JABET
Journal of Advanced Biotechnology and Experimental Therapeutics

ORIGINAL ARTICLE

J Adv Biotechnol Exp Ther. 2025 May; 8(2): 232-241
eISSN: 2616-4760, https://doi.org/10.5455/jabet.2025.19
Published by www.bsmiab.org

Diversity and resistance profile of bacteria associated with washroom surfaces in Bangladesh Agricultural University residence halls

Md. Shafiqul Islam[1], Sabrina Sultana Rimi[1], Md. Nahid Ashraf[1], Sanzila Hossain Sigma[1], Sharika Jahan[1], Mahbubul Pratik Siddique[1], Md. Tanvir Rahman[1, *]

[1]Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

***Corresponding author**
Md. Tanvir Rahman
Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
Email: tanvirahman@bau.edu.bd

Academic editor
Md. Masudur Rahman, PhD
Obihiro University
Japan

Article info
Received: 09 October 2024
Accepted: 14 February 2025
Published: 04 March 2025

Keywords
Antibiotic resistance, Diversity, MRSA, Residence halls, Toilets, Washrooms

ABSTRACT

Public washrooms in shared spaces, such as university residence halls, serve as potential reservoirs for pathogenic and multidrug-resistant bacteria, posing significant public health risks. This study aimed to assess bacterial diversity and evaluate antibiotic resistance profiles on commonly touched surfaces in washrooms and toilets of BAU residence halls. In total, 80 swab samples were obtained from the toilet and bathroom surfaces. Bacterial load was determined from each sample by total viable count (TVC), total coliform count (TCC), and total staphylococcal count (TSC). The bacterial isolates were identified using staining, biochemical testing, and subsequent molecular identification. Afterward, thirteen commonly available antibiotics were used to investigate the antibiotic sensitivity of the isolated organisms by disk diffusion methods. In the washroom samples, the greatest mean values were for TVC (log 5.91), TCC (log 5.75), and TSC (log 5.96). On the other hand, the toilet samples had the lowest mean values for TVC (log 5.39), TCC (log 5.13), and TSC (log 5.47). Notably, the floor surface samples had the highest levels of TVC, TCC, and TSC. The overall prevalence of *E. coli*, *Staphylococcus aureus*, and *Klebsiella* spp. was found to be 71%, 93.75%, and 87.5%. All isolated bacteria were found to be sensitive to chloramphenicol and gentamycin and resistant to ampicillin and amoxicillin. The study also found Methicillin-Resistant *Staphylococcus aureus* (MRSA), which poses a risk to public health. Therefore, the findings of this study can guide better hygiene practices, antibiotic usage, infrastructural improvements, and MRSA control in shared restrooms.

INTRODUCTION

Being the body's most exposed organ, hands play a significant role in physical manipulation and control of the environment; they quickly come into contact with various bacteria, many of which may be pathogens. They spread microorganisms between people and places [1]. According to scientific studies, we use common materials like basins, water taps, toilet door handles, knobs, pans, dirty surfaces, mobile phones, laboratory equipment, paper coins, computers, books, ATM, vending machines, desks, and many others that can spread pathogenic bacteria during daily activities at university halls, workplaces, restaurants, and shopping malls [2-9]. Public toilets are the major source of pathogenic bacteria, including MRSA, *Salmonella, Escherichia, Streptococcus*, and *Klebsiella*. In addition to being characterized by buildings with different purposes, university campuses are a special type of setting with a dense population. Most campus buildings have metal door handles, knobs, water taps, and showers that are often handled by several students, who are likely to exchange some of their own skin microbiota with those surfaces [10].

Commonly transmitted diseases, including the common cold, pneumonia, cold sores, giardiasis, diarrhoea, pinworm disease, conjunctivitis, and meningitis, may also be

transmitted by contact with ambient surfaces such as computers, classrooms, restrooms, sinks, and chairs. Numerous pathogenic bacteria, including *Corynebacterium diphtheriae*, *Streptococcus pneumoniae*, *Shigella dysenteriae*, and *Escherichia coli,* can cause whooping cough, pneumonia, dysentery, food poisoning, and intoxication, respectively [11]. These bacteria are easily spread by using the washroom and toilet and are responsible for many pathogenic diseases, for example, endocarditis, pneumonia (*S. aureus* and *K. pneumonia*), sore throats (*Streptococcus pyogenes*), food-borne illnesses (*S. aureus* and *E. coli*), urinary tract infections (UTI), and diarrhea (*E. coli* and *P. aeruginosa*) [12-14].

Antibiotic-resistant microorganisms are becoming a growing global concern for the health of humans and animals in both acute care and long-term care settings. Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* carbapenemase producing Gram-negatives, Extended-spectrum β-lactamase producing Gram-negative bacteria, Multidrug-Resistant Gram-negative rods (MDR GNR), MDRGN bacteria such as *Enterobacter* species, *E. coli, Acinetobacter baumannii, K. pneumoniae, P. aeruginosa* are common multidrug-resistant organisms [15]. As antibiotic therapy becomes less effective, rising antimicrobial resistance poses the greatest danger to public health, thus increasing the morbidity and mortality rate and the cost of treatment [16].

