Investigated of ampC in Carbapenem Resistant Gram-Negative Bacteria Isolated from Burned Patients

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ABSTRACT

Background: Gram-Negative bacteria are the most cause of nosocomial infection especially in burned patients. Carbapenem resistant strains can limit seriously the choice of antibiotic therapy. AmpC can make resistance to carbapenem and detection of that can be useful for identification of carbapenem resistant. The aim of this study was identification of ampC in most prevalent cause of nosocomial infection.

Methods: boronic acid combined with meropenem in combination disc method was used for phenotypic method and PCR was used for molecular identification of ampC.

Results: Fifty one strains showed positive results in phenotypic method but 119 strains were harbored ampC gene. Combination disc method with meropenem and boronic acid showed 34.4% sensitivity and 78.5% specificity according to the results of this study.

Conclusions: the results of this study were indicated the more prevalent of ampC in carbapenem resistant Gram-Negative bacteria. On the other hand use of boronic acid combined with meropenem showed low sensitivity for detection of ampC.

Introduction

Pseudomonas aeruginosa, Acinetobacter baumannii and Klebsiella pneumoniae are being reported increasingly as the causative nosocomial infections which occur in several countries (1-5). Inappropriate antibiotic therapy and the overuse of broad-spectrum antibiotic have been selected antibiotic resistant bacteria (6). Antibiotic resistant can be association by different mechanism like production of different beta-lactamase enzymes and structurally modified antibiotic target, altered uptake of antibiotics (5, 7). One of the important mechanism of antibiotic resistance is the potential of extended-spectrum beta-lactamase (ESBL) and ampC β-lactamases (ampC) –producing (8-10). ampC b-lactamases are Group I cephalosporinases that can involve in resistance to a wide variety of b-lactam antibiotics include narrow and broad spectrum cephalosporins, aztreonam and in over expression of ampC with porin loss and/or efflux pumps, we can observe resistant to carbapenems (11, 12). One of the ampC properties is poorly inhibition of this enzyme by bata-lactamases inhibitor like clavulanic acid (13). Antibiotic resistance in these bacteria can be a serious threat for antibiotic therapy interventions. Infections caused by ESBL and ampC-producing, Gram-Negative bacteria are often difficult for clinicians to treat and can increase morbidity and mortality especially in hospitalized burned patients. So, detection of ampC-producer microorganisms especially in burned patients that were infected with these antibiotic resistant microorganisms can be helpful for appropriate antibiotic therapy of them. The aim of this study was detection of ampC gene in carbapenem resistant P. aeruginosa, A. baumannii and K. pneumoniae isolated from burned patients.

Bacterial isolations

This cross sectional study was conducted during 2013. One hundred sixty one P. aeruginosa (n=65), A. baumannii (n=63) and K. pneumoniae (n=33) were collected from burned wound infection of hospitalized patients from different wards in Motahari Hospital, a burn care teaching hospital in Tehran, Iran. The plate of bacteria sent to laboratory. These isolates were identified with conventional biochemical and microbiological tests. Like, oxidase, TSI, urea, SIM and citrate etc.

Antibiotic susceptibility testing

Confirmation of carbapenem resistant including imipenem (10µg), ertapenem (10µg) and meropenem (10µg) was done by disc diffusion agar method according to CLSI guide line (14). Standard antibiotic discs were prepared from MAST Company (Mast Diagnostics, UK).

Phenotypic detection of ampC

Two phenotypic tests were used for identification of ampC: The increasing inhibition zone at least 5mm around meropenem (10µg) plus 600 µg/disc boronic acid and meropenem (10µg) plus 750 µg/disc cloxacillin simultaneously in comparison to meropenem (10µg) alone. The stock of boronic acid was prepared 30mg/ml and the stock of cloxacillin was prepared 37.5 mg/ml (15).

Genotypic detection of ampC

PCR was used as a molecular test for detection of ampC gene with primer showed in table 1. The polymerase chain reaction was performed in following condition:

Material and method

60 seconds extension at 72°C for 60 seconds and at last the final extension at 72°C for 5 minutes. Isolates which showed specific band after PCR and electrophoresis have been sent for sequencing to Genfanavaran, Macrogen, and Seoul, Korea.

