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Diversity and Antibiotics Susceptibility of Bacterial Species on Hand Surfaces in Public Buses Plying Kenyatta National Hospital Route 7c in Nairobi

Samson Chebon[1*] and Jackline Chelangat Sonoiya[1]

[1]Department of Medical Microbiology, Jomo Kenyatta University of Agriculture and Technology (JKUAT), Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between both authors. Author SC designed the study, wrote the protocol, wrote the first draft of the manuscript and managed both literature searches and data analysis. Author JCS collected the bacterial isolates, also undertook both morphological, biochemical characterizations of isolates. Both authors undertook antimicrobial susceptibility tests. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the pathogenic and the antimicrobial susceptibility patterns of bacterial isolates on hand surfaces in public buses in Nairobi.
Study Design: A random sampling technique was utilized and targeted public buses plying Nairobi Central Business District (CBD) and Kenyatta National Hospital (KNH) 7C route.
Place and Duration of Study: Bacterial isolates were obtained from hand surfaces in public buses in Nairobi during a three-month period, May-July 2015.
Methodology: A total of 30 swab samples were collected using sterile moist cotton swabs then cultured on three media including MacConkey agar, Mannitol salt agar and Eosin Methyl Blue agar (EMB). Characterization of isolates was by morphological and biochemical features. Antimicrobial susceptibility profile tests using eight antibiotics including tetracycline, sulphamethoxazole, chloramphenicol, kanamycin, gentamycin, ampicillin, co-trimoxazole and streptomycin was also undertaken.

**Corresponding author: E-mail: sambonce06@gmail.com;*

Results: A total of 45 isolates indicated the following prevalence: *Staphylococcus aureus 33%*, *Escherichia coli 24%*, *Staphylococcus epidermidis 18%, Klebsiella* species 11% and *Pseudomonas* species 13%. The antimicrobial resistance profiles indicated *E. coli* isolates had the highest resistance to five antibiotics, *Klebsiella* spp. to four, *Staphylococcus aureus* to three, *Staphylococcus epidermidis* and *Pseudomonas* spp. each to only one antibiotic. Isolates were predominantly resistant to ampicillin (100%) followed by co-trimoxazole and streptomycin but were instead sensitive to gentamycin followed by tetracycline, sulphamethoxazole and chloramphenicol.
Conclusion: These findings demonstrate serious health risks posed to the community by potentially pathogenic and antibiotic resistant bacterial species on hand-touch sites in public buses. Therefore, this study creates awareness of the need for stringent sanitary measures in public buses and hygienic practices among commuters to forestall transmission of community acquired infections.

Keywords: Pathogens; antimicrobial-resistance; hand-touch; surfaces; public-buses, Nairobi.

1. INTRODUCTION

The rural to urban migration, particularly to major cities notably the capital city of Nairobi is still prevalent in Kenya despite the new devolution system of government in 2013. It is estimated that out of the 3 million people residing in Nairobi, among those using vehicles to reach work stations, about 85% use public transport system [1]. The design of Nairobi is such that the southern region including upper hill and community area constitute a critical hub holding the headquarters of numerous governmental ministries. Similarly, both the largest public referral and private health facilities, namely Kenyatta National Hospital (KNH) and Nairobi Hospital, respectively are also located in the community hub. However, due to lack of efficient railway transport system in Nairobi, the upper hill region can only be accessed by road [1]. The main route frequently used by public buses to ferry the thousands of workers to and from their stations is designated 7C. The uniqueness of this route is that it is the only one used by patients and relatives visiting their folks at KNH from Nairobi's CBD. Therefore, public buses plying this route were purposely targeted in this study owing to the possibility of these vehicles acting as a medium of transmitting pathogenic bacteria from the hospital environment to the general community. Public transportation has recently been established as a critical factor contributing to the high disease prevalence particularly tuberculosis transmission in South Africa, notwithstanding its affordability [2].

