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Existence of bacterial contamination on inanimate surfaces and equipment in sub-district health-promoting hospitals in Chiang Rai, Thailand

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Abstract

Hospital environments may serve as habitats for various microorganisms. Here, we show, for the first time, the existence of bacterial contamination on inanimate surfaces and equipment of 12 select sub-district health-promoting hospitals (HPHs) in six districts of Chiang Rai, Thailand. The swabbing technique was used to collect samples from 10 tested points (six from nursing rooms and four from toilets) of different sampling locations. The collected swabbed samples were propagated, isolated, and identified based on their biochemical properties. All 120 samples (100%) were found to be contaminated with 341 isolated bacterial strains, representing a predominance of coagulase-negative staphylococci (35.48%) and *Bacillus* sp. (19.06%). *Staphylococcus aureus* and viridans streptococci accounted for 1.47% and 1.17% of all isolate bacteria, respectively. The majority of gram-negative bacteria belonged to Enterobacteriaceae, including *Escherichia coli* (0.88%), *Klebsiella pneumoniae* (0.29%), and others (9.09%). Less strain of *Pseudomonas aeruginosa* was found (0.29%). The toilet's hand-washing sinks, followed by hospital personnel's computers and keyboards, were identified as surfaces with the largest bacterial colonization. Our results demonstrate promising evidence of environmental contaminants in HPHs. Although they were not considered pathogenic strains, this contamination should be a concern. Appropriate disinfection procedures should be encouraged to reduce the transmission of "unconsidered" bacterial contaminants among HPHs and the community population.

Keywords**:** *bacterial contamination; sub-district health-promoting hospitals; inanimate surfaces and equipment.*

1. Introduction

Hospital environments, particularly those frequently touched surfaces, and equipment involved in the patient treatment may be considered as "secondary reservoir" for contaminated bacteria. Practicing treatment activities and handling of medical equipment during routine care could lead to transmission or cross-contamination of these bacteria to patients, relatives, and even hospital personnel. Several studies have reported the contamination of microorganisms in the hospital environment surfaces and equipment, and a variety of pathogenic and non-pathogenic microorganisms have been observed. The predominant bacteria on hospital surfaces taken from armrest beds, wash sinks, medical tables, and the hands of medical staff were *Klebsiella* sp., *Pseudomonas* sp., and *Escherichia* sp. (Garcia, Najera, & Arroyo, 2012). *Bacillus* sp. and coagulase-negative (CoAg-Neg) staphylococci were found predominantly on surfaces of the emergency unit, central block, and neonatal unit of a provincial public hospital in the city of Fez, Morocco (Lalami, Touijer, Ettayebi, & Benchemsi, 2016). It has been shown that many different bacterial species can persist on hospital surfaces for months and years, related to the presence of humidity and low-temperature conditions (de Abreu, Farias, Paiva, Almeida, &

Morais, 2014). Contamination may occur during hand manipulation, not only by direct patient shedding of bacteria that could survive up to several months on these surfaces but also from the hands of hospital personnel (Russotto, Cortegiani, Raineri, & Giarratano, 2015). Not surprisingly, heavy contamination by bacteria on inanimate surfaces and equipment in intensive care units has been reported (Teng et al., 2009; Russotto et al., 2015; Yusuf et al., 2017). Among various types of hospital surfaces and equipment, computer user interfaces and workstations of healthcare workers have also been implicated. The extent of microbial contamination (*Staphylococcus aureus*, viridans streptococci, enterococci, and gram-negative bacteria) was found on computer user interfaces (keyboard, mouse) in a large tertiary care center under conditions of practice (Engelhart et al., 2008). Some author (Ngonda, 2017) also suggested that door handles or knobs of toilets and bathrooms in hospitals were among the most common sources of bacterial contamination, with the highest quantity of *S. aureus*, followed by *Escherichia coli* and *Pseudomonas aeruginosa*. As mentioned above, numerous bacterial strains from hospital surfaces and equipment of frequent contact are likely to be transmitted to patients, relatives, and hospital personnel. This kind of transmission can cause bacterial infections in hospital environments, commonly known as hospital-acquired infections (HAIs) or "nosocomial infections". Transmission of infectious agents from contaminated hospital surfaces, medical equipment, or hospital personnel may serve as potential sources of these infections (Uneke, Ogbonna, Oyibo, & Onu, 2010). *P. aeruginosa*, *S. aureus*, and *E. coli* play a major role in causing HAIs, whereas *Streptococcus* spp., *Acinetobacter* spp., enterococci, CoAg-Neg staphylococci, *Bacillus cereus*, *Legionella* spp., and other members of Enterobacteriaceae family, including *Proteus mirabilis*, *Klebsiella pneumonia*e, and *Serratia marcescens*, are usually involved (Khan, Ahmad, & Mehboob, 2015). Few data are available concerning bacterial contamination in hospital environments in Thailand. However, there have not been any documents indicating the quantity of bacterial types on contaminated surfaces and equipment of sub-district health-promoting hospitals (HPHs). The HPH, which is located in every sub-district in Thailand, was modified from a traditional public health center and has been established since 2009. HPH is a primary care unit service that provides public health services at the village, sub-district, and local community levels (Chaikoolvatana & Pakasit, 2018). There were still limitations of HPHs, especially the lack of physicians; therefore, HPHs focus mainly on providing only primary care treatment by nurses and public health officers. As a result, there was neither in-patient acceptance nor the infection control department, which was the major difference from higher-level hospitals, including community hospitals, general hospitals, and regional hospitals. Therefore, this study aimed to investigate the existence of bacterial contamination on inanimate surfaces and equipment on frequently touched surfaces from 12 select sub-district HPHs in Chiang Rai, Thailand. Identifying the types and distribution of bacteria on these contaminated surfaces may serve as promising evidence to determine HPH environmental contaminants for the first time. In addition, the results from this preliminary study can be attributable for each HPH to encourage appropriated measures of regular cleaning and disinfection procedures, assisting to reduce transmission of "unconsidered" bacterial contaminants which can lead to HAIs, among HPHs and the community population in Chiang Rai.

