Characterization of beta-lactamases among MDR gram negative bacilli from a tertiary care hospital in central Kerala

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1. Introduction

The discovery of antimicrobial agents to prevent and treat infections is one of the most important developments of modern medicine. Evidence exists that antibiotic-resistance genes were present in the era before antibiotic therapy was available, and they probably originated from antibiotic-producing bacteria. In Gram negative bacteria, the antibiotic susceptibility patterns of pathogens from time to time and place to place has become matter of concern due to the emergence of beta-lactamase, extended spectrum beta-lactamases (ESBL), Amp-C beta-lactamases and Carbapenemases resistance worldwide.

These enzymes collectively can hydrolyze almost all beta-lactam drugs, which are used most frequently for the treatment of serious infections.

Globalization, rapid travel and vanishing borders may have helped the organisms find a safe place to travel via the human body. Uncontrolled infections and irrational use of antibiotics in the hospital are closely related to the emergence of resistant strains. Patients with unrecognized colonization with ESBL producers and Carbapenemase Producing Enterobacteriaceae (CPE) have served as reservoirs for transmission during outbreaks.

As a preventive strategy for arresting the transmission of
CPE, the US CDC recommends for all acute and long-term care facilities the following core measures: hand hygiene, contact precautions, patient isolation and dedicated staff, minimization of the use of invasive devices — particularly urinary catheter-s, promotion or reinforcement of antibiotic stewardship, and screening for CPE.

Recognizing the importance of drug resistance, WHO had selected combating antimicrobial resistance as the theme for World Health Day 2011. i.e. ‘Combat drug resistance: No action today, no cure tomorrow’. The present study was undertaken to find the proportion of different types of multidrug resistance mechanisms and factors associated with multidrug resistance in Gram negative bacilli, from Government Medical College, Thrissur which may have tremendous implications in the infection control and antibiotic policy of the hospital, because knowledge of the status of multidrug resistance in a geographical area is important in formulating the institutional antibiotic policy.

2. Materials and Methods

A prospective study was conducted in the Department of Microbiology, Government Medical College hospital, Thrissur during the period February 2012 to January 2013. The isolates included non-repetitive MDR Gram-negative bacilli recovered from different clinical specimens during the period of study. Biochemical identification of the isolates was done as per standard recommended procedures.

Antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method on Mueller-Hinton agar as per CLSI guidelines. The routine antibiotic discs used were Ampicillin (25 μg/30 μg), Gentamicin (10 μg), Amikacin (30 μg), Ciprofloxacin (5 μg), Cefalexin (30 μg), Cefotaxime (30 μg), Nitrofurantoin (300 μg), Cotrimoxazole (1.25/23.75). For non-fermenters, the antibiotic panel included Piperacillin (100 μg), Ceftazidime (30 μg), Ciprofloxacin (5 μg), Gentamicin (10 μg) and Amikacin (30 μg) (Hi-Media Laboratories Private Limited, Mumbai).

Those isolates which were resistant to three or more classes of antibiotics were designated as MDR and were further evaluated. A random 300 MDR E.coli, Klebsiella spp, Pseudomonas spp and Acinetobacter spp. isolates were selected for this study. A detailed history was taken regarding the risk factors associated with multidrug resistance using a proforma for each isolate. Those isolates resistant to third generation cephalosporins were considered as potential ESBL producers and phenotypic screening of the same was done by double disk synergy test (DDST) using Ceftazidime (30 μg), Cefotaxime (30 μg) and Amoxicillin-clavulanic acid (20 μg/10 μg) combination discs. To the lawn culture of the organism to be tested, ceftazidime and cefotaxime discs were placed 20mm centre to centre from amoxyccillin/clavulanic acid (20 μg/10 μg). A clear extension of the edge of the ceftazidime or cefotaxime inhibition zone toward the disc containing clavulanate was interpreted as synergy, in dicating the presence of an ESBL.

Some of the carbapenem resistant isolates were further checked for susceptibility to Colistin and Tigecycline by E-test.

3. Results

During the one year period, a total of 300 MDR Gram-negative bacilli isolates from various specimens received in the Department of Microbiology, Government Medical College hospital, Thrissur were included in the present study. The highest percentage of specimens in this study were from patients of age group 41-70 years constituting 54.66% (164/300), and mean age was 44 years. Male to female ratio was 1.34:1 (172:128), showing male preponderance in patients included in the present study. Out of 300 samples, 142 (47.3%) were urine, 44 (14.6%) were pus aspirates, 53 (17.7%) were pus swabs, 47 (15.66%) were respiratory specimens including sputum, tracheal aspirates and 14 (4.7%) were blood. The predominant MDR isolate obtained was E.coli constituting 56.7% of the total, and others included 21.7% Klebsiella spp, 11% Acinetobacter spp and 10.7% Pseudomonas spp. The details are given in the Figure 1.

