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Short Communication

Antimicrobial susceptibility of some urinary tract clinical isolates to commonly used antibiotics

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The antimicrobial susceptibility of urinary tract isolates from pregnant women attending antenatal clinics in various hospitals within Kaduna, Nigeria, was carried out using the disc diffusion method. The patterns of inhibition varied with the concentration of the antibiotic used. Escherichia coli was the most sensitive to ciprofloxacillin (33 mm/5 ∝g ml^{-1}) and pefloxacin (15 mm/10 ∝g ml^{-1}). It was resistant to minocycline, nalidixic acid, cefuroxine and cotrimoxazole. Klebsiella spp., Proteus spp. and Staphylococcus spp. were moderately sensitive to ciprofloxacillin (14, 15 and 18 mm/5 ∝g ml^{-1}) respectively. All the organisms were resistant to minocycline and cefuroxine. Ciprofloxacillin appeared to be the drug of choice for the treatment of urinary tract infection.

Key words: Urinary tract infection, clinical isolates, antibiotics.

INTRODUCTION

In the female human subject, the urinary tract has an important association with the reproductive organs because of its proximity. In the non-pregnant state, the uterus lies just behind and partly over the bladder while in the pregnant state; the enlarging uterus affects all the tissues of the urinary tract at various times (Warren et al., 1982). In healthy individuals, the freshly voided urine is sterile and free from microorganisms. The urethra is shorter in females than in males and is more readily transversed by microorganisms. This is why urinary tract infections are more common in females (Uehling, 1995). The highest incidence of urinary tract infection occurs in the child bearing age and this has been linked directly to sexual activity and aging (Blumberg and Abrutyn, 1997). The aims and objectives of this study is to isolate and identify microorganisms implicated in urinary tract infection as well as determining the susceptibility pattern of the isolates.

MATERIALS AND METHODS

Collection of urine samples

Midstream urine samples were collected from pregnant women attending antenatal clinics in Yusuf Dan-tsoho Hospital (40 samples), the Sick Bay, Kaduna Polytechnic (26 samples) and the Army Reference Hospital (24 samples) all in Kaduna, Nigeria. The women were instructed on how to collect the midstream urine samples into sterile bottles. The samples were then transported to the laboratory with ice packs in sterile containers. They were then kept in the refrigerator at 4°C until analysis, which was done within 24 h.

Total aerobic plate count

The bacterial load of the urine samples was determined using surface plating method. Serial dilutions were carried out by pipetting one ml of the urine sample into 9 ml of peptone water and from this 10^{-1} dilution, and further dilutions were carried out up to 10^{-6}. The test tubes were then incubated at 37°C for 24 h after which the count was obtained using a colony counter.

Isolation of specific organisms on selective media

Two selective media were prepared. These were MacConkey agar and cysteine lactose electrolyte-deficient agar. The plates were streaked with the urine samples. The plates were incubated at 37°C for 24 h. The isolates were further identified morphologically and biochemically.

Gram staining technique

The standard procedure for Gram staining technique was carried out on all isolates. The slides were observed under X100 magnification.

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Table 1. Range of bacterial counts in urine samples of pregnant women attending the antenatal clinics of selected hospitals in Kaduna, Nigeria.

Name of hospital	No of urine samples collected	Range of bacterial count (cfu/ml)
Yusuf Dantsoho	40	$1.04x10^4$ to $6.3X10^4$
Kaduna Polytechnic Clinic	26	$1.3x10^3$ to $8.3x10^3$
Army Reference Hospital	24	$1.1x10^4$ to $9.2x10^4$

Table 2. Biochemical characterisation of the isolates from urine samples of pregnant women studied.

Test organism	Growth on MSA	Coagulase	Dnase	Haemolysis on BA
Staphylococcus aureus	+	+	+	+
E coli	Indole +	MR +	VP -	Citrate -
Proteus spp.	Urease +	Lactose +	VP +	Swarming growth on selective agar +
Klebsiella spp.	Lactose +	Glucose +	Mannose +	Hydrogen sulphide production +

Table 3. Susceptibility testing of isolates to antibiotics.

Organisms	Diameter of zone of inhibition (mm)							
	CIP	COT	CF	AMX	AMP	PEF	MIN	NA
E. coli	33	18	-	30	18	15	-	20
Klebsiella spp.	18	12	-	13	20	13	-	22
Staphylococcus	15	9	-	12	13	16	-	14
Proteus spp.	14	13	-	22	18	20	-	13

Cip = Ciproxacillin (5 Jg); Cot = Cotrixozaxole (30 Jg); Cf = Cefuxorine (3.0 Jg); Amx = Amoxycillin (10 Jg); Amp = Ampicillin (20 Jg); Pef = Pefloxacin (10 Jg); Min = Minocycline (10 Jg); NA = Nalidixic acid (30 Jg).

