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# Novel R-Plasmid from *E. coli* Isolated from Diarrheic Patients-Mediated Resistance to the Fluoroquinolones and other Antibiotics in Benin 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 humans and animals. The purpose of this study was to determine the involvement of this organism as cause of diarrhoea. And to screen the resistant strains so isolated for the presence of transferable conjugative R-Plasmid DNA. Three hundred stool samples were collected from patients in various hospitals in Benin City ad and cultured using routine methods in Medical microbiology department of University of Benin teaching hospital. Benin city, Nigeria. One hundred strains of entero-virulent *Escherichia coli* strains were isolated from the samples and were identified to species level using the protocol of Cowan and Steel. Serotyping was also done using antisera obtained from Biotech laboratories UK Antibiotics susceptibility tests was carried out on the isolates, 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 isolated strains belong the following o. Serotypes; 01, 020, 063, 044 (4strains), 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 showed three Plasmid bands greater than that of the reference Plasmid marker *HINDIII*. This study presents, the isolation of R- Plasmid strains conferring resistance to the fluoroquinolone antibacterial agents and other antibiotics in Benin City, Nigeria.

**Keywords:** Plasmids, diarrhoea, antibiotics, strains, entero-virulent, fluoroquinolone

## INTRODUCTION

Infectious enteritis is a major cause of morbidity and mortality throughout the world. WHO reported that diarrhoea diseases were

among the ten killer diseases of children in eleven African and Asian American countries (report 1974) Diarrhoea is relatively common in developing countries and where hygiene and sanitation are poor and excrement contaminate the environment, water and food. In world terms, diarrhoea 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 rota viruses 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 i*s 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* antisera obtained from Biotech laboratories in 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*-1p J62K12. Pro lac- nals while the donor strains were the isolated *Escherichia* strains with very (M I C)

## Plasmid DNA Extration

The alkaline lysis method of Takahashi and Nagano was used to extract Plasmid DNA. Since the PCR machine available to the author, at the time of this experiment

## Agarose Gel Electrophoresis

Electrophoresis was carried on a horizontal apparatus using 0.7 % agarose use gels. A reference molecular weight Plasmid lamda

(Hind11) with a reference molecular weight was included as a control. The gel was stained with ethidium bromide and photographed with a Polaroid camera under a UV trans-illuminator.

## RESULTS

Nine *Escherichia coli* serotypes were isolated, belonging to the following o 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 6.1 KB, 9.05 KB and 23.01 KB greater the molecular weight of the reference Plasmid marker *HIND* III fig 1. .

## DISCUSSIONS

This study presents the isolation of enterovirulent *Escherichia coli* strains exhibiting transferable conjugative R-PLASMID DNA from diarrhoea patients in Benin City Nigeria. Bacterial enteritis has mainly been attributed to infections due to *Salmonella, Shigellae, Camphyllobacter* species and other bacterial 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. (Oronsaye and Oziegbe, 2002) The 3 % incidence rate of enterovirulent strains from the three hundred samples tested is low., but significant.

The low rate of isolation of enterovirulent *of Escherichia coli* even during an outbreak has been previously 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 diarrheoa 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

diarrheoa from all age groups. Therefore, the practise of not screening *Escherichia coli for v*irulence should be reconsidered. More- over, the incidence of the burger bug, came into focus after people that consumed hamburger contaminated with *Escherichia coli 0157H;7* which later had haemolytic uremic symdrome (H U S ) and haemolytic colitis (H C) diseases of previously unknown aeteology .Some of the strains isolated exhibited very high minimum inhibitory concentrations (MICs) to many antibiotics tested against them including the fluoroquinolone group of antibacterial agents, they were accordingly screened for the presence of conjugative transferable R-Plasmid mediated resistance using a horizontal apparatus. The PLASMID DNA analysis result showed R-Plasmid DNA bands with molecular weights of 6.01 KB, A9.03 KB and 23.05 KB as compared with the reference molecular weight Plasmid *lambda HIND*III.

This study presents the characterization of serotypes of *Escherichia coli* exhibiting transferable conjugative R-PLASMID DNA isolated from diarrhoea patients in Benin City Nigeria. *Escherichia coli* is a member of the enterobactereacae family, which basically inhabit 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, outside 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 diarrheoa of babies ,who were bottle fed. This prompted 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, when, some adults that ate hamburger were contaminated with *Escherichia coli* strain; 01 57; H;7 were infected with the organism and suffered from haemolytic uremic symdrome (HUS) (HUS) and haemolytic colitis(HC) diseases with previously an unknown aeteolgy. And thus the burger bug phenomenon was born.

In this study, enterovirulent *Escherichia coli was isolated from,* all age groups This corroborates literature reports that entrovirulent strains of *Escher coli* are involved in diarrheoa 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 of the powerful antibacterial activity of the drug. .

This study therefore, seems to be the first authenticated result known to the author that has shown that bacteria can now exhibit transferable conjugative R-Plasmid mediated and chromosomal sub-mediated resistance to the fluoroquinolone group of antibacterial agents. These 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 is 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. There is always a sigh of relief whenever a new antibiotics is discovered but sooner than

expected, the same antibiotics 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 patents who are the end users must be thoroughly instructed on the use of the prescribed antibiotics.

