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REVIEW ARTICLE

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Detection of Extended Spectrum B-lactamase Producers Species of *E. coli* and *K. pneumoniae* Among Patients with Urinary Tract Infection

Waseem Sameer Kwami, *Najat Mahjoub Ali and *Wejdan Zulfukar Hassan

Department of Microbiology, Faculty of Medical Laboratory Sciences, University of Shendi, Sudan

****Department of Microbiology, Almak Nimer University Hospital, Shendi, Sudan**

ABSTRACT

Urinary tract infection (UTI) is a spectrum of disease caused by microbial invasion of the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus. UTI is an extremely common condition that occurs in both males and females of all ages. Increase in extended-spectrum ß-lactamases (ESBL) producing microbes in recent years has led to limitations of treatment options. This is descriptive cross-sectional study conducted in Shendi town-Sudan, in period from April to August, 2018. The objective of this study was to detect ESBLs producers' species of E. coli and K. pneuomniae among patients with urinary tract infection. A total of 100 urine specimens were collected from patients with symptoms of urinary tract infection in sterile urine container, and then inoculated in CLED medium, incubated at 37c for 16-18 hours. Species of E.coli and klebsiella were identified according to their colonial morphology, indirect gram staining reaction, and biochemical tests. Identified species of E. coli and klebsiella were screened for ESBLs through their susceptibility against Cefpdoxime, Ceftriaxone, Ceftazidime, Ceftaxime. Strains that show positive screening results for ESBLs were further be tested for confirmation by double disk diffusion synergy test. The study revealed that 46% of urinary tract infections were caused by E. coli and 29% of urinary tract infections were caused by Klebsiella spp. The study also showed that 56% of isolated E. coli was ESBLs producers, and only 20 % of isolated Klebsiella spp were ESBLs producers. The study concluded high prevalence of ESBL producing E. coli and Klebsiella spp was observed, warranting that all isolated strains of E. coli and Klebsiella pneumoniae from clinical specimens should be tested for ESBLs production.

Key words: Extended spectrum B-lactamase, E. coli, K. pneumoniae, Urinary Tract Infections, UTI and Shendi.

INTRODUCTION

Urinary tract infection (UTI) is a range of diseases caused by invasion of microbes to the genitourinary tract that extends from the renal cortex of the kidney to the urethral meatus. UTI is common condition that takes places in both genders of all ages. The frequency of infection is higher in women than in men, which is likely the result of several clinical factors including strructural differences, hormonal effects and behavior patterns (Kelvin et al., 2013).

Globally 150 million urinary tract infections occur yearly resulting in more than 6 billion dollar in direct healthcare cost, complicated urinary tract infection include those in patients with stoned or obstructiveuropathies and in patients with catheter_related infection (Emilia et al., 2015).

Urinary tract infection including cyctitis and pyelonephritis are the most common infectious diseases in childhood. *E. coli* is responsible for 90% of the community acquired and 50% of hospital acquired urinary tract infection (Vasudevan, 2014).

Extended spectrum β-lactamases (ESBLs) were first described in 1983. They are able to hydro-lyse oxyimino-cephalosporins like cefotaxime, ceftazidime and ceftriaxone and monobactams (for example, aztreonam), but not cephamycins or carbapenems. The β-lactamases produced by bacteria are known to prevent from the fatal effect of penicillins, cephalosporins and monobactams on their cell wall synthesis. The production of -lactamase is the single most common mechanism responsible for resistance to β-lactams among clinical isolates of the family Enterobacteriaeceae. ESBLs have been found most commonly in uropathogens, like *K. pneumonia* and *E. coli*. Other enterobacteria and non-fermenting Gram negative rods also produce ESBLs but to a lesser extent. A variety of β-lactamases have been classified into class A, B, C and D according to their amino acid homology. ESBLs are Class A enzymes which are inhibited in vitro by β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam, whereas those belonging to class B, C and D are not affected (Hasan et al., 2011).

Most of the ESBLs are derived from SHV and TEM-1 (sulfhydryl variable) by mutations. ESBL producing organisms have broad spread, and have become a common cause of hospital acquired infections associated with high mortality rates, particularly in serious infections such as blood sepsis. The ESBL producing bacteria are causing urinary tract infections both in hospitalized and outpatients. This is making therapy of UTI difficult and promoting greater use of expensive broad spectrum antibiotics, such as carbapenems. Detection of ESBLs using conventional antimicrobial susceptibility methods and delay in the detection and reporting of ESBL production by Gram-negative bacilli are associated with prolonged hospital stay, high morbidity, mortality and health care costs. The failure of treatment of both complicated and uncomplicated UTI is continuously increasing morbidity and mortality among UTI patients. The aim of our study was to evaluate the antimicrobial resistance of ESBL and non-ESBL producing E. coli and K. pneumoniae in patients attending a tertiary care children hospital (Hasan et al., 2011).

MATERIAL AND METHODS

Specimen

A total of 100 urine specimens were collected consecutively from in and out-patients at Shendi Teaching Hospital and Almak Nimer University Hospital in Sudan. The collection was done by trained medical personnel avoiding contamination. Clean-catch mid-stream urine samples were collected from consenting patients. The specimens were immediately transported to the laboratory after collection and processed. All contaminated urine specimens were excluded from the study.

Isolation and identification

A loop-full (0.001 ml) of well mixed uncentrifuged urine was streaked onto the surface CLED agar. The plates were incubated aerobically at 370 C for 18-24 hours and counts were expressed in colony forming units (CFU) per milliliter (mL). A count of 105 CFU/Ml was considered significant bacteriuria. Organisms identified were based on colonial morphology, indirect gram staining reaction, and biochemical tests.

