Detection of OXA-51 Carbapenemase Gene in Klebsiella pneumoniae: A Case Report and a New Dimension on Carbapenem Resistance

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Abstract

We investigated the occurrence of genes associated with the production of carbapenem hydrolysing carbapenemases in Klebsiella pneumoniae isolates recovered from ICU patient. Antimicrobial susceptibility testing was performed and resistance genes were characterized by PCR amplification and sequencing. The Modified Hodge Test (MHT), MBL E-test, EDTA and aminophenylboronic acid (APBA) combined disk diffusion method, and a disk enzymatic assay were performed for the screening of carbapenemases. MICs of carbapenems were as follows (mg/L): ertapenem 0.06, meropenem 0.26 and doripenem 0.5. The isolate demonstrated positive results in the ESBL, EDTA and APBA combined tests, and disk enzymatic assay. PCR and sequencing revealed the presence of blaOXA-51 and blCTX-M-15 beta-lactamase genes. Plasmids were not transferred to recipient E.coli by conjugation and transformation. In conclusion, we report on the first detection of the OXA-51 harboring K. pneumoniae isolate and co-produced a CTX-M-15 beta-lactamase.

Keywords: Klebsiella pneumoniae; OXA-51 carbapenemase; Carbapenems; CTX-M-15 beta-lactamase; OXA genes

Introduction

Carbapenemase related resistance against carbapenems is a serious threat and cause of treatment failure especially for patients infected with hospital strains. Carbapenem resistance was rarely reported in enteric bacteria until recent years. In Enterobacteraeaceae, carbapenemases are diverse problem; these belong to molecular class B (IMP, VIM or NDM), class A (KPC) and class D (OXA-23 and OXA-48) enzymes as well as those combining AmpC enzyme or an extended-spectrum beta-lactamase (ESBL) with porin loss. These carbapenemases are involved in outbreaks in various geographical regions and are increasingly reported in sporadic cases worldwide [1-6]. OXA carbapenemases are responsible for middle level carbapenem resistance mostly among Acinetobacter spp. Previously, OXA-23, OXA-24/40, OXA-58 were responsible for middle level carbapenem resistance mostly among Acinetobacter spp. Previously, OXA-23, OXA-24/40, OXA-58 were reported in sporadic cases worldwide [1-6]. OXA carbapenemases are responsible for middle level carbapenem resistance mostly among Acinetobacter spp. Previously, OXA-23, OXA-24/40, OXA-58 were classified as acquired and OXA-51 was defined as chromosomal. However, in recent years, transmission of resistance via transferrable elements is shown [7,8].

In the present study, we investigated the occurrence of genes associated with the production of carbapenem hydrolysing carbapenemases in Klebsiella pneumoniae isolates recovered from inpatient at Hospital, a 1000-bed training hospital located in Istanbul.

Case Report

At the end of March 2010, a 31-year-old male suffering from Miller-Fisher syndrome was admitted to Neurology intensive care unit (ICU). Nine days after his admission, he suffered from ventilator-associated pneumonia. A strain of Acinetobacter baumannii was isolated from tracheal aspirate sample. The patient was treated with a broad spectrum beta-lactam and beta-lactamase inhibitor combination for Acinetobacter baumannii-related, ventilator-associated pneumonia. However, the patient on prolonged mechanical ventilation, another VAP was diagnosed caused by ESBL producing K. pneumoniae that was resistant to ertapenem was isolated from tracheal aspirate and blood cultures on the 25th day of stay. The patient was successively treated with colistin (10 days) for ertapenem resistant K. pneumoniae. He was discharged. In good health from the hospital 50 days after admission (Table 1).

Methods

Antibiotic susceptibility testing

The Klebsiella pneumoniae strain was identified by conventional and automated methods (VITEC2, bioMérieux France). Antibiotic susceptibility testing was performed by the disk diffusion method. The tested antibiotics were ampicillin, cefazolin, cefotixin, ceftriaxone, cefotaxime cefepime, ceftazidine, aztreonam, amoxicillin/ clavulanic acid, imipenem, ertapenem, meropenem, trimethoprim/ sulfamethoxazol, amikacin, tobramycine and ciprofloxacin. MICs of imipenem, meropenem, ertapenem and doripenem were determined using the agar dilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) [9]. ESBL production was determined by CLSI ESBL confirmatory test. Quality control testing was performed using Pseudomonas aeruginosa ATCC 27853, Escherichia coli ATCC 25922 and K. pneumoniae ATCC 700603.

