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Isolation and Molecular Detection of Bacteria from Frequently Touched Objects of Various Public Places at Sadar Upazila of Mymensingh District

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Abstract

The current research was conducted to investigate the magnitude of bacterial contamination in public buses, different wards of hospitals, and public toilets of bus stations and hospitals in Sadar Upazila, Mymensingh, Bangladesh. A total of 90 swab samples were collected aseptically from the most frequently touched surfaces of the sample area. Identification of the isolated bacteria was done by staining and biochemical tests, followed by molecular detection by PCR using genus or species-specific primers. Isolated organisms were then subjected to an antibiotic sensitivity test using disk diffusion techniques using 13 commonly available antibiotics. Among the samples, 76.67% (n=69/90) were positive for *E. coli*, 80% (n=72/90) were positive for *Klebsiella* spp., and 68% (n=61/90) were positive for *Staphylococcus* spp. Positive *tet*A and *stx*-1 genes were found in 40 and 19 *E. coli* isolates, respectively. 23 positive *mec*A genes, or MRSA, were found in *Staphylococcus aureus* isolates that pose a threat to public health. Toilets of the bus stations were the most contaminated place by the selected bacteria, with the prevalence of *E. coli,Klebsiella* spp., and *Staphylococcus aureus* being 81.48% (n=22/27), 77.78% (n=21/27), and 81.48% (n=22/27), respectively. In an antibiogram study, *E. coli* isolates showed 100% resistance against amoxicillin, azithromycin, tetracycline, and co-trimoxazole. *Klebsiella* spp. was revealed to be 100% resistant to amoxicillin, followed by colistin sulfate (60%). *Staphylococcus aureus* isolates were 100% resistant to methicillin, cefoxitin, and cefixime. The findings can be used to raise public awareness about the possible threat, hence preventing the spread of infectious disease in public places.

Keywords: Isolation, Molecular Detection, Bacteria, Frequently Touched Surfaces, Antibiotic Resistance.

Introduction

Researchers have been increasingly focusing on conducting bacteriological investigations on surfaces that are easily accessible and regularly handled by the general people due to their potential influence on public health. Contaminated surfaces can serve as possible reservoirs of germs of a number of infectious diseases, making them a potential concern to public health and safety. Opportunistic microorganisms may survive and proliferate in a range of settings and cause a broad range of illnesses in both human and animals (Pilipcincova *et al.,* 2010; Akinkunmi and Lamikanra, 2010). Due to healthcare-associated illnesses, patients are contaminated by a significant number of microorganisms found in the hospital environment,

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including pathogens that are resistant to drugs. Overall, germs from hands of medical personnel or from colonized and diseased patients can directly infect surfaces (Adams *et al.,* 2017). Common bacteria from the hand microbiota may infect frequently touched things (such as bed frames, overbed tables, doorknobs, and stethoscopes) (Russotto *et al.,* 2015; Shams *et al.,* 2016). More significantly, medical equipment and touch surfaces, particularly in critical care units, have been shown to harbor multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) (Silva *et al.,* 2012). Transmission may occur via direct contact with things (in hospitals and public areas) and/or individuals who are infected and/or colonized. This can result in morbidity and death (Gravel *et al.,* 2007).

The modern transportation network is progressively growing to accommodate the increased demand for the movement of peoples and products. As a result, since hazardous bacteria have now acquired a better way of multiplication that is faster and more thorough than previously, this networking has received a lot of interest from public health professionals (Stepanovic *et al.,* 2008; Pamela *et al.,* 2011; Mendes *et al.,* 2015). Numerous instances from the previous centuries demonstrate how the development and growth of global transportation networks has enabled the spread of infectious disease pandemics over the globe (Totem *et al.,* 2006).

Reports from the World Health Organization state that a large number of illnesses are brought on by dirty public washroom and may have serious health consequences (Matini *et al.,* 2020). Toilets and washrooms are perfect places for germs to survive because of their warm and humid atmosphere (Suen *et al.,* 2019). Also, due to their frequent and inevitable use, these are often replete with microorganisms (Alonge *et al.,* 2019). They enter public restrooms by human waste, mostly faces and urine. The risks connected to using public restrooms had been identified, but the door handles, knobs, taps, and other inanimate items in these facilities that may contain and spread infectious pathogens had received less attention. It is possible that individuals who touch the same things might spread pathogens to one another.

Numerous investigations have previously been carried out globally, concentrating on the existence and quantity of microbiological contamination on surfaces that are regularly handled, such as those in hospitals, restrooms, train cars, buses, mobile phones, ATM booths, shopping carts, etc. Among the bacteria isolated, *Vibrio cholerae*, *Escherichia coli,* multi-drug resistant *Staphylococcus aureus, Shigella* spp., *Mycobacterium tuberculosis, Salmonella* spp*.,* and *Pseudomonas aeruginosa* are frequently being reported (Flores *et al.,* 2011; Pamela *et al*., 2011; Gavalda*et al.,* 2015). Certain bacterial infections have developed antibiotic resistance, resulting in a significant global public health emergency (Voicu *et al.,* 2017).

