin-resistant staphylococci. The remaining six isolates (6/14; 42.9%) were negative for *mecA* gene as well as *nuc* gene which making them methicillin-susceptible staphylococci. As compared to the tube coagulase test results of the coagulase positive isolates, only two isolates (2/3; 66.7%) were positive for *nuc* gene and *mecA* gene. On the other hand, for coagulase negative isolates, six isolates (6/11; 54.5%) were positive for *mecA* gene and only five of them (5/11; 45.5%) were positive for *nuc* gene (**Tables 4, 5 and Figures 1, 2**).

Oxacillin resistance was confirmed by detection of *mecA* gene by using PCR. [18]. Hence, those isolates that were positive to *mecA* gene were also resistant to oxacillin. In this study, it was found that eight isolates (57.1%) were oxacillin resistant and the remaining six isolates (42.9%) were susceptible to oxacillin. Resistance to erythromycin were observed in two strains of *S. aureus* and six strains of coagulase-negative staphylococci that contained one of the *erm* genes. In this study, only *ermC* gene was detected, which is the most prevalent *erm* gene in *S. aureus* strains and there was no detection of *ermA* gene and *ermB* gene in all 14 isolates (**Figures 3-5**) Other antibiotic resistance genes were detected as follow: 12 isolates (85.7%) were positive for *msrA* gene, *linA* gene and *femA* gene and 4 isolates (14.3%) were negative for *msrA* gene, *linA* gene and *femA* gene (**Table 5 and Figures 6-8**).

**Table 4.** Detection of antibiotic resistance genes among staphylococcal isolates

| Isolate No. | PCR Results | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***Nuc*** | ***MecA*** | ***ErmA*** | ***ErmB*** | ***ErmC*** | ***MsrA*** | ***LinA*** | ***FemA*** |
| **Coagulase-positive *S. aureus*** | | | | | | | | |
| 32y | + | + | - | - | + | + | + | + |
| 70y | + | + | - | - | + | + | + | + |
| 74 | - | - | - | - | - | + | + | - |
| **Coagulase-negative *S. aureus*** | | | | | | | | |
| 7 | + | + | - | - | + | + | + | + |
| 34 | - | - | - | - | - | + | - | + |
| 35w | - | + | - | - | - | - | + | + |
| 39w | + | + | - | - | - | + | + | + |
| 49w | - | - | - | - | - | + | - | - |
| 59w | + | - | - | - | + | + | + | + |
| 61w | + | + | - | - | + | + | + | + |
| 69y | + | + | - | - | + | - | + | + |
| 72w | - | - | - | - | - | + | + | + |
| 78w | - | - | - | - | + | + | + | + |
| 86w | - | + | - | - | + | + | + | + |

+ = presence of the resistance gene, - = absence of the resistance gene

**Table 5.** The prevalence of antibiotic resistance among all staphylococcal isolates

| Genes | ***nuc*** | ***mecA*** | ***ermA*** | ***ermB*** | ***ermC*** | ***msrA*** | ***linA*** | ***femA*** |
|---|---|---|---|---|---|---|---|---|
| Positive results no. (%) | 7 (50) | 8 (57.1) | - | - | 8 (57.1) | 12 (85.7) | 12 (85.7) | 12 (85.7) |
| Negative results no. (%) | 7 (50) | 6 (42.9) | - | - | 6 (42.9) | 4 (14.3) | 4 (14.3) | 4 (14.3) |

n = 14, - = not detected


**Figure 1.** Agarose gel electrophoresis of PCR for *mecA* gene presence. (Sample 27-28, 30, 32, 34-36, 40: positive *mecA* gene)

