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Bacterial Contamination, Antimicrobial Susceptibility Patterns, and Drug Resistance on Frequently Touched Surfaces in Public Transport Vehicles (Hiaces) in Northwestern Tanzania

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Abstract

This study, conducted from March to May 2025 in Mwanza, north-western Tanzania, assessed bacterial contamination, antimicrobial susceptibility, and drug resistance on frequently touched surfaces in public transport vehicles (hiaces). A cross-sectional study of 50 hiaces yielded 385 swab samples from surfaces like seat backs, handrails, and door handles, with data collected via driver questionnaires and analyzed microbiologically within two hours. Pathogenic bacteria were isolated from 266 samples (69.1%), predominantly coagulase-negative Staphylococci (26.7%) and Staphylococcus aureus (24.8%), along with E. coli (14.6%), Enterococcus (8.6%), Klebsiella (8.0%), Salmonella and Shigella (6.1% each), and Pseudomonas aeruginosa (5.1%). S. aureus showed high resistance to ampicillin (94.9%) and clindamycin (42.3%), while coagulase-negative Staphylococci exhibited greater resistance to oxacillin (67.9%) and trimethoprim-sulfamethoxazole (82.0%). Among Gram-negative bacteria, E. coli was notably resistant to ampicillin (91.3%) and ceftriaxone (63.0%), while Klebsiella species demonstrated 100% resistance to ampicillin and substantial resistance to other antibiotics. Multidrug resistance (MDR) was observed in 43.6% of isolates, especially in 67.2% of Gram-negative bacteria, with Klebsiella and E. coli as the most frequent MDR pathogens. Methicillin-resistant S. aureus (MRSA) accounted for 37.2% of S. aureus isolates and extended-spectrum β-lactamase (ESBL) producers were found in 31% of Gram-negative isolates. Significant contamination factors included surface type, uncleanliness, and sampling time. The findings reveal high bacterial contamination and antibiotic resistance in public transport, emphasizing the urgent need for enhanced hygiene and regular sanitation to reduce infection risks.

Keywords: *Antimicrobial Susceptibility Patterns, Bacterial Contamination, Public Transportation Hygiene, Public Transport Vehicle.*

Introduction

Public vehicles are necessary for urban and rural transit, allowing people and products to travel more easily. However, because of their frequent passenger turnover and lax sanitary regulations, these cars also act as hotspots for spreading infectious germs [1].

Studies have indicated that public transportation systems' handrails, seats, and door handles can host a range of germs, including bacteria that could endanger users' health. Studies conducted worldwide suggested

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harmful bacteria, such as *Escherichia coli* and *Staphylococcus aureus*, are frequently detected on high-contact surfaces in public transportation systems, which may result in infectious illness outbreaks [2]. The COVID-19 pandemic has further highlighted these risks, emphasizing the need for effective sanitation measures. Various studies have demonstrated that surfaces in transit vehicles can harbor bacterial loads ranging from 12 to 10^8 colony-forming units (CFU) per cm^2, indicating a serious public health concern [1]. Frequent contact with these surfaces raises the risk of microbiological transmission, particularly in buses and minivans, which are gathering places for people from various backgrounds [3].

In Africa, particularly in urban areas with expanding transportation networks, bacterial contamination is exacerbated by inadequate cleaning protocols and high population density. Research conducted in Mekelle, Ethiopia, identified significant levels of pathogenic bacteria like *E.coli* and *methicillin-resistant S. aureus* (MRSA) on bus surfaces, illustrating the potential for disease transmission among commuters [4]. Similarly, a study in Ghana's Tamale metropolis found that taxis and motorized tricycles were heavily contaminated with pathogens, further underscoring the critical need for improved hygiene practices in public transport [5]. These findings reflect a regional pattern where rapid urbanization and inadequate sanitation measures contribute to heightened risks of infectious diseases. Moreover, studies have shown that environmental factors such as temperature and humidity significantly influence the microbial burden in public transport vehicles, which can affect bacterial survival and proliferation [6].

The differences in the microbial flora on different surfaces further highlight the importance of targeted cleaning methods. Some research indicates that certain types of bacteria are more likely to survive on metal and plastic surfaces than on fabrics [7]. This implies that sanitation plans should be customized for the materials found in public transportation. This highlights how crucial it is to use appropriate disinfectants in addition to routine cleaning that is effective against a wide range of pathogens. Regular evaluation of the bacterial load on these surfaces may be employed as a prophylactic measure to identify contaminated areas and expedite the implementation of cleaning protocols.

Cultural norms and attitudes toward hygiene make keeping Tanzanian public transportation clean more difficult. Depending on socioeconomic position and regional customs, there may be differences in the acceptance and use of hygienic measures [8]. Modifying public health messaging to be interesting and relevant to a certain culture may increase the effectiveness of hygiene campaigns. One way to ensure improved compliance could be to use community leaders or influencers to encourage hygienic behaviors.