Phenotypic characterization

Carbapenemases were phenotypically investigated by the modified Hodge test (MHT) using ertapenem and meropenem disks and by Metallo-beta-lactamase (MBL) Etest, a combined disk test using a meropenem (10 µg) and ertapenem (10 µg) disks which were supplemented with 292 µg of EDTA, 400 µg of aminophenylboronic acid (APBA) for metallo-beta-lactamases (MBL) and KPC [9-12]. ESBL production was determined by the double-disc synergy test.

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NOTE: M; male, VAP; ventilator-associated pneumonia.

(bromide at 120V for 60 minute.

by electrophoresis in a 1.5% agarose gel containing 0.5 mg/L ethidium

V517 as the standard marker.

E. coli

from

Escherichia coli J53 57th as the recipient strain. Mac Conkey and EMB agar plates supplemented with sodium azide (100 mg/L), ceftazidime (0.5 mg/L) and imipenem (0.125 mg/L) were used. Transformants were selected on Mueller-Hinton agar plates supplemented with 100 mg/L ampicilline, 2mM IPTG and 2% X-galactose and screened to harbor corresponding OXA-51 and CTX-M-15 genes multiplex PCR assay.

Plasmid analysis

Plasmid DNA extraction was attempted by the Kado Liu method [28]. Plasmid extracts were subsequently analysed by electrophoresis on a 0.7% agarose gel at 100 V for 4 hours using the plasmids extracted from E. coli V517 as the standard marker.

Transfer of resistance

Conjugation was performed using Escherichia coli J53 57th as the recipient strain. Mac Conkey and EMB agar plates supplemented with sodium azide (100 mg/L), ceftazidime (0.5 mg/L) and imipenem (0.125 mg/L) were used. Transformants were selected on Mueller-Hinton agar plates supplemented with 100 mg/L ampicilline, 2mM IPTG and 2% X-galactose and screened to harbor corresponding OXA-51 and CTX-M-15 genes multiplex PCR assay.

Results

K. pneumoniae isolate was resistant to all β-lactams including ertapenem, to all aminoglycosides, to fluoroquinolones and to trimethoprim/sulfamethoxazol (Table 2). MICs of carbapenems were as follows (mg/L) ertapenem 8, meropenem 1, imipenem 0.25 and doripenem 0.5. It was susceptible to tigecycline and colistin (MICs of ertapenem, imipenem and meropenem are greater than 0.5 and 2.0 mg/L, respectively may indicate isolates with carbapenemase resistance when standardized susceptibility testing methods are employed. Effective treatment and infection control depend upon the rapid and efficient identification of these isolates.

Carbapenemases do not always produce resistant breakpoints for carbapenems when standardized susceptibility testing methods are employed. Effective treatment and infection control depend upon the rapid and efficient identification of these isolates.

MICs of ertapenem, imipenem and meropenem are greater than 0.5 and 2.0 mg/L, respectively may indicate isolates with carbapenemase production (9). Ertapenem resistance is the most sensitive screening test to detect KPC and MBL carbapenemases. On the other hand, there are a number of phenotypic tests which have been developed to detect these enzymes in Gram negative bacteria. The Modified Hodge Test (MHT) has been developed to detect both KPC and MBL enzymes, but it cannot differentiate between them; moreover, there are reports of false-positive results with CTX-M-positive or AmpC-hyperproducing Enterobacteriaceae. Several versions of boronic acid, an EDTA disk test and Etest with imipenem/impinem with EDTA have been used for detections for MBL carbapenemases. However, no currently available test allows detection of CHDLs. In the present study, K. pneumoniae was found resistant to ertapenem (8 mg/L). The isolate demonstrated

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age (year)</th>
<th>Length stay</th>
<th>Infections due to A. baumannii</th>
<th>Day of isolation of A. baumannii</th>
<th>Day of isolation of K. pneumoniae</th>
<th>Infections due to Enteric bacteria</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>M</td>
<td>31</td>
<td>50</td>
<td>VAP</td>
<td>9</td>
<td>25</td>
<td>VAP</td>
</tr>
</tbody>
</table>

Table 1: Clinical features of patient and isolates.

