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Detection of *Staphylococcus aureus* from contact surfaces of public buses in Bangkok and metropolitan area, Thailand

NARUMON BOONMAN[1,♥], JARUWAN CHUTRTONG[1], CHANATE WANNA[1], SIRIPHAN BOONSILP[2], SUPATRA CHUNCHOB[3]

[1]Department of Science, Faculty of Science and Technology, Suan Sunandha Rajabhat University. U-Thong nok Rd., Dusit, Bangkok 10300, Thailand. Tel./Fax.: +66-2160-1143, ♥email: narumon.bo@ssru.ac.th
[2]Department of Clinical Pathology, Faculty of Medicine, Vajira Hospital, Navamindradhiraj University. Kao Rd., Dusit, Bangkok 10300, Thailand
[3]Division of Conservation Biology, School of Interdisciplinary Studies, Mahidol University. Saiyok district, Kanchanaburi 71150, Thailand

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Abstract. *Boonman N, Chutrtong J, Wanna C, Boonsilp S, Chunchob S. 2022. Detection of* Staphylococcus aureus *from contact surfaces of public buses in Bangkok and metropolitan area, Thailand. Biodiversitas 23: 3395-3400.* The purpose of present investigation was to determine the prevalence of different species of genus *Staphylococcus* on the contact surfaces of public buses in Bangkok and metropolitan area. A total of 180 samples were collected from handrails, seats, and window frames of each bus of 2 bus terminals, Bangkhen and Morchit, divided into 15 non-air-conditioned buses and 15 air-conditioned buses from each terminal. The samples were cultivated and identified by Gram's stain and biochemical tests. The results showed that 76.7% from Bangkhen and 80% from Mochit were contaminated with *Staphylococcus* spp. Based on the types of vehicles, *Staphylococcus* spp. was found in 93.3% of air-conditioned buses and 63.3% of non-air-conditioned buses. Considering the sampling locations, *Staphylococcus* spp. was found in 28.3% of handrails, 50% of seats and 51.7% of window frames. Four isolates of *Staphylococcus aureus* were discovered among 294 isolates in a total of *Staphylococcus* spp., including 3 isolates from Bangkhen and 1 isolate from Mochit. Only one isolate was contaminated on the non-air-conditioned bus, whereas 3 isolates were on the air-conditioned buses. Two isolates of them were found from seats and 2 isolates from window frames. Disc diffusion susceptibility test showed that 3 isolates of *S. aureus* resisted fusidic acid and fosfomycin. The results suggested that *Staphylococcus* spp. and *S. aureus* were prevalence in public transport systems which were a source of infections to the persons. Therefore, effectively controlling their distribution is necessary to reduce the risk of bacterial infections in public bus users.

Keywords: Contact surfaces, drug sensitivity, public buses, *Staphylococcus aureus*

INTRODUCTION

The genus *Staphylococcus* contains many species that can cause diseases, especially *Staphylococcus aureus*, which are commonly found on the skin and body parts such as, the nasal cavity, respiratory tract, mucous membranes, and intestines. It can also be found in the environment in the form of dust and air. In healthy people, *S. aureus* stays in the body for a long time without harm but may cause infections if the skin has been injured. On the other hand, immunocompromised persons may be infected at the skin and soft tissues. The manifestation was various, from mild to severe symptoms such as abscesses in the skin infection, and pneumonia that may spread in the bloodstream into the musculoskeletal system causing complications. Moreover, *S. aureus* can also cause food poisoning (Cheung et al. 2021; Evangelista and Oliveira 2015). *Staphylococcus aureus* received a lot of attention since the strains resistant to many antibiotics are found, especially methicillin-resistant *S. aureus* (MRSA) (Khairullah et al. 2022). Healthcare-associated methicillin-resistant *S. aureus* (HA-MRSA) is a major cause of infections in hospitalized patients with a serious problem due to its difficult treatment and high mortality rate. Later, MRSA was reported that it can spread from the hospital to the community called community-acquired methicillin-resistant *S. aureus* (CA-MRSA). The genotypic and phenotypic characteristics of CA-MRSA are different from HA-MRSA, causing infections in normal people without risk factors (Harada et al. 2018; Henderson and Nimmo 2018; Turner et al. 2019).