Mymensingh is the country's twelfth and latest city-corporation area. Day by day, public places are becoming more crowded here. According to the reviewed literature, no studies have been conducted in Mymensingh Sadar Upazila hospitals or public places to investigate the bacterial contamination of frequently handled objects. Therefore, aims of the the current are set to determine the amount of bacteria present on commonly touched surfaces in public places in Sadar upazila of Mymensingh city, isolate and detect bacteria using PCR and culture methods, and analyze the antibiograms of the selected bacterial species.

Materials and Methods

Collection of Samples

A total of 90 swab samples were collected from the bus (*e.g.,* grab rail, indoor handle, outdoor handle, arm rest, vinyl seat, window surface), wards of Mymensingh Medical College Hospital or MMCH (*e.g.,* electric board switches, indoor knob, outdoor knob, bed surface, table surface, floor), toilets of the bus station, and MMCH (*e.g.,* such as door handle, indoor knob, outdoor knob, water tap, ewer, pan, dirty floor, basin tap, shower tap) at Mymensingh Sadar Upazila. The samples were inoculated into nutritional broth and transported to the bacteriology laboratory of the Department of Microbiology and Hygiene, BAU, Mymensingh. The temperature was maintained at 4 ºC using an ice box.

Isolation and Identification

Primary enrichment involved the use of nutrient broth, followed by inoculation and incubation of selective medium at 37 °C for the whole night. Bacterial isolation from collected samples was done using selective media *e.g.,* Eosin Methylene Blue (EMB) agar, MacConkey agar for *Escherichia coli* and *Klebsiella* spp., Mannitol salt (MS) agar for *Staphylococcus* spp. All the isolates were then subjected to subculture on respective agar to obtain pure cultures of the target bacteria (Cheesbrough, 2006). The morphological properties were determined using Gram's staining, following the method outlined by Merchant and Packer (1967).

Molecular Identification

DNA extraction: For each isolate, genomic DNA was extracted using the boiling technique as described by Hussain *et al.* (2016) and Hossain *et al*. (2013).

Molecular Detection of Associated Bacteria

The primers used in the study for molecular detection of the associated bacteria are included in Table 1 along with the appropriate sequences. 12.5 µL of PCR master mixture, 5.5 µL nuclease-free water, 5 µL DNA, and 1 µL of each forward and reverse primer were combined to create 25 µL of the reaction mixture, which was then used for the PCR.

Antibiotic Susceptibility of the Isolates

Antimicrobial drug susceptibility against 13 commonly used antibiotics were performed by disc diffusion or Kirby-Bauer method (Bauer *et al.,* 1966). In short, the turbidity was adjusted to 0.5% McFarland standard by growing all of the isolates in LB broth and inoculating them onto Mueller-Hinton agar (HI media, India) after they had been cultured for around 4 hours at 37 °C in a shaking incubator. The antibiotic discs were then set. To estimate the zone's width, the plates were then incubated for eighteen hours at 37 °C. The zone of inhibition standard interpretation chart from the CLSI (Clinical and Laboratory Standards Institute, 2021) was examined in order to ascertain the resistance or sensitivity of the isolates. Amoxicillin (AMX, 30 µg/disc), Cefoxitin (CX, 30 µg/disc), Azithromycin (AZM, 15 µg/disc), Cefixime (CFM, 5 µg/disc), Ciprofloxacin (CIP, 5 µg/disc), Chloramphenicol (C, 30 µg/disc), Co-Trimoxazole (COT, 25 µg/disc), Colistin sulfate (CS, 10 µg/disc), Gentamicin (GEN, 10 µg/disc), Methicillin (MET, 5 µg/disc), Streptomycin (S, 10 µg/disc), Tetracycline (TE, 10 µg/disc), and Vancomycin (VA, 30µg/disc) were used in the study.

Table 1. List of PCR primers with sequence

Species	List of the primers	Primer's sequence (5'-3')	Amplicon size	References
E. coli	*mal*B F	GACCTCGGTTTAGTTCACAGA	585 bp	Amit-Romach*et al.* (2004)
	*mal*B R	CACACGCTGACGCTGACCA		
	*Stx*1 F	CACAATCAGGCGTCGCCAGCGCACTTGCT	606 bp	Majumder *et al.* (2017)
	*Stx*1 R	TGTTGCAGGGATCAGTCGTACGGGGATGC		
	*tet*A F	GCTACATCCTGCTTGCCTTC	210 bp	Ng *et al.* (2001)
	*tet*A R	CATAGATCGCCGTGAAGAGG		
Klebsiella spp.	*gyr*A F	CGCGTACTATACGCCATGAACGTA	441 bp	Brisse and Verhoef (2001)
	*gyr*A R	ACCGTTGATCACTTCGGTCAGG		
S. aureus	*nuc* F	GCG ATT GAT GGT GAT ACG GTD	279 bp	Kalorey*et al.* (2007)
	nuc R	AGC CAA GCC TTG ACG AAC TAA AGC		
	*mec*A F	ACTGCTATCCACCCTCAAAC	163 bp	Mehrotra *et al.* (2000) and Bitrus *et al.* (2017)
	*mec*A R	CTGGTGAAGTTGTAATCTG		

Molecular Detection of Antibiotic Resistance Genes

PCR was used to detect the tetracycline resistance gene (*tet*A) and the methicillin resistance gene (*mec*A) in *E. coli* and *Staphylococcus aureus* positive isolates, respectively (Table 1).

