CHARACTERIZATION OF SEROTYPES OF *ESCHERICHIA COLI* ISOLATED FROM DIARRHIA PATIENTS IN NENIN CITY NIGERIA

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ABSTRACT  
Diarrhoea disease is a major cause of morbidity and mortality in developing countries especially among children especially infants three years and below. Moreover, *Escherichia coli* has been incriminated as cause of diarrhoea. This organism has been known as a normal biotype in the gut of human and animals. The purpose of this study was to determine the involvement of this organism as cause of diarrhoea. Three hundred stool samples were collected from patients in various hospitals in Benin City and cultured using routine method in Medical microbiology department of University of Benin teaching hospital, Benin City, Nigeria. One hundred strains of *Escherichia coli* were isolated from the samples and were identified to species level using the protocol of Cowan and Steel. Seroyping was also done using the methods of Stokes. R-Plasmid DNA analysis was carried to isolate and characterize the Plasmid DNA using the alkaline analysis method of Tagahashi and Nagano. The strains isolated strains belong the following 0. Serotypes: 01,020,063, 044 (4 strains), 055, 063, 027, 08, 0167, 00153, 0025, 029, 026, 0158, 0018, 014, 015, 0152, 00142, 0028ac, 012ac, 0153, 0156, and 0157 (8 strains). Plasmid DNA analysis revealed three Plasmid bands greater than that of the reference Plasmid marker HINDIII. Conclusion this study presents, the isolation of R-Plasmid strains conferring resistance to the fluoroquinolone antibacterial agents and other antibiotics in Benin City, Nigeria.

INTRODUCTION  
Infectious enteritis is a major cause of morbidity and mortality throughout the world. WHO reported that diarrhea diseases were among the ten killer diseases of children in eleven African and Asian American countries (report 1974) Diarrhoea diseases are relatively common in developing countries and where hygiene and sanitation are poor
and excrement contaminate the environment, water and food. In world terms this disease is estimated to kill about six million people annually and to incapacitate many more in developing countries (Ellis et al., 1984). Most diarrhoeal infections are acquired by ingestion being transmitted through food, drink or contaminated fingers or even surgical instruments. The respiratory route is also important for rotaviruses which have become important causes of diarrhoea in children worldwide, both in developed and developing countries (Cutting and Ellerock, 1998). Factors favouring food poisoning include warm climates which favour rapid multiplication of bacteria, in foods and beverages. Urbanization and travel, which facilitate spread, and problem with food production and preparation which ensures food is free from contamination before consumption. In developed countries these diseases are still common. In Britain in recent years acute diarrhoea and vomiting with severe dehydration have become rare in babies. This is probably due to factors such as improved bottle feeding, increase in breast feeding which is markedly protective, earlier oral rehydration decreased the use of antibiotics which mainly ineffective.

The importance of Escherichia coli diarrhoeal disease has been established through epidemiological studies using serotyping techniques. Evidence gathered through the last 30 years clearly implicated certain serotypes as causative agents of epidemic infantile enteritis in many countries, might be important in diarrhoeal disease of older children and adults’. Escherichia coli is not always a pathogen in the gut. Therefore, its pathogenic role in the G I T is of much significance to the Clinical Bacteriologist.

MATERIALS AND METHODS
Three hundred stool samples were collected from patients having acute diarrhoea attending various hospitals in Benin City, Nigeria. The samples were cultured using routine method of Microscopy culture and sensitivity (MC/S) in medical microbiology department of University of Benin teaching hospital Benin City, Nigeria.

IDENTIFICATION
Escherichia coli strains were identified using the protocol of Cowan and steel.

SEROTYPING
Serotyping was carried out on the strains using somatic Escherichia coli antibacteria obtained from Biotech laboratory UK.