Public areas and public transportation system are the sources of the spread of *S. aureus* in the community. The passengers, who are sick or carriers of *S. aureus* without symptoms are major factors in the dispersion of the bacteria. *Staphylococcus aureus* on their skin causes contamination of various contact surfaces, ready to spread to other passengers when these people are exposed to public things (Ababneh et al. 2022; Chen et al. 2018; Gu et al. 2020; Jaradat et al. 2021). Moreover, *S. aureus* can survive on various things for several days to several months (Jaradat et al. 2020). Many studies have reported the detection of *S. aureus* from exposed surfaces on buses in the various countries. Otter and French (2009) found methicillin-susceptible *S. aureus* 9 (8.0%) from 118 points of contact surfaces on public vehicles and hospitals in London, England but methicillin-resistant *S. aureus* was not found. Conceição et al. (2013) found MRSA on 72 (36.2%) from 199 buses in Lisbon, Portugal. Yeh et al. (2011) found 14 samples (20.0%) of *Staphylococcus* spp. from 70 samples collected from the surface area of the

trains, buses and bus stops in Portland, USA, but no *S. aureus*. Lutz et al. (2014) detected *S. aureus* contaminated on 27 (68.0%) from 40 buses, with MRSA contamination on 25 buses (63.0%) in the Midwestern, United States. Chowdhury et al. (2016) detected 12 MRSA samples (26.7%) from the contact surface of all 45 buses in Chittagong, Bangladesh. The data indicated that the prevalence of *S. aureus* from buses of various countries was different depending on the topography and sanitation of each country. However, the distribution of *S. aureus* on the buses in Thailand still has not been reported.

Therefore, present study aimed to determine the prevalence of *Staphylococcus* spp. and *S. aureus* on the exposed surfaces of public buses in Bangkok and metropolitan areas. The obtained results were compared according to bus terminals, types of buses and the sampling locations. The data is useful for raising awareness of *Staphylococcus* transmission and developing guidelines for infection prevention in public bus users.

MATERIALS AND METHODS

Sample collection

The samples were collected from 2 bus terminals including Bangkhen and Mochit. The public buses from each terminal were divided into 15 air-conditioned buses and 15 non-air-conditioned buses. The samples were collected immediately after serving and before cleaning by using methods modified from Yeh et al. (2011) and Chowdhury et al. (2016). A sterile cotton swab was dipped in the buffered peptone water (BPW), then swiped 2×4 cm^2 section on the surface of handrail, seat and window frame of each bus. The sampling swab was soaked in test tube containing 3 mL BPW and transferred to the laboratory within 2 hours.

Isolation of bacteria

The samples in BPW were incubated at 37°C for 3 hours to recover the bacteria, then the cotton swab was spread onto Baird-Parker egg yolk tellurite agar (BPEY) plate and incubated at 37°C for 24-48 hours. Colonies with dark gray, round, convex, smooth edges, with opaque zones and clear areas on the outer zone were selected for further study.

Staphylococcus aureus identification

The suspected colonies from BPEY were picked up and streaked on mannitol salt agar (MSA) plates. After incubation at 37°C for 18-24 hours, examined the ability to ferment mannitol by observing yellow colonies surrounded by yellow zones on this medium. The isolates with mannitol utilization were stained with Gram's stain and observed under the light microscope. The isolates with Gram-positive, cocci-shaped and cluster arrangements were further identified by the biochemical test including catalase test, coagulase test, hemolysis test and IMViC (Indole, methyl red, Voges-Proskauer, and citrate utilization) tests.

Antibiotic susceptibility assay

The isolates that were identified as *S. aureus*, which were cultivated on nutrient agar (NA) plates and incubated at 37°C for 18-24 hours to obtain the culture of log phase. Then, suspended the bacteria in Mueller-Hinton broth (MHB) and adjusted the turbidity with McFarland standard No. 0.5. Sterilized cotton swab was dipped into the bacterial suspension and spread on Mueller-Hinton agar (MHA) plates. The antibiotic discs were then placed on the surface of bacterial lawn. After incubating at 37°C for 18-24 hours, measured the diameter of clear zone around each disc and interpreted the results by Clinical and Laboratory Standards Institute (CLSI) 2016 guideline.

