

Full Length Research Paper

Diversity and antibiotic susceptibilities of bacterial species from surfaces of publicly used equipment in a medical education setting

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Medical and health science colleges have firm links to hospitals and health services centres through clinical training activities. Such colleges are expected to house microorganisms of nosocomial-infection significance that can be transmitted to and from students' hands and bodies through publicly used equipment, including food and drink vending machines, elevators and computer keyboards. This study aimed to investigate the presence of human body indicator bacterial species on three types of publicly used surfaces in medical and health sciences colleges. Swab samples were collected aseptically from 30 computer keyboards, 10 digital control panels of food vending machines and 10 elevators in the buildings of medicine, dentistry, pharmacy and health sciences colleges over a period of five weeks. Ten surfaces were sampled each week, in the morning and the afternoon (n= 20/week) and cultured in selective and general media. Forty-three percent of surfaces had relatively elevated levels of bacteria, while the rest exhibited no recoverable bacterial growth. Conventional identification methods in addition to 16s rRNA gene sequencing revealed the presence of *Bacillus* spp., *Bhargavaea cecembensis*, *Brevibacterium casei*, *Cellulomonas* spp., *Micrococcus* spp., *Rothia terrae*, *Stenotrophomonas maltophilia*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and other *Staphylococcus* spp. on the examined surfaces. The antibiotic susceptibilities of the isolates were investigated. Most of the isolates were moderately sensitive to commonly used antibiotics. Resistance to β -lactam antibiotics was detected among most of the *Staphylococcus* spp. isolates. High bacterial loads were detected in surfaces of publicly used equipment. The majority of the isolates are of human skin sources (*Staphylococcus* species) and/or soil bacteria (*Bacillus* species) while no coliform bacteria were recovered.

Key words: Bacillus, staphylococcus, elevators, vending machines, surfaces, antibiotic, sensitivity.

INTRODUCTION

Environmentally transmitted diseases and nosocomial infections remain challenging health specialists and/or practitioners. Generally, the medical education sector has

strong links with health service providers. Students of medical or health sciences spend substantial time in hospitals and/or other clinical settings. During their clinical

training, the possibility of acquiring microorganisms from publicly used surfaces and/or clinical tools is worth consideration. Noticeably, little is known regarding the microorganisms and pathogens carried specifically by medical students and shared on publicly used equipment in educational settings. Few studies have investigated the presence of microorganisms among students and/or medical colleges in comparison to numerous studies conducted in clinical or educational settings.

Publicly used equipment are important sources of bacteria transmission. The primary routes of transmission to and from surfaces are through hands. Reynolds et al. (2005) specified that artificial contamination of public surfaces with an invisible fluorescent tracer showed that contamination from outside surfaces was transferred to most of the exposed individuals' hands, and a great majority tracked the tracer to their homes or personal belongings hours later.

Bacteria isolated from surfaces are commonly sourced from hands or soil. The types of microorganisms transferred vary according to people, the body part types, and surface types. Furthermore, scientists have revealed that microorganisms in buildings differ broadly according to the environmental conditions and the individuals who inhabit the space (Arnold, 2014). In a study carried by Hewitt et al. (2012), heterotrophic bacteria were cultivated from nearly every surface in offices. Items such as computer keyboards, light switches, and detergent dispensers are frequently contaminated with microorganisms from hands whenever they are touched (Arnold, 2014).

Flores et al. (2011) observed 19 phylae of bacteria across university restroom surfaces, and most of the isolates belonged to one of four phylae: Actinobacteria (*Micrococcus spp.*), Bacteroidetes (*Bacteroides spp.*), Firmicutes (*Staphylococcus spp.*) or Proteobacteria (*Escherichia spp.*). Doğan et al. (2008) examined computer keyboards used by three groups: medical staff, medical students, and university students in Turkey, and the keyboards were determined to be colonised by coagulase-negative staphylococci followed by Gram-positive spore-forming bacilli and Corynebacteria, while Gram-negative isolates were only found in a hospital setting.

