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A comparative study on the effects of doxycycline, nalidixic acid and meropenem on *Escherichia coli* in inducing urinary tract infections

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Abstract: Urinary tract infections are widely recognized as prevalent bacterial infections frequently encountered in both hospital and community settings. The most common gram-negative bacterial pathogen in humans, extra-intestinal pathogenic *Escherichia coli*, causes these diseases. A retrospective cross-sectional study in Karachi, Pakistan examined *Escherichia coli's* susceptibility to doxycycline, nalidixic acid and meropenem. One hundred isolates of *Escherichia coli* from urine samples of patients were collected. The Kirby Bauer disc diffusion testing method was used following CLSI guidelines. The results showed variability because of differences in patient's gender and age. 84 out of 100 samples were of females and 16 samples were of males. Infection prevalence by age showed that 15% of isolates were from children (0-12 years), 2% from adolescents (13-18 years), 52% from adults (19-59 years) and 31% from seniors. This study concluded that females and adults (aged 19-59 years) are at highest risk of prevalence. Meropenem is a potential antibiotic choice for urinary tract infections due to its demonstrated 100% susceptibility. However, in light of increasing resistance trends, the administration of doxycycline and nalidixic acid should be reserved until antimicrobial susceptibility testing has been conducted. The study ensures that antibiotics are used judiciously and effectively in combating urinary tract infections while minimizing the risk of further antimicrobial resistance development.

Keywords: UTI, Pakistan, retrospective cross-sectional, *Escherichia coli*, Kirby Bauer method.

INTRODUCTION

One of the most frequent bacterial infections commonly encountered in hospitals and communities is Urinary tract infections (UTIs). *Escherichia coli* (*E. coli*) infections typically exhibit a self-limiting course in individuals lacking any discernible physiological or anatomical peculiarities. Nevertheless, it is noteworthy that these occurrences have the potential to manifest repeatedly (Geerlings, 2017). These infections can be categorized as complicated and uncomplicated infections. Several etiological factors are involved in the occurrence of UTIs such as gender, age, bacterial type, pregnancy, etc. (Hooton, 2012; Nielubowicz & Mobley, 2010). The clinical manifestations of characteristic UTI contain infection-induced urethritis, cystitis and pyelonephritis detected by the high bacterial manifestations in the urine (bacteriuria) with associated symptoms (Grabe *et al*., 2015). The most frequent causative agent responsible for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC).

The most frequent gram-negative species responsible for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (Flores-Mireles *et al.*, 2015). It is a highly heterogenous species with certain lineages becoming increasingly resistant to antibiotics (Adams-Sapper *et al.*, 2013; Bengtsson *et al.*, 2012; Nicolas-Chanoine *et al*., 2008).

The proliferation of antimicrobial resistance in *E. coli* is exhibiting a notable surge, thereby necessitating a sense of caution among physicians when considering the prescription of oral antimicrobial agents. In the context of community-outpatient health care facilities, it is customary to administer a substantial quantity of oral antibiotics as part of the standard protocol for managing urinary tract infections (UTIs). Therefore, in order to obtain suitable antimicrobial agents, it is imperative to possess comprehensive understanding of the prevailing predisposition data pertaining to *E. coli* (Kim *et al*., 2016; Lee *et al.*, 2018).

Doxycycline is a broad-spectrum antibiotic that belongs to the tetracycline group, used to treat various bacterial infections. These antibiotics exert their bacteriostatic effect by interrupting with protein synthesis of gram-positive and negative bacteria. They do so by inhibiting aminoacyl-t-RNA binding to ribosomes. The general doxycycline dose for the required potent antimicrobial effect is 50mg-100mg depending upon the severity of infection (Bahrami *et al.*, 2012; Chopra & Roberts, 2001).

A quinolone-based, first-generation antimicrobial with poor pharmacokinetic property and a narrow spectrum is Nalidixic acid (Ahmed & Kelley 2017). It was introduced in 1963 for therapeutic purposes. It exerts a good antimicrobial effect against Gram-negative species and is

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used for the treatment of UTIs (Albrecht, 1977). Quinolones are the only current class of agents that directly inhibit bacterial DNA synthesis and thus are widely used antimicrobials in clinical practice. Since the introduction of nalidixic acid tolerance to quinolone antibiotics has become serious issue in clinical practice, prompting the design of novel molecules to restore there potential efficacy (Pandey *et al.*, 2018).