Statistical analysis

The data of *Staphylococcus* spp. and *S. aureus* detection were compared according to bus terminals, types of vehicles and the sampling locations using the X^2-test method with the IBM SPSS Statistics version 24. A statistically significant difference was determined when $P < 0.05$.

RESULTS AND DISCUSSION

Sample collection

This research collected samples from the contact surface of public buses in Bangkok and metropolitan area. A total of 180 samples were collected from 60 buses of 2 bus terminals, 30 buses from Bangkhen (BK) and 30 buses from Morchit (MC). The public buses from each terminal were divided into 15 air-conditioned buses (BKA or MCA) and 15 non-air-conditioned buses (BKR or MCR). Each public bus was sampled from 3 locations including handrail (BKRR, BKAR, MCRR or MCAR), seat (BKRS, BKAS, MCRS or MCAS) and window frame (BKRW, BKAW, MCRW or MCAW).

Staphylococcus aureus identification

All samples were recovered in BPW and cultivated on BPEY plates. Only samples that revealed black-gray colonies with round shapes, convex, smooth edges, surrounding with opaque zones and clear areas on the outer zone were classified as *Staphylococcus* spp. positive. These colonies were isolated into pure culture and further identified for *S. aureus* by using mannitol utilization test, Gram's stain (Gram-positive, cocci-shaped and cluster arrangement) and biochemical test including catalase test (positive), coagulase test (positive), hemolysis test (β-hemolysis), indole test (negative), methyl red test (positive), Voges-Proskauer test (positive) and citrate test (positive).

Prevalence of *Staphylococcus* spp. and *Staphylococcus aureus*

The prevalence of *Staphylococcus* spp. and *S. aureus* were statistically compared according to bus terminals, vehicle types, and sampling locations as shown in Table 1.

Table 1. Descriptive statistics and comparisons for the prevalence of *Staphylococcus* spp. and *Staphylococcus aureus* according to bus terminals, vehicle types and sampling locations

Variable	Positive *Staphylococcus* spp. n)%(	Negative *Staphylococcus* spp. n)%(	Positive *Staphylococcus aureus* n)%(	Negative *Staphylococcus aureus* n)%(
Bus terminals				
Bangkhen	23)76.7(	7)23.3(	3)10.0(	27)90.0(
Morchit	24)80.0(	6)20.0(	1)3.3(	29)96.7(
	Chi square test)P = 1.00(		Chi square test)P = 0.605(	
Vehicle types				
Non-air-conditioned buses	19)63.3(	11)36.7(	1)3.3(	29)96.7(
Air-conditioned buses	28)93.3(	2)6.7(	3)10.0(	27)90.0(
	Chi square test)P = 0.012*(		Chi square test)P = 0.605(	
Sampling locations				
Handrails	17)28.3(	43)71.7(	0)0.00(	60)100(
Seats	30)50.0(	30)50.0(	2)3.3(	58)96.7(
Window frames	31)51.7(	29)48.3(	2)3.3(	58)96.7(
	Chi square test)P = 0.016*(		Chi square test)P = 0.360(	

Note: Statistically significant x^2 test at $P < 0.05$ level.

There were 78 surface samples (43.3%) contaminated with *Staphylococcus* spp. in a total of 180 surface samples with only 4 isolates (2.2%), BKAS 1/1, BKAW 2/1, BKRS 10/2 and MCAW 1/1, identified as *S. aureus*. When compared with the prevalence of *Staphylococcus* spp. and *S. aureus* from public transport system in various countries, it was found that their contamination on the contact surfaces of the public buses in Thailand was higher than in Portland, United States (Yeh et al. 2011). On the other hand, it was lower than in London, England (Otter and French 2009), Lisbon, Portugal (Conceição et al. 2013), the Midwest United States (Lutz et al. 2014), and Chittagong, Bangladesh (Chowdhury et al. 2016). Thailand is located in a tropical climate, therefore temperature and humidity are suitable for growth and spread of microorganisms. However, the prevalence of *Staphylococcus* spp. on Thai public buses was still lower than many countries.