Studies have identified several common bacteria isolated from frequently touched surfaces in various settings. Globally, *Staphylococcus aureus* is commonly reported as a prevalent pathogen, with a pooled prevalence of approximately 34.34% in diabetic foot ulcer cases in sub-Saharan Africa, followed by *Escherichia coli* and *Pseudomonas aeruginosa* at 21.16% and 20.98%, respectively [9]. In Tanzania specifically, the prevalence of bacterial pathogens such as *E. coli* is notably high; it accounts for 39.1% of urinary tract infections among children [10]. Other studies across sub-Saharan countries have reported similar findings, with *Klebsiella species*, *Salmonella*, and *Shigella* being common isolates [11]. The current situation in Tanzania reflects these trends, with significant antimicrobial resistance observed among these bacteria, posing challenges to effective treatment and control measures [12]. The urgent need for comprehensive surveillance and intervention strategies is evident to mitigate the risks

associated with bacterial contamination in public transport systems [13].

This study aims to address the gaps in current knowledge concerning the occurrence of bacterial contamination and patterns of antimicrobial resistance on commonly touched surfaces in hiaces in northwestern Tanzania.

Materials and Methods

Study Design

The study design was a cross-sectional design. The cross-sectional design enabled data collection at a single point in time while allowing comparisons among different locations and specific conditions that influenced contamination levels [14].

Study Area

The study was conducted in Magu District, Nyamagana City, and Ilemela Municipal, which were the key administrative areas within the Mwanza Region of Tanzania, each with distinct demographic and climatic characteristics. As of 2022, Magu District has a population of approximately 421,119, reflecting its growth and development since the last census. Located to the northwest of Mwanza City, it is bordered by Lake Victoria to the north and shares its western boundary with Ilemela Municipal. Nyamagana City, which includes parts of Mwanza, has a population of around 244,000, while Ilemela Municipal is home to about 200,000 residents. The climate in these areas is characterized by a bimodal rainfall pattern, with annual precipitation ranging from 700 mm to 1,000 mm. The weather typically features two main seasons: the short rains from October to December, where temperatures range from 18°C to 30°C, with October averaging highs of 28°C and November around 27°C; and the heavier rains from March to May, with temperatures averaging between 19°C and 29°C, peaking at about 30°C in March. This temperature variability significantly influences bacterial contamination on surfaces in these regions. This region was selected due to its high population density, reliance on public transportation, and limited studies on microbial contamination in these settings.

Sample Selection

A total of 50 hiaces vehicles were systematically selected from various routes within Mwanza city to ensure diversity in the sampling process. The sampling was conducted at three different times of day: morning (7 AM - 10 AM), afternoon (12 PM - 2 PM), and evening (5 PM - 7 PM) to account for peak and off-peak hours of operation, providing the complete picture of potential contamination rates throughout the day.

Sample Size Calculation

To calculate the sample size for a study, use a formula commonly applied in epidemiological studies. This formula typically considers the expected prevalence of bacterial contamination, the desired confidence level, and the margin of error.

Sample size calculation Steps

1. Define parameters

Confidence level: Typically set at 95%(Z-score=1.96)

Estimated prevalence(p): If no prior data exists, assume p=0.5 for maximum variability.

Margin of error: commonly set at 5% (0.05).3.8416.0.25/0.0025

2. Use the sample size formula for proportions

The formula to estimate the sample size (n) is in below as equation (1)

3. Substituting values

Plugging in the values:

$$n = \frac{(1.96)^2 \cdot 0.5 \cdot (1 - 0.5)}{(0.05)^2}$$

$$= \frac{3.8416 \cdot 0.25}{0.0025}$$

$$=384.16.$$

Thus, the estimated sample size of 385 was needed.

Sample Collection

Surface samples were collected from handrails, seat backs, and door handles. Each sample site was swabbed consistently: swabbing in a back-and-forth motion over an area of approximately 100 cm^2 to ensure adequate collection of microbial contaminants. A special emphasis was placed on the standardization of the swabbing technique to minimize variability in sample collection. Sterile swabs pre-moistened with sterile saline solution were utilized for sample collection, following the guidelines provided by the World Health Organization (WHO) for environmental sampling in public health assessments [15]. All collected samples were put in Stuart transport media and then meticulously labeled with vehicle identification numbers, surface type, and collection time, and they were delivered to the testing laboratory (Sekoutoure Regional Referral Hospital laboratory) within two hours while being kept in a cool box. Immediate inoculation of samples was done on arrival at the laboratory. If the delay was inevitable, specimens were refrigerated at 4°C until time for processing.

Culture and Identification

Swabs collected from distinct surfaces (handrails, seat backs, and door handles) were inoculated on BA and MCA. Culture plates were incubated under aerobic conditions at 37 °C for 24 hours. Conventional methods included Gram stain, biochemical testing, and colony characteristics to identify bacterial species by following standard clinical laboratory methods and CLSI guidelines. Lactose utilization, gas production and hydrogen sulfide gas production in Triple Sugar Iron agar (TSI), Citrate utilization, and urease hydrolyzed, The SIM media was used to test the ability of an organism to liberate hydrogen sulfide (H_2s) from Sulphur-bearing amino acids producing a visible, black color, motility and to split indole from the tryptophan molecule after the additional of Kovacs reagent was used in the identification of gram-negative bacteria. Gram reaction, hemolytic pattern, Catalase and Coagulase tests, mannitol salt agar, Novobiocin test, and Bile esculin agar were used to identify gram-positive bacteria.

