DETECTION OF CARBAPENEM RESISTANCE AMONG ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN CHEST INTENSIVE CARE UNIT AT ASSIUT UNIVERSITY HOSPITALS, EGYPT

Marwa M. Mahmoud¹, Khaled M. Hassanein¹, Tahani Obaid Alshammari², Gaber El-Saber Batiha³ and Helal F. Hetta¹*

¹Department of Medical Microbiology and Immunology, Faculty of Medicine, Assiut University, Assiut 71515, Egypt.
²Clinical laboratory, The College of Applied Medical Sciences, Aljouf University.
³Department of Pharmacology and Therapeutics, Faculty of Veterinary Medicine, Damanhour University, Damanhour 22511, AlBeheira, Egypt.

This study aimed to determine antimicrobial susceptibility pattern, percentage of carbapenem resistance and presence of blaNDM1, blaVIM1, blaIMP, blaKPC genes among Escherichia coli (E.coli) and Klebsiella pneumonia (K. pneumonia) isolated from chest intensive care unit (ICU) at Assiut University Hospitals, Egypt. Antibacterial susceptibility was detected by disc diffusion method. Genotypic detection of carbapenem resistant genes (IMP, NDM, VIM, and KPC) was done by PCR. From totally 200 patient clinical samples, 100 isolates (50%) were identified to be E.coli and K. pneumonia. Various percentages of resistance were reported for oxacillin (80 and 60%), ciprofloxacin (46 and 52%), amikacin (66 and 86%) amoxicillin & clavulanic acid (60 and 90%) lomefloxacin (22 and 18%) gentamicin (70 and 54%) chloramphenicol (54.4 and 66.7%), imipenem (90 and 84%), meropenem (80 and 72%) for E.coli and Klebsiella. The prevalence of KPC, NDM1, IMP, VIM1, and KPC genes was 74, 56, 30, 26 % for E.coli and NDM1,KPC, VIM1, IMP was 66, 64, 54, 50 % For K. pneumoniae. In conclusion, carbapenemases have essential role in antibiotic resistance of E. coli and K. pneumonia.

Keywords: carbapenem resistance; blaNDM1, blaVIM1, blaIMP, blaKPC; E.coli; K. pneumoniae

INTRODUCTION

Because of the rapid emergence and spread of antibiotic resistance, it is critical to track antibiotic use and establish treatment options in order to reduce antibiotic misuse.¹⁻⁵

As a result, it’s critical to keep looking into the genes that cause bacteria to become resistant to various antibiotics. Multidrug resistant Gram-negative bacteria is a growing concern across the Middle East due to several risk factors for acquisition, and treatment failure due to patient’s compliance and duration of treatment.⁶

β-lactam antibiotics are a class of antibiotic which have a broad spectrum of antibacterial activity, including important Gram-positive and Gram-negative pathogens and are the most broadly used antibiotics worldwide.⁷

So, an increasing incidence of resistance to these drugs is a public health concern. β-lactam antibiotics act by inhibiting a set of transpeptidase enzymes called penicillin binding proteins (PBPs), that are crucial for cell wall peptidoglycan synthesis, leading to death of the growing bacteria.⁸⁻⁹

Carbapenems are class of antibiotics of last refuge used for treatment of several infections due to Gram-negative bacilli, as extended spectrum β-lactamase- (ESBL-) producing bacteria. Several clinically relevant
bacteria induce resistance to these life-saving drugs bacteria. Carbapenemases are responsible for those resistance to carbapenems and mediated by several types of those enzyme such as metallo-β-lactamases (MBLs) of Imipenemase (IMP), NDM and VIM types, and serine carbapenemases of K. pneumoniae (KPC) type.

Prevalence of infections due to carbapenem resistant enterobacteriaceae (CRE) has been increased during the last decade. Infections due to these isolates are significantly have morbidity and mortality rate. Several risk factors can associate with those infection that included antibiotic exposure, intensive care unit (ICU) stay, and poor functional status.

Various strains of E.coli have different antibiotic sensitivities. Many drugs that are effective against Gram-Positive bacteria are ineffective against E.coli. Antibiotic resistance is on the rise. Some of this is due to human abuse of antibiotics, but much of it is likely owing to antibiotics being used as growth promoters in animal feeds. Due to widespread β-lactam antimicrobial use, bacterial resistance has been increasing and now represents a serious threat to the continued use of antibiotic therapy.

In this study we aimed to determine the prevalence of E.coli and K. Pneumoniae in chest intensive care units causing pneumonia, the percentage of carbapenem resistance among E.Coli and K. Pneumoniae isolates. Also to determine the presence of blaIMP, blaNDM1, blaVIM1, blaKPC genes by PCR.

