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Research Article

ANTIBIOTIC SUSCEPTIBILITY TESTING FOR *ESCHERICHIA COLI* CAUSING URINARY TRACT INFECTIONS IN SOKOTO METROPOLIS

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ABSTRACT

Objective: The study was designed to diffuse awareness on the prevalence of *Escherichia coli* as a causative agent of urinary tract infection (UTI) in Sokoto metropolis as well as to determine the susceptibility to commonly used antibiotics in Specialist Hospital Sokoto (SHS). This is also to raise awareness of the risk of giving antibiotics and their direct impact on the outcome analysis of UTIs.

Methods: This study was conducted at SHS, and ethical approval to carry out the study was obtained from the Ethical Committee of the hospital. Informed consent was obtained from each participant. Early morning, mid-stream clean catch urine samples were collected by patients in sterile disposable containers. The antibiotic susceptibility of the isolates was determined against 10 commonly prescribed antibiotics in SHS using the modified Kirby–Bauer disc agar diffusion.

Results: A total of 86 urine samples were analyzed over 2 months, and 34 were culture positive giving an isolation rate of 39.5%, while 48 were culture negative giving a rate of 55.8%, and 4 (4.7%) were undecided. A total of 16 isolates were *E. coli* (47.1%), while 18 accounts for others (52.9%). The results of antimicrobial susceptibility profile to 10 antibiotics showed that *E. coli* displayed high susceptibility to vancomycin (91.6%), followed by amikacin (89.2%) and then meropenem (88.0%), while high rate of resistance was found in nalidixic acid (81.2%), followed by co-trimoxazole (73.3%) and then norfloxacin (76.2%).

Conclusion: When there is an adequate detection of *E. coli* and other uropathogens, it will aid in selecting the appropriate antimicrobial therapy and this will also serve as a means of infection control. This will go a long way in reducing the cost of treatment and threat of resistance as witnessed in the management of some uropathogens.

Keywords: Antibiotic susceptibility, *Escherichia coli*, Uri nary tract infections.

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INTRODUCTION

Urinary tract infections (UTIs) have become the most common hospital-acquired infection, accounting for as many as 35% of nosocomial infections, and it is the second most common cause of bacteremia in hospitalized patients [15,18,20,21]. These infections are found commonly in women than in men. The incidence in women within the age of 20–40 years ranges from 25% to 30% and up to 4–43% in elderly women above 60 years of age [3].

It is one of the most common bacterial diseases worldwide [5,8,16,28] that is characterized by a wide range of symptoms from mild irritative voiding to bacteremia, sepsis, or even death [16,26].

Bacteria are the major causative organisms and are responsible for more than 95% of UTI cases [16,25]. About 80–85% of UTIs are caused by Gram-negative bacteria [12,29]. UTIs have different names, depending on which the part of the urinary tract is infected. UTIs are classified as uncomplicated or complicated. It has been observed that despite the widespread availability of antibiotics, UTI remains the most common bacterial infection in the human population [9,22].

It has been reported that UTI can occur in any part of the urinary tract and is caused by the retrograde ascent of bacteria from the fecal flora through the urethra to the bladder and kidney [22]. This is most, especially, in the females who have a shorter and wider urethra and is more transversed by microorganisms [13,17]. In most cases, bacteria travel to the urethra and multiply causing kidney infection if not treated [4].

However, there are some urinary tract diseases that are not associated with urinary infection but often treated with antibiotics, a practice that leads to antibacterial resistance due to improper diagnosis of UTIs. The most commonly reported are interstitial cystitis (IC) and overactive bladder (AOB). IC has been described [25] as the complaint of suprapubic pain related to bladder filling, accompanied by other symptoms such as increased day and night time frequency, in the absence of proven urinary infection or other obvious pathology. The prevalence of IC in the developed countries is 197 and 41 in every 100,000 women and men respectively. AOB has also been described [25] as the symptom complex of urinary urgency, usually accompanied by frequency and nocturia, with or without urgency urinary incontinence, in the absence of urinary tract infection or other obvious pathology. The prevalence of OAB in men and women is 10.8% and 12.8% in a population of 100,000 respectively.

A research by Goldman and Huskins [10] suggested that the improper and uncontrolled use of many antibiotics resulted in the occurrence of antimicrobial resistance, which became a major health problem worldwide. Another author, Manikandan *et al*. [19], also reported that the "widespread use and more often the misuse of antimicrobial drugs have led to a general rise in the emergence of resistant bacteria." Therefore, the diagnosis of UTI is usually made based on the presence of signs and symptoms and confirmed by culture examination with significant bacteriuria supported by high-level pyuria [16,22].

Escherichia coli is the most common cause of UTI among virtually every patient group and accounts for 80–90% of cases of uncomplicated pyelonephritis and cystitis [27].

The current study aimed to determine and disseminate awareness on the prevalence of *E. coli* associated with UTI as well as their susceptibility pattern in Sokoto metropolis.

METHODS

Approval to carry out the study was obtained from the Ethical Committee of Specialist Hospital Sokoto (SHS), and informed consent was obtained from each participant (Appendix I).

Media preparation

The media used in this work include cysteine lactose electrolyte deficient (CLED) agar, and nutrient agar (NA), and Mueller-Hinton Agar (MHA), all sourced from Hi Media, India. The media were prepared based on manufacturer's instruction and sterilized by autoclaving for 15 min at 121°C.

