

The Occurrence of Antibiotic Resistant Bacteria Contamination in Sub-district Health-promoting Hospitals in Chiang Rai, Thailand

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Abstract:

Objective: To determine the antibiotic susceptibility patterns (antibiogram profiles) of the bacterial agents usually involved in hospital-acquired infections found in 12 sub-district health-promoting hospitals (HPHs) in Chiang Rai, Thailand.

Material and Methods: Swabs from 10 different sampling points in each sub-district HPH were aseptically collected. Standard microbiological methods were performed to define the bacterial species. Antibiotic susceptibility was determined by the disk diffusion method following the standard guidelines of the Clinical and Laboratory Standards Institute (2016).

Results: The antibiogram profiles of the 153 isolated bacteria showed that 55.6% of the isolates were resistant to antibiotics. Single drug resistant, double drug resistant, and multi-drug resistant bacteria accounted for 18.3%, 18.3%, and 19.0%, respectively. The *Pseudomonas aeruginosa* isolate was susceptible to all tested antibiotics. MDR phenotypes were most common in coagulase-negative staphylococci (13.1%), followed by members of the family of Enterobacteriaceae (3.9%) and *Staphylococcus aureus* (0.7%).

Conclusion: The MDR rates reported in this study are “worrying”. These results suggest that sub-district HPHs may become sources of HAIs caused by antibiotic-resistant bacteria which can be inevitably transmitted into the wider community. Antibiotic stewardship, antibiotic susceptibility surveillance and hygiene practices may be used to prevent and limit the spread of such bacteria from sub-district HPHs to the community.

Keywords: antibiogram profiles, antibiotic resistant bacteria, sub-district health-promoting hospitals

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Introduction

Bacterial infections in hospital environments are known as hospital-acquired infections (HAIs) or nosocomial infections. The cross-transmission of HAI pathogens occurs via patient-to-patient contact or contact with patient-environments and hospital personnel-patient routes, as a result of hospital contamination and poor hygiene.¹ Several studies have reported that the improper use of antibiotic therapies promote the presence of antibiotic-resistant bacteria (ARB) within hospital settings^{2,3}, although HAI transmission of ARB in hospitals is not typical of epidemic models.⁴ This was supported by a recent study by Cai et al.⁵ which revealed that the abundance and types of ARB as well as their particular antibiotic-resistant genes were significantly determined by the extensive use of antibiotics in hospitals. This co-occurrence reflected clinical antibiotic resistance, and that the ARB which were isolated from hospital environments can be disseminated into the communities to cause health problems.⁵ It could increase the threat to community health because infection caused by ARB contributes to morbidity, mortality, and an increase in medical expenses; as many antibiotics are no longer effective.⁶ In recent years, emergences of ARB contamination in different hospital areas have been reported across the world. The gloves and gowns of healthcare personnel have been found to be contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA) in intensive care units of 4 hospitals in United States of America (USA)⁷; extended-spectrum β -lactamase (ESBL)-producing and carbapenemase-producing Enterobacteriaceae were both found in the bathroom sinks of a university hospital in France.⁸ In Thailand, HAIs are common and are mainly caused by ARB.³ Furthermore, research on ARB have mostly been conducted on clinical samples obtained from hospitalized patients. Clinical MRSA isolates concomitant with multidrug-resistant (MDR) phenotypes were found at a hospital in Bangkok⁹; ampicillin and fluoroquinolones-

resistant *Vibrio parahaemolyticus* isolates were obtained from diarrheal patients of a hospital in southern Thailand.¹⁰ However, limited data exist on the contamination of bacteria as well as ARB in hospital environments. Gram-positive cocci were mainly observed as airborne bacteria contaminating a large hospital center in southern Thailand.¹¹ A high prevalence rate (70.0%) of methicillin-resistant coagulase negative staphylococci (MR-CoNS) producing biofilms were found in a number of areas in a university hospital in Phitsanulok¹²; contamination of ESBL-producing *Escherichia coli* and/or *Klebsiella pneumoniae* in wastewater samples of community, general, and regional hospitals was also reported, even after treatment.¹³ Profiles of contaminated bacteria isolated from 10 different sampling points of inanimate surfaces and equipment of 12 sub-district health-promoting hospitals (HPHs) in six sub-districts of Chiang Rai have also been revealed in one of our previous studies.¹⁴ In addition, that study was the first to report inspection results indicating bacterial contamination in sub-district level hospitals in Thailand as well as to provide primary data to support the further study of ARB occurrences in sub-district HPH environments, which is yet to be documented. To this end, nearly half of bacterial isolates (44.9%; 153/341 isolates) from our prior study were examined as the prime concern, regarding bacterial agents usually involved in HAIs.¹⁵⁻¹⁷ The antibiotic susceptibility patterns (antibiogram profiles) of these bacteria including *Staphylococcus* spp., *Streptococcus* spp., members of the family Enterobacteriaceae, and *Pseudomonas* spp. were determined in this study. We anticipate these antibiogram profiles to be vital for extending trends of ARB from sub-district level hospitals in both Chiang Rai, and Thailand.

Material and Methods

The Ethics Committees of the Chiang Rai Provincial Health Office, Thailand Ministry of Public Health approved the study protocol and all of its procedures and exempted

the study from formal ethics review (CRPPHO No. 75/2563). This research does not contain any studies involving human or animal subjects. In addition, formal permission was obtained from the concerned authorities during sample collection.