In a study conducted at the University of North Carolina, health care computer keyboards from clinical units tested positive for skin organisms, including coagulase-negative staphylococci, diphtheroid, *Micrococcus*, and *Bacillus* species, in addition to nonfermentative Gram-negative species (Rutala et al., 2006). In another study conducted in a large U.S. university, samples were collected from public telephone mouthpieces, water fountain drains, buttons on elevators,

vending machines, photocopiers and student computer keyboards and desks in the morning and afternoon of one day, and significant increases in the bacterial counts occurred on the same surfaces when sampled in the afternoon. *Staphylococcus aureus* was found on telephone mouthpieces, keyboards, and an elevator button and *Stenotrophomonas maltophilia* was found on fountain drains. Telephone mouthpieces had a high bacterial count (Brooke et al., 2009). At Bonn University Hospital, computer samples taken immediately after use had the highest contamination rates and positive growth of different type of bacterial species (*S. aureus*, streptococci, enterococci, and Gram-negative microorganisms) (Engelhart et al., 2008). *Bacillus* spp., *S. aureus*, *Staphylococcus epidermidis* and other gram-negative bacteria were isolated from student-used keyboards in an Iraqi University in Bagdad (Ali et al., 2013).

Hand-touch surfaces within the public transport system and public areas of a hospital, underground trains, and bus stations in central London were investigated for contamination with methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA). MSSA was isolated from 8% of sites, while no MRSA was cultured from any of the sites (Otter and French, 2009).

Roberts et al. (2011) isolated methicillin-resistant *S. aureus* from 11.8% of environmental surfaces in a university campus while its prevalence in student homes and community surfaces were similar and did not exceed 3%.

Environmental surfaces in student communities may receive significant traffic. Such surfaces may support bacterial growth and may or may not receive sufficient cleaning. Determining the number of bacteria and screening for the presence of potential pathogens on publicly used surfaces will provide more information about transmission routes and assist in developing implements for maintaining the health of individuals contacting those surfaces.

To fulfil these purposes, this study aimed to investigate the presence of indicator bacterial species (*Staphylococcus* species and coliform bacteria) on three types of publicly used surfaces in medical and health sciences colleges, including vending machines, keyboards and elevator outside control panels and to compare the bacterial load throughout the day (morning and afternoon). Besides, to test their susceptibilities to commonly used antibiotics as virulence indicators.

MATERIALS AND METHODS

This cross-sectional study was conducted between the 17th of

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February and the 24th of March 2014 at the medical campus of the University of Sharjah, which is located in an eastern suburb of Sharjah city, UAE. The campus houses colleges of dentistry, health sciences, medicine, pharmacy, an administration building, and a library. The number of student was 2599, and about another 500 students from other colleges infrequently use the campus. The facilities available for students were classrooms, computer rooms, libraries, laboratories, lounge rooms, and restaurants. All of these facilities are supplied with washing rooms and hygiene facilities with regular daily cleaning services. There were ten food vending machines, six elevators, and five main student computer rooms with 200 public computers.

Sampling

One hundred specimens were collected aseptically from 30 computer keyboards (K), ten digital vending machine panels (V), and ten outside-panels of elevators (E). Ten surfaces were sampled weekly; and each surface was sampled twice; in the morning and in the afternoon (n=100). The same surface was repeated every week. Sterile plastic templates (10 cm² in area) and cotton swabs moistened with a sterile nutrient broth were used to collect samples. The swabs were transferred within 2 h of collection to the laboratory and immersed in sterile centrifuge tubes containing 1 ml of nutrient broth each. The tubes were vortexed for 30 s and their contents were transferred to tubes containing nine ml of peptone water each, and they were incubated for 2 h at 35°C to help injured cells recovery before culturing them in selective media. Aliquots (0.1 ml) from the diluted tubes were spread onto agar plates either containing MacConkey's agar, Baird- Parker agar or plate count agar. All agar plates were incubated for 24 to 48 h at 35°C. The number of bacterial colony-forming units (CFUs) was counted and expressed as CFU/cm² surface area.

Identification of isolates

Separate colonies were picked for the Gram stain tests and microscopic morphology following sub-culture on nutrient agar plates that were used for catalase testing and DNA isolation. Accuvis's Bacterial Genomic DNA Isolation Kit AV1003 (AccuVis Bio company, Abu Dhabi, UAE) was used to purify DNA from the isolates according to the manufacturer's instructions.

