Antimicrobial Susceptibility Testing of Enterohaemorharrgic *Escherichia coli* Isolated from Rectal Swab of Cattle in Oko Oba Abattoir, Agege, Lagos.

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Abstract

Fifty rectal swab samples were collected from healthy beef cattle at Oko – Oba Agege abattoir in Lagos Metropolis and were screened for the presence of enterohaemorrhagic Escherichia coli (EHEC), using standard media selective for Escherichia coli. Eight different antibiotics such as Gentamicin (Gen), Augmentin (Aug), Tetracycline (Tet), Ofloxacin (Ofl), Cotrimoxazole (Cot), Amoxicillin (Amx), Nalidixic acid (Nal) and Nitrofurantoin (Nitro) were used in this study. Sensitivity test was carried out by the Kirby-Bauer disc diffusion method and the efficacy of a drug was determined by measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Antibiotic susceptibility patterns showed 44% of the isolates to be highly susceptible to the different antibiotics screened, with 56% showing multiple antibiotic resistance.

Key words: *E. coli,* EHEC, efficacy, resistance, rectal swab, cattle, antibiotics

1. Introduction

E. coli is the most common cause of food and waterborne human diarrhea worldwide in developing countries causing 800,000 deaths out of 650 million cases per year primarily in children under the age of five years (Turner *et al.,* 2006).Enterohaemorrhagic *Escherichia coli* (EHEC) is a subset of pathogenic *E. coli* that can cause diarrhea or hemorrhagic colitis in humans. It is an important food and waterborne pathogen of humans that colonizes and is shed in the feces of many animal species (Elder *et al.*, 2000). Human infections result from diverse exposures including contaminated foods of animal (especially bovine) origin, direct contact with shedding or contaminated animals, direct contact with environmental (water) contaminants, and ingestion of contaminated animal products and contaminated vegetables and fruit. Both cattle and sheep are well characterized hosts of EHEC O157:H7 but, while both have been repeatedly linked to human infection, cattle have received much more research attention (Grauke *et al* 2002**).** Numerous epidemiologic studies have described the bovine EHEC O157:H7 reservoir (Hancock et *al*. 2000; LeJeune and Wetzel 2007; Renter and Sargeant 2002; Sargeant *et al*. 2007). Other animals such as rabbits and pigs can also carry this organism. The infectious dose is very low, which increases the risk of disease. EHEC 0157:H7 infections occur worldwide (Griffin, 1995). EHEC are transmitted by the fecal–oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources.

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The reservoir hosts and epidemiology may vary with the organism. A small proportion of the cattle in a herd can be responsible for shedding more than 95% of the organisms. These animals, which are called super shedders, are colonized at the terminal rectum, and can remain infected much longer than other cattle. However, there are investigations which indicate that the carriage of *E. coli* 0157H7 in cattle is an important factor in the emergence of this pathogen in Africa (Effler *et al*., 2001; Renter *et al*., 2003). Consumption of beef, beef products and cow milk contaminated with EHEC is a major cause of human infection worldwide (Elder *et al*., 2000 and Griffin *et al*., 1991). Animals that are not normal reservoir hosts for EHEC O157:H7 may serve as secondary reservoirs after contact with ruminants. Foodborne outbreaks with EHEC O157:H7 are often caused by eating undercooked or unpasteurized animal products, particularly ground beef but also other meats and sausages, and unpasteurized milk and cheese (Gansheroff and O'Brien, 2000).

Oko-Oba Agege abattoir is one of the largest and best organized abattoirs in Nigeria and receives cattle from various part of Nigeria. Although the abattoir has the daily maximum handling capacity of more than one thousand and three hundreds heads of cattle, it presently operates with the slaughter of an average of around 1000 heads of cattle daily (Ibironke *et al*., 2010). Since most abattoir slaughter are done early in the morning in Nigeria due to lack of good storage facilities and good preservation system, the abattoir is congested, a situation that makes it seem incapable of handling the number currently slaughtered on a daily basis, increasing improper food hygiene practice among the abattoir workers. Carcass contamination from hides, skin and gut content of animals can occur during bleeding, handling and processing of meat which include slaughtering, scalding, eviscerating and washing (Ikeme, 1990). Unhygienic floor dressing of carcasses is a common source of contamination and isolation of pathogenic microorganisms from meat and slaughtering facilities in Nigeria. Because of the lack of an efficient commercial vaccine, the control of colibacillosis mainly relies on the use of antimicrobial drugs. However, bacteria have developed strategies for survival within the host during an infection and one of these strategies is the resistance of isolates to the antimicrobial drugs. Antibiotic resistance is a serious problem because it limits the therapeutic possibilities in the treatment of bacterial diseases in domestic animal species in general and poultry in particular (Nicole *et al*., 2000). Global increase in the incidence of antimicrobial resistance in bacterial strains is a major concern to all stakeholders in animal production, veterinary practice and public health (WHO, 2001). Thus, there is the need to study the antibiotics susceptibility patterns of this important organism in which cattle is a principal reservoir host.

