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Research Article
Antimicrobial Resistance Pattern of *Staphylococcus aureus* Strains Isolated from Clinical and Hospital Environment specimens and Their Correlation with PCR-based Approaches

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Abstract

Background and Objective: *Staphylococcus aureus* has long been recognized as one of the most important causes of bacterial disease in humans. This study investigates the prevalence of *Staphylococcus aureus* in clinical and environmental samples and their susceptibility patterns to antibiotics. **Methodology:** A total of 69 samples, 36 clinical specimens obtained from patients and 33 environmental samples from hospital facilities, were screened for *S. aureus* using standard microbiological and biochemical methods and PCR-based assay. Isolates resistant to both cefoxitin and oxacillin were considered to be Methicillin Resistant *S. aureus* (MRSA). **Results:** Of the two categories of samples screened, 31 (86.1%) and 23 (69.7%), respectively tested positive for *S. aureus*. The highest prevalence of MRSA from clinical samples (28.6%) was found in sputum and wounds and the highest (25%) from environmental samples was found in corridor, door handle and patient bed samples. **Conclusion:** In suggesting that healthcare personnel and hospital environments serve as potential reservoirs of *S. aureus*, these findings have practical, clinical and epidemiological importance. Ten of 16 of multi-drug resistant *S. aureus* isolates were MRSA, suggesting a correlation between the results of PCR patterns and their phenotypic multi-drug resistance testing.

Key words: Antibiotic resistant, Hospital environment, MRSA, *Staphylococcus aureus*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Staphylococcus aureus has long been recognized as one of the most important causes of bacterial disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses, furuncles and cellulitis and causes serious infections such as bloodstream infections, pneumonia, or bone and joint infections[1]. The continuing emergence of methicillin resistant *S. aureus* (MRSA), including hospital acquired (HA)-MRSA and community acquired (CA)-MRSA, is a major and increasing threat to public health. Most MRSA isolates are resistant to multiple antibiotics and effective antibiotic treatment of MRSA infections are consequently limited[2]. Clinically, infections caused by HA-MRSA strains are also associated with higher mortality and morbidity[3] and some CA-MRSA strains express additional virulence factors that enable them to cause more serious diseases[4].

Environmental surfaces in communities carry the least risk of disease transmission and can be safely decontaminated using less rigorous methods than those used on medical instruments and devices, especially in public hospital areas. Microbiologically contaminated surfaces can serve as reservoirs of potential pathogens. Hospital environments play an important role in nosocomial infection in that healthcare environments contain a diverse population of microorganisms. Transfer of microorganisms from environment surfaces to hosts can occur indirectly, for example, by hand contact with other surfaces[5]. Jalalpoor has also shown that 54.7% of *S. aureus* isolates among hospital environment specimens were antibiotic resistant strains[6]. This is unacceptably high.

The aims of this study were threefold. Firstly, it aimed to determine the prevalence and antibiotic susceptibility of *S. aureus* and MRSA in clinical and environmental specimens. It also aimed to determine the antibiotic susceptibility levels of isolated *S. aureus* and to evaluate the antimicrobial resistance profiles of *S. aureus* strains isolated from clinical and environmental specimens. Finally, it sought to compare the phenotypic and genotypic methods for detection of *S. aureus* and suggests how the research findings may suggest practical imperatives for infection reduction and control by healthcare professionals.

MATERIALS AND METHODS

Ethics statement: The study was approved by the Committee of the Scientific Study of Humane Technique in Laboratory Animal Experiments and Human Ethics, Faculty of Science, Silpakorn University, Nakhon Pathom, Thailand. No written informed consent was required because all the patients were anonymous and no other personal information was used in this study.

Bacterial isolates: From May to August, 2017, a total of 36 specimens from blood, sputum, pus, wounds, urine and nasopharyngeal swab samples were collected from patients admitted to Nakorn Pathom Hospital and 33 swab samples were also collected from door handles, stair railings, stair floors, corridors, toilet floors and patient beds at the same hospital. *Staphylococcus aureus* isolates were identified in 31 (86.1%) of the clinical specimens obtained from patients and in 23 (69.7%) of the environmental samples from hospital facilities, isolates were examined by conventional methods such as colony morphology on blood agar and mannitol salt agar, Gram stain, catalase production and mannitol utilization and coagulase test. Identified strains were stored at -20°C in Nutrient broth (Oxoid) containing 20% glycerol.

Determination of methicillin resistance: Methicillin resistance was evaluated using three methods: (1) A disk diffusion test using 30 μg cefoxitin disk ($\leq$21 mm indicated MRSA), (2) An oxacillin MIC (Minimum Inhibitory Concentration) test ($\geq$4 μg mL^{-1} indicated MRSA) and (3) A polymerase chain reaction (PCR) for the detection of *mecA* gene (positive indicated MRSA)[7]. Antibiotic disks and Oxacillin powder were obtained from Himedia (Himedia Laboratories, Pvt. Ltd., Mumbai, India). All tests were compared for sensitivity and specificity with PCR for *mecA* gene as a reference method. Sensitivity was calculated by dividing the number of *mecA*-positive isolates detected as resistant using phenotypic methods by the total number of *mecA*-positive strains (ether susceptible or resistant). Specificity was calculated through dividing the number of *mecA*-negative isolates classified as sensitive based on phenotypic criteria by the total number of *mecA*-negative isolates[8].

Antibiotic sensitivity test: Antibiotic susceptibility of the bacteria isolates was assayed according to the Kirby-Bauer disk diffusion method[9]. All the plates were incubated for 20 min before inoculation and placement of antibiotic discs to allow excess moisture to dry. After drying, a single loop of each isolate was inoculated into sterile normal saline and compared

with the 0.5 McFarland standard. The suspension was aseptically swabbed on the surface of Mueller-Hinton plates and antibiotic sensitivity disks that contained penicillin (10 units), cefoxitin (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), erythromycin (15 µg), trimethoprim/sulfamethoxazole (25 µg), gentamicin (10 µg), clindamycin (2 µg), rifampicin (30 µg), linezolid (30 µg) and ciprofloxacin (25 µg). The MICs of oxacillin and vancomycin in both MRSA and MSSA isolates were determined by the broth dilution method. All procedures were carried out and interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI)[10]. *Staphylococcus aureus* ATCC25923 was used as a control strain in both the disk diffusion and broth dilution methods.

PCR amplification: Total bacterial DNA was extracted from *S. aureus* using a modified phenol-chloroform method. Briefly, *S. aureus* was cultured on 5 mL brain heart infusion broth and incubated at 37°C for 24 h. Afterwards, 1.5 mL of the culture was centrifuged for 5 min at a velocity of 14,000 rpm. Each pellet was resuspended in 600 µL Tris-EDTA buffer by repeated pipetting or vortexing. Then 3 µL of 10% SDS and 3 µL of 20 mg mL^{-1} proteinase K were added, mixed and incubated for 30 min at 65°C in a water bath, 600 µL of phenol/chloroform/isoamyl alcohol was added, mixed, centrifuged for 5 min at 14,000 rpm and the supernatant was transferred to a fresh tube. An equal volume of ethanol was then added and mixed gently until DNA precipitated. Each pellet was then centrifuged for 5 min and the supernatant was discarded. The pellet was washed with 1 mL of 70% ethanol, mixed, centrifuged for 5 min and the supernatant was discarded and then dried for 10 min at a velocity vac/45°C. Finally, it was resuspended in 30 µL TE buffer. The DNA concentration was read using 2 µL in a Nanodrop machine using TE buffer as blank. The concentration was made up to 100 ng mL^{-1} in each sample and stored at -20°C.