The samples were collected from inanimate surfaces and equipment in 12 sub-district HPHs in six districts of Chiang Rai, Thailand, from January to March 2018. Ten different sampling points were selected for each sub-district HPH. These 10 sampling points were chosen based on rates of exposure and due to being the most representative points of sub-district HPH services for clients and patients. The sampling points included: (1) bed and bed rails, (2) hand-washing sinks, (3) medical devices/equipment, (4) portable treatment table and medical tray, (5) tables and chairs used by hospital personnel, (6) computers and keyboards used by hospital personnel, (7) toilet bowl dispensers, (8) toilet seat/toilet bowl areas, (9) toilet sinks and taps, and (10) knobs or latches of toilet doors. All sampling locations were found within 10–40 km of the laboratory (Medical Science Laboratory, Scientific and Technological Instrument Center (STIC), Mae Fah Luang University (MFU), Mueang District, Chiang Rai) for convenience. Collected samples were transferred to the laboratory within 2–3 hours (h).

Samples were collected using a sterile technique, by the swabbing method.¹⁸ The collected swab samples were immersed for propagation in a tryptic soy broth and incubated for 24 h at 37 °C. After incubation, these broth cultures were streaked onto differential and selective media: mannitol salt agar, blood agar, and MacConkey agar for the initial screening of suspected staphylococci, streptococci, and gram-negative bacteria (members of the family Enterobacteriaceae and *Pseudomonas sp.*), respectively. The inoculated plates were incubated at 37 °C for 24 h under aerobic conditions. The growth colonies were identified by Gram staining and standard biochemistry tests based on their biochemical properties. Briefly, Gram-

positive isolates (*Staphylococcus* spp., *Streptococcus* spp.) were identified by catalase, and coagulase tests. Susceptibility to novobiocin (OXOID Ltd., Cheshire, United Kingdom) verified to differentiate *S. epidermidis* from *S. saprophyticus*. Members of *Streptococcus* spp. were examined by tests assessing growth in bile esculin medium, catalase and hemolytic reactions. The Optochin (OXOID Ltd.) test was performed to employ differentiation of viridans streptococci and *S. pneumoniae*. Members of the family Enterobacteriaceae and *Pseudomonas* spp. were identified by an oxidase test (Sigma-Aldrich, Missouri, USA), sugar fermentation (lactose and triple sugar iron (TSI); HiMedia Laboratories Pvt. Ltd., Mumbai, India), motile test, indole test, citrate test, urea test (HiMedia Laboratories Pvt. Ltd.), positive OF test, and pigment production on Pseudo F agar and Pseudo P agar (HiMedia Laboratories Pvt. Ltd.).¹⁹ Regular quality control of sterilization was performed according to the requirements of the Medical Science Laboratory, STIC of MFU. All isolated bacterial strains were preserved in a nutrient broth containing 20.0% (v/v) glycerol and were stored at freezing temperature for further analysis.

Antibiotic susceptibility testing was performed on Mueller-Hinton agar which based on the disk diffusion method²⁰ according to the Clinical and Laboratory Standards Institute (CLSI, 2016) guidelines.²¹ The tested antibiotics discs (obtained from OXOID Ltd.) included cefoxitin (FOX; 30 µg), erythromycin (E; 15 µg), clindamycin (DA; 2 µg), gentamycin (CN; 10 µg), trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 µg), cefotaxime (CTX; 30 µg), ceftriaxone (CRO; 30 µg), vancomycin (VA; 30 µg), ampicillin (AMP; 10 µg), ampicillin-clavulanic acid (AMC; 20/10 µg), ampicillin-sulbactam (SAM; 10/10 µg), ciprofloxacin (CIP; 5 µg), amikacin (AK; 30 µg), and ceftazidime (CAZ; 30 µg). The susceptibility pattern to antibiotics (antibiogram profiles) was determined by measuring the diameter of the inhibition zone and comparing with the standards. The zone diameter

interpretive standard recommended by the CLSI (2016) was presented as susceptible (S), intermediate (I), or resistant (R) of CLSI standard stains.²¹

Results

Isolation rates

A total of 153 isolates were examined for their relevant antibiotic resistance profiles. They were found to be contaminated at all sampling points (100.0%) (Table 1). The most frequently contaminated points were the toilet seat/toilet bowl areas (16.3%), followed by toilet sinks and taps (12.4%), and the computers and keyboards of hospital personnel (11.1%). The majority of these samples were gram-positive (85.0%), with a predominance of CoAg-Neg staphylococci (*S. epidermidis* and *S. saprophyticus*, 66.0% and 13.1%, respectively), followed by *S. aureus* (3.3%) and viridans streptococci, a member of the *Streptococcus* species (2.6%). Gram-negative bacteria primarily belonged to members of the family Enterobacteriaceae (*E. coli*, 2.0%; *K. pneumoniae*, 0.7%; others (not specified), 11.8%), and *P. aeruginosa* (0.7%). The estimated rates of contamination among the 10 sampling points of the sub-district HPHs varied from 10.0% to 100.0%, with an average rate of 45.0%.

