Original Article

Incident of the contamination of medical laboratories in four selected universities by pathogenic bacteria in Jordan

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Objective: To determine and identify pathogenic bacteria on solid surfaces in clinical and teaching laboratories in four selected universities in Jordan.
Methodology: This study was conducted in biology, microbiology, hematology, and anatomy laboratories at four Jordanian universities. Solid surfaces (bench tops, seats, sinks, tap water handles, and doorknobs) were swabbed and cultured on nutrient agar as non selective medium and incubated aerobically at 37 °C for 48 hours.
Results: Four types from gram-negative (G-ve) bacteria, and five types of gram-positive (G+ve) bacteria were found to be present in the swabbed surfaces. Identified bacterial included *E. coli spp*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Klebsiella oxytoca*, *Staphylococcus epidermis*, *Staphylococcus Aureus*, *Enterococcus faecalis*, *Bacillus cereus*, and *Bacillus subtilis*.
Conclusion: The identified bacterial species isolated from the exposed surfaces of the teaching laboratories suggests that such surfaces are indeed potential fomites. However, some of the bacterial isolates are not of a pathological concern to healthy individuals. (Rawal Med J 202;46:307-313).
Keywords: Contamination, fomites, medical laboratories, pathogenic bacteria.

INTRODUCTION

It has long been documented that many inanimate surfaces can serve as grounds for the growth and proliferation of various bacterial species. These fomites may include objects that are subject to repetitive touching by hands, things like sinks, doorknobs, cutting boards, and computer keyboards, and all these have been identified as potential fomites. Most Gram-positive (G+ve) bacteria such as *Enterococcus spp*. (including Vancomycin-resistant *Enterococcus*), *Staphylococcus aureus* (including methicillin-resistant *S. Aureus*), or purulent *Streptococcus*, live for several months on dry surfaces. Similarly, many Gram-negative (G-ve) species, such as *Acinetobacter spp*., *E. coli*, *Klebsiella spp*., *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Shigella spp.* can survive on inanimate objects for long periods of time. However, some select bacterial species like *Bordetella*, *Haemophilus influenzae*, *Proteus vulgaris*, or *Vibrio cholerae*, can only survive up to 12 days on inanimate objects.[1-3] Many microorganisms may be transferred to hands or other exposed areas of the body, causing infections.[4]

Several studies have identified objects like cell phones, coins, fabrics, taps, door handles, and plastics as potential fomites that maybe contaminated with, and subsequently serve as sources of infection.[5-7] Medical laboratories are a clinical setting where samples like sputum, blood, urine and stool often come from possibly infected patients and thus may lead to the contamination of surfaces in the laboratory.[8-10] However, there remains little to no investigation of such potential fomites in teaching laboratories at universities. The present work describes an investigation of four Jordanian universities where exposed surfaces in four laboratories (biology lab, microbiology lab, haematology lab, and anatomy lab) were swabbed for the presence of micro-organisms. This is an effort to bridge the knowledge gap about possible fomites in teaching laboratories.

METHODOLOGY

Study area sampling: Four universities (coded University A, B, C, and D) were selected randomly from the list of Jordanian universities. In each

university, four medical laboratories (biology, microbiology, haematology, and anatomy labs) were selected as study sites. Both dry and wet swabs were taken from seats (8 swabs per object), benches (8 swabs per object), water tap handles (8 swabs per object), and doorknobs (4 swabs per object) at various times of the day. The swabs used for sample collection were pre-sterilized, individually packed and sealed cotton swabs. Collected swabs were sealed individually and maintained at ambient temperature. All collected swabs were cultured within one hour after collection.

Bacteria growth and counting: The plates were incubated at 37 °C for 48 hours. After incubation, the plates were examined for bacterial growth, and the resulting colony forming units (CFUs) were counted and converted to CFU/cm^2.

Isolation of pure colonies: Colonies that were observed to be morphologically different were transferred to separate, clean nutritive agar plates in order to obtain pure cultures. The pure cultures were stored in 30% glycerol stock cultures and stored at -20º C for further evaluation.

Morphological characterization of bacteria: The isolated colonies were characterized by their elevation (markedly raised, slightly raised, or flat colonies), form (spreading, irregular, or circular), size (large, medium, small, or pinpoint), pigmentation (white, pink, red, or colourless), and texture.