ESBL Detection Method

Screening Methods

Identified species of *E. coli* and *klebsiella* were screened for ESBLs through their susceptibility against Cefpdoxime, Ceftriaxone, Ceftazidime, Ceftaxime.

This was done using the Kirby Bauer Disc diffusion method with reference to the Clinical Laboratory Standard Institute (CLSI) performance guideline for antimicrobial susceptibility testing. Strains that show positive screening results for ESBLs were further are tested for confirmation by double disk diffusion synergy test (Sharma et al., 2016).

Confirmatory Method

The test was performed as described by Jarlier *et al*. A sterile Mueller-Hinton agar was prepared and a 0.5 McFarland equivalent standard of the test organisms was streaked on the surface of the agar with a sterile loop and allowed for 15-20 minutes to pre-diffuse. An Augmentin which is a combination of clavulanic acid (20 µg) and amoxicillin (10µg) was placed at the center of the plate and cefotaxime (30µg), ceftaxidime (30 µg), aztreonam (30 µg) ciprofloxacin (30 µg) were placed 15 mm a part center to centre on the plates with a sterile forceps ((Sharma et al., 2016).

These were incubated at 35oC for 18-24 h. An enhanced zone of inhibition from 5 mm above in the presence of Augmentin is regarded as positive for phenotypic production of ESBL enzyme (Sharma et al., 2016).

RESULTS

In this study a total of (35) participants were included (47%) were females and (43%) were males**.** Their age ranged from (7) to (95) years, (43%) were within the age group (31-60), (37%) were more than 60 years old and only (20%) were less than 30 years old.

In this study the species of *E. coli* and *Klebsiella spp* isolates were responsible from (46%) and (29%) of urinary tract infections caused by bacterial pathogen (Table 1).

Out of the 35 isolates, 28 (80%) isolates were from in-patients and 7 (20%) from out patients. The prevalence of all *E. coli* isolates in the in-patients was 13/28 (46%) and in out-patients was 3/7 (42%) whereas the prevalence of *Klebsiella spp* isolates in the in-patients was 8/28 (29%) and in out-patients was 2/7 (29%) (Table 2).

Out of the 16 isolates of *E. coli* 12 (75%) were passed screening test for ESBL and only 9 (56%) of them were ESBL producers. And out of the 10 isolates of *Klebsiella spp* 3(30%) were passed screening test for ESBL and only 2 (20%) of them were ESBL producers (Table 3).

DISCUSSION

This study was constructed to detect the urinary tract infections caused by ESBL-producing E. coli and K. pneumonae among patients with symptoms of urinary tract infection.

The study showed that the most predominant isolate from UTI patient was *E. coli* (46%) and *K. pneumonae* (29%). This result is in accordance with results obtained by Pooja *et al*., (2017) who reported that E.coli was the most predominant isolates from patients with urinary tract infection (Pooja et al., 2017).

Table 1. Shows frequency of urinary tract infection caused by *E.coli* and *k. pneumoniae*.

Isolates	No	Frequency
E.coli	16	46%
K. pneumonia	10	29%
Others	9	25%
Total	35	100%

The current study revealed that E. coli was responsible from 46% of UTI cases among inpatients and 42% of UTI cases among outpatients, while *Klebsiella pneumoniae* was responsible from 29% of UTI cases in both inpatient and outpatients. These findings were in difference with results obtained by

Emilia et al., (2015) who indicated that E.coli was responsible from 35.6 % of inpatient UTI cases and 48.3% of outpatient UTI cases. Whereas *Klebsiella pneumonia* was responsible from 33.2% of inpatient UTI cases and 28% of outpatient UTI cases (Emilia et al., 2015).

Table 2. The prevalence of *E.coli* and *K. pneumoniae* isolates in hospitalized and community patients.

Species	Inpatient (n=28)		Outpatient (n=7)	
	No	%	No	%
E. coli	13	46%	3	42%
K. pneumonia	8	29%	2	29%
Others	7	25%	2	29%
Total	**28**	**100%**	**7**	**100%**

Table 3. Distribution of ESBL producers among isolated species of *E.coli* and *K. pneumonia.*

Species	Total number	ESBL screening positive		ESBL confirmed	
		No	%	No	%
E. coli	16	12	75%	9	56%
Klebsiella	10	3	30%	2	20%

In the present study the ESBL producing *E. coli* and *K. pneumoniae* were 56 and 20 %, respectively. In another study conducted in Pakistan, 56.9% isolates of *E. coli* were ESBL positive (Ullah et al., 2009) and in a study from India, nearly 40% urinary isolates of *E. coli* and *K. pneumoniae* were ESBL positive (Babypadmini and Appalaraju, 2004).

ESBL producing *K. pneumonia* was 54.4% in a study from Latin America (Aminzadeh et al., 2008). Mekki et al. (2010) reported ESBL producing 53% *E. coli* and *K. pneumoniae* from the patients suffering from urinary tract infections. The findings of our study are similar to other studies in case of ESBL producing *E. coli* while the number of ESBL producing *K. pneumoniae* was lower in our study as compared to others (Mekki et al., 2010).

CONCLUSION

56% of isolated E. coli strains from UTI patients were found to be extended spectrum beta lactamases producers and only 20% of *Klebsiella pneumoniae* isolates were found to be extended spectrum beta lactamases producers.

Ethical Consideration

The ethical consideration of this study was approved by ethics committee, faculty of graduate studies, Shendi University, Sudan. The participants were informed about the purpose of the research before sample collection, and written consent obtained from them. Privacy and confidentiality of participants were ensured.

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Corresponding author: Waseem Sameer Kwami, Department of Microbiology, Faculty of Medical Laboratory Sciences, University of Shendi, Sudan.
Email: sameerw77@yahoo.com