2. Objectives

The objective of this study was to determine the bacterial contamination on inanimate surfaces and equipment on frequently touched surfaces from 12 select sub-district HPHs in Chiang Rai, Thailand.

3. Materials and methods

3.1 Samples and sampling location

The samples were collected over a period of 3 months, from January 01 to March 31, 2018, from inanimate surfaces and equipment of 12 sub-district HPHs in 6 districts of Chiang Rai, Thailand. The samples were taken from 10 tested points; 6 points from nursing rooms, including (1) bed and bed rails, (2) hand-washing sink, (3) medical devices/equipment (stethoscopes, forceps, bottles of antiseptic solution, and bottles of normal saline solution); (4) portable treatment table and medical tray; (5) table and chair of hospital personnel; and (6) hospital personnel's computer and keyboards, and other 4 points from toilets consisting of (7) toilet bowl dispenser, (8) toilet seat/toilet bowl area, (9) toilet hand-washing sink and tap, and (10) knobs

or latches of the toilet door. The sampling location-targeted the districts located 10-40 km from the laboratory (Medical Science Laboratory, Scientific and Technological Instrument Center [STIC] of Mae Fah Luang University [MFU], Muang District, Chiang Rai) for convenience of traveling, and collected samples were transferred to the laboratory within 2-3 h. The choice of sampling points was made according to the review literature (Garcia et al., 2012; de Abreu et al., 2014; Srion & Nathapindhu, 2015; Chitpirom, 2013; Carvalho, Melo, Melo, Gontijo-Filho, 2007) considering the most exposure and the most representative points of HPH services for clients and patients.

3.2 Samples collection

The swabbing technique, according to (Department of Medical Sciences, Ministry of Public Health, Thailand, 2014), was used to collect samples from 10 tested points. Sterile swabs were moistened with sterile 0.9% normal saline and applied to defined areas (2×2 $inch^2$) by parallel spaced stripes by rotating them slightly on the same areas in perpendicular stripes (three times repeat). The swab samples were collected in sterile 15-ml centrifuge tubes and then delivered quickly, in a cooler kept at 5±3°C, to be analyzed at the Medical Science Laboratory, STIC, MFU.

3.3 Isolation and identification of bacteria

The collected swabbed samples were immersed for propagation in bacterial culture media, tryptic soy broth, and incubated at 37±1°C for 24 h. These broth cultures were used to streak plates containing the differential and selective culture medium; mannitol salt agar for the growth of staphylococci; blood agar (5% Sheep Blood Agar, Professional Nanomed Co. Ltd., Bangkok, Thailand) for the growth of streptococci, and MacConkey agar for the growth of gram-negative bacteria (coliforms; *E. coli* and *Klebsiella* sp., and *Pseudomonas* sp.), and incubated at 37±1°C for 24 h. After incubation, bacterial colonies were isolated and identified based on their biochemical properties (Cappuccino & Sherman, 2005; Brown & Smith, 2012), as shown in **Figure 1** and **Figure 2**. Briefly, staphylococci and streptococci were distinguished by Gram staining and catalase test. Coagulase (Becton, Dickinson and Company, MD, USA) test was used to classify among two groups as coagulase-positive staphylococci (*S. aureus*) and coagulase-negative (CoAg-Neg) staphylococci. The susceptibility to novobiocin (OXOID Ltd., Cheshire, UK) verified CoAg-Neg staphylococci as *S. epidermidis* or *S. saprophyticus*. Identification of β-hemolytic streptococci was approved by the Bacitracin (OXOID Ltd.) test for Group A Streptococcus and by CAMP test for Group B Streptococcus. Non-β-hemolytic streptococci were identified by positive growth on bile esculin agar and ability to grow in 6.5% NaCl broth for Group D streptococci and Group D enterococci. The Optochin (OXOID Ltd.) test was used to classify viridans streptococci and *S. pneumoniae*. The appearance of endospore formation after Gram staining was verified by endospore staining (Wirtz-Conklin method) and positivity for catalase test were used to confirm *Bacillus* sp., among other non-endospore-forming rod-shaped, gram-positive bacteria (not specified). Gram-negative bacteria were separated into cocci shapes (not specified) and rod shapes. Members of the family Enterobacteriaceae were identified by a lactose fermenter, oxidase (Sigma-Aldrich, MO, USA) negative, and production of Acid/Acid and Gas (A/AG) on Triple Sugar Ion (TSI) (HiMedia Laboratories Pvt. Ltd., Mumbai, India), motile and indole (HiMedia Laboratories Pvt. Ltd.) test, citrate (HiMedia Laboratories Pvt. Ltd.) test, and urea (HiMedia Laboratories Pvt. Ltd.) test were used to classify Enterobacteriaceae members as *E. coli* (positive motile and indole test, negative citrate test), *K. pneumoniae* (negative indole test, positive urea test), and other Enterobacteriaceae (not specified). The non-lactose fermenter was verified as *P. aeruginosa* by oxidase positive, the appearance of alkaline slant/no change (K/N) or no change/no change (N/N) on TSI, positive OF test, and pigment production on Pseudo F agar (HiMedia Laboratories Pvt. Ltd.) (yellow pigment of fluorescein), and Pseudo P agar (HiMedia Laboratories Pvt. Ltd.) (blue pigment of pyocyanin). Regular quality control of sterilization operation for culture media (by autoclaving), reagents, materials, and equipment, and the laboratory conditions (temperature and humidity) were performed according to the requirements of the Medical Science Laboratory, STIC, MFU. All isolated bacterial strains were preserved and stored in nutrient broth containing 20% (v/v) glycerol and stored at freezing temperature for further analysis. *Staphylococcus aureus* DMST8840, *Staphylococcus epidermidis* DMST15505, *Staphylococcus saprophyticus* DMST15512,

Escherichia coli DMST4212, *Klebsiella pneumoniae* DMST8216, and *Pseudomonas aeruginosa* DMST4739 (obtained from the Department of Medical Sciences, Thailand; DMST) were used as reference bacterial strains for confirmatory identifications of *S. aureus*, *S. epidermidis*, *S. saprophyticus*, *E. coli*, *K. pneumoniae*, and *P. aeruginosa*, respectively. The results were analyzed using descriptive statistics and were expressed as number and percentage.

