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ANTIBIOTIC SUSCEPTIBILITY OF ORGANISMS CAUSING URINARY TRACT INFECTION IN PATIENTS PRESENTING AT KENYATTA NATIONAL HOSPITAL, NAIROBI
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H. I. MAGALE, I. A. KASSIM, S. A. ODERA, M. J. OMOLO, W. G. JAOKO and P. E. JOLLY

ABSTRACT

***Background*: Changes in susceptibility patterns of bacterial pathogens isolated from urinary tract infections emphasize the need for regional surveillance to generate information that can be used in management of patients. Knowledge on the current status of antimicrobial resistance in uropathogens, and the prevalence of expanding spectrum beta-lactamases (ESBLs) in the isolates will guide policy formulations and encourage prudent use of antimicrobials.**

***Objectives*: To identify bacterial pathogens causing UTI and determine the association between the pathogens isolated from patients attending KNH. Determine antimicrobial susceptibility patterns of the UTI pathogens and the prevalence of ESBL in the isolated pathogens.**

***Design*: Laboratory-based study.**

***Setting*: Department of Medical Microbiology University of Nairobi and Kenyatta National Hospital microbiology laboratory, Nairobi, Kenya.**

***Subjects*: Nine hundred and forty eight patients presenting directly to the Kenyatta National Hospital's diagnostic laboratory. Patients were only classified as in-patients if at the time of specimen collection they were being admitted to one of KNH wards.**

***Results*: Out of the 948 urine samples processed, 189 in-patients and 37 out-patients samples had significant bacterial growth. The uropathogens identified from in-patient specimens were *Escherichia coli* (56), *Klebsiellapneumoniae* (33), *Enterococcus* spp. (34) and Entrobacter (16) making up 30%, 18%, 18% and 9% respectively. ESBL isolates were found to be resistant to the locally administered antibiotics; Augmentin (37%), Levofloxacin (37%), Cefoperazone (37%), Ampicillin (39%), Doxycyline (41%), Gentamicin (30%) and Nalidixic Acid (38%).**

***Conclusion*: The increased prevalence of multidrug resistant ESBL pathogens poses challenges for healthcare providers at KNH and signifies the need for new approach to treat UTI. It would be prudent for laboratories to include specialised tests for detection of ESBL producing pathogens from isolates obtained from in-patients. Further studies on the mechanisms and pathways utilised by these bacteria to cause UTI will highlight other avenues in patient management.**

INTRODUCTION

Urinary Tract Infection (UTI) is a general term that refers to infection/inflammation of any part of the urinary tract caused by bacteria. UTI is one of the most common bacterial infections encountered by clinicians worldwide (1). Moreover, since reporting of antibiotic susceptibility results in suspected cases of UTI takes at least 48 hours following sampling, the condition is treated empirically, based on available data reflecting antibiotic resistance (2).

UTI is classified into two major categories, namely complicated infection or uncomplicated infection. The term complicated refers to the fact that an individual's urinary tract has been in some way obstructed or is abnormal. Uncomplicated infections are far more common and apply to infections within a normal, unobstructed tract (3). If UTI is not detected in early stages and treated adequately, it can result in chronic illness and long term renal damage (4). Uncomplicated UTI in patients can be treated since the causative microbes are highly predictable: 80–90% are caused by *Escherichia coli* (5).

Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial agents in the United States and worldwide (6). However, the evolution of beta-lactam resistance via the extended spectrum beta-lactamase (ESBL), an enzyme produced by beta-lactam resistant bacteria in clinically encountered pathogens has greatly limited their utilization. ESBLs are capable of bacterial resistance against common UTI antibiotics such as penicillin, cephalosporin, cephamycins and monobactams by hydrolyzing the antibiotics (7,8).

Primary ESBL-producing bacteria are from Enterobacteriaceae family of Gram-negative organisms, especially *Klebsiellapneumoniae* and *Escherichia coli* (9,10). *Proteus mirabilis*, *Pseudomonas aeruginosa, Acinetobacter spp., Serratiaspp*, and gram-positive bacteria such as *Enterococcus spp*, and *Staphylococcus spp*. are also known to cause UTI (11). Understanding these common uropathogens and their susceptibility patterns will help to improve prescription decisions for both complicated and uncomplicated UTI (6).