In consideration of the aforementioned facts, the current study's objectives were to identify and isolate bacteria using conventional and molecular techniques, as well as to ascertain the antibiotic resistance profile of the isolated bacteria. It also aimed to quantify the bacteria present on frequently touched surfaces in the washroom and toilet by determining total viable count (TVC), total coliform count (TCC), and total staphylococcal count (TSC).

MATERIALS AND METHODS

Ethical statement

The Animal Welfare and Experimentation Ethics Committee of Bangladesh Agricultural University (BAU), Mymensingh, approved the methods described in this work [approval number AWEEC/BAU/2022(82)].

Collection of specimens

This research comprised a total of eighty (80) swab samples (10 from each hall). Sample was obtained from the door handle, pan, indoor and outdoor knob, dirty floor, water tap of the toilet, and washroom were collected randomly from eight girls' and boy's halls of BAU, Mymensingh. Samples were taken aseptically, and a cool chain was maintained for transportation.

Calculation of total viable count

To evaluate the microbial quality of the target samples, TVC was calculated. The drop plate procedure was employed for this process [17]. To determine the total bacterial count, utilizing a micropipette, 10µl of every 10-fold dilution was placed onto Plate Count Agar (Hi-media, India). The incubation period was overnight at 35-37°C. Following incubation, plates were removed, and colonies were counted on the dilution that produced between three and thirty colonies per 10µl drop. Viable cell counts were expressed as CFU per surface area.

Isolation and identification of associated bacteria

To promote bacterial growth, each sample was inoculated individually with nutrition broth (NB) (Hi-media, India). These media were all incubated overnight at 37°C. Until a pure culture with homogeneous colonies was achieved, the colonies growing on primary cultures were routinely subcultured using the streak plate technique. Subcultures were grown on different selective media such as Eosin Methylene Blue (EMB) agar (Hi-media, India), MacConkey agar (Hi-media, India) for *Escherichia coli* and *Klebsiella* spp., and Mannitol salt (MS) agar (Hi-media, India) for *Staphylococcus* spp. to isolate bacteria from the samples that were collected. The bacteria were identified by the colony morphology, Gram staining reaction, and biochemical tests. Following standard microbiological protocols, biochemical tests were carried out [18].

Molecular detection of associated bacteria

A list of primers, along with their corresponding sequences, is in Table 1. The genomic DNA of bacterial isolates was extracted by boiling methods as described previously [19]. To do the PCR, 25 µl of the reaction mixture was prepared by mixing 5 µl DNA, 1 µl of each forward and reverse primer, 12.5 µl PCR master mixture (Promega, Madison, WI), and 5.5 µl nuclease-free water.

Table 1. PCR primers with sequence.

Species	List of primers	Primer's sequence (5′-3′)	Size	Annealing temperature	Ref.
E. coli	*mal*B F	GACCTCGGTTTAGTTCACAGA	585	58	[20]
	*mal*B R	CACACGCTGACGCTGACCA			
	*Stx*1 F	CACAATCAGGCGTCGCCAGCGCACTTGCT	606	61	[21]
	*Stx*1 R	TGTTGCAGGGATCAGTGGTACGGGGATGC			
	*tet*A F	GCGCCTTTCCTTTGGGTTCT	831	55	[22]
	*tet*A R	CCACCCGTTCCACGTTGTTA			
Staphylococcus aureus	*nuc* F	CGATTGATGGTGATACGGTT	279	58	[23]
	nuc R	ACGCAAGCCTTGACGAACTAAAGC			
	mecA F	AAAATCGATGGTAAAGGTTGGC	533	55	[24]
	mecA R	AGTTCTGGCACTACCGGATTTTGC			
Klebsiella spp.	*gyrA* F	CGCGTACTATACGCCATGAACGTA	441	55	[25]
	gyrA R	ACCGTTGATCACTTCGGTCAGG			

Antibiogram study

Disc diffusion or the Kirby-Bauer technique was employed to test antimicrobial drug susceptibility against 13 commonly used antibiotics on Mueller-Hinton agar (Hi-media, India) [26]. The isolated bacteria were incubated at 37°C for 24 hours after adjusting 0.5 McFarland standard. The antibiotics tested in this study, along with their respective classes, were: Amoxicillin (AMX, 30 µg/disc, Penicillin), Ampicillin (AM, 10 µg/disc, Penicillin), Azithromycin (AZM, 15 µg/disc, Macrolide), Cefixime (CFM, 5 µg/disc, Cephalosporin), Ciprofloxacin (CIP, 5 µg/disc, Fluoroquinolone), Chloramphenicol (C, 30 µg/disc, Chloramphenicol), Co-Trimoxazole (COT, 25 µg/disc, Sulfonamide/Trimethoprim), Colistin sulfate (CS, 10 µg/disc, Polymyxin), Gentamicin (GEN, 10 µg/disc, Aminoglycoside), Methicillin (MET, 5 µg/disc, Penicillin), Streptomycin (S, 10 µg/disc, Aminoglycoside), Tetracycline (TE, 10 µg/disc, Tetracycline), and Vancomycin (VA, 30 µg/disc, Glycopeptide). The findings of antimicrobial testing were categorized as sensitive, intermediate, and resistant based on the CLSI (2021) zone diameter interpretation [27].

