Carbapenem Resistance among Clinical Isolates of Enterobacteriaceae

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Abstract

Aim: This study aimed to determine the presence of carbapenem resistance among Enterobacteriaceae by disk diffusion method and Epsilometer test (E-test).

Methodology: In the present study, 100 Enterobacteriaceae were isolated from different clinical samples and identified by Analytical Profile Index (API) 20 E. Antimicrobial susceptibility testing was done by modified kirby bauer disk diffusion method; using carbapenems; Ertapenem (ETP) and Meropenem (MEM) and third generation cephalosporins; Cefoprazone (CFP) and Cefotaxime (CTX) and followed by Minimum Inhibitory Concentration (MIC) determination by E-test for ETP and MEM.

Results: The disk diffusion method showed that 81% of the isolates were resistant to CTX, 79% were non-susceptible (i.e. resistant and intermediate isolates) to CFP, and the carbapenem non-susceptibility was 68 isolates (68%) to ETP and 57 isolates (57%) to MEM. MIC determination results by E-test method differed from disk diffusion results and showed that the carbapenem non-susceptibility to ETP and MEM was 19% and 9% respectively.

Conclusions: The presence of carbapenem resistance among Enterobacteriaceae isolates causing healthcare-associated infections in the current study emphasizes the necessity for early detection of these isolates and reporting to infection prevention staff to overcome their spread.

Key Words: Enterobacteriaceae – Modified kirby bauer disk – E-test – Carbapenem.

Introduction

THE members of Enterobacteriaceae are among the most common human pathogens causing infections that range from cystitis to pyelonephritis, septicemia, pneumonia, peritonitis, meningitis and device-associated infections. They are the most common source of community and Hospital-acquired Infections (HAIs), with Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae), being by far the most important pathogens for humans [1-5]. Other members include Serratia and Citrobacter which are emerging as significant pathogens [6] but are usually missed by the routine identification methods employed in most of the laboratories [7].

Enterobacteriaceae may account for 80% of clinically significant isolates of Gram-negative bacilli and 50% of clinically significant bacteria in clinical microbiology. They account for more than 70% of Urinary Tract Infections (UTIs), nearly 50% of septicemia cases and constitute a significant percentage of intestinal infections [8]. Enterobacteriaceae spread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons [2-5].

Carbapenem antibiotics, a subclass of the Beta (β)-lactamase agents, are important therapeutic agents in the health care setting. Because these drugs have broad-spectrum activity, they are frequently used for empiric therapy of life-threatening infections, such as sepsis [9]. The rapid emergence and dissemination of Enterobacteriaceae that are resistant to carbapenems such as imipenem and meropenem poses a considerable threat to clinical patient care and public health [10,11].

In Enterobacteriaceae, carbapenem resistance arises from two main mechanisms: (I) A decrease in the uptake of antibiotics by a qualitative or/and quantitative deficiency of porin expression in association with over expression of β-lactamases that possess very weak affinity for carbapenems.
like an Extended-Spectrum $\beta$-Lactamase (ESBL) or Ambler class C (AmpC) or (II) Acquisition of carbapenemase genes that encode for enzymes capable of degrading carbapenems [5]. Carbapenemase-producing strains are characterized by their resistance to virtually all $\beta$-lactam antibiotics, including the cephalosporins and carbapenems, as well as to fluoroquinolones, aminoglycosides and co-trimoxazole. Invasive infections with these strains are associated with high rates of morbidity and mortality [10,11].

The mechanisms of resistance among gram negative Enterobacteriaceae vary widely. The emergence of ESBLs compromised the effect of most $\beta$-lactam antibiotics. In this situation, carbapenem antibiotics remain the drug of choice and have been increasingly utilized to treat infections with ESBL-producing organisms. However, the increasing rates of ESBLs’ isolates are leading to the overuse of carbapenems, creating antibiotic pressure on carbapenems and triggering bacterial resistance against this class of antimicrobial agents. Subsequently, carbapenemases have been increasingly detected among Enterobacteriaceae, limiting the existing antimicrobial tools for treating these resistant isolates [12].

Material and Methods

**Clinical isolates:**

The present study was conducted on 100 different clinical isolates of Enterobacteriaceae collected from hospitalized patients at Kasr El-Aini Cairo University Hospitals during the period from October 2011 through May 2012. These isolates were obtained by cultivation of the following clinical specimens: Urine, endo trachial tube aspirate, sputum, pus. The culture was done using blood agar and MacConkey agar plates incubated aerobically at 37ºC for 24-48 hours.

**Identification of the isolates:**

Identification of the isolates was done according to the conventional microbiological standard tests (Gram’s stain, glucose fermentation test and oxidase test). Isolates identified as gram negative bacilli, glucose fermenters and oxidase negative were considered Enterobacteriaceae [13]. Identification up to the species level was done using API 20E identification system (BioMérieux, France).

