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Microbial Contamination of Environmental Surfaces in An-Najah National University Setting

Ghaleb Adwan[1*], Yousef Salama[1] and Nael Abu Hasan[1]

[1]Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine.

Authors' contributions

This work was carried out in collaboration between all authors. Authors GA, NAH and YS designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GA and YS managed the literature searches and analyses of the study performed. Authors GA and YS managed the experimental process and identified the species of microorganisms. Authors GA and NAH edited and reviewed the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate bacterial contamination of environmental surfaces in An-Najah National University setting. This study focused mainly on staphylococci bacteria in particular detection of some molecular characterization of *Staphylococcus aureus.*
Place and Duration of the Study: Department of Biology and Biotechnology, An-Najah National University, Palestine, March-August 2012.
Methods: A total of 210 environmental surface samples from Faculty of Science, An-Najah National University were collected using cotton swabs. *S. aureus*, biochemical tests and *S. aureus* species-specific identification was used to confirm the isolates. Antimicrobial susceptibility testing for methicillin was performed, *mecA* gene, SCC*mec* typing and *seg*, *seh* and *sei* toxin genes were detected by PCR. Morphology, biochemical tests and selective media were used to identify other microorganisms obtained from contaminated environmental surfaces.
Results: It was found that 31.9% (67/210) of these surfaces were contaminated with *S. aureus*. Only 5 methicillin resistant *S. aureus* (MRSA) isolates were detected and belonged to SCC*mec* IVa type. One isolate of methicillin sensitive *S. aureus* (MSSA) was enterotoxigenic and had genotype

**Corresponding author: E-mail: adwang@najah.edu;*

seg^{+}/*seh*$^{+}$. In addition, 85.7%, 90.5%, 14.3%, 11.9%, 10% and 4.8% of cultures were positive for *S. epidermidis*, fungi, *Bacillus* sp., *Escherichia coli*, *Klebsiella* sp. and *Streptococcus* sp., respectively.
Conclusion: The results show that these different representative environmental surfaces are in daily use and may be a source of infection.

Keywords: Bacterial contamination; Hand/skin contact surfaces; University; MRSA; toxin genes.

1. INTRODUCTION

Pathogenic microorganisms in public areas can be a critical issue in public health, because these pathogens can transfer easily from one person to another. Public or community areas such as public transportation systems, universities, restaurants, schools, daycare centers, parks and other can bring a large number of people together and facilitate the transmission of microbes [1-4]. Therefore, increased attention has been paid to environmental microbes and strains of microorganisms found in these public, non clinical places. Hand/skin contact of non clinical surfaces might serve as a potential source for the transmission of pathogenic microorganisms [1,5,6]. Community surfaces in educational institutions such as universities and schools may act a mechanism for the transmission of pathogenic microorganisms. In previous study [1], *Staphylococcus aureus* (*S. aureus*), methicillin resistant *S. aureus* (MRSA), *S. epidermidis*, methicillin resistant *S. epidermidis,* methicillin resistant *S. hominis* were isolated from computer keyboard swabs in a university setting. Another study showed that multiple-user keyboards in university setting contaminated with different types of microorganisms included *S. aureus*, *Escherichia coli* (*E. coli*), *Enterococcus faecalis*, *Bacillus cereus* (*B. cereus*), yeasts and molds [5]. Recently, samples collected from computer labs were reported to be contaminated with 5 species of bacteria including *S. aureus*, *S. epidermidis*, *Micrococcus* sp*., Streptococcus* sp*.* and *E. coli* [7]. *S. aureus*, *S. epidermidis*, *Enterococcus* sp.*, Streptococcus* sp*.* and fungi were also reported in association with computer keyboards and mouse in a university environment [2]. Investigated air samples in a university setting showed that the predominant bacteria and moulds were: *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Serratia* sp., *E*. *coli*, *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Cladosporium* sp. and *Alternaria* sp [8].

