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Molecular Identification of *Escherichia coli* O157:H7 Isolated from Biomedical Waste in General Hospital Dutse, Jigawa State, Nigeria

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Abstract
Biomedical waste materials produced in hospitals and clinics usually harbour infectious microorganisms, which can pose a threat to societal health and well-being, especially when improperly disposed of. Escherichia coli O157:H7 is a very virulent pathogen of man and animals, with a high prevalence due to a very low infective dose. The present study was conducted between July to September, 2021 on biomedical waste in General Hospital Dutse to detect Escherichia coli O157:H7. A total of 200 samples were randomly collected from the different units of the hospital and analysed using standard microbiological, biochemical and molecular techniques. The frequency of occurrence of Escherichia coli was 73 (36.5%) out of which Escherichia coli O157:H7 were 20 (10%) and others were 127 (63.5%). The detected Escherichia coli O157:H7 was subjected to tests for the virulence genes stx_1 and stx_2, Four (4) of the samples were found to possess the virulent gene stx_2 alone while the other had neither. The presence of presumptive Escherichia coli O157:H7 in the waste samples may directly point to improper waste management practices and may result into outbreak of disease is the waste materials are not disposed of sanitarily. It is therefore recommended that more attention should be placed on the waste management practices employed in the hospital and a critical review of the already existing *practices to tally with international standards.*

Keywords: *Escherichia coli,* O157:H7, Biomedical waste, stx_1, stx_2, STEC, Hospital waste.

INTRODUCTION
Medical waste is generated as a by-product of healthcare workers during surgeries, dental work and laboratory processes (Windfeld & Brooks, 2015). It is likely to be infectious, or potentially infectious, and it is often contaminated with bodily fluids in some way – but the term can also be used to refer to general waste from any medical practice, as well as specific waste streams typically found in the medical industry (WHO, 2018).

WHO (2018) has divided medical waste into various categories that include infectious waste which constitutes anything infectious or contaminated, sharps like needles, scalpels, broken glass and razors, and pathological waste which comprises human or animal tissue, body parts, blood and fluids. The categories also include pharmaceutical waste, genotoxic waste, radioactive waste, chemical waste and other hazardous toxic waste that may

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carcinogenic, mutagenic or teratogenic. The improper management of medical waste could cause serious environmental problems in terms of air, water and land pollution (Jang *et al.,* 2006). Medical waste that is disposed of improperly can contaminate groundwater sources, produce air pollutants and get into the ecosystems of wildlife (Laustsen, 2007). This phenomenon has also been shown to confer antibiotic resistance in bacteria (Anwar *et al.,* 2020). According to Kermode (2004), WHO has also stated that there are at least 16 billion injections administered annually, a great percentage of which are disposed of improperly. The negative impact of improper medical waste disposal on human health is staggering (Anwar *et al.,* 2020). The potential transmission of infectious diseases from needle stick injury or contamination is a primary concern due to medical illnesses and diseases that may be caused by improper disposal of medical waste (Hutin *et al.,* 2003): Respiratory infections such as tuberculosis, *Streptococcus pneumonia*, and viruses like measles, all of which can be transmitted through improper disposal or outright illegal dumping of infectious waste (Chamberlain, 2018). Human Immune-deficiency virus (HIV) and Acquired Immuno- Deficiency Syndrome (AIDS) are both transmitted through items contaminated with blood or body fluids. Furthermore, gastrointestinal infections such as *Salmonella,* helminths (parasitic worms), cholera, and *Shigella* are transmitted through materials contaminated with infected human faeces (PATH, 2021).