Susceptibility patterns of isolates to individual antibiotics

The susceptibility of isolated *Staphylococcus* spp., Viridans streptococci, Enterobacteriaceae, and *Pseudomonas* spp. to antibiotics was tested against 5, 4, 6, and 3 primary categories of selected antibiotic discs, respectively. The results showed that the majority of the investigated isolates (ranging from 50.0% to 100.0%), especially *P. aeruginosa*, were sensitive to several of the antibiotics tested (Table 2). However, CoAg-Neg staphylococci were found to be most resistant to erythromycin (35.5%), followed by clindamycin

(26.5%) and oxacillin (oxacillin-resistant-CoAg-Neg staphylococci; MRCoNS; 19.8%). *S. aureus* isolates were the most resistant to clindamycin, whereas none of them was resistant to oxacillin. Viridans streptococci showed remarkable resistance to cefotaxime, ceftriaxone, and clindamycin (75.0% each). *E. coli* was most resistant to ampicillin-sulbactam and ampicillin (33.3% each). Similarly, one isolate of *K. pneumoniae* showed resistance to both ampicillin-sulbactam and ampicillin. Other members of Enterobacteriaceae (not specified, but excluding *E. coli* and *K. pneumoniae*) showed 44.4% (each) resistance to ampicillin and cefoxitin, and 38.9% to ampicillin-sulbactam but a low resistance to ciprofloxacin, trimethoprim-sulfamethoxazole, and gentamycin. Fortunately, none of the isolated Enterobacteriaceae were ESBL-producing strains (data not shown).

Antibiotic resistance phenotypes

The antibiotic resistance patterns of the investigated bacteria showed that more than half (60.0%) of *S. aureus* and 38.8% of CoAg-Neg staphylococci were sensitive to all of the antibiotics tested. Approximately 8.3% of CoAg-Neg staphylococci showed intermediate susceptibility, mostly to clindamycin and erythromycin. Two patterns of resistance were noted for *S. aureus* isolates, which were resistant to two and four of the drugs tested, respectively (20.0% each), and all isolates of *S. aureus* were not oxacillin-resistant *S. aureus* (MRSA). CoAg-Neg staphylococci appeared to show six different resistance patterns, including resistance to a single drug (19.1%; remarkable resistance to erythromycin (9.9%), clindamycin (5.0%), oxacillin and gentamycin (1.7% each)), double drugs (17.4%; remarkable resistance to clindamycin and erythromycin (8.3%)), three drugs (4.8%), four drugs (3.2%), five drugs (5.0%), and all drugs tested (3.3%) (Table 3).

Table 1 Frequency of examines bacterial isolates at different environmental sampling points in 12 sub-district HPHs

Bacterial isolates, n (%)	Gram-positive (n=130)						Gram-negative (n=23)			
	Staphylococcus spp. (n=126)			Streptococcus spp. (n=4)			Enterobacteriaceae (n=22)		Pseudomonas spp. (n=1)	
	CoAg-Pos			CoAg-Neg						
	S. aureus	S. epidermidis	S. saprophyticus	Viridans streptococci	E. coli	K. pneumoniae	Others*			
Bed and bed rails (16, 10.5%)	3 (18.8)	8 (50.0)	2 (12.5)	-	-	-	3 (18.8)	-	-	-
Hand-washing sink (6, 3.9%)	1 (16.7)	1 (16.7)	-	-	-	-	4 (66.7)	-	-	-
Medical devices/equipment (16, 10.5%)	-	12 (75.0)	4 (25.0)	-	-	-	-	-	-	-
Portable treatment table and medical tray (13, 8.5%)	-	8 (61.5)	3 (23.1)	-	1 (7.7)	-	1 (7.7)	-	-	-
Table and chairs used by hospital personnel (15, 9.8%)	1 (6.7)	10 (66.7)	2 (13.3)	-	-	-	2 (13.3)	-	-	-
Computers and keyboards used by hospital personnel (17, 11.1%)	-	12 (70.6)	4 (23.5)	-	-	-	1 (5.9)	-	-	-
Toilet bowl dispenser (15, 9.8%)	-	11 (73.3)	2 (13.3)	1 (6.7)	-	-	1 (6.7)	-	-	-
Toilet seat / toilet bowl area (25, 16.3%)	-	19 (76.0)	-	2 (8.0)	2 (8.0)	-	2 (8.0)	-	-	-
Toilet sink and tap (19, 12.4%)	-	10 (52.6)	3 (15.8)	1 (5.3)	-	-	4 (21.1)	1 (5.3)	-	-
Knobs or latches of the toilet door (11, 7.2%)	-	10 (90.9)	-	-	-	1 (9.1)	-	-	-	-
Total (153)	5 (3.3)	101 (66.0)	20 (13.1)	4 (2.6)	3 (2.0)	1 (0.7)	18 (11.8)	1 (0.7)	1 (0.7)	1 (0.7)
No. of contaminated points / no. of examined points (10)	3/10	10/10	8/10	3/10	2/10	1/10	8/10	1/10	1/10	1/10
Contamination rates (%)	30.0	100.0	80.0	30.0	20.0	10.0	80.0	10.0	10.0	10.0

10 examined points (isolates, %)

CoAg-Neg=coagulase-negative, CoAg-Pos=coagulase-positive, *not specified but excluding *E. coli* and *K. pneumoniae*

Contamination rates are defined by numbers of contaminated points (from 10 examined points) where each bacterial type was isolated.

