INCIDENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF GRAM - NEGATIVE BACTERIAL ISOLATES FROM UNDERGRADUATE WITH ASYMPTOMATIC BACTERIURIA.

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ABSTRACT

Bacteriuria is one of the major bacterial infections which sexually active men and women often suffer. Undergraduate are more likely to be the high risk group than other young men and women due to the freedom they enjoy. Two hundred and twenty early morning mid-stream urine samples were collected into sterile sample bottles aseptically from undergraduates of the Lagos State University Ojo. They were cultured on MacConkey (MCA) and eosin methylene blue agar (EMB) using standard microbiological techniques and the organisms were identified on the basis of various biochemical tests. Antibiotic susceptibility testing was carried out using the disc diffusion techniques. Seven bacterial genera were identified which included Escherichia Enterobacter, Klesbiella Cetrobacter, Proteus, Pseudomonas and Serratia. About 81.8% of the subjects had bacterial count of over 105 per milliliter. E. coli was recovered from 100 (45.5%) of the 220 subjects constituting 27.8% of the total bacterial isolates. Iseudomonas aezuginosa had an incidence of 32.7%, Serratia spp (29.1%), Enterbacter spp (21.8%), Proteus spp (16.4%) while Klebsiela spp and Citrobacter spp had the least incidence of 9.1% respectively. The in-vitro antibiotic susceptibility testing of the isolates to seven antibiotics showed that hey were moderately resistant with E. coli having the highest resistance to ampicillin (20%) amoxicillin (18%) cotrimixa zole (16%) and cefactor (5%). All isolates were however 100% susceptible to nitrofuration ofloxacin and ciprofloxacin thus suggest their therapeutic efficacy and as the isolates were resistant to. The high incidence of E. coli Pseudomonas aeruginosa and Proteus spp particularly in apparently healthy hazard to the immediate university community and her host communities while taking cognizance of the social implication of same.

INTRODUCTION

The urinary tract is one of the most common sites of bacterial infection particularly in females with between 10-20% of women having a urinary tract infection (UTI) at one time in their life and a significant number have recurrent infections (Mims et al, 1995). Cases of UTI in males have also been reported by several authors. Majority of infections are acute and short-lived but they contribute, to a significant amount of morbidity and severe infections result in loss of renal function and serious long term sequelae (Kunin, 1972).

Bacterial infection is usually acquired by the ascending route from the urethra to the bladder where it often proceeds to the kidney (where it) results in glomerulonephriti. Ascending infections of the urinary tract are most commonly caused by the Gram –negative rod. E. coli and other enteric bacteria like P. mirabilis often associated with urinary stones (calculi) Klebsiella, Enterobacter, Serratia Species and Ps aeruguwsa are more frequently found in hospital acquired UTI. (Mims et al 1998).

Several Gram-positive bacteria such as Staphylococcus saprophyticus, S. epidermidis and Enterococcus species have been associated with UTI in hospital-lized patients Caprophilic species including Corynebacterium and lactobacilli have recently been implicated as possible cause of UTI but obligate anaerobes are very zarely involved (Mims et al, 1998).

In health the urinary tract is sterile although the distal region of the urethra is colonized with commensales such as periurethral and faecal organisms. Bacteriuria is defined as significant when a properly collected mid stream urine specimen is shown to contain >105 organism per ml. Infected urine usually contain only a single bacterial species while contaminated urine usually contain <104 organism per ml and often more than one bacterial species, (Kass, 1956).

Obstruction to normal urine flow or complete emptying of the bladder has been reported to predispose an individual to infection. (Okonofua et at 1989). Sexual intercourse facilitates the movement of organisms up the urethra particularly in females, so that the incidence of UTI is higher among sexually active than celibate women (Okonofua et al; 1989)

The unlimited freedom which is characterized by high sexual activities among undergraduates on campus is likely to contribute to high incidence of asymptomatic bacteriuria. The reported high incidence of sexually transmitted diseases (STDs) including Acquired Imuno-Deficiency (AIDS) appear not to deter them. Most of those with UTI often show no symptoms thus spread the infections unabated. This study investigated the incidence of asymptomatic bacteriuria, its sex distribution, the gram-ve bacteria associated and the antibiotic susceptibility pattern of the isolates among undergraduates at the Lagos State University, Ojo campus.