Meropenem, a broad-spectrum antibacterial agent, part of the carbapenem family, is prescribed as empirical therapy before the identification of the organism. It is also used for the treatment of infections due to single or multiple susceptible bacteria in both children and adults covering a broad range of serious infections. It is an approved antibacterial use for the treatment of intra-abdominal infections, bacterial meningitis, nosocomial pneumonia, complicated UTIs, skin and skin structure infections, septicemia, gynecological and obstetrics infections, cystic fibrosis, and community-acquired pneumonia as well. It exhibits a broad range of action against Gram-positive and Gram-negative pathogens, which includes AmpC-producing Enterobacteriaceae and extended-spectrum β-lactamase (ESBL) as well (Baldwin *et al.*, 2008).

The aim of this study is to provide a suitable antibiotic rationale for the management of recurrent urinary tract infections (UTIs). This study establishes a framework for the identification and utilization of the most suitable antibiotic to optimize treatment efficacy.

MATERIALS AND METHODS

Study design

The retrospective cross-sectional study design was chosen for its suitability in examining the antibiotic susceptibility patterns of *Escherichia coli (E. coli)* isolated from urinary tract infections (UTIs) based on a dataset of 200 isolates. This design accommodates the study's objective efficiently, given the availability of a predefined sample size and the data collected within a specified time.

Inclusion criteria

Urine samples of patients irrespective of age and gender having urinary tract infections due to *E. coli* were included in this study.

Exclusion criteria

The samples of urine other than uropathogenic *E. coli* causing UTI were excluded from this study.

Sample volume calculation

The sample size was calculated through z-score the formula for the analysis of sample volume is:

$$n - \frac{2\times(Z_{a/2} - Z_B)^2 \times \sigma^2}{d^2}$$

Where

n=required sample size per group
$Z_{\sigma/2}$ = Z-score corresponding to the desired significance level (e.g., Z-score of 1.96 for a 5% significance level)
Z_β= Z-score corresponding to the desired power (e.g., Z-score of 0.84 for 80% power)
σ= estimated standard deviation of the outcome
d= desired effect size (minimum detectable difference)

The formula estimates the ample size for comparing means in two groups.

Sample collection

The urine samples analysed in this study were obtained from laboratory of Diagnostic Laboratories located in Karachi, Pakistan. Specifically, Samples containing *E. coli* were requested from the lab and the isolates of *E. coli* were subsequently received from these urine samples for further analysis. As these samples were already screened for the presence of *E. coli* the streamlining data collection process for the study. By targeting samples with known *E. coli*, the study ensured a focused investigation into the antibiotic susceptibility patterns of this pathogen in urinary tract infections.

Method selection

The collected bacterial isolates were tested for sensitivity against different antibiotics in the Pharmaceutical Microbiology Research laboratory at the Faculty of Pharmacy, Jinnah University for Women, Karachi. Kirby Bauer disc diffusion method was employed according to CLSI (Clinical Laboratory Standard Institute) guidelines. Commercially available antibiotic discs of doxycycline, nalidixic acid and meropenem were acquired from Oxoid, UK. Zones of inhibition were observed and interpreted according to CLSI guidelines (Humphries *et al*., 2018).

Antimicrobial susceptibility test

All glassware prepared agar plates and nutrient broth were sterilized at 121ºC for 15 min using an autoclave. Isolated samples of *E. coli* were tested on Mueller Hinton agar (Oxoid, UK) plates. After inoculation of isolates on agar plates, antibiotics were placed at a distance of 15mm and 25mm away from edges and other antibiotic discs respectively. These plates were incubated at 35ºC±2°C for 24 hours. Zones of inhibition were measured according to CLSI guidelines (Hudzicki *et al.*, 2009; Jacobs *et al.*, 1979).

Ethical approval

This study follows proper guidelines (Ref No. JUW/Pharm/EA/01) according to the principles of declaration.