Primers used for detection of the *femA* gene were primers FemA1 and FemA2, leading to an *S. aureus*-specific 450 bp PCR product[11]. The *mecA* gene was detected with the primers MecA1 and MecA2, yielding a 519 bp PCR product for methicillin and oxacillin resistance[12]. The *aac(6')/aph(2'')* gene was detected with the primers aac(6')/aph(2'')1 and aac(6')/aph(2'')2, yielding a 407-bp PCR product for gentamicin resistance[13]. The *blaZ* gene was detected with the primers blaZ1 and blaZ2, yielding a 774 bp PCR product for penicillin resistance[12]. The *ermA* gene was detected with the primers ermA1 and ermA2, yielding a 190 bp PCR product for erythromycin resistance. The *tetK* and *tetM* genes were detected with the primers tetK1 and tetK2, yielding a 360 bp PCR product and primers tetM1 and tetM2, yielding a 158 bp PCR product, respectively[14]. The following reaction mixture was added to each sample: 2 µL DNA (100 ng), 2 µL primer (100 pmol), PCR mixture (1.5 µL $MgSO_4$, 2.5 µL 10xPCR buffer, 0.5 µL dNTPs, 0.2 µL Taq polymerase) and completed to 25 µL volume by H_2O. The primer sequences and the PCR conditions were showed in Table 1. For the visualization of the product, 10 µL of the each PCR reaction was mixed with 5 µL 6x loading dye and loaded on 1.5% agarose gel for electrophoresis and visualization of the amplified PCR products. A 100 bp molecular weight DNA ladder was used to validate the length of the amplified products (Vivantis Technologies).

Table 1: Primer sequences and PCR conditions used

Genes	Primer sequences (5'-3') Forward (F), Reverse (R)	Size (bp)	PCR condition	Accession No.	References
femA	F: CGATCCATATTTACCATATCA	450	94°C 3 min	CP000255	Davoodi *et al.*[11]
	R: ATCACGCTCTTCGTTTAGTT		30x [94°C 30 sec+48°C 30 sec+72°C 30 sec] 72°C 5 min		
blaZ	F: TACAACTGTAATATCGGAGGG	774	95°C 5 min	X52734	Wilailuckana *et al.*[12]
	R: AGGTTCAGATTGGCCCTTAGG		30x [95°C 60 sec+50°C 60 sec+72°C 60 sec] 72°C 7 min		
mecA	F: TGTCCGTAACCTGAATCAGC	519		CP015447	
	R: TGCTATCCACCCTCAAACAG				
aac(6')/	F: TACAGAGCCTTGGGAAGATG	407	95°C 5 min	AF051917	Tsuchizaki *et al.*[13]
aph(2'')	R: CCATTTGTGGCATTATCATCATATC		30x [95°C 30 sec+57°C 30 sec+68°C 60 sec] 72°C 7 min		
ermA	F: TTCGCAAATCCCTTCTCAAC	190	94°C 3 min	X03216	Strommenger *et al.*[14]
	R: AAGCGGTAAACCCCTCTGA		30x [94°C 30 sec+55°C 30 sec+72°C 30 sec] 72°C 4 min		
tetK	F: GTAGCGACAATAGGTAATAGT	360		S67449	
	R: GTAGTGACAATAAACCTCCTA				
tetM	F: AGTGGAGCGATTACAGAA	158		X56353	
	R: CATATGTCCTGGCGTGTCTA				

RESULTS

A total of 54 *S. aureus* isolates including 31 isolates from clinical specimens and 23 isolates from environmental specimens were cultured on nutrient agar (NA) and mannitol salt agar (MSA) medium. All isolates showed yellow-colonies on NA medium. They fermented mannitol in MSA medium and produced yellow-colored colonies surrounded by yellow. These isolates were identified as Gram positive cocci in grape like clusters under microscope with coagulase, catalase and β-hemolysis positive. The PCR amplification of *femA* gene was done for all isolates to detect the species of *S. aureus*. In this study the PCR product appeared as a single band DNA with a size equal to 450 bp fragment corresponding to the *femA* amplicon (Fig. 1).

A total of 31 *S. aureus* isolates including 7 (22.6%) MRSA and 24 (77.4%) MSSA were isolated from different clinical specimens. The prevalence of MRSA was higher in sputum (28.6%) and wound (28.6%) than other specimens (Table 2). As the similar results in MSSA, the majority of isolates were isolated from sputum specimens (n = 11, 45.8%). A total of 23 *S. aureus* isolates including 8 (34.8%) MRSA and 15 (65.2%) MSSA were isolated from different environmental specimens. The prevalence of MRSA was higher in corridor (25%), door handle (25%) and patient bed (25%) than other specimens (Table 3). As the similar results in MSSA, the majority of isolates were isolated from corridor (20%), patient bed (20%) and stair railing (20%).

Table 2: Prevalence of *S. aureus* among clinical specimens

	S. aureus				
	MRSA		MSSA		
Specimen type	No.	Percentage	No.	Percentage	Total
Sputum	2	28.6	11	45.8	13
Wound	2	28.6	5	20.8	7
Blood	1	14.3	3	12.5	4
Pus	1	14.3	3	12.5	4
Nasopharynx	1	14.3	1	4.2	2
Urine	-		1	4.2	1
Total	7	100.0	24	100.0	31

Table 3: Prevalence of *S. aureus* among environmental specimens

	S. aureus				
	MRSA		MSSA		
Specimen type	No.	Percentage	No.	Percentage	Total
Corridor	2	25.0	3	20.0	5
Door handle	2	25.0	2	13.3	4
Patient bed	2	25.0	3	20.0	5
Stair railing	1	12.5	3	20.0	4
Toilet floor	1	12.5	2	13.3	3
Stair floor	-		2	13.3	2
Total	8	100.0	15	100.0	23

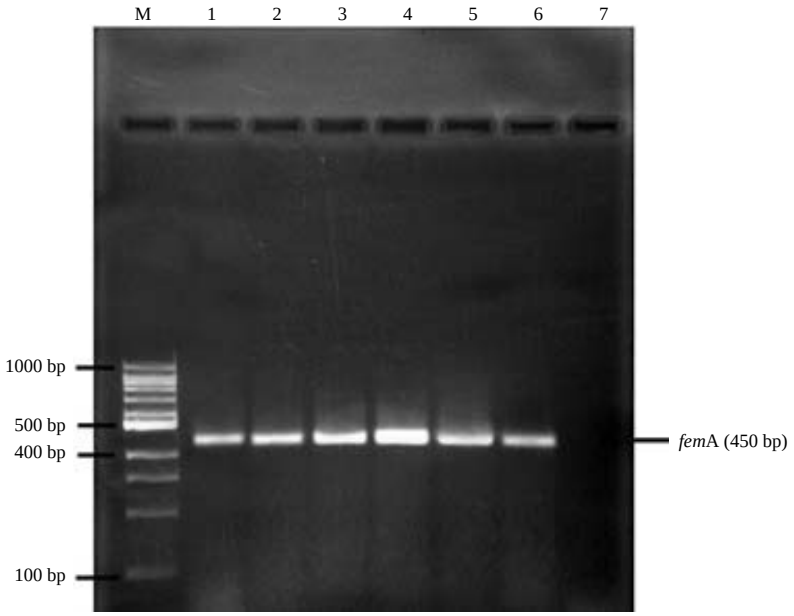

Fig. 1: Amplicon of *femA* gene; lane M: 100 bp DNA marker; Lane 1: *S. aureus* ATCC25923 (positive control), Lane 2-6 are tested isolates with positively amplified *femA* as indicated by 450 bp PCR amplicon, Lane 7 is *femA* negative (*Escherichia coli* ATCC 25922)

Table 4: Frequency and range of oxacillin and vancomycin MICs of *S. aureus* (MRSA and MSSA) isolated from clinical specimens by broth dilution method

	Oxacillin					Vancomycin			
	MRSA		MSSA			MRSA		MSSA	
MIC (μg mL^{-1})	No.	Percentage	No.	Percentage	MIC (μg mL^{-1})	No.	Percentage	No.	Percentage
0.125	-		5	20.80	0.25	-		18	75.0
0.25	-		10	41.60	0.50	3	42.8	4	16.6
0.5	1	14.3	6	25.00	1.00	2	28.6	1	4.2
1	1	14.3	2	8.40	2.00	2	28.6	1	4.2
64	2	28.6	-						
> 64	3	42.8	1	4.20					
Total	7	100.0	24	100.00	Total	7	100	24	100.0