Statistical analysis

The data from this study were entered into Excel 365 for analysis. Data on antibiotic resistance among isolates is reported as frequencies or percentages.

RESULTS

Presence of bacterial load in various samples collected from different halls of BAU

The viable bacterial counts from toilet and washroom samples are shown in Table 2 and Table 3, respectively. By TVC, the highest bacterial load was found in the washroom samples (log 5.91) compared to toilet samples (log 5.39). The TVC in washroom samples ranged between 5.64 log CFU/ml (Outdoor knob) to 6.16 log CFU/ml (Dirty floor). The lowest TVC in toilet samples was 2.76 log CFU/ml (Door handle), and the highest was 6.39 CFU/ml (Dirty floor). By TCC, the highest bacterial load was found in the washroom samples (log 5.75) compared to toilet samples (log 5.13). The TCC in washroom samples ranged between 5.48 log CFU/ml (Outdoor knob) to 6.05 log CFU/ml (Dirty floor). The lowest TCC in toilet samples was 2.64 log CFU/ml (Door handle), and the highest was 6.14 CFU/ml (Dirty floor). By TSC, the highest bacterial load was found in the washroom samples (log 5.96) compared to toilet samples (log 5.47). The TSC in washroom samples ranged between 5.73 log CFU/ml (Outdoor knob) to 6.17 log CFU/ml (Dirty floor). The lowest TSC in toilet samples was 2.89 log CFU/ml (Door handle), and the highest was 6.24 CFU/ml (Dirty floor, Water tap).

Table 2. Bacterial load in toilet samples collected from different halls of BAU, Mymensingh.

Sample ID	TVC						TCC						TSC					
	DH	P	OK	IK	DF	WT	DH	P	OK	IK	DF	WT	DH	P	OK	IK	DF	WT
L.Hall-1	5.54	6.23	6.08	6.20	6.52	6.15	5.30	6.11	5.30	5.90	6.41	6.00	5.95	6.48	6.32	6.36	6.56	6.60
L.Hall-2	-	6.00	5.48	5.54	6.35	6.09	-	5.48	5.18	5.48	6.18	6.02	-	6.44	6.41	5.78	6.40	6.39
L.Hall-3	5.30	6.18	5.90	6.08	6.39	6.15	5.00	6.04	5.30	5.78	6.34	5.90	5.78	6.34	6.20	6.30	6.39	6.50
L.Hall-4	-	6.15	5.74	-	6.35	6.28	-	5.78	5.30	-	5.90	5.78	-	6.35	5.78	-	6.44	6.39
G.Hall-1	5.60	6.08	5.60	6.09	6.30	6.24	5.39	5.78	5.48	5.74	6.09	5.69	5.30	5.54	5.18	5.48	6.11	5.95
G.Hall-2	5.60	5.69	5.78	5.95	6.39	6.00	5.48	5.60	5.00	5.65	6.30	5.78	6.08	6.48	5.69	5.78	6.04	6.00
G.Hall-3	-	6.04	5.54	6.09	6.26	6.02	-	5.48	5.39	6.15	5.85	5.60	-	6.00	6.34	6.39	6.08	6.23
G.Hall-4	-	6.02	5.60	6.24	6.52	6.30	-	5.78	5.54	6.08	6.06	5.95	-	6.09	5.95	5.98	5.93	5.90
Mean	2.76	6.05	5.72	5.27	6.39	6.15	2.64	5.76	5.31	5.09	6.14	5.84	2.89	6.22	5.98	5.26	6.24	6.24
	5.39						5.13						5.47					

DH - Door handle, P - Pan, OK - Outdoor knob, IK - Indoor knob, DF - Dirty floor, WT - Water tap, L.Hall – Lady's hall, G.Hall - Gent's hall. Total viable count (TVC), total coliform count (TCC), and total staphylococcal count (TSC).

Table 3. Bacterial load in washroom samples collected from different halls of BAU, Mymensingh.