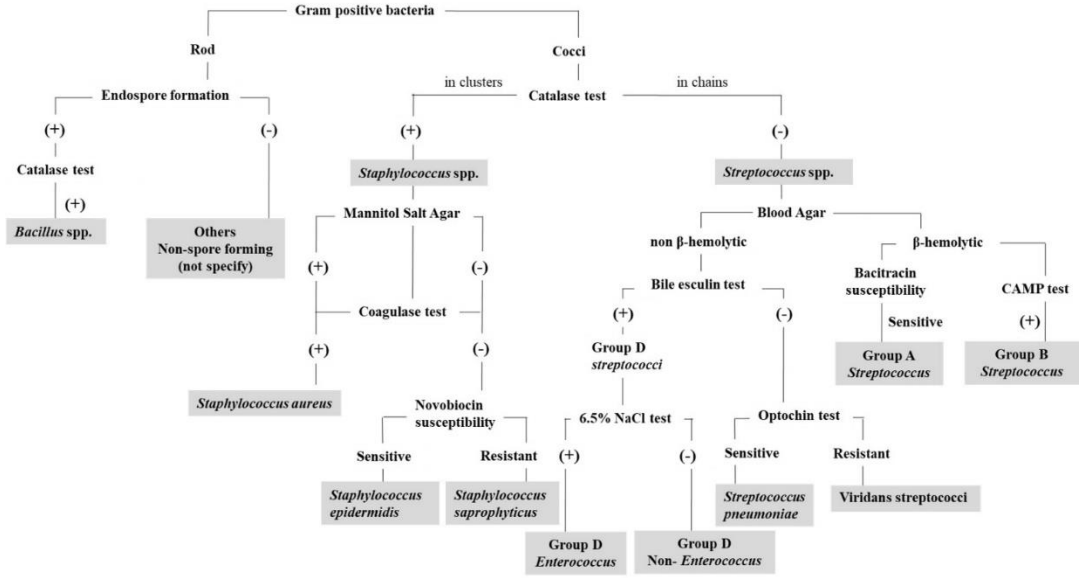

Figure 1 Schema for the identification of Gram-positive bacteria; NOTE : + = *positive test*, - = *negative test*

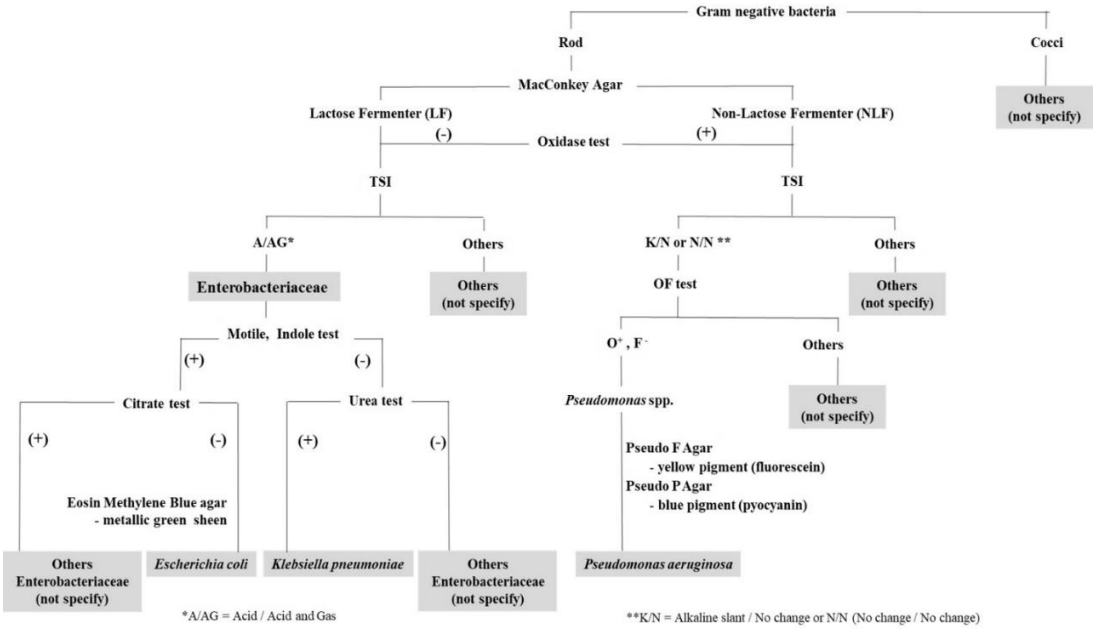

Figure 2 Schema for the identification of Gram-negative bacteria; NOTE : + = *positive test*, - = *negative test*

