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Antimicrobial Resistance of Shigatoxin Producing *Escherichia coli* 0157:NM Isolates from Water Fed to Cattle in Northwestern Nigeria

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Abstract: Shiga toxin-producing *Escherichia coli* 0157:NM (STEC 0157:NM) was isolated from water fed to cattle and its resistance to antimicrobial agents was determined. Five hundred water samples were collected from cattle establishments in northwestern Nigeria from December 2002 to June 2006. Serological confirmation and Shiga toxin production were carried out using kits (Oxoid, Basingstoke England). Resistance to 16 antimicrobials was tested by the standardized disc diffusion method. STEC 0157:NM was isolated from 0.4% (2/500) of the samples. The isolates were resistant to several commonly used antimicrobial agents in livestock health and production. There was no resistance to chloramphenicol and fluoroquinolones but quinolone resistance was observed. The emergence of STEC 0157:NM in water used for feeding cattle in northwestern Nigeria may pose a serious health hazard to livestock and humans. Legislation on import control of livestock, veterinary drug acquisition and use and public education on drug use and its implications are essential to manage this threat to public health and the livestock industry.

Key words: Resistant, Shigatoxin, *Escherichia coli*, water, cattle

INTRODUCTION

Escherichia coli normally reside in the gastrointestinal tract of man and animals. It is a member of the coliform group of bacteria and some serotypes are leading causes of diarrhea in man and animals (Bertolini *et al.*, 2006; Rao *et al.*, 2006).

Shiga toxin-producing *Escherichia coli* (STEC) are an emerging group of pathogens of public health significance on a global scale (Allison *et al.*, 2000; Armstrong *et al.*, 1996; FSAI, 1999; Fukushima *et al.*, 1999; Germani *et al.*, 1998; Riley *et al.*, 1983). *Escherichia coli* 0157:NM is the most common STEC in Africa (Effler *et al.*, 2001). This pathogen contaminates water sources and has been shown to cause death in man and animals. In the outbreak in South Africa and Swaziland, 40,912 human diarrhea cases and a seven-fold increase in cattle death during the period of the outbreak was reported (Effler *et al.*, 2001).

Therefore, this study was design to isolate *E. coli* 0157:NM in water fed to cattle, determine its Shiga toxin production profile and resistance to some commonly used antimicrobial agents in northwestern Nigeria.

MATERIALS AND METHODS

The multistage sampling method (Snedecor and Cochran, 1976) was employed to select Kaduna and Sokoto States from seven states in the northwestern geo-political zone of Nigeria. Four out of the

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six dairy farms and two of the four dairy farms in Kaduna and Sokoto States, respectively were selected as sampling sites. Supplementary water samples were collected randomly from beef cattle farms and nomadic Fulani cattle herds around Zaria, Kaduna State and Sokoto municipal areas from December 2002 to June 2006.

The water was sampled by collecting 100 mL of untreated and 200 mL of chlorinated tap water. The samples were collected using sterile equipment and procedures and stored in cool boxes (Ayres and Mara, 1996).

Isolation

Water samples were assayed for *E. coli* 0157:NM using the membrane filtration technique (Ayres and Mara, 1996), at the Departments of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria and Usmanu Danfodiyo University, Sokoto. Filtration (pore size of 0.45µm) was followed by enrichment in modified tryplone soya broth (MTSB) supplemented with novobiocin (Oxoid Ltd., Basingstoke, England) and incubation at 37±1°C for 24 h. The enriched filters were then cultured on Cefixime-Sorbitol-MacConkey (CR-SMAC) agar (Oxoid Ltd., Basingstoke, England) and incubated for 24 h at 37±1°C. Colourless colonies were selected and stored at 4°C on nutrient agar (NA) slants.