Result

<table>
<thead>
<tr>
<th>Primer AMP</th>
<th>Sequence (5’&gt;&gt;&gt;3’)</th>
<th>bp</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF/A.baumannii</td>
<td>ACTTTACTTCAA CTCGCGAGG</td>
<td>662</td>
<td>16</td>
</tr>
<tr>
<td>CR/A.baumannii</td>
<td>TAAACACCACA TATGTTCG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF/P. aeruginosa</td>
<td>ATGCAGCCAAC GACAAAAGG</td>
<td>1223</td>
<td>17</td>
</tr>
<tr>
<td>CR/P. aeruginosa</td>
<td>CACCCTCGCGA GCGCTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF/K.pneumoniae</td>
<td>ATTCGGGTAT GGCCGT</td>
<td>835</td>
<td>18</td>
</tr>
<tr>
<td>CR/K.pneumoniae</td>
<td>GGGTTTACCCT AACGCC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Carbapenem resistant of 161 tested isolates were confirmed by disc diffusion agar. Fifty one strains showed at least 5mm around meropenem with boronic acid in contrast with meropenem alone. On the other hand, 119 strains (60 P. aeruginosa, 55 A. baumannii and 4 K. pneumoniae) showed specific band after gel electrophoresis. The results of sequencing were confirmed the positive results of PCR assay. Nine strains with positive phenotypic test showed negative result in molecular method and 78 isolates with negative phenotypic test and positive PCR were observed (Table 2).

Combination disc method with meropenem and boronic acid showed 34.4% sensitivity and 78.5% specificity according to the results of this study. Bacterial strains and the percent homology between phenotypic and genotypic were showed in Tabel 3. Bacterial strains and the percent homology between phenotypic and genotypic methods.

Table 2. Results of phenotypic and genotypic ampc detection

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive phenotypic/negative genotypic</th>
<th>Negative phenotypic/positive genotypic</th>
<th>Positive phenotypic/positive genotypic</th>
<th>Negative phenotypic/negative genotypic</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. strains</td>
<td>9</td>
<td>78</td>
<td>41</td>
<td>33</td>
</tr>
</tbody>
</table>

Discussion

P. aeruginosa is the most common Gram-negative bacteria in nosocomial infections in burned patients (1-3) and A. baumannii is the second. K. pneumonia is one of the important enterobacteriaceae which can cause nosocomial infection. Thus, emergence of multi antibiotic resistant isolates led to therapeutic challenge for clinicians (1, 2). These Gram-Negative strains can produce variety type of beta – lactamases enzymes like ampc (1, 2, 4). ampc is one of important betalactamases can cause of resistant not only to cephalosporins but also can cause resistance to carbapenems (11, 12). In regards, sequencing results indicated the high prevalent of ampc – producer, more than 85% of P. aeruginosa and A. baumannii in this study. But we observed low prevalence with the rate of 12% in K. pneumonia according to sequencing results.

These results indicated that over production of ampc have important role in P. aeruginosa and A. baumannii. The results of this study can indicate that one of the most mechanisms in carbapenem
resistant strains which isolated from burned patients in Tehran. The results of study was conducted in Turkey in west-north of Iran were showed the prevalence of 8% ampC-producer K. pneumoniae isolated from blood culture (19). This prevalence is less than our results. This can related to K. pneumoniae were isolated from hospitalized burned patients in our study. In Pakistan that is in east – south border of Iran the rate of ampC – producer – K. pneumoniae was 12% (8). The results of study in China were showed 12% ampC – producer – K. pneumoniae (18). In another study that was conducted in India six out of 44 Klebsiella spp. (13.6%) were positive for ampC (21). These isolates were collected from different clinical specimens (21). The results of these three studies are similar to ours. Six of 22 K. pneumoniae (27.2%) were positive for AmpC in 2014 (22). These two studies (21, 22) were conducted in the same country but the frequency of K. pneumoniae – ampC producer is different between them. It can relate to the influence of antibiotic treatment and their pressure for selection of resistant bacteria can be different in different health care settings. In 2014, in the other study 16% of A. baumannii isolated from burned patients were identified as an ampC-producer strain (23). But in our study 87% of A. baumannii isolated from burned patients were positive strains for ampC. Another study in India and in 2014 was conducted on P. aeruginosa isolated from different clinical specimens. In that study none of the strains were ampC-producer. (24). However, we identified 92% ampC-producer in our tested P. aeruginosa. Of course, our strains were carabapenem resistant and this high prevalence of ampC-producer A. baumannii and P. aeruginosa can be reasonable. Generally, over use of broad spectrum antibiotics for treatment of infection and the pressure of antibiotic can lead to select more resistance bacteria and it can be reason of high prevalence of ampC-producer in A. baumannii and P. aeruginosa in our study. On the other hand, ampC-producer strains should be considered because of plasmid mediated ampC gene can transfer to other bacteria and make complication in treatment of patients (13).

Conclusion

The results suggest that anti-P. aeruginosa OprF antibodies elicited in burn wound sepsis model by active immunization are protective against infection with P. aeruginosa, and provide a rational for further development of the vaccine for prevention against P. aeruginosa infection in burn patients.

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Conflict of interest

None declared conflicts of interest.

References