4. Results

A total of 120 samples taken from 10 tested points of inanimate surfaces and equipment of the 12 select sub-district HPHs, were isolated and identified for specific types of contaminated bacterial strains, using the methods mentioned above. Many bacteria were found in all samples, indicating that all the tested points included bacteria. A total of 341 isolated bacteria, classified into gram-positive 248 isolates (72.73%) and 93 gram-negative isolates (27.27%), were isolated from different inanimate surfaces and equipment. Forty isolates (11.73%) of contaminated bacteria were from HPH H, followed by HPH B (37 isolates, 10.85%). Details of the isolated strains from the inanimate surfaces and equipment of 12 select sub-district HPHs are presented in **Table 1**. The sorts of various bacterial strains isolated from studied hospital surfaces and equipment showed a predominance of species: CoAg-Neg staphylococci (35.49%) and *Bacillus* sp. (19.06%). *S. aureus*, and viridans streptococci represented 1.47% and 1.17%, respectively. The majority of gram-negative bacteria belonged to the Enterobacteriaceae family, including *E. coli* (0.88%), *K. pneumoniae* (0.29%), and others (9.09%). The lowest was found in *P. aeruginosa* (0.29%). In contrast, there were species of isolated bacteria that have not yet been specified. They were represented by gram-positive non-spore-forming, gram-negative cocci, and other gram-negative rods (others excluded from Enterobacteriaceae and *Pseudomonas* sp.) 15.54%, 2.05%, and 14.66%, respectively.

The distribution of the isolated bacteria from different tested points is shown in **Table 2**. Among the 10 tested points, the toilet's hand-washing sink and tap (9th tested point) were the most contaminated points (49 isolates; 14.37%) with *S. epidermidis*, *Bacillus* sp., and other gram-negative rods, as the highest isolation frequency of 20.41% for each, followed by the toilet seat/toilet bowl area (8th tested point; 12.02%) and the hospital personnel's computer and keyboards (6th tested point; 11.44%), which mostly contaminated with *S. epidermidis* (46.34%, and 30.77% of isolation frequency, respectively). The knobs or latches of the toilet door (10th tested point) was the least contaminated (22 isolates; 6.45%), mostly by *S. epidermidis* with an isolation frequency of 45.45%.

The relation between bacterial contamination of 10 tested points examined among 12 select sub-district HPHs is presented in **Table 3.** Of 12 select HPHs, eight (66.67%) had a contamination rate of 100%, with an average rate of 94.17%. Inanimate surfaces and equipment of nursing rooms were more likely to be contaminated (95.83%) compared to those of toilets (91.67%). As measured by isolated bacterial strains (**Table 4**), inanimate surfaces and equipment of nursing rooms were observed to have slightly higher contamination rates (61.11%) compared to those of toilets (60.42%). The results further showed that *S. epidermidis*, *Bacillus* spp., gram-positive rod non-spore-forming, and other gram-negative rods contamination had the highest contamination rate (100%), followed by others Enterobacteriaceae (90%) and *S. saprophyticus* (80%), while the average bacterial contamination rate was 60.83%. The presence of four groups at all tested points examine included CoAg-Neg staphylococci, *Bacillus* sp., gram-positive rod non-spore-forming, and other gram-negative rods, with percentages ranging from 1% to 16%, 6% to 16%, and 2% to 17%, and 4% to 20%, respectively (**Figure 3**).

As determined by strains with a small proportion, *S. aureus* strains (1.47%) were isolated only from inanimate surfaces of nursing rooms (at the bed and bed rails; 1st tested point, hand-washing sink; 2nd tested point, and the table and chair of hospital personnel; 5th tested point), of four HPHs (B, H, K, and L), with a contamination rate of 30%. Viridans streptococci (1.17%) showed a contamination rate of 30% and were isolated only from inanimate surfaces and equipment/objects of toilet areas of four HPHs (B, G, K, and L). *E. coli* was found to be 0.88% at the portable treatment table and medical tray; 4th tested point, and the toilet seat/toilet bowl area; 8th tested point, of two HPHs (G and K), with a contamination rate of 20%. *P. aeruginosa* and *K. pneumoniae*, with a proportion of 0.29% and contamination rate of 10% each, were found only at the toilet's hand-washing sink and tap; 9th test point of HPH B, and the knobs or latches of the toilet door; 10th tested point of HPH G, respectively. Gram-negative cocci (2.05%) found in the knobs or latches of the toilet door (10th tested point), the bed and bed rails (1st tested point), medical devices/equipment (3rd tested point), portable treatment table and medical tray (4th tested point), table and chair of hospital personnel (5th tested point), toilet's hand-washing sink and tap (9th tested point), as well as others Enterobacteriaceae (excluding *E. coli* and *K. pneumoniae*; 9.09%), found in all tested points except the knobs or latches of the toilet door (10th tested point), have not been specified.

Table 1 Isolated bacterial strains from the inanimate surfaces and equipment of 12 select sub-district HPHs