S. aureus is part of the normal flora of human skin and nasal passage; it is known to be associated with several disease conditions. Methicillin resistant *S*. *aureus* is of particular concern. In Palestine, the prevalence of MRSA among clinical *S. aureus* isolates was 56.4% [9], while 4.2% among university student nasal swabs [10]. According to community-associated MRSA (CA-MRSA) strains affect a population distinct from those affected by health care-associated MRSA (HA-MRSA) and cause distinct clinical syndromes. CA-MRSA infections tend to occur in healthy younge patients and predominantly with skin and soft-tissue [11]. *S. epidermidis* is found in large number in the environment and is present on human body as a member of the commensal flora. It can cause serious opportunistic infections especially in immunocompromised individuals, ranging from urinary tract infections to osteomyelitis and endocarditis [12]. *E. coli* is considered as a member of the normal intestinal flora, but may cause urinary tract infection, neonatal meningitis, sepsis and diarrhea [13]. Fungi can be hazardous for health, and can breed allergies, sick building syndrome causing irritation of mucous membranes, dermatosis, respiratory diseases and cancers [8]. *Klebsiella* sp. are common opportunistic pathogens of nosocomial infections and considered as one of the major causes of human morbidity and mortality. It can cause different infections such as pneumonia, septicaemia, urinary tract infection, wound infections and others [14,15]. *Bacillus* sp. other than *B. anthracis,* can cause several complications for immune-compromised patients [16]. *Streptococcus* sp. can cause streptococcal sore throat, scarlet fever, toxic shock syndrome, pneumonia, meningitis, endocarditis, impetigo, urinary tract infection and others [13].

In Palestine, no previous studies concerning microbial contamination of community surfaces and their roles in dissemination and infection development were reported. The aim of this study was to determine the prevalence of bacterial contamination of some non clinical surfaces in An-Najah Naional University, Faculty of Science. These surfaces included computer keyboards and mouse, bathroom door knobs, bathroom sink faucet handles, toilet sprayers, elevator keyboards, faucet handles of water cooler and vending machine keyboards that feature a broad student user base in the Faculty

of Science. The study focused mainly on staphylococci bacteria in particular detection of some molecular characterization of *S. aureus*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

Two hundred and ten swabs were collected from different representative surfaces from the faculty of Science at An-Najah National University, Nablus-Palestine, during March-August 2012. Swab sites included surfaces of computer keyboard and mouse (n=65), bathroom door knob (n=30), bathroom sink faucet handle (n=45), toilet sprayer (n=35), elevator keyboard (n=17), water cooler faucet (n=15), vending machine keyboard (n=3). These surfaces were sampled with a moisture sterile cotton swab, immediately transferred into 5 ml tryptic soy broth and incubated for 18-24 h at 37℃. Thereafter, 10 µl of the tryptic soy broth were sub-cultured on Mannitol salt agar, Malt extract agar (0.01% w/v Chloramphenicol) and MacConkey agar or Eosin-methylene blue and blood agar for identification. Malt extract agar plates were incubated at room temperature and 37℃ for 3 days. Identification of *S. aureus*, *S. epidemidis*, *Bacillus* sp., *Streptococcus* sp., *Klebsiella* sp. and *E. coli* was based on Gram stain, colony morphology and different biochemical tests. Catalse test, coagulase test, Novobiocin sensitivity, grow in 6.5% NaCl were used for the identification of *Streptococcus* sp., *S. aureus* and *S. epidemidis*. Citrate utilization, Indole production, Voges-Proskaur test, Methyl Red test, Nitrate Reduction, Motility test, Urease test and Triple sugar Iron were used for identification *Klebsiella* sp., and *E. coli*. Fungal identification was based on colony morphology, pigmentation and microscopic characteristics using lactophenol-cotton blue dye. All isolates of *S. aureus* were confirmed by PCR using species-specific primers [17]. In case the tactile surface has many buttons all were swapped together and considered as one sample. Samples from computer keyboard and mouse are considered as a single swap.

