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Antibiotic susceptibility of bacteria recovered from university libraries in Jordan

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ABSTRACT

Aims: Due to the growing number of media reports claiming that books contain germs, it is crucial to look into the possibility that contagious diseases could spread through libraries. The aim of the study was to identify bacteria from various fomites in four Jordanian university libraries and to assess the antibacterial resistance pattern of isolates.
Methodology and results: In this study, swab samples were taken from different fomites of four Jordanian university libraries. Samples were then cultivated on nutrient agar and incubated aerobically at 37 °C for 48 h. To identify different types of isolated bacteria, biochemical and conventional biochemical tests were applied using the qualitative RapID™ One System with the help of ERICTM software to identify the bacterial isolates at the species level. Identified bacterial species, including *Escherichia coli*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Staphylococcus epidermis*, *S. aureus*, *Salmonella choleraesuis*, *Bacillus subtilis* and *Citrobacter freundii* were isolated from different library fomites. Seventy-one bacterial isolates from University A were observed to be multidrug-resistant (MDR) (*S. sonnei* and *S. choleraesuis*). This MDR pattern is alarming as those isolates were found in a public environment and that imposes a direct threat on library users, staff and visitors.
Conclusion, significance and impact of study: University libraries' fomites carry live bacterial pathogens, which can contaminate users' hands and serve as an indirect route for spreading antibiotic resistance and microbial illnesses. While more research is required, considering hand hygiene improvement would be the simplest infection control technique at libraries. Additionally, proactive measures should be taken to track the prevalence of harmful microorganisms in these settings and their effects on employees' and the public's health.

Keywords: Contamination, fomites, university libraries, pathogenic bacteria

INTRODUCTION

Microorganisms can be transferred from living things to inanimate environmental sources, which can serve as a potential secondary reservoir in the transmission of pathogens if they meet the pathogens' demands for survival and reproduction (Borkow, 2014; Alsheikh *et al.*, 2021). According to Wißmann *et al.* (2021), most pathogens can persist on inanimate surfaces for weeks or even months and can transmit from these surfaces to humans either directly through surface-to-mouth contact or indirectly by contaminated hand contact with the mouth and eyes or nose (Gerba and Maxwell, 2012). Pathogenic microorganisms can also be transmitted via contact with fomites that are contaminated with infected body secretions such as saliva, mucus, nasal secretions, blood, urine and feces (Gigantesco and Giuliani, 2011).

Single-hand contact with contaminated surface results in a variable degree of pathogen transfer (Borkow, 2014). Transmission from surfaces to hands was most successful with *E. coli*, *Salmonella* spp., *S. aureus* (all 100%), *C. albicans* (90%), *Rhinovirus* (61%), *Hepatitis A* virus (22-33%) and *Rotavirus* (16%). Contaminated hands can also be a source of re-contamination of the surface, as revealed with *Hepatitis A* virus (Kramer and Assadian, 2014). Due to the ability of these germs to move through the air and colonize other surfaces, controlling them might be challenging (Karbowska-Berent *et al.*, 2011).

The library environment provides a suitable condition for microorganisms to grow, as many nutritious elements can be found in books and other archive items. Among them are cellulose contained in book paper and proteins in book binding (Karbowska-Berent *et al.*, 2011; Hempel *et al.*, 2014). That is why there are serious worries about the biodegradation of library materials and the negative impacts of these microorganisms on human health. This complicates the preservation of culturally and historically significant documents frequently kept in libraries (Havermans, 2017).

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Library pathogens have long been a severe public health issue. In an article published in 1985, McClary expressed concern about disease transmission through libraries. This occurred around the same time germ theory became famous and infectious disease was the leading cause of death, with significant outbreaks afflicting many cities and towns. As a result, libraries became a point of concern for disease transmission. Books were believed to have the ability to spread a number of bacterial diseases, such as typhus fever and scarlet fever, as well as fungi that may cause lethal blood poisoning (Jung *et al.*, 2019).

By the 1930s, the fear of libraries being disease harbingers had diminished greatly due to the introduction of more sophisticated public health measures such as immunization and increased sanitary standards. But by the discovery of the herpes virus traces in one of the most popular novels, Fifty Shades of Grey, this health concern has been brought to the fore in the popular media (Hempel *et al.*, 2014; Jung *et al.*, 2019). Rafiei *et al.* (2017) recently found that 20.8 percent of returned books from Isfahan University's Al-Zahra Hospital Library and the Library of Sciences Faculty were culture-positive. There are currently two reports in the United States demonstrating the presence of germs in library books. However, because most reports are based on university course assignments or high school science competitions, the complete procedures and results there is no scientific literature available (Jung *et al.*, 2019). Due to the growing public health concern, it is vital to explore and distribute knowledge on techniques that can inhibit the growth of these organisms in order to reduce their effect on human health. The present study aimed to identify bacteria profiles swabbed from various fomites in four Jordanian university libraries and to assess the antibacterial resistance pattern of bacteria isolated from these libraries along with MDR load. Recommendations regarding future research and health policy relating to this issue are also provided.

MATERIALS AND METHODS

Study area and sampling

Four Jordanian universities (designated A, B, C and D) were randomly selected for the study. The study targeted the library of each university, where seven spots (benches, seats, computer mouse, computer keyboards, bookshelves, books and borrowing books place) were selected for sampling. Two sterile cotton swabs (dry and wet) were used for sampling each surface by swabbing the entire surface of each part from end to end. The surface area was recorded for counting purposes (i.e., calculating colony forming units/cm^2; CFU/cm^2). Swabs were immediately transported (in labeled bags) to the laboratory for processing and cultivation.

