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Prevalence and Antimicrobial Susceptibility Status of Gram-Negative and Gram-Positive Bacteria on Handheld Shopping Trolleys and Baskets in Supermarkets in Ndola, Zambia

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Abstract

Background: Supermarkets are a place visited by individuals with different health conditions daily where microbiological contaminants through touch onto fomites such as trolleys and baskets can be passed on to other people hence potentially spreading infectious diseases. This study aimed to investigate the presence of Gram-negative and Gram-positive bacteria on handheld shopping trolleys and baskets and their antimicrobial susceptibility status against commonly used antibiotics in Zambia. **Methods:** A cross-sectional study was conducted. Trolleys and basket handles were swabbed and standard microbiological methods were used to identify the bacteria and disc diffusion to determine their antimicrobial susceptibility status. Data was collected from December 2021 to April 2022. Data was analysed using IBM Statistical Package for Social Sciences (SPSS) Version 22. **Results:** Twenty-eight percent of the 200 total samples were found to be culture-positive and predominant isolates were *Staphylococcus aureus* (17.3%), *Pseudomonas* species (4.5%), *Escherichia coli* (2%), *Corynebacterium* species (2%), *Staphylococcus* species

(1.5%) and *Enterobacter aerogenes* (0.5%). *Staphylococcus aureus* showed the most resistance to azithromycin (17%) followed by ciprofloxacin (2.8%), nitrofurantoin (2.8%) and chloramphenicol (2.8%). *Escherichia coli* showed 100% resistance to amoxicillin, cloxacillin and ampicillin, 75% resistance to ciprofloxacin and the least resistance to azithromycin (25%) while it was susceptible to nitrofurantoin. *Staphylococcus* species, *Corynebacterium* species, *Enterobacter aerogenes* and *Pseudomonas* species showed no resistance to any antibiotics. **Conclusion:** The study showed the presence of microorganisms with considerable antimicrobial resistance to antibiotics in Zambia on trolley and basket handles indicating the need for more initiatives to address proper hygiene in public environmental sites for better infection prevention and control.

Keywords

Antimicrobial Resistance, Coliform Bacteria, *Staphylococcus aureus, Escherichia coli*, Supermarket, Shopping Trolleys and Baskets

1. Background

Antimicrobial resistance (AMR) is a global threat that is increasing at an alarming rate due to the rapid emergence of antibiotic-resistant bacteria (ARB) and multi-drug resistant (MDR) bacteria. This may be due to the nearly 40% increase in global antibiotic consumption between the years 2000 to 2010 [1]. Initial research in the United Kingdom predicted that a continuous rise in AMR by the year 2050 would lead to 10 million deaths annually and a reduction in Gross Domestic Product (GDP) from 3.5% to 2% and hence AMR may cost the global medical healthcare up to 100 trillion USD if left unchecked indicating that it is a significant public health and economic concern [2].

During an outbreak of cholera in 2016 in Zambia, it was identified that several antibiotics were being misused and several people lacked knowledge of their use as a result of misleading information [3]. These antibiotics include tetracyclines, sulphonamides, trimethoprim, ciprofloxacin, gentamicin and ampicillin. According to the Zambia National Public Health Institute (ZNPHI) report, the University Teaching Hospitals, Lusaka has also identified various multi-drug resistant (MDR) pathogens and superbugs isolated from hospital settings including the Gram-Positive Methicillin-Resistant *Staphylococcus aureus* (MRSA) [4]. MRSA strains are known to cause food poisoning and pose a potential health risk when proper hygiene and sanitation fail [5]. Gram-negative bacteria such as *Escherichia*, *Enterobacter* and *Klebsiella* species are known to cause some of the common infections in Zambia such as urinary tract infections, gastroenteritis, pneumonia and food poisoning [6].

Infection control measures have been widely established in hospital settings to reduce microbial transmission but fewer strategies have been thought of regarding public environmental sites [7]. Previous surveillance findings showed that

commonly used or touched areas such as the washrooms and automatic teller machines (ATMs) in public environmental sites were potential sources of the rapid spread of infection involving bacteria [8] [9]. Previous studies done on shopping carts also identified that some bacterial isolates were antibiotic-resistant [6]. This shows that the prevalence of drug-resistant bacteria is not only commonly found within hospitals but can also be present in other public environmental sites. This statement coincides with a systemic review that reported that many pathogens can survive on inanimate objects such as shopping trolleys and baskets for several months-leading to infections as a result of direct or indirect transmission [10]. Contact with handheld shopping trolleys and baskets is made either directly by surface-to-mouth interaction or indirectly by having contaminated fingers followed by hand-to-mouth interaction [6].

