Detection of Carbapenem-Resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis* clinical isolates in Khartoum; Sudan

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Abstract

**Background:** Carbapenem resistant Gram-negative rods are a group of emerging, highly drug-resistant bacteria causing a spectrum of infectious diseases associated with significant morbidity and mortality. **Objective:** The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of Carbapenem resistant Gram-negative bacilli (*Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*) isolated from clinical specimens.

**Methods:** The current is a cross sectional study. 162 selected Enterobacteria including *E. coli*, *K. pneumoniae*, and *P. mirabilis* strains were isolated and identified from patients suffering from urinary tract infections, bloodstream infections, wound infections and ear infections; using conventional microbiology techniques. Isolated strains were tested for antimicrobial resistance using the disc diffusion technique and carbapenemase production was detected using modified Hodge test. **Results:** Out of 162 clinical isolates, *E. coli*, *K. pneumoniae*, and *P. mirabilis* represented 44.4%, 38.9%, and 16.7% respectively. 10.5% were confirmed as carbapenemase producers, most of them is *K. pneumoniae*. **Conclusion:** In this study, the prevalence of Carbapenem resistant bacteria is high and necessary steps to prevent the spread of resistance should be taken.

**Key words:** *Escherichia coli*; *Klebsiella pneumoniae*; *Proteus mirabilis*; Carbapenem-Resistant Enterobacteriaceae (CRE); Khartoum; Sudan.

Introduction

During the past few years, the spread of carbapenemase producing *K. pneumoniae* has led to an increase in the prevalence of Carbapenem-resistant Enterobacteriaceae (CRE). Infections caused by these bacteria
are difficult to control because they have high levels of antimicrobial resistance and have been associated with a significant morbidity and mortality rates [1]. CRE are group of bacteria that have become resistant almost to all of antibacterial, including Carbapenems, these drugs are typically reserved as the lastline antimicrobial against drug-resistant bacteria [2]. New-Delhi metallo-β-lactamaseis an enzyme present in some Enterobacteriaceae mainly (E. coli, K.pneumoniae and P. mirabilis) responsible for resistance to all β-lactams antibiotics, including Carbapenems [3]. Untreatable or difficult to treat infections caused by CRE are on the rise among patients in clinical facilities [1]. Reports from US estimated that 140,000 hospital acquired Enterobacteriaceae infections occur each year; 9,300 of these are due to CRE, and nearly 600 deaths result from infections due to the two most common types of CRE, Carbapenem-resistant K. pneumoniae and Carbapenem-resistant E. coli [2].

Multiple different mechanisms can lead Carbapenems resistance. One of these mechanisms is the production of Carbapenemase, which are a group of enzymes that can hydrolyze most β-lactam antibiotics including Carbapenems [4,5]. Carbapenemase genes are located on plasmid that can be exchanged between Enterobacteriaceae and other gram negativebacteria [5]. They are also often transmittedtogether with other resistance genes resulting in multi-drug-resistant bacteria [6]. While Carbapenem resistant K.pneumoniae are more frequent to cause hospital acquired out-breaks, E. coli more frequent to cause community acquired out-breaks [5].

In addition to that, many several studies reported an evidence that extra-intestinal E. coli can be transmittedto human via the food supply from animal sources [7]. Laboratory screening of CRE by using disc diffusion techniques is an important for limiting the spread of this resistance mechanism. Confirmatory testing for CRE is important to all Enterobacteriaceae with decreased susceptibility to carbapenems or resistance to most other β-lactams antibiotics by disc diffusion techniques. The modified Hodge test is most easily, sensitive and specific test for CRE confirmation [8]. The aim of this study was to determine the prevalence and antimicrobial susceptibility pattern of Carbapenem resistant Gram-negative bacilli (E. coli, K.pneumoniae and P. mirabilis) isolated from clinical specimens.