Table 2 Antibiotic susceptibility characteristics of examined bacteria

Bacteria and antibiotic (µg/disk)	Susceptibility level		
	Sensitive	Intermediate	Resistant
<i>Staphylococcus</i> spp. (N=126)			
CoAg-Neg (n=121)			
SXT; Trimethoprim-Sulfamethoxazole (1.25/23.75)	79.3 (96/121)	1.7 (2/121)	19.0 (23/121)
FOX; Cefoxitin (30)	88.4 (107/121)	0.0 (0/121)	11.6 (14/121)
CN; Gentamycin (10)	85.1 (103/121)	0.8 (1/121)	14.1 (17/121)
DA; Clindamycin (2)	66.1 (80/121)	7.4 (9/121)	26.5 (32/121)
E; Erythromycin (15)	59.5 (72/121)	5.0 (6/121)	35.5 (43/121)
Oxacillin ^{a,b}	80.2 (97/121)	0.0 (0/121)	19.8 (24/121)
CoAg-Pos; <i>Staphylococcus aureus</i> (n=5)			
SXT; Trimethoprim-Sulfamethoxazole (1.25/23.75)	80.0 (4/5)	0.0 (0/5)	20.0 (1/5)
FOX; Cefoxitin (30)	80.0 (4/5)	0.0 (0/5)	20.0 (1/5)
CN; Gentamycin (10)	80.0 (4/5)	0.0 (0/5)	20.0 (1/5)
DA; Clindamycin (2)	60.0 (3/5)	0.0 (0/5)	40.0 (2/5)
E; Erythromycin (15)	80.0 (4/5)	0.0 (0/5)	20.0 (1/5)
Oxacillin ^{a,b}	0.0 (0/5)	0.0 (0/5)	0.0 (0/5)
Viridans streptococci (N=4)			
CTX; Cefotaxime (30)	25.0 (1/4)	0.0 (0/4)	75.0 (3/4)
CRO; Ceftriaxone (30)	25.0 (1/4)	0.0 (0/4)	75.0 (3/4)
E; Erythromycin (15)	75.0 (3/4)	0.0 (0/4)	25.0 (1/4)
DA; Clindamycin (2)	25.0 (1/4)	0.0 (0/4)	75.0 (3/4)
VA; Vancomycin (30)	75.0 (3/4)	0.0 (0/4)	25.0 (1/4)
<i>Enterobacteriaceae</i> (N=22)			
<i>Escherichia coli</i> (n=3)			
FOX; Cefoxitin (30)	100.0 (3/3)	0.0 (0/3)	0.0 (0/3)
CIP; Ciprofloxacin (5)	100.0 (3/3)	0.0 (0/3)	0.0 (0/3)
SXT; Trimethoprim-Sulfamethoxazole (1.25/23.75)	100.0 (3/3)	0.0 (0/3)	0.0 (0/3)
CN; Gentamycin (10)	100.0 (3/3)	0.0 (0/3)	0.0 (0/3)
SAM; Ampicillin-Sulbactam (10/10)	66.7 (2/3)	0.0 (0/3)	33.3 (1/3)
AMP; Ampicillin (10)	66.7 (2/3)	0.0 (0/3)	33.3 (1/3)
AMC; Ampicillin-Clavulanic acid (20/10)	100.0 (3/3)	0.0 (0/3)	0.0 (0/3)
<i>Klebsiella pneumoniae</i> (n=1)			
FOX; Cefoxitin (30)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
CIP; Ciprofloxacin (5)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
SXT; Trimethoprim-Sulfamethoxazole (1.25/23.75)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
CN; Gentamycin (10)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
SAM; Ampicillin-Sulbactam (10/10)	0.0 (0/1)	0.0 (0/1)	100.0 (1/1)
AMP; Ampicillin (10)	0.0 (0/1)	0.0 (0/1)	100.0 (1/1)
AMC; Ampicillin-Clavulanic acid (20/10)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
Others; not specified (n=18)			
FOX; Cefoxitin (30)	50.0 (9/18)	5.6 (1/18)	44.4 (8/18)
CIP; Ciprofloxacin (5)	94.4 (17/18)	0.0 (0/18)	5.6 (1/18)
SXT; Trimethoprim-Sulfamethoxazole (1.25/23.75)	94.4 (17/18)	0.0 (0/18)	5.6 (1/18)
CN; Gentamycin (10)	94.4 (17/18)	0.0 (0/18)	5.6 (1/18)
SAM; Ampicillin-Sulbactam (10/10)	61.1 (11/18)	0.0 (0/18)	38.9 (7/18)
AMP; Ampicillin (10)	50.0 (9/18)	5.6 (1/18)	44.4 (8/18)
AMC; Ampicillin-Clavulanic acid (20/10)	66.7 (12/18)	0.0 (0/18)	33.3 (6/18)
<i>Pseudomonas</i> spp. (N=1)			
<i>Pseudomonas aeruginosa</i>			
CIP; Ciprofloxacin (5)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
AK; Amikacin (30)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
CAZ; Ceftazidime (30)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)
CN; Gentamycin (10)	100.0 (1/1)	0.0 (0/1)	0.0 (0/1)

Values are % (n/N), CoAg-Neg=coagulase-negative; CoAg-Pos=coagulase-positive

^aOxacillin disk testing is not reliable; 30 µg cefoxitin as a surrogate agent for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result; ^bOxacillin-resistant is considered as methicillin-resistant, CLSI (2016)²¹