Previous studies in other capital cities including Lisbon, Portugal established that contamination of passengers' hands with bacteria from public buses represent a route through which hospital-acquired Methicillin-resistant *Staphylococcus aureus* (MRSA) clones may spread to the community [3]. In another study in the United Kingdom, an increase in respiratory infections including colds and flu was established among persons who travelled in a public bus for five days [4]. Similar findings have been documented from other major cities including Bangkok [5] and London [6]. It has been recognized that overcrowding in small enclosed spaces inadequate ventilation and recirculation of contaminated air increases the duration of exposure and susceptibility of exposed people to the likelihood of airborne disease transmission [7].

In transport built environments, humans and environmental sources (mobile and fixed) are the major reservoirs of biological agents. Respiratory droplets produced by infected individuals during different expiratory activities (talking, coughing, and sneezing) may contain pathogens. These droplets either settle or remain suspended in the air as droplet nuclei depending on their composition and size at the time of release. In addition to the airborne route, the dispersion and transfer of infectious agents deposited on various surfaces/materials/matrix (e.g. skin or in respiratory secretions, to hands and/or to high-touch surfaces, e.g. doorknobs, staircase railings, seats, escalator hand rails, chair arms, grab rails, cash machines, phone, ticket machines) also offer a major transmission pathway [8].

Crowding is a common feature in various urban transport modes and transport hubs [5]. Further, the growing emphasis on energy efficiency and the resultant changes in design, construction and operation of various transport built environments particularly, airtight structured and high space usage efficiency in public buses may lead to increased vulnerability of these environments to airborne disease transmission [9]. For example, transport routes in Nairobi are currently facing chronic overcrowding and traffic jams, especially during rush hours. Therefore, travelling in jams with symptomatic individuals, especially during pandemics, in crowded and poorly ventilated public transport could increase the risk of infection transmission via direct or indirect contact [9]. In a study in Bangkok, Thailand, levels of bacteria of as high as >550cfu/m^3 were established in public buses [5].

The common public transport used by commuters in Nairobi includes small capacity vehicles locally known as *matatus*, minibuses and large buses. Their affordability makes them amenable to both the lower and middle-class Nairobians. Those who may have private cars among the middle class still prefer the public buses over the expensive cabs and also due to the persistent traffic jams in the city and high fuel prices. However, it has been established that besides the tropical warm tropical condition in Nairobi, the design and warm ambient conditions of these public vehicles not only act as an ideal reservoir of pathogenic microbes but also play a critical role in their transmission. The various hand-touch sites within public service buses and the accumulation of bacteria on in-built surfaces and objects such as hand and grab rails, seat fabrics and doors is becoming a great public health concern [5]. Similarly, oils on the human skin surface, dust particles in the vehicles air micro-habitats; grime, moisture and warmth from heat accumulation during traffic jams in tropical cities provide an ideal environment for these microbes to proliferate. Further, the unhygienic habit of carrying beverages and eating food in public vehicles leaves rich particulate substrates ideal for microbial growth on various surfaces of buses [10]. This phenomenon may result in infections owing to the successful interaction between infectious agents, hosts (passengers) and transmission pathways (buses contact surfaces).

Public transport buses may contain a variety of dangerous bacteria, including genus *Escherichia*, *Salmonella* and *Staphylococcus* particularly Methicillin-resistant *Staphylococcus aureus* (MRSA) *Streptococcus* [11]. Hand-touch sites can become contaminated with staphylococci and may be fomites for the transmission of bacteria between humans. Such sites could provide a reservoir for community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA) in high prevalence areas. MRSA and other pathogens are shed by infected patients leading to contaminated bus surfaces. This can contribute to transmission of pathogens from nosocomial to community settings [12]. Findings from a study in London found 9 (8%) of the 112 samples taken from hand-touch surfaces in the public transport system and in public areas of a hospital were positive for *S. aureus*, but no MRSA was isolated [3]. However, these microbes may not only cause nosocomial infections but may cause opportunistic diseases among the general public as well, particularly among the immunocompromised [6].

