Biochemical Identification and Antibiotics Susceptibility Pattern of Bacteria Isolated from Fomites in Dominion University Ibadan, Oyo State – Nigeria

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ABSTRACT

Fomites surfaces (doorknobs) that can harbor and transmit microorganisms have been recognized as potential sources of infectious agents. Hence, understanding the bacterial diversity of fomites is essential to assess the risk of transmission and implement effective control measures. Nutrient, Mannitol Salt and MacConkey agar were used for the isolation of bacteria from fomites using the swab-rinse technique. Biochemical identification of bacterial isolates using catalase, coagulase, oxidase, indole, urea hydrolysis, and sugar fermentation were carried following standard microbiological protocols. Antibiotic susceptibility of the isolates to ten antibiotics was also carried out following the Kirby-Bauer disk diffusion method. A total of twenty (20) bacterial isolates were obtained from forty-five (45) fomites across five (5) locations. The peak microbial count was observed in the afternoon across all surfaces and the total heterotrophic count (THC) during this time ranged from 1.6×10^2 -1.9×10^5, 1.9×10^3 - 1.8×10^6 and 1.7×10^5 – 3.1×10^6 CFU/mL in week 1, 2 and 3, respectively. The Gram's reaction revealed that 9 (45%) of the bacterial isolates were Gram positive while the remaining 11 (65%) were Gram negative. The biochemical characteristics revealed that all the bacterial isolates (100%) were catalase positive, 6 (30%) were coagulase positive, 4 (20%) were indole positive, 13 (65%) were urease positive, and 3 (15%) were oxidase positive. The sugar fermentation pattern showed that glucose and sucrose were the most preferred carbon sources across. The bacterial isolates belonged to five genera viz; Staphylococcus (45%), Proteus (10%), Escherichia (20%), Klebsiella (10%), and Pseudomonas (15%). Pefloxacin (10µg) and Ciprofloxacin (10µg) had the highest antagonistic effect while Zinnacef (20µg), Amoxicillin (30µg), and Erythromycin (10µg) had the least inhibitory effect against the test microorganisms, respectively. This research provides valuable insights into the bacterial composition of fomites within Dominion University, Ibadan. The knowledge gained from this study can serve as a foundation for developing targeted control strategies to ensure a safe and healthy campus environment.

Keywords: Fomites, Dominion University Ibadan, Antibiotic Resistance, Public health.

INTRODUCTION

The existence of life on earth is greatly influenced by the actions of microorganisms (Nwinyi et al., 2022). Microorganisms such as bacteria are known to be ubiquitous, possessing the ability to adapt to new environments and multiply in numerous forms within a limited time (Dawodu and Akanbi, 2021, Wilkie et al., 2022). There are various types of microorganisms, some of which are harmless, while others can be beneficial. However, some can lead to spoilage, sickness, or even produce dangerous toxins that may cause poisoning. Besides the daily interaction of people, a major source and spread of environment-acquired infections are fomites. Fomites are non-living objects that facilitate the transfer of infectious microorganisms. They transfer various microorganisms and infectious human pathogens directly by contact or surface-to-mouth and indirectly by oral transmission or

contaminated fingers (Wilkie et al., 2022). Inanimate objects can transfer microorganisms that come from their surroundings. When these objects touch people or places where harmful microorganisms live, they can spread diseases easily. This is called fomite transmission (Ayuba et al., 2019). Inanimate objects can transfer disease-causing pathogens to humans. These objects can harbor communities of bacteria, fungi, viruses, and toxins that can lead to illness. (Jaradat et al., 2020). Fomites include door handles of lecture halls, hostels, offices, showers, restrooms, toilet seats, conveniences, faucets, lockers, tables, chairs, and hand lockers majorly those present in universities, hospitals, restaurants, public offices, restrooms, and hotels (Ayuba et al., 2019). Among the array of fomites, door handles are presumably the most common routes of microbial contamination and infection. Many organisms make their way onto door handles through contact with skin and hands. Objects that have been contaminated can hold bacteria, which can easily transfer to hands, causing food-borne infections and gastroenteritis (Ayuba et al., 2019). Hence, this research is aimed at isolating and identifying microorganisms on fomites in Dominion University Ibadan that may pose a potential health risk to members of the university community.