Public places such as shopping malls, therefore, provide a suitable environment for the growth of bacteria as they are protected from direct sunlight exposure, dry air and unstable weather conditions. Individuals also visit shopping malls with different health states. Someone infected may not be aware that they are carriers and may unknowingly contribute to spreading pathogenic bacteria in public areas [11]. Accidental contamination might also occur with raw food products such as meat, fish and chicken contact directly [12]. Previous users and staff that handle these fomites can also contribute to contamination. Lack of disinfection of these fomites can also lead to the persistence of colonized microorganisms. Gram-positive bacteria, such as *Staphylococcus aureus* including MRSA, *Streptococcus pyogenes,* or *Enterococci* species survive for a long period on dry surfaces [10]. This is of importance as breastfeeding mothers and people with chronic diseases can contract these organisms easily from shopping carts [13]. Hence, proper hand washing techniques, as well as disinfection, are essential to prevent the transfer of these microorganisms.

Supermarkets are places frequently visited by people who are unaware of harmful pathogens present on commonly used fomites such as shopping trolleys and basket handles that can potentially contaminate and spread infectious diseases. A study done in Japan by Mizumachi *et al. [5]* reported fifty-two strains of *Staphylococcus aureus* isolated from shopping basket handles whereas Alqumber [14] found a prevalence of 0.75% of *Clostridium difficile* on shopping trolleys and baskets in Saudi Arabia that were resistant to commonly used antibiotics. The presence of pathogenic microbes on shopping trolleys and baskets in the findings of these common studies indicates that the public, as well as staff of supermarkets, are constantly exposed to harmful bacteria daily.

While the presence of microorganisms on these fomites has been widely studied in the Asian, European and American regions, there is a lack of knowledge about the status of microbial contamination in supermarkets and their AMR status in Zambia and hence the need to fill the knowledge gap and to further come up with efficient hygiene techniques. Hence this study reports the prevalence and antimicrobial susceptibility status of bacteria isolates from handheld shopping trolleys and baskets in supermarkets in Ndola.

2. Materials and Methods

2.1. Study Site

The study was conducted in Ndola, a city in the Copperbelt province of Zambia. According to Central Statistical Office of Zambia, Ndola has a human population of 455,194.00 and is geographically located within; 12.9906˚S, 28.6498˚E coordinates [15]. The samples from handheld shopping trolleys and baskets were collected from the four most visited supermarkets (Mall A, B, C and D). Thereafter, samples were transported to the Tropical Disease Research Centre (TDRC) for analysis.

2.2. Study Design

A cross-sectional quantitative study design was conducted from December 2021 to April 2022.

2.3. Study Population

The target population of the study included shopping trolleys and baskets in four selected supermarkets in Ndola.

2.4. Sample Size Determination

The following assumptions were made to calculate the sample size; a prevalence of 14% obtained from a previous study [16], a confidence level of 95% and a marginal error of 5%. A minimum sample size of 186 was required and a total of 200 samples were collected (100 shopping trolleys and 100 baskets).

2.5. Sampling Technique

A Multistage sampling technique was adopted. Stratified random sampling was applied to divide the sampling sites (supermarkets) into five (strata), from which swab samples were taken from 25 random shopping trolleys and 25 random baskets in each supermarket. Systematic random sampling was done where every 2nd shopping trolley and basket was selected. One swab sample was taken per trolley and basket.

2.6. Sample Collection

A sterile cotton swab (HiMedia, Mumbai, India) was used to swab surfaces of interest *i.e.* shopping trolleys and basket handles to accumulate bacteria present in each location. The swabs were then immediately placed in collection tubes containing Amies transport medium (HiMedia, Mumbai, India), and then transported in a cooler box to the TDRC laboratory within 24 hours. The samples were then immediately analysed.

2.7. Inclusion and Exclusion Criteria

All shopping trolleys and baskets that were in working condition in the supermarkets were included in the study. Shopping trolleys and baskets that were not

in working condition in the supermarkets were excluded from the study.

2.8. Laboratory Analysis

The analysis was done at the Tropical Disease Research Centre (TDRC) laboratory which is a national health research institution accredited by the Southern African Development Community Accreditation Scheme (SADCAS).