Biochemical Screening

Biochemical screening was carried out as recommended by Cowan *et al.* (1981). This included testing for citrate utilization; urease production; indole, sulphide production and motility using sulphide indole and motility (SIM) medium; Hydrogen sulphide (H₂S) production, fermentation of glucose, sucrose and/or lactose and gas production using Triple Sugar Iron (TSI) agar; methyl red and Voges-Proskauer tests using methyl red and Voges-Proskauer (MRVP) medium; reaction from fermentation of lactose, maltose, manitol, arabinose and sorbitol and gas from glucose fermentation.

Serological confirmation.

The remel Wellcolex *E. coli* 0157:H7 kit (Remel Europe Ltd. Kent UK) was used. It is a rapid latex agglutination test for the identification of the somatic (0157) antigen and flagellum (H7) antigen with specific antibodies. The test was controlled by using a reference strain of *E. coli* 0157:H7 - ATCC 43895.

Detection of Shiga Toxin Production

The production of Shiga toxin 1 (ST1) and/or Shiga toxin 2 (ST2) was detected using VTEC-RPLA kit (Oxoid Ltd., Basingstoke, England). Polymyxin B (Oxoid Ltd. Basingstoke England) at a concentration of 5000 units/mL was used to facilitate the release of the Shiga toxins from the isolates (Karmali *et al.*, 1985) and the solid culture method was carried out. Serologically confirmed isolates of *E. coli* 0157:NM were inoculated onto Brain Heart Infusion (BHI) agar slopes (10 mL) and incubated at 37±1°C for 20 h. The growth was suspended in 1 mL of 0.85% saline containing 5000 units of *Bacillus cereus* selective supplement (Polymixin B) (Oxoid Ltd., Basingstoke, England). Extraction was carried out for 30 min at 37°C in a vibrating water bath. Each culture extract was cooled at 4°C for 30 min in a refrigerator and then centrifuged at 4000 rpm for 20 min.

The extract was used to assay for Shigatoxins following the manufacturer's instructions to the letter and reading agglutination in the v-well microtitre plates.

Identification of STEC 0157:NM

Isolates that were citrate negative; urease negative; indole positive, H₂S negative, non-motile using SIM medium; H₂S negative, produced an acid/acid reaction with gas production on TSI agar; methyl red positive, Voges-Proskauer negative using MRVP medium; produced acid reaction from

fermentation of lactose, maltose, manitol, arabinose and sorbitol; produced gas from glucose fermentation; produced agglutination of 0157 test latex accompanied by a lack of agglutination of the control latex; lack of agglutination of the H7 test latex accompanied by a lack of agglutination of the H7 control latex; agglutination of test sample supernatant and test latex (VT1 and/or VT2), lack of agglutination in the wells containing latex control and no samples respectively, agglutination of verotoxin 1 and 2 controls - assayed as the test latex above; were considered to be STEC 0157: NM

Antimicrobial Resistance Testing

In vitro resistance tests were performed by the standardized disc diffusion method on Mueller-Hinton agar (MHA) (Bauer *et al.*, 1966). From a pure culture of *E. coli* 0157:NM on SMAC agar, 5 colourless colonies of the organism were transferred with a wire loop to a test tube containing 4mL of Tryptone Soya Broth (TSB). The tubes were incubated at 37°C for 5 h. The resulting suspension was standardized by diluting with 0.85% saline to a density visually equivalent to the McFarland Standard of 0.5 (a turbidity standard prepared by adding 0.5 mL of 1% barium chloride solution to 99.5 mL of 1% H₂SO₄). This equated to approximately 10⁸ of *E. coli* 0157:NM per mL of the suspension.

MHA plates were prepared and dried in an incubator. Cotton swabs were immersed into the suspension and the entire surface of the MHA was spread evenly with the suspension. The inoculum was allowed to dry with the plates covered. Using aseptic technique each disc was placed and pressed to ensure full contact with the agar. The inverted MHA plates were immediately incubated at 37±1°C for 14 h. Each disc set was validated by using the control strain-ATTC 43895.