**MATERIALS AND METHODS**

**Sample collection and isolation**

All clinical samples were collected from chest intensive care unit (ICU) at Assiut University Hospital for microbiological diagnosis in Microbiology and Immunology Department throughout the period of November 2018 to November 2019. They were 200 samples, E.coli and K. pneumoniae caused 100 of them. Patients were with a mean age of 47±15 years (SD). They included 45 males (45%) and 55 females (55%). The sputum samples were collected from patients with lower respiratory tract infection.

**Culture characteristics**

On MacConkey medium, E. coli and K. pneumoniae colonies are pink due to lactose fermentation, but K. pneumoniae colonies are large and mucoid dark pink due to slime layer. On Eosin Methyrene Blue (EMB), K. pneumoniae showed large mucoid pink or purple colonies, E.coli showed a characteristic green metallic sheen.

**Antimicrobial susceptibility testing of isolated bacteria**

Antimicrobial susceptibility patterns were determined by disk diffusion method on Muller-Hinton agar. The following antimicrobial disks were used; gentamicin (30 µg), ceftriaxone (30 µg), imipenem (10 µg), chloramphenicol (30 µg), cefotaxime (30 µg), meropenem (10 µg), oxacillin (1 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), oxytetracycline (30 µg), penicillin (10 µg), amoxicillin- clavulanic acid (20/10 µg), amikacin (30 µg), lomefloxacin (10 µg).

**Genotypic detection of carbapenem resistant genes (blaIMP, blaNDM1, blaVIM1 and blaKPC) by Polymerase chain reaction.**

**Extraction of DNA from bacterial Colonies**

Total cellular DNA was prepared as follows: culture grown overnight on Tryptic Soy Broth (TSB) was transferred to a 250 µl Eppendorf vial, and then centrifuged at 10000 rpm for 10 min. The supernatant was discarded and 250 µl of distilled water was added to the pellet and resulting solution was heated for 15 min at 100°C and centrifuged at 10000 rpm for 10 min. The supernatant was transferred to a new microtube and stored at -20°C.

**PCR amplification**

PCR in a 50-µl reaction mixture was performed on 2 µl of extracted DNA. The PCR mixture consisted of 1× PCR buffer (10 mM Tris- HCl [pH 8.3], 50 mM KCl), 1.5 mM MgCl2, 0.125 mM each dNTP, 0.1 µM each primer as shown in table (1), and 2 U of AmpliTaq Gold polymerase (Roche, Meylan, France). The PCR amplification thermal cycling conditions were as follows; 10 min at 94°C; 36 cycles of amplification consisting of 30 s at 94°C, 40 s at 52°C, and 50 s at 72°C; and 5 min at 72°C for the final extension.
Table 1: Primer sequences and product size for bla\text{IMP}, bla\text{NDM1}, bla\text{VIM1} and bla\text{KPC} genes.

<table>
<thead>
<tr>
<th>GENE</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla\text{IMP}</td>
<td>GCGTTTATGTTCATACTTGTCTTT</td>
<td>TCTATTGCCGCCGTTGTCTGT</td>
<td>587</td>
</tr>
<tr>
<td>bla\text{NDM1}</td>
<td>CAACTGGATCAAGCAGGAGA</td>
<td>TCGATCCTAAACGGTGATATT</td>
<td>621</td>
</tr>
<tr>
<td>bla\text{VIM1}</td>
<td>GACCCGTCTGTCATGG</td>
<td>GGCGACTGAGCGATTTTT</td>
<td>748</td>
</tr>
<tr>
<td>bla\text{KPC}</td>
<td>CGTTGACGCCAATCC</td>
<td>ACCGCTGGCAGCTGG</td>
<td>700</td>
</tr>
</tbody>
</table>

**RESULTS AND DISCUSSION**

**Results**

From totally 200 patient clinical samples, 100 isolates were identified to be *E. coli* and *K. pneumoniae* (50%) according to culture characters and biochemical reaction results.

**Isolation and identification of bacterial isolates**

Primary identification of the isolates was conducted by Gram stain.

Isolates were a Gram-negative bacillus arranged in pairs and/or short chains.

Cultivation of the isolates on MacConkey (selective media) showed red colonies due to lactose fermentation, but *K. pneumoniae* colonies are large and mucoid dark pink due to slime layer.

On Eosin Methylene Blue (EMB), *K. pneumoniae* showed large mucoid pink or purple colonies, *E. coli* showed a characteristic green sheen.

**Antimicrobial susceptibility profile for isolated *E. coli* and *K. pneumoniae***

As shown in table 2, all isolates were resistant to penicillin, ampicillin. Various percentages of resistance were reported for oxacillin (80 and 60%), ciprofloxacin (46 and 52%), amikacin (66 and 86%), amoxicillin & clavulanic acid (60 and 90%), lomefloxacin (22 and 18%), gentamicin (70 and 54%), chloramphenicol (54.4 and 66.7%), oxytetracycline (20 and 16%), norfloxacin (26 and 36%), cefotaxime (80 and 90%), cefepime (201 and 0%), imipenem (90 and 84%), meropenem (80 and 72%) for *E. coli* and *Klebsiella* respectively.