Table 3 Antibigram profiles of *Staphylococcus* spp. (N=126)

Resistance phenotypes							Staphylococcus spp. (N=126)								
							N	%	Phenotypes of all (%)	CoAg-Neg (N=121)			CoAg-Pos (N=5)		
										n	%	Pheno- types of type (%)	n	%	Pheno- types of type (%)
.	50	39.7	39.7	47	38.8	38.8	3	60.0	60.0	
.	.	.	DAi	.	.	6	4.8	8.0	6	5.0	8.3	0	0.0	0.0	
.	.	.	.	Ei	.	3	2.4		3	2.5		0	0.0		
.	.	.	DAi	Ei	.	1	0.8		1	0.8		0	0.0		
SXT	1	0.8	18.3	1	0.8	19.1	0	0.0	0.0	
.	.	CN	.	.	.	2	1.6		2	1.7		0	0.0		
.	.	.	DA	.	.	6	4.8		6	5.0		0	0.0		
.	.	.	.	E	.	12	9.5		12	9.9		0	0.0		
.	OX	2	1.6		2	1.7		0	0.0		
SXT	.	CN	.	.	.	2	1.6	17.5	2	1.7	17.4	0	0.0	20.0	
SXT	.	.	.	E	.	4	3.2		4	3.3		0	0.0		
.	FOX	.	.	.	OX	1	0.8		1	0.8		0	0.0		
.	.	CN	.	E	.	2	1.6		2	1.7		0	0.0		
.	.	.	DA	E	.	11	8.7		10	8.3		1	20.0		
.	.	.	DA	.	OX	1	0.8		1	0.8		0	0.0		
.	.	.	.	E	OX	1	0.8		1	0.8		0	0.0		
SXT	.	CN	.	.	OX	1	0.8		1	0.8		0	0.0		
SXT	.	.	DA	E	.	1	0.8	4.8	1	0.8	4.8	0	0.0	0.0	
.	FOX	.	DA	.	OX	1	0.8		1	0.8		0	0.0		
.	FOX	.	.	E	OX	1	0.8		1	0.8		0	0.0		
.	.	CN	.	E	OX	1	0.8		1	0.8		0	0.0		
.	.	.	DA	E	OX	1	0.8		1	0.8		0	0.0		
SXT	FOX	.	.	E	OX	1	0.8	4.0	1	0.8	3.2	0	0.0	20.0	
SXT	FOX	CN	DA	.	.	1	0.8		0	0.0		1	20.0		
SXT	.	CN	DA	.	OX	1	0.8		1	0.8		0	0.0		
SXT	.	.	DA	E	OX	1	0.8		1	0.8		0	0.0		
.	FOX	.	DA	E	OX	1	0.8		1	0.8		0	0.0		
SXT	FOX	CN	DA	.	OX	2	1.6		2	1.7		0	0.0		
SXT	FOX	CN	.	E	OX	1	0.8	4.8	1	0.8	5.0	0	0.0	0.0	
SXT	FOX	.	DA	E	OX	2	1.6		2	1.7		0	0.0		
SXT	.	CN	DA	E	OX	1	0.8		1	0.8		0	0.0		
SXT	FOX	CN	DA	E	OX	4	3.2	3.2	4	3.3	3.3	0	0.0	0.0	
Total							126	100.0	121	100.0		5	100.0		

=sensitive, SXT=Trimethoprim-Sulfamethoxazole-resistant, FOX=Cefoxitin-resistant, CN=Gentamycin-resistant, DA=Clindamycin-resistant, DAi=Clindamycin-intermediate, E=Erythromycin-resistant, Ei=Erythromycin-intermediate, OX=Oxacillin-resistant [Oxacillin disk testing is not reliable; 30 µg cefoxitin as a surrogate agent for oxacillin; report oxacillin susceptible or resistant based on the cefoxitin result and oxacillin-resistant is considered as methicillin-resistant, CLSI (2016)²¹]

The phenotypes of resistance for isolated members of the family Enterobacteriaceae showed that *E. coli* and *K. pneumoniae* exhibited a similar pattern of resistance to double drugs tested with ampicillin–sulbactam and ampicillin (33.3% and 100.0%, respectively). Others (not specified, except *E. coli* and *K. pneumoniae*) displayed five patterns of resistance to a single drug (27.9%; remarkable resistance to ampicillin (16.7%)), double drugs (11.2%), three drugs (16.7%), four drugs (11.1%), and all six drugs tested (5.6%) (Table 4).

Viridans streptococci showed notable resistance to two and three drugs, which covered all categories tested. With the exception of other isolated bacteria, only one isolate of *P. aeruginosa* showed distinctive susceptibility to all the antibiotics tested (Table 5).

Categorization of isolates based on their antibiotic resistance profiles

Among 153 bacterial isolates, 85 (55.6%) were resistant isolates categorized by three profiles: single drug resistant (SDR; 18.3%), double drug resistant (DDR; 18.3%), and multi-drug resistant (defined as resistant to three or more antimicrobial classes²², MDR; 19.0%) bacteria (Table 6). The *P. aeruginosa* isolate was susceptible to all the antibiotics tested. MDR phenotypes were most common in CoAg–Neg staphylococci (13.1%), followed by other Enterobacteriaceae (not specified, except *E. coli* and *K. pneumoniae*) (3.9%), Viridans streptococci (1.3%) and *S. aureus* (0.7%). The distribution of ARB from different sampling points is shown in Figure 1. ARBs were found at all sampling points (100.0%), especially the toilet seat/toilet bowl area (17.6%), followed by the toilet sink and tap (14.1%), and the toilet bowl dispenser (12.9%). MDR isolates were abundant in all sampling points, except the knobs or latches of the toilet doors. Among each sampling points, the highest proportion (60.0%) of MDR isolates were obtained from the bed and bed rails, and hand-washing

sink (equally), followed by the computers and keyboards used by hospital personnel (50.0%).

