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Serology and Sensitivity Analysis of Verocytotoxigenic *Escherichia coli* O157 in Cattle and Humans in Abuja Nigeria

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Abstract

The major natural reservoir of shiga toxin producing *Escherichia coli* (*E. coli*) is cattle. Man gets infected by the consumption of contaminated cattle meat and meat products. Shiga toxin producing *Escherichia coli* O157 is a major cause of haemolytic colitis (HC) and haemolytic uraemic syndrome (HUS) in humans. The cross sectional epidemiologic method was used in this study. Human samples were collected from sick hospital patients, apparently healthy high risk individuals (abattoir workers, cattle herdsmen, milk hawkers) and from members of the public. Freshly voided faeces were collected from cattle in selected abattoirs and cattle herds. The samples were subjected to an enrichment culture and analyzed both bacteriologically and biochemically to confirm typical *Escherichia coli* which were then sub-cultured into plates of cefixine- tellurite sorbitol McConkey (CT- SMAC) agar. The non-sorbitol fermenters stored in nutrient agar slants were further characterized using commercially procured latex agglutination test kits. A total of 572 human samples were tested for the presence of shiga toxin producing *E. coli* and 5 (0.87%) was positive. Of the 718 faecal samples from cattle tested, 17 (2.4%) were positive. The antibiogram of the isolates to 10 most commonly used antibiotics were tested. The isolates from cattle were tested and found to be positive to levofloxacin, streptomycin, chloramphenicol and ciprofloxacin but resistant to erythromycin, gentamycin, augmentin, tetracycline, cotrimoxazole and cloxacillin. The *E. coli* O157 isolated from humans were sensitive to levoflaxacin and ciprofloxacin and resistant to the rest. The study indicated that both cattle and man within the same environment harbour shiga toxin producing *E. coli* O157 proving that cattle play a major role as source of transmission of multi drug resistant shiga toxin producing *E. coli* O157 to humans in Abuja, FCT.

Keywords: *Isolation; Antibiogram; Shiga Toxin Producing E. coli O157; Cattle; Humans*

Introduction

Verocytotoxigenic *Escherichia coli* (VTEC) also known as Shiga toxin-producing *Escherichia coli* (STEC) has emerged and is recognized as an important zoonotic food-borne pathogen and risk to public health [1,2]. The major natural reservoir of shiga toxin producing *E. coli* is cattle [3]. Harbouring of *E. coli* O157 in cattle is a significant concern for Public health because of their transmitting capability to humans through contaminated foods and water with faeces from cattle [4,5]. Enem and Oboegbulem [6], reported that infection of cattle which are major food animal in Nigeria with VTEC O157 portends an epidemiological causal association to infection in humans. Typical illness as a result of an *E. coli* O157 infection can be life threatening and can cause severe disease in humans such as haemorrhagic colitis (HC), haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) [7-10].

Escherichia coli is commonly found in human and animal intestinal tracts and, as a result of fecal contamination or contamination during food animal slaughter, is often found in soil, water, and foods. Shiga toxin-producing *E. coli* (STEC) O157 has emerged as a public health threat following its initial identification as a pathogen in a 1982 outbreak of illness associated with the consumption of undercooked ground beef [11]. The U.S. Centers for Disease Control and Prevention estimates that *E. coli* O157:H7 causes approximately 73,400

illnesses and 60 deaths each year in the United States [12]. Recent reports indicate that antimicrobial resistance of *E. coli* O157 is on the rise [13-15]. Yet the extent to which different antimicrobial use practices have contributed to the increase in antimicrobial resistance is not clear [16].

The magnitude of the public health burden due to resistant food borne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water and proper cooking and home hygiene among others [17]. The major concern on the Public health threat of food borne illness is infection by antimicrobial resistant strains that lead to more intractable and severe disease [18,19]. Antibiotic-resistant bacteria have been found in a surprisingly diverse range of environments, including clinics, animal pens, orchards, aquaculture, food, sewage as well as chlorinated and unchlorinated water supplies [20]. Bacteria are a common contaminant worldwide; and the release of human and animal wastes into the environment exacerbates bacterial contamination. Increased resistance to antibiotics may pose a challenge for the effective treatment of bacterial infections [21].

The use of antimicrobial agents plays a critical role in reducing morbidity and mortality due to communicable diseases. However, the emergence and spread of resistance to many of these antimicrobial agents is reducing their effectiveness [22]. Reports from different parts of Africa have observed temporal trends in the prevalence of antibiotic resistance among enteric organisms, such as *E. coli* and *Shigella* [23,24]. Studies during the last 15 years show increasing resistance to commonly used antimicrobials such as trimethoprim-sulphamethoxazole (TMP-SMX, also known as cotrimoxazole), ampicillin, tetracycline and chloramphenicol [23,24].

