

ENTERIC BACTERIA OF PUBLIC HEALTH SIGNIFICANCE ISOLATED FROM ZARIA METROPOLIS DUMPSITE SOIL

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ABSTRACT

Wastes in dumpsites with no proper waste handling method are a source of pathogens to the soil, which in turn contribute to the emergence of community-acquired infections. Given the lack of data on the pathogens in dumpsites, this study isolated and identified enteric pathogenic bacteria found in dumpsite soils in Zaria Metropolis. Two hundred and twelve samples were collected from four dumpsites in Samaru, Sabon-Gari, Tudun-Wada and Zaria City over a period of twelve months (November 2014– December 2015) for isolation and characterization of enteric pathogenic bacteria. The organisms were isolated by the use of cultural methods on selective media and characterized using a series of biochemical tests and confirmed using microgen identification kits. Results were statistically analysed. Bacteriological analysis of the soil samples revealed a total of 178(84.0 %) isolates among which 46(21.7 %) enteric pathogenic bacteria identified in decreasing order of prevalence, *Escherichia coli* O157:H7 21(9.9 %), *Salmonella* spp. 18(8.5%), *Vibrio cholerae* non-O1 7(3.3 %). Other Gram-negative species were also identified. Samples from Tudun-Wada location have the highest occurrence of the target isolates 15(28.3%). The occurrence of enteric pathogenic species at various dumpsites points out to the faecal contamination of waste buy human or animals. *Salmonella*, *Escherichia coli* O157:H7 and *Vibrio cholerae* are associated to clinical diseases. Prompt attention and immediate action on the appropriate treatment of the dumpsites is recommended.

Keywords: Enteric pathogenic bacteria, Gram-Negative, solid waste management, health risks of waste dumpsite

INTRODUCTION

Zaria Metropolis is an urban city densely populated with a lot of economic and social activities such as markets, institutions and industries. This has created a steady growth in population and human activities which result in the indiscriminate littering and dumping of refuse which constitute a daily nuisance in many of our communities.

Over 5.2 million people, who include 4 million children, die each year from waste related diseases and about 57 to 85 % of the wastes generated worldwide are disposed in dumpsites devoid of effective treatment (Uche *et al.*, 2010). These indiscriminate disposals sometimes contribute to flooding or outbreak of disease epidemic such as gastroenteritis which is a common feature in this city during rainy seasons making pollution a serious problem and a negative impact on the public health (Interim Management Committee, 2011).

Wastes are revealed to be one source of pathogens to soil because most dumpsites do not have leachate collection system.

Bacterial pathogens may develop in wastes undergoing decomposition in soils that suffer from environmental pollution as a result of indiscriminate disposal of pollutants. These bacterial pathogens, when increased in population, pose great risk to human health (Onweremadu *et al.*, 2009; Awisan *et al.*, 2011). Soil-transmitted pathogens play an important role to the emergence of community-acquired infections, contributing to the burden of communicable disease morbidity and mortality.

Waste dumping in Zaria metropolis cut across approved and non-approved dump sites. Composition of these dump sites are generated from, residential, commercial, market, industries, hospitals, etc. After waste is generated, waste workers collect and dispose such waste along road-side, around residential area, on drainages or in a government approved dumpsite which are sometimes close to sources of drinking water or playground of children and their proximity often violate the requirement stipulating the buffer distance (300 mm). It has become a continuous threat to the environment by gradually contaminating the soil, water, the atmosphere and all that depend on the environment for survival such as plants, animals and humans.

During waste collection and disposal most waste workers in developing countries hardly use protective devices. This unproductive condition may make them vulnerable to serious health problems (Odeyemi, 2012).

The bacterial pathogens in the soil and wastes are often not at the centre of public health concern today and little information is available on the types of microorganisms associated with and isolated in waste dumpsite soil (Awisan *et al.*, 2011). Consequently, comprehensive assessments on pathogenic organisms must be established to build local knowledge about public health issues and trends in dumpsites.

This study was aimed to isolate and identify some enteric pathogenic bacteria found in some settlements in Zaria Metropolis dumpsite soil.

MATERIALS AND METHODS

2.1 Study Area

The survey was conducted in Zaria Metropolis, Nigeria. The locations were Samaru, Sabon-Gari, Tudun-Wada and Zaria City. Zaria Metropolis is located on the high plains of Northern Nigeria, on latitudes 11°07' N to 11°51' N and longitudes 7°43'E to 7°45' E (Uba *et al.*, 2013). These are semi-urban with a high population density with the attendant generation of wastes. These wastes are seen disposed within the community owing to the poor waste disposal systems posing a risk of transmission of various disease agents.