Table 5: Frequency and range of oxacillin and vancomycin MICs of *S. aureus* (MRSA and MSSA) isolated from environmental specimens by broth dilution method

	Oxacillin					Vancomycin			
	MRSA		MSSA			MRSA		MSSA	
MIC (μg mL^{-1})	No.	Percentage	No.	Percentage	MIC (μg mL^{-1})	No.	Percentage	No.	Percentage
0.125	-		3	20.0	0.25	2	25.0	2	13.4
0.25	1	12.5	3	20.0	0.50	1	12.5	2	13.4
0.5	2	25.0	1	6.6	1.00	5	62.5	8	53.3
1	1	12.5	2	13.4	2.00	-		3	20.0
2	1	12.5	2	13.4					
4	-		1	6.6					
16	2	25.0	-						
32	-		2	13.4					
64	-		1	6.6					
>64	1	12.5	-						
Total	8	100.0	15	100.0	Total	8	100.0	15	100.0

The MICs for oxacillin and vancomycin from the clinical isolates are listed in Table 4. The MICs for oxacillin were between 0.5 to >64 μg mL^{-1} for MRSA and 0.125-1 μg mL^{-1} (except only one isolate was >64 μg mL^{-1}) for MSSA isolates. The MICs for vancomycin were between 0.5-2 μg mL^{-1} for MRSA and 0.25-0.5 μg mL^{-1} (except 2 isolates were 1-2 μg mL^{-1}) for MSSA isolates.

For the MICs for oxacillin and vancomycin from the environmental isolates are listed in Table 5. The MICs for oxacillin were between 0.25 to >64 μg mL^{-1} for MRSA and 0.125-32 μg mL^{-1} (except only one isolate was 64 μg mL^{-1}) for MSSA isolates. The MICs for vancomycin were between 0.25-1 μg mL^{-1} for MRSA and 0.25-2 μg mL^{-1} for MSSA isolates. Since no isolate had the MIC value for vancomycin greater than 2 μg mL^{-1} that mean these isolates did not fall into vancomycin resistant category according to CLSI.

Table 6 and 7 represent the resistance pattern of *S. aureus* isolates (MRSA and MSSA) from clinical specimens and environmental specimens to the tested antibiotics, respectively. In this study the entire *S. aureus* isolates were susceptible to vancomycin, chloramphenicol, linezolid and rifampicin for the clinical specimens and vancomycin, chloramphenicol, gentamicin, linezolid, ciprofloxacin and rifampicin for the environmental specimens. Among other antibiotics trimethoprim/sulfamethoxazole showed to be the most effective antibiotics against MSSA isolates. Seven of MRSA isolates (100%) and 23 of the MSSA isolates from clinical specimens were resistant to penicillin, while seven of MRSA isolates (87.5%) and 8 of MSSA isolates from environmental specimens were resistant to penicillin. The PCR testing revealed the presence of *mecA* gene in all isolates (Fig. 2) which were determined as methicillin resistant by the phenotypic methods. The sensitivity of oxacillin MIC test and cefoxitin disk diffusion test was 53.3%, whereas, the sensitivity of penicillin disk diffusion test was 93.3% and the specificity of oxacillin MIC test and cefoxitin disk diffusion test was 12.8% and 15.4%, respectively, whereas the specificity of penicillin disk diffusion test was 79.5% (Table 8).

From the 54 positive *S. aureus* isolates, 16 (29.6%) were multi-drug resistant (resistant to three or more antibiotics), 12 (22.2%) were resistant to only two antibiotics, 21 (38.9%) were resistant to only one antibiotic and the remaining five (9.3%) showed no resistance to any of the antibiotics. Nine (56.25%) and 7 (43.75%) out of the sixteen multi-drug resistant *S. aureus* isolates were from clinical and environmental specimens, respectively. Nine (75.0%) of those resistant to only

Table 6: Antibiotic susceptibility profiles of *S. aureus* strains isolated from clinical specimens by disk diffusion method

	MRSA (N = 7)				MSSA (N = 24)			
	Susceptible		Resistant		Susceptible		Resistant	
Antibiotics	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Oxacillin[a]	2	28.6	5	71.4	23	95.8	1	4.2
Vancomycin[a]	7	100.0	-		24	100.0	-	
Cefoxitin	3	42.9	4	57.1	24	100.0	-	
Chloramphenicol	7	100.0	-		24	100.0	-	
Tetracycline	4	57.1	3	42.9	23	95.8	1	4.2
Penicillin	-		7	100.0	1	4.2	23	95.8
Erythromycin	1	14.3	6	85.7	16	66.7	8	33.3
Trimethoprim/sulfamethoxazole	5	71.4	2	28.6	24	100.0	-	
Gentamicin	7	100.0	-		22	91.7	2	8.3
Clindamycin	4	57.1	3	42.9	21	87.5	3	12.5
Linezolid	7	100.0	-		24	100.0	-	
Ciprofloxacin	6	85.7	1	14.3	21	87.5	3	12.5
Rifampicin	7	100.0	-		24	100.0	-	

[a]Oxacillin and Vancomycin susceptibility profiles were determined using broth dilution method

Table 7: Antibiotic susceptibility profiles of *S. aureus* strains isolated from environmental specimens by disk diffusion method

	MRSA (N = 8)				MSSA (N = 15)			
	Susceptible		Resistant		Susceptible		Resistant	
Antibiotics	No.	Percentage	No.	Percentage	No.	Percentage	No.	Percentage
Oxacillin[a]	5	62.5	3	37.5	11	73.3	4	26.7
Vancomycin[a]	8	100.0	-		15	100.0	-	
Cefoxitin	4	50.0	4	50.0	9	60.0	6	40.0
Chloramphenicol	8	100.0	-		15	100.0	-	
Tetracycline	8	100.0	-		12	80.0	3	20.0
Penicillin	1	12.5	7	87.5	7	46.7	8	53.3
Erythromycin	8	100.0	-		13	86.7	2	13.3
Trimethoprim/sulfamethoxazole	6	75.0	2	25.0	15	100.0	-	
Gentamicin	8	100.0	-		15	100.0	-	
Clindamycin	5	62.5	3	37.5	15	100.0	-	
Linezolid	8	100.0	-		15	100.0	-	
Ciprofloxacin	8	100.0	-		15	100.0	-	
Rifampicin	8	100.0	-		15	100.0	-	

[a]Oxacillin and Vancomycin susceptibility profiles were determined using broth dilution method

Table 8: Determination of methicillin resistant sensitivities of clinical and environmental isolates

	PCR testing		
Antibiotic testing	*mecA*+	*mecA*-	Total
Oxacillin[a]			
Resistant	8	5	13
Susceptible	7	34	41
Total	15	39	54
Cefoxitin[b]			
Resistant	8	6	14
Susceptible	7	33	40
Total	15	39	54
Penicillin[b]			
Resistant	14	31	45
Susceptible	1	8	9
Total	15	39	54

[a]Oxacillin susceptibility test was determined using broth dilution method, [b]Cefoxitin and Penicillin susceptibility test was determined using disk diffusion method

two antibiotics were from clinical specimens while the remaining three (25.0%) were from environmental specimens. Twelve (57.1%) of the isolates resistant to only one antibiotic were from clinical specimens, nine (42.9%) were from environmental specimens. Out of the five that were not resistant to any antibiotic, only one (20.0%) was from clinical specimen and four (80.0%) were from environmental specimens (Table 9). Six (66.7%) of multi-drug resistant *S. aureus* from clinical specimens were MRSA and other three (33.3%) were MSSA, while four (57.1%) of multi-drug resistant *S. aureus* from environmental specimens were MRSA and other three (42.9%) were MSSA (Table 9). Of the total 16 (29.6%) multi-drug resistant isolates, two (12.5%) were resistant to six antibiotics, five (31.25%) were resistant to five and four antibiotics and the remaining isolates, four (25.0%) were resistant to three antibiotics. Among the multi-drug