Antimicrobial Susceptibility Test

Isolated bacterial strains were tested antibiotic susceptibility testing using the modified Kirby-Bauer disk diffusion method. The three to five selected pure colonies were taken and transferred into the tube containing 5ml of sterile normal saline and mixed gently to form the homogeneous suspension until the turbidity of the suspension becomes adjusted to 0.5 McFarland standards. Then, using sterile cotton-tipped swabs, the bacteria was distributed evenly over the entire surface of Mueller-Hinton agar (MHA). The inoculated plates were left at room temperature for 15 minutes and then using sterile forceps the set of antibiotic discs were placed on the inoculated MHA plates. Antimicrobials were selected according to Clinical Laboratory Standard Institute guideline [16] and these antibiotic discs for gram-negative bacteria were Ciprofloxacin (5µg), Gentamicin (10µg), Ceftazidime (30µg), Imipenem (10µg), Meropenem (30µg), Amikacin (30µg), Ampicillin (10µg), Amoxicillin/clavulanic(20/10µg), Trimethoprim/Sulfamethoxazole (1.25/23.75), Cefotaxime (30µg), Chloramphenicol (30µg), and Ceftriaxone (30µg), and for the gram positive bacteria were Ciprofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Erythromycin (15µg), Oxacillin (1µg), Trimethoprim/Sulfamethoxazole (1.25/23.75), Chloramphenicol (30µg),), Cefoxitin (30µg), and clindamycin (2µg). After placing these antibiotic discs, the plates were allowed to stand for another 15 minutes at room temperature to dissolve the antibiotics in the media. The plates were then incubated at 370C for 16 to 18h. Finally, zones of inhibition were measured using a ruler and interpreted according to CLSI

2024 guidelines. Bacterial isolates resistant to three or more antibiotics was categorized as being multi-drug resistant (MDR), and the ESBL and MRSA were also tested.

Extended-Spectrum Beta-Lactamase (ESBL) Detection

Antimicrobial susceptibility test on Muller-Hinton media was used for ESBL initial screening, whereby the diameter of the zone of inhibition produced by either of three antimicrobials: cefotaxime (30µg), ceftriaxone (30µg), or ceftazidime (30µg) was measured. The following cut-off points indicated ESBL production: for ceftriaxone ≤25mm, ceftazidime ≤22mm, and cefotaxime ≤27mm. Confirmation of ESBL production was done using the Combined Disk (Double Disk Potentiate) test following the CLSI guideline [16]. A similar procedure for Antimicrobial Susceptibility Testing was used, whereby isolated bacteria were streaked onto the Mueller-Hinton agar plate. Ceftazidime (30µg), cefotaxime (30µg), and clavulanic acid (30µg/10µg) disks were used. After 24 hours of incubation at 37ºC, an increase in zone of inhibition diameter by ≥5mm in either combination of cephalosporin-clavulanate disk versus the zone diameter of the respective Cephalosporin disk was positive, and the isolate was reported as an ESBL producer [17].

Methicillin-Resistant Staphylococcus Aureus (MRSA) Detection

The detection of Methicillin-resistant Staphylococcus aureus (MRSA) using the conventional method were involved the cefoxitin disk diffusion test which were utilized in clinical laboratories due to their established reliability and adherence to Clinical and Laboratory Standards Institute (CLSI) guidelines [18]. In this method, Mueller-Hinton agar plates were inoculated with a standardized bacterial suspension equivalent to a 0.5 McFarland standard, ensuring a consistent inoculum size, which was critical for the accurate interpretations of results [19]. After applying cefoxitin (30 µg) disks the plates were incubated at 35°C for 24 hours, allowing for the growth of bacteria and interaction with the antibiotics [20]. The effectiveness of the antibiotics were assessed by measuring the diameter of the inhibition zones around the discs; larger zones indicate susceptibility, while smaller or absent zones suggest resistance [18]. According to CLSI guidelines, the zone diameter of less than 21 mm for cefoxitin typically indicates MRSA presence, highlighting the importance of using these specific breakpoints for accurate diagnosis [20].

Quality Assurance

Data quality was checked during and after collection to ensure the completeness of the questionnaires. Media and reagents were checked by verifying the expiration date and other parameters indicated in the guidelines. Sample processing and reagent preparation were followed by Standard Operating Procedures (SOPs) for both control and test. The quality of media was assessed using sterility testing and control organisms. Internal quality was done before testing swab samples using reference bacteria strains kept at Sekou Toure Regional Referral Hospital Laboratory. These are *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (ATCC 700603), *Enterococcus faecalis* (ATCC 29212), and *Pseudomonas aeruginosa* (ATCC 27853). Additionally, E. coli (ATCC 25,922) and *K. pneumoniae* (ATCC 700,603) for ESBL detection.