2.2 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility for *S. aureus* was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method [18]. Isolates were examined for resistance using the following antibiotic disks (Oxoid): Ofloxacine (OFX, 5 µg), Tetracycline (TE, 30 µg), Clindamycin (DA, 10 µg), Erythromycin (E, 15 µg), Cefotaxime (CTX, 30 µg), Gentamicin (CN, 10 µg), Streptomycin (S, 10 µg), Vancomycin (VA, 30 µg), Ampicillin (AMP, 25µg). Zones of inhibition were determined in accordance with procedures of the CLSI [18].

Oxacilllin (OX, 1 µg) and Cefoxitin (FOX, 30 µg) antibiotic disks (Oxoid) were used to detect methicilline resistant *S. aureus*. Zones of inhibition were determined in accordance with procedures described by the CLSI [18]. The isolates were categorized as susceptible or resistant. According to Oxacilllin, *S. aureus* isolates were considered resistant if inhibition zones were ≤13 mm after incubation on 2% NaCl Mueller Hinton agar at 35℃ for 24 hours. For Cefoxitin, susceptibility testing was carried out on Mueller Hinton agar at 35℃ for 24 hours. Isolates with inhibition zone diameters ≤21 mm were considered MRSA. Oxacillin resistant *S. aureus* strains, identified by disk diffusion method, were also confirmed by PCR using previously described primers [19]. Methicillin-resistant control strains (department collection) and methicillin-susceptible reference (*S. aureus* ATCC 25923) were used.

2.3 PCR Amplification

S. aureus DNA was prepared for PCR according to method described previously with some modification [10]. Briefly, cells were scraped off an overnight nutrient agar plate with a sterile loop, washed twice with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was re-suspended in 0.5 ml of sterile distilled H_2O, and boiled for 10-15 min. The cells were then incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X *g* for 5 min. DNA was extracted from supernatant using phenol-chloroform method, then DNA was precipitated using 96% cold ethanol. The nucleic acid pellet was washed with 70% cold ethanol, dried and then re-suspended in 300 µl TE (Tris 10 mM, EDTA 1 mM, pH 8), DNA concentration was determined using spectrophotometer and the samples were stored at -20℃ until use.

Gene targets, nucleotide sequences for all PCR primers used in this study and the size of amplified products are listed in Table 1. Multiplex PCR technique was carried out for detection enterotoxin genes and SSC*mec* typing. DNA amplifications were performed using Thermal Cycler (Mastercycler personal Eppendorf, Germany). For *SCCmec* typing, amplification of

toxin genes**,** *mecA* gene and *S. aureus* species-specific sequence as described previously [22,23], each PCR reaction mix (25 µl) was performed using 12.5 µl of PCR premix (ReadyMix^{TM} Taq PCR Reaction Mix with $MgCl_2$, Sigma), 0.2 µM of each primer, and 3 µl (150-200 ng) DNA template. Thermal conditions for amplification are presented in Table 1. Final extension was performed at 72℃ for 5 minutes for all PCR programs. The amplified products were examined by 2% agarose gel electrophoresis to determine the size of amplified fragment. Amplification of non template controls was carried out for each reaction to determine DNA contamination. Control strains for different SCC*mec* types (department collection) were used.

3. RESULTS

Results of the current study showed that tested environmental surfaces were contaminated with different microorganisms; were 31.9% (67/210) were contaminated with *S. aureus*, 5 MRSA belonged to SCC*mec* IVa type, one enterotoxigenic MSSA with genotype *seg*$^+$/*seh*$^+$. In addition, it was found that 85.7% (180/210), 90.5% (190/210), 14.3% (30/210), 11.9% (25/210), 10% (21/210) and 4.8% (10/210) of cultures were positive for *S. epidermidis*, fungi, *Bacillus* sp., *E. coli*, *Klebsiella* sp., and *Streptococcus* sp., respectively. Isolated microorganisms are shown in Table 2. Gram-positive bacteria were more frequent compared to Gram-negative. Data presented in Table 3 shows the antibiotic resistance profile were all MRSA isolates were sensitive to Vancomycin, and all MSSA were sensitive to Oxacillin, Clindamycin, Vancomycin and Ciprofloxacin.