Sample ID	TVC				TCC				TSC			
	IK	OK	DF	WT	IK	OK	DF	WT	IK	OK	DF	WT
L.Hall-1	6.09	5.90	6.34	6.15	6.18	5.78	6.30	5.90	6.18	5.78	6.30	5.90
L.Hall-2	5.69	5.30	6.44	6.18	5.48	5.18	6.31	6.04	6.04	5.88	5.93	6.23
L.Hall-3	5.95	5.78	6.20	6.08	6.01	5.60	6.26	5.78	6.01	5.60	6.26	5.78
L.Hall-4	5.85	5.48	6.52	6.04	5.30	5.00	6.35	5.69	5.78	5.30	6.34	6.18
G.Hall-1	5.69	5.65	5.78	5.74	5.54	5.30	5.65	5.60	6.14	5.48	6.18	5.90
G.Hall-2	5.90	5.81	6.22	5.88	5.74	5.65	6.08	5.74	5.90	5.60	5.69	5.60
G.Hall-3	5.65	5.54	5.81	6.00	5.60	5.69	5.65	5.98	6.32	6.23	6.34	6.19
G.Hall-4	5.78	5.69	6.00	5.88	5.69	5.60	5.78	5.48	5.48	5.95	6.28	6.00
Mean	5.83	5.64	6.16	5.99	5.69	5.48	6.05	5.78	5.98	5.73	6.17	5.97
	5.91				5.75				5.96			

OK - Outdoor knob, IK - Indoor knob, DF - Dirty floor, WT - Water tap. Total viable count (TVC), total coliform count (TCC), and total staphylococcal count (TSC).

Comparison of bacterial load in toilet and washroom samples obtained from Lady's and Gent's halls, BAU, Mymensingh

The highest mean TVC and TCC in toilet samples was found in gent's hall, and the lowest mean TVC and TCC was found in lady's hall (Table 4). The highest mean TVC and TCC in washroom samples were found in the ladies' hall, and the lowest mean TVC and TCC were found in gent's hall (Table 4). In contrast, the highest mean TSC in toilet washroom samples was found in lady's hall, and the lowest was found in gent's hall (Table 4).

Table 4. Comparison of bacterial load in toilet and washroom from Lady's and Gent's halls.

Hall	Sample type	TVC (Mean) in log CFU/ml	TCC (Mean) in log CFU/ml	TSC (Mean) in log CFU/ml
Lady's hall	Toilet	5.28	5.02	5.51
	Washroom	6.00	5.82	5.97
Gent's hall	Toilet	5.49	5.25	5.44
	Washroom	5.82	5.67	5.96

Total viable count (TVC), total coliform count (TCC), and total staphylococcal count (TSC).

Prevalence of *E. coli, Klebsiella* spp., and *Staphylococcus* spp. from toilets and washroom samples of different halls of BAU

The highest prevalence of *E. coli* was found in the swab sample of L. hall-2, that is 90%. The overall prevalence was found to be 71%. The highest prevalence of *Klebsiella* spp. was found in the swab sample of L. hall-1, which is 100%. The overall prevalence was found to be 87.5%. The highest prevalence of *Staphylococcus* spp. was found from swab samples of L. hall-1, L. hall-3, G. hall-1, G. hall-2 is 100%. The overall prevalence was found to be 93.75% (Table 5).

Table 5. Summary of prevalence of *E. coli, Klebsiella* spp., and *Staphylococcus aureus* from Lady's and Gent's Hall in BAU campus.

Name of the hall	No. of samples	*E. coli* (%)	*Klebsiella* spp. (%)	*Staphylococcus aureus* (%)
L.Hall-1	10	50	100	100
L.Hall-2	10	90	90	90
L.Hall-3	10	50	90	100
L.Hall-4	10	80	70	80
G.Hall-1	10	80	90	100
G.Hall-2	10	70	80	100
G.Hall-3	10	80	90	90
G.Hall-4	10	70	90	90
Total	80	71	87.5	93.75

Cultural, morphological, and biochemical characterization

After incubation at 37°C for 24 hours in nutrient broth, a loop full of broth is streaked onto different selective media, and incubated for 24 hours at 37°C. The growth of *E. coli* on EMB agar was indicated by smooth, circular, black or green-colored colonies with metallic sheen. On MacConkey agar the growth of *Klebsiella* spp. was indicated by circular, convex, mucoid, pink to red colored colonies. The growth of *Staphylococcus aureus* on Mannitol Salt agar was indicated by the smooth, circular, yellowish colony, changing the media color from pink to bright yellow. *E. coli* and *Klebsiella* spp. were found as Gram-negative short rods, while *Staphylococcus aureus* was found as Gram-positive cocci by Gram's staining.

Molecular detection of bacteria

A total of 57 culture-positive isolates were screened by PCR using primers specific for *E. coli* targeting the *malB* gene. All the isolates were found positive by PCR, amplifying a band of 585 bp (Figure 1A). All the genus-specific isolates were then screened for *Stx-1*, and 7 out of 57 isolates showed a positive band at 606 bp (Figure 1B). In the case of *Klebsiella* spp., 70 isolates were found to be positive, targeting the Kleb_*gyrA* gene (Figure 1C). Out of 75 culture-positive isolates, 35 were found positive for *Staphylococcus aureus,* targeting the *nuc* gene of 279 bp (Figure 1D).

