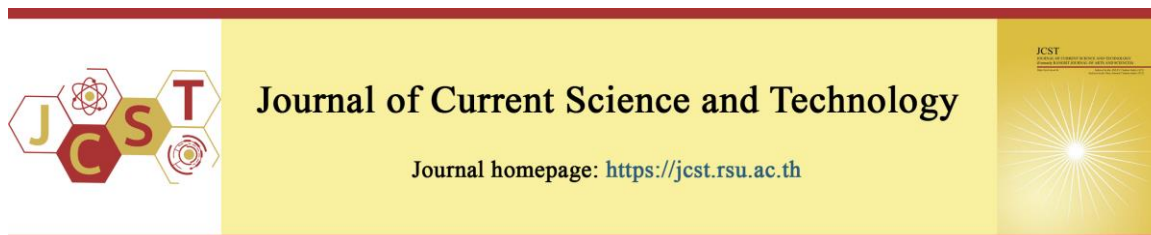


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Multidrug Resistant Staphylococci from Environments of Sub-District Health Promoting-Hospitals in Chiang Rai, Thailand

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Abstract

The presence of multidrug resistant (MDR) bacteria in hospital environments poses a major challenge for infection control, given their role as environmental reservoirs. *Staphylococcus* spp. were the predominant bacteria, accounting for 36.95% of all isolates (126/341), of which 16.67% (21/126) exhibited MDR phenotypes. Accordingly, the present study comprehensively evaluated these 21 MDR isolates to determine their extended antibiotic susceptibility profiles and associated resistance genes. Antibiotic susceptibility was assessed using the disk diffusion method against 12 antibiotics classified into Groups A, B, and C. Polymerase chain reaction was used to detect eight resistance genes: *blaZ*, *mecA1*, *mecA2*, *mecC*, *ermA*, *ermB*, *ermC*, and *aacA-aphD*. In group A, all isolates showed resistance to cefoxitin, and high resistance to trimethoprim-sulfamethoxazole, clindamycin (both at 76.19%), and erythromycin (71.43%). The majority (95.24%) of bacteria were methicillin-resistant coagulase-negative staphylococci (MRCoNS), primarily methicillin-resistant *Staphylococcus epidermidis* (75%) and a small proportion of methicillin-resistant *Staphylococcus aureus* (4.76%). Most isolates remained sensitive to group B and C antibiotics, such as linezolid, doxycycline, and vancomycin, with limited resistance to rifampin (9.52%), gentamicin (33.33%), ciprofloxacin and moxifloxacin (9.52% each), and chloramphenicol (4.76%). MDR staphylococci isolates showed a high prevalence of diverse resistance genes. Notably, the *blaZ* and *mecA1* genes were common (each found in 90.48% of isolates), whereas *ermA* and *mecC* genes were not detected. The *ermC* gene was present in all isolates while *ermB* was rare (4.76%). The *mecA2* and *aacA-aphD* genes were detected only in MRCoNS, each occurring in 61.90% of isolates. These findings reveal distinctive local resistance profiles in resource-limited sub-district health promoting-hospitals and underscore the need for continuous surveillance, targeted antimicrobial stewardship, and rigorous environmental cleaning protocols to control MDR staphylococci in these healthcare settings.

Keywords: antibiotic susceptibility, hospital environments, multidrug resistance, resistance genes, staphylococci, sub-district health promoting-hospitals

1. Introduction

Contamination of hospital environments with multidrug-resistant (MDR) bacteria is a major public health concern because it increases the risk of cross-transmission among patients, visitors, and healthcare workers and contributes to healthcare-associated infections (HAIs) (Chaoui et al., 2019; Salmanov et

al., 2023). Contaminated hospital environments play an important role in HAI transmission because MDR organisms can survive on surfaces and may be transferred to patients, particularly when healthcare personnel do not follow appropriate hand hygiene practices (Facciola et al., 2019; Schinas et al., 2023). In particular, *Staphylococcus aureus* and certain

strains of coagulase-negative staphylococci (CoNS) survive in hospital settings by forming biofilms, enabling them to persist on surfaces and medical devices for long periods (Ledwoch et al., 2018; Socohou et al., 2020; Pietrocola et al., 2022; Rybak et al., 2025) and develop resistance to multiple antibiotic classes (Senobar Tahaei et al., 2021; Alfeky et al., 2022; Rimi et al., 2024). Furthermore, the horizontal transfer of antibiotic resistance genes among MDR staphylococci increases the likelihood that resistance determinants will spread to other bacterial species within hospital environments (Águila-Arcos et al., 2017; Côrtes et al., 2022; Rybak et al., 2025). This process facilitates the emergence and transmission of resistant bacteria and complicates the management and treatment of HAIs. Consequently, staphylococci in hospital environments may serve as reservoirs of antimicrobial resistance, posing a threat to patient safety and treatment effectiveness. Nevertheless, information on the prevalence and antibiotic resistance patterns of staphylococci in hospital environments in Thailand remains limited. Most studies conducted in Thailand have focused on bacterial isolates obtained from clinical specimens (Waitayangkoon et al., 2020; Tribuddharat et al., 2022; Boonsilp et al., 2023; Prommachote et al., 2025; Wassanarungroj et al., 2025), whereas the role of hospital environments as reservoirs of resistant pathogens has received considerably less attention. This knowledge gap may hinder effective infection-control measures and allow transmission pathways within healthcare facilities to remain undetected. The problem may be particularly important in rural hospitals, where limited resources create additional challenges for infection control and antimicrobial stewardship. In Thailand, sub-district health promoting-hospitals (SHPHs) are located throughout the country and provide proactive and primary healthcare services to local communities (Bouphan et al., 2015). Although these facilities are not primarily intended to provide inpatient care, they rely on healthcare personnel who rotate from district hospitals, and their pharmaceutical supplies are limited to medicines included in the National List of Essential Medicines (Kitreerawutiwong et al., 2017). Comprehensive information on the prevalence and antibiotic resistance profiles of staphylococcal isolates in SHPHs in Chiang Rai Province remains scarce. Previous studies found that *Staphylococcus* spp. were the most prevalent bacterial contaminants of inanimate surfaces in 12 SHPHs across six districts of Chiang Rai, accounting for 126 of 341 isolates (36.95%) (Chansareewittaya & Krajangcharoensakul,

2021). Of these isolates, 21 (16.67%) exhibited MDR phenotypes against antibiotics categorized as Group A for primary testing and reporting (Chansareewittaya & Krajangcharoensakul, 2022). These findings indicate a substantial presence of MDR staphylococci in SHPH environments and underscore the need for further investigation. Therefore, the present study comprehensively evaluated the phenotypic and genotypic resistance profiles of these 21 MDR staphylococcal isolates. Phenotypic susceptibility was assessed against commonly prescribed antibiotics classified as Group A for primary testing and reporting, Group B for optional primary testing and selective reporting, and Group C for supplemental selective reporting. Key antibiotic resistance genes were also investigated.

2. Objectives

This study aimed to extend the phenotypic characterization of the 21 MDR staphylococcal isolates identified in previous research and to investigate the presence of key antibiotic resistance genes. As the first report describing local resistance patterns among MDR staphylococci isolated from SHPH environments in Chiang Rai Province, this study provides important evidence supporting adherence to appropriate disinfection protocols. Such measures may reduce environmental reservoirs and help prevent the spread of resistant bacteria within healthcare facilities and surrounding communities.

3. Materials and Methods

Ethical Approval

The study protocol and procedures were exempted from formal ethical review by the Ethics Committee of the Chiang Rai Provincial Health Office, Ministry of Public Health, Thailand (CRPPHO No. 03/2566). The study did not involve experiments with human participants or animals. Formal authorization was obtained from the relevant authorities before sample collection.

Sample Location

Bacterial samples were collected from 12 SHPHs located in six districts of Chiang Rai Province, with two facilities selected from each district. The facilities participated voluntarily, and sampling was conducted without prior notice from January to March 2018 using an accidental sampling approach. At each facility, samples were collected from 10 designated points: six in nursing rooms and four in restroom areas. The nursing-room sampling points comprised

beds and bed rails, handwashing sinks, medical equipment, medical trays and portable treatment tables, staff tables and chairs, and work on computers and keyboards. The restroom sampling points comprise toilet flush handles, toilet seats or bowls, handwashing sinks and taps, and restroom door knobs or locks.

