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POSSIBLE ROUTES OF BACTERIAL TRANSMISSION; A STUDY ON TRICYCLES USED WITHIN ABAKALIKI METROPOLIS

Odo Ikechukwu Ituma,[1] Udu-Ibiam Onyinyechi E,[2] Anger Robin A,[3] Ovuaja Maureen N.[4]

[1]Alex ekwueme federal university Ndufu Alike Ikwo.
[2]Ebonyi State University, Abakaliki.
[3]University of Agriculture Markudi Benue state.
[4]David Umahi federal University of medical Science Uburu.

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***Corresponding Author: Udu-Ibiam, Onyinyechi E.**
Alex Ekwueme FEDERAL University Ndufu Alike Ikwo.

ABSTRACT
Strategic places in tricycles used in Abakaliki metropolis were aseptically swabbed and examined using standard microbiological method. The bacterial count revealed that the examined tricycles analyzed had a bacterial load that ranges from 3.2 x 10^7 to 1.1.2 x 10^7. Morphological and biochemical characteristics of bacteria isolates from the samples shows that a total of three isolates were identified and they includes; *Staphylococcus aureus*, *Eschericia coli* and *Klebsiella* spp. Predominantly *Staphylococcus aureus* was isolated from most of the tricycles examined at a percentage of 38(80%) whereas the least was 1(2%). When the isolates were tested against the local disinfectant it was observed that Germicide Z© had a greater inhibitory effect on the isolates closely followed by De© showing an inhibition zone diameter that ranged between 12mm to 40mm and 20 to 40 mm respectively. Disinfectant IZ© had no inhibitory effect on the isolates.

KEYWORDS: Tricycles, Fomites, Transmission, Infectious Diseases, Disinfectant, Antiseptics.

INTRODUCTION

An infection is the invasion of tissues by pathogens, their multiplication, and the reaction of host tissues to the infectious agent and the toxins they produce (Acevedo et al,. 2019). An infectious disease, also known as a transmissible disease or communicable disease which is an illness resulting from an infection. Infections can be caused by a wide range of pathogens, most prominently bacteria and viruses (Adams et al., 2015). Hosts can fight infections using their immune systems. Mammalian hosts react to infections with an innate response, often involving inflammation, followed by an adaptive response. In certain cases, infectious diseases may be asymptomatic in a given host. In the latter case, the disease may only be defined as a "disease" (which by definition means an illness) in hosts who secondarily become ill after contact with an asymptomatic carrier. An infection is not synonymous with an infectious disease, as some infections do not cause illness in a host (Adam et al., 2013).

As bacterial and viral infections can both cause the same kinds of symptoms, it can be difficult to distinguish which is the cause of a specific infection (Abdulwasiu et al., 2022). Distinguishing the two is important, since viral infections cannot be cured by antibiotics whereas bacterial infections can (AAMC, 2015).

There is a general chain of events that applies to infections, sometimes called the chain of infection (Brown, 1987) or transmission chain. The chain of events involves several steps – which include the infectious agent, reservoir, entering a susceptible host, exit and transmission to new hosts. Each of the links must be present in a chronological order for an infection to develop. Understanding these steps helps health care workers target the infection and prevent it from occurring in the first place (CDC, 2023). Infection begins when an organism successfully enters the body, grows and multiplies. This is referred to as colonization. Most humans are not easily infected. Those with compromised or weakened immune systems have an increased susceptibility to chronic or persistent infections. Individuals who have a suppressed immune system are particularly susceptible to opportunistic infections. Entrance to the host at host–pathogen interface, generally occurs through the mucosa in orifices like the oral cavity, nose, eyes, genitalia, anus, or the microbe can enter

through open wounds. While a few organisms can grow at the initial site of entry, many migrate and cause systemic infection in different organs. Some pathogens grow within the host cells (intracellular) whereas others grow freely in bodily fluids. Wound colonization refers to non-replicating microorganisms within the wound, while in infected wounds, replicating organisms exist and tissue is injured (CDC, 2019). All multicellular organisms are colonized to some degree by extrinsic organisms, and the vast majority of these exist in either a mutualistic or commensal relationship with the host. An example of the former is the anaerobic bacteria species, which colonizes the mammalian colon, and an example of the latter are the various species of staphylococcus that exist on human skin. Neither of these colonizations is considered infections. The difference between an infection and colonization is often only a matter of circumstance. Non-pathogenic organisms can become pathogenic given specific conditions, and even the most virulent organism requires certain circumstances to cause a compromising infection. Some colonizing bacteria, such as *Corynebacteria sp* and *Viridans streptococci*, prevent the adhesion and colonization of pathogenic bacteria and thus have a symbiotic relationship with the host, preventing infection and speeding wound healing. The variables involved in the outcome of a host becoming inoculated by a pathogen and the ultimate outcome include: the route at which the pathogen enter the host to initiate infection, the internal virulence of the particular organism, the microbial load of the organism implicated, the immune status of the host in question. As an example, several staphylococcal species remain relatively non infective on the skin, but, when present in a normally sterile region, such as in the capsule of a joint or the peritoneum, it multiplies rapidly without resistance and cause harm (CDC, 2019).