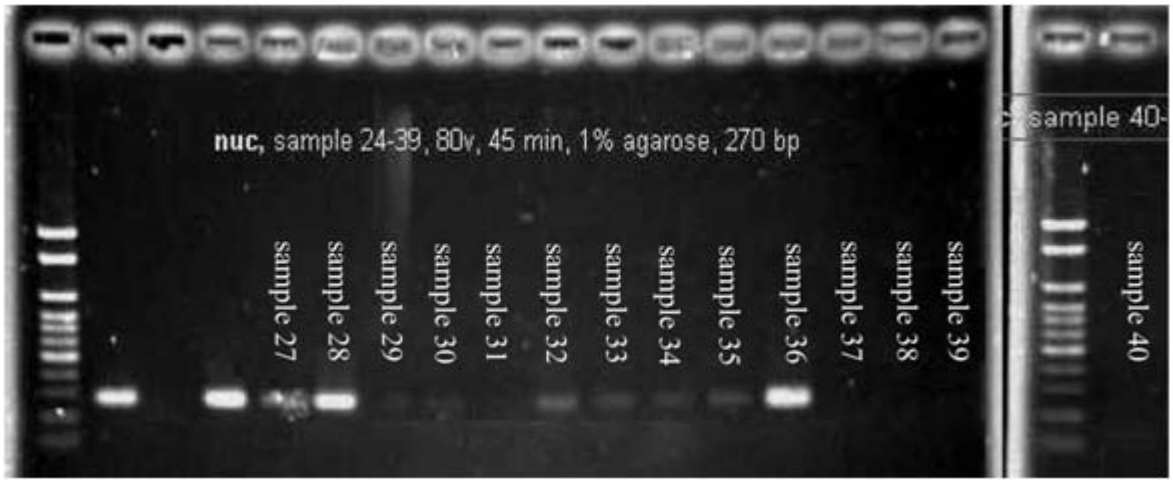


**Figure 2.** Agarose gel electrophoresis of PCR for *nuc* gene presence. (Sample 27-28, 31, 33-36: positive *nuc* gene)

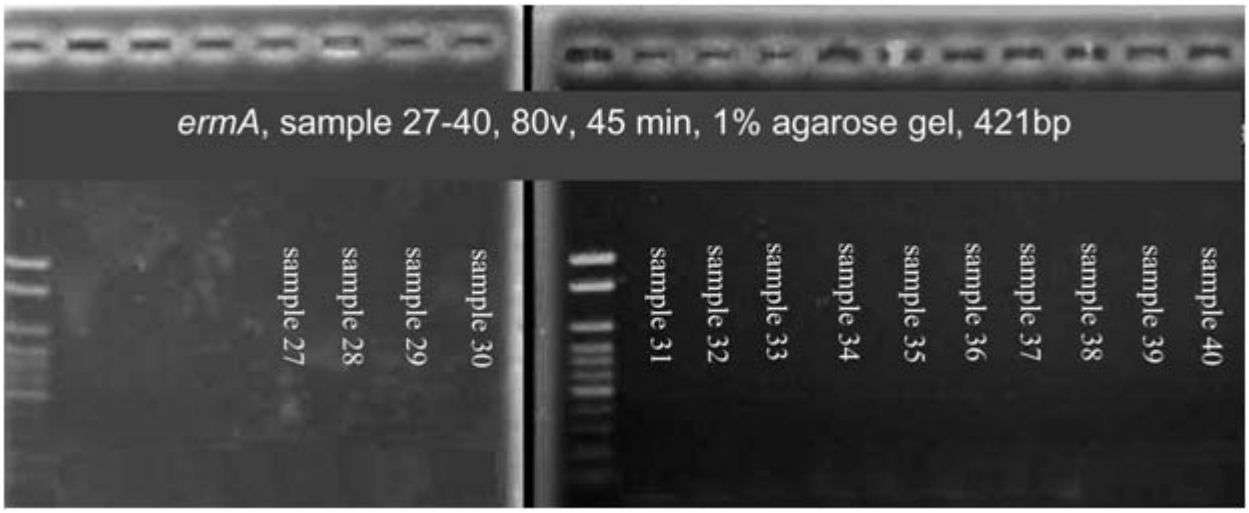


**Figure 3.** Agarose gel electrophoresis of PCR for *ermA* gene presence. (Sample 27-40: negative *ermA* gene)

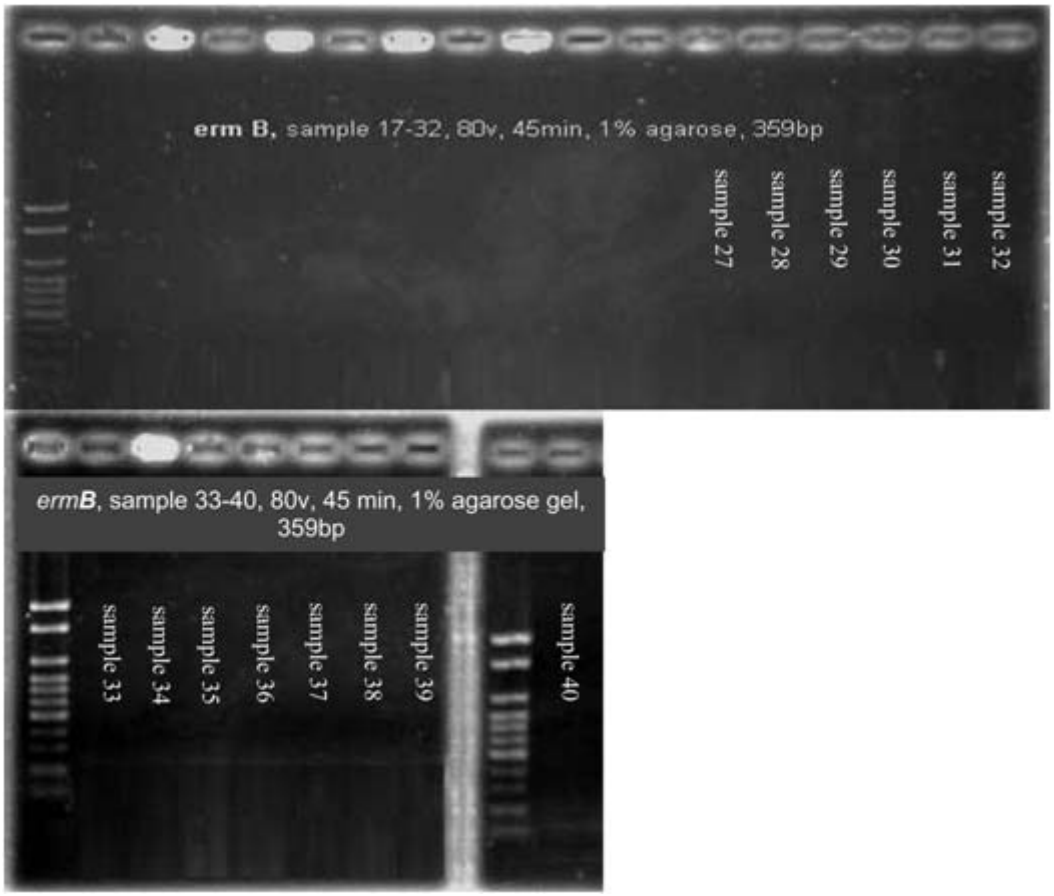