MATERIALS AND METHODS

Study Area and Sample collection

This study was carried out at Dominion University, Ibadan, Oyo State, Nigeria. Fomites (doorhandles and knobs) in various lecture halls were used as the sample for the isolation of bacteria. Using the swab-rinse technique, samples were collected at different time intervals, early hours of the morning and peak afternoon periods. These fomites were selected based on the frequency of usage and accessibility by students and other members of the university environment. The cultivation and isolation of bacteria from the various fomites using Nutrient agar, Mannitol Salt Agar and MacConkey agar was carried out following standard microbiological technique as described by Ayuba et al. (2019).

Colonial characteristics of bacterial isolates

Following the incubation of the various isolation culture media, the plates were examined for bacterial colonies. For each isolation plate, the Total Heterotrophic Count (THC) and the Total Coliform Count (TCC) was recorded and expressed as the colony-forming units per milliliter (CFU/mL). Similarly, the colonial characteristics of the bacterial isolates including color, shape, elevation, texture, edges, consistency, and pigment were assessed and documented as described by Olutiola et al. (2018); Bassey et al. (2022) and Wilkie et al. (2022).

Biochemical characterization of bacteria isolates

Further identification of the bacterial isolates was carried out using various biochemical tests including Catalase, Coagulase, Oxidase, Indole, Urea hydrolysis, and sugar fermentation test following standard microbiological protocols as described by Dawodu and Akanbi (2021).

Gram's reaction of bacteria isolated from fomites

A smear of each bacterial isolate was made on a clean and grease-free glass slide and then heat-fixed. Following successive heat-fixing, each glass slide was flooded with the primary stain, Crystal Violet, for approximately 30 to 60 seconds. Following this, excess dye was drained, and the smear was exposed to Gram's iodine (mordant) and left to stand for 60 seconds. After the iodine was drained, the slide was rinsed under a running tap and then decolorized using 70% ethanol followed by the secondary stain, Safranin red. Air-dried slides were viewed under a microscope with oil immersion and a magnification of x100 (Dawodu and Akanbi, 2021).

Antibiotic Susceptibility Test

To assess the antibiotic susceptibility of the isolates, the Kirby-Bauer disk diffusion method, as outlined by Hudzicki, (2009) was employed. An inoculum was prepared and administered onto Mueller-Hinton susceptibility agar plates utilizing a sterile swab stick. Following this, the plates were allowed to air-dry for a period of 5-10 minutes. The antibiotic susceptibility test was carried out using antibiotic disks containing

different concentrations of various antibiotics; Pefloxacin (PEF) (10µg), Gentamycin (CN) (10µg), Ampiclox (APX) (30µg), Zinnacef (Z) (20µg), Amoxicillin (AM) (30µg), Rocephin (R) (25µg), Ciprofloxacin (CPX) (10µg), Streptomycin (S) (30µg), Septrin (SXT) (30µg) and Erythromycin (E) (10µg) were employed. Using sterile forceps, the antibiotic discs were positioned onto the inoculated plates, and subsequently, the plates were incubated at a temperature of 37 ±2 ℃ for a duration of 24 hours. Following incubation, the plates were carefully examined to identify zones of inhibition, indicative of the susceptibility of each isolate to the antibiotics utilized.

RESULTS AND DISCUSSION

Study Area

This research was conducted Dominion University Ibadan in Oluyole Local Government Area of Oyo Sate, Nigeria and located along the following coordinates 7°11'26.93342"N, 3°48'4.36144"E. Figure 1 is the map of Ibadan showing the location of Dominion University Ibadan.

