A Diversity of Bacterial Spp. on Hand Surfaces in Public Buses Plying Kenyatta National Hospital 7c Route-Nairobi

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ABSTRACT (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

Aims: To determine the pathogenic bacteria and the antimicrobial susceptibility patterns of bacterial isolates obtained from transport service buses operating between Kencom station within Nairobi central business district (CBD) and Kenyatta National Hospital (KNH) stage.
Study design: Purposeful sampling technique was applied targeting public buses plying Central Business District (CBD) and Kenyatta National Hospital (KNH) route in Nairobi.
Place and Duration of Study: Hand and grab rails in public buses operating between Kencom stage in Nairobi's CBD and Kenyatta National Referral Hospital were sampled for bacterial contamination during May and July 2015.
2010.
Methodology: A total of 30 swab samples were collected from 30 hand-touch sites 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 were also undertaken.
Results: The frequency from 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 of the isolates indicated *E. coli* isolates had the highest resistance to five antibiotics, *Klebsiella* spp. to four, *Staphylococcus aureus* to three, *Staphyloccus epidermidis* and *Pseudomonas* spp. to only one antibiotics. 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 revealed the hand-touch sites in public buses are contaminated with potentially pathogenic and antibiotic resistant bacterial spp. Despite the small sample size in this study, the presence of antibiotic resistant isolates in this critical commuter route in Nairobi is an appropriate representation and illustration of the serious public health risks of community acquiring infections from the hospital environments through the buses. Therefore, this study creates awareness for the need of stringent sanitary measures in public bus and hygienic practices among commuters to forestall community acquired infections.

Keywords: Bacteria, pathogens, transmission, 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 still pervades 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. 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. Both the largest public referral and private health facilities, Kenyatta National hospital (KNH) and Nairobi Hospital, respectively are also located in the community hub. Two routes connect the CBD and the community area therefore constitute the artery routes used daily by passenger vehicles ferrying hundreds of workers to and from their stations as well patients and relatives visiting the hospitals. The main route (7C) is frequently used by public buses on CBD-KNH route, and compared to other Nairobi city routes, its uniqueness, for which it was purposely targeted in this study was owing to the possibility of transmission of pathogenic nosocomial bacteria to the community by passenger patients and hospital visitors via the buses.

Previous similar studies in other capital cities including Lisbon, Portugal established contamination of public buses and the transfer of the bacteria to the hands of passengers represent a route through which hospital-acquired MRSA clones may spread to the community [1]. In another study in United Kingdom, an increase in respiratory infections including colds and flus was established among persons who travelled in public bus for five days [2]. Similar findings have been documented from other major cities including Bangkok [3] and London [4]. It has been recognized that overcrowding in small enclosed spaces, inadequate ventilation, recirculation of contaminated air, increased duration of exposure and susceptibility of exposed people increase the likelihood of airborne disease transmission [5]

In transport built environments, humans and environmental sources (mobile and fixed) are the major reservoir 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 [6].

Crowding is a common feature in various urban transport modes and transport hubs. 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. For example, at present, Nairobi is facing chronic overcrowding and traffic jams on public transport, especially during peak 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 [7]. In a study in Bangkok, Thailand, elevated levels of bacteria (>550cfu/m^3) were established in public buses [3].

The common public transport used by commuters in Nairobi includes small capacity vehicles locally known as *matatus*, minibuses and large buses. Their cheapness 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 the persistent traffic jams in the city. 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 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 [3]. 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 habit of carrying beverages and eating food in public vehicles leaves rich particulate substrates ideal for microbial growth on various bus surfaces of buses [8]. This phenomena 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*, cold virus and *Staphylococcus* including Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus* [9]. 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 MRSA (CA-MRSA) in high prevalence areas. MRSA and other pathogens are shed by infected patients leading to contaminated bus surfaces. This can contribute to nosocomial to community transmission of pathogens [10]. 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. However, these microbes may not only cause nosocomial infections but may cause opportunistic diseases among the general public as well, particularly among the immunocompromised [4].

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 and multidrug-resistant TB (MDRTB), extensively drug-resistant TB (XDR-TB) and TB/HIV co-epidemics are serious global health concerns [11]. 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 [12]. At present, we are living with a constant risk of an influenza pandemic, and this could have a significant effect on global public health. Studies from countries with high TB incidence has shown that public transportation, often crowded and poorly ventilated, may play a critical role in transmission and sustaining TB infection [13]. Therefore, it was of great interest in this study to target isolating bacteria from public buses transiting between city center at Kencom bus stage and Kenyatta National Hospital. No such studies have been undertaken or documented before. Previously, a study in 2015 focused only on managerial problems facing public transportation in Nairobi [14]. 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. MATERIAL 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 hub to many ministerial government offices. 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 diverse spectrum ranging from government officers, the sick both from Nairobi and countryside going for treatment at KNH and also those visiting the sick alongside those who have just been discharged from hospital.

2.2 Sample Size and Collection

A total of thirty samples were obtained from hand and grab 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 [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 the JKUAT Medical Microbiology laboratory for analysis.