Sample Collection and Characterization

A total of 120 swab samples were collected as previously described (Thai National Institute of Health, 2014). Briefly, sterile swabs pre-moistened with sterile 0.9% normal saline solution were systematically rotated over specified collection areas measuring 2.54 × 2.54 cm. Sampling was performed with parallel spacing in perpendicular directions and repeated thrice to ensure comprehensive coverage. The collected swab specimens were immersed in tryptic soy broth to facilitate bacterial propagation and incubated under controlled conditions for 24 h at 37 ± 1°C. After incubation, the broth cultures were streaked onto mannitol salt agar (Professional Nanomed Co. Ltd., Bangkok, Thailand) for the presumptive isolation of staphylococcal colonies. Bacterial isolates were subsequently characterized via morphological assessment using Gram staining. To confirm the staphylococcal identity, biochemical profiling was conducted using the catalase test, and the coagulase test (Becton, Dickinson and Company, MD, USA) was employed to differentiate between coagulase-positive staphylococci (*S. aureus*) and CoNS. The susceptibility to novobiocin (OXOID Ltd., Cheshire, UK) confirmed CoNS as either *S. epidermidis* or *S. saprophyticus* (Thai National Institute of Health, 2014). Sterilization quality-control procedures complied with the standards of the Medical Science Laboratory, Scientific and Technological Instrument Center, Mae Fah Luang University. Preserved bacterial isolates were stored at -20°C in nutrient broth supplemented with 20% (v/v) glycerol. During storage, the isolates were evaluated twice annually to confirm their viability and retention of their biochemical characteristics.

Antibiotic Susceptibility Testing

Phenotypic antibiotic susceptibility testing was performed using the disk diffusion method described by Bauer et al. (1966) and interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI) applicable at the time of laboratory analysis (CLSI, 2020). Antibiotics were categorized into three groups: group A (primary testing and reporting), group B (optional primary testing and selective reporting), and

group C (supplemental selective reporting). Group A consisted of cefoxitin (CX; 30 µg), erythromycin (E; 15 µg), clindamycin (DA; 2 µg), and trimethoprim-sulfamethoxazole (COT; 25 µg), provided by OXOID Ltd. (Cheshire, UK). Group B comprised linezolid (LZ; 30 µg), doxycycline (DO; 30 µg), rifampin (RIF; 5 µg), and vancomycin (VA; minimal inhibitory concentration (MIC) was determined using the E-test method). Group C comprised ciprofloxacin (CIP; 5 µg), moxifloxacin (MO; 5 µg), chloramphenicol (C; 30 µg), and gentamicin (GEN; 10 µg). A direct suspension of bacterial colonies was prepared by diluting them in sterile 0.9% normal saline solution, adjusting the turbidity to match a 0.5 McFarland standard ($1-2 \times 10^8$ colony-forming units/mL inoculum). Each inoculum was evenly spread across Mueller-Hinton agar plates and allowed to dry. Antibiotic-impregnated filter paper disks were placed on the agar surface and incubated for 16–18 h (except 24 h incubation for cefoxitin) at 35°C. The susceptibility profile of each bacterial isolate was determined by measuring the inhibition zone diameters on Mueller-Hinton agar, which were then compared to CLSI standards. The results were classified as susceptible, intermediate, or resistant according to the zone diameter interpretive standards recommended by the CLSI (CLSI, 2020). MDR was defined as resistance to three or more distinct classes of antibiotics. The cefoxitin disk diffusion method using a 30-µg disk was employed as a surrogate test for detecting methicillin resistance among the staphylococcal isolates. According to the CLSI guidelines (CLSI, 2020), inhibition-zone diameters of ≤ 21 mm for *S. aureus* and ≤ 24 mm for CoNS were interpreted as indicating cefoxitin resistance.

Detection of Antibiotic Resistance Genes

Staphylococcus isolates were further analyzed to identify antibiotic resistance genes (ARG). Polymerase chain reaction (PCR) was employed to amplify β-lactamase genes (*blaZ*), methicillin resistance genes (*mecA* which consisted of 2 different primer sets; namely *mecA1* and *mecA2*; and *mecC*), erythromycin ribosome methylase genes (*ermA*, *ermB*, *ermC*), and the bifunctional aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase gene (*aacA-aphD*). Specific primers cited in previous studies (Akter et al., 2020; Gan et al., 2021), as detailed in Table 1, were synthesized by GibThai Co., Ltd., Thailand and Macrogen, Inc., Korea. Bacterial DNA was extracted using the Bacterial Genomic DNA Isolation Kit (BIO-HELIX, Taiwan), following

the manufacturer's protocol. PCR amplification reactions were prepared in a total volume of 24 μ L, including 1 μ L of a combined (forward and reverse) primer mix (10 μ mol/L of each primer), 12.5 μ L of OnePCR Ultra (Bio-Helix Co., Ltd.), 1 μ L of the DNA template, and 9.5 μ L of sterile deionized water. Amplification conditions were as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 30 s; annealing for 45 s at 55.5°C (*blaZ*, *mecA1*, and *mecA2*), 49.0°C (*ermA*), 56.0°C (*ermB*), 54.5°C (*ermC*), or 56.5°C (*aacA-aphD*); extension at 72°C for 45 s, and a final extension at 72°C for 15 min. For *mecC*, amplification started with a 15-min initial denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 60 s, extension at 72°C for

60 s, and a final extension at 72°C for 10 min. Negative controls containing sterile PCR-grade water instead of DNA template were included to detect potential reagent contamination. Purified DNA extracted from phenotypically identified MDR staphylococcal isolates expected to contain the target gene was included as a positive control to confirm primer functionality. The PCR products were visualized by electrophoresis on 2% agarose gels stained with 0.5 μ g/mL ethidium bromide in 0.5 $\times$ TBE buffer. The amplified products were commercially sequenced by Macrogen, Inc., Korea. The resulting DNA sequences were analyzed using the Basic Local Alignment Search Tool available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>).

Table 1 Specific primers for PCR amplification

Region	Primer name	Sequence (5'-3')	Size (bp)
<i>blaZ</i> (Gan et al., 2021)	<i>blaZ</i> -F	AACACCTGCTGCTTTCGGTA	314
	<i>blaZ</i> -R	CACTCTTGCGGTTTCACTT	
<i>mecA1</i> (Gan et al., 2021)	<i>mecA1</i> -F	CTTTGCTAGAGTAGCACTCG	533
	<i>mecA1</i> -R	GCTAGCCATTCCCTTTATCTTG	
<i>mecA2</i> (Akter et al., 2020)	<i>mecA2</i> -F	AAAATCGATGGTAAAGGTTGG	533
	<i>mecA2</i> -R	AGTTCTGGCACTACCGGATTTTGC	
<i>mecC</i> (Akter et al., 2020)	<i>mecC</i> -F	GAAAAAAGGCTTAGAACGCCTC	138
	<i>mecC</i> -R	GAAGATCTTTTCCGTTTTCAGC	
<i>ermA</i> (Gan et al., 2021)	<i>ermA</i> -F	CTACACTTGGCTTAGGATGA	311
	<i>ermA</i> -R	AGTGACTAAAGAAGCGGTAA	
<i>ermB</i> (Gan et al., 2021)	<i>ermB</i> -F	TAACGACGAAACTGGCTAA	414
	<i>ermB</i> -R	CTGTGGTATGGCGGGTAA	
<i>ermC</i> (Gan et al., 2021)	<i>ermC</i> -F	GAGGCTCATAGACGAAGAAA	375
	<i>ermC</i> -R	AAGTTCCCAAATTCGAGTAA	
<i>aacA-aphD</i> (Gan et al., 2021)	<i>aacA-aphD</i> -F	ATTGAAGATTTGCCAGAACA	178
	<i>aacA-aphD</i> -R	CACTATCATAACCACTACCG	

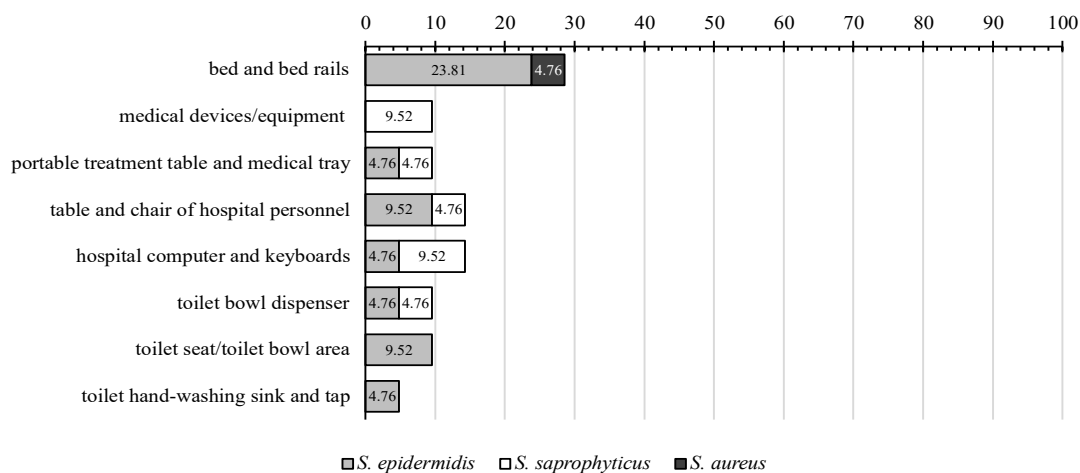


Figure 1 Distribution (%) of MDR staphylococcal isolates in different hospital environments

4. Results

From 120 collected swab samples, a total of 341 isolated bacteria were obtained (Chansareewittaya & Krajangcharoensakul, 2021). There were 126 *Staphylococcus* spp. isolates, which were the most common type (36.95%). This included 121 CoNS isolates (20 with MDR phenotypes) and 5 *S. aureus* isolates (1 with an MDR phenotype) (Chansareewittaya & Krajangcharoensakul, 2022). As a result, this study focused specifically on these 21 MDR staphylococcal isolates. In total, 95.24% (20/21 isolates) of them were CoNS. The isolates were distributed across eight of the ten tested sites, with the highest prevalence observed in beds and bed rails (28.57%; 23.81% for *S. epidermidis*, and 4.76% for *S. aureus*), followed by hospital personnel tables and chairs (14.28%; 9.52% for *S. epidermidis*, and 4.76% for *S. saprophyticus*), and hospital computers and keyboards (14.28%; 4.76% for *S. epidermidis*, and 9.52% for *S. saprophyticus*) (Figure 1).

