Incidence Of Resistant Enterobacteria In Urine Samples Of Some Undergraduates In A Nigerian University

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Abstract: Analysis was carried out on 120 urine samples (60 males and 60 females) obtained from some undergraduate students consulting the University Clinic primarily for the presence of antibiotic resistant enterobacteria. Samples were examined also for presence of pus cells, epithelial cells, crystals and red blood cells. The pH of the samples ranged between 7.8 and 10.7. Seventy samples (58.3%) showed the presence of 10^4cfu/ml and above of total viable bacteria. Seventy four samples (61.67%) showed the presence of Gram negative bacteria rods that were oxidase negative. Out the Gram negative bacteria 26 (35.1%) fermented lactose, producing acid and gas; were indole positive and were identified as *Escherichia coli*. Twenty two of the isolates (29.7%) fermented lactose; producing acid and gas; were citrate positive and were identified as *Klebsiella pneumoniae*. Six of the isolates (8.1%) did not ferment lactose but were positive for urease test and were identified as *Proteus* spp. Antibiotic sensitivity test on the isolates showed that all the Gram negative enterobacteria were resistant to ampicillin and augmentin; 97.3% were resistant to cefuroxine and nalidixic acid and 89.2% were resistant to nitrofurantoin. *E.coli, Klebsiella* spp. and *Protens* spp. were most sensitive to ciprofloxacin followed by gentamycin, norfloxacin, chloramphenicol and tetracycline. The implication of this finding was discussed.
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1. Introduction

Cellular metabolism generates numerous waste compounds, many rich in nitrogen that require elimination from the blood stream. This waste is eventually expelled from the body in a process known as urinations, the primary method for excreting water – soluble chemicals from the body (Woodman, 2005). The urethra does support the growth of some micro-flora, chiefly a virulent species of *Lactobaccllus, Streptococcus, Staphylococcus* (Ronald, 2002).

Microorganisms do contaminate urine during urination. For this reason, normally voided urine contains some bacteria, where as urine collected directly from a urinary bladder via a catheter is typically sterile. A urinary tract infection is a bacterial infection that affects any part of the urinary tract, thereby appearing in the urine. When bacteria get into the kidney or bladder, multiplies in the urine, they cause urinary tract infection.

In females, urinary tract infection (UTI) is caused mostly by gastrointestinal normal flora i.e. enterobacteria because of the shortness of the urethra and its closeness to the anus. In males, UTI is caused mostly by toilet transmission, sharing of towels etc. However, urinary tract infection occurs mostly in females than in males (Mansour et al., 2009).

Although urinary tract infection causes discomfort, it can usually be quickly and easily treated by a short course of antibiotics e.g. ampiclox, penicillin, metronidazol etc (Fishbone, 2006). However, some urinary tract pathogens are able to resist the action of these antibiotics.

Over the past decades, antimicrobial resistance has emerged in all kinds of microorganisms worldwide and this is primarily due to the increase in antibiotic abuse (Sherley et al., 2000; Diel, 2005). The incidence of microbial drug resistance is alarming and in view of its development, pharmaceutical industries are shifting away from traditional strategies to newer approaches in order to cope with the problem (Wiestby et al., 2005; Huelsmann et al., 2006; Jabra-rizk et al., 2006).

In Africa, the problem originated from factors such as indiscriminate use of antibiotics, inappropriate advertisement and erratic prescription by unqualified drug sellers (Al-Jabri, 2005; Chinedum, 2005). Widespread use of antimicrobial agents often leads to the selection of multi – drug resistant microorganisms. Acquired or emerging bacterial resistance to one or several antimicrobial agents is a global problem (Neu, 1992; Gold et al., 1996) and there are numerous reports in the literature on the incidence of growing resistance of urinary isolates to available antibiotics (Chesborough, 1985; Amyes, 1987; Jacoby et al., 1991; Gupto et al., 1999).

The emergence of antimicrobial resistance in the management of urinary tract infection is an important public health issue. While many antibiotics

including penicillin, macrolides and tetracycline were very useful in the treatment of UTI in the past, the rates of bacterial resistance to antimicrobial agent has significantly increased (HO et al., 2004).

In this study, we investigated the incidence and occurrence of enterobacteria in urine samples of some undergraduate and antibiotic resistance pattern of the bacteria in a Nigerian University.