MATERIALS AND METHODS

Sampling Procedure

A total of two hundred and twenty students comprising 120 females and 100 males of the Lagos State university Ojo were enlightened on the purpose and significance of the study, after which each was given a sterile wide mouthed container into which was voided a midstream urine (MSU) sample aseptically. Samples were collected after carefully cleansing of the labia in females or glans penis in males with soap and water so as to wash out contaminants (Jawetz et al 1998 Mims et al 1998). The samples were promptly taken to the laboratory for bacteriological analysis.

Bacteriological Studies:

Urine samples were examined macroscopically to detect colour and bloodstain. microscopically to estimate White Blood Cells (WBC) and Red Blood Cells (RBC) and epithelial cell on wet preparation. Each sample was then diluted aseptically to the 10^5 dilution and a loopful each streaked on MAC and EMB. Similarly O.1 ml was spread on both agar to determine significant and insignificant bacteriuria (Mims et al; 1998). All plates were incubated at 37°C for 18 hours. The bacterial isolates were identified according to standard procedure (Cowan, 1993).

Antibiotic Susceptibility Testing

Susceptibility to antimicrobial agents was performed by the disc diffusion technique using antibiotics prepared with standard concentration of each agent. The antibiotics used included ampicillin (10mcg), amoxicillin (25mcg), cotrimoxazole (25mcg), cefactors ((25mcg), nitrofurantoin (10 mcg), ofloxacin (5mcg), and ciprofloxacin (5mcg).

Five colonies of each bacterial isolates were picked from nutrient agar plates, inoculated into nutrient both and incubated for 2-4 hours at 37°C. The suspension was standardized using 0.5 MacFarland and was inoculated aseptically with sterile swabstick onto Mueller –Hinton agar. The plates were left to dry and the antibiotic discs were placed of the surface of each plates at specific distance to each other and incubated inverted at 37°C for 24 hours. The zone of inhibition of each disc was measured (Cowan 1993). A set of questionnaire was also used for the study.

RESULTS

Two hundred and Twenty apparently healthy undergraduates of the Lagos State University whose ages ranged between 18 and 25 with a mean age of $23^1/_2$ years were studied. Three hundred and Sixty bacterial isolates belonging to seven genera were recovered. They were comprised of one hundred E. coli, 72 Pseudomonas aeruginosa, 64 Serratia spp 48 Enterobacter spp and 20 each of Klebsiella and About 180 (81.8%) of the subjects had >105 organisms per ml of MSU of which 100 (66.7%) were females. Similarly, 202 (56.1%) of the bacterial isolates were recovered from females (Table II). Eighty eight (40%) of the subjects had WBC counts of < 10 but most of the samples appeared normally macroscopically.

Table III shows the susceptibility pattern of the isolates to some antimcrobial agents. Resistance to ampicillin amoxicillin and cotrimoxazole which are the commonly used abused drugs in Nigeria. This trend persisted among all the isolates. This suggests that the isolates are likely from similar source as they exhibited similar antibiotic resistance pattern. All isolates showed multiple resistance. All isolates tested were however susceptible to nitrofurantoin ofloxacin and ciprofloxacin. (Table III).

Table 1 Incidence of Bacterial pathogens among 202 undergraduates.

Organism	Number of positive culture	Percentage
E. coli	100	49.5
Pseudomonas aeruginosa	72	35.7
Serratia spp	64	31.7
Enterobacter spp	48	23.8
Klebsiella spp	20	9.9
Proteus spp	36	17.8
Citrobacter spp	20	9.9

Table II Bacterial incidence on sex

Organism	Female	Male	X2(P < 0.005)
E. coli	62 (28.2)	38 (17.3)	5.76
Pseudomonas spp	50 (22.7)	22 (10)	7.84
Serratia spp	42 (19.1)	22 (10)	6.26
Proteus spp	22 (10)	14 (6.4)	1.78
Klebsiella spp	12 (5.5)	8 (3.6)	0.8
Citrobacter spp	14 (6.4)	6 (2.7)	3.2

DISCUSSION

Urinary tract infections (UTIs) is one of the major health problems amongst young males and females in developing countries. Several predisposing factors have been reported among which are anything that disrupts normal urine flow or complete empting of the bladder sexual intercourse which facilitates the movement of organisms up the urethra particularly in females catheterization, uncircumcrssion in infant males (Mims et al 1998). Pregnancy, prostatic hypertrophy, renal calculi, tumours and strictures of any sort are the major causes of obstruction to complete bladder emptying.