STATISTICAL ANALYSIS

Only descriptive statistics were applied on quantitative data. Frequencies and percentages were used as analytical measures and were calculated using statistical software SPSS version 21.

RESULTS

The findings are represented in the form of figures which shows the comparative analysis in percentages. The findings revealed that all the isolates showed sensitivity to meropenam, with a 100% observed sensitivity rate. Among various samples analyzed, it was found that only 17% demonstrated sensitivity against nalidixic acid and 12% isolates indicated sensitivity to doxycycline.

In fig. 2 the results suggest that among the collected samples, it was observed that the highest occurrence of urinary tract infections (UTIs) caused by *E. coli* was found in the adult population, particularly individuals from 19 to 59 years. The demographic comprised approximately 52% of all isolates obtained. In contrast, the age group of 13-18 years exhibited the lowest frequency of *E. coli* infections.

In fig. 3 of the subsequent analysis, it is evident that E. coli shows complete susceptibility (100%) to meropenem in both male and female subjects. Notably, females demonstrate a higher sensitivity to nalidixic acid against E. coli compared to males. Conversely, when treated with doxycycline, men exhibit a higher resistance rate (81%) against E. coli in comparison to females, who display a resistance rate of 77%.

DISCUSSION

Urinary tract infections (UTIs) are exhibiting a rising prevalence and experiencing a rapid surge, primarily attributed to specific often encountered causes. This study demonstrated that among the isolates obtained from patients diagnosed with urinary tract infections (UTIs), the predominant proportion of samples, specifically 84%, are derived from female patients. Multiple studies that have been conducted demonstrate a consistent association between the female gender and the prevalence of urinary tract infections (UTIs). This association can be attributed to various factors, including but not limited to anatomical characteristics such as shorter urethras, behavioral factors, and the use of contraceptives (Muthulakshmi & Gopalakrishnan, 2017; Tektook *et al.*, 2017).

Carbapenems are considered a highly effective and recommended option for the treatment of urinary tract infections (UTIs). This is due to their exceptional efficacy in combating a wide range of gram-negative uropathogens, particularly those that produce Extended Spectrum Beta-Lactamases (ESBLs). The findings of this study also showed a sensitivity rate of 100% for *E. coli* when treated with meropenem, so reaffirming its status as the recommended therapeutic option for urinary tract infections caused by *E. coli.* Numerous studies have consistently demonstrated the absence of reported resistance against these antibiotics, hence indicating their consistent 100% sensitivity (Agrawal *et al.*, 2017; Sahu *et al.*, 2018).

The present investigation documented a notable elevation in resistance levels against the quinolone derivative nalidixic acid, reaching 76.8%. It is advisable to do antibiotic susceptibility testing before considering the use of nalidixic acid for the treatment of urinary tract infections. The findings of this investigation indicate that a significant proportion of *E. coli* isolates, namely 77%, exhibited resistance to nalidixic acid. Hence, it is imperative for a medical practitioner to possess comprehensive knowledge on the prevailing antibiotic resistance levels prior to administering these medications, since their misuse can potentially result in therapeutic inefficacy. The emergence of antibiotic resistance is not solely attributed to a high level of antibiotic usage. The genetic element is additionally accountable for the emergence of resistant strains within clinical isolates (Asaduzzaman *et al*., 2018; Brown *et al*., 2003; Zhanel *et al*., 2006).

The outcomes of this study reveal a significant level of resistance to doxycycline and nalidixic acid, with rates of 78% and 77% respectively. However, based on the findings of a recent study, it is evident that these antibiotics continue to be the preferred option for selecting antibiotics to effectively eliminate vancomycin-resistant bacteria in urine samples. In the context of carbapenem-associated resistant urinary tract infections (UTIs), the use of doxycycline, nalidixic acid and Meropenam has been observed to exhibit notable rates of microbiological failures and clinical recurrence (Babiker *et al.*, 2018; Kim *et al*., 2020).