Discussion

Reports in the United States and Europe point out the major problem caused by enteric bacteria with metallo-beta-lactamase (MBL) and KPC related carbapenem resistance [1,3-6]. The emergence and the spread of acquired carbapenem-hydrolysing class D beta-lactamases (CHDLs) have been well documented worldwide. There are nine major subgroups of OXA carbapenemases, based on amino acid homologies in Gram negative bacteria. Subgroups 1 (OXA-23-like), 2 (OXA-24-like), 3 (OXA-51-like) and 4 (OXA-58) frequently detected A. baumannii [29]. The plasmid-mediated OXA-48 enzyme, which forms the sixth subgroup, has been found primarily in K. pneumoniae, E. coli, C. freundii and E. cloacae [19, 30]. Among the genes encoding OXA-type carbapenemases; blaOXA-23, blaOXA-40 and blaOXA-58 found in A. baumannii and blaOXA-51 found in K. pneumoniae are plasmid-born, while OXA-51 may be naturally occurring in A. baumannii. However, a plasmid-born ISAb1-bla(OXA-51)-like gene has recently been identified in Acinetobacter genomic species 13TU and several A. baumannii isolates [7,8]. With regard to the mechanism of carbapenem resistance in Turkey, KPC gen was not detected in any of the studied isolates. In contrast, the genes encoding CHDLs (OXA-51, OXA-23, OXA-58) were detected in multidrug resistant Acinetobacter spp in several centers. In addition, OXA-48 carbapenemase is not rare especially among K. pneumoniae strains isolated from hospital infections [30].

Rapid identification of carbapenemase-producing, Gram-negative species is crucial for timely implementation of infection control measures. A previous survey carried out in our hospital in 2010, demonstrated that the blaOXA-23 and blaOXA-51 genes were prevalent enzymes associated with carbapenem resistance in multidrug resistant A. baumannii isolates. All were positive for the blaOXA-23-like genes as well (unpublished data). Data concerning carbapenem resistance among Enterobacteriaceae in our hospital, the percentage of ertapenem-resistant strains of K. pneumoniae was 10% in 2010. OXA-48-type oxacillinase was found responsible for carbapenem resistance.

Carbapenemases do not always produce resistant breakpoints for carbapenems when standardized susceptibility testing methods are employed. Effective treatment and infection control depend upon the rapid and efficient identification of these isolates.

MICs of ertapenem, imipenem and meropenem are greater than 0.5 and 2.0 mg/L, respectively may indicate isolates with carbapenemase production (9). Ertapenem resistance is the most sensitive screening test to detect KPC and MBL carbapenemases. On the other hand, there are a number of phenotypic tests which have been developed to detect these enzymes in Gram negative bacteria. The Modified Hodge Test (MHT) has been developed to detect both KPC and MBL enzymes, but it cannot differentiate between them; moreover, there are reports of false-positive results with CTX-M-positive or AmpC-hyperproducing Enterobacteriaceae. Several versions of boronic acid, an EDTA disk test and Etest with imipenem/impinem with EDTA have been used for detections for MBL carbapenemases. However, no currently available test allows detection of CHDLs. In the present study, K. pneumoniae was found resistant to ertapenem (8 mg/L). The isolate demonstrated
positive results in the ESBL, EDTA and APBA combine tests, and disk enzymatic assay. However, unexpected results were detected as PCR and sequencing revealed the presence of bla_{OXA-51} gene in K. pneumoniae strain for the first time which are known to be A. baumannii-related. The isolates also possess bla_{CTX-M-15} beta-lactamase gene. Acquired AmpC-type beta-lactamases and qnr genes were not found. It is known that OXA enzymes do hydrolyze penicillins, but do not hydrolyze expanded-spectrum cephalosporin. They are responsible for low or middle level carbapenem resistance mostly among Acinetobacter spp. and are not inhibited by clavulanic acid but are inhibited by NaCl in vitro. In the present study, K. pneumoniae strain was found to be susceptible to imipenem meropenem and doripenem on the basis of their MICs, which raises two issues: first, carbapenem-susceptible or decreased susceptibility and intermediate susceptible isolates may go clinically undetected during routine antibacterial drug susceptibility testing, second, the clinical efficacy of imipenem on such strains is uncertain.