The antibiotic susceptibility profiles of the MDR staphylococcal isolates were evaluated using three antibiotic categories: group A, group B, and group C. Among the selected group A antibiotics, the highest resistance was observed for cefoxitin (100%), followed by trimethoprim-sulfamethoxazole, clindamycin (both at 76.19%), and erythromycin (71.43%) (Figure 2). Cefoxitin susceptibility was used as a surrogate marker of methicillin resistance. Based on cefoxitin results, 95.24% (20/21 isolates) were classified as methicillin-resistant coagulase-negative staphylococci (MRCoNS), of which 75% (15/20 isolates) were methicillin-resistant *Staphylococcus epidermidis* (MRSE). A single isolate (4.76%) was identified as methicillin-resistant *Staphylococcus aureus* (MRSA) (Table 2). In contrast, most isolates were susceptible to the antibiotics in group B and group C, with sensitivity rates ranging from 66.67% to 100%. None of the isolates were resistant to linezolid, doxycycline, or vancomycin. Nevertheless, a few notable exceptions were observed. Specifically, resistance to rifampin occurred in 9.52% of the isolates in group B, whereas resistance was detected for gentamicin (33.33%), ciprofloxacin and moxifloxacin (9.52% each), and chloramphenicol (4.76%) in group C (Figure 2). Analysis of the antibiotic resistance

profiles revealed considerable diversity among the isolates, which were grouped into distinct resistance patterns based on the number of antibiotics for which resistance was detected. Three resistance profiles were noted for group A: resistance/intermediate to four antibiotics (10/21 isolates, 47.62%), three antibiotics (8/21 isolates, 38.10%), and two antibiotics (3/21 isolates, 14.29%). In group B, only one resistance pattern was observed, with 2/21 isolates (9.52%) displaying resistance to a single agent, specifically rifampin. In group C, the resistance patterns included isolates resistant/intermediate to one (4/21 isolates, 19.05%), two (2/21 isolates, 9.52%), or three antibiotics (3/21 isolates, 14.29%), as detailed in Table 2.

Figure 3 illustrated the prevalence of ARGs among the 21 MDR staphylococcal isolates. The *blaZ* gene, which confers resistance through β -lactamase production, was detected in 90.48% of the isolates examined. At the group level, this gene was present in 94.74% of MRCoNS isolates and 5.26% of MRSA isolates (Figure 4). Methicillin resistance was primarily attributed to two genetic determinants: *mecA1* (detected in 90.48% of all isolates) and *mecA2* (detected in 61.90% of all isolates). Specifically, *mecA1* was found in 94.74% of MRCoNS isolates and in 5.26% of MRSA isolates, whereas *mecA2* was identified only in MRCoNS isolates (100%) and was absent in MRSA isolates (Figure 4). For genes conferring erythromycin resistance, *ermC* was detected in all isolates (100%), whereas *ermB* was detected in only 4.76% of isolates. The *ermA* gene was not detected in any samples (data not shown). The distribution of *ermC* within the groups showed detection rates of 95.24% for MRCoNS and 4.76% for MRSA. Interestingly, *ermB* was only found in MRCoNS (100%) (Figure 4). In terms of aminoglycoside resistance, the *aacA-aphD* gene was detected in 61.90% of all isolates, with a notable finding in MRCoNS (100%); however, the gene was absent in MRSA (Figure 4). Importantly, *mecC*, which was associated with amoxicillin and penicillin resistance, was not detected in any of the isolates (data not shown). Collectively, these findings highlighted the high prevalence and diverse distribution of resistance genes in MDR staphylococcal isolates from SHPHs.

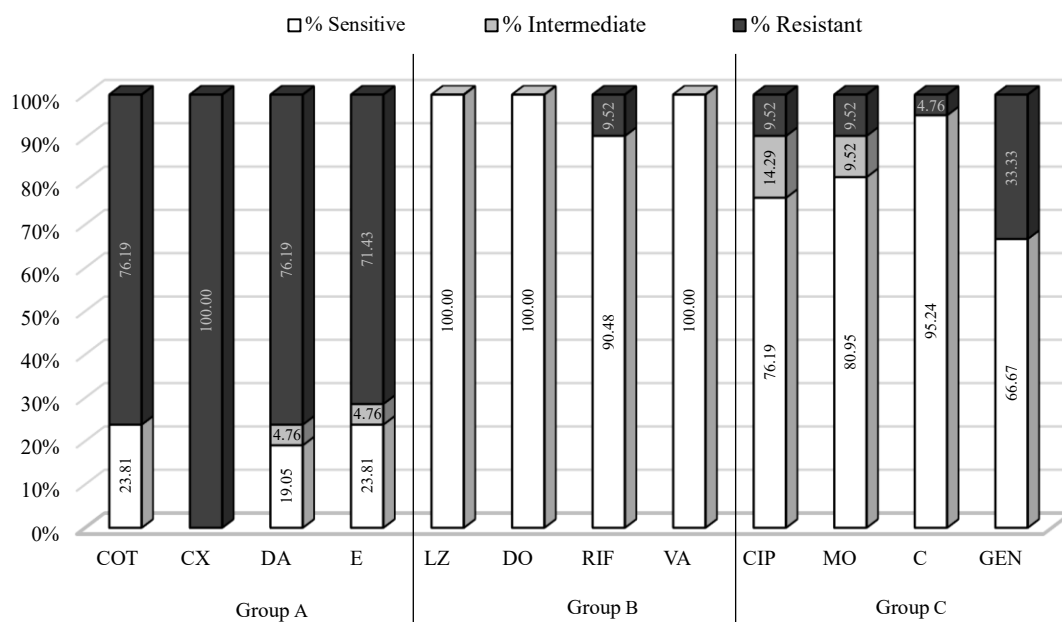


Figure 2 Antibiotic susceptibility profiles of the 21 MDR staphylococcal isolates

COT: trimethoprim-sulfamethoxazole (25 µg), CX: cefoxitin (30 µg), DA: clindamycin (2 µg), E: erythromycin (15 µg), LZ: linezolid (30 µg), DO: doxycycline (30 µg), RIF: rifampin (5 µg), VA: vancomycin (MIC), CIP: ciprofloxacin (5 µg), MO: moxifloxacin (5 µg), C: chloramphenicol (30 µg), GEN: gentamicin (10 µg)

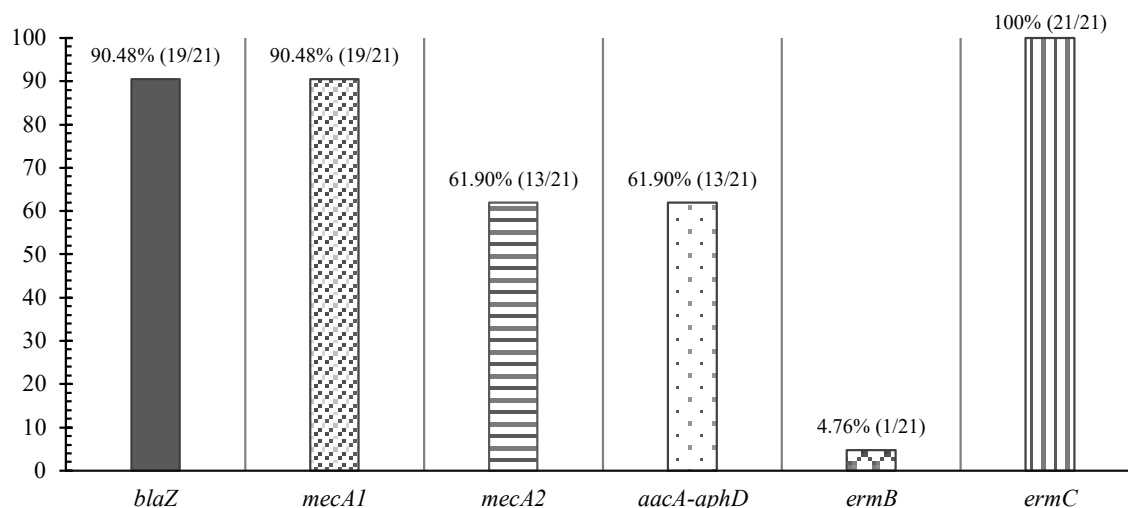


Figure 3 Prevalence (%) of antibiotic resistance genes among the 21 MDR staphylococcal isolates