2. Materials and Methods

2.1 Collection of Samples

Rectal swabs of fifty adult cows were taken using sterile swab sticks at Oko-Oba, Agege abattoir market, in Lagos Metropolis and immediately transported to the laboratory for processing and culture.

2.2 Isolation of *E. coli* (EHEC)

The swab sticks used for the rectal swabs were streaked onto sterile Mac Conkey agar (Oxoid) and incubated at 37^0C for 18- 24 hours. Organisms showing characteristic colony morphology of lactose fermenters were sub cultured onto Eosin methylene blue agar (Oxoid) and incubated at 37^0C for 18- 24 hours. The organisms showing characteristic colony morphology of *Escherichia coli* on EMB as a green metallic coloured sheen were confirmed to be EHEC using the Oxoid Latex Serological test kit. Organisms that gave positive reactions were sub cultured onto Sorbitol Mac Conkey agar (Oxoid) to identify the presence of EHEC 0157: H7 which are non sorbitol fermenters.

2.3 Antibiotic Susceptibility Test

2.3.1 *Antimicrobial discs*

Commercially available antimicrobial discs (ABTEK, Liverpool) were used to determine the drug sensitivity and resistance pattern of the EHEC isolates. A number of 8 different antibiotics with different disc concentration such as Gentamycin (Gen) 10µg/disc, Cotrimazole (Cot)25 µg/disc, Nitofurantoin (Nitro) 300µg/disc, Augmentin (Aug) 30µg/disc, Tetracycline (Tet) 30µg/disc, Amoxicillin (A) 25µg/disc, Nalidixic acid (Na) 30µg/disc and Ofloxacin (Ofl) 30µg/disc were used in this study.

2.3.2 *Antimicrobial sensitivity test of* EHEC *isolates*

The susceptibility of identified *E. coli* isolates to antimicrobial agents was determined by the standard Kirby Bauer disk diffusion method. A single colony of the isolate under test was inoculated unto TSB and incubated for 8 to 12 h. After incubation, the turbidity of the TSB culture was adjusted to 0.5 McFarland standard. A sterile swab was dipped into the adjusted TSB culture and inoculated onto Mueller Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA. Inoculated plates were allowed to dry for approximately 3-5 minutes and then the antibiotic discs were applied aseptically to the surface of the inoculated agar with the help of a sterile forceps. The plates were then inverted and incubated at 37ºC for 24 hours. After incubation, the diameter of the clear zone of inhibition around each antimicrobial disk was measured (in millimeters) and the result was interpreted in accordance with the recommendation of Clinical and Laboratory Standards Institute (CLSI), (2008).

3. Result

3.1 Isolation of *E. coli*

A total of 100 gram negative rod bacterial isolates were got from the 50 rectal swabs analyzed. Fifty (50%) of these isolates were identified as *E. coli* using standard biochemical tests while 48(48%) were identified as EHEC 0157: H7 using the Oxoid serological latex kit and Sorbitol Mac Conkey agar.

3.2 *Escherichia coli* antimicrobial resistance rates

The overall rate of EHEC resistance to the different antibiotics in this study was as follows: Augmentin (41.67%), Cotrimoxazole (50%), Amoxycillin (77%), Tetracycline (33%), Ofloxacin (0%), Gentamicin (0%), Nalidixic Acid (2%) and Nitrofurantoin (1%). (Table 1). The percentage of the overall multiple resistance of EHEC isolates from rectal swab of cattle in this study was 56%.

4. Discussion

Resistance to antimicrobials has increased over the years and normal intestinal microbial flora has become a reservoir for resistant genes. The use of antimicrobial agents in animal production has been identified as an important factor which select for antimicrobial resistant bacterial strains (WHO, 1998). This may be due to an inevitable genetic response to the strong selective pressure imposed by antimicrobial chemotherapy which plays a vital role in the evolution of antibiotic resistance among bacteria. These bacteria then pass the plasmid containing resistance gene among other bacterial cells and species (Chakraborty *et al*., 2001)

Globally, antimicrobial resistant bacteria resident in the gut of carrier animals contribute significantly to environmental contamination and spread of antimicrobial resistant bacterial strains (Kang *et al*., 2005; Lee *et al*., 2006), hence the need to continuously monitor antimicrobial resistance in zoonotic and commensal bacteria of animal origin for the protection of public health (WHO, 2001).