The very low infective dose of *E. coli* O157:H7 of less than ten organisms and several complementing virulence factors have made it the most important Shiga- Toxin-producing *Escherichia coli* (STEC) globally (Maule, 2000). The severity of the clinical symptoms, including chronic infant diarrhoea and kidney failure, as well as the ease of transmission of the organisms from reservoir organisms; mainly cattle as well as the human to human transmission, have further substantiated its place amongst the most clinically important bacteria (Majowicz, 2014). Additionally, it is one of the most common causes of acute or chronic watery diarrhoea, predominantly among infants, especially in a developing country like Nigeria. The constant development of antibiotic resistance by this particular strain has also been found to pose a very serious threat, this is because improperly treated waste and sewage containing these bacteria provides an avenue for the strains to pass on the antibiotic resistance genes to other organisms (Beattiea *et al.*, 2020). Several outbreaks have been reported, resulting from the consumption of contaminated foods which had led to outbreaks that have resulted in thousands of deaths due to chronic and bloody diarrhoea and kidney failure (Callaway.2014). There has also been a documented apparent lack of knowledge amongst hospital workers in Nigeria, coupled with the lack of hygiene and understaffing of most government hospitals which have made it almost impossible to properly address the issues of a possible outbreak as a result of *E. coli* O157H:7. Furthermore, the level of illiteracy amongst the general populace, especially those attending government hospitals and the limited knowledge of low-level hospital staff regarding the hazards associated with improper medical waste disposal, a lack of stringent government policy and other compounding issues may serve as a source of an outbreak of nosocomial origin. Subsequently, there is a critical need to assess the quality of the infectious wastes that are improperly treated or discarded in the hospital environment. This is to ascertain and possibly alert the authorities, the hospital management and the general populace regarding the possibility of an outbreak of massive proportions of diarrhoea, renal failure and other symptoms presented by *E. coli* O157:H7 patients.

MATERIALS AND METHODS

Description of the Study Area

General Hospital Dutse was established in 1972 as Dutse Comprehensive Health Care (CHC) by the old Kano State government. The CHC was converted to Dutse General Hospital in 1985 by the then Military Governor of Kano state Air commander Hamza Abdullahi Fss, Psc. Dutse general hospital covers an area of 500 m by 800 m and is currently a 200-bed capacity secondary healthcare facility serving as a referral centre for all Primary Health Centres (PHCs) in Dutse and Kiyawa Local Government Areas (Dogara and Ocheje, 2016). It is located between latitude 11°72'44"N and longitude 9° 36' 54"E of the equator covering an area of 27.68 acres or 0.11 km^2. It is one of the busiest hospitals in Jigawa state, partly because it is government owned and also because it is situated in the centre of the Dutse metropolis. The general hospital also serves the smaller towns of Kiyawa, Takai, Kachako and Sumaila. With the requirement by the WHO for there to be 5 beds per 1000 population to maintain the 75% optimal occupancy of a hospital, general hospital Dutse is very well overcrowded and over-utilized.

Sample Collection

Biomedical waste samples were collected and swabbed using sterile swab sticks moistened with 0.1% peptone water (Rached *et al.,* 2013). Sharp objects like syringe needles were collected using sterile tongs and placed into sterile sample containers with 250 ml of 0.1% sterile peptone water (Chetan *et al*., 2017). The surfaces of the incinerator and bins were first of all treated with Tween 80, to enable better recovery of organisms (Reitermayer, 2018). A total of six (6) visits were carried out over a period of two months to collect waste samples from the twenty three (23) units and three (3) waste dump sites in the hospital environment. Bandages were collected from Incinerator 2 and the open dumpsite, two (2) biosafety boxes were swabbed from Incinerator 2, two (2) blood transfusion kits, a single drug container, and an injection bottle, rubber drug sachet, three (3) syringes and also four (4) swabs of the incinerator surface. Dustbins were also swabbed from the central laboratory (6), chemical pathology laboratory (1), immunology laboratory (1), microbiology lab laboratory and the hospital side laboratory (3). Several samples were also collected from Incinerator 1, including bandage (1), capillary tube (1), glass slide (3), hand gloves (4), HIV test strip (1), injection bottles (2), rubber drug sachet (4), petri dish (1) and the surface of the incinerator was swabbed three times. As for the dumpsite, several samples were also collected, including blood transfusion kit (2), bandages (2), cotton wool (1), a catheter (1), drug container (2), food remains (2), hand gloves (3), maternity bag (1), plastic carpet (1), sanitary pad (4) and a water sachet. The number and types of samples collected from the wards of the hospital are shown in Table 1. All samples were immediately placed in an icebox and transported to the Microbiology Laboratory at Federal University Dutse for analyses.