Acute infections of the lower urinary tract are characterized by a rapid onset of dysuria, the urgent need to pass urine and frequency of micturition. UTIs encompasses a wide spectrum of bacteria which are both gram-negative and gram-positive but majority of these organisms implicated are enteric bacteria. The most commonly isolated organisms from this study included E. coli, Pseudomonas aeruginosa Serratia spp and Proteus spp. This observation agrees with several other reports that the most implicated bacteria in UTIs are the gram-negative especially E. coli (Burbige et al 1984, Okonofua et al, 1989, Famurewa, 1992).

Other enteric pathogens commonly encountered included Pseudomonas aeruginosa and Serratia spp which contrasted previous reports where Klebsiella spp and Salmonella spp were recovered (Mitchel, 1965, Okonofua et al., 1989). Kass (1956) and Mims et al (1998) reported that a count of 10^5 bacteria per ml of midstream urine (MSU) sample was an indicator of significant bacteriuria especially when only a single bacterial species is contained.

A total of 180 (81.8%) of the subjects had counts of over 10^5 per ml, only 100 (45.5%) has single bacterial species. The high incidence of bacteriologically proven significant bacteriuria calls for serious concern as undergraduates on campuses are known to be very sexually active hence are likely to spread such both sexually transmitted and non-sexually transmitted pathogens. This finding agrees with Adewole et al (1998) and Adekunle et al (1992) who reported that reproductive tract infections are common in Nigeria due to liberal attitudes to sex mobile global population and improved communications is difficult to determine since these diseases are not notifiable.

The prevalence of asymptomatic bacteriuria was more in females than males in this study. Of the 360 bacterial isolates recovered, 232 (64.4%) were from females while 128 (35.6%) were from males. This observation agrees with other reports that UTIs are more common in sexually active females (Burbige et al 1984, Famurewa, 1992). It is noteworthy that no Salmonella spp was isolated as against previous reports where Salmonellae were implicated in UTIs according Mitchel (195). The high incidence of pathogenic gram-negative bacteria in apparently healthy individuals requires the attention of both government and health workers as bacteriuria appears to be a threat to the average Nigerian Public enlightenment as regard personal and environmental hygiene among all Nigerians with emphasis on safe cleaning habit so as to avoid vaginal contamination after defecation. One of the greatest problems confronting the entire world is the wide use of antimicrobial agents. This is compounded by the emergence of multiple resistant bacteria which leads to failure of successfully treating most bacterial infections.

The observation from antibiotic susceptibility testing is quite astonishing. All bacterial isolates were moderately resistant to ampicillin, amoxicillin cotrimoxazole with prexious reports on the susceptibility patterns of bacteria implicated in UTIs according to (Ronald, 1987). The finding suggests that these antibiotics are still effective against asymptomatic bacteriuria after microscopy, culture and sensitivity.

It is interesting to note that the high in-vitro therapeutic efficacy shown by nitrofurantoin ofloxacin and ciprofloxacin is astounding. The efficacy of nitrofurantoin has been reported against UTIs except its limitation due to the principle of selective toxicity as it has been reported not to be safe for pregnant women as it may mediate haemolytic anaemia (Karen et al 1994). This finding suggest that factors that enhance multiple resistance to antibiotics like self medication, indiscriminate use of antibiotics appear to be uncommon as the subjects involved are asymptomatic. This perhaps would have equally contributed to low resistance to other antibiotics such as ampicillin, cotrimoxazole and amoxicillin.

It is also interesting that all isolates tested were 100% susceptible to ofloxacin and ciprofloxacin. A similar finding was reported by Ogunsola et al, 1997. This requires serious attention of all and sundry as the antibiotics with 100% activity are all prohibitively expensive and scarce this our of reach of average Nigerians who are the high risk group. This finding suggests that government in its three tiers should provide free healthcare service through which the fluoroquinolones are either given freely or subsidized so as to boost the health of common Nigerians.

However, susceptibility to the quinolones is still very high and may remain high for a few more years because they are expensive and beyond the reach of most individuals. But their use is rapidly increasing and resistance to these drugs may be more problematic than other antibiotics in the nearest future (Ogunsola et al., 1997) There is therefore the need for continous surveillance of antimicrobial resistance trend in the country. The sensitivity pattern found in this study is a pointer to the value of microscopy, culture and sensitivity it thus suggests that sensitivity report must be obtained before therapy is initiated in clinically suspected cases of UTIs. However where laboratory facilities are not available the use of the fluoroquinolones would save many lives. The incorporation of screening for asymptomatic bacteriuria into the medical examination of all new entrants into every tertiary institution is strongly advocated.

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