Additionally, re-emerging airborne infectious diseases, for instance, tuberculosis (TB), have a worldwide public health impact. In 2013, there were 9 million incident cases worldwide of multidrug-resistant TB (MDRTB), extensively drug-resistant TB (XDR-TB) and TB/HIV co-epidemics which pose serious global health concern [13]. Coincidentally, the majority of TB incidents were in Africa, Southeast Asia and Western Pacific regions. The public transport built environments in such countries with a high burden of TB together with poor airborne disease control measures may become hubs for the airborne spread of disease [2]. At present, we are living with a constant risk of an influenza pandemic, and this could have a significant effect on global public health status. Studies from countries with high TB incidence have shown that public transportation, often crowded and poorly ventilated, may play a critical role in transmission and sustaining TB infections [14]. Therefore, it was of great interest in this study to target isolating pathogenic bacteria from public buses transiting between Nairobi city centre at Kencom bus stage and Kenyatta National Hospital bus termini. No such study has been undertaken or documented before in Kenya. Previously, a study in 2015 focused only on managerial problems facing public transportation system in Nairobi [1]. Therefore findings from this research will lay a critical foundation for future studies on public buses hygiene and microbiological safety not only in Nairobi but other major towns in Kenya including Mombasa, Nakuru, Eldoret and Kisumu.

2. MATERIALS AND METHODS

2.1 Study Site

The study was undertaken at Kenyatta National Hospital bus station whereby samples were collected from the hand and grab rails surfaces within the buses. These buses operate between Kencom and Kenyatta National Hospital bus stations with 7C as the designate route number.

The Kencom-KNH route is one of the main artery linking Central Business District (CBD) and the Community area that is a hub to many ministerial government headquarters. It also links the CBD with the KNH, the largest public teaching and referral hospital in Nairobi. The buses operating this route were chosen because they ferry people of the diverse spectrum ranging from government officers, the sick both from Nairobi and countryside going for referral treatment at KNH and also those visiting the sick alongside those who have just been discharged from the hospital.

2.2 Sample Size and Collection

A total of thirty samples were obtained from hand and grabbed rails in selected 60 seater public buses operating between Kencom and KNH. Samples were obtained from 2 cm × 4 cm sections using sterile cotton swabs moistened with normal saline as described by Otter and French [6]. The swabs then supposedly laden with microbes were put into clean, sterile containers before being capped well to avoid contamination. They were then transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT) department of Medical Microbiology laboratory for analysis.

2.3 Sample Analysis and Morphological Characterization of Isolates

Swab samples were enriched with buffered peptone water then incubated for 24h at 37°C. The enriched swabs were then inoculated by streaking plating method onto three differential solid culture media: MacConkey agar, Mannitol salt agar and EMB agar plates. Incubation was undertaken for 24h at 37°C. The isolates were then sub cultured on nutrient agar to obtain pure colonies for further identification.

Bacterial isolates were first differentiated by macroscopic examination of the colonies. The colonies were differentiated based on size, colour, pigmentation, elevation, surface texture, and margin, and lactose fermentation on MacConkey broth. Gram stain technique to distinguish between Gram-positive and negative isolates was undertaken according methods described by [15].

2.4 Biochemical Characterization of the Isolates

The isolates were subjected to an array of biochemical tests for confirmation of species identity according to methods described by Cappuccino and Sherman [16]. A total of 45 isolates were characterized using the following tests: Indole production, methyl red, coagulase, citrate utilization, triple sugar iron, catalase and hydrogen sulphide, indole and motility (SIM) test.

2.5 Antimicrobial Susceptibility Tests

Susceptibility of the test species to conventional antibiotics was determined by the Kirby–Bauer disk-diffusion technique according to the recommendations of Clinical and Laboratory Standards Institute guidelines [17]. A loopful of test organisms were inoculated into a prepared nutrient agar broth, incubated for 24h at 37°C. From the broth, 0.1 ml of the culture was flooded into a freshly prepared Mueller Hinton agar plate. Using sterile forceps, the sense-disks were placed on top of the agar plate to test for sensitivity of each isolate against the following eight antibiotics; Ampicillin (25 mcg), Tetracycline (25 mcg), Co-trimoxazole (25 mcg), Streptomycin (10 mcg), Kanamycin (30 mcg), Gentamycin (10 mcg), Sulphamethoxazole (200 mcg) and Chloramphenicol (30 mcg). The plates were then incubated at 37°C for 24 h. A total of 45 isolates were screened. Susceptibility test results were interpreted using the Clinical and Laboratory Standards Institute guidelines [16], where the isolates were considered to be either susceptible or resistant. Susceptibility test for each species was undertaken in triplicates.