**Principle:**

API 20E is a standardized biochemical method for biotyping to delineate different spp. of Enterobacteriaceae. The API 20E strip consists of micro cupules containing dehydrated substrates. These cupules were inoculated with a 0.5 McFarland bacterial suspension in sterile saline which reconstitutes the media. During aerobic incubation at 37ºC for 18-24 hours, metabolic activity was revealed by color changes that were detected either spontaneous or by the addition of reagents. The results were recorded according to the reading table and identification was obtained by referring to the Analytical profile index according to the manufacturer’s instructions (BioMérieux, France).

**Testing of antimicrobial susceptibility:**

Isolates were tested by Modified Kirby Bauer disk diffusion method; using carbapenems; ETP and MEM and third generation cephalosporins; Cefoprazone (CFP) and Cefotaxime (CTX) and followed by MIC determination by E-test for ertapenem and meropenem. Results were interpreted according to the standard guidelines [14] (Table 1).

A- Disk diffusion method:

Pure isolates were cultured on blood agar plates for 24 hours at 37ºC. A suspension was prepared using few identical colonies from each pure isolate and then turbidity was adjusted to 0.5 McFarland Standard. A sterile cotton swab was dipped into the suspension, rotated several times and pressed tightly on the inside of the wall of the tube to remove excess inoculum from the swab. The swab was then streaked over the entire surface of Muller-Hinton Agar (MHA) three times with the plate rotated 60º each time and left to dry. The antibiotic disks (Oxoid, UK) were then applied to the plates using a sterile forceps (with 2.5cm apart and 1.5cm from the edge of the plate) pressed well and incubated at 37ºC for 24 hours. The diameters of the inhibition zones were measured in millimeters and compared to a reference table, (Table 1) to differentiate the isolates into Susceptible (S), Intermediate (I) or Resistant (R) [14].

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Disk content (µg)</th>
<th>Zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$S$</td>
</tr>
<tr>
<td>10</td>
<td>≥22</td>
<td>19-21</td>
</tr>
<tr>
<td>10</td>
<td>≥23</td>
<td>20-22</td>
</tr>
<tr>
<td>75</td>
<td>≥21</td>
<td>16-20</td>
</tr>
<tr>
<td>30</td>
<td>≥26</td>
<td>23-25</td>
</tr>
</tbody>
</table>
B- *E-test (BioMérieux, France):*

E-test is a thin, inert and non-porous plastic strip. One side of the strip carries the MIC reading scale in μg/ml. A predefined exponential gradient of antibiotic, dried and stabilized, is immobilized on the other side of the strip. ETP codes for ertapenem (0.002-32μg/ml) and MP codes for meropenem (0.002-32μg/ml). E-test was performed according to the manufacturer's instructions as follows; the E-test strips were allowed to stand for 30 minutes to adapt to room temperature before use. Isolated pure colonies from overnight agar plate were emulsified in saline to achieve turbidity equivalent to 0.5 McFarland Standard. A sterile cotton swab was dipped into the suspension, rotated several times and pressed tightly on the inside of the wall of the tube to remove excess inoculum from the swab. The swab was then streaked over the entire surface of Muller-Hinton agar three times with the plate rotated 60° each time and left to dry completely. The strips were applied using sterile forceps on inoculated agar with the MIC scale facing upwards making sure the whole length of the strip was in complete contact with the agar surface.

*Reading and interpretation:*

- When bacterial growth was visible, the MIC value is read where the pointed end of the inhibition ellipse intersects the side of the strip. Growth along the entire strip i.e. no inhibition ellipse is seen indicated that the MIC as ≥ the highest value on the MIC scale.

- An inhibition ellipse below the strip (does not intersect the strip) indicated the MIC < the lowest value on the MIC scale.

- When macrocolonies were present within the ellipse for bactericidal agents, all macrocolonies within 1-3mm from the strip were read; isolated colonies for carbapenems may represent resistant subpopulations e.g. KPC.

- E-test generates MIC values from a continuous scale and can give results in-between conventional two-fold dilutions i.e. half dilutions. An E-test MIC value which falls between standard two-fold dilutions must be rounded up to the next upper two-fold value before categorization.

*Statistical analysis:*

Data were statistically described in terms of frequencies (number of cases) and percentages. Comparison between the study groups was done using McNemar test. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Table (2): MIC determination by E-test for carbapenems and interpretative standards for Enterobacteriaceae according to CLSI guidelines.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>MIC interpretive standards (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤0.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>≤1</td>
</tr>
</tbody>
</table>

*Results*

*Analysis of clinical isolates:* Out of the 100 Enterobacteriaceae isolated from the study population, 68 isolates were obtained from urine specimens, 20 isolates from endotracheal tube aspirates, 9 isolates from sputum and 3 isolates from pus. They were identified by API 20E (BioMérieux, France) to be 54E. coli, 16 Enterobacter sakazakii, 8K. pneumoniae, 7K. ornithiolytica, 4K. oxytoca, 4 Citrobacter farmer, 3 proteus mirabilis, 2 Enterobacter cloacae, one K. terrigena and one Citrobacter freundii.