4. DISCUSSION

There is increasing evidence that the environment may play a significant role in the spread of pathogens especially antibiotic-resistant microorganisms. The opportunity for the transmission of contaminating microorganisms is potentially great especially in the absence of routine disinfection. The widespread nasal carriage of staphylococci by humans likely facilitated the contamination via hand-to-mouth or hand-to-nose contact while using the keyboard, and/or poor hand-washing habits. CA-MRSA strains have been distinguished from their HA-MRSA strains by molecular techniques. HA-MRSA strains carry a large SCC*mec* types I, II, or III and rarely carry the genes for the Panton-Valentine leukocidin (PVL). These strains are often resistant to many classes of non-β-lactam antimicrobials. In contrast, CA-MRSA carry smaller SCC*mec* element types IV or type V, frequently carry PVL genes and resistant to fewer non-β-lactam classes [11]. In our study, the finding of 31.9% of the surfaces contaminated with *S. aureus* is alarming as this organism is considered as a major causative agent in human diseases. These results were consistent with previous reports concerning *S. aureus* contamination [1-3,5,7,24]. In these studies the incidence was much higher than that observed in the current study and ranged between 38-100%. Our findings were also in agreement with previously reported regarding *S. aureus* among university students of An-Najah National University; were MRSA incidence was 4.2% [10]. Results of this report showed that the MRSA enterotoxigenic strain obtained from nasal swabs. The found strains were typed as SSC*mec* types II, III and IVa [10]. In the same study, enterotoxigenic MSSA with *seg* and/or *sei* genes was reported among 25.3% collected nasal swabs. Contamination of enterotoxigenic *S. aureus* in such community surfaces may play a role in staphylococcal food poisoning. It was reported that newly described enterotoxin types SEH, SEG and SEI have been implicated in staphylococcal food poisoning symptoms [25,26]. On the other hand, no MRSA strains were reported in university setting [24], although, high incidence of CA-MRSA was reported in the same city. The authors explained absence of MRSA as a result of improper technique of MRSA sampling. Previous reports on the level of contamination of MRSA were in agreement with our findings [1,3].

In the current study, contaminated surfaces with *S. epidemidis* were found in most surfaces. This organism is a normal habitat of the human skin and occasionally can cause opportunistic infections especially in immunocompromised patients. These results were consistent with recently published reports, were *S. epidermidis* strains reported to be a major contaminant of all or most community surfaces in university settings [2,5,7]. *S. epidemidis* are considered as important reservoirs for genes that contribute to the evolution of MRSA in both community and hospital settings [1]. The frequent finding of Gram-positive bacteria compared to Gram negative in the current study was in agreement with previous findings [27]. This is an expected result as Gram-positive exhibits long survival time due to differences in cell wall structure.

Table 1. Primers used in this study to identify *S. aureus*, enterotoxigenic genes, *mecA* gene and SCC*mec* typing of MRSA by multiplex PCR