Sample Collection and Isolation of Bacteria

A total number of forty-five (45) samples were collected from five (5) fomite surfaces and these formed the samples from which bacteria were isolated for the research. From the 45 fomites, a total of twenty (20) bacteria isolates were obtained from the various sampling sites. Figure 2 shows the distribution of bacteria isolated from the various surfaces. It was observed that the total heterotrophic and coliform count were higher in the afternoon and evening at the various sampling sites, and this could be attributed to an increased anthropogenic activity around these sites at noon and in the evening. The total heterotrophic and coliform count from the various sampling time and locations are presented in Table 1a and b.

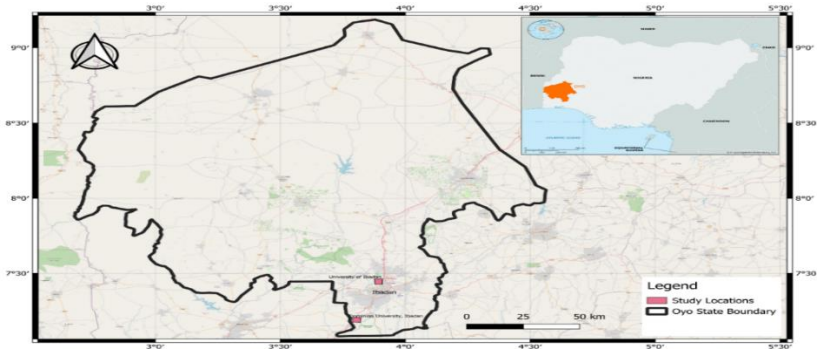

Figure 1: Map of Oyo State showing the location of Dominion University Ibadan.

Key CASF1-F3 = Computing and Applied Science; LAB = Laboratory; LT = Lecture Theatre

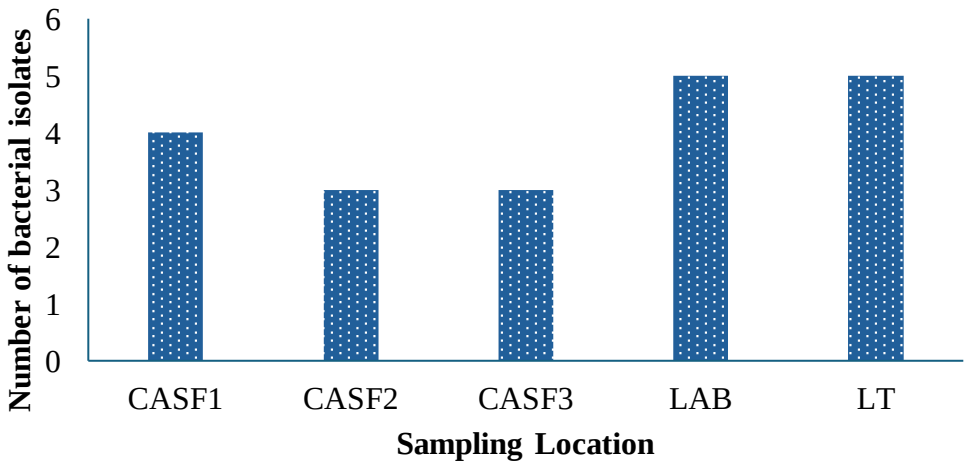

Figure 2: Distribution of bacteria isolated from fomites in Dominion University Ibadan – Nigeria

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Table 1a: Total Heterotrophic Count (CFU/mL) of bacteria isolated from fomites in Dominion University Ibadan - Nigeria