Discussion

In this study, the bacterial agents usually involved in HAIs, namely *Staphylococcus* spp., *Streptococcus* spp. (viridans streptococci), members of the family Enterobacteriaceae (*E. coli*, *K. pneumoniae*, and others), and *Pseudomonas* spp. contaminations on surface areas and inanimate equipment in the 12 sub-district HPHs in Chiang Rai were examined for their antibiotic resistance profiles. CoAg–Neg staphylococci (particularly *S. epidermidis*) were the most frequently isolated, consistent with a previous study.²³ This was not unexpected because they are mainly normal skin flora and especially *S. epidermidis* has a high potential to form biofilms on surfaces.²⁴ In addition, Kramer et al.²⁵ found that most gram-positive bacteria, such as staphylococci and the strains of streptococci, many gram-negative bacteria, notably *E. coli*, *Klebsiella* spp., and *P. aeruginosa* survived for months on dry inanimate surfaces. Although many of these bacteria can be disregarded as harmless commensals, the average contamination rate of 45.0% in the present study was quite high. This could be attributed to bacterial shedding leading into their transmission on inanimate equipment and surface areas in sub-district HPHs, which then become HAIs. To the best of our knowledge, HAIs are common in Thailand and most are caused by ARB.³ Subsequently, the antibiotic susceptibility patterns of the isolated bacteria in the present study were determined.

Over half (61.2%) of CoAg–Neg staphylococci isolates in this study developed resistance to five categories of antibiotics (folate pathway inhibitors, anti-staphylococcal β -lactams, aminoglycosides, lincosamides, and macrolides) (Table 3), notably high resistance to macrolides (erythromycin, 9.9%) and lincosamide (clindamycin, 5.0%), which have been widely used in the treatment of staphylococcal infections.

Table 4 Antibiogram profiles of Enterobacteriaceae (N=2)

Resistance phenotypes		Enterobacteriaceae (N=22)									
		N	%	Phenotypes of all (%)	E. coli			K. pneumoniae			Others*
					n	%	Pheno- types of type (%)	n	%	Pheno- types of type (%)	
.	.	.	.	31.8	2	66.7	66.7	0	0.0	0.0	27.8
FOX	.	1	4.5	22.6	0	0.0	0.0	0	0.0	0.0	5.6
.	SAM	1	4.5		0	0.0	0.0	0	0.0	0.0	27.9
.	AMP	3	13.6		0	0.0	0.0	0	0.0	0.0	16.7
FOX	SAM	1	4.5	18.1	0	0.0	33.3	0	0.0	100.0	11.2
.	SAM	3	13.6		1	33.3		1	100.0		5.6
FOX	AMC	1	4.5	13.6	0	0.0	0.0	0	0.0	0.0	16.7
FOX	AMC	2	9.1		0	0.0	0.0	0	0.0	0.0	11.1
FOX	AMC	2	9.1	9.1	0	0.0	0.0	0	0.0	0.0	11.1
SXT FOX CN	SAM AMP AMC	1	4.5	4.5	0	0.0	0.0	0	0.0	0.0	5.6
Total		22	100.0		3	100.0		1	100.0		18

=sensitive, SXT=trimethoprim-Sulfamethoxazole-resistant, FOX=Cefoxitin-resistant, CN=Gentamycin-resistant, CIP=Ciprofloxacin-resistant, SAM=Ampicillin-Sulbactam-resistant, AMP=Ampicillin-resistant, AMC=Ampicillin-Clavulanic acid-resistant, *not specified but excluding E. coli and K. pneumoniae

Table 5 Antibigram profiles of Viridans streptococci and *Pseudomonas aeruginosa*

Resistance phenotypes					Viridans streptococci (N=4)		
					N	%	Phenotypes of all (%)
.	CRO	.	DA	.	1	25.0	50.0
.	.	E	DA	.	1	25.0	
CTX	CRO	.	DA	.	1	25.0	50.0
CTX	CRO	.	.	VA	1	25.0	
Total					4	100.0	

Resistance phenotypes					Pseudomonas aeruginosa (N=1)		
					N	%	Phenotypes of all (%)
.	1	100.0	100.0
Total					1	100.0	