Primer pair	Target gene	Sequence (5'→3')	PCR program	Size (bp)	Reference
Sa442 F	Species-specific target	AATCTTTGTCGGTACACGATATTCTTC ACG	1	108	[17]
Sa442 R		CGTAATGAGATTTCAGTAGATAATACAACA			
SEG-1	*Seg*	AAGTAGACATTTTTGGCGTTCC	2	287	[20]
SEG-2		AGAACCATCAAACTCGTATAGC			
SEH-1	*Seh*	GTCTATATGGAGGTACAACACT	2	213	[20]
SEH-2		GACCTTTACTTATTTCGCTGTC			
SEI-1	*Sei*	GGTGATATTGGTGTAGGTAAC	2	454	[20]
SE-2		ATCCATATTCTTTGCCTTTACCAG			
mecA1	*mecA*	TGGCTATCGTGTCACAATCG	3	310	[19]
mecA2		CTGGAACTTGTTGAGCAGAG			
Type I F	ORF E008 of strain	GCTTTAAAGAGTGTCGTTACAGG	4	613	[21]
Type I R	NCTC10442	GTTCTCTCATAGTATGACGTCC			
Type II F	kdpE of strain N315	GATTACTTCAGAACCAGGTCAT	4	287	[21]
Type II R		TAAACTGTGTCACACGATCCAT			
Type III F	J1 region of SCCmec type III	CATTTGTGAAACACAGTACG	4	243	[21]
Type III R		GTTATTGAGACTCCTAAAGC			
Type IVa F	ORF CQ002 of strain CA05	GCCTTATTCGAAGAAACCG	4	776	[21]
Type IVa R		CTACTCTTCTGAAAAGCGTCG			
Type IVb F	J1 region of SCCmec type IVb	AGTACATTTTATCTTTGCGTA	4	1000	[21]
Type IVb R		AGTCATCTTCAATATGGAGAAAGTA			
Type IVc F	IVc element of strain 81/108	TCTATTCAATCGTTCTCGTATT	4	677	[21]
Type IVc R		TCGTTGTCATTTAATTCTGAACT			
Type IVd F	CD002 in type IVd	AATTCACCCGTACCTGAGAA	4	1242	[21]
Type IVd R		GAATGTGGTTATAAGATAGCTA			
Type IVh F	J1 region strain HAR22	TTCCTCGTTTTTTCTGAACG	4	663	[21]
Type IVh R		CAAACACTGATATTGTGTCG			
Type V F	ORF V011 of strain JCSC3624	GAACATTGTTACTTAAATGAGCG	4	325	[21]
Type V R		TGAAAGTTGTACCCTTGACACC			

1. 94℃ for 1 min, 37 cycles 94℃ for 40 s, 54℃ fo r 1 min, 72℃ for 1 min.
2. 94℃ for 1 min, 30 cycles 94℃ for 30 s, 55℃, 4 6℃ and 50℃ for 30 s for seg, seh and sei genes , respectively, 72℃ for 30 s.
3. 92℃ for1 min, 40 cycles 92℃ for 20 s, 58℃ for 20 s, 72℃ for 20 s, with increment of 2 s per cyc le for the denaturation and extension.
4. 94℃ for 4 min, 35 cycles 94℃ for 30 s, 55℃ fo r 30 s, 72℃ for 1.5 min

Table 2. Microoganisms isolated from the community surfaces in a university setting

Surfaces	Swabs number	Isolated microorganisms No. (%)							
		S. epidermidis	**Fungi**	***Bacillus* sp.**	***E. coli***	***Klebsiella* sp.**	***Streptococcus* sp.**	***S. aureus***	**MRSA/MSSA**
Computer keyboard and mouse	65	57 (87.7)	60 (92.3)	3 (4.6)	4 (6.2)	4 (6.2)	2 (3.1)	16 (24.6)	MRSA = 2 MSSA = 14
Bathroom sink faucet handle	45	42 (93.3.1)	44 (97.8)	9 (20.0)	6 (13.3)	3 (6.7)	1 (2.2)	19 (42.2)	MRSA = 0 MSSA = 19
Toilet sprayer	35	32 (91.4)	33 (94.3)	10 (28.6)	8 (22.9)	5 (14.3)	1 (2.9)	9 (25.7)	MRSA = 0 MSSA = 9
Bathroom door knob	30	22 (73.3)	27 (90)	5 (16.7)	6 (20.0)	3 (10)	1 (3.3)	7 (23.3)	MRSA = 1 MSSA = 6
Water cooler faucet	15	12 (80)	13 (86.7)	3 (20.0)	0 (0.0)	2 (13.3)	3 (20.0)	7 (46.7)	MRSA = 1 MSSA = 6
Elevator keyboard	17	12 (70.6)	12 (70.6)	0 (0.0)	1 (5.9)	2 (11.8)	1 (5.9)	8 (47.1)	MRSA = 1 MSSA = 7
Vending machine keyboard	3	3 (100)	1 (33.3)	0 (0.0)	0 (0.0)	2 (66.7)	1 (33.3)	1 (33.3)	MRSA = 0 MSSA = 1
Total (%)	210	180 (85.7)	190 (90.5)	30 (14.35)	25 11.9%)	21 (10%)	10 (4.8%)	67 (31.9)	MRSA = 5 MSSA = 62