Table 2 Antibigram profiles of the 21 MDR staphylococcal isolates from SHPHs in Chiang Rai

No.	Hospital no.	CoNS			Antibiotic susceptibility testing											
		SE	SS	SA	Group A				Group B				Group C			
					COT	CX	DA	E	LZ	DO	RIF	VA	CIP	MO	C	GEN
1	Hos#1		●		.	R	R	.	.	.	.	.	.	.	.	.
2		●			.	R	R	R	.	.	R	.	.	.	.	.
3	Hos#2		●		.	R	I	R	.	.	.	.	.	.	.	.
4	Hos#4	●			R	R	R	R	.	.	.	.	R	R	.	R
5		●			R	R	R	R	.	.	.	.	.	.	.	.
6		●			R	R	R	R	.	.	.	.	I	I	.	.
7			●		R	R	R	R	.	.	.	.	.	.	.	.
8			●		R	R	R	R	.	.	.	.	.	.	.	.
9		●			R	R	R	R	.	.	.	.	.	.	R	.
10		●			R	R	R	R	.	.	.	.	.	.	.	.
11	Hos#5	●			R	R	R	R	.	.	.	.	.	.	.	R
12	Hos#6	●			R	R	.	R	.	.	.	.	R	R	.	R
13		●			R	R	.	R	.	.	.	.	.	.	.	.
14		●			R	R	.	.	.	.	.	.	I	.	.	R
15	Hos#7	●			R	R	R	.	.	.	.	.	.	.	.	R
16		●			.	R	.	R	.	.	.	.	.	.	.	R
17	Hos#10		●		.	R	R	R	.	.	.	.	.	.	.	.
18	Hos#11	●			R	R	R	R	.	.	.	.	I	I	.	R
19		●			R	R	R	R	.	.	.	.	.	.	.	.
20				●	R	R	R	.	.	.	.	.	.	.	.	.
21	Hos#12	●			R	R	R	I	.	.	R	.	.	.	.	.

CoNS: Coagulase-negative *Staphylococcus*, SE: *S. epidermidis*; SS: *S. saprophyticus*; SA: *S. aureus*, COT: trimethoprim-sulfamethoxazole (25 µg), CX: cefoxitin (30 µg), DA: clindamycin (2 µg), E: erythromycin (15 µg), LZ: linezolid (30 µg), DO: doxycycline (30 µg), RIF: rifampin (5 µg), VA: vancomycin (MIC), CIP: ciprofloxacin (5 µg), MO: moxifloxacin (5 µg), C: chloramphenicol (30 µg), GEN: gentamicin (10 µg), . = sensitive; I = intermediate; R = resistant

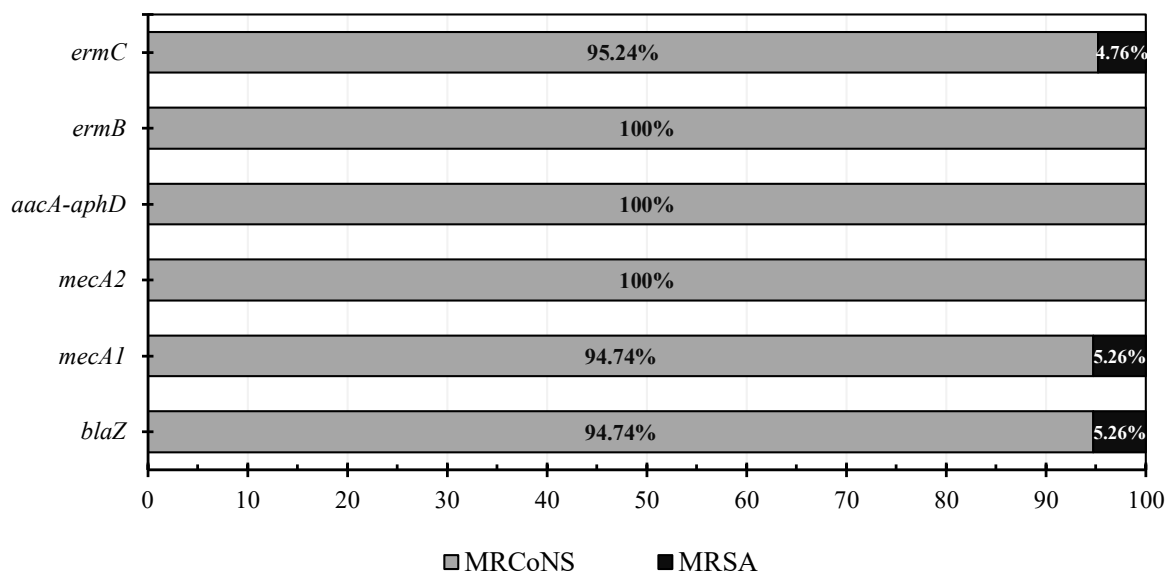


Figure 4 Distribution (%) of resistance genes among methicillin resistance phenotypes

No.	Types	Gene(s) encoding antibiotic resistance									Phenotypic antibiotics resistance patterns		
		β-lactams				Macrolides			Aminoglycosides	Number of detected gene (s)	Group A	Group B	Group C
		<i>blaZ</i>	<i>mecA1</i>	<i>mecA2</i>	<i>mecC</i>	<i>ermA</i>	<i>ermB</i>	<i>ermC</i>	<i>aacA-aphD</i>				
11	MRCoNS	■	■	■				■		5	COT, CX, DA, E		GEN
18	MRCoNS	■	■	■				■		5	COT, CX, DA		CIPi, MOi, GEN
15	MRCoNS	■	■	■				■		5	COT, CX, DA		GEN
12	MRCoNS	■	■	■				■		5	COT, CX, E		CIP, MO, GEN
14	MRCoNS	■	■	■				■		5	COT, CX		CIPi, GEN
2	MRCoNS	■	■	■				■		5	CX, DA, E	RIF	
16	MRCoNS	■	■	■				■		5	CX, E		GEN
3	MRCoNS	■	■	■			■	■		5	CX, DAi, E		
7	MRCoNS	■	■	■				■		4	COT, CX, DA, E		
19	MRCoNS	■	■	■				■		4	COT, CX, DA, E		
21	MRCoNS	■	■	■				■		4	COT, CX, DA, Ei	RIF	
13	MRCoNS	■	■	■				■		4	COT, CX, E		
9	MRCoNS	■	■	■				■		4	COT, CX, DA, E		C
6	MRCoNS	■	■	■				■		4	COT, CX, DA, E		CIPi, MOi
10	MRCoNS	■	■	■				■		4	COT, CX, DA, E		
4	MRCoNS	■	■	■				■		4	COT, CX, DA, E		CIP, MO, GEN
5	MRCoNS	■	■	■				■		4	COT, CX, DA, E		
8	MRCoNS	■	■	■				■		3	COT, CX, DA, E		
17	MRCoNS	■	■	■				■		3	CX, DA, E		
20	MRSA	■	■	■				■		3	COT, CX, DA		
1	MRCoNS	■	■	■				■		1	CX, DA		

Figure 5 Presence of genes encoding antibiotic resistance and phenotypic antibiotic resistance patterns of the 21 MDR staphylococcal isolates from SHPHs in Chiang Rai

COT: trimethoprim-sulfamethoxazole (25 µg), CX: cefoxitin (30 µg), DA: clindamycin (2 µg), E: erythromycin (15 µg), RIF: rifampin (5 µg), CIP: ciprofloxacin (5 µg), MO: moxifloxacin (5 µg), C: chloramphenicol (30 µg), GEN: gentamicin (10 µg), ■ detected gene (s)

Figure 5 shows the integrated resistance profiles for each isolate and combines the phenotypic antibiotic susceptibility patterns and the genotypic detection of ARGs. The relationship between the phenotypic resistance patterns and specific ARGs was observed. Most isolates with multiple ARGs (particularly β-lactam resistance genes; *blaZ*, *mecA1*, and *mecA2*) exhibited resistance to β-lactam antibiotics (cefazolin). The phenotypic resistance to gentamicin was usually observed in isolates that carried the *aacA-aphD* (aminoglycoside resistance) gene. Lower numbers of isolates displayed resistance to erythromycin, which matched the less frequent detection of *erm* genes (*ermA* was absent while *ermB* was found in a single isolate). An exception was noted, as *ermC* genes were present in all isolates.

Co-occurrence of ARGs was observed in 20/21 isolates (95.24%). The most prevalent pattern consisted of five ARGs (*blaZ*, *mecA1*, *mecA2*, *aacA-aphD*, and *ermC*), accounting for 7/21 isolates

(33.33%). This was followed by the four-gene combination of *blaZ*, *mecA1*, *aacA-aphD*, and *ermC* (19.05%; 4/21 isolates). Finally, a three-gene cluster featuring *blaZ*, *mecA1*, and *ermC* appeared in 3/21 isolates (14.29%).