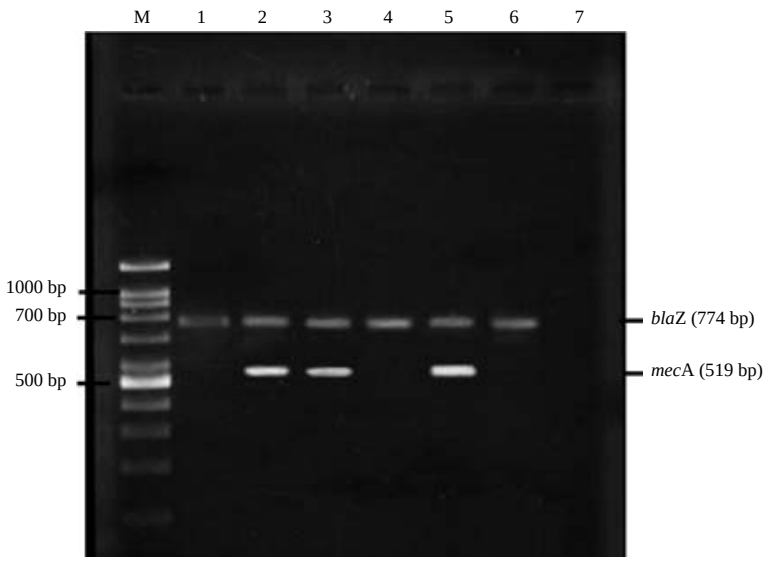

Fig. 2: Amplicon of *blaZ* and *mecA* genes by Multiplex-PCR; M: 100 bp DNA marker, Lane 1-6: *blaZ* (774 bp), Lane 2, 3 and 5: *mecA* (519 bp), Lane 7: *S. aureus* ATCC25923 (negative control)

Table 9: Antibiotic resistant pattern of *S. aureus* isolates from clinical and environmental specimens

		No. of *S. aureus* isolates (%), MRSA/MSSA [type of specimen][b]		
Pattern	Antibiotic resistant phenotype[a]	Clinical specimens (n = 31)	Environmental specimens (n = 23)	Total (n = 54)
1	PEN	12 (38.7)	7 (30.4)	19 (35.2)
2	FOX	-	2 (8.6)	2 (3.7)
3	PEN/ERY	8 (25.8)	1 (4.3)	9 (16.6)
4	PEN/TE	1 (3.2)	-	1 (1.8)
5	FOX/OXA	-	1 (4.3)	1 (1.8)
6	PEN/FOX	-	1 (4.3)	1 (1.8)
7	PEN/FOX/OXA	-	1 (4.3), MRSA [co]	1 (1.8)
8	TE/FOX/OXA	-	1 (4.3), MSSA [co]	1 (1.8)
9	PEN/CIP/DA	1 (3.2), MSSA [sp]	-	1 (1.8)
10	PEN/SXT/DA	-	1 (4.3), MRSA [co]	1 (1.8)
11	PEN/ERY/TE/OXA	1 (3.2), MRSA [bl]	-	1 (1.8)
12	PEN/ERY/SXT/DA	1 (3.2), MRSA [wo]	-	1 (1.8)
13	PEN/GEN/CIP/DA	1 (3.2), MSSA [bl]	-	1 (1.8)
14	PEN/FOX/DA/OXA	-	1 (4.3), MRSA [sr]	1 (1.8)
15	PEN/TE/FOX/OXA	-	1 (4.3), MSSA [tf]	1 (1.8)
16	PEN/ERY/TE/FOX/OXA	1 (3.2), MRSA [sp]	1 (4.3), MSSA [sf]	2 (3.7)
17	PEN/GEN/CIP/DA/OXA	1 (3.2), MSSA [wo]	-	1 (1.8)
18	PEN/ERY/FOX/DA/OXA	1 (3.2), MRSA [wo]	-	1 (1.8)
19	PEN/FOX/SXT/DA/OXA	-	1 (4.3), MRSA [tf]	1 (1.8)
20	PEN/ERY/FOX/CIP/DA/OXA	1 (3.2), MRSA [pu]	-	1 (1.8)
21	PEN/ERY/TE/FOX/SXT/OXA	1 (3.2), MRSA [sp]	-	1 (1.8)
22	Susceptible	1 (3.2)	4 (17.4)	5 (9.2)
-	Multi-drug resistant (resistant to three or more antibiotics, Pattern 7-21)	9 (29.0)	7 (30.4)	16 (29.6)

[a]PEN: Penicillin, ERY: Erythromycin, TE: Tetracycline, FOX: Cefoxitin, GEN: Gentamicin, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole, OXA: Oxacillin, DA: Clindamycin. [b]sp: Sputum, pu: Pus, bl: Blood, wo: Wound, co: Corridor, tf: Toilet floor, sf: Stair floor, sr: Stair railing

resistant MRSA from clinical specimens were from sputum, two isolates (33.3%), wound, two isolates (33.3%); pus, one isolate (16.7%) and blood, one isolate (16.7%), while the multi-drug resistant MSSA from clinical specimens were from sputum, one isolate (33.3%); wound, one isolate (33.3%) and blood, one isolate (33.3%). And among the multi-drug resistant MRSA from environmental specimens were from corridor, two isolates (50.0%); toilet floor, one isolate (25.0%); and stair railing, one isolate (25.0%), while the multi-drug resistant MSSA from environmental specimens were from

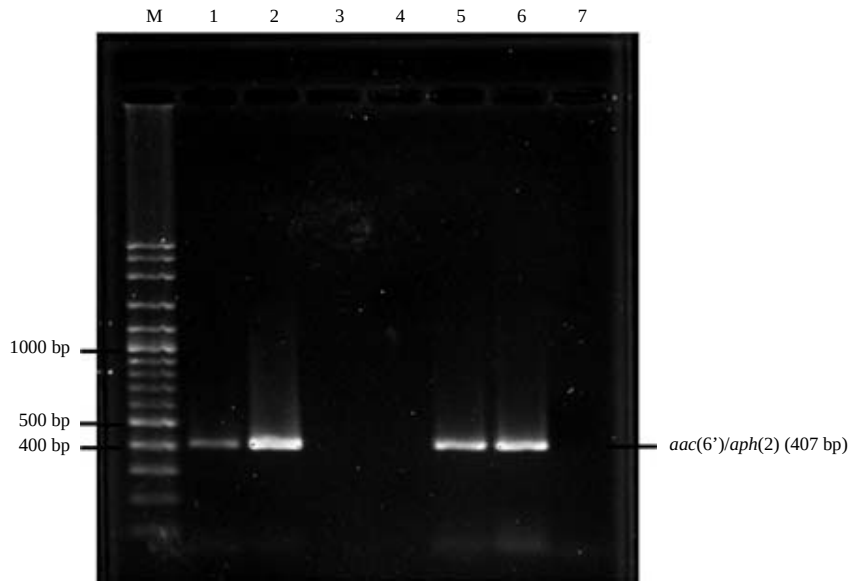

Fig. 3: Amplicon of *aac*(6)/*aph*(2) gene, Lane M: 100 bp DNA marker, Lane 1, 2, 5 and 6: *aac*(6)/*aph*(2) (407 bp), Lane 7: *S. aureus* ATCC25923 (negative control)