Bacterial growth and counting

Cotton swabs were used to inoculate nutrient agar (Oxoid, UK) no more than one hour after sampling to calculate bacterial load (CFU/cm^2). All plates were incubated at 37 °C incubators for 48 h.

Isolation of pure colonies

Bacterial colonies of different morphology (i.e., size, shape, color, margin, elevation and opacity) were sub-cultured on new nutrient agar plates. Different bacteria from pure cultures were kept in 30% glycerol stock cultures and stored at -80 °C freezer for analysis.

Biochemical identification of the isolated bacteria

Pure bacterial cultures were Gram stained and then identified using conventional biochemical tests, including catalase test, oxidase test, decarboxylase test, hydrogen sulphide production test, indole test, phenylalanine deaminase test, nitrate reduction and methyl red test.

Gram-negative bacillus bacteria were first differentiated using an oxidase test, which determines the ability of bacteria to produce the cytochrome oxidase enzyme. The method was adapted from that of Steel (1961). The oxidase test was conducted using an aqueous solution (1%) of N, N, N', N'-tetramethyl-p-phenylenediamine.

The presence of coliform bacteria was assessed by culturing Gram-negative bacillus bacteria on selective media, MacConkey agar (Oxoid, UK) and Eosin methylene blue agar (Oxoid, UK). Bacteria were first streaked on a MacConkey agar plate and incubated for 18-24 h at 30-35 °C. If red-brick colonies of Gram-negative rods surrounded by a reddish precipitation zone were not found, then the result was considered negative. Otherwise, if colonies having the above-mentioned characteristics were seen, then bacteria were sub-cultured on an Eosin methylene blue agar plate and incubated for another 18-24 h at 30-35 °C. Metallic sheen or blue-black color colonies under transmitted light were considered positive for the presence of coliform bacteria. It should be noted that coliform bacteria, as indicators of fecal contamination, were of particular interest in this study.

Oxidase-negative, Gram-negative bacillus bacteria were further analyzed using RapID™ ONE System (Thermo Scientific, USA) according to manufacturer instructions. The resultant numerical micro-codes were entered into the web-based application ERIC$_{TM}$ to obtain the identity of these bacterial isolates to the species level with a high probability percent (>99.9%).

Other identification tests

Samples were further analyzed using several biochemical kits to confirm the identity of isolates, including the Analytical Profile Index (API®kit) (Biomerieux, USA) for Gram-positive and Gram-negative bacteria, Microgen® Bacillus ID Panel (VWR International Company, USA) for *Bacillus* spp. and related genera, and VITEK-2 AES microbial detection system (Biomerieux, USA). All tests were performed according to manufacturer instructions.

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Antimicrobial sensitivity of bacterial isolates

The sensitivity of bacterial isolates to several clinically relevant antibiotics was assessed using the Kirby-Bauer disk diffusion test. Antibiotic discs were placed on Mueller-Hinton Agar (Oxoid, UK) plates inoculated with a bacterial suspension of 0.5 McFarland turbidity standards (BUCH and HOLM, USA) and incubated for 18-24 h at 37 °C. Gram-negative isolates were subjected to the following antimicrobial panel; cefoxitin (30 µg), chloramphenicol (30 µg), norfloxacin (10 µg), ciprofloxacin (15 µg) and gentamicin (10 µg). Whereas Gram-positive isolates were subjected to the following antimicrobial panel: cefoxitin (30 µg), chloramphenicol (30 µg), norfloxacin (10 µg), ciprofloxacin (15 µg), gentamicin (10 µg), vancomycin (30 µg), ampicillin (30 µg) and erythromycin (15 µg). The antibiotic disks used were purchased from BIOANALYSE, Ankara/Turkey.

The diameter of the zones of inhibition was recorded (if found) on the following day and compared to the National Committee for Clinical Laboratory Standards (NCCLS) or the Clinical and Laboratories Standards Institute (CLSI) guidelines. Accordingly, each bacterial isolate was identified as susceptible (S), intermediate (I) or resistant (R) to these antibiotics. Isolates that were resistant to 3 or more different classes of antibiotics were categorized as multi-drug resistant (Magiorakos *et al.*, 2012).

Control strains of bacteria that have known diameter inhibition zones with specific antimicrobial discs were used as positive controls and phosphate-buffered saline (PBS) (Oxoid, UK) and sterile nutrient broth media (Oxoid, UK) were used as negative controls.

Statistical analysis

One-way analysis of variance (ANOVA) tests using SPSS version 23 were 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 university libraries was almost approximately the same for universities A and B (6528 CFU/cm^2 and 7210 CFU/cm^2, respectively) and approximately the same for universities C and D (9430 CFU/cm^2 and 10030 CFU/cm^2, respectively). The site of book and books borrowing place) in four university libraries (A, B, C and D) are the highest total bacterial count of other sites (Table 1).

In this study, the occurrence of coliform and non-coliform bacteria in the parts of libraries was also investigated in all universities. The coliform bacteria were tested by their biochemical properties during growth on MacConkey agar and Eosin methylene blue medium. Three different coliforms were identified, including *E. coli*, *K. pneumonia* and *S. choleraesuis*. One thousand two hundred sixty samples were taken from different fomites in each university library (bench, seats, computer mouse, computer keyboard, bookshelves, books and borrowing books place) presented in Table 2. 22.69% of samples from A university library showed bacterial growth, 22.14% from B university, 23.09% from C university and 23.41% from D university (Table 2). A total of 1151 bacterial isolates were isolated from four different Jordanian university libraries, of which 704 (61.2%) were Gram-negatives and 38.8% were Gram positives (Figures 1 and 2).