3.0 RESULTS AND DISCUSSION

3.1 Results and Discussion on Prevalence of Bacterial species

A total of 45 bacterial isolates belonging to five species were obtained from the 30 samples collected from bus-hand and grab rails. The Gram-negative bacteria isolated included *Klebsiella* species, *Pseudomonas* spp and *Escherichia coli* while *Staphylococcus aureus* and *Staphylococcus epidermidis* were the isolated Gram-positive bacteria. The species identity as revealed from biochemical characterization results is indicated in Table 1.

Staphylococcus spp. was the most predominant isolate (33%), whereas *Klebsiella* spp. was the least isolated with the incidence of 11%. Prevalence for *E. coli* was *24%, S.epidermidis 18%* and *Pseudomonas* spp, 13% (Fig. 1). The presence of high levels of *S. aureus* could be attributed to the fact that it constitutes normal flora, found in the human mucous membrane and skin. The findings on the diversity of organisms isolated in this study which indicated that *Staphylococcus* spp. (33%) as the most prevalent isolate is in agreement with previous similar studies [18,19]. Studies from Colombia on bacterial contamination of public buses also established that *S.aureus* was the most prevalent contaminant [20]. Regarding contamination by enterics, findings from this study established *E.coli* was the most predominant (24%). Similar findings have been established from bacterial contamination on hand surfaces of public buses in Chittang city, Bangladesh where its prevalence was 46.5% [21]. The presence of high levels of *S. aureus* could be attributed to the fact that it constitutes normal flora, found in mucous membrane and skin of 25% healthy individuals. Detection of bacteria of faecal origin on hand and grab rails was similarly high. Though the presence of such bacteria is probably not a health hazard in itself, it is indicative of a failure of hygiene, and more specifically a failure to wash hands after contact with faecal matter [22].

Table 1. Biochemical characterization results of the isolates

Isolate	TSI Agar			SIM						Bacterial spp.
	Slant	Butt	Gas	H_2S	Motility	Indole	MR	Citrate	Urease	Cat	
B1	+	-	-	-	-	-	-	-	+	+	*S. aureus*
B2	+	-	-	-	-	-	-	-	+	+	*S. aureus*
B3	+	-	-	-	-	-	-	-	+	+	*S. aureus*
B4	+	-	-	-	-	-	-	-	+	+	*S. epidermidis*
B5	+	+	+	-	+	+	+	-	-	-	*E. coli*
B6	-	-	-	-	+	-	-	+	-	+	*Pseudomonas spp*
B7	-	-	-	-	+	-	-	+	-	+	*Pseudomonas spp*
B8	+	+	+	-	-	-	+	+	-	-	*Klebsiella*
B9	+	+	+	-	+	+	+	-	-	-	*E. coli*
B10	+	+	+	-	-	-	+	+	-	-	*Klebsiella spp*
B11	+	+	+	-	+	+	+	-	-	-	*E. coli*
B12	-	-	-	-	+	-	-	+	-	+	*Pseudomonas spp*
B13	+	+	+	-	+	+	+	-	-	-	*E. coli*
B14	+	+	+	-	-	-	+	+	-	-	*KlebsiellaSpp*
B15	+	+	+	-	+	+	+	-	-	-	*E. coli*

Key: TSI-Triple sugar iron, Cat-Catalase, SIM- Sulphur indole motility, MR- Methyl red