Sampling Location	Week 1			Week 2			Week 3		
	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening
CASF1	6.8×10^2	1.3×10^4	5.7×10^2	5.0×10^3	2.2×10^3	3.7×10^6	5.0×10^3	2.4×10^5	4.5×10^7
CASF2	7.5×10^3	1.2×10^3	2.5×10^3	5.5×10^2	1.9×10^3	2.8×10^6	5.5×10^2	1.7×10^5	4.6×10^7
CASF3	5.9×10^4	1.6×10^2	2.4×10^3	4.1×10^2	2.7×10^3	3.8×10^2	4.1×10^2	3.1×10^6	3.9×10^8
LAB	7.0×10^4	1.8×10^4	3.8×10^4	6.4×10^2	1.8×10^6	5.6×10^8	6.4×10^3	2.2×10^5	4.6×10^5
LT	7.3×10^3	1.9×10^5	4.6×10^4	5.8×10^2	1.9×10^5	3.4×10^6	5.8×10^4	3.2×10^5	4.8×10^7

Table 1b: Total Coliform Count (CFU/mL) of bacteria isolated from fomites in Dominion University Ibadan – Nigeria

Sampling Location	Week 1			Week 2			Week 3		
	Morning	Noon	Evening	Morning	Noon	Evening	Morning	Noon	Evening
CASF1	5.2×10^3	1.1×10^5	2.7×10^3	3.7×10^3	2.2×10^5	3.2×10^4	3.4×10^2	3.3×10^8	2.3×10^8
CASF2	3.8×10^2	1.6×10^1	2.7×10^2	4.7×10^4	1.7×10^5	2.4×10^5	4.6×10^3	4.1×10^6	3.1×10^6
CASF3	2.3×10^4	1.2×10^4	2.2×10^4	3.0×10^3	2.5×10^6	1.2×10^5	3.6×10^4	3.3×10^8	2.3×10^5
LAB	3.5×10^2	1.4×10^3	2.9×10^6	3.7×10^2	2.3×10^4	2.0×10^3	3.7×10^2	3.7×10^7	2.9×10^6
LT	4.6×10^3	1.3×10^6	3.3×10^7	4.3×10^3	1.6×10^7	3.0×10^4	3.1×10^5	4.1×10^3	3.1×10^5

Colonial and biochemical characteristics of bacterial isolates

The colonial characteristics of the bacterial isolates showed that some colonies had a rough margin while others were smooth. On the basis of the elevation, some of the colonies were either raised, convex or flat. In terms of the color and opacity of the colonies, some were cream, greyish white, yellow, bright yellow, opaque, translucent and transparent, respectively. The Gram's reaction revealed that 9 (45%) of the bacterial isolates were Gram positive while the remaining 11 (65%) were Gram negative. The biochemical characteristics revealed that all the bacterial isolates (100%) were catalase positive, 6 (30%) were coagulase positive, 4 (20%) were indole positive, 13 (65%) were Urease positive, and 3 (15%) were oxidase positive. The sugar fermentation pattern of the bacterial isolates showed that glucose and sucrose were the most preferred carbon sources as there was a highly utilization of these sugars. However, it was observed that cellobiose and sorbitol were the least utilized sugars by the bacterial isolates. Table 2.

Identification of bacteria isolated from fomites in Dominion University Ibadan - Nigeria

Following the results obtained for the biochemical characteristics of the twenty (20) bacterial isolates, it was observed that all the isolates belonged to five genera viz; Staphylococcus (45%), Proteus (10%), Escherichia (20%), Klebsiella (10%), and Pseudomonas (15%). Figure 3 shows the probable identity of the bacterial isolates. The occurrence of Staphylococcus aureus on surfaces holds notable importance, given its recognized role as a frequent trigger of Community-Acquired Infections (CAIs), which encompass various conditions like skin and soft tissue infections, bacteremia, and pneumonia (Jaradat et al., 2020). Furthermore, research conducted by