Nine pure different bacterial isolates were identified as *E. coli*, *S. sonnei*, *P. aeruginosa*, *K. pneumoniae*, *S. epidermidis*, *S. aureus*, *S. choleraesuis*, *B. subtilis* and *C. freundii*. The biochemical properties of these bacterial isolates and morphological were done, and conventional biochemical tests were applied using the qualitative RapID^{TM} One System (Remel, USA) to identify the bacterial isolates to the species level with the help of ERICTM software.

Bacterial profile

Among the different bacterial isolates from four Jordanian university libraries, *E. coli* (16.78%,17.92%, 17.18% and 20.67%) in A, B, C and D university libraries, respectively, was the most predominant Gram-negative isolate, followed by *S. sonnei*. Similarly, among Gram-positive isolates, *S. aureus* (16.08%, 19.35%, 17.18% and 18.64%) in A, B, C and D universities' libraries, respectively, was most commonly isolated, followed by *S. epidermidis* (Table 2).

Nine bacteria were isolated from different library objects (benches, seats, computer mouse, computer keyboard, bookshelves, books and borrowing books place). Among all universities, books were the highest contaminated with bacteria, most of them were *S. choleraesuis*, *S. epidermidis* and *S. aureus*, followed by borrowing books place in A university, borrowing books place and computer keyboard in B university, bookshelves in C university and computer keyboard in D university (Table 3).

The majority of Gram-negative were isolated from books except for C university books borrowing place was the most contaminated fomites with Gram-negative isolates Figure 1. While seat and bench were the least contaminated library fomites with Gram-positive bacteria Figure 2.

Antibiotic susceptibility pattern of isolates

Antibiotic susceptibility pattern of Gram-positive bacteria

Gram-positive isolates' observed drug susceptibility pattern indicated sensitivity toward most tested drugs. The sensitivity pattern to ampicillin was 69%, chloramphenicol 98%, norfloxacin 99%, ciprofloxacin 91% and gentamicin 98%, with only a low level of sensitivity to erythromycin and vancomycin, respectively, of 60% and 48%. Whereas almost all Gram-positive isolates were resistant to cefoxitin (Table 4).

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Table 1: The total count of heterotrophic bacteria on the benches, seats, computer mice, computer keyboards, bookshelves, books and borrowing books place taken from four selected universities in Jordan.

University *	Location	Total count of heterotrophic bacteria load (CFU/cm^2)	Percentage (%)
A	Bench	$1.2*10^2$	1.84
	Seat	$0.97*10^2$	1.49
	Computer mouse	$3.2*10^2$	4.9
	Computer keyboard	$2.91*10^2$	4.46
	Bookshelves	$1.2*10^3$	18.38
	Books	$3.4*10^3$	52.08
	Borrowing books place	$1.1*10^3$	16.85
	Total	6528	100
B	Bench	$1.9*10^2$	2.64
	Seat	$1*10^2$	1.39
	Computer mouse	$2.2*10^2$	3.05
	Computer keyboard	$3*10^2$	4.16
	Bookshelves	$1.8*10^3$	24.97
	Books	$3.6*10^3$	49.93
	Borrowing books place	$1*10^3$	13.87
	Total	7210	100
C	Bench	$2.5*10^2$	2.65
	Seat	$1.4*10^2$	1.48
	Computer mouse	$2.8*10^2$	2.97
	Computer keyboard	$3.6*10^2$	3.82
	Bookshelves	$1.9*10^3$	20.15
	Books	$4.6*10^3$	48.78
	Borrowing books place	$1.9*10^3$	20.15
	Total	9430	100
D	Bench	$2.9*10^2$	2.89
	Seat	$2.2*10^2$	2.19
	Computer mouse	$4.5*10^2$	4.49
	Computer keyboard	$3.7*10^2$	3.69
	Bookshelves	$1.9*10^3$	18.94
	Books	$4.7*10^3$	46.86
	Borrowing books place	$2.1*10^3$	20.94
	Total	10030	100

Table 2: Frequency of bacterial isolates from four universities libraries (A, B, C and D).

Bacterial isolates	A		B		C		D	
	No	%	No	%	No	%	No	%
Gram Negative bacteria								
E. coli	48	16.78	50	17.92	50	17.18	61	20.67
Shigella sonnei	36	12.58	44	15.27	42	14.43	55	18.64
Pseudomonas aeruginos	27	9.44	18	6.45	20	6.87	22	7.45
Klebsiella pneumonia	10	3.49	19	6.81	7	2.4	11	3.72
Salmonella choleraesuis	35	12.23	32	11.49	35	12.02	21	7.11
Citrobacter freundii	13	4.54	10	3.58	21	7.21	17	5.76
Gram Positive bacteria								
Staphylococcus epidermis'	37	12.93	46	16.48	34	11.68	30	10.16
Staphylococcus aureus	46	16.08	54	19.35	50	17.18	55	18.64
Bacillus subtilis	34	11.88	6	2.15	32	10.99	23	7.79
Total	286	100	279	100	291	100	295	100
Total bacterial %	5569		22.14		23.09		23.41	