**Figure 4.** Agarose gel electrophoresis of PCR for *ermB* gene presence (Sample 27-40: negative *ermB* gene)

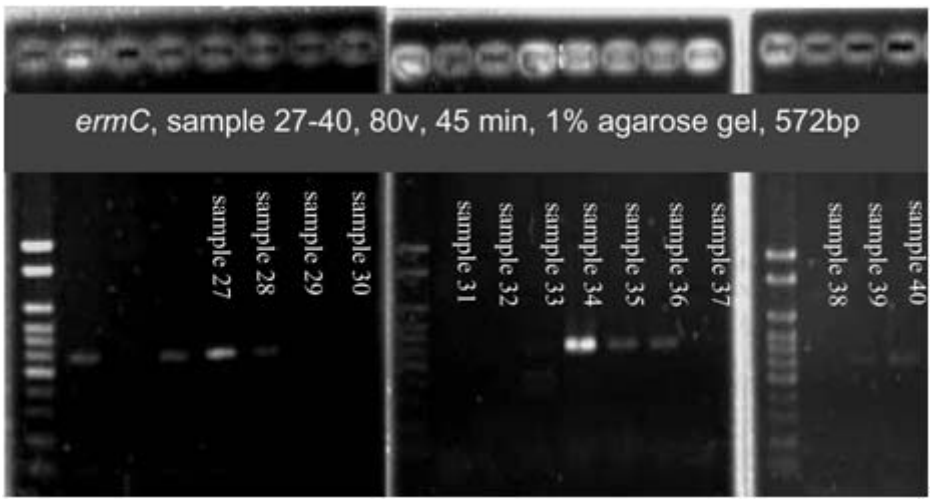


**Figure 5.** Agarose gel electrophoresis of PCR for *ermC* gene presence. (Sample 27-28, 33-36, 39-40: positive *ermC* gene)