OXA enzymes may be widespread rapidly among various Gram-negative bacteria. The dissemination of plasmids, transposons, and integrons among bacteria and species give rise to so-called gene epidemics. Integrons have a fearsome capacity for the recruitment, spread, and expression of resistance genes, and surveys show that they are widespread among gram-negative bacteria [6,7]. Chromosomal mediation of bla_{OXA-23} has been described previously for Proteus mirabilis [1].

In the present study, the patient firstly infected MDR-A. baumannii and treated with a broad spectrum beta-lactam and beta-lactamase inhibitor combination for A. baumannii-related, VAP. However, 15 days later, a etrapenem resistant K. pneumoniae isolate recovered from the same patient with VAP. We suggest that OXA-beta-lactamases remains difficult to detect by phenotypic tests, enhanced surveillance and rapid identification are essential. The ability to limit the spread of these pathogens will require effective laboratory screening methods to quickly identify patients infected with these organisms. Laboratories will need new tools, perhaps molecular techniques in order to make the process quicker and more accurate. It is highly significant to immediately introduce specific infection control measures in hospital settings in order to limit the nosocomial spread of these strains.

Table 2: MIC (mg/L) distribution of imipenem (IP), meropenem (MEM), ertapenem (ETP), doripenem (DOR) tobramisin (TM), cefotaxime (FX), amikacin (AK), ceftriaxone (CTX), amoxicillin/clavulanic acid (XL) ceftazidime (TZ), piperacillin/tazobactam (PTC), cefuroxime (CXM), ampicillin (AM), cefoperazone sulbactam (CPS), cefalothin (CT) ofloxacin (OF) for K. pneumoniae and transformant-38.

<table>
<thead>
<tr>
<th>MIC (mg/L)</th>
<th>K.pn-38</th>
<th>Transformant-38</th>
<th>E.coli DH10B</th>
<th>E.coli DH10B (PUC19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IP</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>MEM</td>
<td>1.00</td>
<td>0.23</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>ETP</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>0.094</td>
<td>0.047</td>
</tr>
<tr>
<td>DOR</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>1.5</td>
</tr>
<tr>
<td>TM</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>3/1.5</td>
<td>0.19</td>
</tr>
<tr>
<td>AK</td>
<td>4.00</td>
<td>4.00</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>TX</td>
<td>1.00</td>
<td>1.00</td>
<td>0.19</td>
<td>0.19</td>
</tr>
<tr>
<td>XL</td>
<td>2.00</td>
<td>2.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>TZ</td>
<td>4.00</td>
<td>4.00</td>
<td>1/4</td>
<td>1/4</td>
</tr>
<tr>
<td>PTC</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>AM</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
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<tr>
<td>CPS</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
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<tr>
<td>CT</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>OF</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

In conclusion, our report affirms the first detection of the OXA-51 harboring K. pneumoniae isolate and co-produced a CTX-M-15 beta-lactamase. As it has already mentioned above, due to the fact that OXA-beta-lactamases remains difficult to detect by phenotypic tests, enhanced surveillance and rapid identification are essential. The ability to limit the spread of these pathogens will require effective laboratory screening methods to quickly identify patients infected with these organisms. Laboratories will need new tools, perhaps molecular techniques in order to make the process quicker and more accurate. It is highly significant to immediately introduce specific infection control measures in hospital settings in order to limit the nosocomial spread of these strains.

References