ANTIMICROBIAL SUSCEPTIBILITY TESTING
Antimicrobial testing was carried out, using the agar diffusion method of Stokes.
MINIMUM INHIBITORY CONCENTRATION (M I C)
Minimum inhibitory concentration was carried out using agar dilution method also of Stokes

MATING EXPERIMENT (CONJUGATION)
Broth method was used for conjugation experiment recipient was Escherichia coli-lp J62K12. prolacnals while the donor strains were the Escherichia strains with very (M I C)

PLASMID DNA EXTRACTION The alkaline lysis method of Takahashi and Nagano was used to extract Plasmid DNA.

AGAROSE GEL ELECTROPHORESIS. Electrophoresis was carried on a horizontal apparatus using 0.7% agar use gels. A reference molecular weight Plasmid lambda (Hind11) with a reference molecular weight was included as a control. The gel was stained with ethidium bromide and photographed with a Polaroid camewra in a transilluminator.

RESULTS
Nine Escherichia coli serotypes were isolated, belonging to the following 0 serotypes 026, 0148, 015, 0142, 015, 0152, 0159, 0115 and 0157. The strains exhibited very high MICS to many antibiotics including the floroquinolone antibacterial agents. DNA Plasmid analysis revealed R-Plasmid bands of 6.1KB, 9.05 KB and 23.01 KB greater the molecular weight reference Plasmid marker HIND 11 fig 1.

DISCUSSIONS
This study presents the isolation of enterovirulent Escherichia coli from diarrhoea patients in Benin City Nigeria. Bacterial enteritis has mainly been attributed to infections due to Salmonella, Shigella and Campylobacter species of Escherichia coli enteritis has mainly been restricted to infection of infants three years old and below. This was wide spread before the advent of baby friendly imitative which greatly reduced morbidity and mortality rate due to enteritis. The 3% incidence rate of enterovirulent strains from the three hundred samples tested is low, but significant. The low rate of isolation of enterovirulent Escherichia coli even during an outbreak has been reported Jones and Roworth, 1996). Seven of the nine isolates were from children three years old and below. This is in agreement with WHO report which reports that infantile diarrhoea has plagued the developing countries for many years. (Report, 1974) Enterovirulent Escherichia coli were also isolated from young adults above three years old, This again is worthy of note because hitherto in this part of the world
adults were excluded from screening for enterovirulent *Escherichia coli* This work, therefore, corroborates literature reports that enterovirulent *Escherichia coli* is incriminated in both bloody and non-bloody diarrhea from all age groups. Therefore, the practice of not screening *Escherichia coli* for virulence should be reconsidered. Moreover, the incidence of the burger bug, came into focus after people that consumed hamburger contaminated with *Escherichia coli* 0157H7 who later had the infections of haemolytic uremic syndrome and haemolytic colitis, diseases with previously unknown etiology. Some of the strains isolated exhibited very high minimum inhibitory concentrations (MICs) to many antibiotics including the fluoroquinolone group of antibacterial agents, they were accordingly screened the presence of conjugative transferable R-Plasmid mediated resistance. The result revealed R-Plasmid DNA with a molecular weight of 6.01 KB, 9.03 KB and 23.05 KB as compared to the reference molecular weight Plasmid lambda Hind11.

This study presents the characterization of serotypes of *Escherichia coli* isolated from diarrhoea patients in Benin City Nigeria. *Escherichia coli* is a member of the enterobactereae family, which basically habit the gastrointestinal tracts of humans and animals. They are also a major group of the normal biotypes of the intestinal tract, where they play a mutualistic role. However onsite this habitat are very pathogenic to the host, for example they are the most notorious cause Urinary tract infection (UTI), wound and infections of blood (septicaemia). Enterovirulent *Escherichia coli* has been the main cause of infantile enteritis world over and was incriminated in diarrhea of babies, who were bottle fed. This promoted the WHO to initiate the baby friendly initiative that stipulates the compulsory breast feeding of babies for the first two years of life. This greatly reduced the infection through contaminated feeding bottles worldwide. Consequently, the morbidity and mortality of babies and children through infection with bottle contamination drastically reduced and is now completely a thing of the past. During this period screening for enterovirulent strains of *Escherichia coli* was restricted to babies and infants three years old and below, thus *Escherichia coli* isolated from stool cultures of adults above three years old were not screened for virulence. In 1983, some adults that ate hamburger were contaminated with *Escherichiacoli* STRAIN 0157H were infected with the organism and suffered from haemolytic uremic syndrome (HUS) and haemolytic colitis (HC) diseases with previously unknown etiology. And thus the burger bug phenomenon was born.