In this study, there was highest resistance of 77% to amoxicillin, followed by 68.8% to Tetracycline, 50% to Cotrimoxazole, and 41.67% to Augmentin among the *E. coli* isolates indicating misuse and abuse of these drugs in animal feed by farm workers. These drugs are widely used in animal production in Nigeria and are readily available over the counter. A study carried out by Amosun *et al*., 2012 showed high resistance of over 70% to amoxicillin, ampicillin and streptomycin among *E. coli* isolates. Likewise, the isolates showed moderate to high resistance (between 30 and 70%) to gentamycin, cotrimazole, nitrofuratoin, erythromycin, chloramphenicol and tetracycline.The present study also revealed that some of the isolates were sensitive to Ofloxacin (100%), Gentamicin (100%), Nalidixic Acid (98%) and Nitrofurantoin (99%). Zinnah *et al*., (2008) reported *E. coli* isolates that were sensitive to Levofloxacin (80%) and Ciprofloxacin (80%); a few number of isolates were sensitive to Azithromycin (30%) and Nalidixic acid (30%) and resistant to Tetracycline (80%), Ampicillin (90%), Erythromycin (90%), Amoxicillin (90%) and Metronidazole (100%). Whereas, Joshi *et al*. (1986) reported that high percentage of isolates were sensitive to Tetracycline (90.90%) and Gentamicin (54.54%) and resistant to Ampicillin (36.36%) and Erythromycin (27.27%), Jordan *et al*. (2005) showed resistance to Tetracycline (3.6%), Amoxicillin (2.2%) and Gentamicin (0.09%), Orden *et al*. (2000) showed resistance to Tetracycline (above 65%), Ampicillin (23 - 50%) and sensitive to Gentamicin (89-95%) and Sawant *et al*. (2007) found resistance to Ampicillin (48%) and Tetracycline (93%).

The percentage of the overall multiple resistance of EHEC isolates from rectal swab of cattle in this study is 56%. There has been increasing concern of the possible development of resistance to antimicrobial agents in the enterobacteriaceae, especially *Escherichia coli* as a result of the use of such agents in animal feed (Willis, 2000). The use of antibiotics in agriculture is contributing to the problem of antibiotic resistance amongst pathogenic bacteria. Resistance in this study was quite high and could be as a result of the wide spread and misuse of such agents in animal feed by the farm and health workers in Agege cattle market, Nigeria.

This study revealed that cattle slaughtered at Oko-Oba, Agege in Lagos are carriers of multidrug resistant EHEC and also suggested that beef could be a vehicle for possible transmission to humans since the beef can get contaminated during the process of slaughtering. As a result of this, measures should be put in place to ensure hygienic practices during slaughtering and during post-process handling of beef to reduce the risk of transmission of multi drug resistant EHEC to humans. Also proper legislation is required to regulate access to and use of antimicrobial agents in animal production in order to prevent the increasing incidence of resistance.

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Table 1: Antibiotics Susceptibility pattern of isolates showing zones of inhibition in mm

Isolates	Aug 30 µg	Ofl 30 µg	Gen 10 µg	Nal 30 µg	Cot 25 µg	Amx 25 µg	Tet 30 µg	Nitro 300µg
2	0	24	13	20	0	0	0	19
3	0	20	10	18	0	0	0	16
4	0	21	10	20	20	0	0	21
5	0	20	17	20	15	0	0	19
6	0	25	17	20	0	0	0	22
7	0	20	18	19	0	0	0	20
8	0	20	15	18	0	0	0	17
9	0	20	17	17	0	0	0	17
10	0	20	10	19	0	0	0	19
11	0	24	15	14	12	0	0	19
12	0	22	15	19	0	0	0	19
13	10	25	19	18	0	9	0	21
14	0	21	16	19	0	0	0	19
15	0	20	16	19	0	0	0	22
16	0	20	15	20	0	0	0	18
17	0	21	16	20	23	0	0	21
18	0	15	16	0	23	0	18	11
19	0	23	15	17	0	0	0	19
20	11	21	16	17	22	0	11	21
21	10	24	17	16	20	0	15	18
22	13	25	16	22	20	10	15	20
23	10	22	17	17	20	7	18	20
24	0	25	17	19	25	0	0	19
25	11	20	16	21	22	10	19	17
26	13	23	16	18	23	11	11	18
27	18	22	19	17	22	0	11	18
28	0	22	18	21	0	0	0	20
29	0	25	20	19	0	0	0	21
30	11	25	18	22	25	14	18	22
31	10	24	17	16	0	15	18	20
32	0	20	16	15	0	0	0	00
33	16	25	18	20	23	16	15	20
34	0	22	13	17	0	0	0	17
35	0	20	15	18	0	0	0	20
36	0	22	14	20	0	0	0	17
37	13	21	19	17	23	0	0	22
38	17	23	17	17	0	0	0	23
39	0	23	15	19	20	0	0	20
40	17	24	18	17	20	17	20	23
41	0	28	18	18	20	0	0	20
42	0	23	15	18	20	0	0	18
43	19	27	16	19	20	17	15	22
44	15	27	16	20	0	0	0	18
45	19	25	16	20	25	18	15	21
46	15	25	17	20	0	0	0	20
47	15	23	18	20	0	0	0	20
48	0	23	16	22	22	0	0	21
49	16	22	16	18	20	0	19	17