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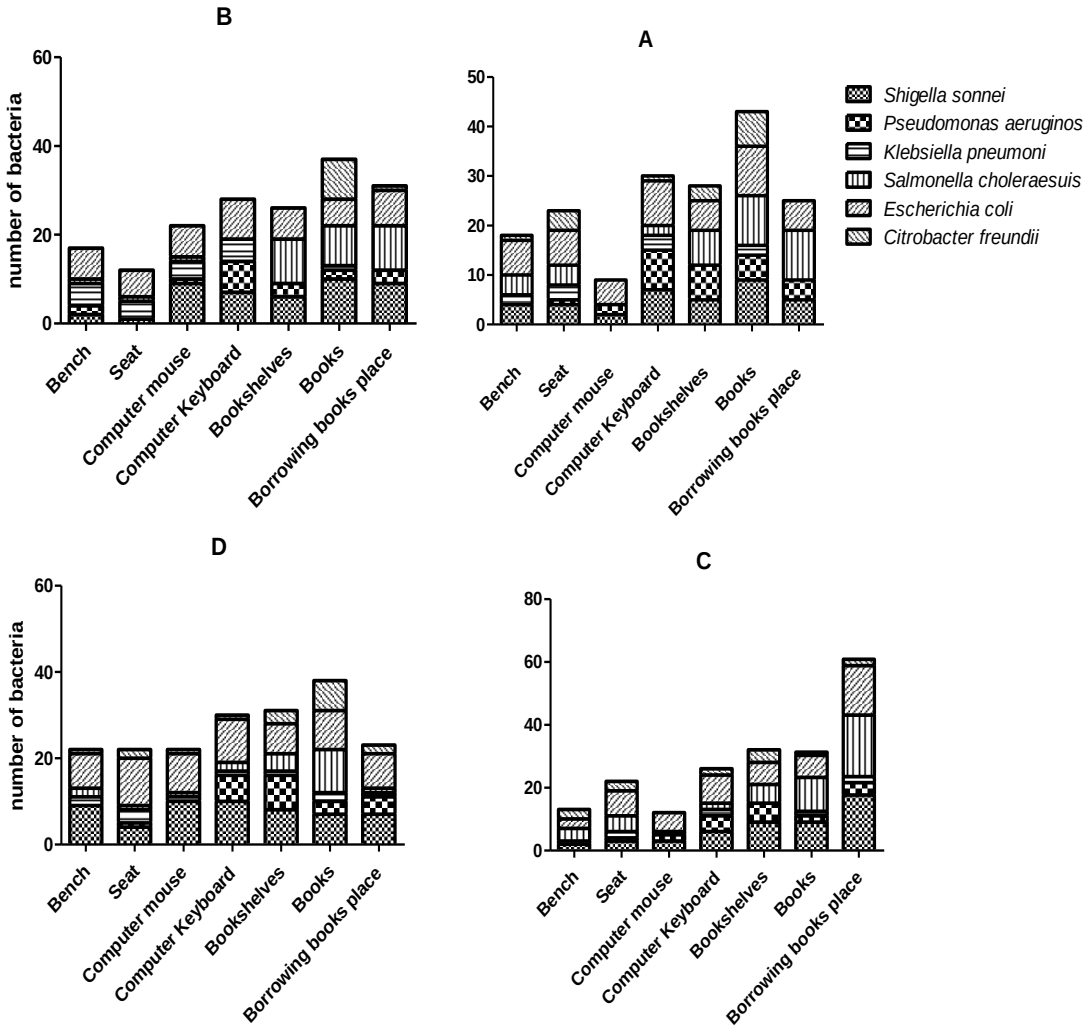

Figure 1: Number of Gram-negative bacterial isolates from different university libraries objects.

Antibiotic susceptibility pattern of Gram-negative bacteria

The antibiotic susceptibility test for Gram-negative bacteria revealed a high level of sensitivity against chloramphenicol 94%, ciprofloxacin 99%, gentamicin 71% and nearly all isolates were sensitive to norfloxacin (100%), whereas almost 93% of Gram-negative isolates were resistant to cefoxitin (Table 5).

The overall antimicrobial susceptibility pattern of Gram-positive and Gram-negative isolates revealed that they have a high level of sensitivity against chloramphenicol, ciprofloxacin, gentamicin and nearly all isolates were sensitive to norfloxacin (100%), whereas almost all isolates were resistant to cefoxitin except *B. subtilis*.

Multidrug-resistant strains

Out of 1151 bacterial isolates from four university libraries, 71 bacterial isolates from A university were MDR *isolates S. sonnei* and *S. choleraesuis.*

DISCUSSION

In this study, four Jordanian universities' libraries (designated as A, B, C and D) were screened for microbial contamination; where in each library, seven sites were inspected (bench, seats, computer mouse, computer keyboard, bookshelves, books and borrowing books place). The colony count for bacteria was found in universities A, B, C and D (6528 CFU/cm^2, 7210 CFU/cm^2, 9430 CFU/cm^2 and 10030 CFU/cm^2, respectively). The site of (Book and Borrowing books place) in four university libraries are the highest in total bacterial count than other sites, and this result is similar to a report by Ayoade and Amona (2018), in which the

