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Antibiogram of Bacterial Isolates from Urine of Male and Female Students in IMO State University

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Abstract

The study was carried out to investigate the antibiogram of bacteria in the urine of both male and female university students. 120 mid-stream urine samples were collected from the students aged 18-25 years using sterile containers. Standard microbiological methods of sterilization and media preparation were observed. Nutrient, MacConkey and Muller Hinton media were prepared, inoculated and incubated at 37°C for 24 hours. Sub-cultured colonies were identified using morphological characteristics and biochemical tests. The total heterotrophic plate bacterial counts ranged from 1.2-5.2 x 10⁶ cfu/ml. The total coliform bacteria plate counts ranged from 1.0-4.5 x 10⁶ cfu/ml, and the total staphylococcal plate counts ranged from 1.2-4.6 x

10⁶ cfu/. Isolates characterized and identified from male students urine samples were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella* spp, while isolates from female urine samples were *Escherichia coli*, *Staphylococcus aureus*, *Proteus* spp, *Pseudomonas aeruginosa*, and *Klebsiella* spp. Urine samples from female students showed more significance to urinary tract infections than those of male students. Antibiogram showed that most isolates were sensitive to Augmentin, Cefazidime, Ciprofloxacin and Cefuroxime, while all bacterial isolates were resistant to Gentamicin and Nitrofurantoin. There is a need to examine students' urine to create awareness of urinary tract infections.

Keywords: Antibiogram, Bacteria, Media, Urinary Tract Infection

Introduction

Urine is a liquid by-product of metabolism in humans and in many animals. Urine flows from the kidneys through the ureters to the urinary bladder (Arthur and John, 2006) [1]. Urinary tract infections (UTIs) are caused by the presence and growth of microorganisms anywhere in the urinary tract (Kolawole *et al.*, 2009) [6]. When more than 10⁵ cells of bacterial colonies are found in urine, of the urinary tract, bacteriuria is considered to exist (Williams, 1996) [10]. Common organisms that are common implicated in asymptomatic bacteriuria and infection of the urinary tract includes species of *Enterobacteriaceae* especially *Escherichia coli* which is predominant followed by *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella*, *pneumonia*, *Proteus specie* and *Pseudomonas aeruginosa* (Nicolle *et al.*, 2005) [7]. Poor hygiene and inadequate sanitary conditions are predisposing factors to urinary tract infection (Ehinmidu, 2003) [5].

Materials and Methods

Collection of Samples: 120 samples of urine from male and female students were collected from the students who were aged 18-25 years, using sterile containers.

Isolation and Enumeration of Microorganisms.

One (1ml) of each urine sample was homogenized and aseptically added to 9ml sterile normal saline and serially diluted up to 10⁻³. 0.1ml of the various dilutions was inoculated into sterile Nutrient agar and MacConkey agar using streak plate method. The plates were incubated at 37°C for 24 hours and observed for bacterial growth. Isolates were sub cultured to obtain pure cultures (Cheesbrough, 2011) [3]. After incubation, the total heterotrophic aerobic bacterial colonies were enumerated and sub-cultured on freshly prepared media for further biochemical identification. Colonies were counted using the electric colony

counter. A bacterial count of (105) cfu/ml was considered significant for urinary tract infection (UTI) and bacterial counts of (102 – 104) cfu/ml were considered as suspected bacteriuria, while counts less than 102 cfu/ml were considered as non-significant bacterial growth (Obirikwurang *et al.*, 2012)^[8].

Cultural Identification of Bacterial Isolates.

Preliminary characterization and identification of bacteria isolates were observed. The bacterial cell morphology as well as biochemical tests were performed (Cheesbrough, 2011)^[3].

Antimicrobial susceptibility test.

Antimicrobial susceptibility test was carried out using the Kirby-Bauer disk diffusion susceptibility test on Muller Hinton agar medium.

The following antibiotics was employed for sensitivity analysis; Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Cefixime (5 µg), Ofloxacin (2 µg), Augmentin (30µg), Nitrofurantoin (30 µg) and Ciprofloxacin (5 µg). The growth was standardized by diluting the culture with normal Saline to match the turbidity of 1.0×10^6 cfu/ml (0.5 McFarland standards). Then 0.1ml was collected and spread on the surface of Muller Hinton agar using sterile glass rod. The antibiotic disc was placed carefully to make good contact with the agar surface using sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zones of inhibition. The agar plates was left on the bench for 30mins to allow for diffusion of the antibiotics and was incubated at 37°C for 24hrs and results was interpreted as sensitive and resistant. The inhibition zone diameters were measured using meter rule after 24 hours incubation and recorded in millimeter (mm). It was further interpreted according to Clinical Laboratory Standards Institute (CLSI, 2006)^[4].

Results and Discussion

A total of 120 samples of mid-stream urine were analyzed from male and female students in Imo state university. The total Heterotrophic plate bacterial count ranged from $1.2 - 5.2 \times 10^6$ cfu/ml. The total coliform bacteria plate count ranged from $1.0 - 4.5 \times 10^6$ cfu/ml and the total Staphylococcal plate count ranged from $1.2 - 4.6 \times 10^6$ cfu/ml

Table 1 showed the characterization of bacterial isolates. Identification and characterization of bacterial isolates revealed that the bacteria isolates from male students were *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella spp* whiles isolates from female students were *Escherichia coli*, *Staphylococcus aureus*, *Proteus spp*, *Pseudomonas aeruginosa*, and *Klebsiella spp*.

