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In-Vitro Susceptibility of Fluoroquinolone Resistance Escherichia Coli to Alkaloid Extracted from Phyllanthusniruri

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Abstract

The antibacterial potency of alkaloid extracted from Phyllanthus niruri was examined on Fluoroquinolone resistant Escherichia coli isolated from different clinical samples using disk diffusion method. Different concentrations (0.1 â?? 5mg/ml) of the alkaloid were used. It was observed that at 0.5mg/ml the extract showed more potency on Escherichia coli isolated from urine than from other samples with a diameter of zone of inhibition of 25.5mm. The percentage susceptibility of the isolated bacterium from urine, blood, semen, swab, and high vagina swab(HVS) to the alkaloid were 75

Index terms— In-vitro, fluoroquinolone, escherichia coli, alkaloid, phyllanthus niruri.

1 Introduction

he increasing prevalence of antimicrobial resistance affecting hospitalized populations has gained prominence. Recent investigations reported that among hospitalized patients, residence in a longterm care facility was a risk factor for colonization or infection with Escherichia coli that was resistant to higher generation cephalosporin and to the fluoroquinolone (FQ) antimicrobial agents **??**Lautenbachet al., 2001; **??**autenbachet al., 2002). Escherichia coli are a common constituent of the gastro-intestinal flora of most vertebrates, including humans, and may be isolated from a variety of environmental sources. While most strains are nonpathogenic, certain ones can cause a variety of intestinal and extra intestinal infections. Pathogenicity is largely determined by gene-encoding virulence factors such as adhesions, toxins, and polysaccharide surface coatings **??**Johnson et al., 2009).

Authors ? ? : Department of Sciences Technology, Microbiology Unit, Federal Polytechnic, P.M.B. 53513, Ado -Ekiti, Nigeria. E-mail : ajibvijay@yahoo.com Phylogenetic analysis showed that most E.coli strains fall into 4 main phylogenetic groups, designated A, B1, B2 and D **??**Arpin et al., 2007). E.coli strains that cause extra intestinal infections derive predominantly from group B2 and, to a lesser extent, group D. Strains of group A and B1 represent most commensal strains and are largely devoid of virulence determinants **??**Johnson et al., 2009). Although strains harboring a robust extraintestinal virulence factors repertoire cluster predominantly in groups B2 and D, isolates within each phylogenetic group can be further classified as extraintestinal pathogenic E.coli (EXPEC) or non-EXPEC depending on whether specific virulence traits are present **??**Johnson et al., 2003; **??**alboet al.,2005).

The fluoroquinolone (FQs) are potent antimicrobial agents used for the treatment and prophylaxis of infections caused by Gram-negative bacteria, including E.coli. FQ-resistant E.coli has been reported increasingly during the last decade in both the hospital environment and the community, which may ultimately limit the utility of these broad-spectrum agents **??**Calboet al., 2005). However, FQs are still the most frequently prescribed antimicrobial class in hospitals at Ado-Ekiti accounting for 25% of all antimicrobial prescriptions. While evidence suggests that the prevalence of FQ-resistant E.coli carriage among residents in Ado-Ekiti is increasing, the present level of risk factors for FQ-resistant E coli colonization has not been studied.

Before the advent of modern medicine of which many drugs were synthetically produced, extract of many plants were known to elicit certain reactions in human body when applied in a prescribed manner. Among such plant is Phyllanthus niruri L., (Syn. P. fraternus. Webster). It belongs to the Euphorbiaceae family and has been claimed to be an excellent remedy for jaundice and hepatitis (Qudhia and Tripathi, 2002; **??**abasumet al., 2005). The plant is considered analgesic, digestive, emmanagogue, laxative stomachic tonic **??**Khannaet al., 2002). It is