5. Discussion

This study established the broadened MDR phenotypic and the genotypic resistance profiles of 21 staphylococcal isolates collected from 12 SHPHs across six districts in Chiang Rai. Given that these hospitals frequently contend with limited resources (Kitreerawutiwong et al., 2017), the prevalence and distribution of MDR not only reflect broader epidemiological trends but also provide essential insights into local resistance patterns within such settings. The distribution of MDR staphylococcal isolates across different hospital environments highlights areas of concern (Figure 1). The highest prevalence was noted in beds and bedrails (28.57%),

which is consistent with studies emphasizing that high-touch surfaces serve as reservoirs for the transmission of antibiotic-resistant pathogens (Facciola et al., 2019; Firesbhat et al., 2021). Beds and bed rails were frequently exposed to both patients and healthcare workers, thereby creating a conducive environment for bacterial persistence and potential transmission. These findings highlight the importance of stringent hand hygiene practices in reducing pathogen transfer from contaminated surfaces to patients. Hospital personnel tables and chairs as well as computers and keyboards accounted for 14.28% of MDR isolates, thereby forming secondary reservoirs. These findings highlight the role of administrative and shared spaces in facilitating bacterial dissemination. Computer keyboards in hospital settings may harbor pathogenic bacteria; hence, effective disinfectants may mitigate risks (Ledwoch et al., 2021). These areas often function as transitional zones that facilitate the spread of MDR pathogens, thereby highlighting the need for enhanced sanitation practices and frequent disinfection. The predominance of CoNS among MDR isolates was noteworthy. Unlike *S. aureus*, CoNS species are traditionally considered less virulent but have emerged as crucial pathogens in immunocompromised patients and those undergoing invasive procedures (Rogers et al., 2009). Their ability to form biofilms on medical devices enhances their resistance to antibiotics and complicates their treatment. CoNS are also adept at acquiring resistance genes through horizontal gene transfer, which further contributes to their MDR profiles (Otto, 2013). This demonstrates their role in HAIs and the importance of targeted interventions to mitigate their spread. The antibiotic susceptibility profiles revealed high resistance to certain antibiotics in group A, whereas groups B and C showed greater sensitivity (Figure 2). This highlights the different resistance patterns among MDR staphylococci in these settings. Notably, all isolates (100%) showed cefoxitin resistance, which also serves as an indicator of methicillin resistance. Correspondingly, methicillin resistance was detected at a markedly higher frequency (95.24%; 20/21 isolates) in CoNS than in *S. aureus* isolates (4.76%) (Table 2), which aligns with Contractor et al. (2025). However, this likely reflects the higher number of CoNS isolates than *S. aureus* isolates. The notably high prevalence (75%) of MRSE among MRCoNS is particularly concerning because of its strong persistence in biofilm production on hospital surfaces. This underscores the role of CoNS, especially *S. epidermidis*, as a reservoir for antibiotic resistance

genes within hospital environments (Chikkaraddi et al., 2022). Because these strains tend to exhibit high MDR, routine *in vitro* antibiotic susceptibility testing is essential to guide effective therapeutic interventions. In addition to methicillin resistance, substantial resistance rates were observed for trimethoprim-sulfamethoxazole, clindamycin (both at 76.19%), and erythromycin (71.43%) (Figure 2). These antibiotics have traditionally served as important alternatives for treating infections caused by methicillin-resistant *Staphylococcus* strains. Both clindamycin (classified as a lincosamide) and erythromycin (classified as a macrolide) inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit. Lincosamide and macrolide resistance is frequently associated with methicillin resistance in staphylococcal strains (Miklasińska-Majdanik, 2021). This co-resistance pattern substantially limits treatment options, complicates patient care, and potentially prolongs the illness. On the other hand, the high prevalence of resistance to group A antibiotics in SHPHs underscores the urgent need to re-evaluate local empirical treatment protocols. In resource-limited settings, continued reliance on these primary antibiotics without regular updates to protocols may result in ineffective treatments and further promote the emergence of multidrug-resistant organisms within hospital environments. Therefore, it is essential to regularly review and modify empirical guidelines to ensure treatment efficacy. In contrast, isolates exhibited promising sensitivity profiles for most antibiotics within groups B and C (66.67% to 100%) (Figure 2). This is similar to recent findings that second-line and supplemental antibiotics, notably linezolid and vancomycin, often retain efficacy against resistant staphylococci strains (Mustafa & Ali, 2025). Exceptions such as rifampin resistance (9.52% in group B) and gentamicin resistance (33.33% in group C) suggest emergent resistance mechanisms that warrant attention. Fluoroquinolones (ciprofloxacin and moxifloxacin) showed lower resistance rates (9.52%), which is consistent with recent studies indicating occasional resistance development linked to horizontal gene transfer and integrative mobile elements (Qv et al., 2025). Chloramphenicol resistance was low (4.76%), indicating that chloramphenicol retained its effectiveness, especially against MDR CoNS (Chopjitt et al., 2025). However, it is important to note that these findings are based solely on hospital environment isolates and included a small number of samples (21 isolates). Therefore, caution should be exercised when applying these results to clinical

treatment recommendations (Rohana et al., 2023), as the results primarily reflect environmental resistance patterns and do not directly inform clinical efficacy or outcomes. A review by Partridge et al. (2018) revealed the role of mobile genetic elements in shaping resistance phenotypes, thereby corroborating the diverse resistance patterns observed among group A (three patterns), B (one pattern), and C (three patterns) (Table 2). These results reflect the genomic adaptability of MDR staphylococci and emphasize the importance of ongoing surveillance to detect shifts in resistant phenotypes and guide empirical therapy. For instance, the study findings demonstrated a correspondence between the phenotypic resistance patterns and genotypic characteristics of MDR staphylococcal isolates. Cefoxitin resistance (indicating methicillin resistance) was found in 100% of all isolates (Figure 2), paralleled by the high prevalence of methicillin resistance genes, *mecA1* and *mecA2*, which were present in 90.48% and 61.90% of the isolates, respectively. Cefoxitin is a more sensitive inducer of the *mecA* gene than penicillin or oxacillin. This makes the cefoxitin diffusion test better than oxacillin for detecting methicillin resistance, as it is easier to interpret and more sensitive (Broekema et al., 2009). Resistance to cefoxitin indicates the presence of the *mecA* or *mecC* gene, which encodes a modified penicillin-binding protein with a low affinity for beta-lactam antibiotics (Ballhausen et al., 2014; Lade & Kim, 2023). The association between cefoxitin resistance and the presence of *mecA* was confirmed by Chopjitt et al. (2025); hence, the gene served as the primary determinant of methicillin resistance among the staphylococci in this study. As *mecA* is located on the staphylococcal cassette chromosome *mec* (SCC*mec*) element, a recent review by Wolska-Gębarzewska et al. (2024) highlighted the variability in SCC*mec* types, thereby supporting the prevalence of both *mecA1* and *mecA2* variants found in the current study. Furthermore, *mecA* is more widely distributed in CoNS than in *S. aureus* (Wolska-Gębarzewska et al., 2024). This is particularly relevant to the current study, where *mecA1* (94.74%) and *mecA2* (100%) were detected predominantly among the MRCoNS isolates (Figure 4). In contrast, the absence of *mecC* (a *mecA* homologue) (data not shown), which is occasionally associated with alternative pathways of methicillin resistance, underscored its minimal contribution to these populations. This finding was consistent with that of Loncaric et al. (2019), who reported a rare detection of *mecC* in CoNS. The *blaZ* gene, encoding β -lactamase and conferring resistance

