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Full Length Research Paper

Multi-drug resistant pattern and plasmid profile of *Escherichia coli* and other gram-negative bacteria isolates from clinical specimen in Benin City, Nigeria

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***Escherichia coli* (*E. coli)* belonging to the family Enterobacteriaceae has been implicated as the causal agent to many gastro-intestinal disorders in man and animal. Seventy samples of clinical origins were collected randomly from patients at Lahour Public Health Research centre, Benin City in Edo state. *E. coli* were found in 4/70 (25%) of the samples; while *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. had percentage abundance of 12.5, 31.25 and 31.25% respectively. All *E. coli* isolates were resistant to the following antibiotics Ampicilin, Nalidixic acid, Chloramphenicol, Tetracycline, Cotrimoxazole, Norfloxacine, Traflox, Ciproval, Nitrofurantoin and Perflacin, while 75% were resistant to Gentamicine, only. *E. coli* isolates were screened for plasmids. It was observed that 3(75%) were plasmid mediated while 1(25%) harbored only the chromosomal DNA. This study indicates the susceptibility pattern of *E. coli* resistance to different antibiotics and the need to contend the continuous spread of the these resistant strains.**

Key words: *Escherichia coli*, antibiotics resistance pattern, plasmid profile.

INTRODUCTION

Escherichia coli is a human pathogen worldwide associated with meat and meat products, dairy products, vegetables, and water (Browning et al., 1990; Obi et al., 2004; Magwira et al., 2005). It is recognized as a bacterium causing hemorrhagic colitis (Olorunshola et al., 2000). Diarrheal diseases linked to *E. coli* infections are characterized by blood, cramping abdominal pain, fever, nausea, and vomiting (Shebib et al., 2003). Resistance of pathogenic organisms to antibiotics is an increasing problem to the treatment of most microbial infection and the rapid dissemination of drug-resistant bacteria is an increasing global problem that seriously complicates the treatment of human infections (Van den Bogaard and Stobberingh, 2000). Different factors could contribute to this increase, as the high use of antimicrobial agents in humans and animals that applies a pressure for selection of resistant bacteria, the capacity of bacteria to disseminate antimicrobial resistance genes to other bacteria mainly by mobile genetic structures, and the facility of dissemination of resistant bacteria in different ecosystems (Martínez, 2008).

Antimicrobial resistance associated with specific antimicrobials may occur in a number of ways. These include: reduced uptake, impermeability or efflux mechanisms for a particular antimicrobial, drug degradation (enzyme attack) or modification of specific target sites by the organism where the drug would normally act. Alternatively, organisms modify specific sites where antimicrobials normally act by duplication of the target site with a site that is non susceptible thus causing a ‘‘bypass’’ of the antibiotic sensitive step (Chopra and Russell and Chopra, 1996; Chopra, 1998; Russell, 1998, 2002). Recently, integrons have been recognized as a mecha-nism for acquiring multiple antimicrobial resistances in some organisms. Integrons are a class of novel,

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Table 1. Percentage Antibiotics Resistance of Pathogenic Isolates from Clinical samples.

Antibiotic	Number of resistant strain				Percentage resistance (%)			
	A	B	C	D	A	B	C	D
Nalidixic acid	4	1	4	5	100	50	80	100
Chloramphenicol	4	NIL	4	5	100	NIL	80	100
Norfloxacin	4	NIL	3	3	100	NIL	60	60
Gentamycin	3	1	1	4	75	50	80	80
Ampicilin	4	1	5	5	100	50	100	100
Tetracycline	4	2	4	5	100	100	80	100
Cotrimoxazole	4	NIL	3	5	100	NIL	60	100
Traflox	4	NIL	1	3	100	NIL	20	60
Ciproval	4	NIL	4	3	100	NIL	80	60
Nitrofurantoin	4	1	5	5	100	50	100	100
Perflacin	4	1	5	5	100	NIL	40	60

Key: A = *E. coli*; B = *Klebsielle* spp.; C = *Proteus* spp. and D = *Pseudomonas* spp.

naturally occurring mobile genetic elements that can capture antimicrobial resistance and other genes and promote their transcription (Stokes and Hall, 1989; Hanau-Bercot et al, 2002). Class 1 type integrons are con-sidered the most common in clinical isolates (Recchia and Hall, 1995; Collis et al., 1998). Resistance genes can be integrated in the form of cassettes, this reaction is catalysed by the integron encoded integrase (int1) gene at the att1 site (Hansson et al., 1997; Partridge et al., 2000).

In many pathogenic bacteria, plasmids frequently carry antibiotics resistance encoding genes allowing bacteria to survive antibiotic treatment besides encoding virulence factors.

The aims of this study are to determine the anti-microbial pattern of some pathogenic bacteria in clinical samples and the relationship of plasmid profile.

MATERIALS AND METHODS

Study areas and sample collection

Seventy clinical samples comprising of stool, urine, sputum and exudates from wound were collected from sick patients at Lahor Public Health Research Center, Benin City Nigeria. The samples were collected in sterile Screw-caped universal container and were taken to the laboratory for analysis.

Identification of bacteria

One gram of the sample was weighed and suspended into 9 ml of peptone water. 10-fold dilution was made. One hundred microlitre of each dilution was plated on Blood agar, MacConkay agar and Chocolate agar and was incubated aerobically at 37℃ for 24 h. Isolates were identified biochemically.