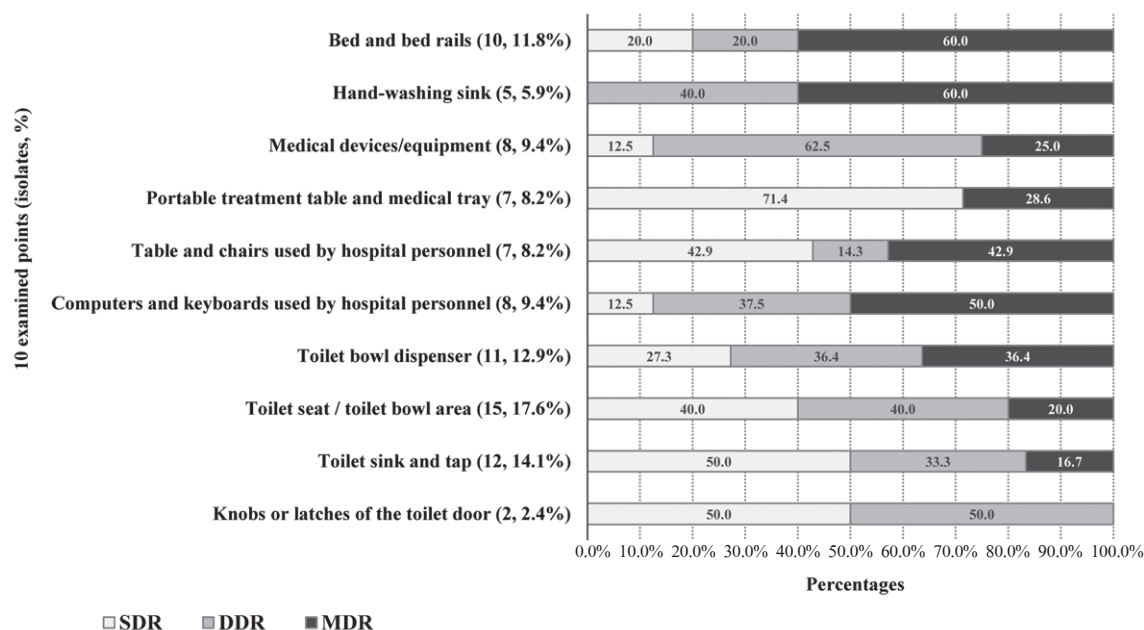
=sensitive, Antibiotic testing for Viridans streptococci: CTX=cefotaxime-resistant, CRO=ceftriaxone-resistant, E=erythromycin-resistant, DA=clindamycin-resistant, VA=vancomycin-resistant

Antibiotic testing for *Pseudomonas aeruginosa*: CIP=ciprofloxacin-resistant, AK=amikacin-resistant, CAZ=ceftazidime-resistant, CN=gentamycin-resistant

Table 6 Frequency distribution of antibiogram profiles of examined bacteria

Isolated organisms	SDR		DDR		MDR	
	% of type	% of all	% of type	% of all	% of type	% of all
<i>S. aureus</i> (5)	0	0	1/5 (20.0)	1/153 (0.7)	1/5 (20.0)	1/153 (0.7)
CoAg-Neg staphylococci (121)	23/121 (19.0)	23/153 (15.0)	21/121 (17.4)	21/153 (13.7)	20/121 (16.5)	20/153 (13.1)
Viridans streptococci (4)	0	0	2/4 (50.0)	2/153 (1.3)	2/4 (50.0)	2/153 (1.3)
<i>Escherichia coli</i> (3)	0	0	1/3 (33.3)	1/153 (0.7)	0	0
<i>Klebsiella pneumoniae</i> (1)	0	0	1/1 (100.0)	1/153 (0.7)	0	0
Others Enterobacteriaceae* (18)	5/18 (27.8)	5/153 (3.3)	2/18 (11.1)	2/153 (1.3)	6/18 (33.3)	6/153 (3.9)
<i>Pseudomonas aeruginosa</i> (1)	0	0	0	0	0	0
Total (153)		28/153 (18.3)		28/153 (18.3)		29/153 (19.0)

Values are % (n/N) for SDR, DDR, and MDR, CoAg-Neg=coagulase-negative, *=not specified but excluding *E. coli* and *K. pneumoniae*, SDR=single drug resistant, DDR=double drugs resistant, MDR=multi-drugs resistant (MDR, resistant to three or more antimicrobial classes²²)



Total ARB isolates=85/153 (55.6%); SDR=single drug resistant, DDR=double drugs resistant, MDR=multi-drugs resistant (MDR=resistant to three or more antimicrobial classes²²)

Figure 1 Distribution of ARB found at different environmental sampling points in 12 sub-district HPHs

This was associated with their DDR profiles, which were remarkable for clindamycin and erythromycin combinations (8.3%). Similarly, 20.0% of the *S. aureus* isolates also showed high resistance to these two drugs. This could be because CoAg-Neg staphylococci are potential sources of antibiotic resistance genes, which are capable of transferring these genes to *S. aureus*.²⁶ Moreover, a previous study demonstrated that macrolide resistance was more prevalent in CoAg-Neg staphylococci.²⁷ Erythromycin and clindamycin are antibiotics that inhibit protein synthesis by binding to the 50S ribosomal subunits of bacterial cells. Resistance to both drugs can occur through the methylation of the ribosomal target site.²⁸ In addition, macrolide resistance may be constitutive or inducible in the presence of either a macrolide or lincosamide inducer.²⁹ The rate of anti-staphylococcal β -lactams (oxacillin) resistance showed that MRCoNS was 19.8%, whereas no MRSA was detected.