**Frequency of some carbapenem resistance genes in the bacterial isolates.**

Virulence genes including (bla\text{IMP}, bla\text{NDM1}, bla\text{VIM1} and bla\text{KPC}) of fifty isolates of *E. coli* and *K. pneumoniae* were amplified by PCR. For *E. coli*, the prevalence of bla\text{KPC}, bla\text{NDM1}, bla\text{IMP}, bla\text{VIM1} genes was 74%, 56%, 30%, 26% respectively. For *K. pneumoniae*, the frequency of bla\text{NDM1}, bla\text{KPC}, bla\text{VIM1}, bla\text{IMP} was 66%, 64%, 54%, 50 % respectively as shown in table 3. Some strains were carrying one and others were carrying more than one carbapenem resistance genes as shown in table 4.

Table 2: Antimicrobial susceptibility profile for isolated *E. coli* and *K. pneumoniae*.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>E. coli</em></th>
<th><em>K. pneumoniae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>50(100%)</td>
<td>50(100%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>40(80%)</td>
<td>30(60%)</td>
</tr>
<tr>
<td>Amoxicillin/ clavulanic acid</td>
<td>30(60%)</td>
<td>45(90%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>35(70%)</td>
<td>27(54%)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>33(66%)</td>
<td>43(86%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>23(46%)</td>
<td>26(52%)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>13(26%)</td>
<td>18(36%)</td>
</tr>
<tr>
<td>Lomefloxacin</td>
<td>11(22%)</td>
<td>9(18%)</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>10(20%)</td>
<td>8(16%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>6(54.5%)</td>
<td>6(66.7%)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>40(80%)</td>
<td>45(90%)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>10(20%)</td>
<td>5(10%)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>45(90%)</td>
<td>42(84%)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>40(80%)</td>
<td>43(86%)</td>
</tr>
</tbody>
</table>
Table 3: Frequency of some carbapenem resistance genes in the isolated E. coli and K. pneumoniae.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>N</th>
<th>blaIMP</th>
<th>N</th>
<th>%</th>
<th>blaNDM1</th>
<th>N</th>
<th>%</th>
<th>blaVIM1</th>
<th>N</th>
<th>%</th>
<th>blaKPC</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>50</td>
<td>15</td>
<td>30%</td>
<td>28</td>
<td>56%</td>
<td>13</td>
<td>26%</td>
<td>37</td>
<td>74%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>50</td>
<td>25</td>
<td>50%</td>
<td>33</td>
<td>66%</td>
<td>27</td>
<td>54%</td>
<td>32</td>
<td>64%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Frequency of one or more than one carbapenem resistance genes in the isolated E. coli and K. pneumoniae

<table>
<thead>
<tr>
<th>Gene(s) in each isolate</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>One gene</td>
<td>17</td>
<td>34%</td>
</tr>
<tr>
<td>Two gene</td>
<td>20</td>
<td>40%</td>
</tr>
<tr>
<td>Three gene</td>
<td>9</td>
<td>18%</td>
</tr>
<tr>
<td>Four gene</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>No gene</td>
<td>2</td>
<td>4%</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100%</td>
</tr>
</tbody>
</table>

Discussion

In this study, we reported nosocomial infections to be 33.3% (200/600). E. coli and K. pneumoniae caused 100 (50%) of these nosocomial infections. Our result was much higher than a study by Amazian, K. et al who reported that prevalence of nosocomial infections was 10.5%; this was higher in non-teaching centers and moderate-sized hospitals. In Amazian, K. et al study, the most commonly isolated organisms were: E. coli [17.2%], Staph. aureus [12.5%], P. aeruginosa and K. pneumoniae [9.2% each]. This difference might be different sample size, different region and infection control policies. The factors associated with acquisition of CRE were often complex.

In this study, the factors associated with acquisition of CRE were recorded. It was found that diabetes, malnutrition, immunodeficiency, peripheral I.V Catheter, artificial feeding, assisted ventilation, previous antibiotic Administration are the major risk factors significantly associated with CR E. coli. In contrast, diabetes, obesity, surgery, peripheral, I.V catheter, artificial feeding, endotracheal intubation, previous antibiotic administration are the major risk factors significantly associated with CRKP. In agreement with this study, several studies reported that hospital environment due to failure of adequate cleaning and disinfection, diabetes, organ/stem cell transplantation, mechanical ventilation, exposure to antimicrobials, and overall longer length of stay in hospitals are major risk factors for acquiring CRE infections.