Results and Discussion

Cultural, Morphological and Biochemical Characterization

The growth of *E. coli* was distinguished by smooth, circular, black or green color colonies with metallic sheen on EMB agar (Figure 1a) and circular, raised, bright pink-colored colonies on MacConkey agar (Figure 1b). The growth of *Klebsiella* spp. on EMB agar was indicated by large, mucoid, pink to purple colonies with no metallic green sheen (Figure 1c) and on MacConkey agarcircular, convex, mucoid, pink to red colored colonies (Figure 1d). The colony characteristics of *E. coli* and *Klebsiella* spp. observed on EMB agar and MacConkey agar were consistent with the findings of Smith (1967) and Lamsal (2015). The growth of *Staphylococcus aureus* on Nutrient agar was indicated by golden yellow and opaque colonies with smooth glistening surface; on Mannitol salt agar smooth, circular, yellowish colony with changing the media color from pink to bright yellow (Figure 1e); on Blood agar smooth, circular, small whitish colony with hemolysis (Figure 1f). These are similar to the findings of Habib *et al.* (2015). In gram's staining, *E. coli* was gram (–)ve, paired or in short chain (Figure 2a); *Klebsiella* spp. was gram (–)ve, in short chains or sometimes in clusters (Figure 2b) and *Staphylococcus aureus*was gram (+)ve and arranged in grapes like cluster (Figure 2c). Result of sugar fermentation test using five basic sugars such as (dextrose, maltose, lactose, sucrose and mannitol) are presented in Table 2. *E. coli*, *Klebsiella* spp., and *Staphylococcus aureus* were catalase positive, as evidenced by the generation of oxygen bubbles. Staphylococcus aureus was coagulase-positive and coagulated rabbit plasma.

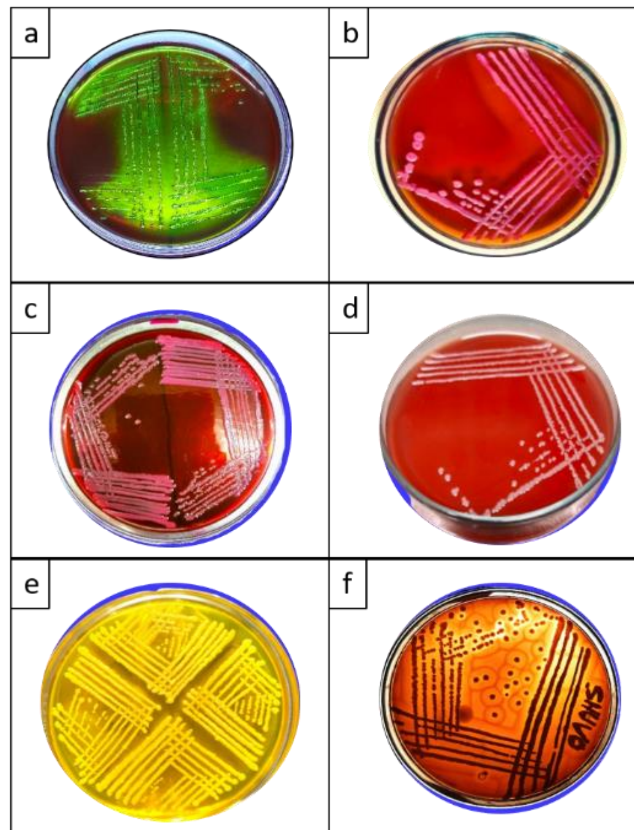

Figure 1. Cultural characteristics of *E. coli* (a, b), *Klebsiella* spp. (c, d) and *S. aureus* (e, f) on different agar media.

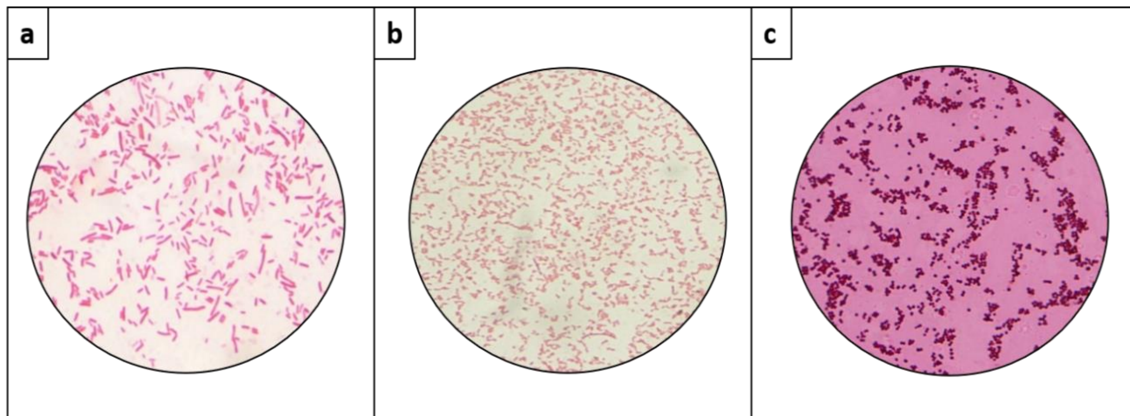

Figure 2. Staining characteristics of *E. coli* (a), *Klebsiella* spp. (b), and *S. aureus* (c) by Gram's staining.