Disease can arise if the host's protection and immune statues mechanisms are compromised and the organism inflicts damage on the host. Microorganisms can cause lots of damages on the tissues by releasing a variety of toxins or destructive enzymes. For example, *Clostridium tetani* releases a toxin that paralyzes muscles, and staphylococcus releases toxins that produce shock and sepsis. Not all infectious agents have the capacity to cause disease in all hosts. For example, less than 5% of individuals infected with polio develop disease (Ericson et al., 1999; Wayback machine, 2010). On the other hand, some infectious agents are known to be highly virulent. The prion causing mad cow disease and Creutzfeldt–Jakob disease is known to kills all animals and people that are infected(Duerkop and Hooper, 2013; Negut et al., 2018). Some of these virulent infections can be persistent, especially when the host is continuously being exposed to the infectious agents through fomites.

Persistent infections occur because the body is unable to expel the organism after the initial infection. Persistent infections are characterized by the continual presence of the infectious organism, often as latent infection with occasional recurrent relapses of active infection. There are some viruses that can continually cause infection by infecting different cells of the body. Some viruses once acquired never leave the body. A typical example is the herpes virus, which tends to hide in nerves and become reactivated when specific circumstances arise (Hector and Booksmythe, 2019; Hotez et al., 2006).

Persistent infections cause millions of deaths around the world each year (CDC, 2019). Chronic infections by parasites shares for a high morbidity and mortality in many underdeveloped countries (CDC, 2019).

For infecting organisms to survive and repeat the infection cycle in other hosts, they (or their progeny) must leave an existing reservoir and cause infection elsewhere. Infection transmission can take place via many known likely routes (GBC, 2014; Microbiology book. Org, 2017) either by; Droplet contact, also known as the *respiratory route*, (Clevland Clinic 2022; NIPA, 2023) and the resultant infection that results is called airborne disease. Tricycles are the most common means of transporting persons and goods within abakaliki, in Ebonyi state. People from all works of life patronize tricycles on daily basis. From the early hours to late ours of the day. This serves as a good means of transferring infectious agent from a carrier to an unsuspecting individual. Fecal-oral transmission, is another means of disease transmission wherein foodstuffs or water become contaminated either (by people not washing their hands before preparing food, or sewage not properly treated going into a drinking water supply) and the people who eat and drink them become infected, Sexual transmission, with the result being called sexually transmitted infection, Oral transmission, diseases that are transmitted primarily by oral means may be caught through direct or indirect contact such as by sharing a drinking glass or a cigarette (Mada and Alam, 2019; NIH, 2007).

MATERIAL AND METHODS

Instruments

The following equipment/instrument was used: Autoclave (Olympic company), Microscope (Olympic company), wire loop, Bunsen burner, autoclave, oven, incubator, weighing balance, spatula.

Chemicals and Reagents

The following reagents and chemicals that were used in this study includes: sterile water, Dimethyl Sulphur Oxide, normal saline.

Media

The following media were used for this research work, Nutrient broth, Nutrient agar and Muller Hinton aga Eosin methylene Blue agar, mannitol salt, Macconkey agar, salmonella-shigella agar. All media were prepared aseptically according to the manufacturers' instruction.

Disinfectants
The following disinfectants were used in this work, De©, IZ©,Z©.

Study Area
The study area is Abakaliki town in Ebonyi State located in south eastern part of Nigeria. It is located 64 kilometers southeast of Enugu. Abakaliki is situated on latitude $6^0$20'N and longitude $8^0$06E.

Sample Collection
Swabs were taken from sensitive area in the commercial tricycles used in abakaliki metroplolis (handles). The samples were transported to the microbiology laboratory of EBSU, Abakaliki where they were analyzed following standard techniques in Microbiology (Anie et al., 2017).

3.3 Analysis of Samples
Samples were streaked on freshly prepared sterile media after undergoing a tenfold serial dilutions. They were all incubated for 18 to 24 hours at 37^0c (AOAC, 1995).

Determination of Aerobic Plate Count
Standard plate count method proposed by AOAC, (2004) was used to determine the total aerobic colony count of the samples. Only plates with moderate growth were counted. The average microbial loads of the samples obtained were expressed as colony forming units per Milliliter (Cfu/ml).