*Testing of antimicrobial susceptibility:*

A- By disk diffusion (Kirby-Bauer) method:

The susceptibility patterns among the 100 Enterobacteriaceae isolates varied; 81 isolates (81%) showed resistance to CTX, 76 isolates (76%) were resistant to CFP, while 56 isolates (56%) exhibited resistance to ETP and 46 isolates (46%) were resistant to MEM Figs. (2-4).

B- MIC determination by E-test:

By the E-test, carbapenem MICs differed among the 100 Enterobacteriaceae isolates; 91 isolates (91%) were susceptible to MEM and the remaining 9 isolates (9%) being either resistant or intermediately resistant. For ETP, 81 isolates (81%) showed susceptibility and the remaining 19 isolates being either resistant (15 isolates, 15%) or intermediately resistant (4 isolates, 4%) as shown in Figs. (5-7).

Out of the 100 isolated Enterobacteriaceae, 72 isolates (72%) were intermediate (13 isolates, 18%) or resistant (63 isolates, 88%) to one or more carbapenems (ertapenem and/or meropenem) and resistant to one or more 3rd generation cephalosporins (cefoprazone and/or cefotaxime).
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Fig. (1): API test strip of E. coli.

Fig. (2): Disk diffusion susceptibilities used for screening the 100 Enterobacteriaceae isolates.

Fig. (3): Disk diffusion test showing resistance to MEM, ETP, CTX and CFP.

Fig. (4): Disk diffusion test showing susceptible isolate to MEM, ETP, CTX and CFP.

Fig. (5): MIC determination for 100 Enterobacteriaceae isolates by E-test.

Fig. (6): Carbapenem-resistant isolate by E-test; MIC for MEM 4µg/ml & 2µg/ml for ETP.

Fig. (7): Carbapenem-susceptible isolate by E-test; MIC 1µg/ml for MEM & 0.5µg/ml for ETP.
Discussion

Healthcare-associated infections caused by multidrug-resistant organisms are increasing. This poses a problem not only to Infection Prevention and Control (IPC) practitioners, but also to clinical diagnostic microbiology laboratories, which must identify the resistant pathogen(s) and disseminate this information to clinicians and IPC teams in a timely fashion [15].

In this study, antimicrobial susceptibility testing was done for all of the 100 isolates of Enterobacteriaceae (by disk diffusion method as an initial screening followed by MIC determination by E-test). By disk diffusion method; 81 isolates (81%) showed resistance to CTX, 79 isolates (79%) were non-susceptible (i.e. resistant and intermediate isolates) to CFP, while the prevalence for carbapenem non-susceptibility was 68 isolates (68%) to ETP and 57 isolates (57%) to MEM. The high prevalence of carbapenem non-susceptibility in the current study could be explained by the fact that the majority of our samples were collected from the Intensive Care Unit (ICU). Patients within the ICU were subjected to invasive procedures, treatment with antibiotic combinations, and were exposed to other patients with multi-drug resistant pathogens. Carbapenems are frequently used as the last choice in treating serious infections caused by multi-drug resistant strains of Gram-negative bacilli in ICUs and in high risk wards [16]. However, lower prevalence rates were reported by another Egyptian study at the Suez Canal University Hospital by Metwally et al., [17] who stated that out of 45 K. pneumoniae isolates, the non-susceptibility to ETP and to MEM was found to be 44.4% (20/45) and 37.8% (17/45), respectively. Also, Priyadarshini et al., [18] stated that out of 2035 Enterobacteriaceae isolates, 468 (22.9%) were resistant to MEM by Kirby Bauer method.

A higher prevalence of MEM resistance was reported in a study conducted by Yusuf et al., [19] in a teaching hospital in Nigeria in Africa where the disk diffusion method revealed that 119 (91%) were non-susceptible to MEM out of 135 Enterobacteriaceae isolates. Also, Priyadarshini et al., [20] detected high resistance to MEM 93.4% (43/46).

In the current study, MIC determination results by E-test method differed from disk diffusion results and showed that 19 isolates (19%) were non-susceptible to ETP while 9 isolates (9%) were non-susceptible to MEM. Determination of carbapenem MICs in organisms producing carbapenemase enzymes can be problematic. There have been studies reporting relatively low reproducibility for most of the conventional methods used, as well as discrepant results among the methods [21-24]. The broth micro dilution and disk diffusion methods are considered to be more reliable for the detection of all types of carbapenemase-mediated resistance [24]. This could be attributed to that the susceptibility tests of carbapenemase producing bacteria using E-test are often difficult to interpret [25]. Moreover, the E-test is not considered to be appropriate for the carbapenemase producing organisms, owing to their heterogeneous growth, which makes the interpretation very difficult [23].

Conclusion:

Carbapenem resistance in Enterobacteriaceae due to continuous selective pressure from the overuse of this class of antibiotics is a growing public health problem. Disk diffusion method using MEM and ETP is more reliable than E-test for detection of carbapenem resistance in Enterobacteriaceae.

References