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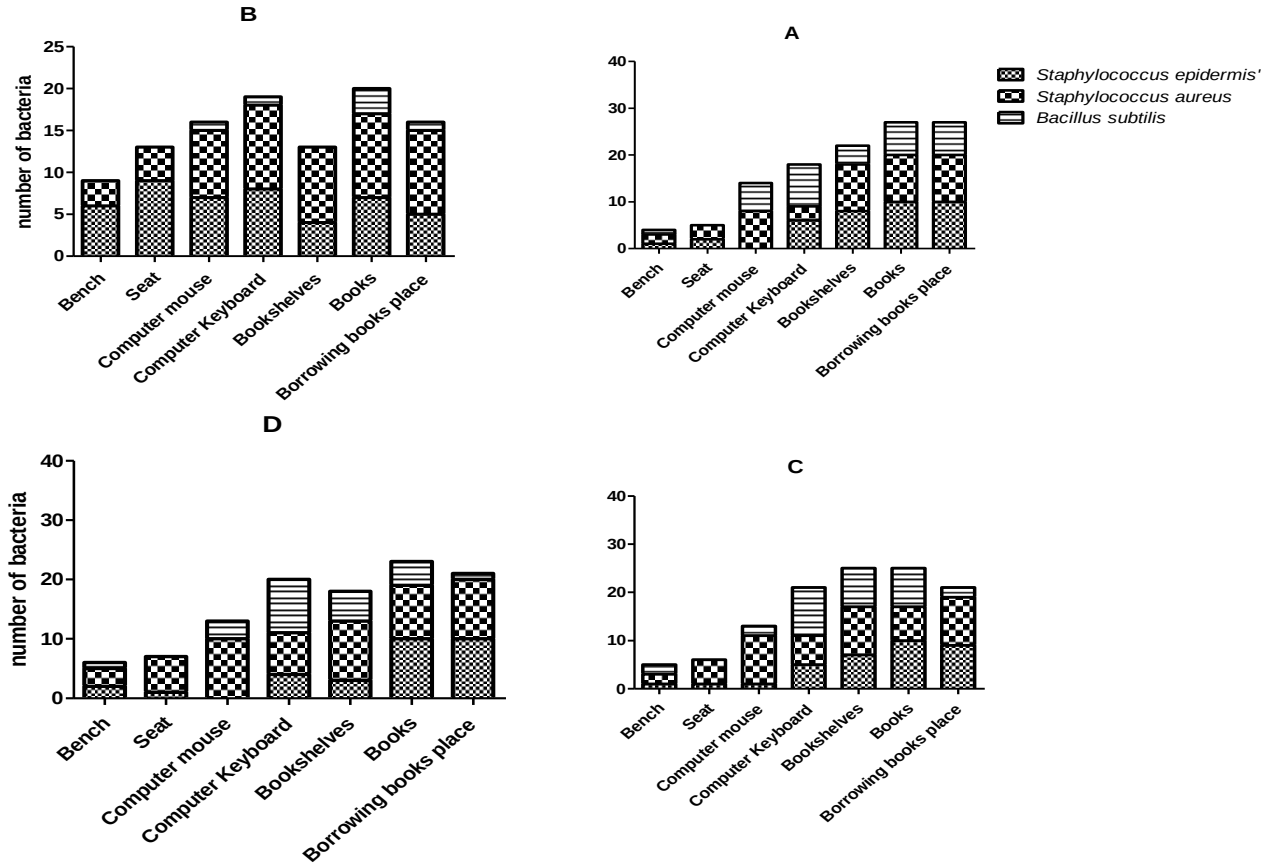

Figure 2: Number of Gram-positive bacterial isolates from different university libraries objects**.**

highest colony count was 1.7×10^{-5} CFU/cm^2 for bacteria was found in books sampled from the library, while the lowest bacterial colony counts (0.2×10^{-5}) were found in printed materials sampled from the clinic and the registry.

A total of 1260 samples were examined, with 22.7%, 22.14%, 23.1% and 23.4% microbial growth in universities A, B, C and D, respectively; there is no significant microbial growth level across the four universities, indicating similar hygiene practices in those universities. This is inconsistent with previous studies, which showed the presence of heterotrophic bacteria on the surface of teaching and medical laboratories were confirmed in all four universities. Thus, the fomites were considered contaminated objects (Alsheikh *et al.*, 2021).

One thousand one hundred fifty-one bacterial isolates, with Gram-positive bacteria dominating (704 isolates; 61.2%). Many similar studies have reported the predominance of Gram-positive isolates over Gram-negative bacteria. In a survey conducted in Dhulikhel hospital, Gram-positive bacteria were accentuated over Gram-negative isolates and the percentages were (79.81%) for Gram-positive isolates and (20.19%) for Gram-negative isolates (Karkee *et al.*, 2017). In another study assessing microbial contamination of the cell phones of healthcare workers, 85% of isolates were Gram-positive and only 15% of isolates were Gram-negative (Ramesh *et al.*, 2008). The higher percentage of Gram-positive isolates may originate from the skin microflora (Gumanju *et al.*, 2019).

Bacterial profile

Among all Gram-negative isolates (from the four universities' libraries), *E. coli* was the predominant isolate, whereas *S. aureus* was the predominant isolate among Gram-positive isolates, followed by *S. epidermidis* (Table 2). *Staphylococci* are considered part of the human microbiome (skin and nose microflora) and spread through mucosal droplets and skin peelings, which explains the prevalence of these bacteria (Stryjakowska-Sekulska *et al.*, 2007). This result is consistent with the literature; in a study conducted in 1994 by Brook and Brook, they found that four out of 15 public library books were contaminated with *S. epidermidis*. In another study, Rafiei *et al*. (2017) found that 20.8% of returned books from the Al-Zahra Hospital Library and the Library of Sciences Faculty of Isfahan University were contaminated with Enterobacteriaceae and *S. epidermidis*. The occurrence of members of the Enterobacteriacease (i.e., *E. coli*, *S. sonnei*, *K.*

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Table 3: Frequency of bacterial occurrence in four different universities libraries objects (benches, seats, computer mouse, computer keyboard, bookshelves, books and borrowing books place).