Considering the bus terminals, *Staphylococcus* spp. was detected from 23 buses of Bangkhen (76.7%) and 24 buses of Morchit (80.0%). *Staphylococcus aureus* was contaminated in 3 buses of Bangkhen (10.0%) and 1 bus of Morchit (3.3%). The public buses of Bangkhen served only in Bangkok with an average of 354 users/day while those of Morchit served in Bangkok and metropolitan area with an average of 762 users/day. The ridership of Morchit public buses was higher than in Bangkhen, therefore, there were more opportunities to find *Staphylococcus* spp. on the contact surfaces on buses from Morchit. However, the discovery of *Staphylococcus* spp. and *S. aureus* from both bus terminals' public buses were not significantly different at $P = 1.00$ and $P = 0.605$, respectively. The results were consistent with Lutz et al. (2014) that the public buses in the Midwest United States with a large number of users (≥200 users/day) and the buses with fewer users (0-199 users/day) did not affect the detection of *S. aureus*.

There are 2 types of public buses in Thailand: non-air-conditioned and air-conditioned. *Staphylococcus* spp. was found in 19 non-air-conditioned buses (63.3%), but only 1 bus had *S. aureus* (3.3%). In contrast, 28 air-conditioned buses (93.3%) were contaminated with *Staphylococcus* spp. which was *S. aureus*, from 3 buses (10.0%). The prevalence of *Staphylococcus* spp. on air-conditioned buses was significantly higher than on non-air-conditioned buses ($P = 0.012$). This may be due to the non-air-conditioned buses having wide-open spaces and better ventilation, which were different from the air-conditioned buses with closed systems and no ventilation from outside. Moreover, the air-conditioned buses consisted of various devices such as air filters, air ducts and open grills which cause dust and microorganisms to stick on. Their temperature and moisture were appropriate for microbial growth and when the air conditioner was turned on, these microbes were allowed to spread out inside the bus. Surprisingly, there were no significant differences in *S. aureus* detection between non-conditioned buses and air-conditioned buses ($P = 0.605$). This may be due to the number of *S. aureus* found in this research being too small to clearly distinguish the differences.

When separated by 3 sampling locations, each of 60 samples from handrails, seats and window frames, *Staphylococcus* spp. was detected from 17 handrails (28.3%), 30 seats (50.0%) and 31 window frames (51.7%) which was *S. aureus* from 2 seats (3.3%) and 2 window frames (3.3%). Seats and window frames were contaminated with *Staphylococcus* spp. significantly higher than handrails ($P = 0.016$). It was consistent with Yeh et al. (2011) that found *Staphylococcus* spp. from seats more than from handrails. Because the handrails had smooth surfaces, therefore they were not suitable for bacterial accumulation and easy to clean whereas fabric or vinyl

seats had rough surface causing bacteria to accumulate and difficult to clean. The window frames were often ignored in cleaning. Kaplan et al. (2014) also reported that MRSA infection increased during the summer. It may be due to people wearing short-sleeved clothes, increasing the chance of skin contact with each other or contact with various surfaces more than any other seasons. The weather in Thailand is quite hot, most people wear short-sleeved clothes. Therefore, when passengers put their arms on the window frames, there was a high chance that *Staphylococcus* spp. from their skin would contaminate the window frames. Similar to vehicle types, the prevalence of *S. aureus* from each sampling location was not statistically different due to a small number was found.

Antibiotic susceptibility of *Staphylococcus aureus*

Four isolates of *S. aureus* isolated from public buses were tested for antibiotic sensitivity with the disc diffusion assay technique. The diameter of the clear zone around each antibiotic disc was measured and compared to the standard table for *Staphylococcus* spp. given by Clinical and Laboratory Standards Institute (CLSI) 2016 (Table 2). All 4 *S. aureus* isolates were susceptible to many antibiotics including cefoxitin, gentamycin, clindamycin, erythromycin, and sulfamethoxazole-trimethoprim. However, BKAS 1/1, BKAW 2/1 and BKRS 10/2 exhibited intermediate resistance against tetracycline. Moreover, BKAW 2/1 was resistant to fusidic acid. BKAS 1/1 and MCAW 1/1 also revealed resistance against fosfomycin. Therefore, if the public bus service providers lack awareness and passengers also have inappropriate behavior or poor hygiene, it will accelerate the spread of drug-resistant *S. aureus* to the community quickly since they can spread easily from contact and can also transfer the drug-resistant genes to other bacteria. During a few years, many natural product extracts exhibited *in vitro* anti-bacterial activity (Chutrtong and Kularbphettong 2019; Liu et al. 2019; Panphut et al. 2020; Wu et al. 2019). They may be the effective alternative agents to overcome the antibiotic-resistant *S. aureus* in the future.