	Characteristics			HPHs												Number of isolates	% of isolate types	% of all isolates
				A	B	C	D	E	F	G	H	I	J	K	L			
+	Cocci	***Staphylococcus* spp. (N=126)**	CoAg-Pos (*S. aureus*)	-	1	-	-	-	-	-	2	-	-	1	1	**5**	2.02	**1.47**
			CoAg-Neg	5	13	12	17	11	17	10	11	7	7	7	4	**121**	48.79	**35.48**
			(S. epidermidis)	(2)	(5)	(12)	(14)	(10)	(17)	(10)	(11)	(7)	(3)	(6)	(4)	**(101)**	(40.73)	**(29.62)**
			(S. saprophyticus)	(3)	(8)	(-)	(3)	(1)	(-)	(-)	(-)	(-)	(4)	(1)	(-)	**(20)**	(8.06)	**(5.86)**
		***Streptococcus* spp. (N=4)**	Viridans streptococci	-	1	-	-	-	-	1	-	-	-	1	1	**4**	1.17	**1.17**
	Rod	**Spore-forming** (*Bacillus* sp.) **(N=65)**		4	-	8	7	7	3	5	9	8	8	3	3	**65**	26.21	**19.06**
		Non-spore forming (not specify) **(N=53)**		3	13	1	8	6	8	1		1	1		11	**53**	21.37	**15.54**
	Total of Gram-positive (isolates)			**12**	**28**	**21**	**32**	**24**	**28**	**17**	**22**	**16**	**16**	**12**	**20**	**248**	**100**	**72.73**
-	Cocci	Not specify		-	-	-	-	1	-	1	2	3	-	-	-	**7**	7.53	**2.05**
	Rod	**Enterobacteriaceae (N=35)**	*Escherichia coli*	-	-	-	-	-	-	2	-	-	-	1	-	**3**	3.23	**0.88**
			Klebsiella pneumoniae	-	-	-	-	-	-	1	-	-	-	-	-	**1**	1.08	**0.29**
			Others (not specify)	1	2	1	-	-	1	6	10	2	3	2	3	**31**	33.33	**9.09**
		***Pseudomonas* spp. (N=1)**	*Pseudomonas aeruginosa*	-	1	-	-	-	-	-	-	-	-	-	-	**1**	1.08	**0.29**
		Others (N=50)	Not specify	5	6	4	4	5	3	1	6	1	5	8	2	**50**	53.76	**14.66**
	Total of Gram-negative (isolates)			**6**	**9**	**5**	**4**	**6**	**4**	**11**	**18**	**6**	**8**	**11**	**5**	**93**	**100**	**27.27**
	Total isolates			**18**	**37**	**26**	**36**	**30**	**32**	**28**	**40**	**22**	**24**	**23**	**25**	**341**		**100**
	(%)			**5.28**	**10.85**	**7.62**	**10.56**	**8.80**	**9.38**	**8.21**	**11.73**	**6.45**	**7.04**	**6.74**	**7.33**	**100**		

NOTE: + = Gram-positive bacteria; - = Gram-negative bacteria; CoAg-Pos = Coagulase-positive; CoAg-Neg = Coagulase-negative

Table 2 Distribution of isolated bacteria from different tested points of the inanimate surfaces and equipment of 12 select sub-district HPHs

		CoAg-Pos	CoAg-Neg						Enterobacteriaceae					
Tested points	**Characteristics**	**StaA**	**StaE**	**StaS**	**VStr**	**Ba**	**posR***	**negC***	**EC**	**KP**	**otEn***	**otnegR***	**PA**	**n (%)**
1 (SN)	Bed and bed rails	3	8	2	-	10	2	1	-	-	5	3	-	**34 (9.97)**
	n (%)	(8.82)	(23.53)	(5.88)	(-)	(29.41)	(5.88)	(2.94)	(-)	(-)	(14.71)	(8.82)	(-)	
2 (SN)	Hand-washing sink	1	1	-	-	5	6	-	-	-	5	9	-	**27 (7.92)**
	n (%)	(3.70)	(3.70)	(-)	(-)	(18.52)	(22.22)	(-)	(-)	(-)	(18.52)	(33.33)	(-)	
3 (EN)	Medical devices/equipment	-	12	4	-	5	8	1	-	-	2	3	-	**35 (10.26)**
	n (%)	(-)	(34.29)	(11.43)	(-)	(14.29)	(22.86)	(2.86)	(-)	(-)	(5.71)	(8.57)	(-)	
4 (SN)	Portable treatment table and medical tray	-	8	3	-	6	7	1	1	-	1	2	-	**30 (8.50)**
	n (%)	(-)	(27.59)	(10.34)	(-)	(20.69)	(24.14)	(3.45)	(3.45)	(-)	(3.45)	(6.90)	(-)	
5 (SN)	Table and chair of hospital personnel	1	10	2	-	4	4	1	-	-	4	3	-	**29 (8.50)**
	n (%)	(3.45)	(34.48)	(6.90)	(-)	(13.79)	(13.79)	(3.45)	(-)	(-)	(13.79)	(10.34)	(-)	
6 (EN)	Hospital personnel's computer and keyboards	-	12	4	-	7	7	-	-	-	3	6	-	**39 (11.44)**
	n (%)	(-)	(30.77)	(10.26)	(-)	(17.95)	(17.95)	(-)	(-)	(-)	(7.69)	(15.38)	(-)	
7 (ET)	Toilet bowl dispenser	-	11	2	1	6	6	-	-	-	3	7	-	**36 (10.56)**
	n (%)	(-)	(30.56)	(5.56)	(2.78)	(16.67)	(16.67)	(-)	(-)	(-)	(8.33)	(19.44)	(-)	
8 (ST)	Toilet seat / toilet bowl area	-	19	-	2	6	3	-	2	-	4	5	-	**41 (12.02)**
	n (%)	(-)	(46.34)	(0.00)	(4.88)	(14.63)	(7.32)	(-)	(4.88)	(-)	(9.76)	(12.20)	(-)	
9 (ST)	Toilet's hand-washing sink and tap	-	10	3	1	10	9	1	-	-	4	10	1	**49 (14.37)**
	n (%)	(-)	(20.41)	(6.12)	(2.04)	(20.41)	(18.37)	(2.04)	(-)	(-)	(8.16)	(20.41)	(2.04)	
10 (ET)	Knobs or latches of the toilet door	-	10	-	-	6	1	2	-	1	-	2	-	**22 (6.45)**
	n (%)	(-)	(45.45)	(-)	(-)	(27.27)	(4.55)	(9.09)	(-)	(4.55)	(-)	(9.09)	(-)	
	Total	**5**	**101**	**20**	**4**	**65**	**53**	**7**	**3**	**1**	**31**	**50**	**1**	**341 (100)**
	n (%)	**(1.47)**	**(29.62)**	**(5.87)**	**(1.17)**	**(19.06)**	**(15.54)**	**(2.05)**	**(0.88)**	**(0.29)**	**(9.09)**	**(14.66)**	**(0.29)**	