	Bench		Seat		Computer mouse		Computer keyboard		Bookshelves		Books		Borrowing books place	
	No	%	No	%	No	%	No	%	No	%	No	%	No	%
Bacterial isolates university A														
Gram Negative bacteria														
E. coli	5	22.77	7	28	5	21.73	9	18.75	6	12	10	14.28	6	11.53
Shigella sonnei	4	22.22	4	16	2	8.69	7	14.58	5	10	9	12.85	5	9.61
Pseudomonas aeruginos	0	0	1	4	2	8.69	8	16.66	7	14	5	7.14	4	7.69
Klebsiella pneumoniae	2	11.11	3	12	0	0	3	6.25	0	0	2	2.85	0	0
Salmonella choleraesuis	2	11.11	4	16	0	0	2	4.16	7	14	10	14.28	10	19.23
Citrobacter freundii	1	5.55	1	4	0	0	1	2.08	3	6	7	10	0	0
Gram Positive bacteria														
Staphylococcus epidermis'	1	5.55	2	8	0	0	6	12.5	8	16	10	14.28	10	19.23
Staphylococcus aureus	2	11.11	3	12	8	34.78	3	6.25	10	20	10	14.28	10	19.23
Bacillus subtilis	1	5.55	0	0	6	26.08	9	18.75	4	8	7	10	7	13.46
Total	18	100	25	100	23	100	48	100	50	100	70	100	52	100
Bacterial isolates university B														
Gram Negative bacteria														
E. coli	7	26.92	6	24	7	18.42	9	19.14	7	17.94	6	10.52	8	17.02
Shigella sonnei	2	7.69	1	4	9	23.68	7	14.89	6	15.38	10	17.54	9	19.14
Pseudomonas aeruginos	2	7.69	0	0	1	2.63	7	14.89	3	7.69	2	3.5	3	6.38
Klebsiella pneumonia	5	19.23	4	16	4	10.52	5	10.63	0	0	1	1.75	0	0
Salmonella choleraesuis	1	3.84	1	4	1	2.63	0	0	10	25.64	9	15.78	10	21.27
Citrobacter s freundii	0	0	0	0	0	0	0	0	0	0	9	15.78	1	2.12
Gram Positive bacteria														
Staphylococcus epidermis'	6	23.07	9	36	7	18.42	8	17.02	4	10.25	7	12.28	5	10.63
Staphylococcus Aureus	3	11.53	4	16	8	21.05	10	21.27	9	23.07	10	17.54	10	21.27
Bacillus subtilis	0	0	0	0	1	2.63	1	2.12	0	0	3	5.26	1	1.12
Total	26	100	25	100	38	100	47	100	39	100	57	100	47	100
Bacterial isolates university C														
Gram Negative bacteria														
E. coli	3	16.66	8	28.57	6	24	9	19.14	7	12.28	10	15.38	7	15.72
Shigella sonnei	2	11.11	3	10.71	3	12	6	12.76	9	15.78	10	15.38	9	17.64
Pseudomonas aeruginos	0	0	1	3.57	2	8	5	10.63	6	10.52	4	6.15	2	3.92
Klebsiell apneumonia	1	5.55	2	7.14	0	0	2	4.25	0	0	1	1.53	1	1.96

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Salmonella choleraesuis	4	22.22	5	17.85	1	4	2	4.25	6	10.52	7	10.76	10	19.6
Citrobacter freundii	3	16.66	3	10.71	0	0	2	4.25	4	7.01	8	12.3	1	1.96
Gram Positive bacteria														
Staphylococcus epidermis'	1	5.55	1	3.57	1	4	5	10.63	7	12.28	10	15.38	9	17.64
Staphylococcus Aureus	2	11.11	5	17.85	10	40	6	12.63	10	17.54	7	10.76	10	19.6
Bacillus s subtilis	2	11.11	0	0	2	8	10	21.27	8	14.03	8	12.3	2	3.92
Total	18	100	28	100	25	100	47	100	57	100	65	100	51	100
Bacterial isolates university D														
Gram Negative bacteria														
E. coli	8	28.57	11	39.28	9	25.71	10	20	7	14.28	9	14.75	8	18.18
Shigella sonnei	9	32.14	4	10.71	10	28.57	10	20	8	16.32	7	11.47	7	15.91
Pseudomonas aeruginos	0	0	1	3.57	0	0	6	12	8	16.32	3	4.91	4	9.09
Klebsiella pneumoniae	2	7.14	3	10.71	1	2.85	1	2	1	2.04	2	3.27	1	2.27
Salmonella choleraesuis	2	7.14	1	3.57	1	2.85	2	4	4	8.16	10	16.39	1	2.27
Citrobacter freundii	1	3.57	2	7.14	1	2.85	1	2	3	6.12	7	11.47	2	4.54
Gram Positive bacteria														
Staphylococcus epidermis'	2	7.14	1	3.57	0	0	4	8	3	6.12	10	16.39	10	22.72
Staphylococcus Aureus	3	10.71	6	21.42	10	28.57	7	14	10	20.4	9	14.75	10	22.72
Bacillus subtilis	1	3.57	0	0	3	8.57	9	18	5	10.2	4	6.55	1	2.27
Total	28	100	28	100	35	100	50	100	49	100	61	100	44	100

pneumoniae, *S. choleraesuisb* and *C. freundii*) family was also reported in similar studies in the literature. In a study conducted at the University of Ibadan Libraries in Nigeria, there was high isolation of Enterobacteriaceae like; *Proteus*, *Klebsiella*, *Yersinia*, *Serratia* and *Providencia* species (Giwa, 2017).

Nine bacterial isolates were fully identified using RapID™ One System (Remel, USA) biochemical testing system and these isolates are *E. coli*, *S. sonnei*, *P. aeruginosa*, *K. pneumoniae*, *S. epidermidis*, *S. aureus*, *S. choleraesuis*, *B. subtilis* and *C. freundii* of special interest in this study were the coliforms, which are a group of Gram-negative bacteria that usually inhabit the colon of humans and other warm-blooded mammals (Warpala *et al.*, 2020). While most the coliform bacteria are not pathogenic, their presence may represent traces of fecal contamination (Mohammed *et al.*, 2017) and some can cause some illnesses, such as gastroenteritis and diarrhea (Seo *et al.*, 2019). Three different coliforms were identified in this study, i.e., *E. coli*, *K. pneumonia* and *S. choleraesuis*.