Table 3. Antibiotic resistance profile of *S. aureus* (MRSA and MSSA) strains isolated from a community surfaces in an-Najah N. University

Antimicrobial tested	Resistance no. (%)	
	MSSA (n=62)	MRSA (n=5)
Oxacillin	0 (0.0%)	5 (100%)
Ofloxacine	5 (8.1%)	2 (40%)
Tetracycline	15 (24.2%)	2 (20%)
Clindamycin	0 (0.0%)	2 (40%)
Erythromycin	5 (8.1%)	5 (100%)
Cefotaxime	9 (14.5%)	5 (100%)
Gentamicin	4 (6.5%)	5 (100%)
Streptomycin	6 (9.7%)	2 (20%)
Vancomycin	0 (0.0%)	0 (0.0%)
Ciprofloxacin	0 (0.0%)	2 (40%)
Ampicillin	19 (28.6%)	5 (100%)

Detection of fungal growth in most of cultures is indicative of the ubiquitous nature of these fungi in the airborne environment [5]. These results were consistent with a previous reports were fungi isolated from most community surfaces [2,5]. Fewer than 200 species have been reported to produce disease in humans, mycoses, which have unique clinical and microbiologic features and are increasing in immunocompromised patients. Fungal cells have typical eukaryotic features. The composition of fungal cell wall differes from that in bacteria and plants and their size varies immensely. The morphologic forms of growth vary from colonies superficially resembling those of bacteria to some of the most complex, multicellular. Fungi may reproduce by either asexual or sexual processes. Reproductive elements vary in size and asexual and sexual spores often actively dispersed by forcible ejection in huge numbers and disseminated by air [28].

The isolation of *E. coli* from different surfaces in the current study could be due to dissemination of this organism through contaminated hands of those working in the Microbiology Laboratories or other sources of fecal contamination. Such finding indicates low level of hygienic conditions. These results were consistent with a previous report were contaminated surfaces with this pathogen in university setting was with an incidence rate of 10% [5]. *Bacillus* sp. commonly exists in the air, soil, dusty environments and normal human intestinal flora. Microorganisms associated to this genus are spore forming bacteria that can survive for long periods in the environment [16].

Expansion of this study is necessary to include molecular characterization of other microorganisms isolated from these surfaces to differentiate between different clones. Investigation of virulence factors in these microorganisms is necessary to detect potential pathogenicity. This study concerned with aspects of public health microbiology and achieving a better understanding of contamination with staphylococci and other microorganisms. Information reported in this study can be used to further expand future studies of contamination with staphylococci or other pathogens in both clinical and non clinical settings in Palestine.

5. CONCLUSION

Our results showed that these different representative environmental surfaces are in daily use and may act as a source of infection. Pathogens like MRSA can remain viable on dry surfaces for long periods of time, decontamination of the skin and these environmental surfaces may limit their spread. The aforementioned data might be important in provoking awareness to these reservoirs as they constitute high exposure risk to such pathogenic bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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