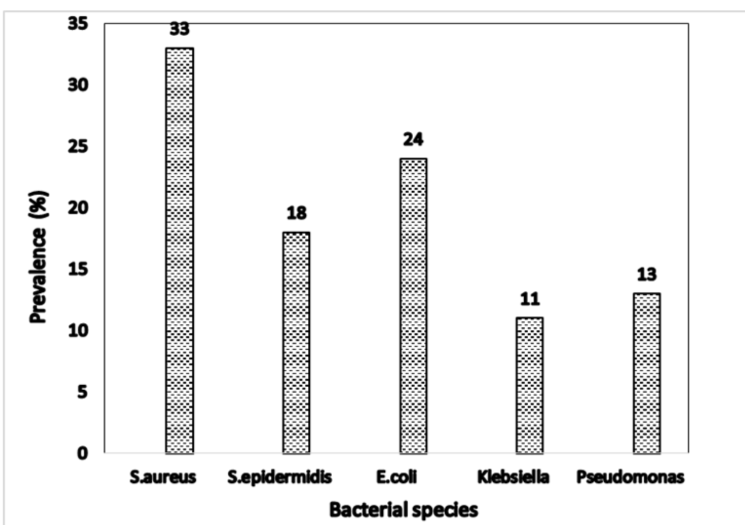

Fig. 1. The incidence of bacterial isolates

Several studies have indicated that various bacteria, including *E. coli, S. aureus* and *Pseudomonas* species survive on hands, sponges/cloths, door knobs etc. for hours or days after initial contact with the microorganism [2]. In a study conducted at Sokoine University, Tanzania, the results on prevalence of bacterial loads from the different surfaces in student's toilets were: *Staphylococcus aureus* 25%, *Escherichia coli* 36.7%, *Pseudomonas aeruginosa* 13.3%, *Proteus mirabilis* 6.7%, *Klebsiella pneumonia* 11.6% and *Streptococcus pyogenes* 6.7% [23]. The predominance of *S.aureus* and *E. coli* in the toilets is similar to the prevalence patterns as demonstrated in the current study, both being crowded public settings.

3.2 Results and Discussion of Antibiotic Sensitivity Profiles

All the 45 bacterial isolates were subjected to antibiotic sensitivity tests where eight antibiotics including: ampicillin, streptomycin, tetracycline, chloramphenicol, sulphamethoxazole, co-trimoxazole, kanamycin, and gentamycin were used.

The results for antimicrobial resistance was profiled according to the five species against the eight antibiotics (Table 2). The most outstanding finding is that none of the 45 isolates recovered from the 30 hand-touch sites demonstrated resistance to all the eight antibiotics. However, *E. coli* isolates indicated predominant resistance recording the highest resistance to five antibiotics including: ampicillin, co-trimoxazole, streptomycin, chloramphenicol and sulphamethoxazole. *Klebsiella* spp. was resistant to four antibiotics including: ampicillin, streptomycin, chloramphenicol and tetracycline. *Staphylococcus aureus* was resistant to three including: ampicillin, co-trimoxazole and streptomycin. *Staphylococcus epidermidis* and *Pseudomonas* spp. were each resistant to only two antibiotics namely ampicillin and co-trimoxazole.

Isolates were predominantly resistant to ampicillin where all the five species representing the 45 isolates indicated resistance. Similarly, all species except *Klebsiella* were resistant co-trimoxazole while three species including *Staphylococcus aureus, Klebsiella* and *E.coli* were resistant to streptomycin. Two species namely *Klebsiella* and *E. coli* showed resistance to chloramphenicol while *E. coli* and *Klebsiella* were each resistant to one antibiotic, namely sulphamethoxazole and tetracycline, respectively. In contrast, all the five spp. indicated sensitivity to gentamycin and Kanamycin while sulphamethoxazole and Tetracycline indicated the lowest resistance (Table 2).

Table 2. Resistance profiles of the five bacterial spp. against eight antibiotics

Bacterial spp.	**Antibiotic**								
	Gentamycin	**Kanamycin**	**Tetracycline**	**Sulphamethoxazole**	**Chloramphenicol**	**Streptomycin**	**Co-trimoxazole**	**Ampicillin**	**Total: resistance out of 8 antibiotics N=8**
	R	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Pseudomonas n=6	-	-	-	-	-	-	+	+	2
S. epidermidis n=8	-	-	-	-	-	-	+	+	2
S. aureus n=15	-	-	-	-	-	+	+	+	3
Klebsiella n=5	-	-	+	-	+	+	-	+	4
E. coli n=11	-	-	-	+	+	+	+	+	5
Total *(n=45)*	0	0	1	1	2	3	4	5	

Key: +: Resistance; -: Sensitivity

The findings from this study indicated that *E. coli* isolates recorded the highest resistance to five out of the eight antibiotics tested (ampicillin, co-trimoxazole, streptomycin, chloramphenicol and sulphamethoxazole). This is in agreement with findings of Chowhury et al. [21] which also revealed that more than 90% of *E. coli* isolates were resistant to both amplicin and chloramphenicol. The resistance patterns of *E. coli* as revealed in this study indicates that the isolates are difficult to control by administration of commonly prescribed drugs. Similarly, findings from this study demonstrating all the five species were sensitive to gentamycin and kanamycin are agreement with studies in public buses in Chittagong city, Bangladesh by Chowhury et al. [21] which also established that all isolates were susceptible to gentamycin and ciprofloxacin.