2.2 Collection of Samples

Soil samples were collected from around the refuse dump sites as suggested by Isirimah *et al.* (2005). At each sampling site, surface debris was removed and soil was dug to a depth of 15cm using a hand trowel. Soil was then scooped into a sterile low density polythene bag and transported in cool boxes to the Bacteriology Laboratory in the Department of Microbiology, Ahmadu Bello University, Zaria for analysis. Samples were stored at 4 °C if not analysed immediately.

2.3 Cultural Isolation of the Enteric Pathogenic Bacteria

Twenty five grams (25 g) of soil was suspended in 225 ml sterile distilled water and mixed using a flame sterilized glass rod. 1ml of the resultant solution was inoculated into 9ml of sterile alkaline peptone water (pH 8.6), tryptone soy broth and selenite-F-broth in duplicate in sterile McCaurney bottles and incubated at 37 °C for 24 h. Aseptically, inoculations were made from the overnight samples on prepared sterile plates of Thiosulphate-Citrate-Bile-Salt-Sucrose (TCBS), Eosin Methylene Blue (EMB) *Salmonella Shigella* (SSA) agar. All inoculated plates were incubated aerobically at 37 °C for 24 h and observations were taken thereafter. Any distinct colonies formed were Gram-stained. Pure isolates were maintained on Nutrient Agar (NA) slants at 4 °C for further laboratory investigation. Biochemical tests were performed using the procedures of Cheesbrough (2006). All media were prepared using instruction manuals of their manufacturers. Discreet colonies showing greenish metallic sheen with dark centres, small red and/or colourless colonies with black centres, yellow (sucrose-fermenting) and blue-green (non-sucrose fermenting) colonies were picked as presumptive *E. coli* O157:H7, *Salmonella* spp. and *Vibrio* spp. respectively. The presumptive isolates were subjected to routine IMViC tests (Indole, Methyl red, Voges Proskaur and citrate utilization tests), oxidase test, string test among other tests. Isolates giving atypical responses for any of the above named tests were examined further using MICROGEN GNA+B-ID test kit. The data obtained by the Microgen GNA+B-ID microwell strip was designed to generate a 4 digit octal code for Enterobacteriaceae and 9 digit octal code for Vibrionaceae which was used to interpret the result from the Micogen Identification System Software (World Health Organization/Centers for Disease Control and Prevention/ United States Agency for International Development, WHO/CDC and P/USAID, 2003). It is worth noting that not all the presumptive discreet colonies that gave atypical responses for any of the above named tests that were identified as target pathogens. Micogen Identification System Software identified some as other Gram-negative pathogenic bacteria species among which *Acinetobacter Baumannii*, *Citrobacter sakazakii*, *Citrobacter freundii*, *Citrobacter sakazakii*, *Enterobacter Liquefaciens*, *Salmonella* Typhi, *Salmonella* Arizonae, *Salmonella* Pullorium, *Hafnia alvei*, Non-O157:H7 *Escherichia coli*, *Proteus mirabilis*, *Morganella morganii*, *Pseudomonas* spp., *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Klebsiella ozaenae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*

2.3.1 Identification of *E. coli* Serogroup O157 using the M44 MICROGEN[®] E. COLI O157

A smooth suspension of the presumptive *E. coli* O157:H7 grown on Sorbitol MacConkey agar for 24 h at 37°C was prepared in two wells of an agglutination slide. The slide was rocked gently for 30 seconds and observed for agglutination. If there was no

agglutination in either well, a drop of MicrogenR *E. coli* O157:H7 Test Latex (M44 a) was added to one well and one drop of control Latex (M44 b) to the other. The slide was then rocked gently for 2 min. An obvious agglutination only in the well containing the test latex indicates a positive result.

2.3.2 Serological identification of *Vibrio cholerae* using *Vibrio cholerae* O1 and *Vibrio cholerae* O139 Antisera

Three (3) colonies of the overnight bacterial growth on Nutrient agar at 37 °C were suspended in 0.5 ml physiological saline and use antigenic suspension. A drop of antiserum and physiological saline (30 ul) as a control were placed onto a clean glass slide partitioned into several parts. The antigenic suspension was placed onto the serum and the physiological saline on the glass slide. The reagents were then mixed by tilting the glass slid back and forth for 1 min to see if there was agglutination. Only strong agglutination observed within 1min in the reaction with each serum was regarded as positive. Delayed or weak agglutination was regarded as negative (Cheesbrough, 2006). All the test organisms with negative polyvalent sera were retested by heating antigen suspension as follows. Three colonies of the bacterial growth was suspended in 3 ml physiological saline and heated at 121 °C for 15 minutes. The heated solution was then centrifuged at 900 rpm for 20 mins, the supernatant was discarded and the precipitate was suspended with 0.5ml physiological saline and used as heated cell suspension. Only Polyvalent sera that showed negative results with the heated antigen suspension were identified as *Vibrio cholerae* non-O1.