The samples were cultured onto Columbia Blood Agar (Mast Group Ltd, Bootle, United Kingdom) and MacConkey Agar (Mast Group Ltd, Bootle, United Kingdom) plates for Gram-positive and Gram-negative bacteria respectively and incubated for 16 - 24 hours.

The media was prepared in-house following the manufacturer's instructions and the laboratory's standard operating procedure (SOP). Quality controls (QC) were performed on each prepared batch that is sterility test and checking of the quality of the media where standard QC organisms were inoculated. MacConkey agar was inoculated with *Escherichia coli* American type culture collection (ATCC) 25922 and *Proteus mirabilis* ATCC 12453. Blood agar was inoculated with *Staphylococcus aureus* ATCC 25923 and *Streptococcus pneumoniae* ATCC 49619.

1) Bacterial colony identification

Bacterial colonies were then subjected to colony and cellular morphological identification following incubation. Bacterial colony representation was tabulated in a morphology sheet indicating colony colour, haemolytic property, form, margin, and elevation. For cellular morphological analysis, the Gram staining procedure was conducted on all bacterial colonies and viewed under a light microscope at ×100 magnification (oil immersion) to elucidate the cellular form of the colony [17].

2) Biochemical tests

All colonies were then subjected to biochemical tests depending on the type of cellular morphology (*i.e.* Catalase test and Coagulase test for Gram-positive isolates, Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), Sulphur Indole Motility (SIM), Citrate, Urease and Oxidase test (HiMedia, Mumbai, India) for Gram-negative isolates;) to further characterize these isolates before proceeding to antibiotic resistance profiling [17]. The media for biochemicals was prepared in-house following the manufacturer's instructions and the laboratory's SOP. QCs were performed on each prepared batch that is sterility test and checking of the quality of the media where standard QC organisms were inoculated. All the media were inoculated with *Escherichia coli* ATCC 25922.

3) Antimicrobial susceptibility profiling

The Kirby-Bauer Disc Diffusion Assay was used to determine the susceptibility status of the isolates according to the protocol described by Hudzicki [18]. A panel of antibiotics that were used to determine resistance patterns included ciprofloxacin (5 μg), nitrofurantoin (300 μg), azithromycin (15 μg), chloramphenicol (30 μg), amoxicillin/clavulanic acid (30 μg), sulfamethoxazole/trimethoprim (30 μg), ampicillin (10 μg), piperacillin-tazobactam (100/10μg), penicillin (10 μg), imipenem (10 μg), tetracycline (30 μg), and gentamicin (10 μg). Drug selection

was made based on the 2020 Clinical Laboratory Standards Institute (CLSI) standard guidelines [19].

A sterile inoculating loop was used to pick colonies and then suspended in 2 ml sterile saline in a tube to make 0.5 MacFarland standard which was measured using a MacFarland densitometer. The MacFarland cell suspension was prepared following the institutional SOP and CLSI standard guidelines. A sterile swab was dipped into the inoculum tube and rotated against the side of the tube to drain excess liquid. The dried surface of the Mueller-Hinton (HiMedia, Mumbai, India) agar plate was inoculated by streaking the swab three times over the entire surface. A pair of sterile forceps was used to place the antimicrobial-impregnated discs (HiMedia, Mumbai, India and Oxoid Ltd., Basingstoke Hampshire, United Kingdom) on the surface of the agar plates. The plates were then placed inverted in an incubator for 18 - 24 hours. The zones of inhibition were then measured in millimetres. The results scored as susceptible, intermediate or resistant based on the 2020 CLSI Performance Standards for Antimicrobial Disc Susceptibility Tests [19].

The media was prepared in-house following the manufacturer's instructions and the laboratory's SOP. QCs were performed on each prepared batch that is sterility test and checking of the quality of the media where standard QC organisms were inoculated. Mueller-Hinton agar was inoculated with *Escherichia coli* ATCC 25922.

2.9. Data Analysis

After collecting the data, data entry and analysis were conducted using IBM Statistical Package for Social Sciences (SPSS) Version 22 (IBM Corp, Armonk, New York). Descriptive analysis was done and presented in graphs and tables.

