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Case Report

MOLECULAR, SEROLOGICAL AND MICROBIOLOGICAL PROFILING EVIDENCE OF THE FORMIT TRANSMISSION OF *ESCHERICHIA COLI* O157: NM TO A 1-YEAR OLD NIGERIAN CHILD

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INTRODUCTION

Transmission of *Escherichia coli* O157 by the formit route is under reported since interest in *E. coli* O157 is usually arisen during outbreak situations which are mostly linked to consumption of food of bovine origin (Armstrong *et al.*, 1996; Fukushima *et al.*, 1999 and Allison *et al.*, 2000). In December 2005 while working on the isolation of *E. coli* O157 from cattle herds in Zaria, Kaduna State, a 1 year old child of a member of the team came down with a mild watery diarrhea. He was not presented to any Physician, since he was observed to be alert, active and with a normal appetite. However, stool specimens were collected from the child on days 2 and 3 of the diarrhea, (which was self limiting on day 3). Stool specimens were also collected from all the five members of his family. All items that the boy had contact with including a laboratory coat, bunch of keys and shoes were swabbed. Finally samples of all the boy's food and drinks were taken. Microbiological, Serological and Polymerase Chain Reaction (PCR) Profiling Assays. The samples were cultured on Sorbitol - MacConkey (SMAC) agar, after enrichment using Modified Tryptone Soya Broth (MTSB) supplemented with Novobiocin (Oxoid Ltd, Basingstoke, England). All colourless colonies on SMAC agar from the samples were biochemically confirmed as *E. coli* by the methods described by (Cowan, 1981) and serologically as *E. coli* O157:NM using the Remel Wellcolex *E. coli* O157:H7 kit (Remel Europe Ltd, Kent UK). Shigatoxin profiling was done using the VTEC-RPLA kit (Oxoid Ltd, Basingstoke England). Antimicrobial resistance profiling was carried out by the method of Bauer *et al.* (1966) using 16 antimicrobial agents: Ampicillin (AMP10), Ceftazidime (CAZ30), Cefuroxime (CXM30), Cephazolin (KZ30), ciprofloxacin (CIP5), compound Sulphonamides (S3300), gentamicin (CN10), Kanamycin (K30), Chloramphenicol (C30), Nalidixic acid (NA30), Neomycin (N10), Norfloxacin (NOR2), streptomycin (S10), Sulphamethoxazole (RL25), Co-trimoxazole (SXT25) and tetracycline (TE30) (Oxoid Ltd, Basingstoke, England). The assays were controlled using a control strain of *E. coli* O157:H7 (ATCC 43895). Total DNA was isolated from 1 ml of brain heart infusion (BHI) broth culture grown overnight based on the method of Silhavy *et al* (1984). The Eae primer (Operon Ltd, Dusseldorf, Germany) was used (Germani *et al.*, 1997). The PCR mix consisted of 2.50l 10 x buffer, 1.50l $MgCl_2$, 0.50 dNTPs, *eae*-f 0.40l, Taq polymerase (Ampli Taq Gold, Applied Biosystems, California, U.S.A.) 0.50l, and 4.0l of template DNA. The volume of the mix was adjusted to 25l by addition of 15.2l of nuclease free sterile water. DNA amplification was carried out in a Gene Amp PCR System 9700 Thermocycler (Applied Biosystems, California, U.S.A.) using an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of amplification with denaturation step at 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 72°C for 10 minutes and cooled to 4°C.

KEYWORDS: *Escherichia coli* O157: NM, Formit, Transmission.

RESULTS AND DISCISSION

E. coli O157: NM was confirmed from the stool specimens of the child on day 1 and 2 of the diarrhea, and from swabs of the bunch of keys. This two isolates and a milk-borne isolate of *E. coli* O157: NM from a dairy farm that had been visited a day before the onset of the diarrhea all produced Shigatoxin 2 (Stx2) were resistant to only sulphamethoxazole and harboured the *eaeA* gene (Fig. 1).

Profiling is a standard epidemiological technique often use in investigation of outbreaks (Allison *et al.*, 2000; Bidet *et al.*, 2005). The results of this study have led to the conclusion that the boy was infected through a formit carrier of the pathogen. This conclusion seems to be backed by the isolation of *E. coli* O157:NM strains of the same resistance pattern, habouring the enterohaemorrhagic gene from the boy, bunch of keys and the cattle reservoir.

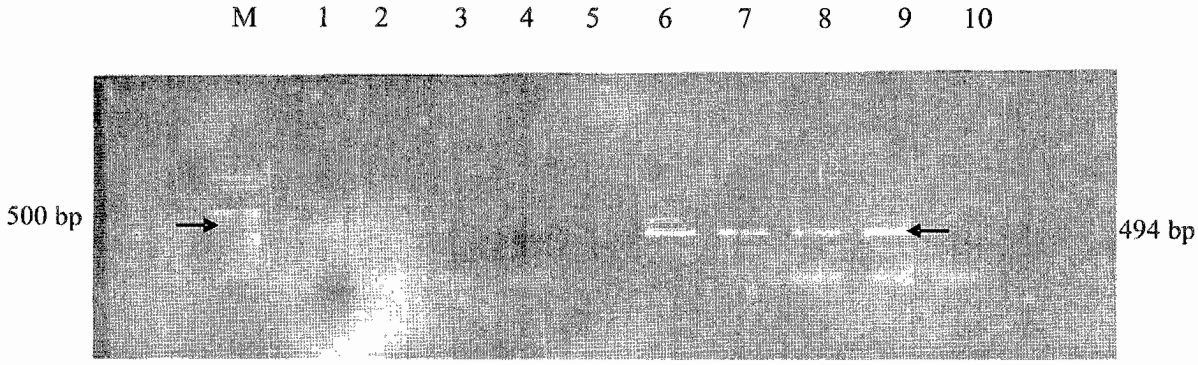

Fig. 1: Results from the PCR amplified DNA products by using Eae primer

Lane M, 1kb DNA ladder (Roche, Indianapolis U.S.A.); lanes 1-5, strains of *E. coli* O157 from food; lanes 6-8 isolates from the boy's stool sample, milk and swab sample of the bunch of keys; lane 9 and 10, positive and negative controls (ATTC 43895 and *Salmonella tyhphimurium*). The bands in lanes 6-9 are just below the 500 bp mark on the DNA ladder and correspond to 494 bp (arrows) and are for the amplicons of the *eae* gene from the 3 isolates and the positive control ATTC 43895.

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