Apart from the therapeutic use of antimicrobials in human and veterinary medicine, they are routinely used for disease prevention and growth promotion in animal production. This practice leads to the inevitable selection of antimicrobial resistance among commensals in the intestinal tracts of food animals, which poses a public health threat [25]. For instance, antimicrobial-resistant bacteria from food animals may colonize the human population via the food chain, contact through occupational exposure, or waste runoff from animal production facilities [25,26]. Food animals, in particular mature cattle, which may be asymptomatic carriers of *E. coli* O157, including STEC [27], when exposed to antimicrobial agents in the animal production environment, may serve as a reservoir of antimicrobial-resistant bacteria.

Antibiograms are used by clinicians to assess local susceptibility rates as an aid in selecting empiric antibiotic therapy and in monitoring resistance trends over time within an institution. The most common methods utilized to measure the *in vitro* vulnerability of microorganisms to antimicrobial operators include the disk diffusion method, agar dilution, broth micro-dilution, and testing by antimicrobial gradient agar strips (E-test method) [22].

This study investigated the prevalence of *E. coli* O157 in cattle and humans in Abuja, Federal Capital Territory, Nigeria. The antimicrobial susceptibility profiles of *E. coli* isolates obtained were also analyzed.

Materials and Methods

Study area and design: The study was carried out in the Federal Capital Territory, Abuja, Nigeria which is located at the centre of the country between latitude 8° and 9°25' North of the equator and longitude 6°45 and 7°45 East of Greenwich Meridian [28]. A cross sectional epidemiological study and a multi staged sampling method were used in this research which was carried out between May, 2011 and April, 2012. The area has a tropical climate marked with two distinct seasons – rainy season (April – October) and dry season (November – March).

Sample collection: Five hundred and seventy-two human faecal samples were collected from populations at risk (abattoir workers, cattle herdsmen and milk hawkers), apparently healthy people and diarrhoeic patients in the hospital. Faecal samples were collected from 718 cattle in selected abattoirs and cattle herds. The samples were collected using sterile plastic universal bottles and transported to the laboratory for analysis under aseptic conditions.

Enrichment Culture for *E. coli* O157: An enrichment media of buffered peptone water (BPW) supplemented with 8 mg/litre vancomycin, 10 mg/litre cefsulodin and 0.05 mg/litre cefixime (BPW-VCC) was prepared to suppress the growth of gram positive organisms About 0.5g of faecal sample was inoculated into 5 ml of the BPW-VCC and incubated at 37°C for 6 - 8 hours [29].

A loop full of each stool specimen was cultured for *E. coli* on Eosin Methylene Blue (EMB) Agar (Oxoid) and incubated at 37°C for 20 hours. The cultured isolates exhibited the typical greenish sheen colouration characteristic of *E. coli* on EMB agar.

Biochemical Tests: The *E. coli* suspected colonies ex – EMB were subjected to biochemical tests for confirmation as typical *E. coli*. Such tests as indole production test; voges proskauer test; methyl red test; citrate utilization test; urease production test and hydrogen sulphide production test were carried out [30,31].

Isolation of VTEC O157: *E. coli* isolates ex – EMB were subcultured into plates of CT – SMAC and incubated at 37°C for 24 hours [32]. Non-sorbitol fermenting isolates that appear as colourless or neutral gray with smokey centre (1 – 2mm in diameter) were presumptive of *Escherichia coli* O157 [33].

Serological Test: Isolates that were sorbitol negative were stored at 4 - 8°C in nutrient agar slants in bijou bottles for further characterization. Commercially procured latex agglutination test kits from Oxoid ltd, Hampshire, England were used. The isolates in nutrient agar slants were subcultured into plates of CT – SMAC for the serology test according to the manufacturer's instructions.

Antimicrobial sensitivity test: The *E. coli* O157 isolates were tested for antimicrobial susceptibility by disk diffusion technique in accordance to Clinical and Laboratory Standards Institute (CLSI) criteria [34] using multi-antibiotic discs (Maxicare Medical Laboratory, Nigeria) containing the following antimicrobials and disc content (in µg): Levafloxacin (30 µg), Cotrimoxazole (30 µg), Streptomycin (30 µg), Ciprofloxacin (10 µg), Cloxacillin (30 µg), Erythromycin(30 µg), Augmentin (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg). The degree of sensitivity was measured and expressed in percentages while resistant was not expressed.

Results

Five hundred and seventy-two human samples were tested and a prevalence of 5 (0.87%) was found. Among the number tested, were 372 samples from Sick hospital patients with a prevalence of 4 (1.08%), 150 from population at risk with a prevalence of 1 (0.7%) and 50 from apparently healthy public with no positive results (Table 1). The prevalence for the 718 cattle samples tested was 17 (2.4%). Three hundred and fifty-eight were from cattle herds with a prevalence of 8 (2.23) while 360 were from abattoir with the prevalence of 9 (2.25%) (Table 2).