Data Analysis

Laboratory findings were subjected to Chi-square (χ^2) analysis with the IBM SPSS Statistics Version 21 at p=0.05. Results were simplified in tables.

RESULTS

There were 178 total isolates grown from the 212 soil samples collected (Table 1). Three target species were confirmed to be enteric pathogenic bacteria, *Escherichia coli* O157:H7, *Salmonella* spp. and *Vibrio cholerae* non-O1 with 9 other different Gram-Negative species identified. *Enterobacter cloacae*, *Citrobacter* spp., *S. Typhi*, *Pseudomonas aeruginosa*, *Proteus vulgaris* *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella* spp. and *Vibrio* spp. (Table 2).

The highest occurrence of the target isolates was obtained in Tudun-Wada 15(28.3 %), followed by Zaria City 13(24.5 %) and Sabon-Gari 11(20.8 %). Samaru had the least target bacterial isolates 7(13.5 %) [Table 3]

Table 1: Occurrence of Bacterial isolates at Various Sampling Locations in Zaria Metropolis

Bacteria spp. Isolated	Sampling Locations				Total Frequency N=212 (%)
	Sabon-Gari N=53 (%)	Samaru N=53(%)	Tudun-Wada N=53 (%)	Zaria City N=53 (%)	
<i>Acinetobacter iwoffii</i>	0(0.0)	1(1.9)	0(0.0)	2(3.8)	3(1.4)
<i>Acinetobacter Baumannii</i>	2(3.8)	1(1.9)	2(0.9)	1(1.9)	6(2.8)
<i>Citrobacter sakazakii</i>	1(1.9)	2(3.8)	0(0.0)	1(1.9)	4(1.9)
<i>Citrobacter freundii</i>	2(3.8)	0(0.0)	1(1.9)	2(3.8)	5(2.4)
<i>Enterobacter Liquefaciens</i>	0(0.0)	2(3.8)	1(1.9)	0(0.0)	3(1.4)
<i>Escherichia coli O157:H7</i>	5(9.4)	3(5.7)	9(17.0)	4(7.5)	21(9.9)
<i>Salmonella spp.</i>	3(1.4)	2(0.9)	7(13.2)	6(11.3)	18(8.5)
<i>Salmonella Typhi</i>	0(0.0)	0(0.0)	2(3.8)	1(1.9)	3(1.4)
<i>Salmonella Arizonae</i>	1(1.9)	1(1.9)	3(5.7)	1(1.9)	6(2.8)
<i>Salmonella Pullorium</i>	1(1.9)	2(3.8)	1(0.9)	3(5.7)	7(3.3)
<i>Hafnia alvei</i>	0(0.0)	3(5.7)	0(0.0)	1(0.5)	4(1.9)
<i>Non-O157:H7 E. coli</i>	8(15.1)	6(11.3)	14(26.4)	9(17.0)	37(8.7)
<i>Proteus mirabilis</i>	3(5.7)	1(1.9)	4(7.5)	3(5.7)	11(5.2)
<i>Morganella morganii</i>	0(0.0)	0(0.0)	1(1.9)	1(1.9)	2(0.9)
<i>Pseudomonas spp.</i>	2(3.8)	3(5.7)	2(0.9)	2(3.8)	9(4.2)
<i>Vibrio cholerae non-O1</i>	1(1.9)	0(0.0)	3(5.7)	3(5.7)	7(3.3)
<i>Vibrio parahaemolyticus</i>	2(3.8)	2(3.8)	1(1.9)	1(0.5)	6(2.8)
<i>Vibrio vulnificus</i>	2(3.8)	0(0.0)	0(0.0)	1(0.5)	3(1.4)
<i>Vibrio alginolyticus</i>	0(0.0)	1(0.5)	1(1.9)	2(3.8)	4(1.9)
<i>Klebsiella oxytoca</i>	1(1.9)	2(0.9)	2(3.8)	1(1.9)	6(2.8)
<i>Klebsiella pneumonia</i>	2(3.8)	1(1.9)	3(5.7)	3(5.7)	9(4.2)
<i>Klebsiella ozaenae</i>	1(1.9)	1(1.9)	2(3.8)	0(0.0)	4(0.9)
Total	37(69.8)	34(64.1)	59(111.3)	48(90.6)	178(84.0)