ESBL infections also are more aggressive than non-ESBL infections. Patients suffering from ESBL induced UTI often encounter: delay in the initiation of appropriate therapy, significantly longer hospital stays, higher medical costs, recurrent UTI and increased risk of clinical failure and death in comparison to non-ESBL induced UTI (12).One class of drugs successfully used to treat ESBL bacteria are carbapenems (13). However, due to the widespread use of these antibiotics in the treatment of UTI, researchers and clinicians fear that ESBLs will soon become resistant to the drugs.

In Africa, there is no comprehensive data regarding ESBL producing *Enterobacteriaceae* (10). However, there is sufficient evidence to suggest that there is ESBL-producing uropathogens in Africa (10).Also, there had been reports of UTIs owing to ESBL-producing bacteria being on the rise. One longitudinal study in Turkey showed an increase from 13 UTI cases due to ESBLs to 111 ESBL cases at the end of five years (13). These numbers suggest that ESBLs are out competing non- ESBLs and are replacing the latter as the dominant uropathogens. Thus, studies to increase knowledge on pathogens causing UTI and their antibiotic resistance patterns regionally are very important to guide clinicians in empiric treatment (2).

MATERIALS AND METHODS

The study protocol was approved with a waiver of informed consent by the Institutional Review Board of the University of Alabama at Birmingham and the Kenyatta National Hospital Ethical Review Committee before its implantation. Study investigators had no direct contact with the patients and were provided with urine samples containing only identification numbers by the hospital laboratory staff. All urine samples had undergone routine processing by microscopy and culture on the urinalysis bench and patients were treated according to the results obtained.

This laboratory-based study was conducted in the Department of Medical Microbiology University of Nairobi from May to August 2013. Urine samples from both in and outpatients were cultured and subjected to antibiotics susceptibility testing. Antimicrobial agents used were: Augmentin, Levofloxacin, Meropenem, Ceftazidime, Nalidixic Acid, Gentamicin, Cefoperazone, Ceftriazone, Imipenem, Doxycycline and Ampicillin. Sensitivity and resistance were determined by measuring the diameter of zones around each drug according to Clinical Laboratory Standard Institute (CSLI) guidelines (14).

Isolates were streaked on CHROMagar ESBL media plates to determine whether they were ESBL positive or negative, according to the manufacturer's instructions. CHROMagar ESBL was obtained directly from the manufacturer (CHROMagar, New Jersey, U.S.A). All components were provided as dehydrated powders and media were prepared in-house according to the manufacturer's instruction; CHROMagar Orientation (15) was used as the base medium for preparation of CHROMagar ESBL CHROMagar. Quality control for the media was performed using stock organisms of *Klebsiellapneumoniae, Escherichia coli, Proteus spp.,Pseudomonas spp.* and *Staphylococcus aureus*. Samples streaked on CHROMagar media were incubated overnight at 37 °C. ESBL producing pathogens were determined by full growth on plates, that is, growth past initial inoculation streak coupled with colonies. ESBL

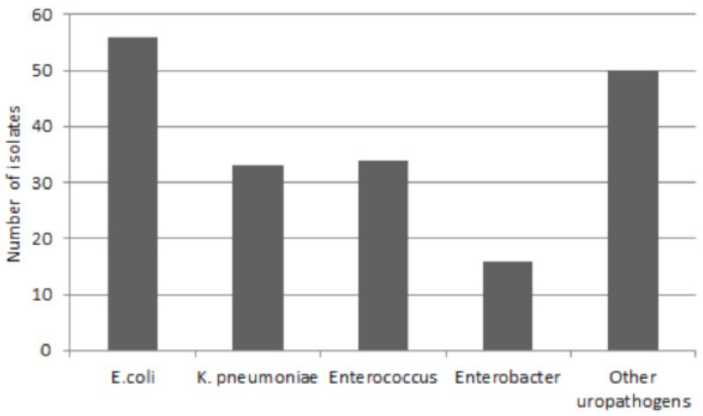
negative samples failed to form colonies and / or grow past the initial inoculation streak.