Isolation and Identification of Bacteria

Samples collected on solid waste materials and surfaces using moistened swab sticks were inoculated onto already prepared Eosin Methylene Blue (EMB) agar plate by streaking (Rijal, 2021). After which it was incubated at 37 ^{o}C for a maximum of 48 hours. The plates were observed for green metallic sheen colonies (Divya *et al.*, 2016). The green metallic sheen colonies were obtained in pure cultures and also Gram stained as described by Cheesebrough (2006). Biochemical tests including indole, vogues-proskeur, methyl red and citrate utilisation were carried out as presumptive tests to identify *E. coli*. The positive strains were further screened for *E. coli* O157H:7 strain using cefixime-infused Sorbitol MacConkey agar. This differentiates the *E. coli* O157H:7 strains from other *E. coli* strains as well as *Shigella* spp which

may have the same biochemical characteristics. The plates were incubated at 37 ^{o}C checked for a period of 20 hours after which it was observed for colourless colonies (non-sorbitol fermenters) (Müller and Ehlers, 2005). The Polymerase chain reaction (PCR) technique was employed for the confirmation of *E. coli* O157H:7 by detecting the virulence genes stx_1 and stx_2 present in the *E. coli* O157H:7 as described by Dabo and Saleh (2018). The primer sequences used for the molecular analyses are shown in Table 2.

Table 1: Biochemical characterization of bacterial isolates

Cultural Characteristics			Biochemical Characterization				SF	Inference
Colour	Shape	GR	Indole	MR	VP	Citrate		
GMS	Rod Shaped	-	+	+	-	-	-	*E. Coli* O157H:7

KEY:- GMS: green metallic sheen, GR: Grams Reaction, MR: Methyl Red Test, VP: Vogues Proskeur Test, Citrate: Citrate Utilization Test, SF: Sorbitol Fermentation.

Table 2: The primer sequences used for the molecular analyses (Source: Dabo and Saleh, 2018)

Primer	**Sequence (5`-3`)**	**Target Gene**	**AS(bp)**
Stx1	ACACTGGATGATCTCAGTGG CTGAATCCCCCTCCATTATG	stx_1	641
Stx2	CCATGACAACGGACAGCAGTT CCTGTCAACTGAGCAGCACTTTG	stx_2	779

KEY:- AS (bp): Amplicon Size (base pair)

RESULTS

A total of eighty-five (85) dustbin contents were analysed, twenty-two (22) samples of grey water and seventeen (17) samples of toilet effluents were also collected and analysed from the different sections, making the total number of wastewater samples thirty-nine (39). The remaining samples were randomly collected from the three (3) different dumpsites in the hospital; swabs of two (2) blood transfusion kits, one (1) maternity bag, two (2) remains of food, two (2) used cotton wool balls, four (4) sanitary pads, three (3) empty drug containers, four (4) bandages, seven (7) hand gloves, one (1) water bottle and one (1) capillary tube were all collected and processed. Furthermore, four (4) sample containers of stool and urine, four (4) glass slides, one (1) HIV test strip, 3 syringes, 3 injection bottles, one (1) drug sachet, fifteen (15) biosafety boxes, one (1) catheter, one (1) plastic carpet and one (1) petri dish were all swabbed for processing. The surfaces of the different incinerators were also swabbed 7 times during different visits to check for the presence of the bacteria.

As depicted in Figure 1, out of the two hundred (200) samples collected only ninety-three (93) returned positive results for *E. coli*. These were confirmed using biochemical tests as previously described, and twenty (20) of the total *E. coli* were found to be *E. coli* O157:H7.