Total p<0.05

KEY: N=Total number of samples analysed

Table 2: Target Enteric Pathogenic Bacteria and other Gram-negative Bacteria Isolates in Zaria Metropolis dumpsite soil

Target Enteric Pathogenic Bacteria	Other Gram-negative Pathogenic Bacteria
<i>Escherichia coli O157:H7</i>	<i>Acinetobacter iwoffii</i>
	<i>Acinetobacter Baumannii</i>
	<i>Citrobacter sakazakii</i>
	<i>Citrobacter freundii</i>
<i>Salmonella spp.</i>	<i>Enterobacter Liquefaciens</i>
	<i>Salmonella Typhi</i>
	<i>Salmonella Arizonae</i>
	<i>Salmonella Pullorium</i>
	<i>Hafnia alvei</i>
	<i>Non-O157:H7 Escherichia coli</i>
	<i>Proteus mirabilis</i>
	<i>Morganella morganii</i>
	<i>Pseudomonas spp.</i>
	<i>Vibrio cholerae non-O1</i>
<i>Klebsiella pneumonia</i>	
<i>Klebsiella ozaenae</i>	
<i>Vibrio parahaemolyticus</i>	
<i>Vibrio vulnificus</i>	
	<i>Vibrio alginolyticus</i>

Table 3: Occurrence of the Target Enteric Pathogenic Bacteria in Waste Dumpsite in Zaria Metropolis

Source	No of samples	No of isolates	Percentage organisms isolated			Total Frequency
			<i>Escherichia coli O157:H7</i>	<i>Salmonella spp.</i>	<i>Vibrio cholerae non-O1</i>	
Samaru	53	7	3(5.7 %)	4(7.5 %)	0(0.0 %)	7(13.5 %)
Sabon-Gari	53	11	4(7.5 %)	6(11.3 %)	1(1.9 %)	11(20.8 %)
Tudun-Wada	53	15	8(8.0 %)	4(7.5 %)	3(5.7 %)	15(28.3 %)
Zaria City	53	13	6(11.3 %)	4(7.5 %)	3(5.7 %)	13(24.5 %)
Total	212	46	21(9.9 %)	18(8.5 %)	7(3.3 %)	46(21.7 %)

DISCUSSION

The occurrence of enteric pathogenic and other Gram-negative bacteria in Zaria Metropolis dumpsite soil suggests a potential risk to the public health. *Escherichia coli O157:H7*, *Salmonella spp.* and *Vibrio cholerae* are among the most common and notorious pathogens causing variety of infectious diseases responsible for significant morbidity and mortality (Wachukwu *et al.*, 2002); Barkocy-Gallagher *et al.*, 2004; Cheesbrough, 2006). Other bacterial species such as *Citrobacter spp.*, *Proteus spp.*, *Klebsiella spp.*, *Enterobacter spp.*, *Escherichia coli*, *Pseudomonas* etc. were also isolated, which implied that the waste dump soils were heavily contaminated.

Vibrio cholerae and some species of *Salmonella* are often excreted out of the body through stool. *Escherichia coli* is one of the most predominant organisms that is excreted out of the body through stool and sometimes urine. Their presence in waste is an indicator of possible contamination of the refuse dumps by human or animal faeces since it was common practice to dump human excreta in the sites which could also be used as latrines at night. These organisms have also been isolated by other researchers who worked with waste dumps in other parts of the country like (Mbata, 2008; Oviasogie *et al.*, 2010; Wachukwu *et al.*, 2010; Ikpeme *et al.* 2011) Osunwoke & Kuforiji, 2012). There are a number of major risks and impacts of solid waste on the environment. Improper disposal of untreated municipal solid wastes is not only harmful to human health but also constitute a threat to the ecology of any environment (Obire *et al.*, 2002). In many dumpsites, the waste is directly increasing global concern over the public health impacts attributed to environmental pollution, in particular, the environmental quality and human health risks associated with the waste dumps. The air emissions and leachates generated as a result of decomposition of waste may contaminate air, surface and groundwater sources. The World Health Organization estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution (WHO, 2011).

