

Carbapenem Resistance Enterobacteriaceae Among Wound Isolates, Kosti City, Sudan

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Abstract

Background: Bacterial wound infections are a major health problem worldwide and it has dangerous complications. This study aim to investigate the frequency and antibiotics susceptibility of carbapenem-resistant Enterobacteriaceae (CRE).

Methods: A total of 100 Enterobacteriaceae were isolated from patients with wound infections. Phenotypic detection of CRE was confirmed by modified Hodge test (MHT) and antibiotics susceptibility testing were performed according to clinical and laboratory standard institute (CLSI) guidelines 2011.

Results: The frequency of CRE was 6% the frequency was higher in Beta-lactam users and lower in non-Beta-lactam antibiotics users. In this study, most isolates are sensitive to (gentamicin 57%, meropenem 68%, and imipenem 96%) and resistant to (Ceftriaxone 100%, and Cefotaxime 79%).

Conclusion: Continuous and regular surveillance programs can aid in the development of guides for the treatment, control, and prevention of wound infections caused by carbapenem resistant Enterobacteriaceae.

Keywords: Antimicrobial resistance; CRE; MHT; Sudan; Wound infection

Introduction

A wound is a damaged area of the body, usually involving a break to the skin. Wounds can be surgical or due to traum [1]. Wound infections may cause by viruses, fungi, parasite, and bacteria. Enterobacteriaceae family is a one of the major cause of bacterial wound infection [2]. Bacterial wound infections are a major health problem worldwide and it has dangerous complications [2]. Enterobacteriaceae are a large, heterogeneous group of Gram negative rods whose are natural habitat in the intestinal tract of humans and animals [3]. There are many genera of Enterobacteriaceae causes wound infections but this study restricted on *E. coli*, *K. pneumoniae*, *P. mirabilis*, *E. aerogenes*, and *C. freundii* [4]. Enterobacteriaceae developed resistance to many antibiotics, although this study was interesting in the resistance of Enterobacteriaceae to carbapenem antibiotics. Carbapenem resistant occur by two mechanisms, either production of carbapenemases that breakdown carbapenem or production of extended spectrum Beta lactamases (ESBLs) together with porin loss [5]. This study aim to investigate the frequency and antibiotics susceptibility of CRE that isolated from patients with wound infections in order to provide guidance for the treatment of wound infections caused by those pathogen in Kosti city, White Nile state, Sudan.

Materials and Methods

This study is a cross sectional, hospital based study, carried out in Kosti Teaching Hospital-Kosti city during the period from October 2016 to August 2017. A total of 100 Enterobacteriaceae isolates (*P. mirabilis* 33, *K. pneumoniae* 25, *E. coli* 23, *E. aerogenes* 11, and *C. freundii* 8) were collected from patients with wound infections. All isolates were presumptively identified based on their culture characteristics, Gram stain, and conventional biochemical tests [6]. All isolated Enterobacteriaceae were further subjected to antibiotics susceptibility testing using modified Kirby Bauer disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2011, and phenotypic detection for carbapenem resistance using modified Hodge test [7].

Antibiotics Susceptibility testing were performed by modified Kirby Bauer disk diffusion technique according to CLSI guidelines 2011 using Bioanalyse antibiotics which includes amoxycylav 30 µg, ceftriaxone 30 µg, cefotaxime 30 µg, and gentamycin 10 µg. Inoculum suspension of test organism equivalent to 0.5 McFarland standards was prepared, and by using sterile cotton tipped swab the inoculum suspension was inoculated in Mueller Hinton agar (Himedia media), then with sterile forceps the antimicrobial discs were placed on inoculated media, incubated aerobically at 35°C for 18 h. The diameter of inhibition zones were measured in mm with ruler, and interpreted according to Clinical and Laboratory Standards Institute guidelines 2011 [7]. *E. coli* ATCC 25922 strain was used as control strain.

The screening for carbapenem resistance were performed a long with susceptibility testing by using meropenem 10 µg, and imipenem 10 µg discs. Each isolate showed resistant to meropenem 10 µg or/and imipenem 10 µg was confirmed for Carbapenem resistance by using modified Hodge test [7,8].

Modified Hodge test was performed for each isolate showed resistant to meropenem or imipenem. Inoculum suspension of *E. coli* ATCC 25922 strain equivalent to 0.5 McFarland standard were prepared, diluted in 1:10 with normal saline, then using sterile cotton tipped swab the diluted suspension were inoculated in Mueller Hinton (MH) agar. Using sterile forceps, meropenem 10 µg disk was placed on the center of inoculated plate, then with sterile wire loop the colonies of test organism were streaked from edge of disk to edge of MH agar plate, incubated aerobically at 35°C for 18 h. Positive strain shows a 'cloverleaf shaped' zone of inhibition due to carbapenemase production, while the negative strain shows an undistorted zone of inhibition [7,9].

All the Data were analyzed using statistical package for social sciences (SPSS version 16) software, and P value ≤ 0.05 were considered significant in comparative data.