A commercial identification service of the bacterial 16S rRNA gene was purchased from AccuVis Bio Company, Abu Dhabi-UAE under the supervision of the researchers. In this service: for amplification of the bacterial 16S rRNA gene, the 16S universal PCR primers: 27F-5'-AGAGTTTGATCMTGGCTCAG-3' (forward) and 1492 R-5'-TACGGYTACCTTGTACGACTT-3' (reverse) were used. The reaction started by initial incubation at 95°C for 2 min followed by 35 cycles of melting at 95°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 2 min and 10 min of final extension at 72°C. The amplified DNA products were purified using Norgen's PCR Purification Kit (Norgen Biotek Corp., Canada) according to the manufacturer's instructions. The amplification products were kept at 4°C before sequencing.

Sequencing analysis was performed with a BigDye Terminator v1.1 Cycle Sequencing Kit (Life technologies, Thermo Fisher Scientific, USA) and the Applied Biosystems 3130 Genetic analyser (Life Technologies) sequencing platform. The sequencing reactions were performed with 518F (forward) and 800R (reverse) primers. Data analysis was performed using Sequencing Analysis Software v5.2.3. For the identification of isolates, the produced sequences were converted to FASTA format per the National Center for Biotechnology Information (NCBI) and the final sequences were trimmed followed by performing NCBI Basic Local Alignment Search Tool (BLAST) searches for similar sequences.

Antibiotics susceptibility of the isolates

The bacterial isolates from publicly used surfaces were tested for their sensitivity to 18 antibiotics using a disk diffusion susceptibility test protocol following the Clinical and Laboratory Standards Institute (CLSI) approved standards: M02-A11 titled *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, Eleventh Edition* (Cockerill et al., 2012). The antibiotic sensitivity testing disks (HiMedia, Bombay, India) used in this study included ampicillin (10 µg), chloramphenicol (10 µg), ciprofloxacin (30 µg), clindamycin (2 µg), colistin (50 µg), co-trimazine (25 µg), erythromycin (15 µg), gentamycin (30 µg) kanamycin (30 µg), levofloxacin (5 µg), methicillin (10 µg), nitrofurantoin (200 µg), norfloxacin (5 µg), oxacillin (1 µg), penicillin (10 µg), rifampicin (15 µg), tetracycline (10 µg) and vancomycin (10 µg). To interpret the isolates' susceptibilities, the results were matched against the twenty-fourth informational supplement tables of the M100-S24 (Patel et al., 2014).

Statistical analysis

Statistical analysis was performed using IBM-SPSS software (version 22) for regression plotting of variables associated with bacterial counts. Excel (Microsoft Corporation) was used to calculate and compare percentages of the isolates.

Qualitative and non-parametric data collection

Information regarding cleaning schedules and observations of cleaning patterns and methods in addition to the patterns of use and traffic differences in the morning and afternoon were recorded by the researchers at several intervals throughout the study period.

Observations of the availability and condition of hygiene facilities and infrastructure on the campus were also noted.

Information concerned with availability of students' orientation sessions regarding good hygiene practices during the clinical training was obtained from the chair of the clinical training committee at College of Health Sciences.

The cleaning process of each surface of equipment was done as follows: dusting by mopping using a towel, followed by wiping with liquid detergent and a clean towel then spraying the target area with surface sanitizer followed by waiting for few minutes as contact times before wiping the surface with another clean towel. For the purpose of this study, the observations of surface cleaning degree were rated into very clean, clean, or unclean.

RESULTS

Among the 100 specimens collected from surfaces, indicator organisms and other bacterial species were recovered from 43% of samples. Over half (57%) of samples exhibited no recoverable indicator organism growth in selective media. The highest average bacterial counts (CFUs) were obtained from vending machines at both the morning and afternoon sampling intervals (Figure 1). In a comparison of CFU from surfaces sampled in the mornings with those sampled in the afternoons, 50% of morning samples had bacterial growth compared to 36% of afternoon samples. The mean CFU values of these samples are presented in Figure 2.