Data Analysis

The questionnaire used in the study underwent a translation process to ensure consistency with the local language, Swahili. Initially, professional linguists translated the questionnaire from English to Swahili. To validate the accuracy of the translations, the questionnaire was then translated back into

English by a different team of linguists. Before the commencement of data collection for this study. The data from the microbiological analyses were compiled and statistically analyzed using Microsoft Excel. Descriptive statistics were summarized to show the frequency and times of bacterial contamination across various surfaces. To investigate potential correlations between contamination levels and multiple factors (e.g., surface type), the Adjusted Odds Ratio (AOR) was utilized. Statistical significance was set at $p < 0.05$ [21].

Ethical Approval

The study obtained ethical clearance reference no 2025/TOC/BC/0011 from the Texila American University of the Research and Ethics Committee. In addition, permission was obtained for data collection from the Ministry of TAMISEMI and Sekou Toure RRH Medical Officer. Before sample collection, all participants were thoroughly informed about the study's objectives and voluntarily provided written consent. Strict measures were implemented to ensure the confidentiality of participant information, and positive results were communicated to the respective vehicle drivers. Comprehensive information about the study, including its objectives, methodology, potential risks, and benefits, was shared with each driver involved. Participants were informed of their right to decline participation or withdraw at any point during the research process. Furthermore, results were shared with local health authorities and transport operators to promote awareness and advocate for improved sanitation practices in public transportation.

Results

The study involved 50 vehicles and their drivers where by yield to 385 swab samples, achieving a 100% response rate. The mean age of the participants was 33.5 years (SD ± 8.3). All participants were male (100%), with a majority in the age group of between 30-34 years old (40.0%). Samples were collected from door handles, handrails, and seat back surfaces with 26.0%,33.2% and 40.8% respectively were recorded. Buhongwa stand recorded the highest number of vehicles of 23(46.0%) and Airport stand recorded with the least number of 7 (14.0%). In terms of educational background, 76.0% had completed secondary school, while 20.0% held a higher education. Additionally, 157 (40.7%) of the swab samples were collected in the afternoon time followed by evening time of 119 (31.0%) where the vehicles stayed for a long time at the stands (Table 1).

Table 1. Sociodemographic Characteristics of Public Transport Drivers in North-Western Tanzania, from March to May 2025

Variables	Category	Frequently(N)	Percentage (%)
Sex of participants	Male	50	100.0%
	Female	-	0.0%
Age of participants	20-24 years	4	8.0%
	25-29 years	10	20.0%
	30-34 years	20	40.0%
	>35 years	16	32.0%
Vehicle (Hiace) Location	Kisesa stand	20	40.0%
	Buhongwa stand	23	46.0%
	Airport stand	7	14.0%
Educational background	No formal education	-	0.0%
	Primary	2	4.0%
	Secondary	38	76.0%

	Higher education	10	20.0%
Sample location	Door handles	100	26.0%
	Handrails	128	33.2%
	seat backs	157	40.8%
Clean	Regularly cleaned	23	34.0%
	Regularly not cleaned	27	46.0%
Sample collection time	Morning	109	28.3%
	Afternoon	157	40.7%
	Evening	119	31.0%

Prevalence of Bacterial Contamination on Frequently Touched Surfaces

A total of 385 swab samples were collected from which pathogenic bacteria were isolated from 266 (69.1%) swab samples, resulting in an isolation rate of 74.4%. The highest isolation rate was observed on handrails (74.0%), followed by seat backs (69.0%), and door handles (62.0%), as shown below in Table 2, and Figure 1.

This Table 2 summarizes the overall bacterial contamination found on the different surfaces sampled.

Table 2. Prevalence of Bacterial Contamination on Frequently Touched Surfaces

Surface Type	Number of samples	Number of samples positive for bacteria	Percentage of samples positive for bacteria
Door Handles	100	62	62%
Seat backs	157	109	69%
Handrails	128	95	74%
Total	385	266	69.1%