NOTE : CoAg-Pos = Coagulase-positive, CoAg-Neg = Coagulase-negative; S= Inanimate surfaces, E=Equipment/objects, N = Nursing rooms, T= Toilets; StaA= *Staphylococcus aureus*, StaE= *Staphylococcus epidermidis*, StaS= *Staphylococcus saprophyticus*, VStr= Viridans streptococci, Ba = Gram-positive rod spore-forming (*Bacillus* sp.), posR= Gram-positive rod non-spore forming, negC = Gram-negative cocci, EC= *Escherichia coli*, KP= *Klebsiella pneumoniae*, otEn= others Enterobacteriaceae, otnegR = other Gram-negative rods**,** PA= *Pseudomonas aeruginosa*, *= Not specify, n (%) represented as % of contamination at each tested point

Table 3 Relation between bacterial contamination of different tested points among 12 select sub-district HPHs

Area	Tested point	HPHs	A	B	C	D	E	F	G	H	I	J	K	L	
		No. of examined	No. of contaminated												Average
Nursing rooms	Inanimate surfaces	4	2	4	3	4	4	4	4	4	4	4	4	4	**3.75**
	Equipment/objects	2	2	2	2	2	2	2	2	2	2	2	2	2	**2.00**
	Total	**6**	**4**	**6**	**5**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**5.75**
	Contamination rate (%)		**66.7**	**100**	**83.3**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**95.83**
Toilets	Inanimate surfaces	2	2	2	2	2	2	2	2	2	2	2	2	2	**2.00**
	Equipment/objects	2	0	1	2	2	2	2	2	2	2	1	2	2	**1.67**
	Total	**4**	**2**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3.67**
	Contamination rate (%)		**50**	**75**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**75**	**100**	**100**	**91.67**
	Total	**10**	**6**	**9**	**9**	**10**	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**	**9.42**
	Total contamination rate (%)		**60**	**90**	**90**	**100**	**100**	**100**	**100**	**100**	**100**	**90**	**100**	**100**	**94.17**

Table 4 Relation between bacterial contamination of different tested points defined by isolated strains

Area	Tested point		CoAg-Pos	CoAg-Neg						Enterobacteriaceae					
			StaA	StaE	StaS	VStr	Ba	posR*	negC*	EC	KP	otEn*	otnegR*	PA	
		No. of examined	No. of contaminated												Average
Nursing rooms	Inanimate surfaces	4	3	4	4	0	4	4	3	1	0	4	4	0	**2.58**
	Equipment/objects	2	0	2	2	0	2	2	1	0	0	2	2	0	**1.08**
	Total	6	3	6	6	0	6	6	4	1	0	6	6	0	**3.67**
	Contamination rate (%)		**50**	**100**	**100**	**0**	**100**	**100**	**67**	**17**	**0**	**100**	**100**	**0**	**61.11**
Toilets	Inanimate surfaces	2	0	2	1	2	2	2	1	1	0	2	2	1	**1.33**
	Equipment/objects	2	0	2	1	1	2	2	1	0	1	1	2	0	**1.08**
	Total	4	0	4	2	3	4	4	2	1	1	3	4	1	**2.42**
	Contamination rate (%)		**0**	**100**	**50**	**75**	**100**	**100**	**50**	**25**	**25**	**75**	**100**	**25**	**60.42**
	Total	10	3	10	8	3	10	10	6	2	1	9	10	1	**6.08**
	Total contamination rate (%)		**30**	**100**	**80**	**30**	**100**	**100**	**60**	**20**	**10**	**90**	**100**	**10**	**60.83**

NOTE : CoAg-Pos = Coagulase-positive, CoAg-Neg = Coagulase-negative; S= Inanimate surfaces, E=Equipment/objects, N = Nursing rooms, T= Toilets; StaA= *Staphylococcus aureus*, StaE= *Staphylococcus epidermidis*, StaS= *Staphylococcus saprophyticus*, VStr= Viridans streptococci, Ba = Gram-positive rod spore-forming (*Bacillus* sp.), posR= Gram-positive rod non-spore forming, negC = Gram-negative cocci, EC= *Escherichia coli*, KP= *Klebsiella pneumoniae*, otEn= others Enterobacteriaceae, otnegR = other Gram-negative rods**,** PA= *Pseudomonas aeruginosa*, *= Not specify

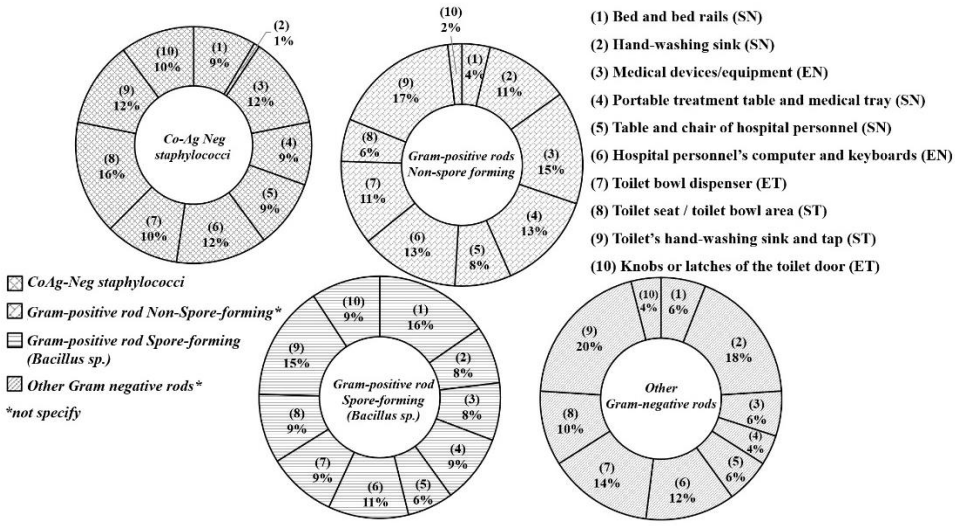

Figure 3 Percentage of 4 bacterial groups found in all tested points; CoAg-Neg staphylococci (left top), Gram-positive rod non-spore forming (right top), Gram-positive rod spore-forming *(Bacillus sp.),* (left bottom), and other gram-negative rods (right bottom); NOTE : CoAg-Neg = Coagulase-negative; S= Inanimate surfaces, E=Equipment/objects, N = Nursing rooms, T= Toilets.