![Fig. 1: Frequencies of MDR gram negative isolates](image)

In this study various risk factors associated with multidrug resistance were analyzed. 57.7% (173/300) patients had prior antibiotic usage, 29.4% (88/300) patients had previous hospitalization and 20.8% (62/300) had history of admission to ICUs. Immunosuppressive and interventional risk factors associated with drug resistance were also analyzed. Diabetes and chronic disease conditions affecting kidney, lung, liver and cardiovascular system...
predominated with 13% (39/300) and 8% (24/300) respectively. Steroid intake (14/300, 4.7%), chronic non healing ulcers (9/300, 3%), malignant conditions (7/300, 2.3%) and HIV patients (3/300, 1%) were the other immunosuppressive conditions. Among the interventions, 77.3% (232/300) patients had peripheral venous lines, 31% (93/300) had bladder catheterization, 28% (84/300) had history of blood transfusion, 21% (63/300) had recent surgery.

Among the 300 MDR GNBs, resistance patterns to various antibiotics were also analyzed (Figure 2). All the 300 isolates were resistant to Ampicillin, Cephalexin. Ceftriaxone resistance was 98.3% (295/300). Resistance percentage to Ciprofloxacin, Cotrimoxazole and Gentamicin were 95% (285/300), 90.3% (271/300), 87% (261/300) respectively. In urinary isolates Nitrofurantoin resistance was found to be 66.7% (200/300). Antibiotics Amikacin (37.7%, 113/300), Meropenem (16.3%, 51/300), Imipenem (13.66%, 43/300) showed minimum resistance among the 300 samples.

![Fig. 2: Antibiotic resistance pattern of MDR GNB isolates](image)

Multidrug resistance mechanisms screened in these MDR isolates were ESBL, AmpC beta lactamases and Carbapenemases. Their frequency is given in Table 1.

Distribution of these resistance mechanisms among the MDR isolates analysed. E.coli were the predominant organism with ESBL and AmpC Beta lactamases. Acinetobacter spp outnumbered Enterobacteriaceae in carbapenemase production. The details are given in Table 2.

Carbapenemase enzyme distribution varied among various specimens and departments in the present study. Isolates from 7% of total urine samples (10 out of 142), 22.7% of pus aspirates (10 out of 44), 18.86% of pus swabs (10 out of 53), 21.27% of respiratory specimens (10 out of 47) and 28.57% of blood (4 out of 14) were found to be carbapenemase producing. Specimens received from Medicine and Newborn ICU department were harbouring the highest number of carbapenemase producing isolates in the present study.

Out of 44 carbapenemase producers, 25 isolates were tested for Colistin susceptibility using E test. All the isolates were in the susceptible MIC range for Colistin according to EUCAST. Tigecycline susceptibility E test was tested on 20 carbapenemase producing isolates. The MIC of E.coli isolates was in the susceptible range constituted 30% (6/20) of the total carbapenemase producing isolates tested for tigecycline MICs whereas Acinetobacter spp. showed resistant (71%) and intermediate (29%) MIC values. The details are given in Table 3.

4. Discussion

The emergence of multidrug resistant (MDR) nosocomial pathogens, resistant to all currently available antibiotics has been recognized as a public health threat in recent times. The spread of these resistant strains coupled with the decline in the discovery and development of newer effective antibiotics over the last two decades poses a severe impact on our health care system by the depletion of most of the available therapeutic options for MDR bacterial infections. Currently there is renewed interest in the usage of polymyxins, as they are the only treatment option for these MDR and pan-drug resistant (PDR) Gram-negative infections.

To understand the resistance pattern in our hospital, 300 non-repetitive MDR GNB were screened for other mechanisms of antibiotic resistance like ESBL, AmpC beta lactamases and carbapenemase production along with their clinicoepidemiological details. According to Lortholary et al. and Masaeli Milad et al., the risk factors associated with multidrug resistance were found to be prior exposure to antibiotics, immunosuppressive conditions like DM, ICU hospitalization. Previous antibiotics and urinary catheter were identified as independent risk factors for MDR GNB acquisition in Gudiol et al. study. In the present study the findings related to risk factors were in accordance with the above authors’ data.

In the present study, by the initial screening technique the predominant resistant mechanism observed was due to ESBL production (56%, 168/300), followed by AmpC (24%, 72/300) and carbapenemase (14.6%, 44/300) production. Meyer & Picoli in 2011 in their study with fifty-eight bacterial isolates K. pneumonia with reduced susceptibility to third generation cephalosporins and/or cefoxitin detected a high frequency of ESBL (48.3%), followed by the AmpC plasmid/ESBL (15.5%), and two carbapenemase strains and the resistance frequencies were similar to the present study.