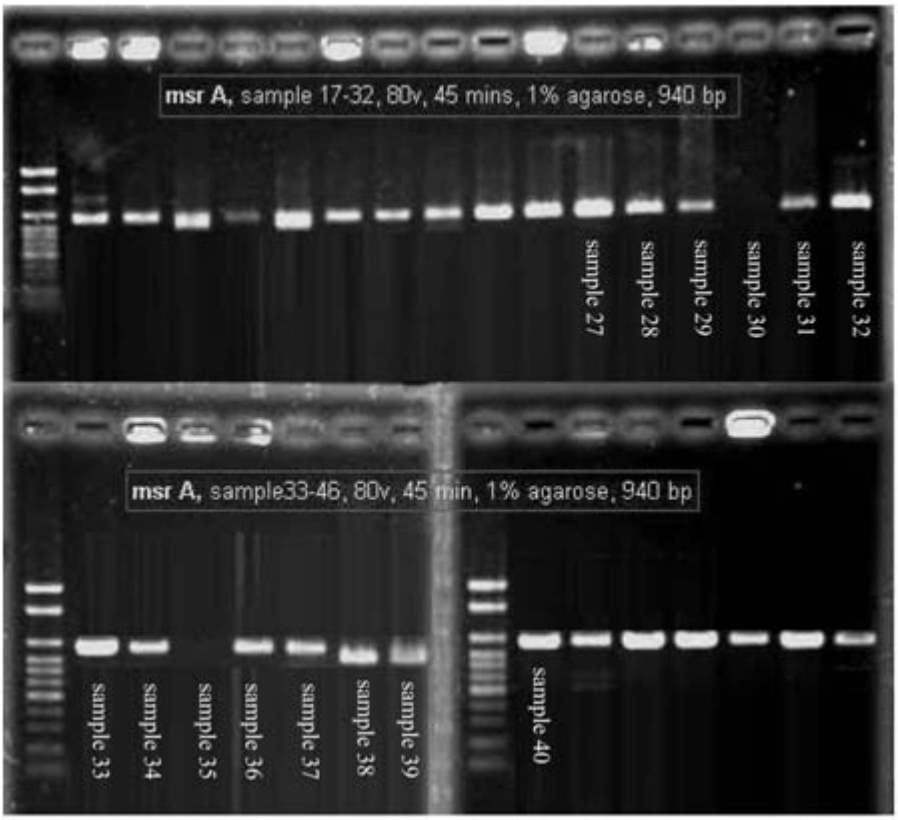


**Figure 6.** Agarose gel electrophoresis of PCR for *msrA* gene presence (sample 27-29, 31-34, 36-40: positive *msrA* gene)

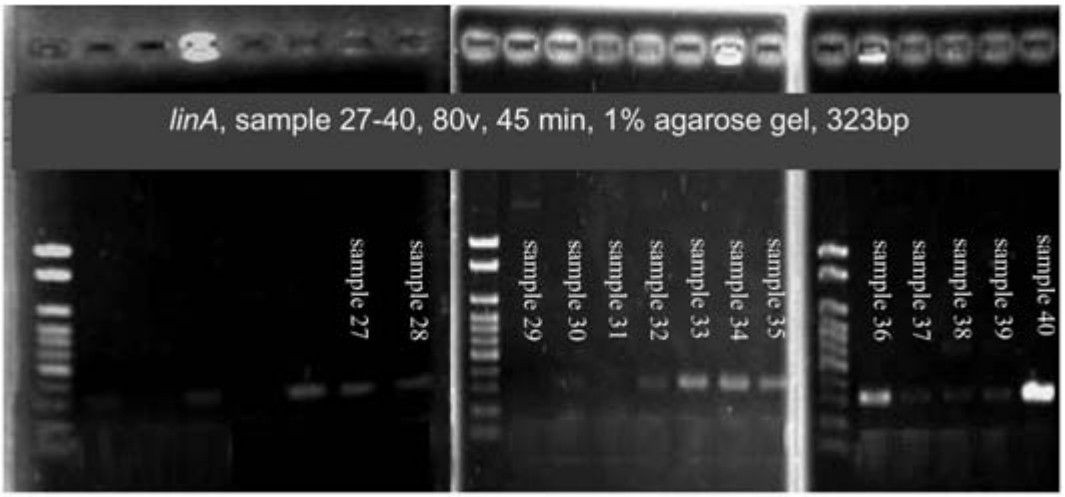


**Figure 7.** Agarose gel electrophoresis of PCR for *linA* gene presence (sample 27-28, 30, 32-40: positive *linA* gene)