Table 2. Results of sugar fermentation test of the isolated bacteria

Sugar fermentation reaction profiles					Interpretation
Dextrose	**Maltose**	**Lactose**	**Sucrose**	**Mannitol**	
AG	AG	AG	AG	AG	*E. coli*
AG	AG	AG	AG	AG	*Klebsiella* spp.
A	A	A	A	A	*Staphylococcus aureus*

A = Acid; AG = Acid and Gas

Molecular Detection of Isolated Bacteria

Molecular detection of *E. coli* was performed by PCR using specific primer *mal*B gene amplifying a band of 585 base pair (bp) (Figure 3). Using a particular primer for the *Kleb_gyr*A gene and amplifying a band of 441 bp, PCR was used to identify *Klebsiella* spp. molecularly (Figure 4). *Staphylococcus aureus* was detected molecularly by PCR amplification of a 279 bp band using a particular primer *nuc* gene (Figure 5).

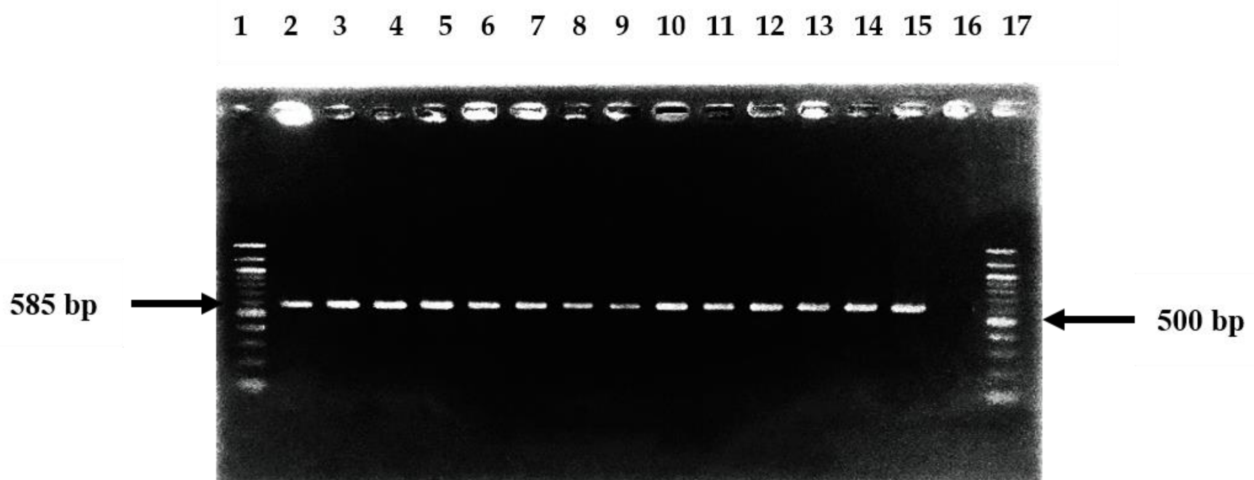

Figure 3. Results of amplification of *mal*B gene of *E. coli* isolates by PCR. Lane 1 and 17 = 100 bp size DNA marker; lane 15 = Positive control; lane 16 = Negative control without DNA and lane 2–14 = representative *E. coli* isolates.

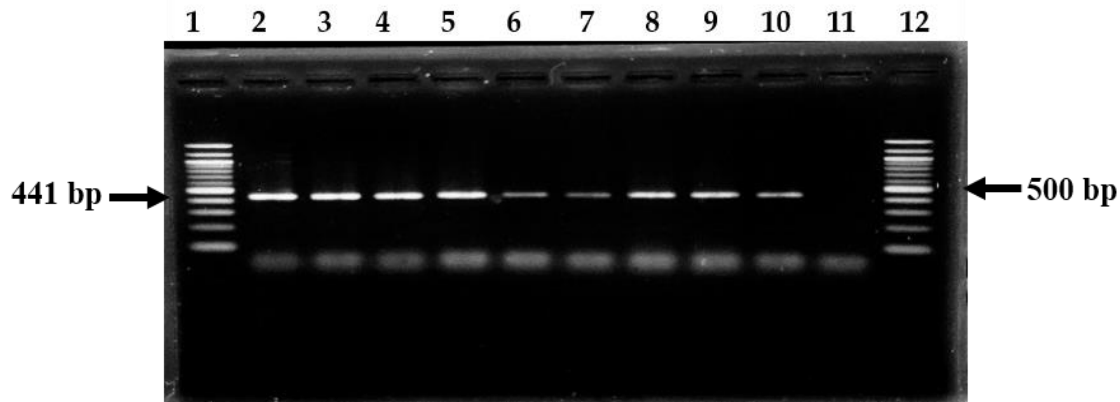

Figure 4. Results of amplification of *Kleb_gyrA* gene of *Klebsiella* spp. by PCR. Amplicon size = 441 bp, lane 1 and 12 = 100 bp size DNA marker; lane 2 = Positive control; lane 11 = Negative control without DNA; and lane 2–10 = representative *Klebsiella* spp. isolates.