also helpful in treating edema, anorexia and diabetes (George and Roger, 2002.). According to Ayurvedic system of medicine it is considered acrid, cooling, alexiopharmic and useful in thirst, bronchitis, leprosy, anemia, urinary discharge, anuria, biliousness, asthma, for hiccups, and as a diuretic. According to Unani system of medicine, the plant is stomachic and good for sores and useful in chronic dysentery (Unander, 1990;Raphael et al., 2000;Halim and Ali, 2002). A poultice of the leaves with salt cures scabby infection. The bark yields a bitter principle phyllanthin **??**Tabasumet al., 2005). Many of the active constituents found in the plant are biologically active lignands, glycosides, flavonoids, saponins, alkaloids, ellagitannins and phenylpropanoids (Tiwaladeet al.,2000; **??**hir et al.,2002), common lipids sterols and flavonoids also occur in the plant (Barros et al.,2003). Alkaloids are organic nitrogen-containing compound found in 20%-30% of vascular plants (**??**Tabasumet al., 2005) and at lower doses they are useful pharmacologically. Some have important clinical use such as analgesics, antimalarial, antispasmodics, for pupil dilation, and treatment of hypertension, mental disorders and tumors. Morphine, codeine, atrophine and ephedrine are just a few of the plant alkaloid currently used in medicine (Naik and Juvekar, 2003). Other alkaloids, including cocaine, nicotine and caffeine, enjoy a widespread non-medical use as stimulants or sedatives (Naik and Juvekar, 2003). Some alkaloids are medically useful for the cure of human diseases e.g. atrophine in treatment of bronchial asthma (Tabasumet al., 2005); intestinal and biliary colic, and to dilate pupil of the eye (Naik and Juvekar, 2003).

The aim of this work is to study the potency of alkaloid extracted from Phyllanthus niruri on Escherichia coli found to be resistant to fluoroquinolone.

2 II.

3 Materials and Methods

4 a) Collection of Plant Material

Phyllanthusniruri was collected from shrubs around the Federal Polytechnic compound, Ado-Ekiti, Nigeria between the months of July and September, 2008 and was identified at the Department of Plant Science, University of Ado-Ekiti, Nigeria. A voucher specimen was deposited at the herbarium of the Department of Science Technology, Federal Polytechnic, Ado-Ekiti. The sample used for the analysis were air-dried at room temperature of (28 ± 2 o C) and pulverized.

5 b) Collection of Specimens and detection of FQresistant E.coli

FQ-resistant E.coliwas detected by antimicrobial activity against Nalidixic acid multo-disk and a 1-step screening procedure (Maslow et al., 2004). Species identification and FQ resistance were confirmed by automated testing (Vitek, bioMerieux, USA). Urine, blood, High vaginal swab (HVS), semen and rectal swab samples were obtained from the University Teaching Hospital, Ado-Ekiti.

6 c) PCR Amplifications

Template DNA was prepared by boiling. Briefly, from 59 initial patient samples, 37(62%) colonies of E. coli were suspended thoroughly in 1mL DNase-and RNase-free water and boiled for 10minutes. After centrifugation, supernatant was used as template DNA.TheampC was amplified in theupstream region, bla TEM , bla SHV , bla CTX-M , bla OXA-I , and bla CMY by PCR, using specific oligodeoxynucleotides. PCR was performed in a 25-?L mixture of 1 x buffer, 2.5 mmol/L MgCl 2 2.5U of FIREPol DNA polymerase, 200?mol/L of each deoxynucleoside triphosphate, and 25 pmol of each primer. The PCR mixture was subjected to a 5min denaturation step at 94 o C, followed by 30cycles of 45s at 94 o C, 45s at 55 o C, and 60s at 72 o C, and a final elongation step of 5min at 72 o C. PCR products were separated by 100V elkectrophoresis in a 2% agarose gel for 30min, after which they were stained with ethidium bromide **??**Maslow et al., 1993

7 d) Extraction of Crude Alkaloid

The method of Naik and Juvekar (2003) was employed for the extraction. The dried, coarsely powdered whole plant of P. niruri (200g) was moistened with 25% ammonium hydroxide, allowed overnight standing and then Soxhlet extracted with 95% ethanol. After concentration under vacuum, the syrup residue (30g) was treated with concentrated hydrochloric acid. The acidic filtrate was washed with benzene, made basic (pH 10) with 25% ammonium hydroxide and extracted with chloroform to afford the alkaloidal fraction.

8 e) Bacteriological Assay

Isolates were removed from stocks, streaked onto Nutrient agar (LAB) incubated overnight at 37 o C to resuscitate the cultures. The organisms were identified by Gram's reaction, colony characteristics and biochemical reactions.

9 f) Determination of antibacterial potency

The disk Diffusion method described by Odetola and Okorosobo (1996) was employed. Various concentrations of the extracts (10-30mg/ml) were prepared and spread evenly on Nutrient agar. Nalidixic disc was placed on the

dried agar and incubated for 24hrs at 37 o CThe diameter of zones of inhibition was measured with a meter rule. The plates were examined in triplicate according and the average diameter recorded. Zone measuring ?5.0mm was recorded as susceptible and ?4.0 as resistant. The percentage resistance/sensitive was also calculated.

