Incidence of *Escherichia coli* among patients presenting urinary tract infections

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Bacteriological examination was carried out on urine samples collected from patients visiting the in-patient and out-patient clinics at the University of Benin Teaching Hospital. A semi-quantitative methods using streak plate technique was employed. Of the 78 (42.3%) samples with significant bacteriuria, *Escherichia coli* constituted 16 (20.5%), *Klebsiella* species 8 (10.3%), *Staphylococcus aureus* 13(16.6%), *Staphylococcus epidermidis* 33 (42.2%), Diphtheroids 2(2.6%), *Alcaligenes faecalis* 2(2.6%), *Pseudomonas aeruginosa* 1(1.3%), *Streptococcus faecolis* 1 (1.3%) and *Candida* species 1 (1.3%). The results indicated high occurrence of *E. coli* species among pathogens isolated from the affected individuals. .

Key words: Bacteriuria *Escherichia coli*, urinary tract infection (UTI).

INTRODUCTION

Urinary tract infection (UTI) is an infection that occurs in kidneys, bladder or urethra. It is usually presented with burning sensations, serious pain without vaginal discharge (Nicolle, 2008). These symptoms range from stern to mild pain in women; and in men (Lane and Takhar, 2011; Colgan and Williams, 2011). Persons who experience pyelonephritis, bacteria that caused the kidney infection have travelled to the bladder. This is common in women than men because the distance to the bladder from skin where bacteria live is quiet short and direct. They could develop nausea, fever and vomiting (Lane and Takhar, 2011). Hardly ever the urine could appear bloody or contain noticeable pyuria (Salvotore et al., 2011).

This infection is very common in women (Colgan and Williams, 2011). It occurs frequently in women between the ages of 16 and 35 years, with 10% infection rate yearly and 60% at some point in their lives (Nicolle, 2008; Salvotore et al., 2011). Pyelonephritis occurs between 20-30 times in children less than three months of age and females less than one year. The frequency of the infection varies widely among children (Amdekar and Singh, 2011).

Most women are prone to UTIs than men as women's oestrogen levels usually decrease with the onset of menopause and the decline of vaginal flora (Dielubanza and Schaeffer, 2011). During menopause women have low level of oestrogen and antimicrobial peptides as well as a damaged lining of lumen in the urinary tract. Thus, bacteria have the opportunity to reach the underlining tissues where they can cause a new infection (Luthje et al., 2013).

In the elderly, urinary tract systems are commonly leaking otherwise known as urinary incontinence in men (Woodford and George, 2011). This may be cause by a painful inflammation of the prostate glands, injury or damage to nerves or muscles from surgery and enlarged prostate gland which can lead to cancer of the prostrate (National Institute of Ageing, 2017). *Escherichia coli* is the main cause of 80-85% of bacterial infections, *Staphylococcus saprophyticus* causes 5-10% (Nicolle, 2008). *E.*

coli is a gram-negative, rod-shaped bacterium resident in the lower intestine of man. Most strains are harmless, but some serotypes are pathogenic in human (Singleton, 1999). Harmless strains are normal flora, and are a source of vitamin K_2 (Centre for Disease Control, 2012), which prevents the establishment of pathogenic bacteria in the intestine (Vogt and Dippol, 2005). Some other bacterial pathogens that could result to infections include: *Proteus* sp. *Klebsiella* sp. and *Pseudomonas* sp. which are usually not common and classically related to abnormalities of urinary catheterization (Salvotore et al., 2011). This study investigated the incidence rate of *E. coli* bacterium in urinary tract infection.

MATERIALS AND METHODS

Semi-quantitative, aerobic culture method using standard streaking technique was employed for significant bacteriuria. Although, cultures are done routinely in the hospital laboratory using this method but incident rate of *E. coli* in patient presenting urinary tract infection has not been investigated. Hence, there is need for this study to assist clinicians in the management of their patients.

Collection and preparation of samples

Mid-stream samples of urine were collected from 184 males and females suspected of urinary tract infection in the in-patients and out patient's clinics using random sampling techniques after informed consent to determine the incident of *E. coli* infection. Samples were preserved by the addition of 0.5 g boric acid into the sterile containers and kept at 4°C until they were examined to avoid bacterial proliferation which may produce misleading results.