## CONCLUSION

This study presents the isolation of enterovirulent *Escherichi* 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 fluoroquinilone antibacterial agents.

**Table 1: Minimum Inhibitory Concentrations of all Antibiotics to the Isolates in ug/ml**

**Antibiotics: PN, SXT, S, TE, OFX, CIP, AU, PEE, NA, CXM**

| ISOLATE | 10 | 20 | 25 | 50 | 100 | 200 | 400 | 5000 | 1000 | 1200 | 1400 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 0148 | + | + | + | + | + | + | + | + | + | - | - |
| 0149 | + | + | + | + | + | + | + | + | + | - | - |
| 014 | + | + | + | + | + | + | + | + | + | - | - |
| 07 | + | + | + | + | " | + | + | + | + | - | - |
| 0152 | + | + | + | + | " | + | + | + | + | - | - |
| 044 | + | + | + | + | + | + | + | + | + | - | - |
| 026 | + | + | + | + | + | + | + | + | + | - | - |
| 0126 | + | + | + | + | + | + | + | + | + | - | - |
| 044 | + | + | + | + | + | + | + | + | + | - | - |

**Keys:**

Legend + = Growth - = NO Growth PN-ampicillin, SXT-cotrimoxazole, S –stromycine, TTE-tetracycline, CIP-ciprrofloxacin, AU- augumetin, OFX-ofloxoxacin, CXM-zinacef, PEF-pefloxacin, NA-nalidixic acid

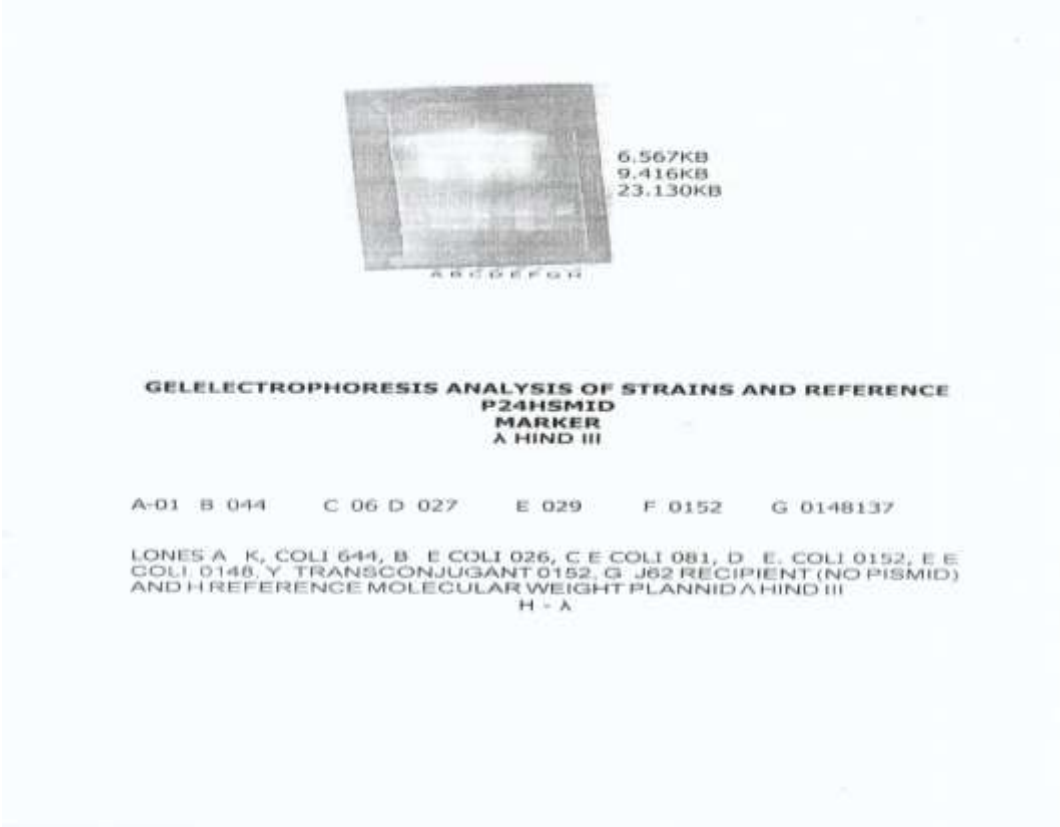


Figure 1:

Table 2: Antibiotic susceptibility pattern of cured strains

| Serotype | OFX | CXM | CPX | AU | S | COL | NA | SXT | PN |
|---|---|---|---|---|---|---|---|---|---|
| 026 | S | S | S | S | S | S | S | R | R |
| 015 | S | S | S | S | S | S | S | R | R |
| 0143 | S | S | S | S | S | S | S | R | R |
| 0148 | S | S | S | S | S | S | S | R | R |
| 0115 | S | S | S | S | S | S | S | R | R |
| 0152 | S | S | S | S | S | S | S | R | R |
| 0148 | S | S | S | S | S | S | S | R | R |
| 0159 | S | S | S | S | S | S | S | R | R |
| 015 | S | S | S | S | S | S | S | S | R |

Gel-electrophoresis analysis of plasmid DNA of trans-conjugant and donor strains

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