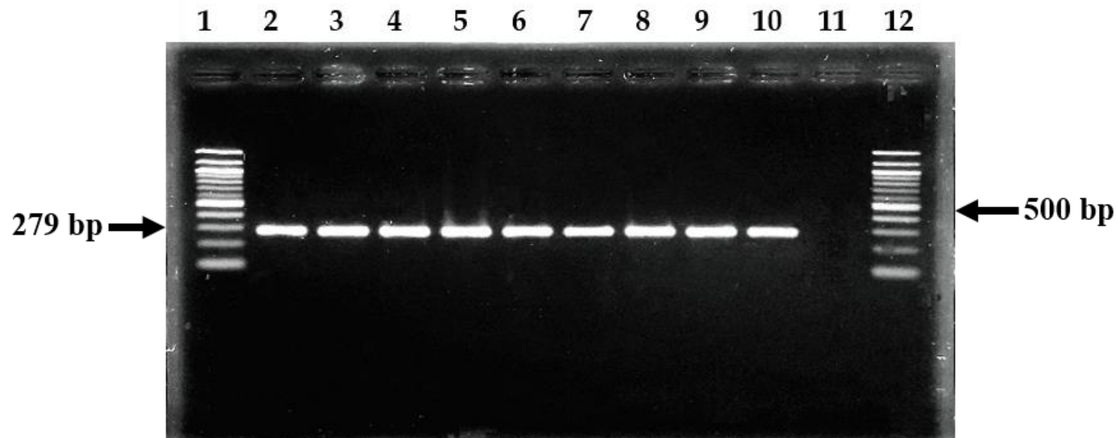

Figure 5. Results of amplification of *nuc* gene of *S. aureus* by PCR. Lane 1 and 12 = 100 bp size DNA marker; lane 2 = Positive control; lane 11 = negative control without DNA; and lane 2–10: represent *Staphylococcus aureus* isolates.

Prevalence of E. coli, Klebsiella spp. and Staphylococcus aureus in Sample Collection Area

The total frequency of *E. coli* was 76.67%. The highest prevalence of *E. coli* was found in the samples obtained from the public bus of Bridgemor Bus Station (100%) and the toilet of the medicine ward, MMCH (100%). The lowest prevalence was observed on the public bus at Trishal Bus Station (50%). *Klebsiella* spp. had an overall prevalence of 80%. The highest prevalence (100%) was identified in the toilet of the medicine ward at MMCH, while the lowest (50%) was found in the bus at Trishal Bus Station and the gynecology ward at MMCH. The total prevalence of *Staphylococcus aureus* was 68%. The highest prevalence (88.89%) was found in the medicine ward toilet and at the Bridgemor Bus Station. The lowest prevalence (16.67%) was identified on public bus at Trishal Bus Station (Table 3). Our findings were higher compared to those reported in similar microbial investigations conducted by several researchers. Arhin *et al.* (2020) identified *Staphylococcus aureus* (33.1%), *Escherichia coli* (30.8%), and *Klebsiella* spp. (3.55%) as common isolates from washroom and toilet objects. Similarly, Matini *et al*. (2020) reported *Escherichia coli* (28.48%), *Staphylococcus aureus* (8.52%), and *Klebsiella* spp. (1.98%).

Table 3. Summary of prevalence of *E. coli*, *Klebsiella* spp. and *Staphylococcus aureus*in the sample collection area

Name of sample area(n)	**No. of *E. coli* +ve isolates**	**Prevalence of *E. coli* (%)**	**No. of *Klebsiella* spp. +ve isolates**	**Prevalence of *Klebsiella* spp. (%)**	**No. of *S. aureus* +ve isolates**	**Prevalence of *S. aureus* (%)**
Public bus from Bridgemor Bus Station (6)	6	100.00	4	66.67	4	66.67
Public bus from Maskanda Bus Station (6)	4	66.67	4	66.67	4	66.67
Public bus from Trishal Bus Station (6)	3	50.00	3	50.00	1	16.67
Toilet of Bridgemor Bus Station (9)	7	77.78	8	88.89	8	88.89
Toilet of Maskanda Bus Station (9)	6	66.67	6	66.67	7	77.78
Toilet of Trishal Bus Station (9)	8	88.89	7	77.78	7	77.78
Medicine patient's ward (6)	4	66.67	5	83.33	3	50.00
Surgery patient's ward (6)	5	83.33	4	66.67	4	66.67
Gynecology patient's ward (6)	4	66.67	3	50.00	3	50.00
Toilet of Medicine ward (9)	9	100.00	9	100.00	8	88.89
Toilet of Surgery ward (9)	6	66.67	4	44.45	6	66.67
Toilet of Gynecology ward (9)	7	77.78	6	66.67	6	66.67
Total no. of sample collected (90)	**69**	**76.67**	**72**	**80.00**	**61**	**68.00**

Antimicrobial Susceptibility Profile of the Isolated Bacteria

The results of the study showed that *E. coli* isolates were 100% sensitive to gentamicin, chloramphenicol, ciprofloxacin and 100% resistance against amoxicillin, azithromycin, tetracycline, co-trimoxazole followed by colistin sulfate (73.34%) (Figure 6). The results were in agreement with Bag *et al.* (2021) and Nwankwo *et al.* (2022) who isolated *E. coli* and found higher resistance against amoxicillin and tetracycline. The bacteria *Klebsiella* spp. exhibited 100% susceptibility to gentamicin, tetracycline, chloramphenicol, ciprofloxacin, co-trimoxazoie, azithromycin, and streptomycin, but 100% resistance to amoxicillin, followed by colistin sulfate (60%) (Figure 7). Our results were more or less similar with Farhadi *et al.* (2021) who mentioned that 62% carbapenem-resistant *K. pneumoniae* were resistant to colistin antibiotic. The study found that *Staphylococcus aureus* was completely susceptible to ciprofloxacin, co-trimoxazole, chloramphenicol, gentamicin, and vancomycin, while 80% of isolates were sensitive to azithromycin, 70% to streptomycin, and 100% were resistant to methicillin, cefoxitin, and cefixime (Figure 8). Our isolates were 100% resistant to cefoxitin which was miserably higher than the findings of Abdelmalek *et al.* (2022) who found 45.5% resistance against cefoxitin. Our research matched with the research outcomes of Benrabia *et al.* (2020) and Okorie-Kanu *et al.* (2020) who didn’t find any resistance against vancomycin.