Statistical data analysis was performed to determine the significance in our findings. Data from the study were inserted into two separate MS Excel spreadsheets that were later on merged usingDigDB to ensure correct match of specimen information with unique patient ID. The present study focused on analysing the 189 in-patient samples that had bacterial growth. Goodness-of-fit statistics (Pearson's Chi-square test) was used to compare the fits of different variables using p-values of α-level of 0.05. Descriptive statistics was also employed to explain the outcome of variables.

RESULTS

In our study, we recorded significant bacterial growth among the most common uropathogens. Out of the 948 urine samples that the KNH lab processed, 189 in-patient samples had bacterial growth. Among the isolates, the most common organisms identified were *Escherichia coli* (56),*Klebsiella pneumoniae* (33), *Enterococcus spp*. (34) and *Entrobacter* (16) making up 30%, 18%, 18% and 9% respectively (Figure 1).

Figure 1
Frequency of uropathogens isolated in the study (n = 189)



The other uropathogens in the chart represents *Acinebacter spp., Citrobacter spp., Protes spp., Pseudonomas spp.* and *Staphylococcus spp*.

Among *E.coli*, 34 specimens be were found to ESBL positive while 22 were ESBL negative. *Enterobacter spp*. Showed 13 ESBL positive and 3 ESBL negative samples. *K. pneumoniae* contained 26 ESBL positive and 7 ESBL negative samples. Within *Enterococcus spp*., 3 samples were ESBL positive while 31 samples were ESBL negative. Of the less common organisms isolated including: *Acinebacter spp., Citrobacter spp., Proteus spp., Pseudomonas spp.* and *Staphylococcus spp*. 29 were ESBL positive and 21 were ESBL negative isolates. These data are further illustrated in Figure 2.

Figure 2
Prevalence of ESBLs and non-ESBL producing uropathogens isolated in the study

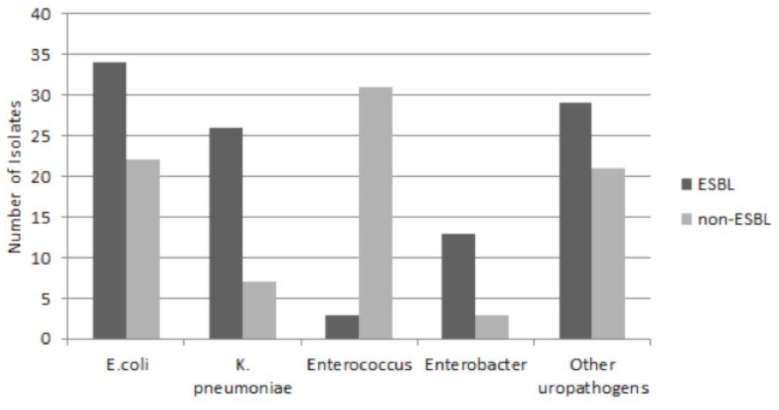


Figure 3
Antibiotic susceptibility profile of ESBLs and non-ESBLs uropathogens to commonly used antibiotics at KNH, Nairobi