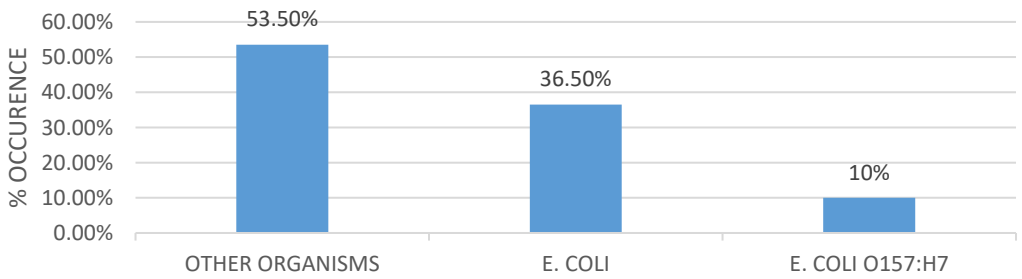

Fig 1: Total number of isolates obtained

The 10% *E. coli* O157H:7 strains was obtained from different samples, positive samples were obtained from dustbins each from the male, paediatric and dressing rooms of the accident and emergency ward, others were from the dustbins in the microbiology laboratory, chemical pathology laboratory, side laboratory, dental surgery unit, female medical ward, immunology laboratory and labour room. The remaining positive samples were obtained from an assortment of other samples, a bandage each from incinerator 2 and the dressing room dustbin, hand gloves from the open dump and incinerator. Others were isolated from an empty drug container from the open dump, toilet effluents from the female medical ward, dental surgery and three (3) from the paediatric ward. The samples from the paediatrics unit had the highest positive result for the bacteria, with three (3) positive samples from the eight (8) samples collected in the unit. This represents 37.5% positive results from the unit and also 15% of the total positive results from the entire hospital. Several other units turned up no positive results while other bacteria were isolated but not *E. coli* O157H:7. The frequency distributions of the samples and the isolates are represented in Tables 3 and 4.

Finally, five (5) of the twenty (20) positive isolates of *E. coli* O157H:7 were taken for molecular analysis. DNA was successfully obtained from all the samples through the process described, PCR and gel electrophoresis were respectively carried out. The results obtained indicate that four (4) of the selected five (5) samples possessed the virulent gene stx_2 only, while the remaining one (1) had neither stx_1 nor stx_2. This was indicated by the movement of the band from the four (4) isolates up to the 779 bp line as indicated by the DNA ladder on Plate I shown below.

Table 2: Frequency of occurrence of the isolates from biomedical waste

S/N	Unit	*E.coli 0157:H7*	*E.coli*	Other bacteria
1.	Accident and Emergency	2	2	5
2.	Antenatal	0	2	5
3.	Central Lab	0	2	4
4.	Chem. Pathology	1	0	0
5.	Compound	0	13	8
6.	Dental Surgery	2	5	4
7.	Dressing Room	2	4	3
8.	Ear, Nose and Throat Clinic	0	0	6
9.	Family Planning	0	1	0
10.	Female Medical Ward	2	0	4
11.	Female Surgical Ward	0	1	4
12.	Immunology	1	0	0
13.	Incinerator 1	1	7	12
14.	Incinerator 2	1	3	11
15.	Infectious Disease Clinic	0	0	4
16.	Isolation Unit	0	6	4
17.	Labour Room	1	0	5
18.	Male Medical Ward	0	2	2
19.	Male Surgical Ward	0	1	2
20.	Maternity	0	0	3
21.	Microbiology Laboratory	1	7	5
22.	Open Dump	2	8	10
23.	Paediatrics Unit	3	3	2
24.	Retainership Clinic	0	0	2
25.	Side Lab	1	1	1
26.	Theatre	0	5	1
	Total	20 (10%)	73 (36.5%)	107 (53.5%)