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Agarwal et al. (2018) spotlighted the presence of bacteria on mobile phones belonging to healthcare personnel. In this study, the predominant bacteria identified on these devices were Gram-positive cocci, including Staphylococcus aureus. This finding aligns with prior research demonstrating the high occurrence of Staphylococcus aureus in diverse environmental settings (Chen et al., 2020). The prevalence of Staphylococcus aureus might be attributed to their Gram-positive nature, allowing them to endure for extended periods on dry surfaces and thrive in conditions of lower humidity (Arhin et al., 2020). Similarly, the presence of Escherichia coli on fomites gives rise to notable concern, as it serves as a well-known indicator of fecal contamination and can be linked to gastrointestinal infections (Iskandar et al., 2022). The elevated presence of Escherichia coli could potentially be attributed to poor hand hygiene practices and the plausible dissemination of fecal contamination within university environments (Jones et al., 2019).

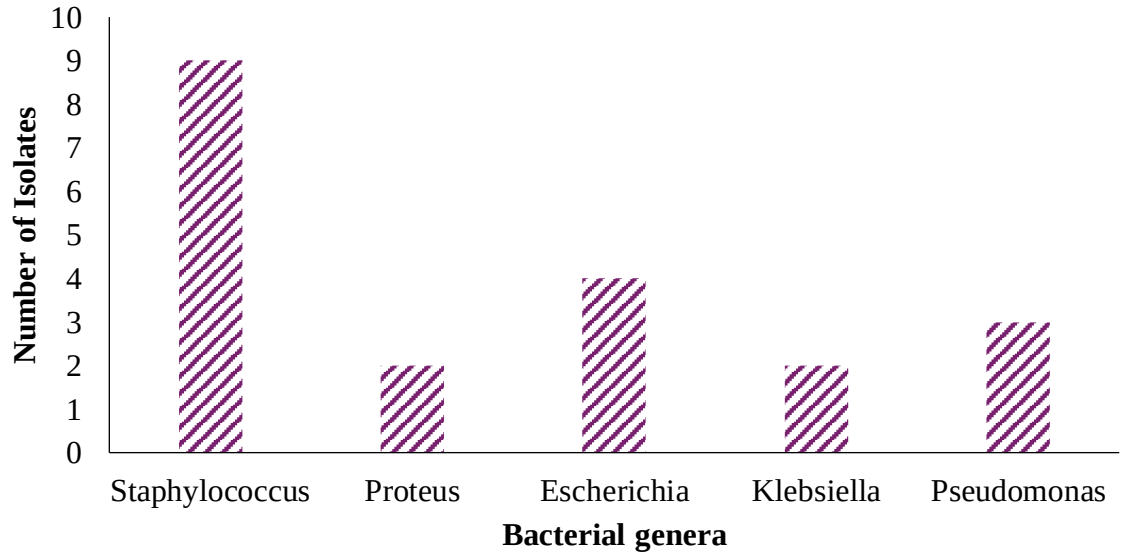

Figure 3: Bacterial genera isolated from fomites in Dominion University Ibadan, Nigeria

Table 2: Biochemical characteristics of bacteria isolated from fomites in Dominion University Ibadan

S/N	Isolate code	Gram's reaction	Catalase	Coagulase	Indole	Urease	Oxidase	Sugar fermentation							Probable Identity
								Glucose	Sucrose	Lactose	Maltose	Galactose	Sorbitol	Cellobiose	
1.	HS1	+	+	-	-	+	-	+/+	+/-	+/-	+/+	-/-	-/-	-/-	Staphylococcus epidermidis
2.	LSB	+	+	+	-	+	-	+/+	+/-	+/-	+/+	+/+	-/-	-/-	Staphylococcus aureus
3.	PLB	+	+	+	-	+	-	+/+	+/+	+/-	+/+	+/+	-/-	-/-	Staphylococcus aureus
4.	C3B	-	+	-	+	-	-	+/+	-/-	+/-	-/-	-/-	+/+	-/-	Escherichia coli
5.	C2B	-	+	-	+	-	-	+/+	-/-	+/+	-/-	-/-	+/+	-/-	Escherichia coli
6.	C1A	-	+	-	-	+	-	+/+	+/+	-/-	-/-	-/-	-/-	-/-	Proteus mirabilis