The public transport system is necessary for people in almost every country. Because it helps facilitate travel, energy-saving, and can save more money than using a private car. On the other hand, contact with objects on public buses is a risk of *Staphylococcus* infections, especially MRSA that is resistant to vancomycin, which is the last antibiotic used for treatment. Unfortunately, *S. aureus* isolates with complete resistance to vancomycin have emerged in recent years (Cong et al. 2020). Therefore, this may be a major public health problem worldwide. If the public bus has a route through the hospital, it is also a source of HA-MRSA from the hospital to spread to the community as CA-MRSA.

The public bus services in Bangkok and metropolitan area are under the supervision of the Bangkok Mass Transit Authority (BMTA). During rush hours, there are many passengers therefore, they have to provide continuous service, cleaning with disinfectant after each round of service is impossible. BMTA has guidelines for cleaning by sweeping and the wiping floor, seats and handrails but without using chemicals or active substances to kill microorganisms, resulting in the accumulation of microorganisms on the surface area of the public buses. Therefore, creating an understanding and awareness of the employees about the importance of efficient bus cleaning should help reduce the chance of *Staphylococcus* spp. distribution into the community. In addition, passengers should also protect themselves from infections after using public transportation. Conceição et al. (2013) reported that 15 medical students had MRSA contamination on their hands after traveling by public transportation. The strains of bacteria found on their hands consistent with the bacteria contaminated on the buses which indicated temporary MRSA contamination after using the service. Therefore, cleaning hands during and/or after using public transportation immediately will reduce the chance of infections. However, washing hands with soap may be inconvenient. Therefore, using alcohol gel to clean hands is more practical.

In conclusion, the prevalence of *Staphylococcus* spp. and *S. aureus* on public bus contact surfaces was 43.3% and 2.2%, respectively in this investigation. The detection of *Staphylococcus* spp. and *S. aureus* from Bangkhen and Morchit were not significantly different. Interestingly, *Staphylococcus* spp. were more common in the air-conditioned buses than in the non-air-conditioned buses. In addition, the seats and window frames were contaminated with *Staphylococcus* spp. significantly higher than the handrails. There were 3 isolates of *S. aureus* resisted to fusidic acid and fosfomycin. This finding indicated that the public buses may be a potential source of *S. aureus* infections in humans.

Table 2. Antibiotic susceptibility patterns of *S. aureus* isolated from public buses according to Clinical and Laboratory Standards Institute (CLSI) 2016

Isolate no.	Diameter of clear zone (mm)																							
	Cefoxitin)30 µg(			Gentamycin)10 µg(			Tetracycline)30 µg(			Fusidic acid)10 µg(			Fosfomycin)200 µg(			Clindamycin)2 µg(			Erythromycin)15 µg(			Sulfamethoxazole - trimethoprim)25 µg(		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
BKAS 1/1	-	-	27.6	-	-	23.3	-	14.6	-	-	-	32.7	30.5	-	-	-	-	27.1	-	-	28.7	-	-	34.1
BKAW 2/1	-	-	28.1	-	-	22.9	-	15.0	-	28.8	-	-	-	-	45.3	-	-	24.0	-	-	26.1	-	-	30.6
BKRS 10/2	-	-	29.0	-	-	23.6	-	15.1	-	-	-	31.1	-	-	58.8	-	-	26.4	-	-	29.2	-	-	32.8
MCAW 1/1	-	-	26.5	-	-	25.1	-	-	18.7	-	-	31.1	24.0	-	-	-	-	26.8	-	-	25.9	-	-	29.6

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