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RESEARCH PAPER

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Frequency of Carbapenem Resistance Enterobacteriaceae among Urinary Isolates in Kosti City, Sudan 2017

Babiker Saad Almugadam^{1,*}, Hassan AlmadaniMohamed¹,
Hamid Omer Hamid¹ and Abdelhakam G.Tamomh²

^{1,*}Department of Microbiology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti City, Sudan

²Department of Medical Parasitology and Entomology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti City, Sudan

ABSTRACT

Urinary tract infections (UTIs) is an infection of any part of the urinary system, it mainly cause by bacteria. The emergence of antibiotics resistant among urinary isolates was observed worldwide. This study was conducted to determine the frequency of carbapenem resistant among isolated Enterobacteriaceae from patients with urinary tract infections. A total of 100 Enterobacteriaceae (85 *E. coli* and 15 *K. pneumoniae*) were isolated from patients with urinary tract infections during period from November 2016 to February 2017. Phenotypic detection of carbapenems resistant and antibiotics susceptibility testing (AST) were performed according to clinical and laboratory standard institute (CLSI) guidelines 2011. The frequency of carbapenems resistant among Enterobacteriaceae was 12% (12/100), the frequency of carbapenems resistant was higher in *K. pneumoniae* 33.3% (5/15) than *E. coli* 8.2% (7/85). Also the frequency of carbapenem resistant is higher in 6-

*lactam antibiotics users 16.1% (5/31) than non β -lactam users 10.1% (7/69). Most *E.coli* and *K. pneumoniae* isolates were resistant to co. trimoxazole 87% and sensitive to (amikacin 85%, gentamycin 85%, and nitrofurantoin 83%). The emergence of carbapenem resistant was became a major therapeutic and health problem worldwide. The continuous and regular evaluation of the susceptibility of urinary isolates to antibiotics can help in elimination and eradication of this problem.*

Key words: Antibiotics resistant, CRE, UTIs, MHT and Sudan.

INTRODUCTION

Urinary tract infections (UTIs) is an infection of any part of the urinary system, urinary tract infections may involve the lower or upper urinary tract (Sobel JD and Kaye D 2010). Urinary tract infections are second most common type of infection in the body (Mittal R *et al.*, 2009). Women are more likely to develop UTIs than men due to anatomical differences (Rahn DD 2008).

Enterobacteriaceae are a family of Gram negative bacilli that occur naturally in the gastrointestinal tract of human and animals. These organisms can spread outside the gastrointestinal tract and cause serious infections such as UTIs. Clinically important members of *Enterobacteriaceae* related to UTIs include *Escherichia coli* (*E.coli*), *Klebsiella pneumoniae* (*K.pneumoniae*), and *Proteus mirabilis* (*P.mirabilis*). The occurrence of antimicrobials resistance in *Enterobacteriaceae* is increasingly reported worldwide and has become a one of major health problems (CDC, 2012).

Carbapenems is a group of β -lactam antibiotics including imipenem, meropenem, doripenem, and ertapenem which are considered as last line therapy and first line agents for treatment of serious infections caused by multidrug resistant *Enterobacteriaceae* (Sidjabat H *al.*, 2011; Hu F *et al.*, 20125). The resistance of carbapenems increased recently worldwide among the members of *Enterobacteriaceae* family (Hu F *et al.*, 2012). This study aimed to investigate the frequency and antibiotics susceptibility of carbapenem resistance *Enterobacteriaceae* (CRE) isolated from urinary tract infected patients in order to provide guidance's for treatment of urinary tract infections cause by those pathogen in Kosti city, White Nile state, Sudan.

MATERIALS AND METHODS

This study is across sectional, hospital based study, conducted in Kosti teaching Hospital during period from November 2016 to February 2017. A total of 100 *Enterobacteriaceae* isolates were collected from patients with urinary tract infections attending Kosti Teaching Hospital. All isolates were identifying base on culture characters, Gram stain, and standard biochemical tests (Almugadam B S *et al.*, 2016). Each isolate was subject to phenotypic detection of carbapenems resistant and antibiotics susceptibility testing (AST). All Data were analyzed using statistical package for social sciences (SPSS version16) software, and *P* value ≤ 0.05 were considered significant in comparative data.