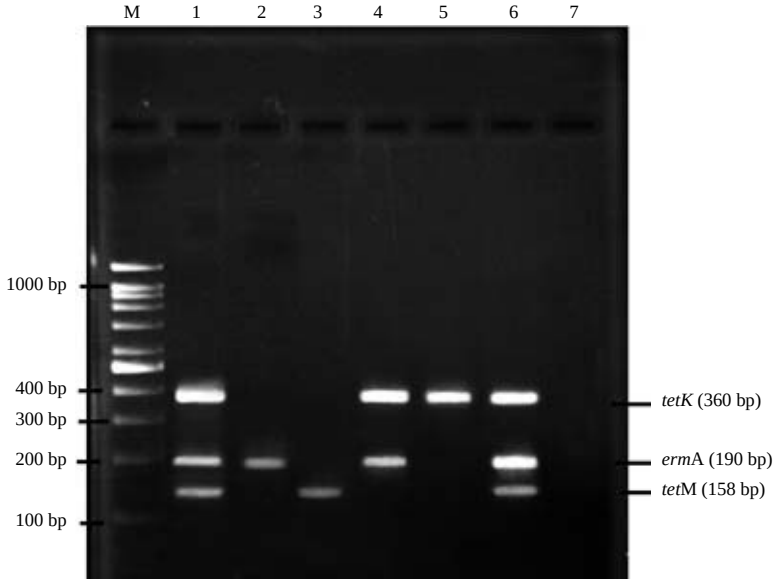

Fig. 4: Amplicon of *ermA*, *tetK* *tetM* genes by Multiplex-PCR, M: 100 bp DNA marker, Lane 1, 2, 4 and 6: *ermA* (190 bp), Lane 1, 4, 5 and 6: *tetK* (360 bp), Lane 1, 3 and 6: *tetM* (158 bp), lane 7: *S. aureus* ATCC25923 (negative control)

corridor, one isolates (33.3%); toilet floor, one isolates (33.3%); and stair floor, one isolate (33.3%). Of the six multi-drug resistant MRSA isolates from clinical specimens, the four that were resistant to five to six antibiotics were from sputum, 2 isolates (33.3%); pus, 1 isolate (16.7%) and wound, 1 isolate (16.7%), while the total four isolates of multi-drug resistant MRSA from environmental specimens, only one isolate (25.0%) that was resistant to five antibiotics was from toilet floor (Table 9). For 16 phenotypic multi-drug resistance *S. aureus* isolates determined by disk diffusion and broth microdilution assay, we compared with the results of PCR-based assay for the simultaneous detection of antibiotic resistance genes (Fig. 1-4). These isolates, we found a correlation between the results of the PCR patterns and those of classical resistance testing (Table 10).

Table 10: Correlation between multi-drug resistant pattern of *S. aureus* isolates and PCR results

		Presence of fragment						
S. aureus strain[a]	Multi-drug resistant phenotype[b]	*fem*A	*mec*A	*bla*Z	*erm*A	*aac*(6')/*aph*(2'')	*tet*K	*tet*M
Sw2	PEN/FOX/OXA	+	+	+	-	+	-	-
Sw9	TE/FOX/OXA	+	-	+	-	-	+	+
Sp9	PEN/CIP/DA	+	-	+	-	-	-	+
Sw11	PEN/SXT/DA	+	+	+	-	-	-	-
Sp10	PEN/ERY/TE/OXA	+	+	+	+	-	+	+
Sp7	PEN/ERY/SXT/DA	+	+	+	+	+	+	-
Sp4	PEN/GEN/CIP/DA	+	-	+	-	+	+	-
Sw13	PEN/FOX/DA/OXA	+	+	+	-	-	-	-
Sw20	PEN/TE/FOX/OXA	+	-	+	-	-	+	+
Sw10	PEN/ERY/TE/FOX/OXA	+	-	+	+	-	-	+
Sp2		+	+	+	+	-	+	-
Sp11	PEN/GEN/CIP/DA/OXA	+	-	+	-	+	-	-
Sp8	PEN/ERY/FOX/DA/OXA	+	+	+	+	-	-	-
Sw8	PEN/FOX/SXT/DA/OXA	+	+	+	-	-	-	-
Sp3	PEN/ERY/FOX/CIP/DA/OXA	+	+	+	+	+	-	-
Sp6	PEN/ERY/TE/FOX/SXT/OXA	+	+	+	+	-	+	+
ATCC25923	Susceptible	+	-	-	-	-	-	-

[a]Sw: Swab specimen from environmental samples, Sp: Specimen from clinical samples, [b]PEN: Penicillin, ERY: Erythromycin, TE: Tetracycline, FOX: Cefoxitin, GEN: Gentamicin, CIP: Ciprofloxacin, SXT: Trimethoprim-sulfamethoxazole, OXA: Oxacillin, DA: Clindamycin

Table 11: Correlation between phenotypic cefoxitin and oxacillin susceptibility tests and PCR results of *mec*A gene

	Number of strain for cefoxitin	Number of PCR results (%)		Number of strain for oxacillin	Number of PCR results (%)	
Samples (number)	susceptibility (%)	*mec*A-positive	*mec*A-negative	susceptibility (%)	*mec*A-positive	*mec*A-negative
Clinical sample (31)	R = 4 (12.9)	4 (12.9)	-	R = 6 (19.3)	5 (16.1)	1 (3.2)
	S = 27 (87.0)	3 (9.7)	24 (77.3)	S = 25 (80.6)	2 (6.5)	23 (74.1)
Environmental sample (23)	R = 10 (43.5)	4 (17.4)	6 (26.1)	R = 7 (30.4)	3 (13.0)	4 (17.4)
	S = 13 (56.5)	4 (17.4)	9 (39.1)	S = 16 (69.5)	5 (21.7)	11 (47.8)
Total (54)	R = 14 (25.9)	8 (14.8)	6 (11.1)	R = 13 (24.1)	8 (14.8)	5 (9.3)
	S = 40 (74.1)	7 (13.0)	33 (61.1)	S = 41 (76.0)	7 (13.0)	34 (63.0)

R: Resistant, S: Susceptible

Table 12: Correlation between phenotypic penicillin susceptibility test and PCR results of *bla*Z gene

		Number of PCR results (%)	
Samples (number)	Number of strain for penicillin susceptibility (%)	*bla*Z-positive	*bla*Z-negative
Clinical sample (31)	R = 30 (96.7)	30 (96.7)	-
	S = 1 (3.2)	-	1 (3.2)
Environmental sample (23)	R = 15 (65.2)	15 (65.2)	-
	S = 8 (34.8)	2 (8.7)	6 (26.1)
Total (54)	R = 45 (83.3)	45 (83.3)	-
	S = 9 (16.7)	2 (3.7)	7 (13.0)

R: Resistant, S: Susceptible

Correlation between phenotypic antibiotic resistance and PCR results. For 54 *S. aureus* isolates, we compared susceptibility results determined by disk diffusion and broth microdilution assay with the results of PCR-based assay for the simultaneous detection of antibiotic resistance genes. Of 13 oxacillin-resistant isolates, 8 isolates carried a *mec*A gene, while 14 cefoxitin-resistant isolates, 8 isolates carried a *mec*A gene. And of 41 oxacillin-sensitive isolates, 7 isolates carried a *mec*A gene, while 40 cefoxitin-sensitive isolates, 7 isolates carried a *mec*A gene (Table 11). All penicillin-resistant isolates were shown to have the *bla*Z gene, while 2 of 9 penicillin-sensitive isolates were shown to have this gene (Table 12). A total of 16 isolates were resistant to erythromycin, 14 of these isolates, the *erm*A gene was present, while 5 of 38 erythromycin-sensitive isolates were shown to have this gene (Table 13). All two gentamicin-resistant isolates were shown to have the *aac*(6')/*aph*(2'') gene, while 5 of 52 gentamicin-sensitive isolates were shown to have this gene (Table 14). A total of 7 isolates were resistant to tetracycline, 6 and 5 isolates carried *tetK* gene and *tetM* gene, respectively, while a total of 47 isolates were sensitive to tetracycline, 5 and 4 isolates were carried *tetK* gene and *tetM* gene, respectively (Table 15). Thus, the results of the PCR-based assay did not completely correlate with the results of the phenotypic antibiotic resistance determination.