Zones of inhibitions around the disc were measured to the nearest millimeter. A disc set was considered valid if it produced zones of inhibition ≥ 15 mm on MHA agar using ATTC 43895. A strain was considered stable (resistant) if the diameter of the zone was ≤ 10 mm, moderately sensitive being 11-14 mm and sensitive ≥ 15 mm. Zones of ≥ 26 mm indicated high sensitivity (Krivoshin *et al.*, 1989). A total of 16 antimicrobial disc (Oxoid Ltd. Basingstoke England), were used for the tests. The antimicrobials tested were the following: ampicillin, cephalosporin, gentamicin, kanamycin, neomycin, streptomycin, tetracycline, sulphamethoxazole, compound sulphonamides, co-trimoxazole, ceftazidime, cefuroxime, chloramphenicol, ciprofloxacin, nalidixic acid and norfloxacin (Shroeder *et al.*, 2002; Ramon-Yusuf *et al.*, 1990).

RESULTS

STEC 0157:NM Isolation

Shiga toxin-production was detected in 2 isolates making the isolation rate of Shiga toxin-producing *E. coli* 0157:NM to be 0.4% (2/500). Only Stx2 was detected from the two isolates (Table 1).

Antimicrobial Resistance among STEC 0157:NM Isolates

The two isolates were resistant to several antimicrobials including ampicillin, streptomycin, sulphamethoxazole, compound sulphonamides, tetracycline and nalidixic acid. One isolate was resistant to neomycin and co-trimoxazole. There was no resistance to chloramphenicol, kanamycin, gentamicin, ceftazidime, cefuroxime, cephalosporin, norfloxacin and ciprofloxacin. High sensitivity was observed to chloramphenicol, norfloxacin and ciprofloxacin (Table 2).

Antibiograms of STEC 0157:NM from Water

There were two antimicrobial resistance patterns. Ampicillin-Streptomycin-Sulphamethoxazole-Compound Sulphonamides-Tetracycline-Nalidixic acid and Ampicillin-Neomycin-Streptomycin-Sulphamethoxazole-Co-trimoxazole-Compound Sulphonamide-Tetracycline-Nalidixic acid (Table 3).

Table 1: Shigatoxin-production in *E. coli* 0157: NM isolates from water

| CR-Smac | | Serology | | Shigatoxinnt | |
|---------|-------|----------|--------|--------------|--------|
| NT | NN | NT | NP | ST1 | ST2 |
| 500 | 20(4) | 5 | 2(0.4) | 0 | 2(0.4) |

The figures in parenthesis are percentages; NT, Number tested; NN, CR-SMAC Negative; NP, number positive

Table 2: Antimicrobial resistance among two STEC 0157:NM isolates from water

| Class/Antimicrobial | Level (µg) | Isolates ¹ | |
|--|------------|-----------------------|-----|
| | | D10 | D15 |
| B-lactams | | | |
| Ampicillin | 10 | R | R |
| Cephalosporins | | | |
| Ceftazidime | 10 | S | S |
| Cefuroxime | 30 | S | S |
| Cephazolin | 30 | MS | MS |
| Phenicols | | | |
| Chloramphenicol | 30 | S* | S* |
| Aminoglycosides | | | |
| Kanamycin | 30 | MS | MS |
| Gentamycin | 10 | MS | MS |
| Neomycin | 10 | MS | MS |
| Streptomycin | 10 | R | R |
| Sulphonamides and/or trimetoprim | | | |
| Sulphamethoxazole | 25 | R | R |
| Sulphamethoxazole/trimetoprim (co-trimoxazole) | 25 | MS | R |
| Compound sulphonamides | 300 | R | R |
| Tetracycline | | | |
| Tetracycline | 30 | R | R |
| Quinolones | | | |
| Nalidixic acid | 10 | R | R |
| Fluoroquinolones | | | |
| Norfloxacin | 2 | S* | S* |
| Ciprofloxacin | 5 | S* | S* |