In spite of the apparent differences in averages of

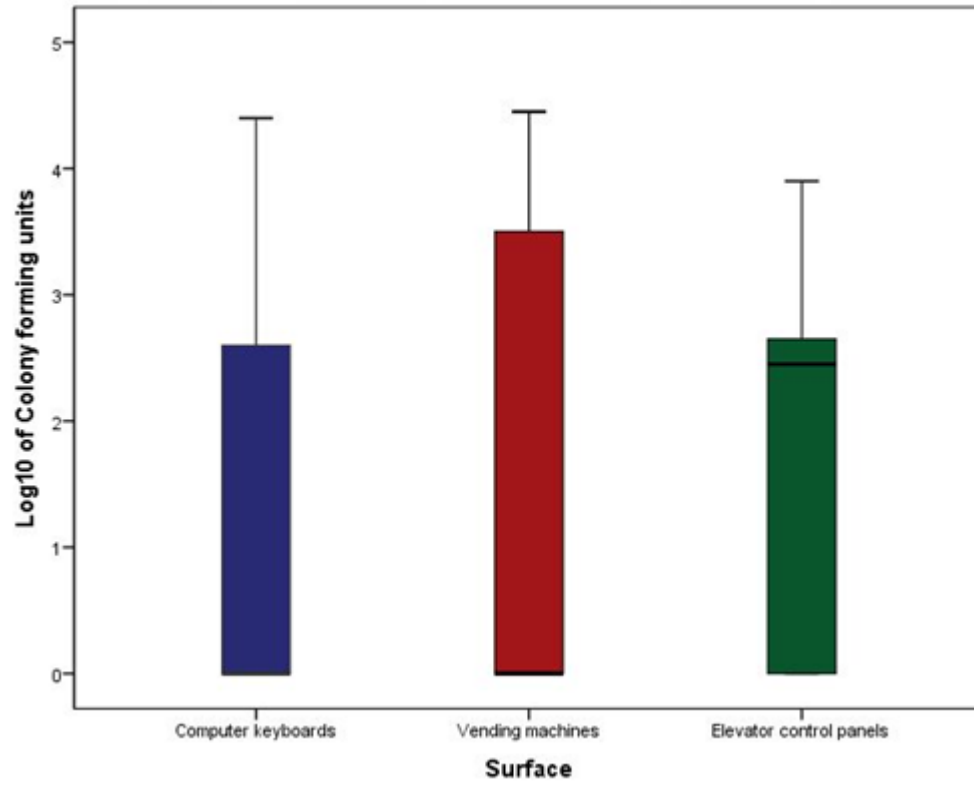


Figure 1. Means of CFU /10 cm² of publicly used surface expressed as log₁₀ values.

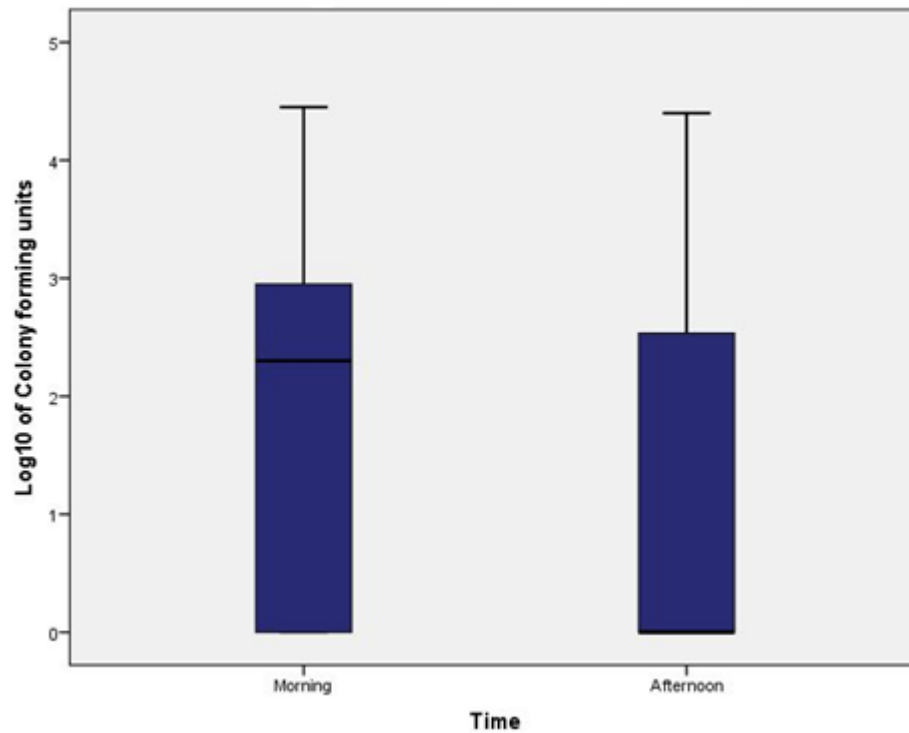


Figure 2. The mean log₁₀ of CFU values per 10 cm² of surfaces sampled in the morning and afternoon.