Table 13: Correlation between phenotypic erythromycin susceptibility test and PCR results of *erm*A gene

Samples (number)	Number of strain for erythromycin susceptibility (%)	Number of PCR results (%)	
		*erm*A-positive	*erm*A-negative
Clinical sample (31)	R = 14 (45.1)	13 (41.9)	1 (3.2)
	S = 17 (54.8)	2 (6.4)	15 (48.4)
Environmental sample (23)	R = 2 (8.7)	1 (4.3)	1 (4.3)
	S = 21 (91.2)	3 (13.0)	18 (78.2)
Total (54)	R = 16 (29.6)	14 (25.9)	2 (3.7)
	S = 38 (70.4)	5 (9.3)	33 (61.1)

R: Resistant, S: Susceptible

Table 14: Correlation between phenotypic gentamicin susceptibility test and PCR results of *aac*(6')/*aph*(2'') gene

Samples (number)	Number of strain for gentamicin susceptibility (%)	Number of PCR results (%)	
		aac(6')/*aph*(2)-positive	*aac*(6')/*aph*(2)-negative
Clinical sample (31)	R = 2 (6.4)	2 (6.4)	-
	S = 29 (93.5)	3 (9.6)	26 (83.9)
Environmental sample (23)	R = 0 (0)	-	-
	S = 23 (100)	2 (8.7)	21 (91.3)
Total (54)	R = 2 (3.7)	2 (3.7)	-
	S = 52 (96.3)	5 (9.3)	47 (87.0)

R: Resistant, S: Susceptible

Table 15: Correlation between phenotypic tetracycline susceptibility test and PCR results of *tet*K and *tet*M genes

Samples (number)	Number of strain for tetracycline susceptibility (%)	Number of PCR results (%)				
		*tet*K-positive	*tet*K-negative	*tet*M-positive	*tet*M-negative	*tet*K and *tet*M-positive
Clinical sample (31)	R = 4 (12.9)	4 (12.9)	-	3 (9.7)	1 (3.2)	3 (9.7)
	S = 27 (87.1)	4 (12.9)	23 (74.2)	2 (6.4)	25 (80.7)	2 (6.4)
Environmental sample (23)	R = 3 (13.9)	2 (8.6)	1 (4.3)	2 (8.6)	1 (4.3)	2 (8.6)
	S = 20 (87)	1 (4.3)	19 (82.6)	2 (8.6)	18 (78.3)	1 (4.3)
Total (54)	R =7 (12.9)	6 (11.1)	1 (1.8)	5 (9.3)	2 (3.7)	5 (9.3)
	S = 47 (87)	5 (9.3)	42 (77.7)	4 (7.4)	43 (79.6)	4 (7.4)

R: Resistant, S: Susceptible

Table 16: Cefoxitin and oxacillin susceptibility test for nmrMSSA, mrMSSA, nmrMRSA and mrMRSA

Organism	Number of strains for oxacillin susceptibility	Number of strains for cefoxitin susceptibility (%)
nmrMSSA	R = 1	R = 1 (3.0)
		S = 0 (0.0)
	S = 32	R = 3 (9.1)
		S = 29 (87.9)
mrMSSA	R = 4	R = 3 (50.0)
		S = 1 (16.7)
	S = 2	R = 0 (0.0)
		S = 2 (33.3)
nmrMRSA	R = 0	R = 0 (0.0)
		S = 0 (0.0)
	S = 5	R = 1 (20.0)
		S = 4 (80.0)
mrMRSA	R = 8	R = 7 (70.0)
		S = 1 (10.0)
	S = 2	R = 0 (0.0)
		S = 2 (20.0)

R: Resistant, S: Susceptible

Correlation of oxacillin and cefoxitin susceptibility results and multi-drug and nonmulti-drug resistant *S. aureus* is presented in Table 16. Most of nonmulti-drug resistant MSSA (nmrMSSA) isolates were sensitive to both oxacillin and cefoxitin (87.7%), three isolates (9.1%) were sensitive to oxacillin but resistant to cefoxitin and only one isolates (3.0%) was resistant to both oxacillin and cefoxitin, while most of multi-drug resistant MSSA (mrMSSA) isolates were resistant to both oxacillin and cefoxitin (50.0%), two isolates were sensitive to both oxacillin and cefoxitin (33.3%) and only one isolate (16.7%) was resistant to oxacillin but sensitive to cefoxitin. These results were similar to those MRSA isolates which most nonmulti-drug resistant MRSA (nmrMRSA) isolates were sensitive to both oxacillin and cefoxitin (80.0%), only one isolate was sensitive to oxacillin but resistant to cefoxitin (20.0%), while most of multi-drug resistant MRSA (mrMRSA) isolates were resistant to both oxacillin and cefoxitin (70.0%), two isolates were sensitive to both oxacillin and cefoxitin (20.0%) and only one isolate (10.0%) was resistant to oxacillin but sensitive to cefoxitin.

DISCUSSION

Staphylococcus aureus is major causes of community-acquired and nosocomial infection, it has spread throughout the world and has become highly endemic in many geographical areas[15]. This study was carried out to determine the prevalence and antibiotic resistance of *S. aureus* isolated from clinical and hospital environmental samples. In this study the results of bacterial culture for a total 54 isolates on BA and MSA revealed *S. aureus* with all isolates appeared as Gram-positive. The frequency of *fem*A in *S. aureus* was 100%, but neither of this gene was found in other bacteria. Thus, there was 100% agreement between the conventional identification results and the amplification of the 450 bp fragment of the species-specific gene, *fem*A. The gene product of *fem*A has been suggested to have a role in cell wall metabolism and is reported to be present in all *S. aureus* species during the active growth phase[16]. There was perfect correlation between the conventional phenotypic tests and molecular technique results for identification of *S. aureus*[17]. The prevalence of *S. aureus* isolation from clinical and hospital environmental samples was 86.1 and 69.7%, respectively. The prevalence of *S. aureus* isolation from hospital environment in this study is higher than those of Saba *et al.*[18] for the public hospitals in Ghana where the *S. aureus* prevalence was 39%, Newman[19] for a hospital in Accra, Ghana was 44% and Hammuel *et al.*[20] for a hospital in Zaria, Nigeria was 50.8%. The highest frequency of MRSA isolation from clinical specimens was noted from sputum and wound samples (28.6%). This study's findings are in agreement with a study done in Windhoek, Namibia which found from sputum (41.3%) and pus swab (35.0%)[21]. Contamination of corridors, door handles and patient beds with MRSA occurred more than the other of the surface samples. Although routine cleaning procedures are undertaken in these areas, they are not completely effective and improved methods of disinfecting these environments are recommended[22]. During the course of this study, it was observed that more attention was paid to cleaning floors rather than corridors, door handles or knobs and patient beds, which may have allowed a build-up of *S. aureus* on these surfaces. Subsequently, the high contamination rate of door handles and surfaces of patient bed by *S. aureus* in hospitals is likely to be a contributing factor for these bacteria being implicated in blood stream infections[23,24]. Preventive measures such as improved personal hygiene and the regular cleaning and disinfection of hospital corridors, door handles, patient beds, stair railings, floors and other points of contact are highly recommended, which recorded the highest *S. aureus* contamination rate. The MRSA prevalence of this study are similar to those of Carvalho *et al.*[25] for a Brazilian university hospital which was 33.3% (versus 34.8% in our study). In a similar study by Oie *et al.*[26] for 27% (versus 17.4% in this study) of the door handles in a University hospital in Japan were contaminated with *S. aureus* of which 20.9% (versus 65.2% in this study) were MSSA and 8.7% (34.8% in this study) were MRSA. In this study, oxacillin susceptibility by broth dilution method and cefoxitin disk diffusion test could not detect all MRSA isolates. The oxacillin and cefoxitin susceptible *mecA*-positive *S. aureus* are on the rise. Because of their susceptibility to oxacillin and cefoxitin, it is very difficult to detect them by using phenotypic methods. This finding is in agreement with Chambers[27], who found that among the MRSA isolates, only few express homogeneous oxacillin resistance while the majority show heterogeneous drug resistance. Phenotypically oxacillin and cefoxitin susceptible and *mecA*-positive *S. aureus* clinical isolates are being increasingly reported[28-35]. Dependence on growth conditions like temperature and osmolarity of the medium for phenotypic expression of resistance further complicates susceptibility testing of MRSA by standard microbiological methods[27]. On the basis of the Clinical and Laboratory Standards Institute (CLSI) guidelines, a method based on agar containing 6 μg mL^{-1} of oxacillin was developed to screen *S. aureus* isolates[36]. Though it can detect true MRSA effectively, it is likely to miss *mecA*-positive *S. aureus* having an oxacillin MIC of <2 μg mL^{-1}. Such isolates have been considered to be extremely hetero resistant (<1 in 10^8 of the population is highly resistant to methicillin), but there are also reports documenting the existence of nonheterogeneous phenotypically oxacillin-susceptible *mecA*-positive *S. aureus*[31]. The use of β-lactams to treat such isolates may cause an increase in the MIC of oxacillin well above the established breakpoint for resistance (oxacillin MIC, ≥4 μg mL^{-1}), ultimately leading to failure of therapy[37]. Using PCR for *mecA* gene detection for MRSA as reference method, the sensitivity for disk diffusion test using cefoxitin and oxacillin MIC test was lower than penicillin disk diffusion test. These results are difference to those previous reports by Dibah *et al.*[38] and Farahani *et al.*[39] that cefoxitin disk diffusion and oxacillin MIC tests showed the sensitivity equal to PCR for MRSA detection. However, the emergence of *mecA*-positive oxacillin susceptible and *mecA*-negative oxacillin resistant *S. aureus* strains reduces the sensitivities of both the phenotypic and genotypic methods [30,40,41]. Thus, combination of genotypic and phenotypic tests is necessary to detect the methicillin resistance in *S. aureus* accurately. In this study, the incidence rate of MRSA detection in clinical and environmental specimens was similar, with an estimated prevalence 22.6 and