Individual's state	No tested	No positive	% positive
Sick Hospital patients	372	4	1.08
Population at risk	150	1	0.7
Apparently Healthy Public	50	-	-
Total	572	5	0.87

Table 1: *Prevalence of VTEC O157 in Humans.*

Cattle type	No tested	No positive	% positive
Cattle herds	358	8	2.3
Abattoir	360	9	2.5
Total	718	17	2.4

Table 2: *Prevalence of VTEC O157 in Cattle.*

The antibiotic susceptibility test of *E. coli* isolates in human showed that the isolates were sensitive to Levofloxacin (100%) and Ciprofloxacin (80%) and resistant to the other antibiotics. The isolates from cattle were sensitive to Lefloxacin (100%), Streptomycin (67%), Chloramphenicol (53%) and Ciprofloxacin (60%) (Table 3) and resistant to other antibiotics.

Antibiotics	Concentration (µg)	Humans		Cattle	
		Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)
Levofloxacin	30	0	100	0	100
Cotrimoxazole	30	0	80	100	0
Streptomycin	30	100	0	0	85
Ciprofloxacin	30	100	0	0	60
Cloxacillin	30	100	0	100	0
Erythromycin	30	100	0	100	0
Augmentin	30	100	0	100	0
Tetracycline	30	100	0	100	0
Chloramphenicol	30	100	0	0	75
Gentamycin	10	100	0	100	0

Table 3: *Antibiogram of E. coli in Humans Cattle.*

Discussion

In recent years, *E. coli* has gained public health significance due to its association with life threatening human diseases like HC, HUS, TTP syndromes. Foods of animal origin are one of the important routes for the disease transmission from animals to human. In Nigeria, Cattle roam freely in every part of the country (urban and rural inclusive) dropping cattle dung along the line which come in contact with people thereby increasing the chances of enteric infections such as VTEC O157 among people. Nontongana., *et al.* [22] explained that fresh and dry cattle and human excreta were spotted along the shores of Kat River which has given rise to the high coliform counts obtained in his study.

In the current study, a total of 572 faecal samples from humans and 718 from cattle were analyzed for the presence of VTEC O157. A prevalence rate of 5 (0.87%) was obtained for humans and 17 (2.4%) was recorded for cattle. The estimated annual incidence of VTEC O157 in 2004 reported in Scotland, the US, Germany, Australia, Japan and the Republic of Korea ranged from 0.08 to 4.1 per 100,000 populations with the highest incidence in Scotland (CSFPU, 2009). Complications and fatalities are particularly common among children, the elderly and immune-suppressed or have debilitating illnesses. HUS was fatal in 3 - 10% of children and TTP in up to 50% of the elderly (Chase-Topping., *et al.* 2008). The prevalence in cattle in this study is within the range of published research findings which ranged from 1.8% (Hancook., *et al*. 1997) to 15.7% (Chapman., *et al*. 1998).

In this work, VTEC O157 was found to be sensitive to Levofloxacin and Ciprofloxacin and resistant to all the other 8 antibiotics tested in humans. There was sensitivity for Levofloxacin, Streptomycin, Chloramphenicol and Ciprofloxacin but resistant to others in the case of cattle samples. This indicated that VTEC O157 obtained in this study expressed high levels of resistance to antimicrobials that are commonly used in clinical practice. According to a previous report, sulfisoxazole has the most common antimicrobial resistance, followed by tetracycline, streptomycin, ampicillin, trimethoprim, chloramphenicol, and neomycin [35]. Moreover, it has been reported that over 50% of their isolates displayed antimicrobial resistance against sulfamethoxazole, cephalothin, and tetracycline, and 20% of them against ampicillin and gentamicin [36].

The resistance to these specific antimicrobials is sometimes encoded by plasmids, which may distribute resistance in susceptible bacteria through horizontal gene transfer [37,38]. The use of antibiotics for prevention and control of bacterial infections as a whole and in *E. coli* infections in particular has always been a matter of investigation as a large number of isolates have been reported to be resistant to a group of antibiotics [39].

Nontongana., *et al*. [22] in their work, reported that about 98% of the VTEC O157 isolates were 100% susceptible to norfloxacin, while susceptibility to the other antibiotics were in the following order: amikacin (97%), ciprofloxacin (93%), streptomycin (77%), tetracycline (75%) and chloramphenicol (73%). All the isolates were 100% resistant to penicillin G, while 98% of the isolates were resistant to ampicillin. A number of the isolates exhibited resistance to streptomycin, tetracycline, trimethoprime-sulphametoxazole and the β-lactam class of antimicrobials.

The VTEC O157 isolates tested in this work showed multidrug resistance to the antibiotics at various percentages. Pandey., *et al.* [39] reported that the most commonly used antibiotics kanamycin, ampicillin, penicillin, cephalexin, neomycin, streptomycin, ofloxacin were found resistant for all the isolates of human being and cattle tested in their work. Some other researchers have reported this same pattern of antibiotic resistance in their research [40-42].

Conclusion

As antibiotics are continually used against pathogenic infections, the spread and persistence of antimicrobial resistant bacteria and resistance determinants in animals and humans become an important problem in clinical practice. The outcome of the research findings showed the isolation of multiple antibiotic resistant strains of VTEC in both cattle and human faecal samples highlighting the human health risk associated with exposure to contamination from infected cattle. This suggests the need for adequate risk prevention strategies to protect the foods of animal origin, water and environment from contaminants which will consequently lead to sound public health state.

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