¹Isolates from a dairy farm watering points; *High sensitivity

Table 3: Patterns of antimicrobial resistance of 2 isolates of STEC 0157: NM from a dairy farm watering point

| Resistance Pattern ^a | No. of isolates |
|---------------------------------|-----------------|
| AMP, S, RL, S3, TE, NA | 1 |
| AMP, N, S, RL, SXT, S3, TE, NA | 1 |

^aAMP, ampicillin; S: Streptomycin; RL: sulphamethoxazole; S3: compound sulphonamides; TE: tetracycline; NA: nalidixic acid; N: neomycin and SXT: sulphamethoxazole/trimethoprim (co-trimoxazole)

DISCUSSION

The isolation of STEC 0157:NM should be of interest and concern to the public, dairy and fast food industries in Nigeria because this pathogen causes bloody diarrhea and death in man and animals respectively. A huge reservoir of this zoonotic pathogen could be building up in the cattle population from infected calves through consumption of contaminated water. The farm from which these isolates were obtained is stocked with imported exotic breeds from countries where *E. coli* 0157:NM has been incriminated as a cause of epidemics (Armstrong *et al.*, 1996). Cattle, especially dairy cattle have been implicated as the source of the organisms in outbreak situations (Allison *et al.*, 2000; Armstrong *et al.*, 1996; Effler *et al.*, 2001). Contamination of water and food by organisms from bovine reservoirs is the major mode of transmission to animals and man (Armstrong *et al.*, 1996). Though the low percentage of isolation is deceptive but this could have been due to the organism's inability to survive for extended periods outside its host. Moreover, the infective dose of STEC is extremely low. Consumption of food with 2 cells/25 g has been reported to have caused illness in man (Sekla *et al.*, 1990). Studies have shown that cattle can harbour the organism without showing any overt or clinical signs including faecal

shedding (Armstrong *et al.*, 1996; Effler *et al.*, 2001). Shedding has been shown to commence when the host is subjected to stress such as changes in diet and drought (Effler *et al.*, 2001). Effler *et al.* (2001) reported that *E. coli* 0157:NM could not be found in South African waters one year after the outbreak due to its inability survive outside its natural host.

The finding that the two isolates were resistant to ampicillin, streptomycin, sulphamethoxazole and tetracycline has serious implication on the continued use of sub-therapeutic levels of these drugs in animal production, particularly where these un-recommended levels are often used by feed manufacturers as growth promoters. This use has often been blamed for the wide spread resistance of bacteria to antimicrobial agents in animals and man (Weber and Courvalin, 2005). The result of this study, though very limited supports this view since 100% resistance was observed to antimicrobials ampicillin, streptomycin, tetracycline, compound sulphonamides and sulphamethoxazole used commonly in animal production and veterinary practice in Zaria (Ramon-Yusuf *et al.*, 1990). Kabir *et al.* (2002) reported that the failure to observe withdrawal time, un-recommended drug usage and disregard of meat inspection procedures and laws, are responsible for the abuse/misuse of drugs in ruminant and intensive poultry production in Zaria. This may have contributed greatly to the resistance exhibited by the organism to the drugs.

Resistance to nalidixic acid is inherent, caused mostly by mutations and is important mainly from a public health view point, because it is the type quinolone- a class of frontline antibiotics that offer hope in infectious disease therapy against resistant pathogens. This may explain the resistance since nalidixic acid is not recommended for therapeutic purposes in veterinary practice. The quinolones are antibacterial agents that target two essential bacteria enzymes; DNA gyrase and DNA topoisomerase IV. These enzymes are essential in DNA synthesis in bacterial and by preventing their production quinolones destroy bacterial cell walls leading to death of the bacteria. Mutations remove the site of quinolone activity from the *gyrA* and *gyrB* genes of *E. coli*. These mutations can be induced by substances and proteins that possess quinolone- like activity such as bacteria proteins like Microcin B17 (Drlica and Zhao, 1997), making the organisms resistant to quinolones.