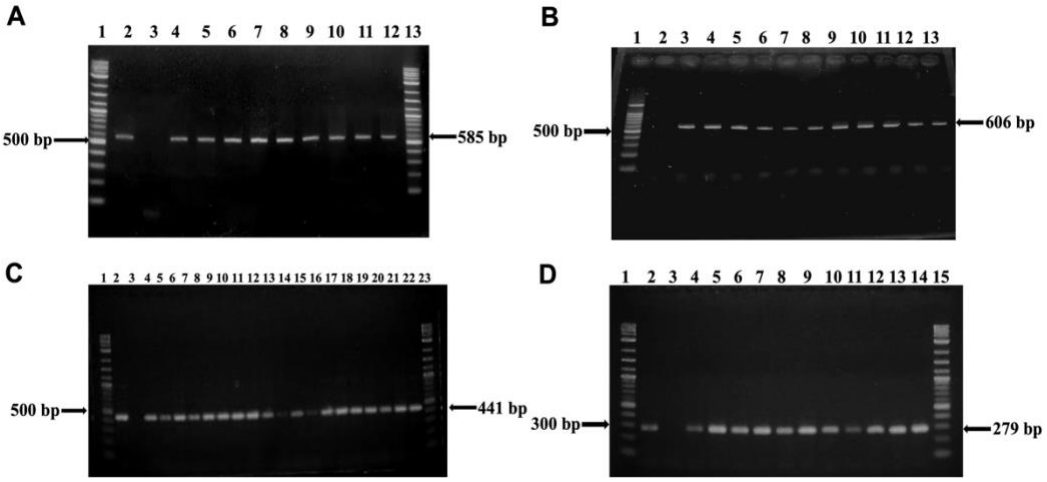

Figure 1. Agarose gel electrophoresis results of PCR amplification targeting the (A) *malB* [585 bp] and (B) *stx-1* [606 bp] gene of *E. coli* isolates, (C) Kleb_*gyrA* [441 bp] gene of *Klebsiella* spp. isolates, (D) *nuc* [279 bp] gene of *Staphylococcus aureus* isolates. In all cases, Lane 1: 100 bp DNA ladder; blank lane represents negative control; and lanes with specific band represent positive isolates.

Antimicrobial resistance profile of the isolated bacteria

While 100% of the *E. coli* isolates were found to be resistant to Amoxicillin, 86.67% to Cefixime, Co-trimoxazole, and Tetracycline, 75% to Colistin sulfate, and 66.66% to Azithromycin, the isolates were shown to be 100% responsive to Gentamicin and Streptomycin, 92% to Chloramphenicol, and 62% to Ciprofloxacin (Figure 2A). *Klebsiella* spp. were completely susceptible to Ciprofloxacin and Gentamicin, 95% to Co-trimoxazole, 93% to Chloramphenicol, 88% to Tetracycline, and 66.66% to Streptomycin. Amoxicillin had a complete resistance rate of 100%, whereas Cefixime, Azithromycin, and Colistin sulfate showed resistance rates of 92%, 87.67%, and 53.33%, respectively (Figure 2B). *Staphylococcus aureus* exhibited complete sensitivity (100%) to Vancomycin, with high sensitivity rates to Tetracycline (81.5%), Co-Trimoxazole (77.17%), Gentamicin (93.5%), and Chloramphenicol (93.25%). It showed moderate sensitivity to Azithromycin (52.5%), Ampicillin (80.5%), and Ciprofloxacin (56.16%). However, it was completely resistant (100%) to Methicillin (Figure 2C).