Biochemical identification and confirmation of the isolated bacteria: This was conducted using enzymatic identification tests (catalase, decarboxylase, and oxidase enzyme), polymer metabolism tests, indol tests, hydrogen sulphide production capacity, phenylalanine deamination test, nitrate reduction test, and the methyl red test. Pure bacterial isolates were at first Gram stained and treated with biochemical tests. As described previously and the oxidase test[11] was used to determine if the bacteria includes certain cytochrome C oxidase. The oxidase test was carried out using an aqueous solution (1%) of N, N, N', N'-tetramethyl-p-phenylenediamine. For the biochemical tests, certain identification kits, and software were used; the diagnostic RapID ONE system (Remel, USA), ERICTM software, and Biomeriux VITE-2 AES. Microgen® Bacillus ID Panel, for the identification of *Bacillus spp*. and related genera, 20 tests per kit, by Microgen® Bioproducts were used to identify the isolated strains.

Statistical analysis: One-way Analysis of variance (ANOVA) tests using SPSS version 23, was performed to determine whether the bacterial counts from the four universities and between the laboratories were statistically different ($p \leq 0.05$).

RESULTS

The total count number of heterotrophic bacteria within the teaching laboratories was almost approximately the same for the universities B, C, and D (837 CFU/cm^2, 851 CFU/cm^2, and 891 CFU/cm^2 respectively). Notably, university A showed a slightly lower number than the other universities (627 CFU/ cm^2). In University A, no significant difference was seen in the total number of heterotrophic bacteria observed in the microbiology lab and hematology lab (52 CFU/cm^2 vs. 90 CFU/cm^2, respectively). However, a significantly higher number of colony forming units was seen in the biology and anatomy labs (184 CFU/cm^2 and 301 CFU/cm^2 respectively) (Table 1).

Nine different bacterial species were isolated and identified, namely E. coli spp, Shigella sonnei, Pseudomonas aeruginosa, Klebsiella oxytoca, Staphylococcus epidermis, Staphylococcus Aureus, Enterococcus faecalis, Bacillus cereus, and Bacillus subtilis. Two different coliforms were identified including E. coli and Klebsiella oxytoca. All laboratory area was contaminated with both coliform and non-coliform bacteria. The Frequency of various bacterial contaminants in the laboratory area (fomites) is different from lab to another in the university (Table 2). Table 3 contains a summary of the frequency of the bacterial contaminants in the laboratory area in the four universities.

Table 1. Total number of bacterial colonies counts according to place from which the swab was acquired.

University		Total count number of heterotrophic bacteria (CFU/cm^2)				
		Microbiology	**Hematology**	**Biology**	**Anatomy**	**Total**
A	Bench	27	33	50	80	190
	Seat	06	22	44	47	119
	Sink	13	24	39	87	163
	Tap water handle	03	09	42	79	133
	Knob	03	02	09	08	22
	Total	52	90	184	301	627
B	Bench	22	39	81	120	262
	Seat	10	27	53	112	202
	Sink	09	28	73	75	185
	Tap water handle	05	11	62	88	166
	Knob	01	04	08	09	22
	Total	47	109	277	404	837
C	Bench	29	47	64	112	252
	Seat	15	35	75	109	234
	Sink	07	34	59	90	190
	Tap water handle	07	13	55	79	154
	Knob	02	04	07	08	21
	Total	60	133	260	398	851
D	Bench	21	51	79	103	254
	Seat	13	43	81	106	243
	Sink	10	40	68	89	207
	Tap water handle	06	12	65	76	159
	Knob	06	04	09	09	28
	Total	56	150	302	383	891
Total		215	482	1023	1486	3206

Table 2. Frequency of various bacterial contaminants in the laboratory area (fomites) in university A, B,C & D.