2.3 Laboratory culture of the isolates and Morphological Characterization

The swabs were then enriched with buffered peptone water then incubated for 24hrs at 37^{o} C. The enriched swabs were then inoculated onto three differential culture media: MacConkey agar, Mannitol salt agar and EMB agar plates. Incubation was undertaken for 24 hours at 37^{o} 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 agar. Gram stain technique to distinguish between gram positive and negative isolates was undertaken acccording 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 [16]. A total of 45 isolates were characterized using the following tests:

2.4.1 Indole production Test

The bacterial isolates were tested for their ability to degrade amino acid tryptophan with the production of indole. The test organisms were inoculated into tryptone broth by means of stab inoculation. The tubes were then incubated at 37^{0}C for 24hrs. After incubation, 3 drops of Kovac's reagent are added to all the cultures and observed for red coloration. Formation

of a red ring indicated a positive indole test.

2.4.2 Methyl red test

The test was carried out on recovered organisms to determine their ability to oxidize glucose with the production and stabilization of high concentration of acid end products. The isolated organisms were inoculated into tubes with MR-VP broth by means of loop inoculation. The tubes were then incubated at 37^0C for 24hrs. Three drops of methyl red indicator were added later to all the tube cultures and colour change observed. A red coloration signified a positive reaction.

2.4.3 Coagulase test

This test was done to confirm the presence of *Staphylococcus aureus*. The isolates were inoculated in plasma and incubated at 37^0C for 24hrs. After incubation, a clot formation is observed. Clot formation indicates production of coagulase enzyme which is an enzyme that clots blood plasma and is a virulence factor of *S. aureus.*

2.4.4 Citrate utilization test

The microorganisms were tested for the ability to utilize citrate as the sole source of carbon and energy for growth and ammonia salt as the sole source of nitrogen. The microorganisms were inoculated on the surface of Simmon's citrate agar slants by means of a stab-and-streak inoculation medium and incubated at 37^0C for 24hrs. Growth and colour change of the cultures were observed. A deep Prussian blue colour confirmed a positive reaction

3.4.5 Triple sugar iron agar test

The organisms were tested for their ability to utilize glucose and lactose or sucrose fermentative and produce hydrogen sulphide. The test organisms were inoculated into agar slants by means of stab-streak inoculation then incubated for 24hrs at 37^0C and observed for growth and colour changes. A yellow butt and slant signaled utilization of glucose / or lactose.

3.4.6 Catalase test

The microorganisms were tested for the ability to degrade hydrogen peroxide by producing the enzyme catalase. The evolution of gas causes bubbles to form and this is an indicative of a positive test. Each test isolate was placed on clean glass slides to which 3 drops of 3% H_2O_2 was added, observations were made for the effervescence.

3.4.7 SIM Test

This test is for hydrogen Sulphide, Indole and Motility of the organism. The isolates were inoculated onto the medium by a swab and stab type methods then Incubated for about 24 hours. The Indole aspect of the test was performed by adding Kovac's reagent to the inoculated medium. The Motility aspect of the test was done by checking the medium for turbidity.

3.5 Antimicrobial susceptibility tests

Susceptibility of the test strains 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 24 hours at 37^0C. From the broth, 0.1ml of the culture was flooded into a freshly prepared Mueller Hinton agar plate. Using sterile forceps, the sensi-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), Sulphamethxazole (200 mcg) and Chloramphenicol(30 mcg). The plates were then be incubated at 37^0C for 24 hours. 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 susceptible or resistant.

3. RESULTS AND DISCUSSION

3.1 Results and Discussion on Prevalence of Bacterial spp.

A total of 45 bacterial isolates belonging to five spp. 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 are indicated in Table 1.

The prevalence of the bacteria isolated demonstrated *Staphylococcus* spp. had the highest frequency of contamination (33%), whereas *Klebsiella* spp. was the least isolated with incidence of 11%. Prevalence for *E.coli* was *24%, S.epidermidis 18%* and *Pseudomonas* spp, 13% (Figure 1).The presence of high levels of *S. aureus* could be attributed to the fact that is part of normal flora, found in the mucous membrane and skin of human. The findings on 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 by [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* as 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 is part of normal flora, found in the mucous membrane and skin of human. In fact it is found in 25% of healthy individuals. Detection of bacteria of faecal origin on hand and grab rails was 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].

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 pneumoniae* 11.6% and *Streptococcus pyogenes* 6.7% [23]. The predominant prevalence 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.