3. Results

3.1. Background Characteristics

A total of 200 samples were processed (100 trolleys and 100 baskets) with each supermarket contributing 25 trolleys and 25 baskets. 56 (28%) out of 200 samples were culture-positive and the highest number of organisms was isolated from shops around Mall D (32.1%) and the least from Mall B (14.3%) as shown in Table 1. In Mall A, there was an equal number of isolates (50% apiece) between

Table 1. Number of bacteria isolated per site.

Characteristics Study Site	Total n (%)	Bacteria	
		Basket n (%)	Trolley n (%)
Mall A	16 (28.6)	8 (50)	8 (50)
Mall B	8 (14.3)	3 (37.5)	5 (62.5)
Mall C	14 (25)	4 (28.6)	10 (71.4)
Mall D	18 (32.1)	5 (27.8)	13 (72.2)

trolleys and baskets. In Mall B 62.5% of organisms were isolated from trolleys and 37.5% from baskets. In Mall C 71.4% of organisms were isolated from trolleys and 28.6% from baskets. In Mall D, 72.2% of organisms were isolated from trolleys and 27.8% from baskets. Hence more organisms were isolated from trolleys as compared to baskets.

3.2. Prevalence of Isolates

The most frequent isolate recovered was *Staphylococcus aureus* (17.3%), followed by *Pseudomonas* species (4.5%), *Escherichia coli* (2%), *Corynebacterium* species (2%), *Staphylococcus* species (1.5%) and *Enterobacter aerogenes* (0.5%) (Table 2). *Staphylococcus aureus* isolates were more prevalent in Mall D (37.1%) than in Mall B (14.3%) while *Escherichia coli* was more prevalent and only isolated from Mall C (100%).

3.3. Antimicrobial Resistance Patterns

Staphylococcus aureus showed the most resistance to azithromycin (17%) followed by ciprofloxacin (2.8%), nitrofurantoin (2.8%) and chloramphenicol (2.8%). *Escherichia coli* showed 100% resistance to amoxicillin, sulfamethoxazole/trimethoprim, Chloramphenicol and ampicillin, 75% resistance to ciprofloxacin and 25% resistant to azithromycin, but showed no resistance to nitrofurantoin. *Staphylococcus* species, *Corynebacterium* species, *Enterobacter aerogenes* and *Pseudomonas* species showed no resistance to any antibiotics tested (Table 3).

4. Discussion

Shopping trolleys and baskets are used every day by shoppers to transport their purchases. However, studies have implicated shopping trolleys and baskets as one of the most biologically contaminated public surfaces [6]. During the time of this manuscript write-up, there was no published study examining shopping trolley and basket contamination in Zambia. The occurrence of bacteria on the handles of shopping trolleys and baskets was assessed in four different supermarkets located in Ndola City Zambia. The results of this study demonstrated

Table 2. Type of organisms isolated per site.

Study site	Organism identified					
	Corynebacterium sp. n (%)	*Escherichia coli* n (%)	*Enterobacter aerogenes* n (%)	*Pseudomonas* sp. n (%)	*Staphylococcus aureus* n (%)	*Staphylococcus* sp. n (%)
Mall A	1 (25)	0	1 (100)	3 (33.3)	10 (28.6)	1 (33.3)
Mall B	0	0	0	3 (33.3)	5 (14.3)	0
Mall C	1 (25)	4 (100)	0	1 (11.1)	7 (20)	1 (33.3)
Mall D	2 (50)	0	0	2 (22.2)	13 (37.1)	1 (33.3)
Total	4 (100)	4 (100)	1 (100)	9 (100)	35 (100)	3 (100)

Table 3. Antibiotic resistance patterns of organisms isolated.

Microorganism	n	Antimicrobial agents											
		CIP n (%)	NFN n (%)	AZM n (%)	C n (%)	AMC n (%)	SXT n (%)	AMP n (%)	TZP n (%)	P n (%)	IPM n (%)	TE n (%)	CN n (%)
Staphylococcus aureus	35	1 (2.8)	1 (2.8)	6 (17)	1 (2.8)	ND	ND	ND	ND	ND	ND	ND	ND
Staphylococcus species	3	0	0	ND	ND	ND	ND	ND	0	ND	ND	ND	ND
Escherichia coli	4	3 (75)	0	1 (25)	4 (100)	4 (100)	4 (100)	4 (100)	ND	ND	ND	ND	ND
Corynebacterium species	4	0	ND	ND	ND	ND	ND	ND	ND	0	0	0	ND
Enterobacter aerogenes	1	0	ND	0	ND	ND	ND	0	ND	ND	ND	0	ND
Pseudomonas species	9	0	ND	ND	ND	ND	ND	0	0	ND	0	ND	0