Out of the genera of organisms isolated, *Escherichia coli* have the highest prevalence 58(27.4 %). Van Elsas *et al.* (2011) in a separated study conducted in India, also reported high incidence of *Escherichia coli*. The high prevalence of *Escherichia coli* could be linked to its ability to withstand competition from the other indigenous microorganisms with higher growth rates. Majority of *Vibrio cholerae* strains in the environmental reservoir have been observed to belong to the non-O1/non-O139 serogroups (Smritikana *et al.*, 2012). The ability of *Vibrio cholerae* to survive within and outside the aquatic environment makes cholera a complex health problem to manage (Osei & Duker, 2008). Cheesbrough (2006) reported that cholera is endemic in parts of Africa, South and Southeast Asia where sanitation and hygiene

are inadequate and water supplies are contaminated and not treated.

The occurrence of these pathogenic Gram-negative bacteria isolated and identified suggest that there is a significant sanitary risk, especially to the waste collectors, scavengers and those leaving in close proximity to the dumpsites. There is a risk for these people to develop gastrointestinal infections. However, diseases such as bacteraemia, typhoid fever, urinary tract infections, cholera, respiratory infections and other opportunistic infections can also occur, especially for immunosuppressed individuals.

The cattle, dogs and chicken living nearby could also be at risk. There are farms very close (less than one kilometre) to the waste dumps and even cattle graze and drink a few meters away from some of these waste dumps. Several bacteria strains isolated from these waste dumps have been reported to be pathogenic for bovine, pigs and birds (Flores-Tena *et al* 2007). These animals could also be a potential risk as pathogen dispersers. Much of the pathogenic bacteria are transmitted by infected hosts. However several pathogenic microorganisms are transmitted by environmental carriers. Osei & Duker (2008) hypothesized a high rate of contact of these pathogens through flies. Flies are attracted by the odour emanating from refuse dumps, especially the common housefly. This fly lives in close association with man feeding on all kinds of human food, garbage and excreta, and will travel no farther from its breeding site (refuse dumps) to the nearest resting place. Osunwoke & Kuforiji (2012) opined that truly pathogenic forms may survive in waste. In line with this statement, all the bacterial genera isplated from dumpsites in this study have been reported by Wachukwu *et al.* (2002) and Cheesbrough (2006) as potential pathogens. That is, they are capable of causing diseases. Therefore the occurrence of 46 enteric pathogenic bacteria and other Gram-negative pathogens in Zaria Metropolis dumpsite soil is a matter of public health:

Salmonella spp. that were isolated from all the dumpsites locations could have the potential of causing typhoid fever and food poisoning. Similarly, *Escherichia coli* cause Urinary Tract Infection (UTI) and gastroenteritis in children (Nazzal *et al.*, 2012; Matthew *et al.*, 2013). Another major organism is *Vibrio cholerae*, which is one of the organisms that cause cholera and vomiting (Fakhruddin, 2012) Infection caused by this pathogen can proceed to dehydration, electrolyte disturbances, renal failure, hypovolemic shock and subsequent death if immediate and proper management is not effected (Cheesbrough, 2006). A large number of *Vibrio cholerae* non-O1/non-O139 strains are able to produce cholera like toxins, i.e., heat-stable enterotoxin, thermostable direct haemolysin, hemaagglutinin/protease, shiga-like toxin play significant role in the enteropathogenicity (Butt *et al.*, 2004). *Pseudomonas aeruginosa* causes wound and burns infections and are recalcitrant to treat with some antibiotics (Wachukwu *et al.*, 2002). Enterohaemorrhagic *Escherichia coli* (EHEC) refers to a subset of Shiga toxin-producing *Escherichia coli* (STEC) strains is found to cause human and sometimes animal disease (Matthew *et al.*, 2013)

Mortalities can occur with these bacterial organisms in the form of secondary infections (Wachukwu *et al.*, 2002). The number of these types of infections can directly link to dumpsite thereby leading to death. Proper disposal and effective management of waste is therefore needed that will reduce morbidity and mortality.

Conclusion

Bacteriological analyses results confirmed the presence of *Escherichia coli* O157:H7, *Salmonella* spp. and *Vibrio cholerae* non-O1 in Zaria Metropolis dumpsite soil. Other important isolates include *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli*.

To avoid further microbial contamination, the dumpsite facility should include features for control and containment of wastes. This includes aseptic collection, transport and disposal of waste. Waste collectors must also be equipped with personal protective equipment such as coat, gloves, hat and mask while garbage trucks must have covers to ensure a lack of contaminating microorganisms during the transport of garbage.

Future studies should be conducted utilizing other tests, performing antimicrobial profile of each bacterial pathogen isolates. These studies should also prove fly and animal transmission of the pathogens to humans and correlating the bacterial pathogens isolated from the dumpsite soil to the soil-borne diseases present among dumpsite villagers and waste collectors in the transmission of diseases.

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