Isolates were predominantly resistant to ampicillin where all the five species, comprising of the 45 isolates (100%) indicated resistance. This is an interesting finding from this study. This is consistent with findings by Dancer [11] which established that 45% of *S. aureus* isolates were resistant to ampicillin. Another remarkable observation from this study is the isolates, *S. epidermidis* and *Pseudomonas* spp. which had the lowest resistance was towards two antibiotics. The alarmingly high multi-drug resistance of these isolates clearly illustrates the grave dangers of nosocomial drug-resistant isolates transfer to the community setting via the public buses. Since the samples from this study were isolated from public buses ferrying passengers exiting directly from the hospital, it strongly demonstrates that the source of these isolates were hospital wards, structures and appliances within the hospital buildings with which people get into contact during their stay [24]. Subsequently, transfer of bacterial pathogens to the bus hand-touch sites occur through health workers, out-patients and visitors upon leaving the hospital. This poses a great danger in the event of a horizontal transfer of virulent resistance genes from nosocomial to community. *Staphylococcus aureus* is primarily transmitted through direct contact with a colonized or infected individual or through a fomite intermediate [25,26].

Among the isolates that indicated the lowest resistance to the eight antibiotics, *S. epidermidis* was resistant to only two antibiotics namely ampicillin and co-trimoxazole. Although *S. epidermidis* is exclusively opportunistic, lacking many of the toxins produced by *S. aureus,* it can present a serious threat to immununocompromised individuals. Further, *S. epidermidis* also easily forms biofilms in catheters and intubation devices, subsequently causing infections that are difficult to treat within the patients [24].

Hands are the critical disseminators, particularly the hands of healthcare workers. Healthcare workers are important in transmission for several reasons. First, they care for multiple patients throughout the day, going from ward to ward, patient to patient. In this process, there are countless occasions to touch infected or colonized patients and contaminated fomites. It has been estimated that an ICU healthcare worker has an average of 43.4 opportunities for hand washing per hour, per patient. This high demanding standard of hygiene is laboriously unattainable among health workers in the general public wards thereby enhancing dissemination of multi-drug resistant isolates [27]. The scenario is even more precarious among the general community members exiting the hospital who board the buses yet not having washed their hands. Eating or drinking during travel, hands laden with bacterial loads acerbates the health condition with high possibility of contracting foodborne, respiratory and skin diseases.

The crowded condition in public buses due to the engineering design of the sitting arrangement further acerbates the possibility of pathogens' transmission. Instead of the conventional two rows of seats, an extra third row has been added on the right-hand side of the large sitting capacity buses in Nairobi. It should be reduced to two to avoid overcrowding and possibility of the spread of pathogens. Similarly, use of small-holder capacity buses of 25 passengers should be encouraged instead of the 60 capacity. Further, the use of antimicrobial coatings on different high-touch surfaces in public buses could also be adopted to prevent inanimate surfaces acting as reservoirs of pathogenic organisms. Further, the Nairobi county government in collaboration with the management of KNH should install water taps at exit points from the hospital premises to enable washing off of any microbes contracted in the hospital from contaminating touch surfaces in buses.

4. CONCLUSION

The findings from this study strongly demonstrate that hand-touch sites in public buses in Nairobi contain diverse bacterial pathogens. The finding that the isolates were predominantly resistant to some commonly used chemotherapeutic antibiotics including ampicillin, co-trimoxazole and streptomycin further illustrates the great danger that public buses pose as a transmission medium of multi-drug resistant isolates from the hospital environment to the community. These findings from this pioneer study fundamentally create awareness among stakeholders in the public transport and health industry in the importance of enhancing hygienic safety measures.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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