5. Discussion

In this study, a total of 120 samples were collected to monitor the existence of bacterial contamination on inanimate surfaces and equipment of 12 select sub-district HPHs in Chiang Rai. All samples (100%) were found to be positive, contaminated with 341 isolated bacterial strains from 10 different tested points, remarkably greater than those of previous studies (>50% [Sergent et al, 2012], 96% [Lalami et al., 2016], and 98.7% [Cataño, Echeverri, & Szela, 2012]). The types of the isolated bacterial strains varied, but the majority of them were *Staphylococcus* spp. (126 isolates; 36.95%). Among the strains isolated from samples, a pattern of quantitative contamination by the dominance of CoAg-Neg staphylococci (*S. epidermidis* and *S. saprophyticus*; 35.48%) and *Bacillus* sp. (19.06%) was noted, consistent with previous studies (Lalami et al., 2016; Nabila et al., 2014). This could be explained by the fact that staphylococci are part of the normal skin flora, and CoAg-Neg staphylococci, especially *S. epidermidis*, had a high potential to form biofilms on hospital surfaces (Shaheen & Baqai, 2016). Meanwhile, *Bacillus* sp. bacteria are abundant in the environment because they possess endospore forming, which provides the ability to constantly survive in the environment and withstand bio-cleaning accessibility (Meunier et al, 2005). The largest colonization by bacteria on equipment located in the nursing rooms was the hospital personnel's computer and keyboards. This was not unexpected since computer use was very regular in hospitals. This finding was in agreement with a previous study (Karbasizade, Sichani, & Parsafar, 2014) in which computer keyboard contamination was common, and the contaminant bacteria mostly included *Bacillus* sp. or CoAg-Neg staphylococci, which are widely dispersed in air or soil. Therefore, computers and keyboards may become reservoirs for bacteria. Moreover, the highest contamination rates were found immediately after the user touched the computer workstation (Engelhart et al., 2008). Touching computers and keyboards with hospital personnel's hands increased the likelihood of bacterial transmission. Therefore, it has been suggested that computers and keyboards in hospitals should be adequately disinfected daily with 70% ethanol.

Types of contaminated bacteria found on surfaces and equipment of four patient treatment areas of the nursing room (bed and bed rails, hand-washing sink, medical devices/equipment, portable treatment table, and medical tray) were almost identical. The patterns of contaminated bacterial types included CoAg-Neg staphylococci (*S. epidermidis*), *Bacillus* sp., gram-positive rods non-spore-forming, others Enterobacteriaceae, and other gram-negative rods, while *S. aureus* and *E. coli* were found only on surfaces of those areas (**Table 2**). These findings were interesting because surfaces and equipment in the patient treatment areas must be cleaned and disinfected daily. Similar findings were also observed for the contamination of *Bacillus* sp. (30%) and CoAg-Neg staphylococci (24%), *S. aureus* (9%), other gram-negative rods (5%), and *E. coli* (2%) on hospital surfaces in Morocco (Lalami et al., 2016), CoAg-Neg staphylococci (87.6%) (*S. epidermidis* as the predominant) on stethoscopes of a tertiary hospital in Greece (Leontsini, Papapetropoulos, & Vantarakis, 2013), *S. aureus* (50%) on bedside table, bed rail, and door handle of a hospital in Brazil (Carvalho et al., 2007), gram-negative rods (*Klebsiella* sp.; 50.45%, *Pseudomonas* sp.; 32 %, and *E. coli*; 9.17%) on the wash sink and medical table surfaces of a hospital in Mexico (Garcia et al., 2012), CoAg-Neg staphylococci and *Bacillus* sp. in the room and four corners of operating beds in a hospital operating room in Thailand (Tankaew, Komolmal, Jainoonwong, Intayot, & Sutabhaha, 2009). Although most of these bacteria were not considered pathogenic bacteria, contamination found on non-critical objects and surfaces should be considered. This could indicate that these contaminated bacteria could survive and withstand daily disinfection based on surfaces for months or years, and they might constitute a bacterial reservoir. Patient treatment or patient surrounding areas could represent a marker of ubiquitous bacterial contamination in hospital inanimate objects and surfaces, especially hospital bed-control handsets where both hospital personnel and patients frequently come in direct contact (Brady, Kalima, Damani, Wilson, Dunlop, 2007). It has been also suggested that routine daily disinfection practices on particular surfaces might not be adequate. The use of disinfectants should be considered as well as more effective or new cleaning and decontamination practices might be required. Identifying frequent-contact surfaces and objects that lead to a higher risk of bacterial transmission should be undertaken. Lei, Jones, and Li (2017), reported the cleaning strategies of hospital surfaces

approved by a mathematical model. Using a wipe or cloth containing disinfectant, daily whole treatment room cleaning, especially cleaning just before the first patient care activities of the day, and supplemented with frequent targeted cleaning of high and frequent-touch surfaces were shown to be more effective than the whole room cleaning at other times. Regarding toilet areas, the greatest number of bacterial colonization was found in the toilet's hand-washing sinks and tap (Lei et al., 2017). This result was supported by Chitpirom (2013), who reported that hand-washing sinks and taps in toilet areas are a bacterial contamination risk area. Furthermore, wet conditions (with moist and humid) in toilet areas, such as hand-washing sinks and non-covered toilets, could facilitate the growth of contaminant bacteria and serve as sources of fecal bacteria. These bacteria were then capable of being spread in the air through aerosols from toilet flushing and transmitted through contact with hand-washing sinks by fingers and skin (Pesevska et al., 2016).