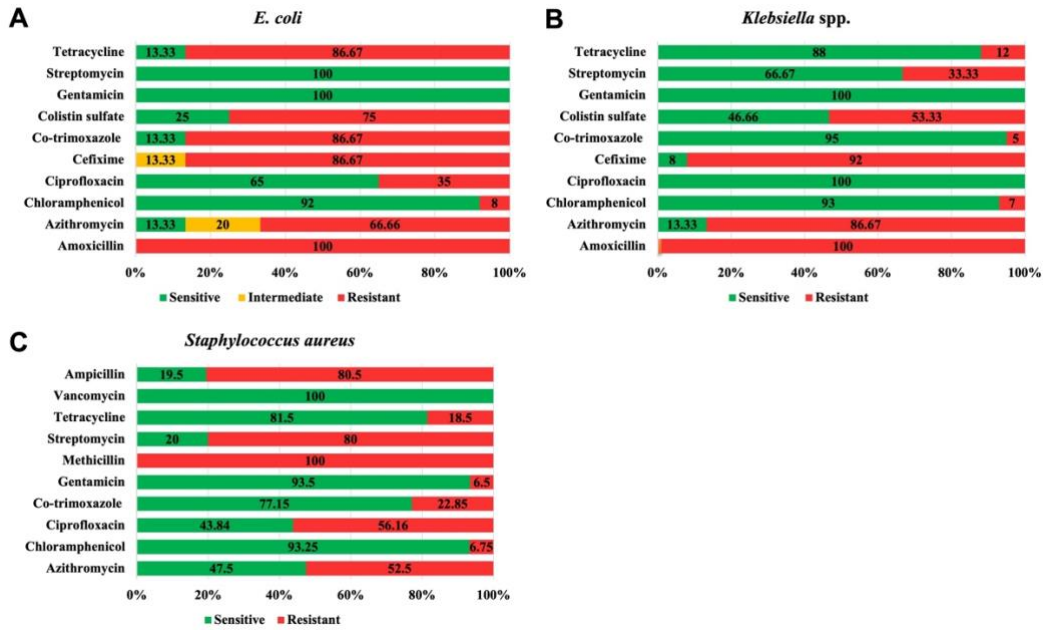

Figure 2. Sensitivity pattern of selected (A) *E. coli*, (B) *Klebsiella* spp., and (C) *Staphylococcus aureus* isolates.

Detection of antibiotic-resistant genes by PCR

On antimicrobial resistance gene screening, 31 out of 57 *E. coli* isolates were found to contain *tetA* genes (Figure 3A) by PCR amplifying a band of 831 bp, and 23 out of 35 *S. aureus* isolates were found to contain *mecA* genes (Figure 3B) by PCR amplifying a band of 533 bp.

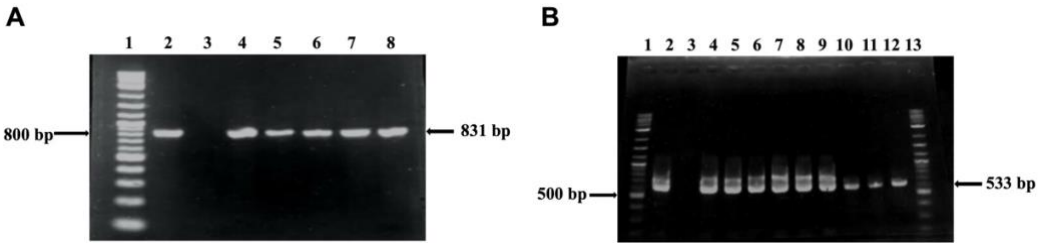

Figure 3. Agarose gel electrophoresis results of PCR amplification targeting the (A) *tetA* [831 bp] gene of *E. coli* isolates and (B) *mecA* gene of *Staphylococcus aureus* isolates. In all cases, Lane 1: 100 bp DNA ladder; blank lane represents negative control; and lanes with specific band represent positive isolates.

DISCUSSION

In the present work, 80 swab samples of different toilets and washrooms were used to determine microbial load by TVC, TCC, and TSC. The maximum mean TVC was determined in the washroom samples (log 5.91) compared to toilet samples (log 5.39). The highest mean of TVC and TCC in toilet samples was found in Gent's Hall, and the lowest mean of TVC and TCC was found in Lady's hall. The highest mean of TVC and TCC in washroom samples was found in Lady's Hall, and the lowest mean TVC and TCC was in Gent's Hall, whereas the highest mean TSC was found in Lady's Hall and lowest in Gent's Hall in both toilets and washroom samples. One study reported that the bacteria population density varied across different sampling points, with walls having a density of 6.3×10^{5} CFU/ml to 2.35×10^{11} CFU/ml, while floors of female hostel had a maximum density of 2.13×10^{9} CFU/ml and door handles had a density of 1.12×10^{8}

CFU/ml in male hostel at Ambrose Alli University Ekpoma Nigeria [28]. Another study obtained that the gas station bathroom doorknobs showed the most bacterial count, measuring 2.8 log10 CFU/ml, while the hospital pantry had the lowest at 1.3 log10 CFU/ml [29]. In our study, we found the highest bacterial load on floor surfaces from toilets (log 6.39) and washrooms (log 6.16) and similar findings have also been described in previous study, where the highest bacterial load was (5.8×10^4- 2.96×10^7) CFU/ml on the washroom floor surface and (3.2×10^4 - 2.24×10^6) CFU/ml in toilet seat, (3.1×10^4 - 2.96×10^7) CFU/ml on door handles in the student's hostel toilets at Sokoine University of Agriculture, Morogoro, Tanzania [30].