Bacteria name	University	Number of isolates along with their percentage					
		Bench	Seat	Sink	Tap water handle	Knob Door	Total
E. coli spp.	**A**	5 (35.71)	7 (50)	1 (7.14)	0	1 (7.14)	14 (31.11)
	B	4 (30.7)	7 (53.84)	1 (7.69)	**0**	1 (7.69)	13 (25)
	C	2 (13.33)	9 (60)	2 (13.33)	1 (6.66)	1 (6.66)	15 (19.23)
	D	3 (17.64)	7 (41.17)	4 (23.52)	2 (11.76)	1 (5.88)	17 (20)
Shigella sonnei	**A**	2 (40)	3 (60)	0	0	0	5 (11.11)
	B	4 (44.44)	4 (44.44)	1(11.11)	0	0	9 (17.30)
	C	1 (50)	1 (50)	0	0	0	2 (2.56)
	D	1 (33.33)	2 (66.66)	0	0	0	3 (3.52)
Pseudomonas aeruginosa	**A**	0	1 (100)	0	0	0	1 (2.22)
	B	0	1 (100)	0	0	0	1 (1.92)
	C	2 (33.33)	1 (16.66)	1 (16.66)	0	2 (33.33)	6 (7.69)
	D	2 (33.33)	3 (50)	1 (16.66)	0	0	6 (7.05)
Klebsiella oxytoca	**A**	4 (44.44)	4 (44.44)	1 (11.11)	0	0	9 (20)
	B	2 (25)	3 (37.5)	3 (37.5)	0	0	8 (15.38)
	C	4 (36.36)	4 (36.36)	2 (18.18)	0	1 (9.9)	11 (14.10)
	D	2 (14.28)	6 (42.85)	4 (28.57)	1 (7.14)	1 (7.14)	14 (16.47)
Staphylococcus epidermis	**A**	1 (33.33)	2 (66.66)	0	0	0	3 (6.66)
	B	1 (33.33)	2 (66.66)	0	0	0	3 (5.76)
	C	3 (30)	4 (40)	1 (10)	1 (10)	1 (10)	10 (12.82)
	D	4 (44.44)	3 (33.33)	1 (11.11)	0	1 (11.11)	9 (10.58)
Staphylococcus Aureus	**A**	2 (50)	1 (25)	1 (25)	0	0	4 (8.88)
	B	2 (33.33)	3 (50)	1 (16.66)	0	0	6 (11.53)
	C	5 (45.45)	4 (36.36)	1 (9.09)	0	1 (9.09)	11 (14.10)
	D	6 (46.15)	5 (38.46)	1 (7.69)	0	1 (7.69)	13 (15.29)
Enterococcus faecalis	**A**	2 (33.33)	3 (50)	1 (16.66)	0	0	6 (13.33)
	B	3 (37.5)	4 (50)	1 (12.5)	0	0	8 (15.38)
	C	3 (33.33)	4 (44.44)	1 (11.11)	0	1 (11.11)	9 (11.53)
	D	4 (44.44)	3 (33.33)	1 (11.11)	0	1 (11.11)	9 (10.58)
Bacillus cereus	**A**	1 (100)	0	0	0	0	1 (2.22)
	B	2 (66.66)	1 (33.33)	0	0	0	3 (5.76)
	C	2 (33.33)	3 (50)	1 (16.66)	0	0	6 (7.69)
	D	4 (50)	4 (50)	0	0	0	8 (9.41)
Bacillus subtilis	**A**	1 (50)	1 (50)	0	0	0	2 (4.44)
	B	1 (100)	0	0	0	0	1 (1.92)
	C	4 (50)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	8 (10.25)
	D	2 (33.33)	3 (50)	1 (16.66)	0	0	6 (7.05)
Total	**A**	18	22	4	0	1	45
	B	19	25	7	0	1	52
	C	26	31	10	3	8	78
	D	28	36	13	3	5	85

Table 3. Frequency of various bacterial contaminants in the laboratory area (fomites) in the four universities.

Name	Number of isolates along with their percentage				
	D	C	B	A	Total
E. coli spp.	17 (28.81)	15 (25.42)	13(22.03)	14(23.72)	59(22.69)
Shigella sonnei	3 (15.78)	2 (10.52)	9 (47.36)	5 (26.31)	19(7.30)
Pseudomonas aeruginosa	6 (42.85)	6 (42.85)	1 (7.14)	1 (7.14)	14 (5.38)
Klebsiella oxytoca	14 (33.33)	11 (26.19)	8 (19.04)	9 (21.42)	42 (16.15)
Staphylococcus epidermis	9 (36)	10 (40)	3 (12)	3 (12)	25 (9.61)
Staphylococcus Aureus	13 (38.23)	11(32.35)	6 (17.64)	4 (11.76)	34 (13.07)
Enterococcus faecalis	9 (28.12)	9 (28.12)	8 (25)	6 (18.75)	32 (12.30)
Bacillus cereus	8 (44.44)	6 (33.33)	3 (16.66)	1 (5.55)	18(6.92)
Bacillus subtilis	6 (35.29)	8 (47.05)	1 (5.88)	2 (11.76)	17 (6.53)
Total	85	78	52	45	260