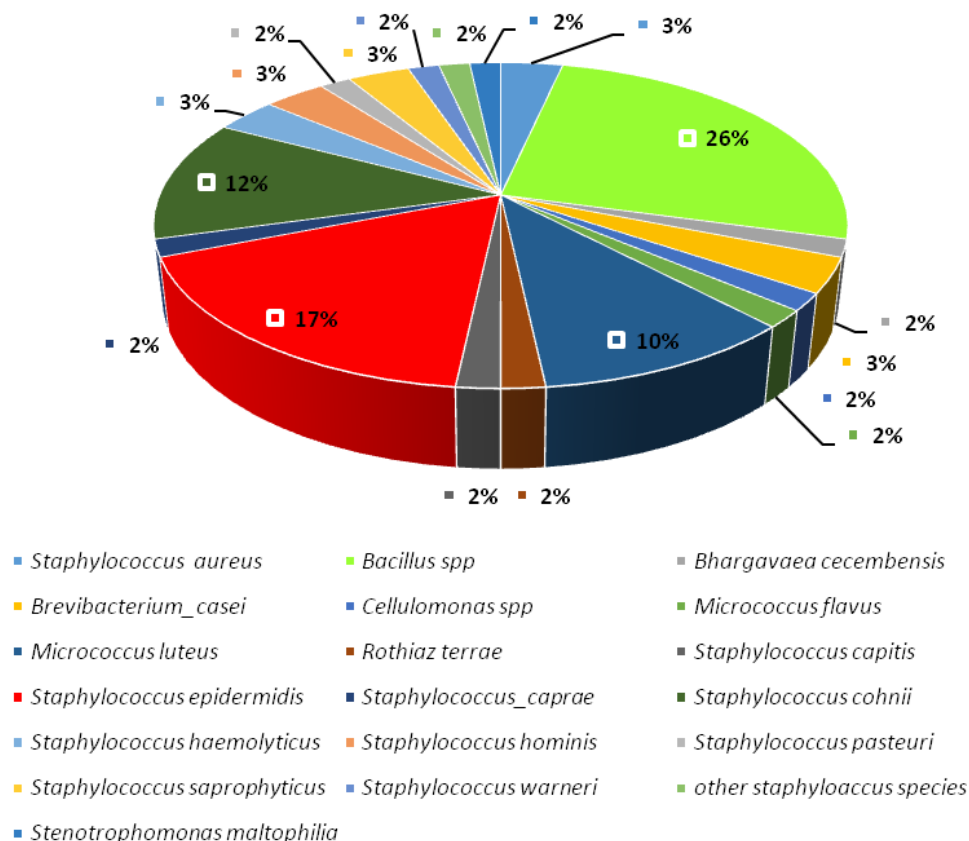


Figure 3. The percentages of bacterial genera and species among the isolates.

bacterial counts among surfaces, the differences in the bacterial load between computer keyboards, vending machines and elevator buttons were not significant ($p = 0.092$).

Nineteen bacterial species were isolated from the surfaces. These include *Bacillus persicus* and other *Bacillus spp.* (26%) in addition to *Bhargavaea cecembensis*, *Micrococcus luteus*, *Rothia terrae*, *S. aureus*, *Staphylococcus caprae*, *Staphylococcus cohnii*, *Staphylococcus epidermidis* (17%), *Staphylococcus haemolyticus* and *Staphylococcus pasteurii*. Three percent of the isolates were *S. aureus*, which is a highly hazardous microorganism. Two percent of the samples were *Bravibacterium casei*. Figure 3 shows the percentages of each species out of the total number of isolates.

The specimens from the elevator panel and vending machines were mostly colonised by *Staphylococcus spp.* (35 and 20%, respectively). Five different types of *Staphylococcus spp.* were identified (*S. hominis*, *S. epidermidis*, *S. haemolyticus*, *S. saprophyticus*, and *S. aureus*). One-third (33.3%) of computer keyboards ($n=60$) were positive for *Staphylococcus spp.* followed by *Micrococcus spp.* (6.7%), *Stenotrophomonas maltophilia* (1.7%) and *B. casei* *Cellulomonas spp.* (1.7%).

The majority of isolates had high rates of sensitivity to

antibiotics (Table 1). The majority of *Staphylococcus spp.* isolates were resistant to penicillin and ampicillin, and most of the isolates were resistant to colistin. No methicillin-resistance strains were observed among the *Staphylococcus spp.* isolates. Table 1 shows the isolates' sensitivities and resistance patterns.