**Materials and Methods**

**Isolation and Identification of strains:** A total of 162 clinical isolates of E. coli, K.pneumoniae, and P. mirabilis isolated from patients suffering from bloodstream infections, urinary tract infections, ear infections and wound infections from different Khartoum hospitals during the period of November 2015 and January 2016 were included in this study. Strains were isolatedby inoculation of collected specimens on CLED, Brain Heart Infusion Broth or Blood agar and MacConkey agar (Depending on the specimen) after
overnight incubation at 37°C. The isolates identified based on colony morphology, Gram’s stain, KIA test, citrate utilization test, urease production test, indole production test and motility test, according to standard microbiological procedures. The isolates sub-cultured onto nutrient agar and incubated at 37°C for approximately 18 to 24 hours prior to testing.

**Antimicrobial susceptibility Testing:** The antimicrobial susceptibility pattern of the isolated strains was determined by the modified Kirby Bauer disc diffusion method on Muller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to Amoxyclav (30µg), Cefotaxime (30µg), Cefoxitin (30µg), Ceftriaxone (30µg), Ceftazidim (30µg), Aztroname (30µg), Cefepime (30µg), Ciprofloxacine (10µg), and Imepinem (10µg) according to Clinical and laboratory standards Institute (CLSI).

**Screening of Carbapenem resistant strains:** Carbapenem resistant strains were defined as any strain that shows resistance to Carbapenem antibiotics tested [9].

**Confirmation of Carbapenem resistant strains (Modified Hodge Test):** This test is performed first by culturing a susceptible E. coli isolate (ATCC 25922) on a Mueller-Hinton plate, after which a Carbapenem disk is placed in the center. Isolates suspected of carbapenemase production, then are streaked from the disk to the outer margin of the plate. Growth of E. coli near the disk or along the isolate streak (Characteristic cloverleaf-like indentation) indicates that carbapenemase is present [9].

**Results**

During the study period a total of 162 clinical isolates of E. coli, K.pneumoniae, and P. mirabilis isolated from different clinical specimens: 25 (15.5%) blood, 83 (51.2%), urine, 4 (02.4%) ear swab, and 50 (30.8%) wound swab, from different Khartoum hospitals during the period of November 2015 and January 2016 were included in this study. E. coli accounted for 72 (44.4%), K.pneumoniae 63 (38.9%) and P. mirabilis 27 (16.7%) (Table 1). Out of 162 isolates, 17(10.5%) strains were found to be Carbapenem resistant. E. coli accounted 08/72(11.1%), K.pneumonia 08/63 (12.7%) and P. mirabilis 01/27(03.7%) (Table 2). About 53% (9/17) of CRE strains isolated from urine samples followed by blood samples 35% (6/17) and no CRE strain isolated from ear swabs or discharges (Table 3).

Antibiotic susceptibility of Carbapenem resistant E. coli, K.pneumoniae, and P. mirabilis isolates was evaluated for 12 antimicrobial agents. However, these strains are 100% resistant to Amoxyclav, Cefotaxime, Cefoxitin, Ceftriaxone, Ceftazidim, Aztroname, Cefepime, Ciprofloxacine, Gentamicin and Imepinem. About 12% (2/17) CRE strains were Colistin resistant while resistant to Tigecyclinerepresent about 6% (1/17) (Table 4).
Table (1) Distribution of Bacterial Isolates according to specimens types (No. 162)

<table>
<thead>
<tr>
<th>Isolates/Specimens</th>
<th>Urine No. (%)</th>
<th>Wound No. (%)</th>
<th>Blood No. (%)</th>
<th>Ear No. (%)</th>
<th>Total No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>52 (32.1)</td>
<td>15 (09.2)</td>
<td>04 (02.5)</td>
<td>01 (00.6)</td>
<td>72 (44.4)</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>23 (14.2)</td>
<td>19 (11.7)</td>
<td>21 (13.0)</td>
<td>00 (00.0)</td>
<td>63 (38.9)</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>08 (04.9)</td>
<td>16 (09.9)</td>
<td>00 (00.0)</td>
<td>03 (01.8)</td>
<td>27 (16.7)</td>
</tr>
<tr>
<td>Total</td>
<td>83 (51.2)</td>
<td>50 (30.8)</td>
<td>25 (15.5)</td>
<td>04 (02.4)</td>
<td>162 (100)</td>
</tr>
</tbody>
</table>