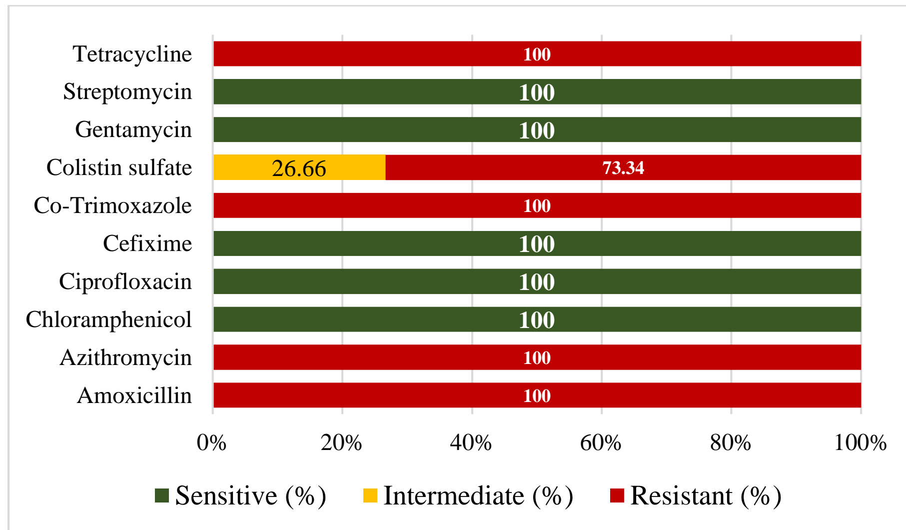

Figure 6. Sensitivity pattern of the selected *E. coli* isolates.

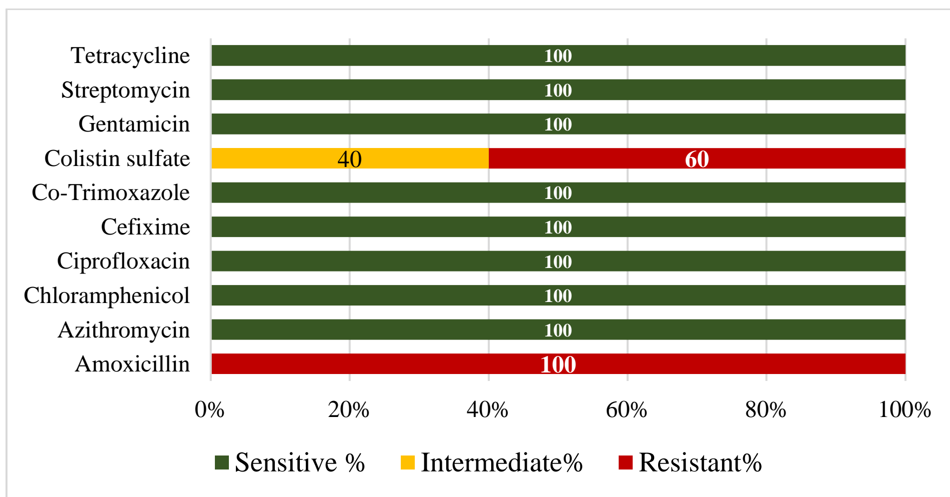

Figure 7. Sensitivity pattern of the selected *Klebsiella* spp. isolates.

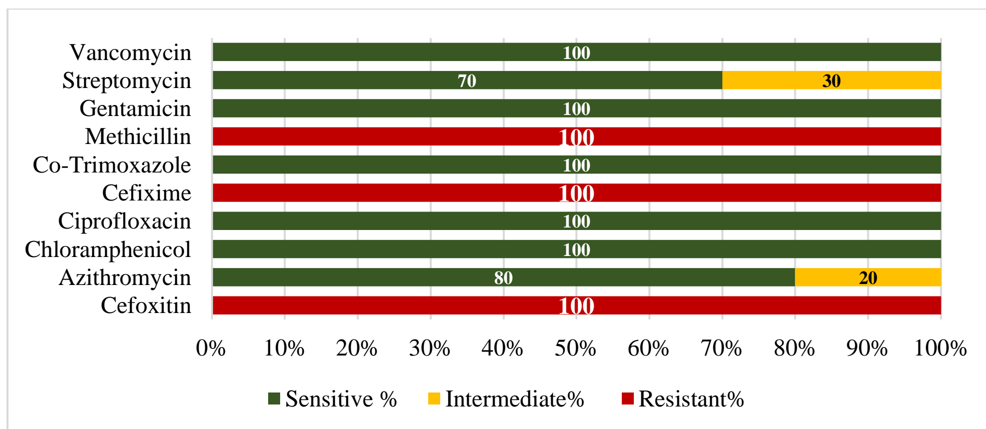

Figure 8. Sensitivity pattern of the selected *S. aureus* isolates.