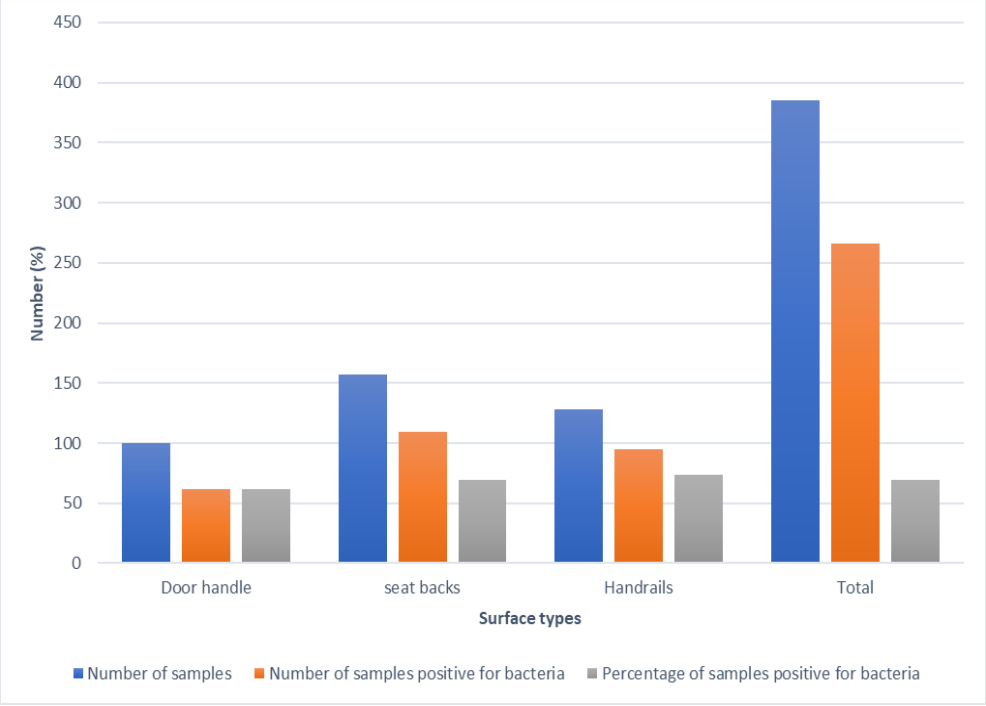

Figure 1. Prevalence of Bacterial Contamination on Frequently Touched Surfaces

Of the 314 bacterial isolates, 189 were Gram-positive, while 125 were Gram-negative bacteria. Among the Gram-positive isolates, *coagulase-negative staphylococci* (CoNS) were the most frequently isolated (n = 84), followed by Staphylococcus aureus (n = 78), and 27 were *enterococcus species*. Of the 125 Gram-negative isolates, 46 were E. coli, 25 were *Klebsiella species*, 19 were *Salmonella species*, 19 were *Shigella species*, and 16 were *Pseudomonas aeruginosa*. The frequencies of pathogens varied considerably between surface types; the bacterial pathogens on bus surfaces included CoNS at 26.7%, S. aureus at 24.8%, Enterococcus species 8.6%, E. coli at 14.6%, *Klebsiella species* at 8.0%, *Salmonella species* at 6.1%, *Shigella species* at 6.1%, and *Pseudomonas aeruginosa* at 5.1%, as shown below in Table 3, and Figure 2.

This table 3 breaks down the types of bacteria identified on each sampled surface.

Table 3. Distribution of Bacterial Isolates by Surface Type

Surface Type	**Staphylococcus aureus**	**Coagulase negative staphylococci (CoNS)**	**Enterococcus species**	**Escherichia coli**	**Klebsiella species**	**Pseudomonas aeruginosa**	**Salmonella species**	**Shigella species.**
Door Handles	15	17	7	14	8	3	5	4
Seat backs	28	39	14	17	9	7	5	8
Handrails	25	28	16	15	8	6	9	7
Total	78	84	27	46	25	16	19	19
% of the total	24.8%	26.7%	8.6%	14.6%	8.0%	5.1%	6.1%	6.1%