It has been shown that both Gram-positive and Gram-negative transient bacteria can survive for months in dry environments such as fomites (Russotto *et al.*, 2015). This study was inspired by many studies investigating microbial contamination in healthcare facilities (Russotto *et al.*, 2015), public places, such as libraries (Landry *et al.*, 2018) and everyday use objects; e.g., cell phones (Gumanju *et al.*, 2019), shopping carts, e.g. (Irshaid *et al.*, 2014), currency notes (Al-Ghamdi *et al.*, 2011) or waiting room magazines (Charnock, 2005).

Those studies investigated microbial contamination in libraries; some of them investigated bacterial contamination; e.g. (Brook and Brook, 1994; Singh *et al.*, 2011) and other studies investigated fungal contamination, e.g. (Júnior *et al.*, 2012; Leite *et al.*, 2012) and many studies investigated both; bacterial and fungal contamination in libraries; e.g.(Karbowska-Berent *et al.*, 2011; Pasquarella *et al.*, 2012).

The most heavily contaminated areas tested among all universities were books (mostly with *S. choleraesuis*, *S. epidermidis* and *S. aureus*), followed

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Table 4: Antimicrobial susceptibility pattern of gram-negative isolates from university libraries A, B, C and D.

Gram Negative bacterial isolates	COX			C			NX			CIP			GEN		
University A	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
E. coli n=48	0	0	0	43	5	0	48	0	0	48	0	0	33	5	10
Shigella sonnei n=36	0	0	36	33	3	0	36	0	0	36	0	0	30	3	3
Pseudomonas aeruginosa n=27	0	0	27	1	1	25	27	0	0	27	0	0	24	1	2
Klebsiella pneumonia n=10	0	0	10	10	0	0	10	0	0	10	0	0	10	0	0
Salmonella choleraesuis n=35	0	0	35	35	0	0	35	0	0	35	0	0	30	5	0
Citrobacter freundii n=13	0	0	13	7	2	4	13	0	0	13	0	0	10	3	0
University B	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
E. coli n=50	0	0	50	50	0	0	50	0	0	50	0	0	40	7	3
Shigella sonnei n=44	0	0	44	44	0	0	44	0	0	44	0	0	35	7	2
Pseudomonas aeruginosa n=18	0	0	18	18	0	3	18	0	0	18	0	0	12	4	2
Klebsiella pneumonia n=19	0	0	19	19	0	0	19	0	0	19	0	0	17	2	0
Salmonella choleraesuis n=32	0	0	32	32	0	0	32	0	0	32	0	0	28	4	0
Citrobacter freundii n=10	0	0	10	10	0	0	10	0	0	10	0	0	10	0	0
University C	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
E. coli n=50	0	0	50	50	0	0	50	0	0	50	0	0	31	4	15
Shigella sonnei n=42	0	0	42	42	0	0	42	0	0	42	0	0	32	5	5
Pseudomonas aeruginosa n=20	0	0	20	20	0	0	20	0	0	20	0	0	12	1	7
Klebsiella pneumonia n=7	0	0	7	6	1	0	7	0	0	5	1	1	0	1	6
Salmonella choleraesuis n=35	0	0	35	35	0	0	35	0	0	35	0	0	10	10	15
Citrobacter freundii n=21	0	0	21	21	0	0	21	0	0	21	0	0	18	1	2
University D	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
E. coli n=61	0	0	60	61	0	0	61	0	0	61	0	0	54	7	10
Shigella sonnei n=55	0	0	55	55	0	0	55	0	0	55	0	0	32	18	5
Pseudomonas aeruginosa n=22	0	0	22	22	0	0	22	0	0	22	0	0	8	5	9
Klebsiella pneumonia n=11	0	0	11	10	1	0	11	0	0	8	2	1	1	1	9
Salmonella choleraesuis n=21	0	0	21	21	0	0	21	0	0	21	0	0	5	2	14
Citrobacter freundii n=17	0	0	17	17	0	0	17	0	0	17	0	0	15	1	1

Note: Cx: Cefoxitin; AMP: Ampicillin; ERY: Erythromycin; Va: Vancomycin; C: Chloramphenicol; Nx: Norfloxacin, CIP: Ciprofloxacin; GEN: Gentamicin. R=Resistance, I=Intermittent and S=Sensitive.

by borrowing books place in A university, borrowing books place and computer keyboard in B university, bookshelves in C university and computer keyboard at D university (Table 2). Books represent a good environment for microbial growth; as they include cellulose and lignin (from papers) (Baty *et al.*, 2010; Ayoade and Amona, 2018) and protein (included in the bindings), which explains the predominance of microbial contamination within books compared to other fomites investigated. Other contributing factors are stacking books on shelves in a way that hinders airflow and increases the amount of microbial sedimentation (Hempel *et al.*, 2014).

It was not until 1985 that the issue of indoor contamination of libraries and books was brought to attention by McClary in his article “Beware the Deadly Books’’, which raised the issue of the hazards of contaminated books passing on microbes between individuals (McClary, 1985). Gram-negative bacteria were predominantly isolated from books (except for C university, where book borrowing place was the most contaminated area).

Antibiotic susceptibility pattern of isolates

The presence of microbial contamination within public premises, such as libraries, may impose a direct threat on the health of the employees and visitors of these buildings, especially in case of pathogenic and/or antimicrobial-resistant microorganisms (Kramer *et al.*, 2006), because resistant microbes are potentially pathogenic and can cause diseases (Giwa, 2017).