CONCLUSION

In conclusion, urinary tract infections (UTIs) caused by *E. coli* are currently among the most frequently reported infections. Following the initial susceptibility screening, it is imperative to exercise caution in the selection of antibiotics due to the emergence of increasingly resistant species. Women and individuals between the ages of 19 and 59 who are classified as adults are more susceptible to increased risk. The development of treatment strategies should be carefully researched and the introduction of more potent analogues should be considered to enhance the selection criteria. Carbapenems have demonstrated a 100% response rate in combating these infections, making them a potential preferred antibiotic.

The prescription of doxycycline and nalidixic acid is contingent upon the results of an antimicrobial susceptibility test, as these drugs have demonstrated an elevated level of resistance. It is imperative to avoid disregarding the notion of perceiving it as a commonplace illness, since failure to address it may result in the development of more severe disorders.

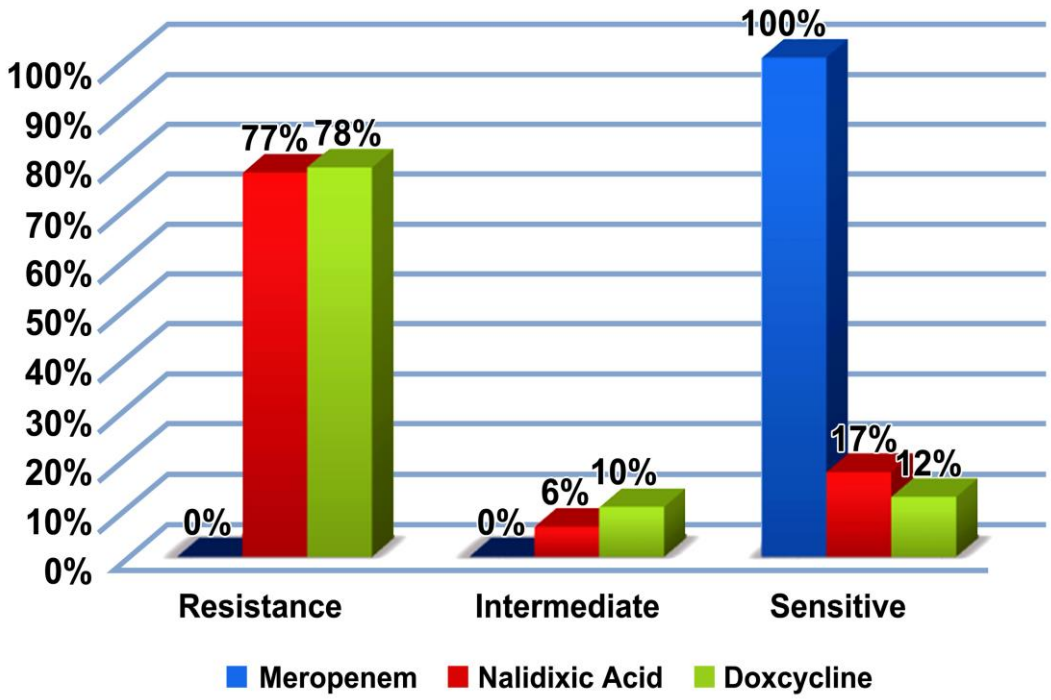

Fig. 1: Overall antimicrobial susceptibility results showing comparative sensitivity.