Susceptibility testing

Antibiotics susceptibility test for the isolated organisms was conducted by disc diffusion using Muller Hintin agar plates according to modified method of Bauer et al. (1966). The plates were incubated at 37℃ for 24 h. The antibiotics tes ted includes Ampicilline, Nalidixic acid, Chloramphenicol, Tetracycline, Cotrimoxazole, Norfloxacine, Traflox, Ciproval, Nitrofurantoin, Gentamicin and Perflacin. The zones of inhibition around the disc were measured.

Plasmid profiling

The modified method of Birmboin and Doly (1979) was used to screen for the presence of Plasmid in the resistant isolates.

Plasmid DNA electrophoresis

Plasmid DNA was electrophoresed on 1.3% agarose gel, stained with ethidium bromide. Hind III digested plasmid was used as a control marker. The gel was visualized with UV transilluminator and photograph of the bands were taken using the pollaroid.

RESULTS

Drug susceptibility testing

Seventy samples from clinical origins were collected randomly from patients at Lahour Public Health Research centre, Benin City in Edo state. Percentage abundance of *E. coli* isolated was (25%). While other Gram-negative bacteria comprising of *Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. had percentage abundance of 12.5, 31.25 and 31.25% respectively.

All *E. coli* isolates were resistant to Ampicilin, Nalidixic acid, Chloramphenicol, Tetracycline, Cotrimoxazole, Norfloxacine, Traflox, Ciproval, Nitrofurantoin and Perflacin while 75% were resistant to Gentamicin (Table 1).

Two *Klebsiella* spp. were isolated and tested for antibiotic resistance; only one (50%) was resistant to

Table 2. Plasmid profile.

Isolate number	Niurofurantom	Traflox	Norfloxacin	Cotrimoxazeole	Gentamicin	Tetracyclin	Ciproval	Nalidixil acid	Chloramphenicol	Ampicillin	Peflacin	Plasmid number	Plasmid size (kb)
E1	-	-	-	-	-	-	-	-	-	-	-	1	3.3
E2	-	-	-	-	-	-	-	-	-	-	-	1	3.4
E3	-	-	-	-	+	-	-	-	-	-	-	1	3.3
PR1	-	+	-	-	-	-	+	-	-	-	+	2	3.7, 3.4
PR2	-	-	-	-	-	-	-	-	-	-	-	1	3.4
P1	-	+	+	-	+	-	+	-	-	-	+	2	3.5, 3.3
P2	-	-	-	-	-	-	-	-	-	-	-	2	3.5, 3.3
P3	-	+	+	-	-	-	+	-	-	-	+	2	3.5, 3.3
P4	-	-	-	-	-	-	-	-	-	-	-	2	3.5, 3.3
P5	-	-	-	-	-	-	-	-	-	-	-	1	3.2

+, Positive; _, Negative.

Nitrofurantoin, Gentamicin, Nalidixic acid and Ampicilin. While the two isolates were resistant to Tetracyclin. For the rest of other antibiotics (Chloramphenicol, Cotrimoxazole, Norfloxacine, Traflox, Ciproval and Perflacin), the *Klebsiella* spp. is 100% sensitive (Table 1).

The five isolates of *Pseudomonas* spp. tested for susceptibility to multi-antibiotics activity were 100% resistant to Nitrofurantoin, Cotrimoxazole, Tetracycline, Nalidixic acid, Chloramphenicol, Ampicilin; 80% were resistant to Gentamicin and 60% were resistant to Norfloxacine, Traflox, Ciproval and Perflacin (Table 1).

Five *Proteus* spp. isolated and tested for antibiotics resistance, 100% were resistant to Nitrofurantoin and Ampicilin; 80% were resistant to Gentamicin, Tetracycline, Nalidixic acid, Chloramphenicol, and Ciproval. 60% were resistant to Cotrimoxazole and Norfloxacine; 40% were resistant to Perflacin and 20% were resistant to Traflox (Table 1).

Plasmid profilling

E. coli and other Gram-negative bacteria isolates were screened for plasmids. Table 2 shows the plasmid number and size from the isolated *E. coli, Klebsiella* spp., *Proteus* spp. and *Pseudomonas* spp. Gram-negative bacteria from clinical samples. It was observed that 3(75%) of *E. coli* isolates were plasmid mediated while 1(25%) harbored only the chromosomal DNA.

DISCUSSION

The study reveals that multiple antibiotics resistance exists among the clinical pathogens examined. *E. coli* isolates were observed to be resistant to commonly used antibiotics in clinical medicine.

There has been a growing concern of the possible emergences of antimicrobial resistant Enterobacteriaceae strains especially *E. coli* as a result of possible neglect of treatment procedures. The level of antibiotic resistance of these pathogens is quite high and it could be as a result of antibiotic drug abuse, in appropriate dosage administration and duration, wrong diagnosis before treatment has also contributed to the spread of antibiotic resistant strains.

Plasmid profiling analysis of the isolates revealed that 3(75%) of *E. coli* resistant strain were Plasmid mediated, thus each harboring one plasmid. The other Gram-negative bacteria isolates harbored either one or more plasmids (Table 2). The implication of this is that these isolates may be containing either resistant or virulent plasmids. Smith et al. (2003) revealed the presence of plasmid (47%) in eight *E. coli* isolates from animal origin.

The resistance upsurge of these isolates to commonly used antibiotics were very high and in view of the burden they pose to medical practitioners, as well as the limited availability of antimicrobial agents for the treatment of infections caused by these organisms, there is need to contend the spread of the antibiotic resistant strains, since multidrug resistant strains can transfer resistance through their plasmid to other enteric organisms.

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