2. Materials and methods

Sampling

Samples were collected from 60 male and 60 female undergraduate students consulting the Bowen University clinic, Iwo, Nigeria, in sterile universal tubes and taken to the microbiology laboratory for examination. In cases where the sample was not used immediately, it was kept it in the freezer for not more than 24 hours. The pH of the samples was measured using a pH meter (Jenway, model 6501).

Microscopy of urine: 2 – 3ml of urine samples were centre tugged at 3000rpm for 5 minutes. A wet preparation of the sediment was made on clean glass slides and was observed under the microscope for pus cells, epithelial cells, casts, crystals, red blood cells.

Culturing of urine: A sterile wire loop was used to pick a loopful of the urine sample and this was streaked appropriately on CLED (Cystein lactose electrolyte deficient) medium and nutrient medium. The plates were then incubated at 37^0C for 24 hours to obtain discrete colonies that were identified by their colour, shape, size and lactose fermenting properties.

Colony Counting: This was achieved by serial dilution of each urine sample (1ml) into 9ml of sterile de-ionized water i.e. 1 in 10 dilution (10^{-1}). One ml of each dilution was pipette into sterile Petri dishes and covered. Nutrient agar (20ml) was than added to it and swirled to prevent clogging. All plates were incubated after the medium had set at 37^0C and the plates were examined after 24 hours to count the number of colonies. Plates with significant growth were then separated and identified using standard bacteriological and biochemical tests as described by Cowan and Steel (1974).

Antibiotic Sensitivity testing: This was done by the disc diffusion technique using nutrient agar as growth medium. Using a sterilized wire loop, a portion of the pure isolate was taken and streaked on every space covering the nutrient agar. Antibiotics discs were placed on the nutrient agar and the plates were incubated at 37^0C for 24 hours.

The antibiotics used were both positive and negative discs, the negative antibiotics discs used were gentamicin, augmentin, nalidixic acid, nitrofurantion, norfloxicin, tetracycline, ciprofloxacin, chloramphenicol, ampicillin, cefuroxime; the positive antibiotic discs used are perfloxcin, gentamicin, ampliclox, zinnacef, amoxillin, rocephin, ciprofloxacin, streptomycin, septrin and erythromycin. The diameter of zone of inhibition produced by each antibiotic disc were measured in 'mm' and the result interpreted as earlier described as sensitive(s) or resistance (r) to the antibiotic agent used, depending on the length of zone of inhibition produced, compared to reported standard length (NCCLS, 1993).

3. Results and discussion

The pH values obtained for the urine samples ranged between 7.8 and 10.7 indicating a tilt towards the alkaline region.

Urinary tract infection (UTI) is a common infection found in both males and females most especially as a result of improper hygiene. Out of the 120 samples analysed 70 had significant levels of bacteria i.e. above 10^4 cfu/ml in urine while 50 samples had below this standard (Table 1). According to Burton (2000), urinary tract infection is indicated if the number of bacteria in the urine sample equals or exceeds 10,000 (10^4 cfu/ml). This high level of bacterial counts in urine has been attributed to increased sexual activity among the age group sampled i.e. between 18 and 28 years (Olaitan, 2006).

A total of 74 Gram positive enterobaceria were isolated from the urine samples (Table 2). Relative occurrence of these enterobacteria is shown in table 3. *Escherichia coli* was the most frequently encountered (35.1%), followed by *Klebsiella* sp (29.7%) and *Proteus* sp .11%). Previous studies had enterobacteria present in urine samples with a relative percentage (35%) followed by *Klebsiella* sp. (24%) and *proteus* sp. (16%), *Citrobacter* (15%) and Enterobacter (10%) (Trevor, 1993). *E. coli* in its pathogenic form in the urine i.e. uro-pathagenic *E. Coli* has the highest incidence in the sampled urines as well as those in previous studies because it had certain pathogenic factors that enables it to colonise the bladder easily. According to Todar (2008), *E. coli* being a normal flora of the large intestine can be passed to the vigina or bladder from the anus, thereby casing urinary tract infections.

A closer look at table 3 showed that urine samples from female students had more enterobacteria when compared to their male counterparts. Previous reports had shown that females (93.3%) were more prone to urinary tract infection by enterobacteria compared to males (6.99%) – (Hanson, 2004; Kothari and Sagar, 2008). Prescott (2005) had also reported that urinary tract infection is 14 times more common in females than in males. This is because in females the urethra is much shorter and closer to the anus than in males (Hanson, 2004).