In this study, the overall prevalence of *E. coli, Klebsiella* spp., and *Staphylococcus* spp. were 71%, 93.75%, and 87.5%, respectively. The prevalence is much higher than in certain other studies [15,31]. This variation might be due to geographic location, population density, environment setup, etc. On the antibiogram, *E. coli* was 100% susceptible to Gentamicin and streptomycin and 100% resistant to Amoxicillin, followed by 86.67% to Cefixime and co-trimoxazole and Tetracycline, 75% to Colistin sulfate, and 66.66% to Azithromycin. Out of 57 *E. coli* isolates, 27 (47.37%) were *tet*A gene positive, which was greater than one study, which detected 30.4% from wastewater treated effluents in Eastern Cape, South Africa [32]. Isolated *Klebsiella* spp. showed 100% sensitivity to Ciprofloxacin and Gentamicin, 95% to Co-trimoxazole, 88% to Tetracycline, and 100% resistance to Amoxicillin as well as 92%, 87.67%, and 53.33% against Cefixime, Azithromycin, and Colistin sulfate, respectively. A previous study also showed *Klebsiella* spp. sensitive to Gentamicin and tetracycline, and resistant to Amoxicillin [33]. *Staphylococcus aureus* was sensitive to Vancomycin (100%), Gentamicin (93.5%), Chloramphenicol (93.25%) and resistant to Methicillin (100%), Ampicillin (80.5%), and Streptomycin (80%). This result matched previous studies, where they identified *Staphylococcus* spp. resistant to methicillin, sensitive to co-trimoxazole (100.00%), gentamycin (90.00%), and ciprofloxacin (80.00%) [34]. In this investigation, 60% of *nuc* gene-positive *S. aureus* demonstrated *mecA* gene positivity, comparable to one previous report, where they isolated 49.3% *mecA*-positive from Shanghai hospital patients and personnel [35].

However, differences in geographic location, population density, sampling technique, environmental conditions, local antibiotic usage, and research design may all be responsible for the reported variances in microbial load, prevalence, and antibiotic resistance patterns. Therefore, the study's main drawback is the use of fewer samples, and each sample came from a single location.

CONCLUSIONS

The study conducted on swab samples from toilets and washrooms of BAU, Mymensingh, highlights the prevalence of antibiotic-resistant bacteria, particularly *Staphylococcus* spp., *E. coli*, and *Klebsiella* spp., posing a significant health risk due to potential transmission through bathrooms. The identification of the *stx*-1 gene in some *E. coli* isolates indicates the existence of pathogenic strains. The antibiotic sensitivity profiles demonstrated diverse susceptibilities among the isolated bacteria, with several strains exhibiting concerning levels of resistance to frequently used medicines such as Amoxicillin. These results emphasize the immediate need for enhanced hygiene practices and infection control measures in student facilities to reduce the transmission of antibiotic-resistant bacteria and decrease related health hazards.

ACKNOWLEDGMENTS

We thank the Ministry of Science and Technology, Government of the People's Republic of Bangladesh, for providing financial support (SRG-221106 for 2022–2023) to carry out this research.

AUTHOR CONTRIBUTIONS

MSI was responsible for conceptualization, funding acquisition, project administration, supervision, reviewing, and editing the manuscript; SSR, MNA, and SHS were involved in methodology, data curation, formal analysis, and writing the original draft; SJ was involved in sample collection, methodology, investigation, and data curation; MPS was involved in validation, supervision, writing – review & editing; MTR was responsible for formal analysis, supervision as well as writing, reviewing and editing the manuscript. The manuscript's final submitted version was reviewed and approved by all authors.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

REFERENCES

[1] Fakhoury S, Nawas T. Contamination of the Internal Handles/Knobs of Public Restroom Doors with Potentially Pathogenic Bacteria. Int J Curr Microbiol Appl Sci. 2018; 7:3434-3440.
[2] Nworie A, Ayeni JA, et al. Bacterial contamination of door handles/ knobs in selected public convenience in Abuja metropolis, Nigeria: A Public Health Threat. Cont J Med Res. 2012; 6:7 – 11.
[3] Joshaline CM. Automated teller machine (ATM) - a "pathogen city" – a surveillance report from locations in and around Madurai city, Tamil Nadu, India. Int. J Public Health Sci. 2014; 3:51-56.
[4] Memišević S, Jerković-Mujkić A, et al. Bacterial contamination of public telephones in the downtown area of Sarajevo. Afr J Microbiol Res. 2013; 7:1664-1667.
[5] Reynolds KA, Watt PM, et al. Occurrence of Bacteria and Bacteria Markers on Public Surfaces. Int J Environ Health Res. 2005; 15:225-234.
[6] Selim HS, Abaza AF. Microbial contamination of mobile phones in a health care setting in Alexandria, Egypt. GMS Hyg Infect Control. 2015; 10:2-3.
[7] Hamze F, Nawas T. Computer Mice in Public Centers: A Source of Pathogenic Bacteria. ARPN J Sci Technol. 2015; 5:537-539.
[8] Sani NM. Isolation and Identification of Bacterial Contaminants from Door Handles of Public Toilets in Federal University Dutse, Jigawa State Nigeria. IOSR J Pharm Biol Sci. 2016; 11:53-57.
[9] Dakroub R, Nawas T. Vending machine buttons and touch screens. A surface colonized by pathogenic bacteria. Int J Innov Appl Res. 2017; 5:82-88.
[10] Ashley AR, Josh DN. Microbial biogeography of a university campus. Microbiome. 2015; 3:1-12.
[11] Tsaku PA, Ehinmidu JO, et al. Antibiotic Susceptibility and Plasmid Profile of Escherichia coli from Door Handles in Two Tertiary Institutions in Nasarawa State, Nigeria. J Adv Microbiol. 2017; 3:1-13.
[12] Vogt RL, Dippold L. Escherichia coli O157:H7 outbreak associated with consumption of ground beef, June-July 2002. Public Health Rep. 2005; 120:174-78.
[13] Khan MF. Brief History of *Staphylococcus aureus:* A Focus to Antibiotic Resistance. EC Microbiology. 2017; 5:36-39.
[14] Martin RM, Bachman MA. Colonization, infection and accessory genome of *Klebsiella pneumonia.* Front cell infect microbiol. 2018; 8:1-15.
[15] Ajayi A, Ekozien M. Sensitivity profile of bacterial flora isolated from bathroom. Elite Res J Biotechnol Microbiol. 2014; 2:1–3.
[16] Coast J, Smith R, et al. Superbugs: should antimicrobial resistance be included as a cost in economic evaluation? Health Econ. 1996; 5:217-26.
[17] Herigstad B, Hamilton M, et al. How to optimize the drop plate method for enumerating bacteria. J Microbiol Methods. 2001; 44:121–129.
[18] Cheesbrough M. Microbiology. In: Medical laboratory manual for tropical countries. English Language Book Society, London. 1985; 2:225-392.