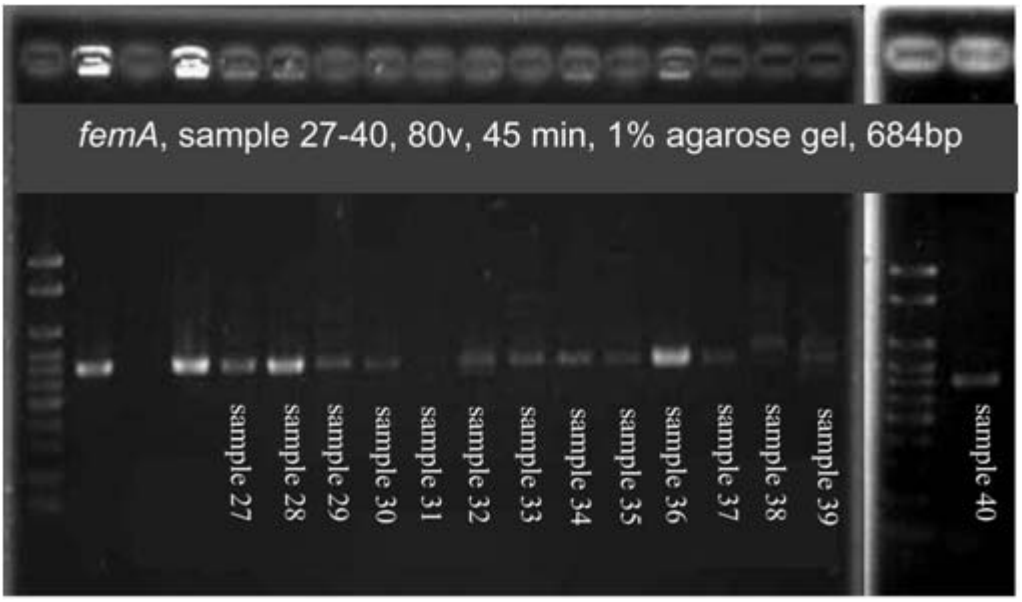


**Figure 8.** Agarose gel electrophoresis of PCR for *femA* gene presence (sample 27-30, 32-37, 39, 40: positive *femA* gene) sample 27 = isolate 7, sample 28 = isolate 32y, sample 29 = isolate 34, sample 30 = isolate 35w, sample 31 = isolate 39w, sample 32 = isolate 49w, sample 33 = isolate 59w, sample 34 = isolate 61w, sample 35 = isolate 69y, sample 36 = isolate 70y, sample 37 = isolate 72w, sample 38 = isolate 74, sample 39 = isolate 78w, sample 40 = isolate 86w

***The specificity and sensitivity of tests conducted***

The specificity of antibiogram test in the detection of methicillin resistance was only 55.6% and the sensitivity was 40%. There were three false positive (positive in methicillin disk diffusion test but no *mecA* gene was detected) and four false negative results (susceptible to methicillin in disk diffusion test but positive for *mecA* gene) obtained in methicillin disk diffusion test. Similarly, erythromycin disk diffusion test yielded six false positive (resistant to erythromycin when tested in disk diffusion test but *erm* genes were not detected) and two false negative results (susceptible to erythromycin in disk diffusion test but *erm* gene were detected) giving sensitivity of 50% and specificity of 0%. Oxacillin resistance was confirmed by the detection of *mecA* gene using PCR. Upon comparing the results of antibiogram typing using oxacillin disc with *mecA*-gene results, four false positive and six false negative results were detected. The sensitivity of oxacillin antibiogram typing was 66.7% and its specificity was 75% as shown in **Table 6**.

In addition, 50% (3 isolates) of PCR-tested MRSA were obtained from cell phones of male students while the other three isolates obtained from female students. In addition, 66.7% of them were Malay, 33.3% were Chinese and none of them was Indian. Of the six MRSA isolates, the age of the carriers ranged between 19 and 25 years; however, the age 21 years was shown to be associated with the highest percentage (33.3%) of MRSA carriage while only one carrier was associated with age 20 years, 23 years, 24 years and 25 years, respectively. Most of the MRSA carriers study in the field of Science (33.3%) and Education (33.3%) while minority of them study in Computer Science (16.7%), and Human Development and Management (16.7%). On the other hand, Most of the MRSA carriers identified in this study (83.3%) do not have the history of cold or flu in the past one month. Therefore, it was revealed that history of flu or cold in the past one month is not associated with the colonization of MRSA in college students.

In addition, the sensitivity and specificity of rabbit plasma, human plasma, DNase, MSA, catalase, glucose oxidation and fermentation tests were compared with the PCR-based detection of nuc gene as shown in **Table 7**.

**Table 6.** Sensitivity and specificity of antibiogram typing compared to the PCR detection of resistance genes in *Staphylococcus aureus* isolates

| Antibiogram typing results (n=14) | True positive | False positive | True negative | False negative | % sensitivity | %specificity |
|---|---|---|---|---|---|---|
| Methicillin | 2 | 3 | 5 | 4 | 40.0 | 55.6 |
| Erythromycin[a] | 6 | 6 | 0 | 2 | 50.0 | 0 |
| Oxacillin[b] | 2 | 4 | 2 | 6 | 66.7 | 75.0 |

[a] = comparison of erythromycin-resistant with *erm* genes, [b] = molecular detection of oxacillin-resistant is determined by detection of *mecA* gene