This may be because of the greater proportion of CoAg-Neg staphylococci isolates than *S. aureus* isolates in the present study. In addition, these rates are lower than those of previous studies which reported 70.0% of MRCoNS¹², and 50.0% of MRSA strains found in hospital environments.³⁰

Viridans streptococci isolated from this study showed 100.0% (4/4) resistance to four categories of antibiotics: cepheems, macrolides, lincosamides, and glycopeptides. High levels of resistance to individual antibiotics were observed against cefotaxime, ceftriaxone, and clindamycin (75.0% each) (Table 2), which was higher than the rates of resistance to ceftazidime and ceftriaxone (53.0% and 22.0%, respectively), and the 18.0% resistance to clindamycin reported by another study.³¹ DDR and MDR profiles were noticeably aligned with a combination of these three distinct classes (ceftriaxone-clindamycin, cefotaxime-ceftriaxone-clindamycin, 25.0% each) (Table 5). In contrast, low

resistance to erythromycin and vancomycin was observed. This was in agreement with the findings of a prior study³², which suggested the use of glycopeptide (vancomycin-based regimens) for the empirical antibiotic treatment of viridans streptococcal infection.

For gram-negative isolates, 67.9% of Enterobacteriaceae appeared to be resistant to six categories of antibiotics: cephamycins, fluoroquinolones, folate pathway inhibitors, aminoglycosides, penicillins (and penicillins + β -lactamase inhibitors (Table 4). *E. coli* and *K. pneumoniae* isolates were notable for their resistance to ampicillin and ampicillin-sulbactam (Table 2), which is in agreement with a previous study.³³ Their DDR patterns were associated with these two antibiotic combinations (Table 4). In addition, different resistance profiles were observed among other members of Enterobacteriaceae (not specified, except *E. coli* and *K. pneumoniae*) because of the higher proportion and variation in strains, which was limited to identification in this study. They were most resistant to ampicillin (16.7%), followed by cefoxitin and ampicillin-sulbactam (5.6% each), in combination with their DDR and MDR profiles for these three different classes, together with ampicillin-clavulanic acid (Table 4). This might be because members of Enterobacteriaceae are highly resistant to commonly used β -lactam-based antibiotics (including ampicillin, ampicillin-sulbactam, and ampicillin-clavulanic acid).³⁴ Beyond limited information about the susceptibility profiles of common Enterobacteriaceae, it should be noted that ESBL production was not observed in any of the Enterobacteriaceae isolates in this study (data not shown). This may be because of the limited types of antibiotics used in sub-district HPHs, since their services focus generally on providing only primary care treatments for local community people, with no in-patient services.

Unlike other isolates in our study, *P. aeruginosa* was 100.0% sensitive to all categories of antibiotics tested. This was exceptional because *P. aeruginosa* is well known for its ability to be multidrug resistant, as supported by an

earlier study.³⁵ The small number of *P. aeruginosa* isolates in this study may have limited its resistance profile (only one isolate was detected in samples collected from 12 sub-district HPHs).

Nearly half of all isolates (85/153; 55.6%) were resistant to the selected antibiotic categories (Table 6). These ARB were found distributed in all sampling points, mainly on the surfaces of toilet areas, whereas most of MDR isolates was abundant on surfaces and equipment located in the patient treatment areas of the nursing rooms (Figure 1). This could reflect that routine daily disinfection practices on particular surfaces and equipment might not be adequate. The overall rates of SDR, DDR, and MDR seem to be associated with the proportion of isolated types, indicating that CoAg-Neg staphylococci (13.1%) were notably MDR phenotypes. Although the MDR rate of 19.0% reported in this study was much lower than the MDR rates reported by others (77.0%³⁶, 79.4%³⁰, and 81.5%³⁷), the occurrence of MDR phenotypes should be taken into consideration. In regards to this study, “worrying” rates of antibiotic resistance in bacteria contaminating different environmental samples of the sub-district HPHs were observed. This could indicate that sub-district HPHs may serve as sites of HAIs caused by bacteria which are capable of resistance to multiple categories of antibiotic drugs. Transmission of these ARB from sub-district HPHs to the community via contact between sub-district HPH personnel and their patients or clients, was inevitable. As a result, antibiotic resistance, especially MDR phenotypes, could become a problem in local communities since infections caused by these bacteria are more difficult or impossible to treat with currently available antibiotics, leading to greater morbidity and mortality, and resulting in higher healthcare costs.³⁶ It is worth noting that antibiotic stewardship and other preventive strategies are recommended to prevent the ARB transmission.³⁴ Practicing infection control standards to ensure the cleaning and use of cleaning agents on sub-district HPH environments, and keep checking on

antibiotic use and its resistance might be essential. For sub-district HPHs personnel, maintaining personal hygiene especially hand decontamination is required with proper hand disinfectants before and after being in contact with their patients or clients.³⁸ Furthermore, the surveillance of antibiotic susceptibility should be implemented in sub-district HPHs, since these are necessary to provide healthcare services to the community.³⁹

Conclusion

This study is the first to provide original data on the antibiogram profiles of the bacterial agents usually involved in HAIs found on surface areas and inanimate equipment of sub-district level hospitals in Thailand. By collecting samples from 12 sub-district HPHs in Chiang Rai, we highlighted the occurrence of ARB, especially MDR phenotypes, and pointed out the high chance of ARB cross-contamination and cross-infection, which poses a threat to the health of individuals in the nearby community when no preventive measures to control or limit bacterial spread are implemented. This study provides a basis for further studies on antibiotic stewardship and the surveillance of antibiotic susceptibility in sub-district HPHs in order to allow the development of measures that support consequential community healthcare in the near future.

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Conflict of interest

None declared.

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