Sample collection

Our study included patients (male and female) of all ages who attended the outpatient department with evidence or symptoms of UTI as determined by the physician. Early morning, mid-stream clean catch urine samples were collected by patients in sterile disposable containers with screw caps. Before urine collection, patients were counseled on how to collect the urine sample by observing all aseptic conditions to avoid contamination.

Isolation and culturing of urine samples

Sterile Petri dishes containing 20 mL prepared CLED agar were allowed to set and their surfaces dried in an incubator at 37°C for 5 min. Urine samples were inoculated on CLED agar using calibrated wire loop and allowed to stay for 30 min and incubated in aerobic condition for 18–24 h at 37°C. Plates without any colony at the end of 18–24 h incubation were discarded. Samples with counts up to and $>10^5$ CFU/mL were counted microscopically and considered positive for further analysis.

Characterization of isolates

Isolates were purified by single colony isolation unto NA plates and incubated at 37°C for 18–24 h. Isolates from pure culture were characterized by Gram-staining followed by different biochemical tests (indole production test, motility test, and triple sugar iron agar test) were performed to confirm the *E. coli* causing UTI.

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates was determined against 10 commonly prescribed antibiotics in SHS using the modified Kirby–Bauer disc agar diffusion [6,7]. The discs (Oxoid, UK) were meropenem (MER, 10 µg), amikacin (AMK, 30 µg), vancomycin (VA, 10 µg), amoxicillin/clavulanic acid (AMC, 30 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), cotrimoxazole (SXT, 25 µg), nitrofurantoin (F, 300 µg), gentamicin (CN, 30 µg), and nalidixic acid (NA, 30 µg).

A fresh subculture of isolates was prepared on MHA and incubated at 37°C for 18–24 h. With the aid of a wire loop, 4–5 well-isolated colonies of similar appearance were picked and transferred into the tube of sterile normal saline. The inoculum was emulsified inside the tube to avoid clumping of the cells. The inoculums were adjusted to 0.5 McFarland (McFarland 0.5 equals approximately 10^8 CFU/mL).

Within 15 min of preparing the adjusted inoculums, a sterile cotton swab was dipped into the inoculums. The swab was rotated several times and pressed firmly on the inside of the tube above the fluid level to remove excess inoculums from the swab.

The swab was streaked over the entire surface of the MHA plate, rotating the plate approximately 60° 3 times to ensure confluent growth. Inoculation was completed by running the swab around the rim of the agar. Excess moisture on the agar surface was allowed to be absorbed before applying the antimicrobial discs.

The disc was placed 20 mm center to center on the surface of the agar using a sterile needle. The plates were allowed to stay for 20–30 min to allow for pre-diffusion. The plates were incubated at 37°C for 18–24 h.

Following incubation, the diameter of the zones of growth inhibition was measured to the nearest millimeter using a ruler, including the diameter of the disc in the measurement. Results were interpreted using the CLSI Guidelines (2006).

RESULTS AND DISCUSSION

A total of 86 urine samples were analyzed over 2 months' period and 34 were culture positive giving an isolation rate of 39.5%, while 48 were culture negative giving a rate of 55.8%, and 4 (4.7%) were neither decided, due to probably contamination as shown in Fig. 1. A total of 16 isolates were *E. coli* (47.1%), while 18 accounts for others (other Gram-negative, 11 and Gram-positive, 7) as shown in Fig. 2.

The percentage susceptibility of *E. coli* causing UTI is shown in Table 1. The antimicrobial susceptibility profile results were interpreted according to the CLSI, 2006 interpretative chart.

Different bacterial pathogens were reported to cause UTI with many reporters concluded that *E. coli* and *Klebsiella* spp. were found to be predominant in causing the UTI among patients. Hence, the present study was conducted and focused only on *E. coli* to determine its sensitivity and antibiotic resistance against ten commonly prescribed

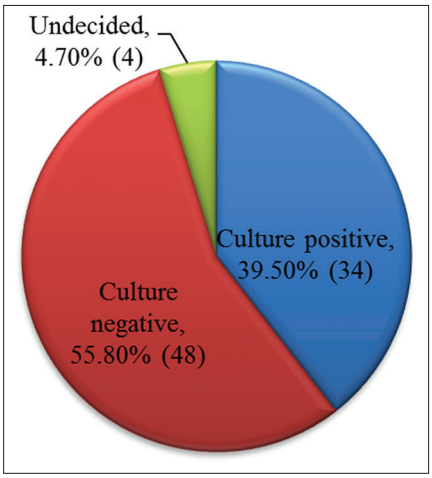

Fig. 1: Percentage distribution for the presence or absence of uropathogens from urine culture

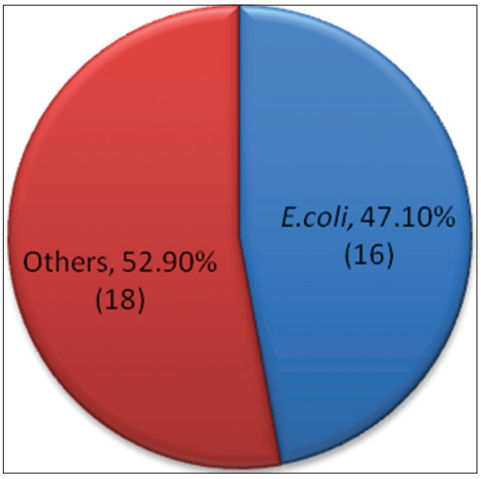

Fig. 2: Percentage distribution of uropathogens among culture positive