**Table 7.** Identification of *S. aureus* with common tests compared to the PCR detection of *nuc* gene

| Tube coagulase test | PCR detection of nuc gene | | % sensitivity | % specificity |
|---|---|---|---|---|
| | Positive | Negative | | |
| Rabbit plasma | | | | |
| Positive | 2 | 1 | 66.7 | 54.6 |
| Negative | 5 | 6 | | |
| Human plasma | | | | |
| Positive | 3 | 1 | 75.0 | 60.0 |
| Negative | 4 | 6 | | |
| DNase | | | | |
| Positive | 5 | 2 | 71.4 | 71.4 |
| Negative | 5 | 2 | | |
| MSA | | | | |
| Positive | 6 | 6 | 50.0 | 50.0 |
| Negative | 1 | 1 | | |
| Catalase test | | | | |
| Positive | 7 | 7 | 50.0 | 0 |
| Negative | 0 | 0 | | |
| Glucose oxidation and fermentation test | | | | |
| Positive | 7 | 6 | 53.9 | 100.0 |
| Negative | 0 | 1 | | |

## Discussion

Within the genus of staphylococci, MRSA and *S. aureus* are the major pathogens that caused severe skin infections and bacteremias in humans. They are clinically more important than MRCoNS and CoNS because of increased virulence and resistance [19-21]. Recently, cell phones have become a modern home for MRSA and many other bacteria [22]. It is crucial to detect MRSA carriers as early as possible, not only for infection control but also for therapeutic decision with last-line antibiotics against MRSA. Hence, this study was conducted to investigate the circulation of MRSA in the carrier itself and its transmission to others in the community. Screening of MRSA from cell phones of college students aimed at identifying cell phones as a hidden reservoir of MRSA. This study also compares among different methods of Staphylococcal identification and evaluates the performance of each test.

Results of the current study revealed that eight out of one hundred (8%) cell phones collected were contaminated with MRSA, six of which were shown to be MRSA by using PCR amplification of specific gene sequences. Most reports have proven that PCR-based assays for MRSA identification potentially deliver rapid, specific and sensitive results [23-25]. All MRSA strains have a staphylococcal cassette chromosome mec (SCC mec) which is a genetic mobile component where the *mecA* gene resides in it. The *mecA* gene encodes for an altered penicllin-binding

protein (PBP2a), a cell wall enzyme, which has a low affinity for all β-lactam antibiotics, including methicillin [26]. Hence, *mecA* gene is recognized as the Staphylococcal methicillin-resistant determinant gene and the *nuc* gene was detected to confirm that the strain under test was in fact *S. aureus* [27].

Furthermore, detection of genes conferring resistance to the older standard antibiotics like macrolides and lincosamides were included in this study. The *ermA*, *ermB* and *ermC* genes were responsible for erythromycin resistance, *msrA* gene responsible for macrolides antibiotics resistance and *linA* responsible for lincosamide antibiotics resistance [28]. When erythromycin resistance genes were examined, *ermC* genes were observed most frequently in MRSA isolates (100%) and neither of the *ermA* nor *ermB* genes was detected in *S. aureus* and CoNS isolates. Ardic et al. detected *ermA* genes primarily in MRSA (71.4%) and the *ermC* gene in *S. aureus* and CoNS isolates (64.3%) [28]. Whereas, Lim et al. detected *ermA* gene mainly in *S. aureus* isolates (82.5%) and *ermC* gene primarily in CoNS (47.2%) [29]. These findings were different with our findings. *ermA* gene must be present in MRSA strains, but *ermC* gene may also be present in the resistant strains. As described in the report of Eady et al., the *ermB* gene was present in a minority strains but was formerly found in only animal strains [30]. The *erm* genes were also detected in combination with other resistance genes such as *msrA* and *linA* gene [14]. In the current study, there were 12 staphylococcal strains detected with *msrA* and *linA* genes and eight of them were detected with *ermC* gene.

The *femA* gene is species-specific oligonucleotides which is found in all *S. aureus* and *S. epidermidis* strains [19]. On the other hand, nuc gene is specific for all *S. aureus* strains [27]. Both *femA* and *nuc* genes were selected and included in our study to identify and differentiate staphylococci into *S. epidermidis* and *S. aureus* strains. Of the 12 stapylococcal isolates detected with *femA* gene, five were identified as *S. epidermidis* and seven were identified as *S. aureus* with positive *nuc* gene as well.