7.	HS2	+	+	+	-	+	-	+/+	+/+	+/-	+/-	+/+	-/-	-/-	Staphylococcus aureus
8.	SSF2	-	+	-	-	+	-	+/+	+/+	+/+	+/-	+/+	+/+	+/+	Klebsiella species
9.	C1C	-	+	-	-	-	+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	Pseudomonas aeruginosa
10.	C2C	-	+	-	-	-	+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	Pseudomonas aeruginosa
11.	LTSF	+	+	-	-	+	-	+/+	+/+	+/-	+/+	-/-	-/-	-/-	Staphylococcus epidermidis
12.	C2A	+	+	+	-	+	-	+/+	+/+	+/+	+/+	+/+	-/+	-/-	Staphylococcus aureus
13.	C1B	-	+	-	-	+	-	+/+	+/-	+/+	+/+	+/+	+/-	+/+	Klebsiella species
14.	LSF	+	+	+	-	+	-	+/+	+/-	+/+	+/+	+/+	-/-	-/-	Staphylococcus aureus
15.	HS3	-	+	-	-	+	-	+/+	+/-	-/-	-/-	-/-	-/-	-/-	Proteus species
16.	SSF1	+	+	+	-	+	-	+/+	+/+	+/+	+/+	+/+	-/-	-/-	Staphylococcus aureus
17.	C3C	-	+	-	+	-	-	+/+	-/-	+/+	-/-	-/-	+/+	-/-	Escherichia coli
18.	C2A	-	+	-	-	-	+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	Pseudomonas aeruginosa
19.	C3A	-	+	-	+	-	-	+/+	-/-	+/+	-/-	-/-	+/+	-/-	Escherichia coli
20.	HS4	+	+	-	-	+	-	+/+	+/+	+/-	+/+	-/-	-/-	-/-	Staphylococcus epidermidis

Key: + = Positive, - = Negative, +/+ = Utilization/Gas evolution, +/- = Utilization/No gas evolved

Antibiotic susceptibility pattern of bacteria isolates

The antibiotic susceptibility pattern of the bacterial isolates showed that bacterial strains belonging to the genera Staphylococcus and Proteus were highly inhibited by most of the antibiotics tested against as they were susceptible to 70% of the antibiotics used. Conversely, there was a high level of antibiotic resistance amongst bacterial strains in the genera Escherichia, Pseudomonas and Proteus with an average resistance to over 80% of the antibiotics used. Out of the ten (10) antibiotics used against the bacterial isolates, Pefloxacin (10µg) and Ciprofloxacin (10µg) had the highest antagonistic effect while Zinnacef (20µg), Amoxicillin (30µg), and Erythromycin (10µg) had the least inhibitory effect against the test microorganisms, respectively. Table 3 and plate 1. The findings in this study corroborates the reports of Arhin et al. (2020) where a wide array of bacterial isolates demonstrated resistance to a substantial number of antibiotics, including Zinnacef and Amoxicillin, which could complicate the treatment of infections caused by these bacterial groups.

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Table 3: Antibiotic susceptibility pattern of bacteria isolated from fomites in Dominion University Ibadan