34.8%, respectively. Previously, it has been documented that MRSA accounted for 25.5% of total isolates of CA *S. aureus* infections and 67.4% of HA infections in Asian countries, whereas, 2.5% of CA *S. aureus* and 57.0% of HA infections in Thailand[42]. However, recent reports indicate declining clinical acquired MRSA infection with applying appropriate infection control measures, rapid and reliable detection of methicillin resistance and effective antibiotic therapy[43,44]. In this study, all isolates were susceptible to vancomycin, chloramphenicol, linezolid and rifampicin. The absence of resistance to these antibiotics may be related to the low usage of these antibiotics in the study setting. The vancomycin is the drug of choice for the treatment of infections due to MRSA[45]. Several studies reported emergence of vancomycin resistant clinical MRSA isolates around the world[46-48]. In our study all of the isolates displayed MICs of $\leq$2 μg mL^{-1} to vancomycin and were susceptible to vancomycin. This is in agreement with previous studies[22,49]. And all isolates of *S. aureus* were sensitive to rifampicin and the rifampicin was an important antibiotic in treatment *S. aureus* infection and tuberculosis[50,51]. We speculate that the effectiveness observed with the drug might be due to its high cost in our environment making, it less readily available and hence less misused. Most of the reports suggested vancomycin as a credible drug for treating *S. aureus* infection[52,53]. However, regular monitoring of the drug's sensitivity is of importance because resistance has been reported in the USA, Japan and Korea[49,54]. The other reports indicated that most clinical samples of *S. aureus* were resistant to vancomycin[55,56], while the 28.57% of MRSA from clinical samples were rifampicin-resistant[57]. Multi-drug resistant characteristics of MRSA and emergence of glycopeptide resistant strains have been frequently caused treatment failure of MRSA infections[58]. These findings have promoted researchers to seek new antibiotics for the treatment of MRSA infection[59]. Linezolid showed good activity *in vitro* and *in vivo* and are promising therapeutic options against staphylococcal infections[60]. In this study all isolates were susceptible to linezolid. Similar to those previous study on *S. aureus* strains isolated from clinical specimens in Aralabil, Iran by Dibah *et al.*[38], that all of MRSA and MSSA isolates were found to be susceptible to linezolid. This antibiotic is not generally used in Thailand for treatment of staphylococcal infections. Therefore, emergence of linezolid susceptible *S. aureus* strains in this finding could be reasoning. There is also a similar finding that has been reported from Iran[38]. In this study, 0% of the tested isolates were resistant to chloramphenicol which is comparable to China and Iran where 0.8 and 0% of the isolates were resistant, respectively[61,62]. The studies carried out in European countries have revealed that in Greece 100% of the MRSA isolates from community acquired infections were susceptible to chloramphenicol[63] while in UK almost 92.3% of such isolates recovered from patients of otitis externa were sensitive to this antimicrobial[64]. The results from studies carried out in Japan and Korea have also revealed similar pattern as 91.6 and 100% of MRSA isolates were susceptible to this compound, respectively[65,66]. The published literature from USA has revealed that although *in vitro* efficacy of chloramphenicol against MRSA is still very encouraging but there is declining trend noted from different regions of the country[67,68]. Only one study was found from African country of Uganda where 88.2% of the isolates were susceptible[69]. The most significant finding of our results was 100% of the isolates susceptible to chloramphenicol. This result could be attributed to the fact that chloramphenicol is not being routinely used in clinical practice to treat majority of infections caused by Gram positive and Gram negative bacteria. Bone marrow toxicity is the major complication of chloramphenicol. This side effect may occur as either due to dose related bone marrow suppression or idiosyncratic aplastic anaemia. Keeping in view the low cost and oral preparation of chloramphenicol coupled with very high rate of *in vitro* susceptibility makes this antimicrobial an ideal choice for wide variety of infections caused by MRSA. Further studies focusing more on the clinical outcome of patients of MRSA treated with chloramphenicol would definitely give icing on cake for *in vitro* results achieved for this compound. It is also imperative that since this compound has shown very promising results against MRSA isolates, the availability of this antibiotic must be ensured in the market for the benefit of patients.

The high susceptibility rate for most commonly used antibiotics was observed among MSSA isolates in comparison to MRSA. Most MSSA isolates were susceptible to nearly all antimicrobial agents used in this study. In contrast, in the case of MRSA, multiple-drug resistance was common and only few antibiotics were active against these isolates. Except for above mentioned antibiotics, ciprofloxacin showed the lowest resistance rate for MRSA isolates, tetracycline and oxacillin showed the lowest resistance rate for MSSA isolates. However, MRSA strains from clinical specimens should be considered as resistant to all β-lactam agents other than the cephalosporins with anti-MRSA activity as stated by CLSI[10]. Resistance of MRSA to ciprofloxacin in general is low. According to a study published by Zabielinski *et al.*[70], MRSA became more sensitive to ciprofloxacin, while MSSA demonstrated increased antibiotic resistance to ciprofloxacin.

The pattern of resistance observed might be due to the fact that antibiotics are used in auto-therapy in this locality, which may result in a multitude of antibiotics used at sub-therapeutic levels heralding the emergence of resistant strains. Antimicrobial resistance patterns revealed a total of twenty-one phenotype patterns of which the most prevalent was penicillin which accounted for 19 (35.2%) of the isolates. The second resistance pattern was noted for the resistance indices designated pattern 3 (which penicillin and erythromycin) exhibited by 9 (16.6%) of the isolates. Approximately 16(29.6%) of the isolates were resistant to three or more antibiotics (multi-drug resistant). The majority of these multi-drug resistant isolates originated from clinical samples which was 9 (56.25%) and most of them were MRSA, while 7 (43.75%) of the isolates were of environmental samples. The multi-drug resistant strain may occur when the uncontrolled disposing of antibiotics and chemicals into the hospital, which turns to create selective pressure on the drugs. Pellegrino *et al.*[71] reported that the uncontrolled use of antibiotics in hospital and the community created a reservoir of bacteria that could become resistant. Therefore, we are constrained to hypothesize that this situation may be a reason for the multi-drug resistance MRSA was observed in this study.

Resistance determinants may be included the clinical and technical considerations. Since the PCR amplification of the gene fragments is limited, we chose genes most frequently associated with resistance of *S. aureus* to clinically relevant anitbiotics[72-75].