Because PCR assays can detect conserved DNA sequences within bacterial genomes, PCR is considered a promising and specific assay [19, 26]. The comparison among the methods of MRSA identification was based on the results of the gold standard, namely PCR assay. Methicillin resistance is expressed heterogeneously in which the expression of resistance varies according to the culture media, the salt and antibiotic concentration of the media, and the temperature of incubation. Only about 1% of MRSA population expresses resistance at any given time and conditions [31]. Thus, discrepant results may be detected in some settings. In this study, discordance among resistance genes and their phenotypic sensitivity, represented by the conventional antibiogram testing using disk diffusion method was detected.

Phenotypically methicillin-sensitive, mecA-positive isolates lead us to think that methicillin resistance can be overlooked when disk diffusion method was performed and the presence of the gene but lack of the phenotype is possible. Similar to us, Francois et al. also found discordance among erythromycin genes and phenotypic sensitivity [19] According to their reports, this discordance might be brought about by mutations in the coding or promoter region of the PCR-detected genes.

Other than genotypic and phenotypic tests used to identify MRSA isolates and to confirm the antibiotics resistance patterns in *S. aureus*, some conventional biochemical tests such as coagulase test, DNase test, MSA screening medium, glucose oxidation and fermentation test, and catalase test were included in the current study to differentiate staphylococci from other Gram-positive cocci. Tube coagulase test remains the most widely used test for the identification of *S. aureus* [32]. However, the results of TCT can vary depending on the types of plasma used, anticoagulant factors, and lot-to-lot variation of plasma [33]. Zarzour and Belle noted that false results can also occur with the tube coagulase test despite of the types of plasma used [34]. In addition, poor performance may obtain when aged (several weeks) rehydrated plasma was used. Therefore, coagulase plasma should not be stored more than 5 days under refrigerated conditions before use. The formation of clots in TCT seems to involve the conversion of fibrinogen to fibrin with the enzyme complex, coagulase and coagulase-reacing factor [35]. Thus, the performance of each types of plasma in TCT were well depending on the amount of coagulase reacting factor, the amounts of fibrinogen and the inhibitory factors present in the plasma (rabbit, pig, sheep, human, etc.) [35].

In this study, discrepant results were obtained in the tube coagulase test that was performed using rabbit plasma and human plasma. This discrepant result cannot be related to technical problems as these results

were confirmed by repeated testing. The specificity of TCT with rabbit plasma in this study was 54.6% and its sensitivity was 66.7%, which have five false negative results and one false positive result. On the other hand, when human plasma was used, the specificity was 60% and sensitivity was 75%. Interestingly, human plasma was more sensitive than rabbit plasma, implying that using human plasma in TCT may detect less false negative isolates. The result of this study is different from that of previous studies in which rabbit plasma was shown to be more sensitive than human plasma with sensitivity of 94-100% [29;28, 30;29]. Most investigators prefer using rabbit plasma in TCT, which is a "gold standard", than human plasma as the specificity of human plasma was found to be relatively low and may not be appropriate for the TCT in some settings [36]. Furthermore, human plasma is inefficient, risky, and contributed much errors with poor performance in some settings that is due to the composition of human plasma is different from rabbit plasma [10]. However, it was shown in this study that human plasma was not too bad in performance and reliability. The quality of the plasma determines its performance and reliability. Human plasma that was used in this study was fresh that have been stored in refrigerated condition for only one day while the rabbit plasma that was used have been stored in refrigerated condition for 4 days. Aged plasma most probably gives inefficient agglutination and hence poorer results.

Sperber and Tatini [36] reported that 99% of the *S. aureus* isolates that were tested in their study produced 4+ clots within 2 hours and the clots might become less distinct after extended incubation when fibrinolysin enzymes are produced by some *S. aureus* strains. Their findings are in agreement with the findings of the current study. Since the clots may dissolve by the action of fibrinolysin enzymes and give false-negative, the test was examined periodically at a half hour interval so that false-negative results can be avoided. In this study, four fibrinolysin-positive reactions were observed in the coagulase test with rabbit plasma while only one fibrinolysin-positive reaction was detected in coagulase test using human plasma. This result shows that rabbit plasma is more reliable than human plasma.

Despite of using tube coagulase test to identify *S. aureus*, DNase test was included in this study as well. Although DNase test is not as widely used as coagulase test, it is very useful to check the accuracy of the coagulase test and the screening agar, mannitol salt agar, and for identification of coagulase-negative *S. aureus* [37]. Out of 14 presumptive *S. aureus* isolates, seven were DNase-positive and four of them were found to be CoNS. Three DNase-negative *S. aureus* were also detected; this is similar to the findings of Rao et al, [38] but no explanation for these findings. DNase test gave a sensitivity of 71.4% and a specificity of 71.4% (Table 7). In this study, all coagulase-positive cultures (100%) were also DNase-positive and 36.4% of coagulase-negative cultures were DNase-positive. This result is similar to Morton and Cohn [39], which demonstrated that 98% of coagulase-positive and 13.5% of coagulase-negative cultures produced DNase. Zarzour and Belle claimed that these results were due to the presence of *S. epidermidis* strains that may be DNase positive, whereas some *S. aureus* may not produce DNase [34].