Figure 1 shows the antibiotic sensitivity pattern of the isolated enterobacteria. All the isolates were resistant to both augmentin and ampicilins. Many of the isolates showed some resistance to nalidixic acid, cefuroxime and nitrofurantoin. High antibiotic sensitivity of the isolates was observed in ciproflaxin, gentamycin, norflacin and chloramphenicol. Increase in bacterial resistance to antibiotics has been widely reported in the literature widely reported in the literature. For example the sensitivity of *E. Coli* (Isolated from urine) to gentamicin, ciprofloxacin, norfloxacin, tetracycline and nalidixic acid previously reported to be 92.3%, 100%, 84.62%, 46.15% and 23.10% respectively (Mansouri *et al.,* 2002) has declined considerably and this does not correlate with Turpin *et al.*, (2007) whose study showed that nitrofurantoin, cefuroxine and gentamicin were very effective against most of the urinary isolates.

Many reasons have been adduced for the increasing resistance of urinary pathogens to antibiotics. Most prominent among this is the prevalence of fake and sub-standard drugs available in many developing countries like Nigeria (Kothari and Sagar, 2008). Also attached to this is the in-appropriate use of antimicrobial agents without proper prescriptions from qualified medical personnel.

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Table 1: Occurrence of bacteria in Urine Samples

	Number of Occurrence	Percentage of Occurrence
Significant bacteriuria (>10^4 cfu/ml)	70	58.3
Non-significant bacteriuria (<10^4cfu/ml)	50	41.6
Total	**120**	**100**

Table 2: Biochemical reactions of 74 isolated Gram negative rods – summary

Biochemical Test	Reactions		Percentage of +ve reactions
	negative	positive	
Urease	6	68	91.9
Citrate	22	55	71.4
Indole	26	48	64.9
Oxidase	0	74	100.0
Lactose	20	48	70.6

Table 3: Relative occurrence of Enterobacteria encountered relative to sex.

Bacteria	Occurrence by sex	Percentage Occurrence
Escheriachia coli	M – 11 F – 15	35.1
Klebsiella sp	M – 16 F – 6	29.7
Proteus sp	M – 2 F – 4	8.11
Unidentified	M – 45 F – 15	27.0
Total	74	100

M-male, F-female

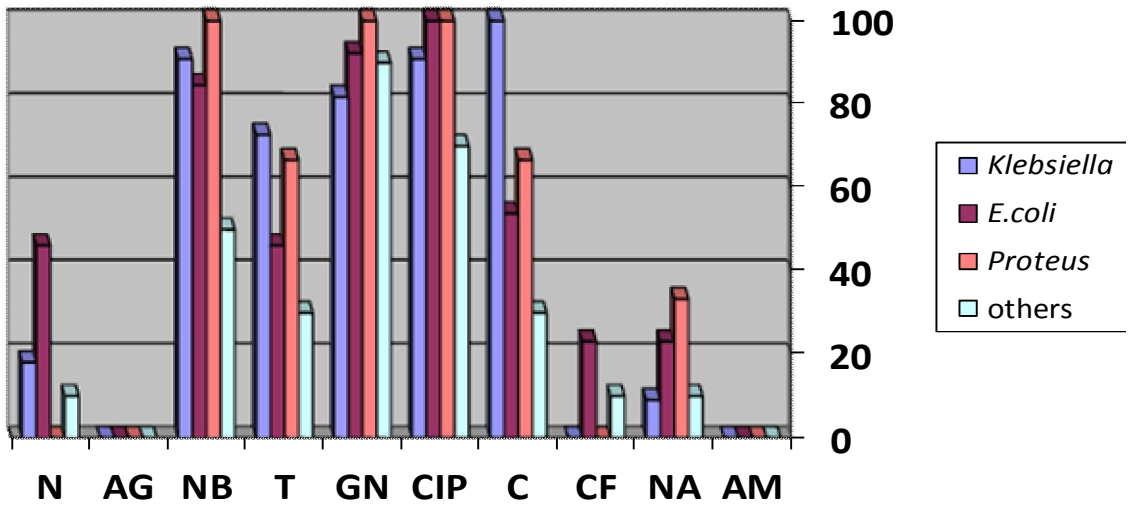

Figure 1: Antimicrobial susceptibility pattern of isolated Gram negative bacteria.
Key: N: Nitrofurantoin, AG: Augmentin, NB: Norfloxacin T: Tetracycline, GN: Gentamicin, CIP: Ciprofloxacin, C: Chloramphenicol, CF: Cefuroxime, NA: Nalidixic acid, AM: Ampicillin.

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