The two isolates predictably showed high sensitivity to the fluoroquinolones used. The fluorinated quinolones such as norfloxacin and ciprofloxacin are exceptionally potent in attacking bacteria, because their action is at several sites and thus avoiding point specific mutations effect associated with quinolone action (Ozeki, 1997). Sensitivity to chloramphenicol may be due to the fact that it is not recommended for use in food animals and is unavailable in preparation suitable for veterinary use in northwestern Nigeria. This viewpoint is supported by Kabir *et al.* (2000) and Ramon-Yusuf *et al.* (1990) who have reported that resistance to antimicrobial agents is strongly linked to abuse/misuse in animal health and production practices. Sensitivity to fluoroquinolones and chloramphenicol is an important finding from a public health viewpoint. These drugs are available in Nigeria and are fairly affordable since they are manufactured locally. However, it is important to chlorinate water that is used for human consumption and to prevent faecal contamination of water that is intended for cattle consumption to forestall the outbreak in the human population and the devastating effect that outbreaks can have on the cattle industry. Effler *et al.* (2001) reported that the South African and Swaziland outbreak wiped out calves and young stock on most farms in the affected areas.

To control the spread of resistance we must heed the warning of Alexander Fleming in his 1945 Noble Prize acceptance speech: Dr. Fleming noted the danger of sub-therapeutic use of antimicrobial agents and said: If you use penicillin, use enough (Fleming, 1945).

CONCLUSION

This study has exposed the magnitude of the problem of indiscriminate importation of livestock and the association between importation and emergence of new pathogens. *Escherichia coli* 0157: NM

is present in water sources consumed by cattle in northwestern Nigeria. The future of food safety and security lies in education and legislation. Education is required so that the public is aware of the risk associated to certain animal production practices like indiscriminate drug use and its consequences. Legislation is required to deter offenders concerning the sale, acquisition and administration of antimicrobial agents in animals.

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REFERENCES

- Allison, L.J., P.E. Carter and F.M. Thomson-Carter, 2000. Characterization of a recurrent clonal type of *Escherichia coli* 0157:H7 causing major outbreaks of infection in Scotland. *J. Clin. Microbiol.*, 38: 1632-1635.
- Armstrong, G.L., J. Hollingsworth and J.G. Morris, 1996. Emerging food-borne pathogens. *E. coli* 0157: H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.*, 18: 29-51.
- Ayres, R.M. and D. Mara, 1996. Analysis of waste Water for agricultural use-a laboratory manual of parasitological and bacteriological technique. World Health Organisation, Geneva, pp: 46.
- Bartoloni, A., L. Pallecchi, M. Benedetti, C. Fernandez, Y. Vallejos, E. Guzman, L. Villagran, A. Mantella, C. Luchetti, F. Bartalesi, M. Strohmeier, A. Bechini, H. Gamboa, H. Rodriguez, T. Falkenberg, G. Kronvall, E. Gottuzzo and G.M. Paradisi and Rossolini, 2006. Multi-drug-resistant commensal *Escherichia coli* in children, Peru and Bolivia. *Emerg. Infect. Dis.*, 12: 907-913.
- Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Truck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 45: 493-496.
- Cowan S.T., 1981. Manual for the Identification of Medical Bacteria. 2nd Edn., Cambridge University Press, Cambridge, pp: 238.
- Drlica, K. and X. Zhao, 1997. DNA gyrase, topoisomerase IV and the 4- quinolones. *Microbiol. Mol. Biol. Rev.*, 61: 377-392.
- Effler, P., M. Isaacson, L. Arntzen, R. Heenan, P. Canter, T. Barrett, L. Lee, C. Mambo, W. Levine, A. Zaidi and P.M. Griffin, 2001. Factors contributing to the emergence of *Escherichia coli* 0157 in Africa. *Emerg. Infect. Dis.*, 7: 812-819.
- Fleming, A., 1945. Penicillin. Nobel lecture, December 11, 1945. Available at: <http://nobelprize.org/nobel-prizes/medicine/laureates/1945/fleming-lecture.pdf> [cited March 17, 2007, 10.06 a.m.].
- Food Safety Authority of Ireland, 1999. The prevention of *E. coli* infection: a shared responsibility. Food Safety Authority of Ireland, Abbot Court Lower Abbey Street, Dublin.
- Fukushima, H., T. Hashizume, Y. Morita, J. Tanaka, K. Azuma, Y. Mizumoto M. Kaneno, M. Matsuura, K. Korma and T. Kitani, 1999. Clinical experiences in Sakai City Hospital during the massive outbreak of enterohemorrhagic *E. coli* 0157:H7 infections in Sakai City 1996. *Paediat. Int.*, 41: 213-217.
- Germani, Y., P. Cunnin, L. Tedjouka, C.B. Ncharre, J. Morvan and P. Martin, 1998. Enterohaemorrhagic *Escherichia coli* in Ngoila, Cameroon during an outbreak of bloody diarrhea. *Lancet (Letter)* 22: 625-626.