to β -lactam antibiotics, was identified in 90.48% (Figure 3) of isolates. This aligned with substantial cefoxitin resistance (100%) (Figure 2) and widespread abundance of β -lactamase enzymes in staphylococci. Remarkably, the *blaZ* gene was found in 94.74% of MRCoNS, which was consistent with a previous study showing high detection rates of *blaZ* in CoNS (95.70%) in Tanzania (Charles et al., 2024). Therefore, *blaZ* may be a dominant β -lactam antibiotic resistance marker in the MDR staphylococci investigated in the current study. Furthermore, clindamycin and erythromycin resistance, reported at 76.19% and 71.43%, respectively (Figure 3), were strongly supported by the presence and distribution of key resistance determinants, specifically *erm* (erythromycin resistance methylase) genes. The *erm* genes, including *ermA*, *ermB*, and *ermC*, are commonly responsible for resistance to macrolides and clindamycin in staphylococci by regulating the methylation of the 23S rRNA-binding region (Mazloumi et al., 2021). The *ermC* gene was predominantly present in all isolates (Figure 3) (95.24% in MRCoNS and 4.76% in MRSA; Figure 4). The low prevalence of *ermB* (4.76%) (notably 100% in MRCoNS) (Figure 3), and the absence of *ermA* (data not shown) suggested that the *ermC* gene was the principal contributor to macrolide resistance in these MDR staphylococcal isolates. This is supported by recent surveillance studies demonstrating the dominance of *ermC* among clinical isolates, particularly in hospital-associated *S. aureus* and CoNS (Mahdi Al-Buhilal et al., 2021; Mazloumi et al., 2021). In staphylococci, aminoglycoside resistance is mediated by enzymatic modifications that render these antibiotics ineffective. The bifunctional enzymes, aminoglycoside N-acetyltransferase and aminoglycoside phosphotransferase, which are encoded by *aacA-aphD*, are among the most frequent aminoglycoside-modifying enzymes found in methicillin resistant staphylococci (Perumal et al., 2016). This is particularly relevant in the current study, where the *aacA-aphD* gene was present in 61.90% of the isolates (Figure 3) and was only found in MRCoNS (100%) (Figure 4). This *aacA-aphD* prevalence in MRCoNS was higher than that in previous studies (83.10%) (Sabzehali et al., 2017). This highlights the importance of aminoglycoside-modifying enzymes in MDR beyond β -lactams and macrolides, particularly in these hospital settings. Interestingly, this study identified a single isolate that was resistant to cefoxitin and methicillin, although *mecA* was absent; the only resistance gene detected was *ermC*, although the isolate remained phenotypically

sensitive to erythromycin (Figure 5). On the other hand, the 100% prevalence of *ermC* (Figure 3), despite only 71.43% phenotypic resistance to erythromycin (Figure 2), suggests the existence of "silent resistance." This phenomenon occurs when bacteria possess ARGs that remain inactive or "silent," causing them to appear sensitive during susceptibility testing. This finding aligns with Kime et al. (2019), who indicated that staphylococcal isolates frequently harbored silent resistance genes due to mutations. The presence of silent resistance indicates a hidden genetic reservoir that may revert to active resistance under certain conditions, potentially leading to therapeutic failures (Deekshit & Srikumar, 2022). This observation suggests that a comprehensive understanding of antibiotic resistance requires both phenotypic and genetic analysis, as relying solely on disk diffusion for phenotypic testing may not always accurately reflect underlying genetic resistance patterns and may overlook the existence of silent resistance genes. Such inconsistencies are often linked to methodological factors, laboratory conditions, and limitations of using a single gene for PCR-based species identification, all of which can influence the expression of resistance traits (Contractor et al., 2025; Mustafa & Ali, 2025). Therefore, combining genotypic and phenotypic approaches with standardized laboratory protocols is essential to achieve precise detection and characterization of antibiotic resistance in staphylococcal isolates. However, the detection of the co-occurrence of ARGs is a critical concern in the evolution of MDR. Figure 5 also shows that 20/21 isolates (95.24%) exhibited co-occurrence of ARGs. The cluster consisting of *blaZ*, *mecA1*, and *ermC* genes represents a foundational resistance pattern that may serve as a precursor to more complex combinations. The predominant five-ARG pattern (*blaZ*, *mecA1*, *mecA2*, *aacA-aphD*, and *ermC*) reflects a robust resistance mechanism against multiple classes of antibiotics, including β -lactams, macrolides, and aminoglycosides. The *blaZ* and *mecA* genes are well-documented contributors to β -lactam resistance, with *blaZ* encoding β -lactamase and *mecA* mediating methicillin resistance through altered penicillin-binding proteins, thereby offering remarkable resistance to this antibiotic class (Chopjitt et al., 2025). The coexistence of these genes with *aacA-aphD* (aminoglycoside resistance) and *ermC* (macrolide resistance) enhances their MDR profiles. Our study revealed that *mecA* commonly occurs in conjunction with other ARGs. Similarly, numerous studies have established a link between methicillin resistance and resistance to aminoglycosides (Perumal et

al., 2016; Sabzehali et al., 2017). Furthermore, *ermC* was frequently associated with *mecA* (Fasihi et al., 2017), and was found more often in methicillin-resistant staphylococcal isolates (Mahdi Al-Buhilal et al., 2021). The prevalence of ARG co-occurrence patterns in this study reflects the complexity and adaptability of MDR staphylococcal isolates acquired from the hospital environments. These findings are relevant for understanding local resistance trends within environmental reservoirs, even as antibiotic resistance patterns may change over time. ARGs within environmental samples tend to be more stable and persist longer than those found in clinical samples, which can change rapidly due to direct antibiotic pressure on patients (Domingues et al., 2026). Despite their stability, ARGs retain the ability to acquire new traits through horizontal gene transfer (e.g., via plasmids) (Sassi et al., 2025). Therefore, an urgent need for surveillance and intervention strategies is suggested. Advanced genomic analyses can reveal the genetic interplay between ARGs and offer insights into the mechanisms driving MDR. Targeted therapeutic approaches, such as combination therapies and novel antimicrobials, can mitigate the effects of these resistance patterns. Therefore, addressing this challenge requires a multidisciplinary approach that encompasses genomic research, clinical interventions, and antimicrobial stewardship.

6. Conclusion

This study provides the first comprehensive phenotypic and genotypic characterization of MDR staphylococcal isolates recovered from sub-district health promoting-hospitals in Chiang Rai, Thailand. By focusing on resource-limited primary healthcare settings, the study identified local resistance patterns that may not be captured in larger-scale surveillance programs. The uneven distribution of MDR staphylococci across hospital areas highlights the importance of environmental contact surfaces and sanitation practices. High-touch surfaces, particularly beds and bed rails, should therefore be prioritized in routine cleaning and disinfection protocols. The high prevalence of resistance to Group A antibiotics also emphasizes the need for prudent antimicrobial use and periodic review of local empirical-treatment guidelines. However, because the isolates were obtained from environmental rather than clinical samples, treatment decisions should continue to be based on patient-specific susceptibility results. The isolates carried diverse resistance determinants, particularly *ermC*, *blaZ*, and *mecA*, with a notable

absence of *mecC* and *ermA*. Although some genotypic findings may not correlate with phenotypic resistance, isolates with more ARGs are more likely to be resistant to a wider range of antibiotics, while those with fewer genes are less resistant. This indicates the need for focused local surveillance and future studies to assess the risk of resistance spread by investigating the transferability of these genes (such as via plasmids) to the surrounding community. These actionable insights are vital for the control of MDR staphylococci and antimicrobial stewardship in these healthcare environments.

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8. Abbreviations

Abbreviation	Full Term
.	Sensitive
ARG	Antibiotic resistance gene
C	Chloramphenicol
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase-negative staphylococci
COT	Trimethoprim-sulfamethoxazole
CX	Cefoxitin
DA	Clindamycin
DO	Doxycycline
E	Erythromycin
GEN	Gentamicin
HAIs	Healthcare-associated infections
Hos	Hospital
I	Intermediate
LZ	Linezolid
MDR	Multidrug resistant
MIC	Minimal inhibitory concentration
MO	Moxifloxacin
MRCoNS	Methicillin-resistant coagulase-negative staphylococci
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MRSE	Methicillin-resistant <i>Staphylococcus epidermidis</i>
PCR	Polymerase chain reaction

Abbreviation	Full Term
R	Resistant
RIF	Rifampin
SHPHs	Sub-district health promoting-hospitals
VA	Vancomycin

9. CRediT Statement

Korakot Chansareewittaya: Conceptualization, Methodology, Investigation, Data Curation, Validation, Formal Analysis, Writing – Original Draft, Writing – Review & Editing, Supervision, Project Administration, Funding Acquisition
Sirikarnnapa Krajangcharoensakul: Methodology, Investigation, Resources, Validation

10. References

- Águila-Arcos, S., Álvarez-Rodríguez, I., Garaiyurrebaso, O., Garbisu, C., Grohmann, E., & Alkorta, I. (2017). Biofilm-forming clinical *Staphylococcus* isolates harbor horizontal transfer and antibiotic resistance genes. *Frontiers in Microbiology*, 8, Article 2018. <https://doi.org/10.3389/fmicb.2017.02018>
- Akter, S., Sabuj, A. A. M., Haque, Z. F., Kafi, M. A., Rahman, M. T., & Saha, S. (2020). Detection of antibiotic-resistant bacteria and their resistance genes from houseflies. *Veterinary World*, 13(2), 266–274. <https://doi.org/10.14202/vetworld.2020.266-274>
- Alfeki, A. A. E., Tawfick, M. M., Ashour, M. S., & El-Moghazy, A. N. A. (2022). High prevalence of multi-drug resistant methicillin-resistant *Staphylococcus aureus* in tertiary Egyptian hospitals. *The Journal of Infection in Developing Countries*, 16(05), 795-806. <https://doi.org/10.3855/jidc.15833>
- Ballhausen, B., Kriegeskorte, A., Schleimer, N., Peters, G., & Becker, K. (2014). The *mecA* homolog *mecC* confers resistance against β -lactams in *Staphylococcus aureus* irrespective of the genetic strain background. *Antimicrobial Agents and Chemotherapy*, 58(7), 3791-3798. <https://doi.org/10.1128/aac.02731-13>
- Bauer, A. W., Kirby, W. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4 ts), 493-496. https://doi.org/10.1093/ajcp/45.4_ts.493