Table 3: Frequency of occurrence of the isolates for each sample type

S/N	Sample type	*E.Coli* O157:H7	Non *E.Coli* O157:H7	Other Bacteria
1.	Bandage	1	2	1
2.	Biosafety Box	0	1	14
3.	Blood Transfusion Kit	0	1	1
4.	Capillary Tube	0	0	1
5.	Catheter	0	1	0
6.	Cotton Wool	0	1	1
7.	Drainage	1	5	2
8.	Drug Sachet	0	1	0
9.	Dustbin	10	31	44
10.	Empty Drug Container	1	0	2
11.	Food Remains	0	2	0
12.	Glass Slide	0	3	1
13.	Grey Water	1	9	12
14.	Hand gloves	2	2	3
15.	HIV Test Strip	0	0	1
16.	Incinerator Surface	0	1	6
17.	Injection Bottle	0	0	3
18.	Maternity Bag	0	1	0
19.	Petri Dish	0	1	0
20.	Plastic Carpet	0	0	1
21.	Sample Container	0	1	3
22.	Sanitary Pad	0	1	3
23.	Syringe	0	0	3
24.	Toilet Effluent	4	9	4
25.	Water Sachet	0	0	1
	Total	20 (10%)	73 (36.5%)	107 (53.5%)

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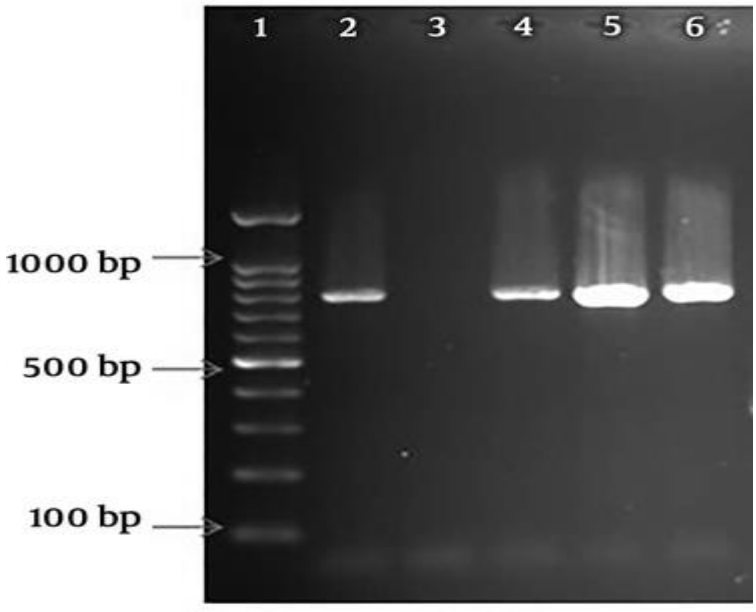

Plate I: Agarose gel showing PCR products of stx_1 and stx_2 virulence genes isolated from *E. coli* O157: H7
Lane 1= DNA 1000 bp marker; Lanes 2,3,4,5 and 6 =*E. coli* O157: H7strain

DISCUSSION

Apart from *E. coli* majority of the samples obtained in this research were positive for several other bacteria. *E. coli* was not isolated from one hundred and seven (107) samples. Other research carried out on hospital waste, especially those that undergo similar handling and disposal techniques have also recorded a very high microbial load (Manzoor and Sharma, 2019). Research conducted in Ghana reported a microbial load ranging from 0.036×10^3 CFU/mg to 0.167×10^3 CFU/mg in hospital waste. Moreover, several pathogenic organisms including *Escherichia coli* were all isolated from the waste (Egbenyah *et al.,* 2021). This high microbial load might be a result of the different types of waste from different sources that come into contact with the waste. The bacterial load in the rubber bins was high because there was a mixture of waste disposed of by patients that included used maternity bags, blood transfusion kits, infectious waste such as stool and urine samples, HIV test strips, leaves from the compound, sand and a lot of other assorted waste products. Additionally, initial objects that came into contact with the wounds, pus, stool, sputum, urine or other possibly pathogenic samples had not been mixed up with other waste materials that might be harmful to the microorganisms, such as chemicals or substances such as pharmaceutical waste, no exposure to extraneous situations, such as ultraviolet light from the sun, desiccation and drying due to low humidity. In addition, the high load of the waste in the dumpsite and incinerators may be attributed to further exposure of the waste to the environment, like aerosols and surface runoffs. The contents of these dumpsites were sometimes observed to be mixed up by patients or errant staff, thereby increasing the microbial load from units whose waste would have originally been quickly processed because of its contagious nature. It was also observed that some of the patients' relatives take their baths and also cook food very close to the area. Animals were grazed and rodents were also seen in the proximity. These are all possible vectors, carriers or shedders of microorganisms, though not all of the organisms may prosper or are pathogens of humans.