Molecular Detection of Shiga Toxin Producing Gene and Antibiotic Resistant Genes

All the *mal*B gene positive isolates were screened for *stx*-1 and *stx*-2. Out of 69 PCR positive *E. coli*, 19 isolates showed *stx*-1 positive band at 606 bp (Figure 9) and no isolates showed positive results to *stx*-2. These findings were in agreement with the results of Bag *et al.* (2021) and Ripon *et al.* (2021). All the PCR positive *E. coli* isolates were subjected to molecular detection targeting tetracycline resistance gene (*tet*A) and 40 isolates were showed positive to *tet*A genes by PCR amplifying a band of 210 bp (Figure 10). This result was higher than the result of Adefisoye and Okoh (2016), who identified 30.4% *tetA* gene positive *E. coli* from treated waste water effluents in Eastern Cape, South Africa. Twenty-three MRSA were identified through PCR using *mec*A gene specific primer amplifying a band of 163 bp (Figure 11), which was more than the findings of Otter *et al.,* (2009) who found no MRSA in public transport and hospital surface; Bhatta *et al.,* (2018) who identified 36.3% *mecA* positive isolated from frequently touched objects in a tertiary care hospital and the findings of Benrabia *et al.* (2020) who identified 30% MRSA in poultry farm.

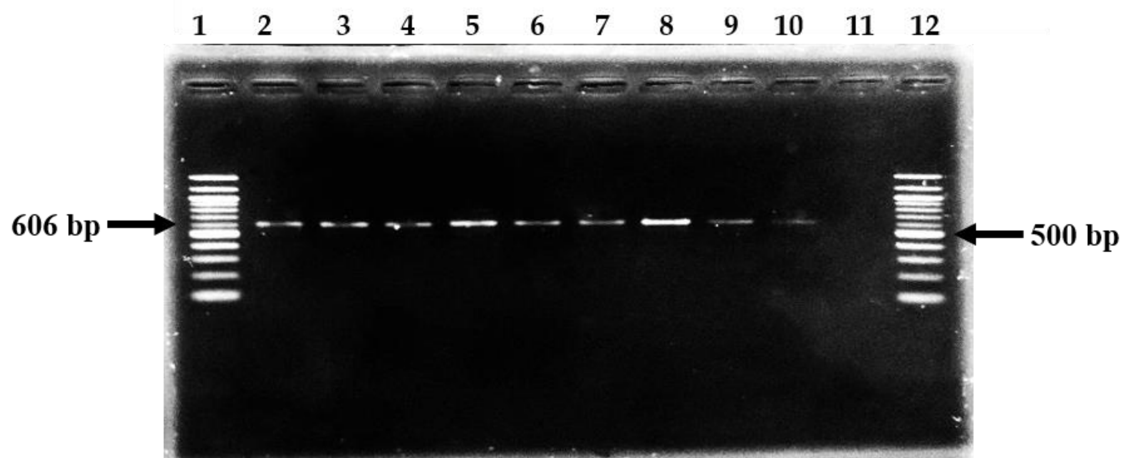

Figure 9. Result of amplification of *stx*-1 gene of *E. coli* isolates by PCR. Lane 1 and 12 = 100 bp size DNA marker; lane 1 = Negative control; lane 5 = Positive control; and lane 2–4 and 6–9 = representative *stx*-1positive *E. coli* isolates.

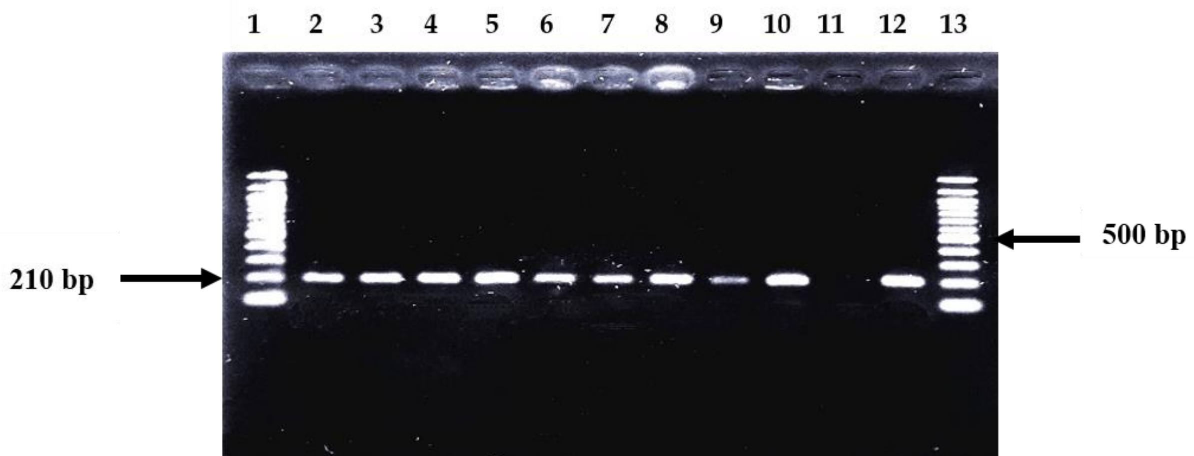

Figure 10. Results of amplification of *tet*Agene of *E. coli* isolates by PCR. Lane 1 and 13: 100 bp size DNA; lane 12 = Positive control; lane 11 = Negative control without DNA; and lane 2-9 = *tet*A gene positive *E. coli* isolates.