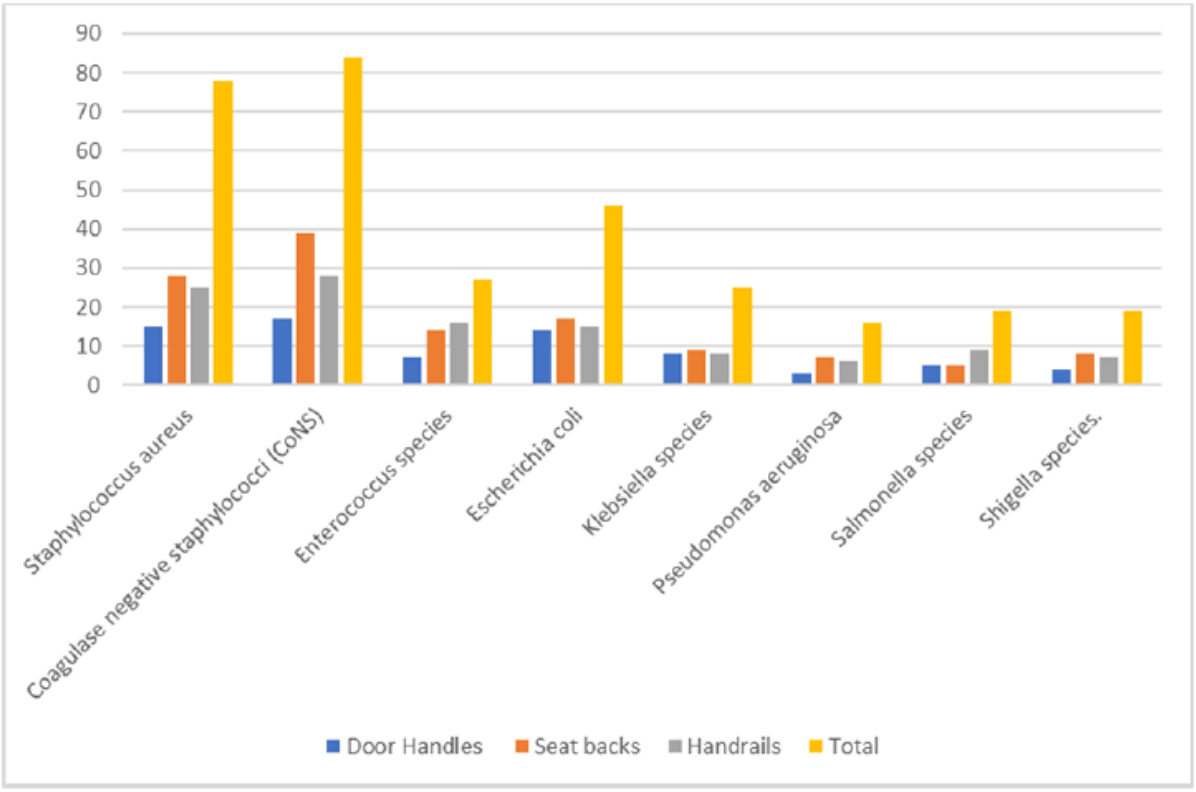

Figure 2. Distribution of Bacterial Isolates by Surface Type

Antimicrobial Susceptibility Test

A total of 314 pathogenic bacterial isolates were tested against different antibiotic disks, revealing higher proportions of resistance rates among Gram-positive bacteria to ampicillin (88.9%) and oxacillin (67.2%). The resistance rate of S. aureus to ampicillin (94.9%) and Clindamycin (42.3%) is greater than that of CoNS, which shows resistance rates of (89.7%) to ampicillin and (41.0%) to Clindamycin. In contrast, CoNS exhibits a higher resistance rate to oxacillin (67.9%), and Trimethoprim-sulfamethoxazole (82.0%) compared to S. aureus (62.8%) to Oxacillin, and (59.0%) to Trimethoprim-sulfamethoxazole.

Escherichia coli was found to be the most resistant Gram-negative bacterium among the investigated antibiotics: ceftriaxone 63.0% (29/46), ampicillin 91.3% (42/46), chloramphenicol 15.2% (7/46), and cefotaxime 23.9% (11/46). Klebsiella species exhibited the highest resistance rates, including ampicillin (100%), ceftriaxone (73.7%), ciprofloxacin (6.6%), cefotaxime (48.0%), and chloramphenicol (24.0%). Salmonella species showed resistance to chloramphenicol at 28.7% (6), 61.9% to ceftriaxone, and 38.1% to cefotaxime. Shigella species exhibited resistance to ceftriaxone (36.8%), cefotaxime (36.8%), and chloramphenicol (21.1%). The resistance rate in Pseudomonas aeruginosa was 25.0% to chloramphenicol, 68.8% to Trimethoprim-sulfamethoxazole, and 43.8% to ceftazidime (Table 4).

This table 4 presents the susceptibility of the most commonly isolated bacteria to various antibiotics. This would typically be presented for each significant bacterial species.

The antibiogram of Gram-positive bacterial isolates (58.8%) did not show resistance to any of the antibiotic classes tested (Oxacillin, Trimethoprim-sulfamethoxazole, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, amikacin, and Clindamycin); however, none of the isolates exhibited resistance to all the antibiotics tested. MDR was observed in 137 out of 314 (43.6%) isolates. Notably, a higher rate of MDR was detected in 84 out of 125 (67.2%) Gram-negative isolates. More frequently, Klebsiella species were multidrug-resistant at 24/137 (17.5%) followed by E. coli at 21/137(15.3%). Overall, among the total isolates (n = 137/314), multidrug resistance, defined as resistance to ≥3 antibiotic classes, is shown in Table 5.

Table 4. Antimicrobial Susceptibility Patterns of Bacterial Isolates

Antibiotic	**Staphylococcus aureus**			**Coagulase negative staphylococci**			**Enterococcus species**			**Escherichia coli**			**Klebsiella species**			**Pseudomonas aeruginosa**			**Salmonella species**			**Shigella species**		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	0	4	74	0	14	70	0	3	24	0	4	42	0	0	25	-	-	-	0	2	17	0	3	16
Cefazolin	-	-	-	-	-	-	-	-	-	1	5	40	0	0	25	-	-	-	2	1	16	0	1	18
Ciprofloxacin	78	0	0	84	4	0	20	7	0	43	3	0	20	3	2	16	0	0	19	0	0	14	5	0
Gentamycin	78	0	0	84	0	0	27	0	0	46	0	0	23	2	0	16	0	0	19	0	0	19	0	0
Oxacillin	20	9	49	17	14	53	0	2	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Erythromycin	25	9	44	22	19	43	0	8	19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Clindamycin	30	15	33	28	24	32	10	5	12		-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ceftriaxone	-	-	-	-	-	-	-	-	-	5	12	29	18	5	2	8	3	10	4	1	14	10	2	7
Amox/clav	-	-	-	-	-	-	-	-	-	3	7	36	15	6	4	-	-	-	7	5	7	9	5	5
Chloramphenicol	47	6	25	80	4	0	24	2	3	35	4	7	19	0	6	10	2	4	17	0	2	15	0	4
Imipenem		-	-	-	-	-	-	-	-	40	2	3	15	7	3	8	7	1	15	3	1	12	5	2
Cefotaxime	-	-	-	-	-	-	-	-	-	24	10	11	8	5	12	14	3	8	5	3	11	10	2	7
Meropenem	-	-	-	-	-	-	-	-	-	46	0	0	25	0	0	14	2	0	19	0	0	19	0	0
Ceftazidime	-	-	-	-	-	-	-	-	-	20	8	18	9	5	11	5	4	7	5	7	7	3	9	7
Amikacin	78	0	0	84	0	0	27	0	0	46	0	0	25	0	0	16	0	0	19	0	0	19	0	0
Trimethoprim-sulfamethoxazole	7	15	46	4	18	64	3	9	15	17	20	9	10	4	11	5	4	11	4	7	8	2	7	10