Observations made by the researchers at different intervals indicated that relatively higher vending machine traffic occurred in the mornings compared to the afternoons. Additionally, extensive traffic in the computer rooms was observed in the mornings compared to the afternoons.

The cleaning schedules in the university campus were maintained daily and were performed by contractors who were double-checked by supervisors from the university bodies dedicated to this purpose. Cleaning checklists were displayed in several places and were checked daily by cleaning staff and their supervisors. The hygiene facilities, infrastructure, and conditions appear in acceptable hygienic condition. All the investigated surfaces were described as clean by the researchers.

All students attend compulsory orientation sessions regarding good hygiene practices during the clinical training provided by their departments and/or the clinical training preceptors as stated by the chair of the clinical training committee at College of Health Sciences.

Table 1. Contd.

	S	17	2	2	1	7	1	1	2	11	18
Kanamycin (30)	R	1	0	0	0	0	0	0	0	1	0
	I	4	0	0	1	1	0	0	0	1	1
	S	12	2	2	0	6	1	1	2	9	17
ciprofloxacin (30)	R	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0
	S	17	2	2	1	7	1	1	2	11	18
chloramphenicol (10)	R	0	0	0	0	0	0	0	0	1	0
	I	0	0	0	0	0	0	0	0	0	0
	S	17	2	2	1	7	1	1	2	10	18
Ampicillin (10)	R	17	1	1	1	7	1	1	0	8	12
	I	0	0	0	0	0	0	0	0	0	0
	S	0	1	1	0	0	0	0	2	3	6
Rifampicin (15)	R	0	0	0	0	0	0	0	0	0	0
	I	8	0	0	0	0	0	0	0	0	0
	S	10	2	2	1	7	1	1	2	11	18
Vancomycin (10)	R	0	0	0	1	2	0	1	0	0	0
	I	1	0	1	0	1	0	0	0	4	5
	S	16	2	1	0	4	1	0	2	7	13
Methicillin (10)	R	17	1	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	1	0
	S	0	1	2	1	7	1	1	2	10	18
Nitrofurantion (200)	R	1	0	0	0	0	0	0	0	1	0
	I	2	0	0	0	0	0	0	0	0	3
	S	14	2	2	1	7	1	1	2	10	15

R = resistant, I = intermediate resistant, O = susceptible.

DISCUSSION

In this study, the aerobic and facultative aerobic bacterial loads on surfaces of publicly used

equipment in medical and health sciences colleges were assessed in addition to an exploration of the diversity of isolates with an emphasis on indicator organisms that can indicate

human sources of microbial contamination.

The highest bacterial counts were obtained from vending machines in both morning and afternoon

sampling intervals (Figure 1). This could be attributed to higher numbers of users. Extensive user traffic was observed in the morning samples, which had a higher count of microorganisms compared to the afternoon samples, when the traffic of students reduced substantially due to the students' study plans, lecture schedules, and off-site practical sessions. Fifty percent of morning samples had bacterial growth compared with 36% of afternoon samples.

The high rate of positive bacterial cultures found on computer keyboards (53%) might be related to the fact that keyboards are frequently used by numerous students and experience prolonged contact time with a person's hands. Similar observations have been described before by Bright et al. (2010). These levels of contamination are notably common in such facilities because the equipment is displayed in open spaces and are thus more susceptible to environmental sources of bacteria in the forms of soil (dust), air and natural bacterial species on human skin (users). Apparently, the health significance of the number and level of bacterial species originates from two factors; the first is the presence of pathogenic isolates and the second is the bacteria count. The major patterns of bacterial isolates observed during this study belong to the *Staphylococcus species* and *Bacillus species*. The first genus is attributed to contamination by humans while the second is mostly native to soil. In this study, isolated *S. aureus* was in only 3% of isolates. However, this highly hazardous bacterium is more common skin and nares coloniser among hospital workers and related subjects (Miller et al., 2012).

Several of the isolates identified in this study have rarely been encountered in similar studies, including *B. casei*, *B. cecembensis*, *Rothia terrae*, *Cellulomonas* spp., *S. maltophilia* and *B. casei*. The latter species is a pathogenic bacterium in immune-compromised and immune-competent persons (Kumar et al., 2011). *B. cecembensis* is a relatively novel species isolated from sea sediment in the Indian Ocean (Manorama et al., 2009). No data regarding its pathogenicity are available. *Rothia terrae* is a soil bacterium (Chou et al., 2008).