Ethical approval

Ethical consideration and approval was obtained from the ethical committee of the University Teaching Hospital, Benin city in Edo State.

Standardization of wire loop

This is achieved by winding nichrome wire once round a metal rod of an approximate diameter 4 mm. A scissor was used to cut one arm of the wire at the junction and the loop bent back to centre it. It was inserted into a metal holder. Using the loop, drops of water were counted while dropping at a standard rate into a graduated cylinder until the 1 ml mark was reached. From our standardization 3 readings were taken and an average obtained (50, 49 and 51). 50 drops give 1 ml:1 drop=1/50=0.0 ml. This was done to standardize the inoculums.

Three different culture media namely; MacConkey, Cystine lactose electrolyte deficient and blood agar were prepared according to manufacturer's instruction. Prior to usage, to avoid contamination and proper and absorption of inoculums the plates were allow to dry in a safety cabinet with the media dishes hangling down for about one hour at 37°C.

(i). With a grease pencil, the bottom of the CLED, MacConkey and blood agar plates were divided into four and each of the plates carried same four serial numbers for four samples.

(ii). Using a standardized wire loop, samples were plated out ensuring the wire loop was flamed at interval. The culture plates were incubated at 37°C. A culture preparation containing known *E. coli* cells was prepared along with each batch of specimen as a positive control.

Microscopy

About 10 ml urine was centrifuged and a drop of unstained deposit examined under x40 objective for pus cells, epithelial cells and other things such as yeast cells, *Trichomonas vaginalis*. Pus cells of 3-5 and above are considered meaningful in this study. The gram-stained smear of the deposit was also made and examined for Gram-negative rods as described by Cheesbrough (2000). All the samples were screened for *E. coli* and compared with positive control. The bacterial isolates were identified and confirmed with biochemical tests which include indole, citrate, oxidase, motility, catalase, coagulase, urease, glucose fermentation test and vogesproskaeur as adapted from Cowan and Steele (1974).

RESULTS

A total of 184 males and females were examined during the period under study, but of the 184 samples only (78) 42% showed significant bacteria and were identified. Of the 78 samples

with significant bacteria, *E. coli* was isolated from 16 samples representing (20.5%), *Klebsiella* species 8 (10.3%), *Staphylococcus aureus* 13 (16.6%), *Staphylococcus epidermidis* 33 (42.2%), Diptheriods 2(2.6%), *Alkaligenes faecalis* 2(2.6%), *Peusdomonas aeruginosa* 1 (1.3%) *Streptococcus faecalis* 1(1.3%), *Providencia* species 1(1.3%) and *Candida* species 1(1.3%) (Table 1).

Table 2 showed the occurrence of *E. coli* between sex. Of the 16 samples (20.5%) that were positive for *E. coli*, 14 (87.5%) were from females and 2 (12.5%) from males. Although accurate ages of patients were not available, the Medical Record Department showed that ages below 16 years are regarded as paediatrics and ages of 16 years and above as adult; therefore, adults were regarded as 16 years of ages and above.

Table 1. Incidence of *E. coli* and other isolates.

Isolates	No. of occurrence Isolates	% Incidence of isolates
Escherichia coli	16	20.5
Klebsiella species	8	10.3
Staphylococcus aureus	13	16.6
Staphylococcus epidermidis	33	42.2
Diphtheriods	2	2.6
Alcaligenes faecalis	2	2.6
Pseudomonas aeruginosa	1	1.3
Streptococcus faecalis	1	1.3
Providencia species	1	1.3
Candida species	1	1.3
Total	78	100.0

Table 2. Sex and gender related distribution of patients with *E. coli* infections.

Age (years)	0-15		16 and above	
Sex	No. infected	% Incidence of *E. coli*	No. infected	% Incidence of *E. coli*
Female	2	66.7	12	92.3
Male	1	33.3	1	7.7
Total	3	100.0	13	100.0

DISCUSSION

This study was not intended as an epidemiological survey, but to enhance planning of a service for general practitioners. Thus, visible colony counts on culture plates were done since we are interested in the occurrence rates of *E. coli*. Bhat et al. (2011) postulated that the bacterial colony forming unit (CFU) can be used to estimate viable bacteria in a clean catch mid-stream urine when streak on culture plate.