Table 5. Patterns of MDR (resistance to three or more classes of antibiotics) bacteria isolated on public transport vehicle surfaces

Antimicrobial agent	**N(%)**	**Staphylococcus aureus**	**Coagulase negative staphylococci**	**Enterococcus species**	**Escherichia coli**	**Klebsiella species**	**Pseudomonas aeruginosa**	**Salmonella species**	**Shigella species**
AMP, C, TS	48	18	10	5	3	4	0	5	3
AMP, C, CIP	25	7	5	2	2	4	0	3	2
AMP, CIP, TS, C	23	6	2	3	4	3	0	3	2
AMP, KEZ, IM	12	0	0	0	3	4	0	3	2
AMP, CXT, C, AMC	14	0	0	0	5	4	0	3	2
TS, CXT, CAZ	15	0	0	0	4	5	7	3	1
MDR, n (%)	137/314(43.6%)	31(22.6%)	17(12.4%)	10(7.3%)	21(15.3%)	24(17.5%)	7(5.1%)	20(14.6%)	12(8.8%)

Keynote: *AMP-ampicillin, TS-trimethoprim-sulfamethoxazole, CIP-ciprofloxacin, IM-Imipenem, CAZ-ceftazidime, AMC-amoxicillin clavulanic acid, C-chloramphenicol, KEZ Cefazolin, CXT-Cefotaxime.*

The MRSA was 29/78 (37.2%) recorded in a total of 78 Staphylococcus aureus isolates, shown in Table 6.

Table 6. Distribution of methicillin-resistant Staphylococcus aureus (MRSA) among Staphylococcus aureus isolated on surfaces

Type of Staphylococcus aureus strains	Number of isolates	Percentage (%)
MRSA	29	37.2%
MSSA	49	62.2%

The overall Gram-negative bacteria, ESBL bacteria, 31.0% were recorded, where Escherichia coli was at 21.1% higher than Klebsiella species at 9.9% as shown in Table 7.

Table 7. Distribution of ESBL bacteria among Enterobacteriaceae isolated on surfaces

Type of Enterobacteriaceae	N of ESBL bacteria	Percentage (%) ESBL bacteria
Escherichia coli	15	21.1%
Klebsiella species	7	9.9%
ESBL bacteria N(%)	22	31.0%

An Adjusted Odds Ratio (AOR) Analysis

AOR analysis was conducted with door handles as the reference category. Among 100 door handle samples, 62 were positive for bacterial contamination. For seat backs, 109 out of 157 samples tested positive, and for handrails, 95 of 128 samples were positive. Calculating the odds of contamination, seat backs showed an increased odds of bacterial contamination compared to door handles, with an AOR of approximately 1.39, indicating a 39% higher likelihood of contamination (not statistically significant). Handrails exhibited an even higher adjusted odds ratio of about 1.76, suggesting they are 76% more likely to be contaminated than door handles (statistically significant). These findings highlight that surface type is correlated with bacterial contamination risk, with handrails posing the greatest risk among the three surfaces studied (Table 8).

Table 8. Statistical analysis of correlation between Surface Type and Bacterial Contamination

Surface type	Number of Samples	Positive Samples	Odds of Positivity	Adjusted Odds Ratio (AOR)	95% CI	p-value	Interpretation
Door Handles	100	62	62/38=1.63	1(Reference)	NA	NA	Baseline
Seat backs	157	109	109/48=2.27	1.39	[1.002, 1.133]	0.049	39% higher odds than door handles (not statistically significant)
Handrails	128	95	95/33=2.88	1.76	[-0.197, 0.858]	0.219	76% higher odds than door handles (statistically significant)

Discussion

This study examines the prevalence of pathogenic bacterial contamination in public transportation and its implications for public health. The current results reveal that 69.1% of public transport vehicle surfaces harbor pathogenic bacteria. However, this rate is higher than findings from Nigeria (67%) (Otu et al., 2020). This discrepancy may be due to various factors, such as geographical location, cleaning protocols, and public sanitation awareness (Ly et al., 2024). Consistent with findings from India (80%) (33), this rate is lower than the results reported from Bangladesh (100%) [22], possibly due to differences in cleaning protocols and public sanitation awareness. Contamination levels varied significantly across different surfaces. Contamination was significantly higher on handrails and seats compared to door handles, with bacterial prevalence reaching 74.0%, 69.0%, and 62.0%, respectively. Regularly not cleaned vehicles had prevalence of 54% and 46% for regularly cleaned were recorded.