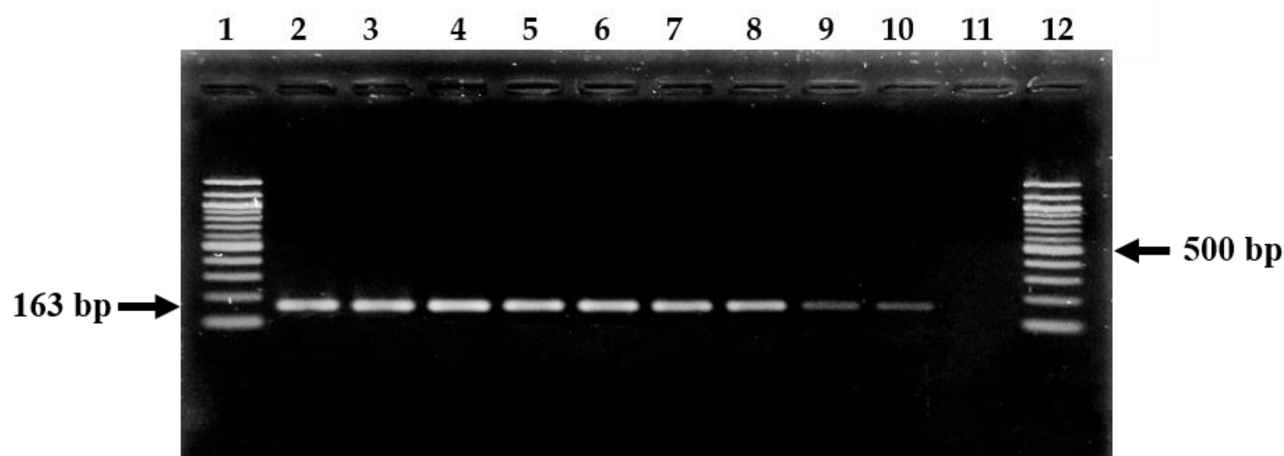

Figure 11. Results of amplification of *mec*A gene of methicillin resistant *S. aureus*. Amplicon size = 163 bp; lane 1 and 12 = 100 bp size DNA marker; lane 1 = Positive control; lane 11 = Negative control without DNA; and lane 2−10 = represents *mec*A gene positive *S. aureus* isolates.

Prevalence of MRSA Among the Staphylococcus aureus Isolates

The overall prevalence of MRSA was 37.70%. The highest prevalence (57.14%) was found in the toilet of Trishal Bus Station. The lowest prevalence (0%) was found in public bus at Trishal Bus Station. The comprehensive prevalence of MRSA was concisely outlined in Table 4.

Table 4. Summary of prevalence of MRSA among the *Staphylococcus aureus* isolates

Name of sample area	No. of *S. aureus* positive isolates	MRSA positive	Prevalence (%)
Public bus from Bridgemor Bus Station	4	2	50.00
Public bus from Maskanda Bus Station	4	2	50.00
Public bus from Trishal Bus Station	1	0	0
Toilet of Bridgemor Bus Station	8	3	37.5
Toilet of Maskanda Bus Station	7	2	28.57
Toilet of Trishal Bus Station	7	4	57.14
Medicine patient ward of MMCH	3	1	33.33
Surgery patient ward of MMCH	4	1	25.00
Gynecology patient ward of MMCH	3	1	33.33
Toilet of medicine ward	8	3	37.5
Toilet of Surgery ward	6	2	33.33
Toilet of Gynecology ward	6	2	33.33
Total no. of *S. aureus* positive isolates	**61**	**23**	**37.70**

Conclusions

The results of the current study indicate that 76.67%, 80%, and 68% of the 90 samples tested positive for *E. coli*, *Klebsiella* spp. and *Staphylococcus aureus*, respectively. *E. coli* isolates were completely resistant to amoxicillin, azithromycin, tetracycline, and co-trimoxazole. Isolates of *Klebsiella* spp. were completely resistant to amoxicillin, while *Staphylococcus aureus* isolates were completely resistant to methicillin, cefoxitin, and cefixime. Ninteen and forty *E. coli* isolates were identified to be positive for the *stx-1* and *tetA* genes, respectively. Twenty-three MRSA were found in *Staphylococcus aureus* isolates. However, the findings of our study provide crucial information about the presence of specific pathogenic bacterial strains in public settings, as well as their antibiotic resistance profile. More importantly, this discovery will raise public awareness about bacterial pollution and antibiotic resistance in public settings, as well as encourage the implementation of appropriate hygienic measures.

Acknowledgments

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Declaration

The authors declare no conflicts of interest.

Authors' contributions

MSI was in responsible for conceptualization, funding acquisition, and project administration; SB was involved in sample collection; MNA, SSR, and SA were in charge of the experiment, data curation, formal analysis, methodology, and writing the original draft; JH, MK, and MSI were involved in methodology validation, visualization, supervision, as well as writing, reviewing and editing the manuscript.

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