Isolation and Identification of Bacteria Isolated from Tricycles
Mac Conkey agar, Eosin methylene Blue agar, mannitol salt and salmonella – shigella agar were employed for the isolation of bacteria for the purpose of identification. Mac Conkey agar was used to isolate lactose fermenting gram negative bacteria, Eosin methylene Blue agar was used for the selective isolation of enteric coliforms, mannitol salt agar was used for the selective isolation of salt-tolerant bacteria and salmonella – shigella agar was used for the isolation of enteric bacilli particularly *Salmonella and Shigella* species. All plates were incubated at 37^0C for 24 hours. Identification of bacteria isolates was based on the standard culture, morphological and biochemical methods (CLSI, 2015).

RESULTS
Out of the fifty (50) tricycles sampled only thirty Seven (37) had visible growth. The colony count is as shown in Table 1 below. The bacterial count revealed that the examined tricycles analyzed had a bacterial load that ranges from 3.2 x 10^7 to 1.1.2 x 10^7.

Table 1: Colony count from commercial Tricycles.

S/no	Sample code	Colony count/colony forming unit
1	K1	16 (3.2x 10 ^{4})
2	K4	48 (9.6 x 10 $^{4)}$
3	K6	56 (1.12 x 10^5)
4	K7	104(2.08 x 10^5)
5	K8	80(1.6 x 10^5)
6	K9	56(1.12 x10^5)
7	K10	68 (1.36 x 10^5)
8	K11	112 (2.24 x10^5)
9	K12	88 (1.76 x 10^5)
10	K13	92 (1.84 x 10^5)
11	K14	96 (1.92 x 10^5)
12	K15	54 (1.08 x10^5)
13	K16	119(2.38 x 10^5)
14	K17	86 (1.72x 10^5)
15	K18	45(9.0 x 10^4)
16	K19	43(8.6 x 10^4)
17	K20	23(4.6 x 10^4)
18	K21	54 (1.08 x 10^5)
19	K23	64 (1.28 x10^5)
20	K27	98 (1.96 x 10^5)
21	K28	105 (2.10 x 10^5)
22	K30	26 (5.2 x 10^4)
23	K31	28 (5.6 x 10^4)
24	K32	80 (1.6 x 10^5)
25	K33	56 (1,12 x10^5)
26	K34	68 (1.36 x 10^5)
27	K35	112 (2.24 x10^5)
28	K36	88 (1.76 x 10^5)
29	K37	92 (1.84 x 10^5)
30	K38	96 (1.92 x 10^5)

31	K40	115(2.3x10^5)
32	K41	98 (1.96 x10^5)
33	K45	45 (9.0x10^5)
34	K47	43 (8.6 x 10^5)
35	K48	23 (4.6x10^4)
36	K49	54 (1.08 x 10^5)
37	K50	64(1.28 x 10^5)

The percentage distribution of isolates from the commercial tricycles was determined as shown in Table 2 below. *S. aureus* was the highest with 35(77.7) followed by *Eschericia coli* with value 9(20). The least was *Klebsiella pneumonia* with 1(2.22).

Table 2: Percentage distribution of isolates from the Commercial Tricycles.

S/No	ISOLATES FROM MOBILE PHONES	PERCENTAGE DISTRIBUTION
1	*S. aureus*	35(77.7)
2	*Klebsiella pneumonia*	1(2.22)
3	*Eschericia coli*	9(20)
	TOTAL : 45	

The inhibition Inhibition Zone Diameter of Isolates from Commercial Tricycle with IZ© was determined. The entire isolates were resistant to this particular dis infectant, this result is as shown in Table 3 below.

Table 3: Inhibition Zone Diameter OF Isolates from Commercial Tricycle With IZ©.

Isolates	Inhibition zone diameter (mm)/ concentration (mg/ml) 100 50		25
S. aureus	Nil	Nil	Nil
E. coli	Nil	Nil	Nil
K. pneumonia	Nil	Nil	Nil

In Table 4 below, it shows the result of the isolates against another disinfectant DE©. It was observed that the antibacterial agent was able to inhibit the growth of the isolates giving the highest inhibition zone diameter of 40mm at 100mglml and 25mm at 50mg/ml for *S. aureus*. For E. coli 100mg/ml concentration gave an IZD of 20mm whereas 25mg/ml equally showed an IZD of 20mm. *K. pneumoniae* had the same value for 100mg/ml and 50mg/ml which is 20mm whereas 25mg/ml exhibited an IZD of 25mm.

Table 4: Inhibition Zone Diameter Of Isolates from Commercial Tricycle With De©.