Table 1: Biochemical characterization result 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	+	+	+	-	-	-	+	+	-	-	*KlebsiellaSpp*
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

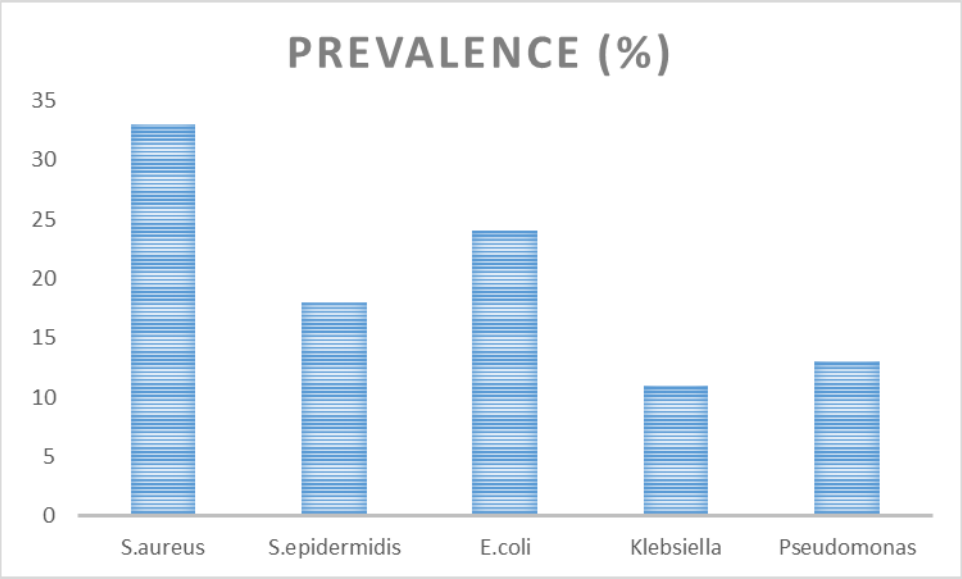

Figure 1. Incidence of bacterial isolates obtained.

3.2 Results and Discussion on Antibiotic Sensitivity Profiles

All the 45 bacterial isolates were subjected to antibiotic sensitivity tests where 8 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 8 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. *Staphyloccus 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 spp., representing the 45 isolates indicated resistance. Similarly, all spp. except *Klebsiella* were resistant co-trimoxazole while three spp. including *Staphylococcus aureus, Klebsiella* and *E.coli* were resistant to streptomycin. Two spp. namely *Klebsiella* and *E.coli* showed resistance to chloramphenicol while *E.coli* and *Klebsiella* were each resistant to one antibiotic, namely sulphamethoxazole and Tetracyline, respectively. In contrast, all the five spp. indicated sensitivity to gentamycin and Kanamycin while sulphamethoxazole and Tetracyline 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	-	-	-	-	-	-	+	+	
S. epidermidis n=8	-	-	-	-	-	-	+	+	2
S.aureus n=15	-	-	-	-	-	+	+	+	3
Klebsiella n=5	-	-	+	-	+	+	-	+	4
E.coli n=11	-	-	-	+	+	+	+	+	5
Total spp (n=5)	0	0	1	1	2	3	4	5	

Key: + :Resistance -: Sensitivity

The findings from this study indicating that *E. coli* isolates recorded the highest resistance to five out of the eight antibiotics tested including: ampicillin,co-trimoxazole,streptomycin, chloramphenicol and sulphamethoxazole are in agreement with findings by [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 thus study that demonstrated that all the five spp. were sensitivity to gentamycin and Kanamycin are agreement with studies in public buses in Chittagong city, Bangladesh by [21] which also established that all isolates were susceptible to gentamycin and ciprofloxacin.

An outstanding feature from this study was Isolates were predominantly resistant to ampicillin where all the five spp., representing the 45 isolates (100%) indicated resistance. This is consistent with findings by [7] 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. that 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 via the public buses. Since the samples from this study were isolated from public buses ferrying passengers exiting directly from hospital it strongly demonstrates that the original source of these isolates were hospital wards, structures and appliances within the hospital buildings with which people get into contact during their stay. Subsequently, transfer the bacteria to the bus hand-touch sites occur by health workers, out-patients and visitors upon leaving the hospital. This poses a great danger in the event of horizontal transfer of virulent resistance genes from nosocomial to community *Staphyloccus* isolates.

Staphylococcus aureus is primarily transmitted through direct contact with a colonized or infected individual, or through a fomite intermediate [24, 25]. 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 room to room, 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 [26]. This high demanding standard of hygiene is laboriously unattainable among health workers in the general wards thereby enhancing dissemination of multi-drug resistant isolates. 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, respiratary and skin diseases.

Among the isolates that indicated the lowest resistant 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 in immununocompromised individuals. Further, has been found to easily form biofilms on such as catheters and intubation devices, subsequently causing infections that are difficult to treat within the patients [27].

4.0 Conclusion

The findings from this study, though are from a small sample size is a great contribution owing to its being a pioneer study in Nairobi. It will fundamentally create awareness among stallholders in the public transport industry in enhancing hygienic safety measures including adoption of environmental and engineering controls focusing on reducing the concentration of infectious agents. For instance, instead of the common three rows on the right side of the buses, it should be reduced to two to avoid overcrowding and possibility of spread of pathogens. Similarly use 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 in order to prevent inanimate surfaces acting as reservoirs of pathogenic organisms. The Nairobi county government in collaboration with the management of KNH should install water taps at exit points from the hospital premises so at to enable washing off of any microbes contracted in the hospital from contaminating touch surfaces in buses

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