Note: CIP = Ciprofloxacin, NFN = Nitrofurantoin, AZM = Azithromycin, C = Chloramphenicol, AMC = Amoxicillin/Clavulanic acid, SXT = Sulfamethoxazole/Trimethoprim, AMP = Ampicillin, TZP = Piperacillin-Tazobactam, P = Penicillin, IPM = Imipenem, TE = Tetracycline, CN = Gentamicin, ND = Not done.

that the majority of swabbed trolleys and baskets were contaminated with bacteria, most of which were *Staphylococcus aureus* (17.3%) followed by *Pseudomonas* species (4.5%), *Escherichia coli* (2%), *Corynebacterium* species (2%), *Staphylococcus* species (1.5%), *and Enterobacter aerogenes* (0.5%). The rate of shopping trolley and basket contamination was 28%. The presence of bacterial isolates in this study was lower than the results obtained in Jeddah by Al-Ghamdi *et al.* [16], in the USA by Gerba and Maxwell [20], in Saudi Arabia by Ashgar and El-Said [21] and in Spain by Carrascosa *et al.* [11], who reported the contamination rate as 95.5%, 72%, 48% and 35% respectively. The reduced prevalence obtained in our study could be due to the small sample size and availability of resources. The study being done during the COVID-19 pandemic when mandatory hand-sanitising before entering shopping malls was implemented along with lower human traffic in Zambian malls as compared to the malls in other countries could also have led to the reduced prevalence.

The Gram-positive isolates recovered in this study were *Staphylococcus aureus, Staphylococcus* species and *Corynebacterium* species. *Staphylococcus aureus* showed the highest prevalence of 17.3%, which was higher than the 14% reported in a study in Jeddah by Al-Ghamdi *et al.* [16]. The prevalence level in the present investigation was slightly higher than these observations, which could be caused by the variation in environmental factors and hygienic conditions. In the same study, the prevalence of *Staphylococcus* species was significantly higher (87%) than in this study (2%). Furthermore, 2% of *Corynebacterium* species were isolated, however, no studies are showing its prevalence on shopping trolleys and baskets to be compared. The above being Gram-positive bacteria agrees with a study by Scott and Bloomfield [22] that observed that Gram-positive bacteria survive for a longer period on laminate surfaces than Gram-negative organisms. This data also correlates with the findings of Al-Ghamdi *et al.* [16] where more Gram-positive bacteria were frequently isolated compared to Gram-

negative organisms. The high numbers of *Staphylococcus aureus* indicate the level of extreme unsanitary conditions of the trolleys that the public is exposed to and the increased risk of contracting disease-causing organisms.

The Gram-negative bacteria isolated in this study were *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas* species. Isolation of *Escherichia coli* is well documented by several studies [9] [11] [16] [20] [21], and it has been observed that the prevalence levels of *Escherichia coli* on shopping trolleys and baskets vary from study to study [16]. In the present study, we found 2% of *Escherichia coli* contamination and similar results were shown by Carrascosa *et al.* [11] who determined 2.45% of *Escherichia coli* contamination. Studies done in the USA, Saudi Arabia and Jeddah reported higher prevalence; Gerba and Maxwell [20] 21.17%, Reynolds *et al.* [9] 21%, Ashgar and El-Said [21] 16.7% and Al-Ghamdi and others [16] 7%. Carrascosa *et al.* [11] isolated Enterobacteria (41.17% from shopping trolleys and 50.6% from baskets), whereas only 0.5% were isolated from this study. Our study found a 4.5% prevalence of *Pseudomonas* species which is slightly higher than the 4.25% and 2.55% prevalence of *Pseudomonas rhodesiae* and *Pseudomonas fluorescens* respectively which was reported in Spain by Carrascosa *et al.* [11]. Species such as *Pseudomonas aeruginosa* have been found to survive for months on inanimate surfaces which can potentiate individuals to easily come in contact with them and cause nosocomial infections such as pneumonia [10].