The figure obtained for bacterial infection in this work was considered important because most patients misuse antibiotics without prescription since antibiotics can be obtained outside of recognised treatment centres and taken without medical authorization or supervision. This leads to the inappropriate taken of wrong dosages and for an insufficient length of time. Thus, pyuria with a sterile or scanty growth on routine culture maybe found when a patient with urinary tract infection has been treated with antimicrobial agents as earlier stated by Cheesbrough (2000).

Consequently, our results showed that the incidence of *E. coli* among patients with significant bacteriuria was 20.5%. This was far lower than the 80-85% reported by Amdekar and Singh (2011), but agreed with the findings that *E. coli* is the commonest organism isolated out of 1000 women screened for urinary tract infection. They obtained 79.7% out of the 64 patients with significant bacteriuria. The high percentage obtained in their study is not comparable with our results since our study was based on male and female subjects and the large sample size used in

their work However, the percentage of *E. coli* infection recorded for female 87.5% in our study is slightly higher than the 79.7% obtained in their study. This difference may be because of age variation of patients screened and the disparity in sample population used during their study.

The higher rate of infection in female 66.7% than in male 33.3% of the ages between 0-15 years can be attributed to the females' wide urethra, short and close to the anus which is a suitable medium for bacterial multiplication. Thus, *E. coli* isolated from urinary tract infection are similar to the species found in the gastrointestinal tract which indicates the source of infection as earlier stated by Dielubanza and Schaeffer (2011). They also added that women's oestrogen levels decline with menopause, which leads to high risk of urinary tract infections and loss of vaginal flora. The low percentage of 12.5% of *E. coli* observed in males can be as a result of their personal hygiene and the position of urethra which creates opportunities for reduction in bacterial contamination. The increased percentage of infection in female (92.2%) of the age 16 years and above is yet to be ascertained. Kelsy et al. (1979) found that urinary tract infection in females rises after the attainment of sexual maturity.

However, Nicolle (2008) stated that in young sexually active women, sexual activity is responsible for 75-90% of bladder infections. The risk of infection is closely related to the frequency of sex. *E. coli* have the ability of forming biofilm because of the possession of pili for adherence to host cells which prevent from phagocytosis as stated by Salvatore et al. (2011). Other likely factors include personal hygiene such as bad cleaning up habit, sitting on toilet sinks that had already been contaminated and the closeness of the premium to the urinary orifice in women. A great reduction in *E. coli* infection (7.7%) in male adult from the age of 16 years and above could be that adults are better placed to taken care of themselves, whereas children in their habit of playing in floor dust, wearing of contaminated clothes and dirty napkins by their parents could be the reasons for high percentage of 33.3% obtained as earlier stated by Baht et al. (2011). From our findings, *S. epidermidis* appeared to have the highest percentage, but it is however not important. The role of *S. epidermidis* in causing acute disease of the urinary tract had not been established (Yusuf, 1988). However, when Gram stained and urine samples reveals gram-positive cocci clinicians should taken into consideration that *S. epidermidis* in children with urinary abnormalities, without urinary catheter or those receiving continuous antibiotic prophylaxis can develop pylonephritis (Kanai et al., 2014).

Conclusion

The high frequency of infection observed occurred in both children and adults. This congruence in properties strongly suggest that the evolution of the bacteria induced acute pyelonephritis is similar in non-compromised individuals of all ages and may provide the basis for prophylactic and therapeutic measures against such infections. However, the 20.5% rate of occurrence of *E. coli* infection obtained in the present study corresponded with the highest number of isolated pathogen in urinary tract infections. Therefore, the public should be enlightened about the importance of basic hygiene practices, with more emphasis on females in relation to the habit of cleaning backward. Also cleaning of toilets sinks before sitting on them should be encouraged. The habit of using unwashed napkins for children should be discouraged or preferably disposable napkins should be used.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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