- Boonsilp, S., Sikora, J., Rupprom, K., Acilioğlu, S., Homkaew, A., Nutalai, D., ... & Wongsuk, T. (2023). Molecular characterization and antibiotic resistance of *Staphylococcus aureus* isolated from clinical specimens in an urban university hospital in Bangkok, Thailand. *Acta Microbiologica et Immunologica Hungarica*, 70(1), 61-72.
<https://doi.org/10.1556/030.2023.01942>
- Bouphan, P., Apipalakul, C., & Ngang, T. K. (2015). Factors affecting public health performance evaluation of sub-district health promoting hospital directors. *Procedia-Social and Behavioral Sciences*, 185, 128-132.
<https://doi.org/10.1016/j.sbspro.2015.03.421>
- Broekema, N. M., Van, T. T., Monson, T. A., Marshall, S. A., & Warshauer, D. M. (2009). Comparison of cefoxitin and oxacillin disk diffusion methods for detection of *mecA*-mediated resistance in *Staphylococcus aureus* in a large-scale study. *Journal of Clinical Microbiology*, 47(1), 217-219.
<https://doi.org/10.1128/jcm.01506-08>
- Chansareewittaya, K., & Krajangcharoensakul, S. (2021). Existence of bacterial contamination on inanimate surfaces and equipment in sub-district health-promoting hospitals in Chiang Rai, Thailand. *Journal of Current Science and Technology*, 11(3), 355-366.
<https://doi.org/10.14456/jcst.2021.36>
- Chansareewittaya, K., & Krajangcharoensakul, S. (2022). The occurrence of antibiotic resistant bacteria contamination in sub-district health-promoting hospitals in Chiang Rai, Thailand. *Journal of Health Science and Medical Research*, 40(4), 459-473.
<https://doi.org/10.31584/jhsmr.2021857>
- Chaoui, L., Mhand, R., Mellouki, F., & Rhallabi, N. (2019). Contamination of the Surfaces of a Health Care Environment by Multidrug-Resistant (MDR) Bacteria. *International Journal of Microbiology*, 2019(1), Article 3236526.
<https://doi.org/10.1155/2019/3236526>
- Charles, A. J., Majigo, M., Makupa, J. E., Kibwana, U., Mwazyunga, Z., Mwandigha, A. M., ... & Joachim, A. (2024). Occurrence of virulence genes *icaADBC* and antibiotic resistance genes *blaZ*, *mecA*, and *aac (6')-Ie-aph (2'')-Ia* in coagulase-negative staphylococci isolates from neonates with sepsis at a regional referral hospital in Dar es Salaam, Tanzania. *Bulletin of the National Research Centre*, 48(1), Article 115.
<https://doi.org/10.1186/s42269-024-01271-6>
- Chikkaraddi, U., Nandihal, N. W., & Smitha, N. R. (2022). Clinico-bacteriological profile and antibiogram of *Staphylococcus epidermidis* with special emphasis on Methicillin resistance and hospital acquired infections in a tertiary care center south India. *Indian Journal of Microbiology Research*, 9(1), 34-40.
<https://doi.org/10.18231/j.ijmr.2022.006>
- Chopjitt, P., Tangthong, P., Kongkaem, J., Wonkyai, P., Charoenwattanamaneechai, A., Khankhum, S., ... & Sunthamala, N. (2025). Molecular characterization and genotype of multi-drug resistant *Staphylococcus epidermidis* in nasal carriage of young population, Mahasarakham, Thailand. *Biomolecules and Biomedicine*, 25(2), 461-471. <https://doi.org/10.17305/bb.2024.11116>
- Clinical and Laboratory Standards Institute (CLSI). (2020). *Performance standards for antimicrobial susceptibility testing*. (30th ed.). CLSI supplement M100. Wayne, US: Clinical and Laboratory Standards Institute.
- Contractor, S. S., Kulkarni, R. D., & Arakera, S. B. (2025). Prevalence, antibiotic susceptibility pattern and the detection of *mecA* gene among *Staphylococcus* spp. isolates from a tertiary care hospital in North Karnataka. *Indian Journal of Microbiology*, 65(2), 1019-1027.
<https://doi.org/10.1007/s12088-024-01403-6>
- Côrtés, M. F., André, C., Martins Simões, P., Corvec, S., Caillon, J., Tristan, A., ... & Laurent, F. (2022). Persistence of a multidrug-resistant worldwide-disseminated methicillin-resistant *Staphylococcus epidermidis* clone harbouring the *cf* linezolid resistance gene in a French hospital with evidence of interspecies transfer to several *Staphylococcus aureus* lineages. *Journal of Antimicrobial Chemotherapy*, 77(7), 1838-1846.
<https://doi.org/10.1093/jac/dkac119>
- Deekshit, V. K., & Srikumar, S. (2022). 'To be, or not to be'-The dilemma of 'silent' antimicrobial resistance genes in bacteria. *Journal of Applied Microbiology*, 133(5), 2902-2914.
<https://doi.org/10.1111/jam.15738>
- Domingues, C. P. F., Rebelo, J. S., Dionisio, F., & Nogueira, T. (2026). Clinical and environmental plasmids: Antibiotic resistance,

- virulence, mobility, and ESKAPEE pathogens. *Antibiotics*, 15(1), Article 29.
<https://doi.org/10.3390/antibiotics15010029>
- Facciola, A., Pellicano, G. F., Visalli, G., Paolucci, I. A., Venanzi Rullo, E., Ceccarelli, M., ... & La Fauci, V. (2019). The role of the hospital environment in the healthcare-associated infections: A general review of the literature. *European Review for Medical & Pharmacological Sciences*, 23(3), 1266-1278.
https://doi.org/10.26355/eurrev_201902_17020
- Fasihi, Y., Saffari, F., Kandehkar Ghahraman, M. R., & Kalantar-Neyestanaki, D. (2017). Molecular detection of macrolide and lincosamide-resistance genes in clinical methicillin-resistant *Staphylococcus aureus* isolates from Kerman, Iran. *Archives of Pediatric Infectious Diseases*, 5(1), Article e37761.
<https://doi.org/10.5812/pedinfect.37761>
- Firesbhat, A., Tigabu, A., Tegene, B., & Gelaw, B. (2021). Bacterial profile of high-touch surfaces, leftover drugs and antiseptics together with their antimicrobial susceptibility patterns at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *BMC Microbiology*, 21(1), Article 309.
<https://doi.org/10.1186/s12866-021-02378-w>
- Gan, T., Shu, G., Fu, H., Yan, Q., Zhang, W., Tang, H., ... & Lin, J. (2021). Antimicrobial resistance and genotyping of *Staphylococcus aureus* obtained from food animals in Sichuan Province, China. *BMC Veterinary Research*, 17(1), Article 177.
<https://doi.org/10.1186/s12917-021-02884-z>
- Kime, L., Randall, C. P., Banda, F. I., Coll, F., Wright, J., Richardson, J., ... & O'Neill, A. J. (2019). Transient silencing of antibiotic resistance by mutation represents a significant potential source of unanticipated therapeutic failure. *MBio*, 10(5), Article e01755-19.
<https://doi.org/10.1128/mbio.01755-19>
- Kitreerawutiwong, N., Jordan, S., & Hughes, D. (2017). Facility type and primary care performance in sub-district health promotion hospitals in Northern Thailand. *PLoS One*, 12(3), Article e0174055.
<https://doi.org/10.1371/journal.pone.0174055>
- Lade, H., & Kim, J. S. (2023). Molecular determinants of β -lactam resistance in methicillin-resistant *Staphylococcus aureus* (MRSA): An updated review. *Antibiotics*, 12(9), Article 1362.
<https://doi.org/10.3390/antibiotics12091362>
- Ledwoch, K., Dancer, S. J., Otter, J. A., Kerr, K., Roposte, D., Rushton, L., ... & Maillard, J. Y. (2018). Beware biofilm! Dry biofilms containing bacterial pathogens on multiple healthcare surfaces; A multi-centre study. *Journal of Hospital Infection*, 100(3), e47-e56.
<https://doi.org/10.1016/j.jhin.2018.06.028>
- Ledwoch, K., Dancer, S. J., Otter, J. A., Kerr, K., Roposte, D., & Maillard, J. Y. (2021). How dirty is your QWERTY? The risk of healthcare pathogen transmission from computer keyboards. *Journal of Hospital Infection*, 112, 31-36.
<https://doi.org/10.1016/j.jhin.2021.02.021>
- Loncaric, I., Kuebber-Heiss, A., Posautz, A., Ruppitsch, W., Lepuschitz, S., Schauer, B., ... & Spargser, J. (2019). Characterization of *mecC* gene-carrying coagulase-negative *Staphylococcus* spp. isolated from various animals. *Veterinary Microbiology*, 230, 138-144.
<https://doi.org/10.1016/j.vetmic.2019.02.014>
- Mahdi Al-Buhilal, J. A., Saad, M., & AL-Rubaey, N. K. F. (2021). Molecular detection of *ermA*, *ermB* and *ermC* genes among methicillin resistant *Staphylococcus aureus* isolated from patients with ocular infections. *Biochemical & Cellular Archives*, 21, 1443-1448.
<https://connectjournals.com/03896.2021.21.1443>
- Mazloumi, M. J., Akbari, R., & Yousefi, S. (2021). Detection of inducible clindamycin resistance genes (*ermA*, *ermB*, and *ermC*) in *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Microbiology and Biotechnology Letters*, 49(3), 449-457.
<https://doi.org/10.48022/mbi.2103.03011>
- Miklasińska-Majdanik, M. (2021). Mechanisms of resistance to macrolide antibiotics among *Staphylococcus aureus*. *Antibiotics*, 10(11), Article 1406.
<https://doi.org/10.3390/antibiotics10111406>
- Mustafa, H. A., & Ali, S. M. (2025). Prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) in acute and chronic sinusitis. *Molecular Biology Reports*, 52(1), Article 882.
<https://doi.org/10.1007/s11033-025-10994-2>
- Otto, M. (2013). Coagulase-negative staphylococci as reservoirs of genes facilitating MRSA