10 g) Statistical Analysis

Statistical analysis was performed by using SPSS software version 13.0. The Mantel-Haenszel x 2 test was used for trend analysis.

11 III.

12 Results and Discussion

The results of the resistant pattern of E. coli to fluoroquinolone are shown in table 1. It was observed that all the isolates from urine, blood, semen, rectal swab and HVS were resistant to fluoroquinolone. Table 2 represents the susceptibility pattern of E. Colii solated from different samples to different concentration of alkaloid. The highest susceptibility was seen in urine sample (18) while the lowest was seen in rectal swab and HVS. (3). the percentage susceptibility pattern of the isolates to different concentration of alkaloid is shown in table 3; it was observed that all the isolates were susceptible with the highest susceptibility showed in isolates from semen. Thirty-seven (37) strains were tested for extended-spectrum beta-lactamase (ESBL) identification. They were all positive for bla CTX-M in 37(100%) of the ESBL-carrying strains. CXT-M-14 was the most frequently isolated ESBL (n=15), followed by CTX-M-27 (n=12) and CTX-M-15(n=5), one strain (CEC7) was carrying both bla CTX-M-14 and bla CTX-M-15. Strain CEC14 was carrying a bla CTX-M-14 variant, which differed from the parental enzyme by a single transversion Although the patients included in this work came from the clinical, antimicrobial drug resistance was prevalent among UTI-causing strains and those isolated from blood, particularly to ?-lactamase producing strains (including extended-spectrum cephalosporins). The findings of this work suggest that CTX-M type ?-lactamase is widespread in Ado Ekiti (the area where the research was undertaking). CTX-M production was significantly associated with resistance to quinolones. The spread of CTX-M in the community has already been described through prospective studies in industrialized countries such as Canada **??**Etienne et al., 2009). The excessive use of ?-lactam antimicrobial drugs has led to the emergence of resistant strains worldwide. ?-lactam resistance is mostly mediated through acquisition of ?-lactamase genes located on mobile genetic elements such as plasmids or transposons.

Epidemiologic surveillance of antimicrobial resistance is indispensible for empirically treating infections, implementing resistance control measures, preventing the spread of antimicrobial-resistant microorganisms and most significantly revisiting nature that is cheap and affordable for treating ailments from these pathogens.

The use of alkaloid extracted from the whole plant of P. niruri showed high veracity and potency on E. coli at the various concentration tested, and especially at 0.5 mg/ml. The data obtained in this study have led to the conclusion that the alkaloid extracted from P. niruri is potent on fluoroquinolone resistant E. coli and may be responsible for the significant antibacterial effect of this plant on a wide range of organisms. This mayexplain some of the ethno pharmacological claims thatthis plant, especially its application as poultice for the treatment of chronic dysentery is effective (George and Pamplona-Roger, 2002).

Because the resistance patterns are continually evolving and E. coli invasive isolates undergo progressive antimicrobial resistance, continuously updated data on antimicrobial susceptibility profiles will continue to be essential to ensure the provision of safe and effective empiric therapies by using herbs. Moreover, results obtained from these surveillance systems must be used to implement prevention programs and policy decisions to prevent emergence and spread of antimicrobial resistance and most importantly embrace phytotherapy.

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[1] [2]

[2]In-Vitro Susceptibility of Fluoroquinolone Resistance Escherichia Coli to Alkaloid Extracted from Phyllanthusniruri

Figure 1:

Figure 2:

1

Samples	Sensitive (%)	Resistant (%)
Urine	0(0)	24(100)
Blood	0(0)	12(100)
Semen	0(0)	5(100)
Rectal Swab	0(0)	5(100)
HVS	0(0)	5(100)

Figure 3: Table 1 :

2

Samples (No) Number of sensitivities	Concentration (mg/ml)				
	0.1	0.2	0.3	0.4	0.5
Urine (24)	9	10	14	17	18
Blood (12)	8	8	9	9	10
Semen (4)	3	3	3	3	4
Rectal swab (5) 2		2	3	3	3
HVS (5)	0	2	3	3	3

Figure 4: Table 2 :

3

	coli to
alkaloid at 0.5mg/ml	
Samples	Sensitive (%)
Urine	18(75)
Blood	10(84)
Semen	4(100)
Rectal Swab	3(60)
HVS	3(60)

Figure 5: Table 3 :

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