Isolates	Inhibition zone diameter (mm)/ concentration (mg/ml) 100 50		25
S. aureus	40	25	30
E. coli	20	23	20
K. pneumonia	20	20	25

In Table 5 below, it shows the result of the isolates against another disinfectant Z©. It was observed that the antibacterial agent was able to inhibit the growth of the isolates giving the highest inhibition zone diameter of 40mm at 100mglml and 25mm at 50mg/ml for *S. aureus*. For E. coli 100mg/ml concentration gave an IZD of 20mm whereas 25mg/ml equally showed an IZD of 35mm. *K. pneumoniae* had the value of 33mm for 100mg/ml and 50mg/ml the IZD value was 15mm whereas 25mg/ml exhibited an IZD of 12mm.

Table 5: Inhibition Zone Diameter OF Isolates from Commercial Tricycle Z ©.

Isolates	Inhibition zone diameter (mm)/ concentration (mg/ml) 100 50		25
S. aureus	40	25	19
E. coli	20	35	25
K. pneumonia	33	15	12

DISCUSSION

This research work examined 50 tricycles that ply the Abakaliki metropolis to determine possibility of transmission of diseases through fomites like tricycle. Out of the 50 tricycle examined only 37 tricycles had visible growth. The total viable count ranged from3.2 x 10^7 to 1.1.2 x 10^7. Morphological and biochemical characteristics of bacteria isolates from the samples after

comparison with the Bergey's Determinative Bacteriology Manual (1994) shows that a total **of** three isolates were identified and they include; *Staphylococcus aureus, Eschericia coli* and *Klebsiella* spp. Predominantly *Staphylococcus aureus* was isolated from most of the tricycles examined. This is observed from the result of the percentage distribution of isolates from the commercial tricycles, *S. aureus* was the highest with 35(77.7) followed by *Eschericia coli* with value 9(20). The least was *Klebsiella pneumonia* with 1(2.22). This result obtained is similar to that carried out by Abdulwasiu et al (2022) on handles of doors from buildings. Similar research reported found out that 84.6% of isolates from faucets examined were gram positive cocci with only 15.4%-gram negative cocci. Vehicle transmission by an inanimate reservoir (food, water, soil)(Acevedo et al., 2019) has been equally reported by some credible researchers which mainly washing one's hands appears to be an effective way to prevent the spread of infectious disease(Reddy et al., 2012), wearing gowns, and wearing face masks can help prevent infections from being passed from one person to another. Aseptic technique was introduced in medicine and surgery in the late 19th century and greatly reduced the incidence of infections caused by surgery. Frequent hand washing remains the most important defense against the spread of unwanted organisms. The isolates from the tricyles were tested against locally known and used disinfectant to determine its efficacy against the bacterial isolates. The result of the isolates against disinfectant DE© showed that the antibacterial agent was able to inhibit the growth of the isolates giving the highest inhibition zone diameter(IZD) of 40mm at 100mglml and 25mm at 50mg/ml for *S. aureus*. For E. coli 100mg/ml concentration gave an IZD of 20mm whereas 25mg/ml equally showed an IZD of 20mm. *K. pneumoniae* had the same value for 100mg/ml and 50mg/ml which is 20mm whereas 25mg/ml exhibited an IZD of 25mm. The result of the isolates against another disinfectant Z©. It was observed that the antibacterial agent was able to inhibit the growth of the isolates giving the highest inhibition zone diameter of 40mm at 100mglml and 25mm at 50mg/ml for *S. aureus*. For E. coli 100mg/ml concentration gave an IZD of 20mm whereas 25mg/ml equally showed an IZD of 35mm. *K. pneumoniae* had the value of 33mm for 100mg/ml and 50mg/ml the IZD value was 15mm whereas 25mg/ml exhibited an IZD of 12mm. This finding is in line with Olowe et al., (2004) that observed inhibitory effect on some of the disinfectants examined. Disinfectant IZ© had no inhibitory effects on the isolates from the tricycles. This is likely to be a case of bacterial resistance to the disinfectant used. Antimicrobial substances in form of antiseptics, (which are applied to living tissue/skin), disinfectants(which destroy microorganisms found on non-living objects), antibiotics, called prophylactic when given as prevention rather as treatment of infection. However, long term use of antibiotics leads to resistance of bacteria. While humans do not become immune to antibiotics, the bacterium does become immune to antibiotics as it develops resistance to the antibacterial agent. Thus, avoiding the usage of antibiotics longer than necessary helps to prevent bacteria from forming mutations that aide in antibiotic resistance (Peterson, 1990; Pinsky and Hayden, 2019).

This findings has further showed that indeed fomites harbor bacteria and can serve as a means of transferring infectious materials but constant cleaning of the surfaces of this commercial tricycle can prevent some communicable diseases and hence control epidemic in the long run.

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