- infection: Staphylococcal commensal species such as *Staphylococcus epidermidis* are being recognized as important sources of genes promoting MRSA colonization and virulence. *Bioessays*, 35(1), 4-11.
<https://doi.org/10.1002/bies.201200112>
- Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews*, 31(4), Article e00088-17.
<https://doi.org/10.1128/cmr.00088-17>
- Perumal, N., Murugesan, S., & Krishnan, P. (2016). Distribution of genes encoding aminoglycoside-modifying enzymes among clinical isolates of methicillin-resistant staphylococci. *Indian Journal of Medical Microbiology*, 34(3), 350-352.
<https://doi.org/10.4103/0255-0857.188339>
- Pietrocola, G., Campoccia, D., Motta, C., Montanaro, L., Arciola, C. R., & Speziale, P. (2022). Colonization and infection of indwelling medical devices by *Staphylococcus aureus* with an emphasis on orthopedic implants. *International Journal of Molecular Sciences*, 23(11), Article 5958.
<https://doi.org/10.3390/ijms23115958>
- Prommachote, W., Deeudom, M., Koonyosying, P., Srichomphoo, P., Somnabut, R., Khamnoi, P., ... & Srichairatanakool, S. (2025). Drug susceptibility, siderophore production, and genome analysis of *Staphylococcus aureus* clinical isolates from a university hospital in Chiang Mai, Thailand. *Antibiotics*, 14(5), Article 521.
<https://doi.org/10.3390/antibiotics14050521>
- Qv, M., Dai, D., Wu, Q., Wang, W., Li, L., & Zhu, L. (2025). Metagenomic insight into the horizontal transfer mechanism of fluoroquinolone antibiotic resistance genes mediated by mobile genetic element in microalgae-bacteria consortia. *Journal of Environmental Management*, 380, Article 124946.
<https://doi.org/10.1016/j.jenvman.2025.124946>
- Rimi, S. S., Ashraf, M. N., Sigma, S. H., Ahammed, M. T., Siddique, M. P., Zinnah, M. A., ... & Islam, M. S. (2024). Biofilm formation, *agr* typing and antibiotic resistance pattern in methicillin-resistant *Staphylococcus aureus* isolated from hospital environments. *PLoS One*, 19(8), Article e0308282.
<https://doi.org/10.1371/journal.pone.0308282>
- Rogers, K. L., Fey, P. D., & Rupp, M. E. (2009). Coagulase-negative staphylococcal infections. *Infectious Disease Clinics of North America*, 23(1), 73-98.
<https://doi.org/10.1016/j.idc.2008.10.001>
- Rohana, H., Hager-Cohen, A., Azrad, M., & Peretz, A. (2023). Trend of changes in chloramphenicol resistance during the years 2017–2020: A retrospective report from Israel. *Antibiotics*, 12(2), Article 196.
<https://doi.org/10.3390/antibiotics12020196>
- Rybak, B., Werbowy, O., Debowski, K., Plotka, M., & Kocot, A. M. (2025). Coagulase-negative staphylococci determined as blood culture contamination have high virulence characteristic including transfer of antibiotic resistance determinants to *Staphylococcus aureus* and *Escherichia coli*. *International Journal of Molecular Sciences*, 26(9), Article 4424. <https://doi.org/10.3390/ijms26094424>
- Sabzehali, F., Goudarzi, M., Goudarzi, H., & Azimi, H. (2017). Distribution of aminoglycoside resistance genes in coagulase-negative Staphylococci isolated from hospitalized patients. *Archives of Pediatric Infectious Diseases*, 5(3), Article e57297.
<http://doi.org/10.5812/pedinfec.57297>
- Salmanov, A., Shcheglov, D., Artyomenko, V., Svyrydiuk, O., Maliarchuk, R., Bortnik, I., ... & Loskutov, O. (2023). Nosocomial transmission of multi-drug-resistant organisms in Ukrainian hospitals: Results of a multi-centre study (2019–2021). *Journal of Hospital Infection*, 132, 104-115.
<https://doi.org/10.1016/j.jhin.2022.12.008>
- Sassi, A., Basher, N. S., Kirat, H., Meradji, S., Ibrahim, N. A., Idres, T., & Touati, A. (2025). The role of the environment (Water, Air, Soil) in the emergence and dissemination of antimicrobial resistance: A one health perspective. *Antibiotics*, 14(8), Article 764.
<https://doi.org/10.3390/antibiotics14080764>
- Schinas, G., Polyzou, E., Spernovasilis, N., Gogos, C., Dimopoulos, G., & Akinosoglou, K. (2023). Preventing multidrug-resistant bacterial transmission in the intensive care unit with a comprehensive approach: A policymaking manual. *Antibiotics*, 12(8), Article 1255.
<https://doi.org/10.3390/antibiotics12081255>

- Senobar Tahaei, S. A., Stájer, A., Barrak, I., Ostorházi, E., Szabó, D., & Gajdács, M. (2021). Correlation between biofilm-formation and the antibiotic resistant phenotype in *Staphylococcus aureus* isolates: A laboratory-based study in Hungary and a review of the literature. *Infection and Drug Resistance*, 14, 1155-1168. <https://doi.org/10.2147/IDR.S303992>
- Socohou, A., Sina, H., Degbey, C., Nanoukon, C., Chabi-Sika, K., Ahouandjinou, H., ... & Baba-Moussa, L. (2020). Antibiotics resistance and biofilm formation capacity of *Staphylococcus* spp. strains isolated from surfaces and medicotechnical materials. *International Journal of Microbiology*, 2020(1), Article 6512106. <https://doi.org/10.1155/2020/6512106>
- Thai National Institute of Health, Department of Medical Sciences, Thailand Ministry of Public Health. (2014). *Laboratory manual for bacteriology laboratories at regional and general hospitals* (1st ed.). Bangkok, Thailand: Thana Press.
- Tribuddharat, C., Pummangura, C., Srifuengfung, M., Pipobwatthana, P., Thuncharoon, H., Rodjun, V., ... & Srifuengfung, S. (2022). Prevalence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* clones: A study at Taksin Hospital, Bangkok, Thailand. *ScienceAsia*, 48, 159-164. <http://doi.org/10.2306/scienceasia1513-1874.2022.022>
- Waitayangkoon, P., Thongkam, A., Benjamungkalarak, T., Rachayon, M., Thongthaisin, A., Chatsuwat, T., ... & Chiewchengchol, D. (2020). Hospital epidemiology and antimicrobial susceptibility of isolated methicillin-resistant *Staphylococcus aureus*: A one-year retrospective study at a tertiary care center in Thailand. *Pathogens and Global Health*, 114(4), 212-217. <https://doi.org/10.1080/20477724.2020.1755550>
- Wassanarungroj, P., Nobthai, P., Ruekit, S., Srijan, A., Sukhchat, P., Serichantalergs, O., ... & Lertsethtakarn, P. (2025). Molecular characterization of clinical isolates of methicillin-resistant *Staphylococcus aureus* from Chonburi, Thailand. *Pathogens*, 14(5), Article 406. <https://doi.org/10.3390/pathogens14050406>
- Wolska-Gębarzewska, M., Międzobrodzki, J., & Kosecka-Strojek, M. (2024). Current types of staphylococcal cassette chromosome *mec* (SCC *mec*) in clinically relevant coagulase-negative staphylococcal (CoNS) species. *Critical Reviews in Microbiology*, 50(6), 1020-1036. <https://doi.org/10.1080/1040841X.2023.2274841>