Biochemical identification of isolates

All isolates from the urine samples were subjected to biochemical tests. Escherichia coli was identified by using the ImVic tests. These included indole production, Voges-Proskauer reactions and citrate utilization tests. Staphylococcus aureus was identified using the coagulase, haemoglobin, and lecithinase tests. Other tests conducted were Dnase and gelatinase tests. Proteus spp. was identified using the characteristic ammonia liberation from urea in 4 h and production of hydrogen sulphide from TSI agar. Klebsiella spp. was identified using sugar fermentation test (lactose, glucose, mannitol, adonitol, saccharose), gelatin liquefaction and hydrogen sulphide tests.

Disc susceptibility test

Disc sensitivity test agar was prepared and poured into sterile plates. The medium was allowed to solidify. The pure isolates were then streaked on well-dried plates. The antibiotic discs were then placed on the agar plates, which were then left at room temperature for 1.5 h to allow for diffusion of the antibiotic into the medium. The plates were then incubated at 37°C for 24 h. The zones of inhibition were then measured to the nearest millimeter and recorded. The antibiotic discs and the concentrations used were ciprofloxacillin (5 ∝g), cotrimozaxole (30 ∝g), cefuxorine (30 ∝g), amoxycllin (10 ∝g), ampicillin (20 ∝g), pefloxacin, minocycline (10 ∝g) and nalidixic acid (30 ∝g).

RESULTS AND DISCUSSION

The range of bacterial counts in urine of the pregnant women attending various antenatal clinics in the three hospitals selected for the study is recorded in Table 1. The counts ranged between $1.3x10^3$ to $9.2x10^4$ cfu/ml. Table 2 shows the biochemical characterisation of the isolates from urine samples of pregnant women studied. Table 3 shows the susceptibility testing of isolates. E. coli was most sensitive to ciproflaxicin, followed by Klebsiella spp., Staphylococcus spp. and Proteus spp.

The incidence of urinary tract infections is far more frequent in women than in men by reason of their fundamental physiological differences. The results showed that the organisms isolated from urine samples of pregnant women studied were E. coli, Staphylococcus aureus, Proteus spp. and Klebsiella spp. The viable count of these organisms ranged from $1.3x10^3$ to 9.2×10^4 cfu/ml. These results indicate that infection is present

in these women.

The apparent distribution of these organisms in the pregnant women studied gives cause for concern, as they are prone to several complications in labour as well as risk to the foetus. Some of these pathogens have been observed to bring about miscarriages, prevent future conception and may cause blindness in the newborn. They are also known to gradually weaken the immune system of the mother and lead to secondary infection (Kunin, 1997).

E. coli had the highest percentage occurrence (58.8%) and was highly sensitive to ciprofloxacin, followed by pefloxacin, cotrimoxazole, amplicox, ampicillin and nalidixic acid. Resistance to minocylcline and cefuroxine was observed. Resistance is usually transferable especially amongst the members of the family Enterobacteriaceae (Gilman, 1996). Similar studies by Olusi et al. (2004) and Esan et al. (2004) also indicate that the members of the family Enterobacteriaceae are the predominant organisms implicated in urinary tract infections. The predominant bacteria isolated were Escherichia coli, Staphylococcus aureus and Klebsiella spp.

Proteus spp. are incriminated in urinary tract infections, although infection may be endogenous but there is a possibility of infection being introduced from an exogenous source such as diagnostic or therapeutic instrumentation. The urease activity of Proteus spp. is an important factor determining its pathogenicity in the urinary tract. The organism rapidly forms ammonia from the urea in the urine. The kidney tissues become saturated with ammonia, which promotes infection. The alkalinity may cause deposition of phosphate stones which promote the retention of urine (Gilman, 1996). All strains of Proteus spp. are resistant to polymyxins while susceptibility to chloramphenicol and tetracycline varies (Lowbury, 1994).

All species of the microorganisms were susceptible to ciprofluoxacillin. This is a bacterial antibiotic that interferes with nucleic acid synthesis by inhibiting the enzyme gyrase. This antibiotic has several binding sites on the enzyme and thus decreases the likelihood of resistance (Nester et al., 1998). The drug of choice for urinary tract infection treatment depends on the susceptibility test. Where the causative agent is purely E. coli, then ciprofluxacillin is the drug of choice. With increasing antibiotic resistance, cotrimoxazole is becoming less effective for therapy as all the uropathogens showed evidence of resistance. Antibiotic therapy should be commenced only after thorough culture and sensitivity tests have been carried out to avoid emerging drug resistance amongst bacteria. This will discourage the indiscrimate use of the antibiotics.

In conclusion, ciprofloxacin and pefloxacin should be the drugs of choice in the treatment of urinary tract infection in pregnant women. A high level of personal hygiene is expected from pregnant women as they are more exposed to urinary tract infections. Self-medication should be avoided in order to prevent spread of multiple drug resistant strains of bacteria.

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