Numerous studies have shown that mannitol salt agar (MSA) is a promising selective screening medium that enhance the recovery of MRSA in surveillance specimens [40, 41]. Out of 14 presumptive *S. aureus*, six mannitol positive isolates were nuc-confirmed *S. aureus* and other six mannitol positive isolates were negative for nuc gene (non-*S. aureus*). These findings were similar with the reports published by other investigators in which there were staphylococci other than nuc-confirmed *S. aureus* isolates produced yellow colonies on MSA [40, 42]. The sensitivity of MSA was 50% and the specificity was 100% (**Table 7**). MSA is inefficient for the identification of *S. aureus* and must be coupled with other "gold standard" tests such as coagulase test and DNase test. A combination of all common phenotypic tests improves the performance in the identification of *S. aureus*. Nevertheless, genotypic test remains the most important test with high specificity and sensitivity. It specifically detects the specific genes in the bacterial genome and yielded accurate and reliable results. In short, no single phenotypic test can be used to identify *S. aureus* precisely.

Glucose oxidation and fermentation test is the most widely accepted diagnostic test for the differentiation of staphylococci from micrococci in which it is based on the ability of staphylococci to ferment glucose anaerobically while micrococci are not able to do so [43]. The standard procedures were recommended by the International Association of Microbiological Societies Subcommittee on Taxonomy

of Staphylococci and Micrococci. In short, this test can aid in separating staphylococci from micrococci and hence helps in the identification of *S. aureus*. However, this test owes a disadvantage whereby it somewhat has some difficulties in interpreting the results, particularly when the indicator partly turns yellow and hence gives rise to a false negative results [44]. Furthermore, other genera of Gram-positive cocci, such as streptococci and pediococci, are not readily distinguished from staphylococci, so catalase test should be included. In this study, six false positive results were reported as compared with the results of PCR amplification of *nuc* gene. The sensitivity of glucose oxidation and fermentation test was 53.9% and its specificity was 100% (without any false negative results).

Catalase test was incorporated in this study to aid in the identification of *S. aureus* by differentiating staphylococci from other Gram-positive cocci. In this study, all 14 presumptive *S. aureus* isolates were catalase-positive. This test has a sensitivity of 50% and a specificity of 0% (without any false negative results). Notably, seven false positive results were detected which indicates that catalase test only aids in the differentiation.

A rare finding was noticed in this study; an isolate negative to nuc-gene was shown to be coagulase-positive staphylococci and able to produce fibrinolysin enzyme (fibrinolysin-positive). Besides, out of six methicillin-resistant *S. aureus*, only two of them were coagulase-positive *S. aureus* and the remaining four were coagulase-negative *S. aureus*. In this case, it has been shown that MRCoNS is prevalent and predominant and have steadily increased in Malaysia. Interestingly, two methicillin-resistant *Staphylococcus epidermidis* were detected (positive femA gene but negative nuc gene [19, 27]. Since *S. epidermidis* is a normal flora in human body, its resistance to methicillin has highlighted the emergence of non-*S. aureus* resistant strains with undefined clinical significance in future.

## Conclusion

Taken together, it is obvious that contamination of MRSA on cell phones could be risky as these organisms have the potential to spread to the public silently. MRSA could colonize and circulate within the individual and spread and eventually reside in the community. Public should be aware of this issue and strict infection control procedures, and hand hygiene; environmental disinfection should be regularly practiced in the community to prevent the rate of MRSA transmission to be increased. Although it seems not common, this study evaluated the high risk of cell phones as a mobile spreading vector and the public should be aware of limiting its usage and/or taking the precautions to limit its risk for MRSA transfer.

We have evaluated the performance of each common phenotypic tests used for the identification of *S. aureus* and its antibiotic resistance patterns using antibiogram typing. In this study, a molecular method, amplification of specific genes using polymerase chain reaction (PCR), was performed for confirmation. The identification of *Staphylococcus aureus* still largely relies on genotypic test whereby no single phenotypic test can guarantee a reliable result. Furthermore, human plasma was shown to be more sensitive than rabbit plasma where the quality of the plasma is mainly dependent on the freshness of plasma and the storage time. Aged plasma gave poorer performance.

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