- Kabir, J., J.U. Umoh and V.J. Umoh, 2002. A survey of veterinary drug use among animal health workers and livestock owners and its implication in the occurrence and control of drug residues. *Sahel J. Vet. Sci.*, 1: 40-47.
- Karmali, M.A., M. Petric, C. Lim, P.C. Fleming, G.S. Arbus and H. Lior, 1985. Infections by Verocytotoxin-producing *Escherichia coli*. *J. Infect. Dis.*, 151: 775-782.
- Krivoshain, Y.S., 1989. Handbook on Microbiology. Laboratory Diagnosis of Infectious Diseases. Mir Publishers, Moscow.
- Ozeki, H., 1997. Mutations in *gryA* and *parC* genes and fluoroquinolone resistance. *J. Clin. Microbiol.*, 35: 2315-2319.
- Ramon-Yusuf, S.B., Y.O. Aliu and A.E. Lawal, 1990. Frequency of polytherapy in Veterinary practice at Ahmadu Bello University Teaching Hospital [Zaria]. *Zariya Vete.* 5: 66-70.
- Rao, A.N., M. Barlow, L.A. Clark, J.R. Boring, F.C. Tenover and J.E. McGowan, 2006. Class 1 integrons in resistant *Escherichia coli* and *Klebsiella* spp., US hospitals. *Emerg. Infect. Dis.*, 12: 1011-1014.
- Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. David, R.J. Herbert, E.S. Oclott, L.M. Johnson, N.T. Hargrett, P.A. Blake and M.L. Cohen, 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *New Eng. J. Med.*, 308: 681-685.
- Sekla, L., D. Milley, W. Stackiw, J. Sisler, J. Drew and D. Sargent, 1990. Verotoxin-producing *Escherichia coli* in ground beef, Manitoba Canada. *Dis. Weekly Rep.*, 16: 103-105.
- Shroeder, C.M., C. Zhao, C. DebRoy, J. Torcolini, S. Zhao, D.G. White, D.D. Wagner, P.F. McDermott, R.D. Walker and J. Meng, 2002. Antimicrobial resistance of *Escherichia coli* 0157 isolated from humans, cattle, Swine and food. *Applied and Environ. Microbiol.*, 68: 576-581.
- Snedecor, G.W., W.G. Cochran, 1976. *Statistical Methods*. 6th Edn., Iowa State University Press, Iowa, pp: 201.
- Weber, J.T. and P. Courvalin, 2005. An emptying quiver: antimicrobial drugs and resistance. *Emerg. Infect. Dis.*, 11: 971-793.