[19] Queipo-Ortuno MI, De Dios Colmenero J, et al. Preparation of bacterial DNA template by boiling and effect of immunoglobulin G as an inhibitor in real-time PCR for serum samples from patients with brucellosis. Clin Vaccine Immunol. 2008; 15:293–296.
[20] Fratamico PM, Bagi LK, et al. A multiple polymerase chain reaction assay for rapid detection and identification of Escherichia coli 0157: H7 in foods and bovine feces. J Food Prot. 2000; 63:1032-1037.
[21] Talukdar PK, Rahman M, et al. Antimicrobial resistance, virulence factors and genetic diversity of Escherichia coli isolates from household water supply in Dhaka, Bangladesh. Plos one. 2013;8:e61090.
[22] Chen S, Zhao S, et al. Characterization of multiple-antimicrobial-resistant Salmonella serovars isolated from retail meats. Appl Environ Microbiol. 2004; 70:1–7.
[23] Kalorey DR, Shanmugam Y, et al. PCRbased detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. J Vet Sci. 2007; 8:151-154.
[24] Lee JH. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol. 2003; 69:6489-94.
[25] Brisse S, Verhoef J. Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC genes sequencing and automated ribotyping. Int J Syst Evol Microbio. 2001; 51:915–924.
[26] Bauer AW, Kirby WM, et al. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966; 45:493–496.
[27] CLSI. Performance Standards for Antimicrobial Susceptibility Testing (31st Ed). CLSI Supplement M100. Wayne, PA: Clinical and laboratory standards institute; 2021.
[28] Al-Harbi M, Anderson A, et al. Evaluation of microbial contamination in frequently used Fomites in Kuwait. Biodivers Int J. 2017; 1: 80–86.
[29] Chengula A, Lushino A, et al. Determination of bacterial load and antibiotic susceptibility testing of bacteria isolated from students toilets at Sokoine University of Agriculture, Morogoro, Tanzania. J Health Med Nurs. 2014; 5:1-11.
[30] Sampson T, Esheyigba AP, et al. Bacteriological Assessment of Toilet Seats in a Nigerian University. J Adv Microbiol. 2019; 19:1-11.
[31] Adefisoye M, Okoh AI. Identification and antimicrobial resistance prevalence of pathogenic *Escherichia coli* strains from treated wastewater effluents in Eastern Cape, South Africa. Microbiologyopen. 2016; 5:143-151.
[32] Chakraborty S, Mohsina K, et al. Prevalence, antibiotic susceptibility profiles and ESBL production in *Klebsiella pneumoniae* and *Klebsiella oxytoca* among hospitalized patients. Period Biol. 2016; 118:53–58.
[33] Thaker HC, Brahmbhatt MN, et al. Study on occurrence and antibiogram pattern of Escherichia coli from raw milk sample in Anand, Gujarat, India. Vet World. 2012; 5:556-559.
[34] Soomro AH, Arain MA, et al. Isolation of Staphylococcus aureus from milk products sold at sweet meat shops of Hyderabad. Online J Bio. Sci. 2003; 3:91-94.
[35] Wang X, Lin O, et al. Methicillin-resistant Staphylococcus aureus isolates in a hospital of Shanghai. Oncotarget. 2017; 8:6079-6084.