The results of this study confer a high probability of harbouring highly pathogenic organisms which agrees with those reported by others [9] [16] [20] [21]. Our results revealed the importance of cleaning and disinfecting shopping trolleys to avoid the presence of *Staphylococcus aureus* which is responsible for invasive skin infections and toxin-mediated shock caused by *Staphylococcus aureus* and diarrheal diseases associated with the coliforms such as *Escherichia coli*. It is reported that coliform bacteria often originate from faeces and are associated with poor sanitary conditions. *Escherichia coli* isolated may have originated from contact with raw foods, birds (while the trolleys were sitting in the parking lots between use), other sources of animal faeces, and contact with faecal-contaminated hands or other body parts (diaper-aged infants) [20]. The in-shop handling of different items is another factor that determines hand hygiene. The fluctuation between items such as fresh vegetables, fruits and then fresh dripping chicken, fish or frozen items would subject the hands to dampness and make them suitable for picking up microbes [16].

The emergence of resistant bacteria represents a substantial global health crisis that has led to long hospital stays and overuse of drugs. In the present study, the antimicrobial resistance patterns of the six isolated microorganisms were evaluated to commonly used antibiotics.

Staphylococcus aureus was found to be highly resistant to azithromycin (17%) and these results agree with a study by Hema-Ouangraoua [23] which showed increased resistance of *Staphylococcus aureus* to azithromycin of 24.42%. This

has been attributed to the increasing use of the drug to reduce bacterial diseases in children. Further resistance was seen to ciprofloxacin, nitrofurantoin and chloramphenicol.

Escherichia coli showed a 100% resistance to amoxicillin, sulfamethoxazole/trimethoprim and ampicillin, 75% resistance to ciprofloxacin and 25% resistance to azithromycin. A study done in Nigeria by Akingbade *et al.* [24] reported a high resistance of *Escherichia coli* to cloxacillin (92.5%), amoxicillin (90.8%) ampicillin (90.8%) and ciprofloxacin (27.5%). Aibuni *et al.* [25] further found a 100% to ampicillin. Similar findings of high resistance have been reported by Daini and Adesemowo [26], Ogbolu *et al.* [27] and Stelling *et al.* [28], and Khan and Ahmed [29]. This confers to the indiscriminate and uncontrolled use of antibiotics which has further severe implications for the empiric therapy of infection caused by *E.coli*.

Conversely, *Staphylococcus* species, *Corynebacterium* species, *Pseudomonas* species and *Enterobacter aerogenes* showed no resistance to any antibiotics which could be due to a reduced prevalence due to the small sample size.

With the emergence of global infectious diseases including COVID-19, many supermarkets have been implementing hygienic measures by providing disinfectants at entry points. This can be a step forward to minimize hand contamination and reduce exposure to pathogens and transmission of bacterial infections among shoppers. Such approaches should be undertaken in parallel with community education for hygienic standards, respiratory etiquette and handwashing together with regular cleaning and disinfection of contaminated sites to minimize bacterial growth.

5. Limitations

The information obtained could not explain microbial contamination in other towns in Zambia and the information obtained could not highlight the source of bacteria in these fomites. The study had a small sample size and did not cover all the supermarkets available in Ndola.

6. Conclusions

Shopping trolleys and baskets of the study supermarkets in Ndola were contaminated with different microorganisms with *Staphylococcus aureus* being the most prevalent and these are common objects that the public comes into contact with daily. Furthermore, the isolated microorganisms were also resistant to commonly used drugs in human medicine. This could have significant public health consequences if these resistant microorganisms are transmitted to humans through the food chain.

What is already known on this topic:

1) Knowledge of the prevalence and antimicrobial status of bacteria on shopping trolleys and baskets has been reported in other countries.

2) Pathogenic bacteria have been isolated from the handles of shopping trol-

leys and baskets in other countries.

What this study adds:

1) To our knowledge, this is the first-ever study conducted in Zambia on types of microbial contamination found on fomites in supermarkets. Our study, therefore, generates critical information for infection prevention in public spaces.

2) The study identifies the resistance patterns of the bacteria isolated using commonly used antibiotics.

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Authors' Contributions

SP and RLM conceptualised the study. SP and RLM did the data collection. SP, EC and MS performed the experiments. SP, VD, SM[2], IM and TH conducted data analysis. SP, MC, WC, TM, SM[5] and SM[8] drafted the initial manuscript. All authors reviewed and approved the final version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Annex

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	7
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider the use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders	11
		(b) Indicate number of participants with missing data for each variable of interest	
Outcome data	15*	Report numbers of outcome events or summary measures	11

Continued

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g. 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorised (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—e.g. analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	11
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	12

*Give information separately for exposed and unexposed groups.