Gram-negative isolates were subjected to the following antimicrobial panel; cefoxitin, chloramphenicol, norfloxacin, ciprofloxacin and gentamicin. Whereas Gram-positive isolates were subjected to the following antimicrobial panel: cefoxitin, chloramphenicol, norfloxacin, ciprofloxacin, gentamicin, vancomycin, ampicillin and erythromycin.

The antibiotic susceptibility revealed that norfloxacin was the most effective antibiotic on the bacteria in this study (against both Gram-positive and Gram-negative isolates). Generally, most isolates had high sensitivity levels against chloramphenicol, ciprofloxacin, gentamicin and norfloxacin. Almost all isolates were resistant to cefoxitin except *B. subtilis.* This result agrees with a recent study in 2019, conducted to assess microbial contamination of cell phones, where all Gram-positive (except *Micrococcus* spp.) and Gram-negative (except *Neisseria* spp.) isolates were resistant to cefoxitin (Gumanju *et al.*, 2019).

The only concern about the antimicrobial susceptibility profile of the isolates is the issue of multi-drug resistant

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Table 5: Antimicrobial susceptibility pattern of Gram-positive isolates from university libraries A, B, C and D.

	COX			AMP			ERY			Va			C			NX			CIP			GEN		
Bacterial isolates from university A																								
Gram Positive bacteria	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus epidermis' n=37	0	0	37	36	1	0	37	0	0	10	11	16	37	0	0	37	0	0	37	0	0	15	8	14
Staphylococcus aureus n=46	0	0	46	40	6	0	7	4	35	14	20	12	42	4	0	45	1	0	10	26	10	10	14	22
Bacillus subtilis n=34	0	0	34	30	4	0	34	0	0	25	0	9	34	0	0	34	0	0	34	0	0	34	0	0
Bacterial isolates from university B																								
Gram Positive bacteria	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus epidermis' n=46	0	0	46	36	**3**	**7**	28	12	6	28	14	4	46	0	0	46	0	0	46	0	0	**35**	**11**	0
Staphylococcus aureus n=54	0	0	54	**30**	11	13	11	34	9	20	23	11	54	0	1	54	0	0	53	0	1	**32**	15	7
Bacillus subtilis n=6	0	0	6	**6**	**0**	0	**6**	**0**	0	4	2	0	6	0	0	6	0	0	6	0	0	**6**	0	0
Bacterial isolates from university C																								
Gram Positive bacteria	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus epidermis n=34	0	0	34	27	6	1	27	6	1	27	5	2	34	0	0	34	0	0	34	0	0	30	3	1
Staphylococcus aureus n=50	0	0	50	23	21	6	19	12	19	24	17	9	48	1	1	49	1	0	49	0	1	21	7	22
Bacillus subtilis n=32	0	0	32	22	7	3	23	8	1	30	1	1	32	0	0	32	0	0	32	0	0	31	1	0
Bacterial isolates from university D																								
Gram Positive bacteria	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Staphylococcus epidermis' n=30	0	0	30	27	2	1	27	3	0	17	10	3	30	0	0	30	0	0	30	0	0	25	3	2
Staphylococcus aureus n=55	0	0	55	11	33	11	27	5	23	2	3	50	50	4	1	53	2	0	54	1	0	20	18	17
Bacillus subtilis n=23	0	1	22	20	2	1	20	1	2	14	7	2	23	0	0	23	0	0	23	0	0	19	3	1

Note: Cx: Cefoxitin; AMP: Ampicillin; ERY: Erythromycin; Va: Vancomycin; C: Chloramphenicol; Nx: Norfloxacin, CIP: Ciprofloxacin; GEN: Gentamicin.
R=Resistance, I=Intermittent and S=Sensitive.

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microbes and it was found that 71 bacterial isolates from A university were MDR Enterobacteriaceae isolates (i.e., *S. sonnei* and *S.choleraesuis*). Enterobacteriaceae (e.g., *S. sonnei* and *S. choleraesuis*) can cause intestinal infections that might pass to the bloodstream causing life-threatening conditions. In addition, it may cause infections in surgical sites, the urinary tract and the respiratory tract. This multi-antibiotic resistance pattern of these isolates is alarming as those isolates were found in a public environment and that imposes a direct threat on library users, staff and visitors (Wassmer *et al.*, 2006). And from a broader perspective, these multi-antibiotic-resistant environmental microbes represent a reservoir of novel antibiotic-resistance genes that need to be explored and dealt with (Gaze *et al.*, 2008).

The other problem with microbial contamination within public premises is that these microbes could adhere to dry, inanimate surfaces for quite a long time (Kramer *et al.*, 2006). For example, *S. aureus*, including MRSA can stick to surfaces for up to 7 months (Wagenvoort *et al.*, 2000), *E. coli* for up to 16 months (Williams *et al.*, 2005), *P. aeruginosa* for up 16 months (and 5 weeks on the dry floor) (Panagea *et al.*, 2005) and *Shigella* spp. for up to 5 months (Panagea *et al.*, 2005).

CONCLUSION

It might be challenging to control microbial contamination within public premises as these microorganisms can spread very easily through air or contact. However, some ways could be used to suppress microbial contamination, such as adapting more strict and more frequent cleaning regimes, using high-level disinfectants, encouraging visitors to use hand sanitizers before handling books and after using the rest room, and adapting new sterilization technologies, e.g., UV sterilizing machines to deep clean the books people borrow from libraries (such machines are used in Japan, Okayama city library).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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