The total number of samples that were positive for *E. coli* was 73 (36.5%), thirteen (13) of these were found in the hospital compound representing 17.8% of the total number of samples positive for *E. coli*. This percentage is higher than what was obtained in similar research by Alwabr *et al.* (2016) who detected up to 12% prevalence of *E. coli* in dustbins and other hospital waste in Sana'a. It is also higher than the prevalence (10%) reported by Sharmin *et al.* (2016). Anitha and Indira (2012) in India, who equally reported a 15% prevalence of *E. coli* in biomedical waste. The prevalence of samples with *E. coli* was however lower than the 59.1% that was obtained by Atta *et al.* (2021). The presence of the bacterium can be attributed to its ability to resist difficult environmental conditions due to one of three methods of defence; the thickness of the cell wall, the ability to produce chemicals from inside the cell to oxidise and reduce the toxic substances probably present in the direct environs, and its ability to store food inside the cell which gives them a greater chance to survive for long periods and potentially reproduce in extra-intestinal environments (Jang *et al.,* 2017).

In 10% of the samples collected, *E. coli* O157:H7 was isolated. This incidence is a bit lower than the 15% incidence of *E. coli* obtained by Anitha and Indira (2012) in a similar research. The high prevalence of *E. coli* O157:H7 isolated in the paediatric samples is also similar to another research by Getaneh *et al.* (2021), where there was a 15.3% prevalence in children from a paediatric ward in Ethiopia. The high prevalence of *E. coli* O157:H7 may be due to the nature

of waste that was generated in the hospital, coupled with the fact that *E. coli* O157:H7 is one of the commonest causes of food poisoning globally. Moreover, in some cases, certain asymptomatic humans tend to shed the bacteria in their faeces (Ahn *et al.,* 2008). Furthermore, the paediatric ward having the highest prevalence of all the hospital wards may be associated with the fact that *E. coli* O157:H7 infections are most common in children under five (5) years and adults that are immune-compromised like old people. The high prevalence and mortality rate due to *E. coli* O157:H7 coupled with the low immunity of infants may also be a contributing factor (Belongia *et al.,* 1993). The prevalence of the bacterium in children under the age of two (2) has been associated with the high milk content of their diet. It has also been reported that children infected with *E. coli* O157:H7 excrete 10,000,000–100,000,000 viable cells in their stool (Cornick *et al.,* 2002). With children known to have several bowel movements daily and the infective dose of E. *coli* O157:H7 low, it is very easy for the bacterium to spread among children (Ahn *et al.,* 2008).

The presence of stx_2 but not stx_1 in three (3) of the five (5) isolates may not be unrelated to the findings of Gyles (2007) who interjected that the majority of strains of *E. coli* O157 produce *stx*2 only. The lack thereof of the two (2) genes, namely *stx*1 and *stx*2 is also substantiated by the findings of Schmidt *et al.* (1999), who were able to isolate one (1) strain of non-sorbitol fermenting *E. coli* O157:H7 that did possess neither *stx* gene.

The treatment methods, as well as the pre-treatment handling of the waste produced in General Hospital Dutse, are grossly insufficient as indicated by the results of this research. The sanity and hygiene of certain units of the hospital were quite good, as there were no positive isolates of *E. coli* O157:H7. However, the treatment and management processes in the hospital were inadequate, with the low infective dose of the bacterium and its ability to survive in some very extreme conditions, it poses a risk of causing an outbreak due to the proximity with humans, especially children (Asime *et al.,* 2020).

CONCLUSION

Samples of waste collected from General Hospital Dutse and screened for *E. coli* O157H:7 indicated that 53.5% of the samples did not have the presence of any *E. coli* isolates, 36.5% had other *E. coli* strains and only 10% of the samples was positive for *E. coli* O157:H7. Subsequently, only four (4) of the tested bacterial isolates possessed the virulence genes stx_1 *and* stx_2. Majority of the positive samples were obtained from the paediatric ward.

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