Results

A total of 100 Enterobacteriaceae isolates (*P. mirabilis* 33, *K. pneumoniae* 25, *E. coli* 23, *E. aerogenes* 11, and *C. freundii* 8) were collected from wound infected patients. The frequency of CRE among isolates is 6%; the frequency was higher in females 8.3% (2/24) than males 5.2% (4/76) as shown in table 1. The frequency of carbapenem resistant among isolates was 6% (2/33) in *P. mirabilis*, 12% (3/25) in *K. pneumoniae*, and 9% (1/11) in *E. aerogenes* as shown in table 2.

In this study, most isolates were sensitive to (IPM 96%, MEM 68%, and CN 57%) and resistant to (CTR 100%, CTX 79%, and AMC 69%) as seen in table 3.

Sex	Frequency		Total
	Carbapenem resistant	Carbapenem sensitive	
Male	4 (5.3%)	72 (94.7%)	76
Female	2 (8.3%)	22 (91.7%)	24
Total	6 (6%)	94 (94%)	100

Table 1: The frequency of CRE isolates among sex.

The frequency of carbapenems resistant was higher in females 8.35% (2/24) than male 5.3% (4/76).

Type of bacteria	Frequency		Total
	Carbapenem Sensitive	Carbapenem Resistant	
<i>P. mirabilis</i>	31 (94%)	2 (6%)	33
<i>K. pneumoniae</i>	22 (88%)	3 (12%)	25
<i>E. aerogenes</i>	10 (91%)	1 (9%)	11
<i>C. freundii</i>	8 (100%)	0 (0%)	8
<i>E. coli</i>	23 (100%)	0 (0%)	23

Total	94	6	100
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Table 2: The frequency of carbapenem resistance among Enterobacteriaceae isolates.

The higher frequency of carbapenem resistant was shown in *K. pneumoniae* 12% (3/25) followed by *E. aerogenes* 9% (1/11) and *P. mirabilis* 6% (2/33) while the frequency was zero in *E. coli* and *C. freundii*.

Antibiotics		Frequency		P value
		CRE	Non CRE	
CTX	S 7 (7%)	0	7	0.639
	R 93 (93%)	6	87	
CTR	S 0 (0%)	0	0	0.639
	R 100 (100%)	6	94	
AMC	S 23 (23%)	1	22	0.58
	R 77 (77%)	5	72	
GEN	S 53 (53%)	0	53	0.009
	R 47 (47%)	6	41	
MEM	S 76 (76%)	2	74	0.028
	R 24 (24%)	4	20	
IMP	S 96 (96%)	5	91	0.222
	R 4 (4%)	1	3	

S=Sensitive, R=Resistant. The frequency of CRE were higher in Beta lactam and GEN resistant isolates. P value showed significant difference between the susceptibility of CRE and non CRE isolates for MEM, and GEN.

Table 3: The susceptibility of isolated CRE and non CRE to antibiotics.

Discussion

Regardless of advances techniques and technology in wound management, wound infections are still a major health problem particularly in the developing world, so the frequent detection and evaluation of the susceptibility of pathogens to antibiotics empirically prescribed for eradication of infections [10].

In this study, the frequency of CRE among all isolates was 6%, and it was slightly lower in males 5.4% (4/76) when compared with females 8.3% (2/24). This result was disagreeing with Henkhoneng M study (India, 2014) which report the frequency of CRE was 70% [11]. Our result was lower when compared with Henkhoneng M study; these differences in results may arise from the differences in sample sizes or study areas.

In our study, the frequency of carbapenem resistance among *K. pneumoniae*, *E. aerogenes*, *P. mirabilis*, *E. coli*, and *C. freundii* isolates were 12% (3/25), 9% (1/11), 6% (2/33), 0% (0/23), and 0% (0/8) respectively. This result agree with Irmak B study (turkey, 2016) which report the frequency of carbapenem resistance among *C. freundii* was 0% and disagree with it, as it reported the frequency of CRE among *E. aerogenes* was 0% [12]. The higher frequency of carbapenem resistance

in *E. aerogenes* in our study when compared with Irmak B may be due to differences in study area and sample size.

In this study, all isolates were resistant to Ceftriaxone 100%; and the most isolates were resistant to (Cefotaxime 79% and Amoxicillin-clavulnic acid 69%) and sensitive to (Imipenem 96%, Meropenem 68%, and Gentamycin 57%). Also all *E. aerogenes* isolates were sensitive to Amoxycylav and gentamicin. And all *P. mirabilis* isolates were resistant to Ceftriaxone and Cefotaxime, so our data suggests that these drugs are of very limited value in the prophylaxis or empiric treatment of wound infections.

Conclusions

CRE isolates were more resistant to other antibiotics than non CRE that may suggest the carbapenem resistant is associated with resistant to other antibiotics. Since carbapenem are the drugs of last resort in the treatment of infections caused by multidrug resistant Enterobacteriaceae, the increase of CRE isolates can limit the therapeutic options. Proper wound care and regular update of antibiotics susceptibility through continuous surveillance is essential to maintain good infection control program.

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