In this study, all of the isolates were identified as *S. aureus* based on morphological and biochemical characteristics and all *S. aureus* isolates were positive by PCR for the species specific *femA* gene. Methicillin resistance in *S. aureus* has been reported to be associated with the presence of penicillin-binding proteins, encoded by the *mecA* gene. The heterogeneous expression of *mecA* gene may not be the completed and, therefore, these strains may not be detectable with phenotypical methods[76-79]. Although, methicillin resistance was observed in 27.8% of total isolates when tested by oxacillin and vancomycin MIC methods which was equal to 27.8% of total isolates had *mecA* gene by PCR assay. Phenotypically methicillin susceptible 7 isolates also carried the *mecA* gene. Comparison of conventional method and PCR assay did not show a good agreement. Additionally, the absence of *mecA* gene within resistant staphylococcal isolates was presented in the various studies[80-82]. Furthermore, moderate methicillin resistance was observed in isolates that lacked the *mecA* gene mutation[83,84]. A previous report in Nigeria found that the complete absence of five major SCCmec types, *mecA* gene and the gene product of PBP2a in the isolates of MRSA, suggested that a probability of hyper production of β-lactamase as a cause of the phenomenon[85]. Moreover, Ba *et al.*[86] pointed out specific mutations of amino acids in penicillin binding proteins (PBP1, 2 and 3) which may play a role in antibiotic resistance. These mutations were found to include three amino acid substitutions which were identical and were present in PBPs 1, 2 and 3[86]. In additionally, the same amino acid was found to have two other different substitutions in PBP1. Both the identical and different amino acid substitutions were observed in isolates from different multilocus types. These findings distributed that there are other mechanisms responsible for β-lactam resistance of MRSA than the presence of *mecA* gene that molecular methods alone are not enough for confirmed characterization of MRSA isolates.

The *blaZ* gene encoding β-lactamase that inactivates the β-lactam antibiotics by hydrolysis of the β-lactam ring[87]. In this study, all the penicillin-resistant *S. aureus* isolates exhibited genotypic resistance to penicillin, but in various studies, no resistance genes could be detected in some resistant isolates[88-90].

In some isolates, phenotypic resistance may be caused by point mutations rather than gene acquisition. Additionally, except for the general resistance mechanisms[91],other pathways such as biofilm formation may play a major role in the resistance mechanisms[92]. These results suggest that the *blaZ* gene carried by penicillin-resistant *S. aureus* may play a major role in the penicillin-resistant phenotype, but the resistance gene cannot be used alone as a diagnostic indicator for penicillin resistance. Mechanisms of resistance to antibacterials are so complex that the presence or absence of a certain resistance gene does no certainly indicate that the particular isolate is resistant or sensitive to the corresponding antimicrobial agent[93].

The *aac(6')/aph(2'')* gene encodes the AAC(6')-APH(2'') enzyme, a bifunctional enzyme with kinase activity, that inactivates a broad range of aminoglycosides and confers concomitant resistance to gentamicin and the majority of aminoglycosides commonly used in medical practice[94,95]. As also reported in other studies, the *aac(6')/aph(2'')* gene was the most common amino glycoside modifying enzyme (AME) genes among the *S. aureus* isolates and was found either alone or with other AME for example; *aph(2'')-Ib*, *aph(2'')-Ic*, *aph(2'')-Id*, *aph(3')-IIIa*, *ant(4')-Ia* and *ant(6)-Ia* genes[95,96]. In this study, drug-susceptibility testing showed rates of gentamicin-resistant *S. aureus* strains of 3.7% (all staphylococcal strains resistant to gentamicin in the present study carried the *aac(6')/aph(2'')* gene), surprisingly, this gene was detected in five gentamicin susceptible isolates. In some studies have also reported similar findings[78,97,98]. The detection of resistance genes in antibiotic susceptible isolates

may be due to the amplification of repressed antibiotic resistance gene[97] or AME of these strains display lower enzymatic activity, detected also in other study of *S. aureus*[78]. Vanhoof *et al.*[78] also showed that the *aac(6')/aph(2'')* gene is the most prevalent gene encoding AME enzymes among clinical MRSA isolates in European countries and Choi *et al.*[77] obtained similar results in South Korea. They present study's results were similar to the predominant gene. In this case, 100% of gentamicin-resistant *S. aureus* isolates carried the *aac(6')/aph(2'')* gene.

Erythromycin resistance in staphylococci is predominantly mediated by erythromycin resistance methylase encoded by *erm* genes[99]. In human infections caused by staphylococci *ermA* and *ermC* are the most common methylase genes[100]. In the present study, the incidence of *ermA* was 35.2% for *S. aureus*. A similar incidence for *ermA* has been reported for *S. aureus* isolated from various sites and clinical specimens[56] and 87.5% of erythromycin-resistant *S. aureus* from the present study carried *ermA*. Similar results have also observed high incidences (82-94%) for *ermA* in erythromycin-resistant *S. aureus* isolated from blood[100,101]. However, two of erythromycin-resistant *S. aureus* strains were absent *ermA* gene which have the other *erm* or *msrA* genes[99,102,103]. Lim *et al.*[104] reported that the *ermA* gene was more prevalent than the other erythromycin resistance genes in *S. aureus* isolates and *ermC* gene was found mostly in coagulase-negative staphylococci (CoNS)[104]. Similarly, in a study performed by Martineau *et al.*[32] the ermC gene has been reported to be more prevalent in CoNS.

Tetracycline resistance is the common resistance phenotype in MRSA strains isolated worldwide. Two main mechanisms of resistance to tetracycline have been described in *S. aureus*; active efflux, resulting from the acquisition of the plasmid-located *tet*K and *tet*L genes and ribosomal protection by elongation factor-like proteins that are encoded by chromosomal or transposonal *tet*M or *tet*O determinants[105,106]. In the present study, the phenotypic resistance to tetracycline in *S. aureus* was observed as 12.9%. Whereas 9.3% *S. aureus* isolates carried the *tet*K and *tet*M genes. Tetracycline resistance genes *tet*K and *tet*M were found positive by PCR method in four isolates which were phenotypically sensitive to tetracycline. A majority of tetracycline-resistant strains harboured *tetK* gene followed by *tetM* gene. This is similar to those reports by Trzcinski *et al.*[107], Jones *et al.*[108] and El-Mahdy *et al.*[109] where *tet*K gene was the predominant gene in tetracycline-resistant strains. In addition, this study showed that a strain which harboured *tet*K gene also harboured *tet*M gene. This is in agreement with the report of Schmitz *et al.*[74] as their MRSA strains also harbour both *tet*K and *tet*M genes.

CONCLUSION

This is the first study conducted on *S. aureus* on clinical and hospital environment samples in Thailand. There were high levels of contamination of *S. aureus* and MRSA on corridor, door handle and patient bed. Isolates of *S. aureus* and MRSA had high rates of resistance to the antibiotics used in this study. There is a need for periodic surveillance and monitoring of *S. aureus* and MRSA in clinical and hospital environment samples as well as regular and effective cleaning of contact surfaces in hospital. Vancomycin, chloramphenicol, linezolid and rifampicin have shown very good *in vitro* susceptibility against MRSA and MSSA and are likely to have a key role in the treatment of infections caused by *S. aureus*. These antimicrobials can serve as an alternative to new expensive antimicrobials in resource poor countries. There is need to further evaluate these antimicrobials for determining the *in vitro* as well as *in vivo* efficacy before broad usage of this compound can be undertaken. And this study has shown that some of *S. aureus* isolates recovered from clinical and environmental specimens contain a variety of β-lactam, erythromycin, tetracycline and aminoglycoside resistance genes were common *blaZ*, *ermA*, *tet* and AME genes among the *S. aureus* isolates, respectively.

SIGNIFICANCE STATEMENT

Staphylococcus aureus is one of the important bacteria that cause infections acquired in the community and hospital, its ability to develop resistance to many antibiotics. The high prevalence of MRSA was found in hospital environment. It is suggested that improved personal hygiene, effective cleaning of contact surfaces and disinfection in hospital environment are highly recommended. Since, The phenotypic antibiotic susceptibility patterns of *S. aureus* isolates were not similar to those obtained by genotyping done by PCR. Rapid reliable methods for antibiotic susceptibility are important to determine the appropriate prophylaxis and therapy. The PCR can be used for confirmation of the results obtained by conventional phenotypic methods.

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