S/N	Test bacteria	Antibiotics susceptibility pattern (µg)										Antibiotic resistance profile	Antibiotic Resistance Index (%)
		PEF (10)	CN (10)	APX (30)	Z (20)	AM (30)	R (25)	CPX (10)	S (30)	SXT (30)	E (10)		
1.	Staphylococcus epidermidis	+	+	+	-	-	+	+	+	+	-	Z, AM, E	30
2.	Staphylococcus aureus	+	-	-	-	+	+	+	+	+	+	CN, APX, Z,	30
3.	Staphylococcus aureus	+	-	-	-	-	+	+	+	+	-	Z, AM, E	50
4.	Escherichia coli	-	+	-	-	-	-	+	-	-	-	PEF, APX, Z, AM, R, S, SXT, E	80
5.	Escherichia coli	+	-	-	-	-	+	+	+	-	-	CN, APX, Z, AM, SXT, E	60
6.	Proteus mirabilis	+	+	+	-	-	+	+	+	+	-	Z, AM, E	30
7.	Staphylococcus aureus	+	+	+	-	-	-	-	+	-	+	Z, AM, R, CPX, SXT	50
8.	Klebsiella species	+	-	-	+	-	+	+	+	+	-	CN, APX, AM, E	40
9.	Pseudomonas aeruginosa	-	-	+	-	+	-	-	-	-	-	PEF, CN, Z, R, CPX, S, SXT, E	80
10.	Pseudomonas aeruginosa	+	+	+	-	-	+	+	-	-	-	Z, AM, S, SXT, E	50
11.	Staphylococcus epidermidis	+	+	-	-	-	-	-	-	-	-	APX, Z, AM, R, CPX, S, SXT, E	80

12.	Staphylococcus aureus	+	-	+	-	-	-	+	-	-	+	CN, Z, AM, R, S, SXT	60
13.	Klebsiella species	+	-	+	-	-	-	+	-	+	-	CN, Z, AM, S, E	60
14.	Staphylococcus aureus	+	+	+	-	-	+	+	+	+	-	Z, AM, E	30
15.	Proteus species	-	-	-	-	-	-	-	-	-	-	PEF, CN, APX, Z, AM, R, CPX, S, SXT, E	100
16.	Staphylococcus aureus	+	+	-	-	-	-	+	+	-	+	APX, Z, AM, R, SXT	50
17.	Escherichia coli	-	-	+	-	+	-	-	-	-	-	PEF, CN, Z, R, CPX, S, E	80
18.	Pseudomonas aeruginosa	+	-	+	-	-	-	+	-	-	-	CN, Z, AM, R, S, SXT, E	80
19.	Escherichia coli	+	+	-	-	-	-	-	-	-	-	APX, Z, AM, R, CPX, S, SXT, E	80
20.	Staphylococcus epidermidis	+	+	+	-	-	-	+	+	-	-	Z, AM, R, SXT, E	50

Key: PEF = Pefloxacin, CN = Gentamycin, APX = Ampiclox, Z = Zinnacef, AM = Amoxicillin, R = Rocephin, CPX = Ciprofloxacin, S = Streptomycin, SXT = Septrin, E = Erythromycin, + = Susceptible.

Plate 1: Antibiotic sensitivity disc showing the antibiotic resistance pattern of Escherichia coli on Mueller Hinton agar

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CONCLUSION

The isolation and biochemical identification of bacteria on fomites provided valuable insight into the presence and characteristics of bacteria on commonly touched surfaces within the campus and it is crucial in understanding the potential risks of infectious diseases. This study allowed for a better understanding of the different species present and effectively demonstrates the existence of diverse pathogenic bacterial strains on fomites at Dominion University, with Staphylococcus aureus exhibiting the most predominant prevalence. The presence of enteric bacteria on the fomites indicates the potential for fecal contamination, which is associated with human infections. These bacteria serve as causative agents of diarrhea and can lead to outbreaks among members of the university community. The findings underscore the indispensable necessity of adhering to robust disinfection practices, heightened awareness campaigns and regular surveillance within the university community to effectively attenuate the risk of transmitting infectious diseases through contact with inanimate surfaces. Hence, it recommended routine sanitary steps should be taken to reduce the presence of potential harmful microorganism on fomites within the university community.

Conflict of interest

The authors declare no competing interest.

Credit authorship contribution statement

JA: Conceptualization, Investigation, Supervision; OTD: Validation and Supervision; SAO: Conceptualization, Investigation and Writing; GEO: Supervision, Writing and Editing; VIA: Writing and Editing.

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