In this study enterovirulent *Escherichia coli* was isolated from all age groups invested in this study. This corroborates literature reports that entrovirulent strains of *Escher coli* are involved in diarrhea of all age groups worldwide. The strains isolated in this study exhibited high degree of MICs, and the screening for R[Plasmid resistance. The strains
exhibited transferable conjugative R-Plasmid mediated resistance to many antibiotics tested including the fluoroquinolones. Hitherto, Scientist and researchers have opined that bacteria cannot exhibit R-Plasmid mediated resistance to the fluoroquinolones, because the powerful antibacterial activity of the drug. This study therefore, seems to be the first authenticated result known to the author that has revealed that bacteria can now exhibit transferable conjugative R-Plasmid mediated and chromosomal mediated resistance to the fluoroquinolone group of antibacterial agents. This findings are very significant because the fluoroquinolones are the latest group of antibacterial agents available for treating life threatening infections in this part of the world. The result of this seemingly reduction of activity the fluoroquinolones could be as a result of abuse and misuse of drugs which very rampant in developing countries. The issue of abused, and misuse of drugs particularly antibiotics is responsible for treatment failures and it should be addressed by regulatory bodies especially WHO, NAFDAC in Nigeria and other agencies in various countries. Antibiotic resistance has been in the world from the discovery of antibiotics, even during the discovery of penicillin some bacteria were resistant to it. It said to note that when a new antibiotic is discovered people he a sigh of relief but sooner than none the same antibiotic becomes of little effect because of development of resistance. Some have opined that to prevent development of resistance one should stop the administration of antibiotics. This is a difficult decision to take, however with recent advent of probiotics this might be effective, but there are many challenges associated with it. Another important role in the prevention of the development of resistance is to reduce the use of broad spectrum antibiotics and for clinicians to avoid blind therapy and use antibiotics as prophylaxis. If antibiotics have to be used the laboratory susceptibility of the antibiotics by the bacteria one intends to eradicate must be received before a particular antibiotic is prescribed. The patients who are the end users must be thoroughly instructed on the use of the prescribed antibiotics.

CONCLUSION
This study presents, the isolation of enterovirulent Escherichia coli from stools of diarrhoeic patients in Benin City Nigeria, which also harboured transferable conjugative R-Plasmid mediated resistance to may be antibiotics tested including the fluoroquinolone antibacterial agent.

TABLE 1: MINIMUM INHIBITORY CONCENTRATIONS OF ALL ANTIBIOTICS TO THE ISOLATE IN ug/ml

<table>
<thead>
<tr>
<th>ANTIBIOTICS: PN, SXT, S, TE, OFX, CIP, AU, PEE, NA, CXM</th>
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<td>ISOLATE</td>
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### Legend

+ = Growth  - = NO Growth

### Table 2: Antibiotic susceptibility pattern of cured strains

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<th>Serotype</th>
<th>OFX</th>
<th>CXM</th>
<th>CPX</th>
<th>AU</th>
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<th>COL</th>
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Gel-electrophoresis analysis of plasmid DNA of trans-conjugant and donor strains

REFERENCES
Obaseiki Ebor ERE and Smith K C (1992) Properties of R-Plasmid PEB017 which confers both enhanced UV irradiation and immutability into wild type UMUC strains of E coli K12 Mutation research 256; 67-76.
Oronsaye F E (1997) Novel R-Plasmid mediated resistance to nalidiixic acid and the new 45-quinolones. MSC, THESIS.