S. maltophilia is a species of increasing importance as a nosocomial infection pathogen in hospitals and other clinical settings. A reported increase in the number of patients infected with this pathogen was previously declared by Samonis et al. (2012).

Bacillus spp. comprised 26% of the total isolates, and these bacteria are widely present in nature in the soil, air, and plants. Therefore, they may have settled on surfaces via dust particles.

None of the sampled surfaces was positive to coliform species, enterobacteria were relatively less detected in touched surfaces compared to other bacterial groups. This could be supported by data presented by Anderson and Palombo (2009) who studied computer keyboard in Australian University and isolated Enterobacteriaceae and *E. faecalis* from one keyboard out of 17 computers.

Egert and his colleagues detect Enterobacteriaceae members from only 3 out of 60 uncleaned touch screens of smart phones and did not detect any enterobacteria from cleaned screens, although smartphones has a known repeated touch pattern (Egert et al., 2014). Their mere deference from other touched surfaces is that they are mostly used personally.

Undetectable coliform in this study could be attributed to an efficient cleaning process. This hypothesis can be supported by the findings of Joga and Palombo (2012) who declared that their disinfected keyboards showed a reduction in coliforms count between 40% and 60%. Furthermore, no coliforms on all computers surfaces could be detected after two weeks of the commencement of their study. They stated that even simple cleaning procedures were very effective in eliminating coliforms.

The health risks of bacterial contamination are minimal when pathogenic bacteria are not present.

This study assessed the bacterial profiles from surfaces of medical colleges in a UAE university. The campus had a well-maintained hygienic infrastructure and the regular cleaning schedules might have contributed to the absence of recoverable bacterial species observed in other studies. Nevertheless, using selective media in isolation from surfaces may limit the precision of the detection of bacterial contamination on surfaces, especially for enterobacteria and species other than bacillus and Staphylococci species. However, the effect of effective cleaning and the maintenance of proper hygiene measures by cleaners and users cannot be overlooked.

The bacterial profile of publicly used equipment in the studied medical and health sciences colleges resemble the bacterial profiles of surfaces in settings used by the general population. This is demonstrated by the moderate and high sensitivities of the isolates to commonly used antibiotics in the medical field. The isolate susceptibilities to the tested antibiotics are still high compared to hospital and clinical isolates highlighted in the scientific literature concerned with the resistance and sensitivity of bacterial isolates to antibiotics. Macahilig et al. (2015) stated that epidemiologic reports of methicillin-resistant *S. aureus* (MRSA) burden in the Middle East vary by country in the rates of 20–60% in blood cultures positive for *S. aureus* isolates. No data were available about MSRA or other Staphylococcus species concerning antibiotic resistance in UAE (Aly and Balkhy, 2012). Thus, this study contribute in forming a baseline data about other staphylococcus species pattern of resistance to antibiotics in general population represented by university students and staff.

Further follow-up and concurrent comparative studies in hospitals, medical colleges, and non-clinical settings are needed to understand the antibiotic resistance patterns further.

One limitation of this study was the absence of a simultaneous study assessing surface bacteria in

hospitals where students complete their practical training. However, such a study would require a large sample size of hospitals and medical colleges in addition to samples for non-clinical settings. The hygienic practices of the students also require further study.

Conclusion

This study determines the bacterial counts and identities of species on publicly used surfaces located in medical and health science educational institutes. The majority of the isolates are Gram-positive species that are commonly associated with human body, including *Staphylococcus* species followed by those associated with soil; comprising *Bacillus* spp. Vending machine revealed the highest bacterial count compared to elevator buttons and computer keyboards. The latter showed the most diverse group of species among the surfaces sampled. Most of the staphylococci groups are resistant to penicillin, ampicillin, colistin, and sensitive to methicillin. The antibiotic resistance patterns of isolates in the educational environment still resemble patterns observed previously in bacterial isolates of general community settings. The count, species, and antibiotic susceptibility patterns of the isolates suggest that students of medical and health specialities contact with publicly used equipment and shared devices might not form a significant route of dissemination of nosocomial infections' causative agents. Nevertheless, more studies are still needed in this concern.

Conflict of interests

The author(s) did not declare any conflict of interest.

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