A uniform pattern of contaminated bacteria compared to patient treatment areas was isolated from toilet areas, except for viridans streptococci, *K. pneumoniae*, and *P. aeruginosa*. Our results showed that viridans streptococci were abundant on all surfaces of toilet areas. Viridans streptococci represent a member of *Streptococcus* species and are commensals of the oral cavity, upper respiratory, gastrointestinal, and genitourinary tracts (Han, Kamana, & Rolston, 2006). The remaining of them on public toilet surfaces, especially surfaces routinely touched with hands, was unexceptional, supported by a previous study (Flores et al., 2011). In contrast, the presence of *E. coli* and other gram-negative rods, including members of Enterobacteriaceae (others than *E. coli* and *K. pneumoniae*) was predictable. However, the appearance of *E. coli*, known as fecal coliform bacteria, is a reasonable indication of fecal contamination. It should be noted that individuals (hospitals' clients or even hospital personnel) performed toilet-use behaviors that might not be in accordance with sanitation principles. As a result, hospital surfaces and toilet areas may become the potential accumulation and spread of opportunistic pathogens (Chitpirom, 2013). Transmission between individuals by touching toilet surfaces was probable. The greater number of toilet users daily would subsequently lead to higher toilet surface contamination. The only site detected in *K. pneumoniae* was the knobs or latches of the toilet doors, in agreement with a previous study (Ngonda, 2017). The isolation of *E. coli* and *P. aeruginosa* on door handles/knobs of hospital toilets has been reported. Nevertheless, in the present study, *P. aeruginosa* was found on the toilet's hand-washing sinks and tap. Unexpectedly, heavy contamination with the *P. aeruginosa* strain was found on the triclosan soap dispenser at the hand-washing sink (Lanini et al., 2011). Although this contamination on soap dispensers is uncertain, *P. aeruginosa* is intrinsically unsusceptible to triclosan. In addition, they recommended the use of clorexidine-based soap owing to its highest bactericidal activity, provided with disposable, non-refillable cartridges, and implemented the use of alcohol-based hand rub when appropriate.

The results from this study imply the importance of the hands as a role of bacterial transmission. Concurrently, hospitals inanimate surfaces and equipment could become an "unrealized" source of certain contaminated bacteria and serve as a source of HAIs. Poor hand cleanliness would then obviously expand the opportunity of cross-contamination between person-to-person and environment-to-person. Of the ten tested points examined among 12 select sub-district HPHs, rates of bacterial contamination were up to 60%-100%, with an average rate of 94.17%. Concerning defined isolated bacterial strains, CoAg-Neg staphylococci; *S. epidermidis*, *Bacillus* sp., gram-positive rod non-spore-forming, and other gram-negative rods showed the highest rate of contamination (100%) at all examined points, while the average rate of contamination was 60.83%. Although similar results have not been reported, the high contamination rate may be associated with shed-off contaminated bacteria transmitted into HPHs' environment. As HPH personnel attend to patients, cross-contamination is highly likely. The services of HPHs that focus on primary treatments for people living in local communities and infectious control departments have not been precisely set up. Cross-contamination could finally become cross-infection from secondary reservoirs directly to the community population receiving services at HPHs. To be more specific, HPH personnel could contract contaminated bacteria by being in constant contact with their patients or clients. Therefore, both patients and HPH personnel could transmit infection through direct and indirect contact with inanimate surfaces, equipment, and

even objects at HPHs (Mwamungule et al., 2015). This is supported by Wang and Ruan, 2017, which indicated that hospital environmental contamination may contribute to bacterial transmission, when hospital's personnel contaminate their hands or gloves by touching contaminated surfaces or when patients come into direct contact with contaminated surfaces. Transmission of these cross-infections should then be restricted to prevent HAIs' possibility, because HAIs could lead to serious consequences, including long term disability, increased antimicrobial resistance, increased socio- economic disturbance, and an increased mortality rate (Khan, Baig, & Mehboob, 2017). Although transmission of contaminating bacteria from hospital surfaces and equipment is not always directly attributable to hand hygiene, hand hygiene is required as part of an integrated approach to infection control (Jumaa, 2005). Appropriate standards for infection control should be developed and supported as a prominent priority, as well as the surveillance of HAIs. Limiting the scope of patients' activities to avoid non-essential contacts with the environment and increasing hand hygiene compliance of HPHs personnel, particularly before contacting any patient, could be two basic recommendations assisted to remarkably reduce bacterial cross-transmission (Wang and Ruan, 2017). The results of this preliminary study could help to demonstrate bacterial contamination of inanimate surfaces and equipment/objects of sub-district HPHs in Chiang Rai. However, more expanded examination for molecular typing of non-specified isolated strains from our study should be performed.

6. Conclusion

The present study demonstrates, for the first time, the types of bacteria isolated from environmental contamination at 12 select sub-district HPHs in Chiang Rai. This study highlights that the samples were 100% positive, contaminated at 10 different tested points with the predominance of CoAg-Neg staphylococci (*S. epidermidis* and *S. saprophyticus*) and *Bacillus* sp. The hospital personnel's computer and keyboards and the toilet's hand-washing sinks and tap were identified as the largest bacterial colonization on equipment located in the nursing rooms and toilet areas, respectively. Although certain bacteria were not considered to be pathogenic and found ordinarily in the environment, some strains were associated with humans as commensal flora on the skin and digestive tract. Contamination found on such non-critical equipment and surfaces should be considered because it could reflect hospital cleaning/disinfection routines as well as individual hygiene. This should allow further study on the susceptibility to related antibiotics used to treat infections caused by particular isolated contaminated bacteria. Furthermore, since this study was conducted in HPHs in only 6 out of 18 (33%) districts of Chiang Rai, investigation of this theme should be encouraged.

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