In our study, we reported that all isolates were resistant to penicillin, ampicillin. Various percentages of resistance were reported for oxacillin (80 and 60%), ciprofloxacin (46 and 52%), amikacin (66 and 86%), amoxicillin & clavulanic acid (60 and 90%), lomefloxacin (22 and 18%), gentamicin (70 and 54%), chloramphenicol (54.4 and 66.7%), oxytetracycline (20 and 16%), norfloxacin (26 and 36%), cefotaxime (80 and 90%), cefepime (201 and 0%), imipenem (90 and 84%), meropenem (80 and 72%) for E. coli and K. pneumoniae, respectively. Our results came in agreement with this study those of Kotsoanas et al., (2013) who reported that 85–100% of the isolates were Ampicillin resistant, (66.7–100%) were resistant to Piperacillin, Ceftazidime, Cefotaxime and Aztreonam. (90%) of the isolates were resistant to Amoxicillin, Piperacillin and Cephalothin. In our study the resistance rate was much higher than one study, reported that high antibiotic resistance patterns were detected among E. coli and K. pneumoniae isolates.

Also, other study, stated that, Ceftriaxone resistance is increased in E. coli (from 48% to 70.5%) and in K. pneumoniae up to 81%, whereas Ciprofloxacin resistance in both organisms is in the range of 60-70%. Carbapenem resistance is also started increasing for both organisms.

In this study, the prevalence of blaKPC, blaNDM1, blaIMP, blaVIM1 genes
Our findings were much higher than several studies included Egyptian where reported that the most prevalent gene was blaVIM (21,10.7%), followed by blaOXA-48 (19, 9.7%), blaIMP (12, 6.1%), blaKPC (10,%) and blaNDM-1 (5, 2.6%). Also, carbapenemases genes, 62.1 % were blaKPC positive, 20.7 % were blaVIM-positive, 3.4 % were blaNDM-positive, 13.8 % were blaOXA-48-positive and none was blaIMP-positive. In addition, the most prevalent gene was blaKPC 47.8% followed by blaVIM-1 21.7%, blaIMP 15.2%, blaOXA-48-like 10.9% and blaNDM-1 4.3%

The rapid emergence and spread of antibiotic resistance makes it vital to keep track of antibiotic use and develop therapeutic solutions to decrease antibiotic misuse. As a result, it’s vital to continue researching the genes that drive bacteria to become antibiotic-resistant.

Conclusion
Our results revealed a high level of antimicrobial resistance among the studied clinical isolates of E. coli and K. Pneumoniae. The prevalence of carbapenemases producing isolates and their isolation from life threatening infections is increasing at an alarming rate worldwide. IMP, NDM1, VIM1, KPC play a vital role in the generation of this antimicrobial resistance phenotypically and genotypically. So, clinicians should understand the drug resistance of E.coli and K. Pneumoniae in order to control further propagation of these severely resistant bacterial strains in community and hospital settings.

Acknowledgments
The authors would like to thank the Medical Research Center at the Faculty of Medicine, Assiut University for providing the research equipment.

Conflicts of Interest
The authors declare no conflict of interest.

REFERENCES
11. B. Suay-García, M.T.Pérez-Gracia, "Present and future of carbapenem-


birds as the first report in Egypt”, *BMC microbiol*, 21(1), 237(2021).
الكشف عن مقاومة الكارباميبين بين الإشريكية القولونية والكلبيسلا الرئوية في وحدة العناية المركزة للصدر بمستشفيات جامعة أسيوط بمصر

مرارة محمد، خالد م. حسنين، تهاني عبيد الشمري، جابر الصاير، بطالة

هلالي ف. حنة

قسم الميكروبيولوجيا والمناعة الطبية، كلية الطب، جامعة أسيوط

المختبر السريري، كلية العلوم الطبية التطبيقية، جامعة الجوف

قسم الأدوية والعلاجات، كلية الطب البيطري، جامعة دميتور، دمنهور

في هذه الدراسة، تم تجميع 200 عينة إكلينيكية من مرضى العناية المركزة الصدرية. تم عزل 100 ععترا الإشريشيا كولاي والكلبيسلا رئوية من 100 مريض يعانون من عودي المستشفيات المكتسبة. تبين أن العديد من عوامل الخطر يمكن أن تشارك في اكتساب العدوى. فيما يتعلق بماقاومة المضادات الحيوية كانت الإشريشيا كولاي والكلبيسلا الرئوية مقاومة بالكامل لمعظم المضادات الحيوية التي تم اختبارها. تم فحص الجينات المسؤولة عن المقاومة لمجموعة كارباميبين (blaIMP، blaKPC، blaVIM1، blaNDM1) من خمسة عزلة من كل من الإشريشيا كولاي والكلبيسلا. البلازما M1 أعلى نسبة في الإشريشيا الكولاي بينما كان M1 أعلى نسبة في الكلبيسلا الرئوية.