DISCUSSION

The presences of heterotrophic bacteria on the surface of teaching and medical laboratories was confirmed in all four universities. Thus, the fomites were considered as contaminated objects. The results also showed that there were variations in types of heterotrophic bacteria present on the surfaces of working areas inside the laboratories. Furthermore, a notable variation in the numbers of heterotrophic bacteria was observed. These variations and fluctuations are more likely a result of degrees of the cleanliness of these fomites and may be attributed to hygiene practices and general health of the students using the laboratories.

This is inconsistent with previous studies which showed that bacterial communities can be transported by dust, and the total bacterial cells concentrations tend to increase in the presence of dust, moisture, and nutrients from leftovers of the food items.[12,13] Therefore, clean surfaces are milestone to reduce bacterial contamination.[14] However, since little data is available about the bacterial contamination of the working area in the teaching laboratories, it is difficult to establish a baseline for the allowed bacterial contamination level in teaching laboratories.

Environmental contamination of laboratories differs between teaching laboratories due to the difference experimental work and geographical distribution. A study on clothes samples showed *Bacillus subtilis* was isolated from 28.5% of the samples along with *Stahylococcuss aerous* (21.5% of the samples swabbed), with 50% of the samples being *Staphylococcus epidermidis*.[15,16] Other reported results showed bacterial contaminants in microbiology laboratory in different areas of laboratory including table, floor, clothing, air, and incubator, the following ratios; 36.36% was *Staphylococcus epidermis* which was the most common contaminant, 31.81% was *Bacillus subtilis* which was the second most frequent contaminant, 18.18% was *Staphylococcus aureus,* while 13.63% was *Deptheriods,* which showed minimum bacterial contaminant.[8]

In a study, over 50% of the bacteria isolated from the swabbed fomites in the selected environments was found to be *S. aureus*.[1] Similarly, a study in which the fomites swabbed were the persons and belongings of healthcare workers in a selected hospital found multiple bacterial contaminants, including *Staphylococcus aureus* (57.6%), *Klebsiella pneumoniae* (19.2%), and *E. coli* (6.4%).[2] A similar study examining fomites in intensive care units showed 68% of the swabbed surfaces showed bacterial growth before and 63% bacterial growth after fumigation, mainly growth of *Bacillus spp*. (15%), *Klebsiella pneumoniae* (13.2%), *E. coli* (10.5%), *Klebsiella oxytoca* (7.9%), and *Streptococcus pyogenes* (5.3%).[3] Other studied reported prevalence of fomites in biological laboratories, with a significantly higher incidence of *S. aureus* and *S. epidermidis* (58.57% and 26.84%, respectively), with a lesser incidence of *Klebsiella spp. And Protus spp.* (11.98% and 4.29%,

respectively).[17]

Unlike results reported in the literature, (14.44%) the bacterial contaminants observed in this study were *E. coli spp* (22.69%), *Shigella sonnei* (7.30%), *Pseudomonas aeruginosa (*5.38%)*, Klebsiella oxytoca (*16.15%)*, Staphylococcus epidermis* (9.61%), *Staphylococcus aureus* (13.07%), *Enterococcus faecalis* (12.30%)*, Bacillus cereus* (6.92%), and *Bacillus subtilis* (6.53%). This leads to the conclusion that the incidence of fomites appears to be largely dependant on personnel to the highest extent, as evidenced by the significantly different distribution of bacteria relative to the area studied.

CONCLUSION

The surfaces in teaching laboratories can indeed act as potential fomites. The isolated bacterial colonies found contaminating teaching laboratories at the select universities were largely of little pathological concern to healthy individuals, which is a notable difference to other findings reported in the literature. However, some bacterial colonies were identified that may indeed be a pathological hazard, albeit having a lower occurrence than some of the findings reported in the literature.

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