A study by Gudiol et al. also stated that the most frequent mechanism of resistance was extended-spectrum β-lactamase (ESBL) production (45%), mainly by Escherichia coli, followed by Amp-C cephalosporinase hyper production (24%). In the present study, E.coli constituted 73% (122/168) of the ESBL producers, 19% (32/168) by Klebsiella spp, 5% (9/168) by Pseudomonas...
Table 1: Results of phenotypically detected multidrug resistance mechanisms

<table>
<thead>
<tr>
<th>Beta-lactamase enzymes</th>
<th>No of isolates</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL</td>
<td>168</td>
<td>56%</td>
</tr>
<tr>
<td>AmpC</td>
<td>72</td>
<td>24%</td>
</tr>
<tr>
<td>Carbapenemases</td>
<td>44</td>
<td>14.6%</td>
</tr>
</tbody>
</table>

Table 2: Frequencies of MDR mechanisms among GNB

<table>
<thead>
<tr>
<th>Resistance mechanisms</th>
<th>E.coli</th>
<th>Klebsiella spp</th>
<th>Pseudomonas spp</th>
<th>Acinetobacter spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESBL</td>
<td>122(73%)</td>
<td>32 (19%)</td>
<td>9 (5%)</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>AmpC</td>
<td>33 (46%)</td>
<td>18 (25%)</td>
<td>11 (15%)</td>
<td>10 (14%)</td>
</tr>
<tr>
<td>Carbapenemases</td>
<td>9 (21%)</td>
<td>9 (21%)</td>
<td>8 (18%)</td>
<td>18 (40%)</td>
</tr>
</tbody>
</table>

Table 3: Frequencies of Tigecycline susceptible carbapenemase producing isolates

<table>
<thead>
<tr>
<th>Tigecycline MIC</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.coli (6)</td>
<td>6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acinetobacter spp (14)</td>
<td>-</td>
<td>4</td>
<td>10</td>
</tr>
</tbody>
</table>

spp and 3% (5/168) Acinetobacter spp. Similarly E.coli and Klebsiella spp were the predominant AmpC producers with 46% (33/72) and 25% (18/72) respectively in the present study.

According to Gaur et al\textsuperscript{23} and Wattal et al\textsuperscript{24} carbapenem resistance were found to be ranging from 6% to 57% in Acinetobacter spp. In the present study, carbapenemases constituted 14.6% (44/300) of the total MDR organisms screened and Acinetobacter spp predominated with 40% (184/44) of carbapenemase producing MDR bacteria, followed by 21% (94/44) E.coli, 21% (94/44) Klebsiella spp and 18% (8300) Pseudomonas spp.

Among the 44 carbapenemase producing organisms, fourteen Acinetobacter spp, six E.coli, five Pseudomonas spp, as a treatment option were tested with Colistin E tests. All the tested isolates were sensitive to Colistin. Acinetobacter spp and E.coli strains were also tested for Tigecycline susceptibility using E-tests, all the E.coli were in the sensitive range for Tigecycline while none of the Acinetobacter strains came in the sensitive range of MIC values. A study by Behera et al\textsuperscript{25} in 2009, tigecycline was found to be highly effective against Gram-positive bacteria (35/35) and Gram-negative members of Enterobacteriaceae (11/11), but a high prevalence of resistance was reported in members of Acinetobacter spp (20/26). According to Datta et al\textsuperscript{26} in a ten year analysis of multi-drug resistant blood stream infections caused by Escherichia coli and Klebsiella pneumoniae in a tertiary care hospital, tigecycline was introduced in the hospital formulary from 2007 and in the same year a resistance of 14 percent was observed which further increased to 20 per cent in 2009 in K. pneumoniae. However, in E. coli the resistance to Tigecycline remained 1.7 percent in 2008 and increased marginally to three per cent in 2009. All the isolates remained sensitive to Colistin. This suggests that Tigecycline may not be as effective as Colistin in treatment of carbapenem resistant isolates. In the battle against rapidly emerging bacterial resistance we can no longer rely entirely on the discovery of new antibiotics; we must also pursue rational approaches to the use of older antibiotics such as colistin.

5. Conclusion
The present study reveals a change in antibiotic susceptibility patterns of pathogenic Gram-negative bacteria and emerging newer drug resistance mechanisms in this tertiary care centre. Carbapenems were the only active antibiotics against many multidrug resistant Gram-negative pathogens, particularly those with extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes. The emergence and spread of carbapenemase-producing strains has become a major concern in health care institutions. So vigilance and timely recognition of infections with resistant bacteria and appropriate antibiotic therapy, is highly recommended. For integration of laboratory and clinical practice, auditing of antibiotic reports along with evaluating the impact of the report on treatment policy in the hospital could be a first step. Careful attention to barrier precautions and hand hygiene by all health care providers which can be monitored by Institutional infection control team can help in preventing the spread of these, multidrug resistant Gram-negative microorganisms.

6. Source of Funding
None.

7. Conflicts of Interest
There are no conflicts of interest.

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