Antibiotics susceptibility testing was performed using modified Kirby Bauer disk diffusion technique according to clinical and laboratory standards institute (CLSI) guidelines 2011 (CLSI M100-S21, 2011). Using sterile cotton tipped swab the inoculum suspension (equivalent to 0.5 McFarland STD) of test organism was inoculated in Mueller Hinton agar (Himedia) plate, and with a sterile forceps the antimicrobial disks was placed on inoculated Mueller Hinton (MH) agar, then incubated aerobically at 37°C for 18 hours (CLSI M100-S21, 2011). Bioanalyse antibiotics were used, which includes meropenem 10 μ g (MEM),

imipenem 10µg (IPM), gentamycin 10µg (GEN), amikacin 30µg (AK), ciprofloxacin 5µg (CIP), co.trimoxazole 125 µg (COT), and nitrofurantoin 100 µg (NIT).The diameters of inhibition zone was measured by ruler from the back of plate and interpreted according to CLSI 2011 guidelines(CLSI M100-S21, 2011). *E.coli* ATCC 25922 strain was used as control strain.

The screening for CRE was performed along with antibiotics susceptibility testing, each isolate showed resistant to meropenem 10µg or/and imipenem 10µg disc were subjected for confirmation of carbapenems resistant using modified hodge test as recommended by CLSI guidelines 2011(CLSI M100-S21, 2011).

Modified hodge test was performed for each isolate showed resistant or decrease of susceptibility to meropenem 10µg or/and imipenem 10µg. Inoculums suspension of *E.coli* ATCC 25922 strain (equivalent to 0.5 McFarland) was prepared and diluted in 1:10 by normal saline, then with sterile cotton tipped swab the inoculums suspension was inoculated on MH agar plate (Sathya P *et al.*,2015 and Lee K *et al.*, 2001). And with sterile forceps, meropenem10µg disk was placed on the center of MH agar plate, then with sterile wire loop the test organism was streaked in a straight line, from the edge of the disk to the edge of plate (Lee K *et al.*, 2001; Sathya P *et al.*, 2015).All plates were incubated aerobically for 18 hours at 35C° (Lee K *et al.*, 2001; Sathya P *et al.*, 2015).The positive test indicate by the presence of a cloverleaf like indentation of *E.coli* ATCC 25922 growth along the test organism streaking growth within the disk diffusion zone(Lee K *et al.*, 2001; Sathya P *et al.*, 2015).

RESULTS

A total of 100 *Enterobacteriaceae* isolates were included in this study (85 *E.coli* and 15 *K.pneumoniae*).The frequency of carbapenems resistant was 12% (12/100). The frequency of carbapenems resistant was higher in *K.pneumoniae* 33.3% (5/15) than *E.coli* 8.2% (7/85) as shown in table 1. The frequency of carbapenem resistant was higher in β-lactam antibiotics users 16.1% (5/31) than non β-lactam users 10.1% (7/69). In this study most *E.coli* and *K.pneumoniae* isolates were resistant to co. trimoxazole 87% and sensitive to (amikacin 85%, gentamycin 85%, nitrofurantoin 83%, imipenem 70%, and meropenem 66%) as seen in table 2.

P value showed there is no significant difference between the susceptibility of carbapenems and non carbapenems resistant isolates to other antibiotics as shown in table 3.

Table 1. Frequency of carbapenem resistant among *E.coli* and *K.pneumoniae* isolates.

Type of bacteria	Number	Frequency		P value
		Carbapenem resistant	Carbapenem sensitive	
<i>E.coli</i>	85 (85%)	8.2% (7/85)	91.8% (78/85)	0.016
<i>K.pneumoniae</i>	15 (15%)	33.3%(5/15)	66.7%(10/15)	
Total	100(100%)	12%(12/100)	88%(88/100)	

The frequency of carbapenem resistant are higher in *K.pneumoniae* 33.3% (5/15)than *E.coli* 8.2 % (7/85), *P* value showed significant difference (.016).

Table 2. Susceptibility of *E. coli* and *K.pneumoniae* isolates to antibiotics.

Antibiotics		Isolate		Total
		<i>E.coli</i>	<i>K.pneumoniae</i>	
Amikacin 30ug	S	82.3%(70/85)	100%(15/15)	85%(85/100)
	R	17.7%(15/85)	0%(0/15)	15%(15/100)
Ciprofloxacin 5ug	S	34.1%(29/85)	86.7%(13/15)	42%(42/100)
	R	65.9%(56/85)	13.3%(2/15)	58%(58/100)
Co.trimoxazole 25ug	S	15.3%(13/85)	0%(0/15)	13%(13/100)
	R	84.7%(72/85)	100%(15/15)	87%(87/100)
Nitrofurantoin 100ug	S	80%(68/85)	100%(15/15)	83%(83/100)
	R	20%(17/85)	0%(0/15)	17%(17/100)
Gentamycin 10ug	S	88.2%(75/85)	66.7%(10/15)	85%(85/100)
	R	11.8%(10/85)	33.3%(5/15)	15%(15/100)
Imipenem 10ug	S	74.1%(63/85)	46.7%(7/15)	70%(70/100)
	R	25.9%(22/85)	53.3%(8/15)	30%(30/100)
Meropenem 10ug	S	73%(62/85)	26.7%(4/15)	66%(66/100)
	R	27%(23/85)	73.3%(11/15)	34%(34/100)

Most *E.coli* and *K.pneumoniae* isolates were resistant to co. trimoxazole 87% and sensitive to (amikacin 85%, gentamycin 85%, and nitrofurantoin 83%). All *K.pneumoniae* isolates were sensitive to amikacin, and nitrofurantoin.