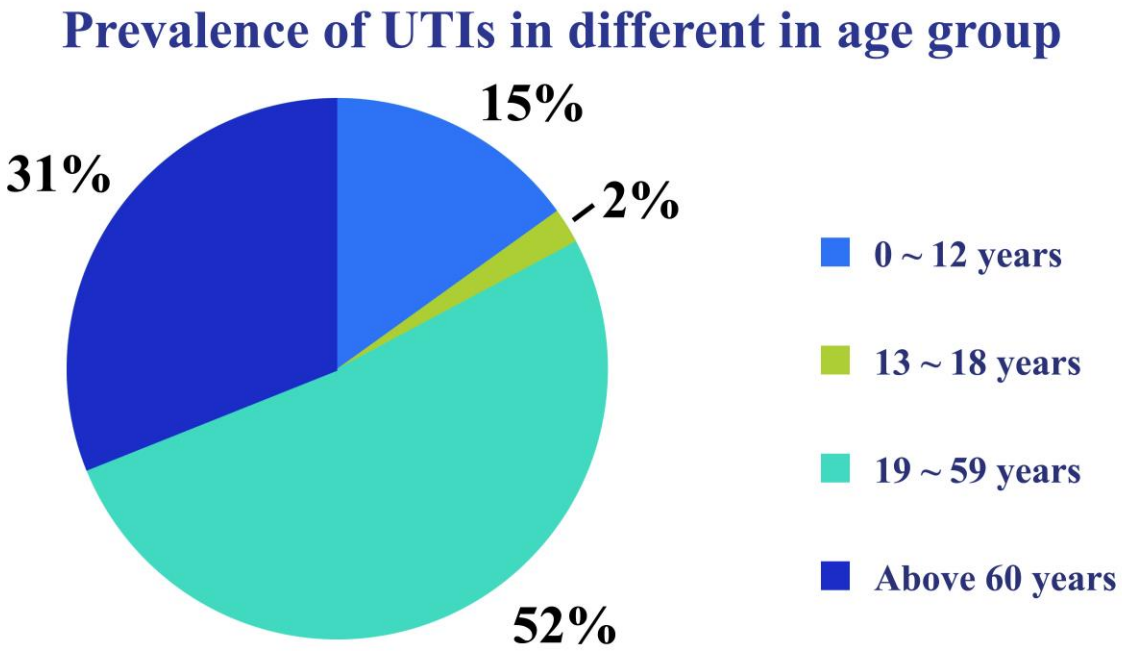

Fig. 2: Age-wise prevalence of UTIs in the collected isolates

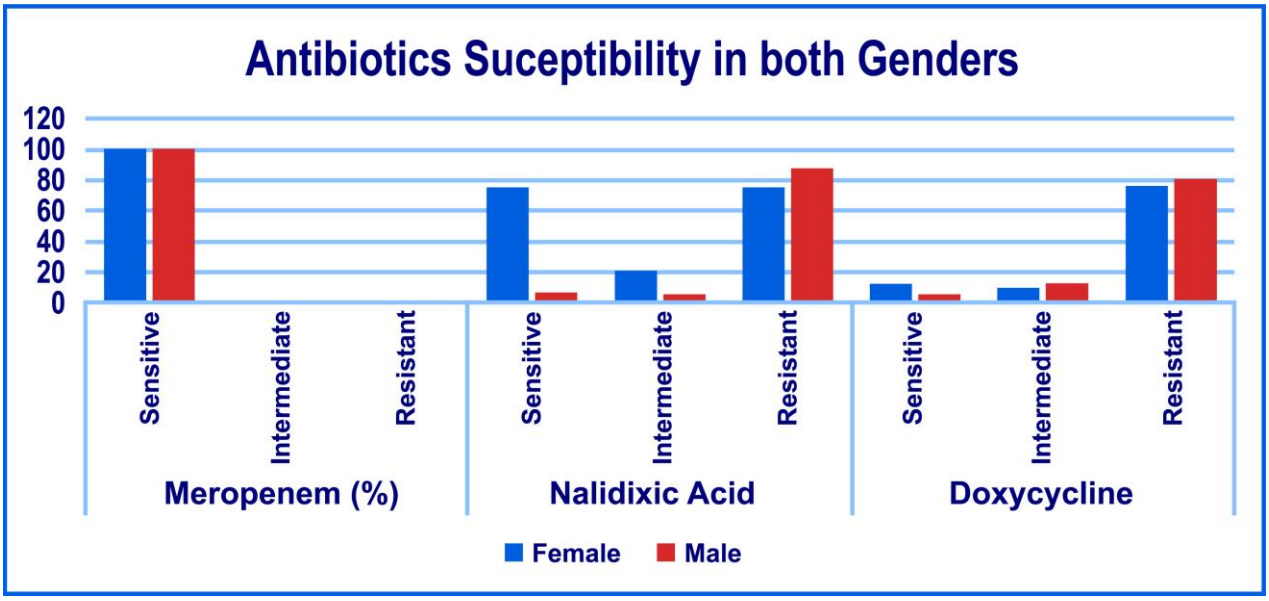

Fig. 3: Antimicrobial susceptibilities of meropenem, nalidixic acid and doxycycline in males and females

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