Table 2 shows the bacteriological significance, suspected and non-significance of urine according to the gender of the students. Out of the 60 urine samples for males, 31 urine samples were significant for urinary tract infection while out of 60 urine samples for females, 43 urine samples were significant for urinary tract infection. Female students showed more significance with a percentage of 72 to urinary tract infections more than male students which had a percentage of 52. Table 3 shows the antibiotic susceptibility test for the isolated organisms. It was carried out using the following drug disc; Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Cefixime (5 µg), Ofloxacin (2 µg), Augmentin (30µg), Nitrofurantoin (30 µg) and Ciprofloxacin (5 µg) for the isolated bacteria. Antibigram showed that most of the isolates were sensitive to Augmentin, Ceftazidime, Ciprofloxacin and Cefuroxime while all bacterial isolates were resistant to Gentamicin and Nitrofurantoin.

Table 1: Physiological properties of bacterial isolates identified

Isolates	Bacteriological tests			Biochemical tests										Probable organism
	Gram reaction	Cellular arrangement	Motility	Catalase	Citrate	Indole	Oxidase	Coagulase	Voges-Proskauer test	Methyl red test	Glucose	Lactose	Sucrose	
1	-	Rod	+	+	-	+	-	-	-	+	A/G	A/G	A/G	<i>Escherichia coli</i>
2	+	Cocci	-	+	+	-	-	+	+	+	A	A	A	<i>Staphylococcus aureus</i>
3	-	Rod	+	+	+	+	-	-	+	-	A/G	-	A/G	<i>Proteus spp</i>
4	-	Rod	+	+	-	-	-	-	-	-	-	-	-	<i>Pseudomonas aeruginosa</i>
5	-	Rod	+	+	+	-	-	-	+	-	A/G	A/G	A/G	<i>Klebsiella spp.</i>

Key: = Negative, + = Positive, A = Acid, A/G = Acid and Gas production

Table 2: Bacteriological significance of the urine according to gender of students

Students	Number of samples	Significant (%)	Suspected (%)	Non-significant (%)
Males	60	31 (52)	13 (22)	16 (26)
Females	60	43 (72)	7 (12)	10 (16)

Table 3: Antibigram zone of inhibition for isolated organisms in mm

Antibiotics	<i>Escherichia coli</i> (mm)	<i>Staphylococcus aureus</i> (mm)	<i>Proteus spp</i> (mm)	<i>Pseudomonas aeruginosa</i> (mm)	<i>Klebsiella spp</i> (mm)
Ceftazidime (30µg)	25 ± 1.000	26 ± 1.000	24 ± 0.577	25 ± 1.154	25 ± 1.000
Cefuroxime (30 µg)	23 ± 1.000	25 ± 1.000	23 ± 1.527	23 ± 1.000	24 ± 1.000
Gentamicin (10 µg)	7 ± 0.577	12 ± 1.154	4 ± 0.577	9 ± 1.000	7 ± 0.577
Cefixime (5 µg),	16 ± 1.527	18 ± 0.577	17 ± 1.000	16 ± 1.000	18 ± 1.000
Ofloxacin (2 µg),	15 ± 1.527	9 ± 0.577	16 ± 1.000	10 ± 0.577	8 ± 1.000
Augmentin (30µg),	26 ± 1.000	27 ± 1.154	26 ± 1.527	25 ± 1.000	27 ± 1.000
Nitrofurantoin(30µg)	5 ± 1.154	10 ± 0.577	6 ± 1.000	8 ± 1.527	3 ± 0.577
Ciprofloxacin (5 µg)	25 ± 1.000	25 ± 1.527	24 ± 0.577	23 ± 1.000	24 ± 1.000

Key: Breakpoint table for *Staphylococcus aureus* Susceptible (S) ≥ 25 mm, Intermediate (I) 18-24 mm, Resistance (R) ≤ 19mm. Enterobacteriaceae Susceptible (S) ≥ 23mm, Intermediate (I) 15-22 mm, Resistance (R) ≤ 14mm (CLSI, 2006)^[4]

Urinary tract infections (UTIs) are fast growing infections in the world due to poor hygiene, inadequate sanitary conditions and resistance among antimicrobials. This findings show that *Escherichia coli* for males and for females was the predominant organism. Other organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella spp* were identified. This concurred with the research of (Ojo and Anibijuwon, 2010)^[9]. The result of the susceptibility tests in Table 3 shows that most of the isolates were sensitive to Augmentin, Ceftazidime, Ciprofloxacin and Cefuroxime which concurred with the report of (Chaudhary *et al.*, 2014)^[2].

Conclusion

This study showed that the urine samples collected from both male and female students of Imo State University Owerri showed significant urinary tract infections. There is need to examine the urine of students to create awareness of the presence of urinary tract infection as well as to educate students on preventive measures.

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