The predominant bacterial isolates in this study were *coagulase negative staphylococci* (CoNS; 26.8%), likely reflecting its preponderance on normal skin [23]. Consequently, *Staphylococcus aureus* (24.8%), *Enterococcus species* (8.6%), *Klebsiella species* (8.0%), *Escherichia coli* (14.6%), *Pseudomonas aeruginosa* (5.1%), *Salmonella spp*. (6.1%), and *Shigella spp*. (6.1%) were isolated.

These findings align with earlier research in Ethiopia [23]. CoNS (with a prevalence of 26.8%), was frequently isolated from transport surfaces, similar to findings in Ethiopia (40.3%) [24]. *S. aureus*, identified in 24.8% of samples, showed isolation rates consistent with studies from Kenya (33%). This bacterium is known for colonizing mucous membranes in humans [25]. However, this rate was higher than that reported from Makelle city, Ethiopia (18.0%) [6]. The most common gram-negative isolates, *Escherichia coli* (14.6%) and *Klebsiella species* (8.0%) were also significant contaminants linked to poor hygiene practices among passengers and drivers, as observed in studies from Kenya (24%) (Karami et al., 2019). *Pseudomonas aeruginosa*(5.1%), *Salmonella spp*. (6.1%), and *Shigella species* (6.1%) were other notable isolates, consistent with global studies, but varying in prevalence due to environmental factors and sanitation standards, as the study revealed in Malawi [11].

Antimicrobial resistance profiles revealed high resistance to ampicillin (87.8%) and oxacillin (67.1%) among Gram-positive bacteria. These results align with the study (Bhatta et al., 2018), indicating that bacterial resistance is an evolving phenomenon arising from genetic mutations and/or acquired genome. Gentamicin and Amikacin remained the most effective treatment, showing 99.4% efficacy and aligning with studies conducted in Tanzania [26]. However, multidrug resistance was observed in 43.6% of isolates, emphasizing the need for stricter antibiotic usage protocols and hygiene practices in public transport environments. Gram-negative bacteria also exhibited resistance rates, with 28.1% showing resistance to at least one class of antibiotics, including *Escherichia coli* and *Klebsiella* species. This demonstrates significant resistance and underscores the challenges of treating infections linked to contaminated transport surfaces. The high prevalence of multidrug-resistant organisms indicates that the irrational use of antibiotics and inadequate infection control measures are contributing factors.

In this study, the overall prevalence of ESBL-producing bacteria was 31.0%, comparatively lower than the 34% reported in a previous systematic review of a pooled prevalence study conducted in the same region in community settings [8]. The prevalence of MRSA in this study is 37.2 % higher than in previous studies conducted in India, which reported 24.7% among *S. aureus* from samples collected from vehicles that tested positive for

MRSA, underscoring a significant risk of transmission in communal environments where hygiene practices may be inadequate, further complicating public health efforts [25].

The study has possible weaknesses. It was limited to aerobic bacteria, thus excluding anaerobic and non-cultivable pathogenic bacteria. Additionally, observational bias may have occurred during cleaning practices.

Equation Formulas Used

1. **For calculating sample size**

$$n = (Z^2.P(1+P))/\mathrm{E}^2 \qquad (1)$$

Whereby

n: Number of samples size

Z: a statistical measure that indicates how many standard deviations a data point is away from the mean of a distribution

P: Estimated prevalence

E: Margin of error

2. **For calculating the Odds ratio (OR)**

$$OR = \frac{a/b}{c/d} \qquad (2)$$

Whereby

a: represents the number of individuals who are both exposed and have the outcome.

b: represents the number of individuals who are both exposed and do not have the outcome.

c: represents the number of individuals who are not exposed and have the outcome.

d: represents the number of individuals who are not exposed and do not have the outcome

3. **For calculating prevalence**

$$P\% = \frac{C}{N} X\ 100 \qquad (3)$$

Whereby

P = prevalence,

C = number of existing cases of the outcome,

N = total population at risk.

Conclusion

This study revealed that public transport in north-western Tanzania could be a means of transmission for pathogenic bacteria. Thus, public transport vehicles (hiaces) especially should be used with care to avoid the possible contraction of diseases. Routine cleaning and disinfection of vehicles alongside the encouragement of the practice of personal hygiene is necessary to keep a safer and healthier urban population and environment. The Regional health authorities, health professionals, transport authorities, and other stakeholders should collaborate to improve awareness among drivers, vehicle owners, and the community about the potential health risks of infectious diseases related to public transport. Environmental health should collaborate with transport authorities to ensure regular cleaning inspections and improved control measures.

Data Availability

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

CSD: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. BM: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing– original draft, Writing – review & editing. TS: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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