Table 3. Susceptibility of carbapenem and non carbapenem resistance isolates to antibiotics.

Antibiotics		Carbapenem		P value
		Carbapenem resistant (12 isolates)	Carbapenem sensitive (88 isolates)	
Amikacin	S	83.3%(10/12)	85.2%(75/88)	0.57
	R	16.7%(2/12)	14.8%(13/88)	
Ciprofloxacin	S	41.7%(5/12)	42%(37/88)	0.61
	R	58.3%(7/12)	58%(51/88)	
Co.trimoxazole	S	8.3%(1/12)	13.6%(12/88)	0.51
	R	91.7%(11/12)	86.4%(76/88)	
Nitrofurantoin	S	83.3%(10/12)	83%(73/88)	0.66
	R	16.7%(2/12)	17%(15/88)	
Gentamycin	S	91.7%(11/12)	84%(74/88)	0.43
	R	8.3%(1/12)	16%(14/88)	

P value showed, there is no significant difference between the susceptibility of carbapenems and non carbapenems resistant isolates to other antibiotics.

DISCUSSION

Carbapenemase producing Gram negative rod have been associated with increasing mortality and with serious hospital, and community outbreaks that represent major therapeutic and infection control challenges (Patel G and Bonomo RA, 2011). The frequency of CRE among urinary isolates began to elevate worldwide and in Kosti city that represents a serious problem to public health.

In this study the frequency of carbapenem resistant *Enterobacteriaceae* was 12% (12/100). This result disagree with Nair P study (Mombai, India 2012) and Mate H study (North-East India 2014) which reported the frequency were 46%, and 24% respectively (Nair P K and Vaz M S, 2013; Mate H *et al.*, 2014). The lower frequency of this study when compared with Nair P and Mate H studies may arise from the differences in study area or population.

One of the main predisposing factors for the emergence of CRE is the abuse of antibiotics, as observed in this study, the frequency of carbapenem resistant is higher in β -lactam antibiotics users 16.1% (5/31) than non β -lactam users 10.1% (7/69). This high prevalence may be mainly due to the availability of non prescribed antibiotics as reported by Amer W H study, which reported about 76.6% of cases receiving previous empirical antibiotics were infected with CRE (Amer W H *et al.*, A 2016). The frequency of CRE was higher in *K.pneumoniae* 33.3% (5/15) than *E.coli* 8.2% (7/85), this result disagree with Muhammad M H study (Jordan 2015) which reported the frequency of CRE amonguropathogenic *E.coli* isolates was 23% (Muhammad M H and Swedan S, 2015). The higher frequency in this study when compared with our study, may be due to the difference in study area or population.

Also in this study most isolates were sensitive to (Amikacin 85%, Gentamycin 85%, Nitrofurantoin 83%, and Imipenem 70%), and resistant to (Co.trimoxazole 87%, and Ciprofloxacin 40%). All *K.pneumoniae* isolates were sensitive to amikacin, and nitrofurantoin. The resistant pattern of *E.coli* isolates to ciprofloxacin and nitrofurantoin were 65.9%, and 20% respectively. This result disagree with Muhammad M H study (Jordan 2015) which reported the resistant of *E.coli* isolates to ciprofloxacin and nitrofurantoin were 40%, and 67.1% respectively (Muhammad M H and Swedan S, 2015). Our data suggests, this difference may arise from the difference of study population.

CONCLUSIONS

The emergence elevation of carbapenem resistant *Enterobacteriaceae* (CRE) frequency in Kosti city, was become a major problem. Updating and implementation of therapy protocol together with continuously and regular evaluation of the susceptibility of urinary isolates to antibiotics can help in elimination and eradication of this problem.

RECOMMENDATIONS

Urine for culture and susceptibility test should be performing as routine test for patient with recurrent UTIs or non response to antibiotics therapy. The national surveillance programs for evaluation, and monitoring of antibiotics resistance should be establishing.

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Corresponding author: Babiker Saad Almugadam, Department of Microbiology, Faculty of Medical Laboratory Sciences, University of El Imam El Mahdi, Kosti city, Sudan.

Email: Babiker888@yahoo.com Mobile: 00249917717034