Table (2) Frequency of Carbapenem Resistant among isolates (n= 162)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>No.</th>
<th>Carbapenem resistant No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>72</td>
<td>08 (11.1)</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>63</td>
<td>08 (12.7)</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>27</td>
<td>01 (03.7)</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>17 (10.5)</td>
</tr>
</tbody>
</table>

Table (3) Carbapenem resistant isolates among specimens (n=17)

<table>
<thead>
<tr>
<th>Isolates/Specimens</th>
<th>Urine</th>
<th>Wound</th>
<th>Blood</th>
<th>Ear</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>

Table (4) Resistance of isolates to Colistin and Tigecycline (n=17)

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Colistin No. (%)</th>
<th>Tigecycline No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>1 (05.9)</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>1 (05.9)</td>
<td>1 (05.9)</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0 (00.0)</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (11.2)</td>
<td>1 (05.9)</td>
</tr>
</tbody>
</table>
Discussion
The introduction of Carbapenems (Meropenem and Imipenem) into clinical practice represents a significant progress for the treatment of serious bacterial infections caused by β-lactamaes producing bacteria. These antimicrobials have a broad spectrum activity and great stability to destroyed by most β-lactamases. Carbapenems have been used as a last line antimicrobial to treatlife threatening infections caused by β-lactamase producing bacteria since 1985 [2,4,9]. Recently the morbidity of CRE strains has increased in both community and hospitals through out the world [10,11]. WHO Global report on antimicrobial resistance surveillance reported high mortality from 30 - 75% for patients with severe CRE infections [12,13]. In this study, 10.5% (17/162) of our Enterobacteriaceae isolates were Carbapenem resistant using modified Hodge test (MHT). Comparable result were reported in study from Turkey by Nazik et al [14] 2012 (10.9%). Our result is lower thanthat obtained in studies from Uganda by Okoche et al [15] 2015(22.4%) using MHT, Nigeria by Yusuf et al [16] 2012 (33.5%), Sudan by Satir et al [17] 2016 (45.7%) using PCR, Tanzania by Mushli et al [18] 2014 (35%) using PCR, and Pakistan by Amjad et al [19] 2011 (69%) using (MHT). Our prevalence is higher than that obtained in studies from Turkey by Irmak et al [20] 2016 (3%) United States by Pollett et al [21] 2014 (4.2%) and Malaysia by Hammoudi et al [22] 2014 (4%). CRE prevalence varies substantially among countries, these differences may be due to restricted use of antibiotics in some countries compared to other where antimicrobials are available without prescription by a clinician. CRE prevalence variation may also due to variation instudy populations, nosocomial pathogens tend to be more resistant than community acquired pathogens, and or differences in the hospital hygiene guidelines applied and/or antibiotic use. CRE has been among the most frequently identified antimicrobial drug resistant pathogens worldwide This study revealed that all CRE isolates were multi-resistant (i.e. resistant to almost all of the antibiotics tested) this finding is similar to results that published by Mushi et al [18] 2014 and Okoche et al [15] 2015. The current study and similar studies concluded that; the antimicrobial treatment options for CRE are very limited, as these pathogens are overwhelming multi-drug resistant. In this study, Tigecycline and Colistin are drugs of choice to treat Carbapenem resistant E. coli, and K.pneumoniae strains, same reports showed that the in vitro susceptibility toTigecycline and Colistinamong clinical carbapenemase producing isolates ranges from 90-100%[23].

Conclusion
Our study found a high percentage of Carbapenem resistantamong E. coli, K.pneumoniae strains. These isolates showed resistance to all common used antimicrobials, while 11.8% were Colistin resistant and 5.9% were Tigecycline resistant. According to these results we recommend routine testing for carbapenem resistance using the simple modified Hodge
test form multi-drug resistant *E. coli* and *K. pneumoniae* strains. In addition, Tigecycline and Colistin should be tested routinely to provide an alternative treatment option for infections caused by these strains.

**References**