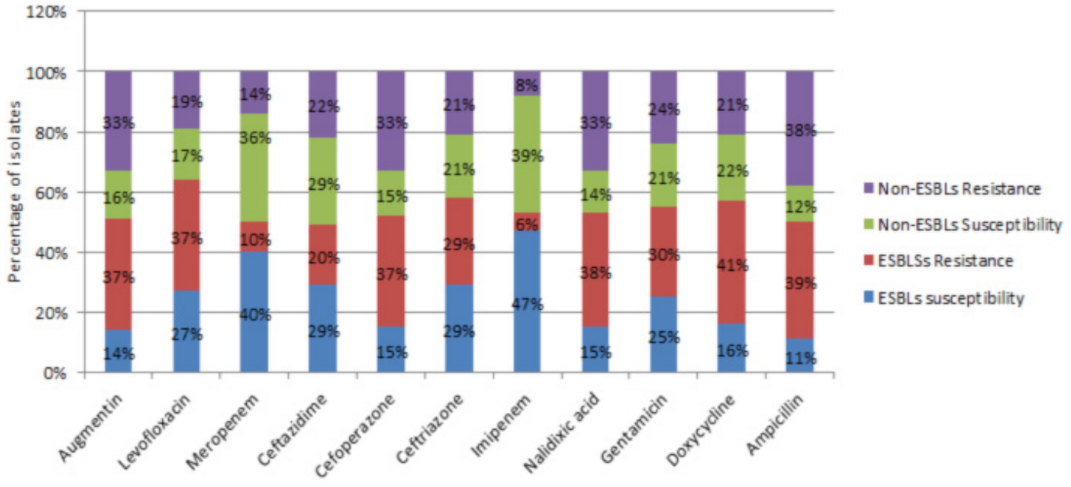


Figure 3 outlines patterns of susceptibility and resistance among ESBL and non-ESBL pathogens to 11 commonly used antibiotics. In 8 out of 11 antibiotics, ESBLs had a greater percentage of resistance when compared to non-ESBL resistance. Meropenem, Ceftazidime and Imipenem has about the non-ESBLs resistance representing 14%, 22% and 8% versus the10%, 20% and 6% of ESBLs respectively. The highest percentages of susceptibility among ESBLs were in response to Imipenem (47%), Meropenem (40%), Cefoperazone (29%), Levofloxacin (27%), Gentamicin (25%) and Ceftazidime (29%). Susceptibility patterns also show that non-ESBL pathogens are susceptible to Imipenem (39%), Meropenem (36%), Cefoperazone (21%), and Doxycycline (22%), Gentamicin (21%) and Ceftazidime (29%).

DISCUSSION

E.coli, K. pneumoniae and *Enterococcus spp*. were the primary isolates found to cause UTI from our study at KNH. These findings are in agreement with the results from previous studies from Cameroon, Pakistan, Israel and Turkey (2,7,16,13) that found *E. coli* and *K. pneumoniae* were the most significant causers of UTIs. However, the development of *Enterococcus spp*. as a cause of UTI shows that other pathogens may be starting to cause UTI.

Additionally, ESBL positive species within *E.coli* and *Enterobacter spp*. constituted 61% and 81% respectively while *K. pneumoniae* had a 79% to 21% split between ESBL and non-ESBL isolates respectively. Our results indicate that ESBL pathogens were very significant (P<0.0001) across these groups: *E.coli, Enterobacterspp*. and *K.pneumoniae*. Even though there is no national surveillance figures for ESBL-producing uropathogensin Kenya, there have been reports of ESBL caused UTI cases (17,18). The fact that some of the uropathogens investigated in this study; *E.coli, Enterobacter* and *K. pneumoniae* have a higher prevalence of ESBL pathogens in comparison to the non-ESBLs within the same bacterial species indicates that ESBL pathogens out-compete their non-ESBL counterparts in order to cause UTI. The high percent of those ESBL producing uropathogens especially *E. coli* and *K. pneumoniae* have also been reported in previous similar studies (7,16).

ESBL pathogens are often known to be multidrug resistant. Our results showed ESBLs were highly resistant (over 30%) to Augmentin, Levofloxacin,Ce foperazoneAmpicillin, Doxycycline, Nalidixic Acid and Gentamicin.The resistant to these antibiotics: Augmentin, Levofloxacin and Ampicillin were found to be significant (P<0.005) among the ESBL-producing uropathogens. These data shows that ESBLs are multidrug resistant and pose challenges to clinicians in determining which drugs to use to effectively treat UTI. The emergence of ESBL-producing uropathogens demonstrates the remarkable ability of bacteria to evolve and survive in the world of antibiotics. Our Clinicians face clinical and economic challenges on managing UTI due to such resistant isolates, the need to perform specialised laboratory tests for detection, and treatment with more expensive antibiotic agents will be necessary in some cases.

Our study focused primarily on the in-patient samples mainly because of the smaller outpatient population size (15.7%). A comparatively higher outpatient population size is necessary to determine if UTI caused by ESBLs from our in-patient study resulted from nosocomial or was community acquired infection. A large scale study with balanced in-patients and out-patients is needed to resolve the cause of the infection.

Lack of access to previous patient history of UTI hindered our assessment of how current ESBL causing pathogens differ from previously acquired ESBL strains.

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