Frequency of Extensively Drug-Resistant Gram-Negative Pathogens in a Tertiary Care Hospital in Pakistan

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Abstract

Background
Gram-negative bacteria are frequently involved in nosocomial infections. These bacteria have a particular tendency to develop antibiotic resistance and may become extensively drug-resistant (XDR). This study aimed to detect the prevalence of XDR Gram-negative bacteria in a tertiary care hospital in Pakistan.

Materials and methods
Clinical samples were obtained from patients admitted to different inpatient wards and sent for microbial analysis and culture. Antibiotic susceptibility testing of isolates was performed by the disk diffusion method to detect XDR strains.

Results
Antibiotic susceptibility patterns of a total of 673 clinical samples were studied. Of all bacterial isolates, 64% were extensively drug-resistant. Klebsiella pneumoniae had the highest percentage of XDR isolates (68.4%), followed by Pseudomonas aeruginosa (67.6%) and Escherichia coli (56.1%). Most XDR pathogens were isolated from the burn unit (87.7%), followed by the intensive care unit (69.2%) and surgical unit (68.9%).

Conclusions
The rate of extensive drug-resistance is alarmingly high, which calls for strict surveillance and control measures to prevent the development of further resistance. Proper sanitation and rational prescription of antibiotics should be ensured.

Introduction
Gram-negative bacteria and its subgroup Enterobacteriaceae constitute the normal intestinal flora in most human beings. These bacteria are mainly involved in nosocomial infections and have a particular propensity to develop antibiotic drug resistance [1]. This phenomenon has rendered treatment of these infections challenging, often resulting in increased morbidity and costs of healthcare. Research has identified various mechanisms by which Gram-negative bacteria develop resistance. These include extended-spectrum β-lactamases (ESBLs), carbapenemases, and other antimicrobial resistance genes (ARGs) that are circulated among bacteria via horizontal gene transfer or acquired through mobile genetic elements such as plasmids, integrons, and transposons [2].

Based on the patterns of antibiotic resistance, bacteria can be classified as multidrug-resistant (MDR), extremely drug-resistant (XDR), or pan drug-resistant (PDR). The Center for Disease Control & Prevention (CDC) and the European Center for Disease Control (ECDC) have developed standardized definitions of all these entities, which are now universally accepted [3]. According to these definitions, XDR bacteria are resistant to all but two or fewer antimicrobial categories. In other words, bacteria that remain susceptible to only one or two classes of antimicrobial drugs are extensively drug-resistant [3].

Extensively drug-resistant bacteria are a significant healthcare concern, with their incidence increasing worldwide. The World Health Organization has categorized ESBL-producing Enterobacteriaceae (XDR Enterobacteriaceae) and carbapenem-resistant Pseudomonas aeruginosa as ‘critical’ threats and has called for urgent development of new and effective antibiotic treatments against these pathogens [4]. XDR Gram-
negative pathogens pose a serious threat to the economy of developing countries like Pakistan, where rates of antibiotic resistance are much higher due to the excessive and non-judicial use of antibiotics [5].

Gram-negative bacilli are implicated in the majority of healthcare-associated infections (HAI) in our setting. Various studies have been done in the past to report resistance patterns, and reports from all over the country have provided evidence of increasing resistance of Gram-negative bacteria in Pakistan [6,7]. Strict surveillance of antibiotic resistance is necessary to be able to take timely measures and devise control strategies for the control of this significant health issue. Such surveillance is also essential to improve patient care, by influencing the choice of empirical antibiotics used as treatment or prophylaxis of infections [8]. Consequently, our study aims to recognize and appreciate the popularity of XDR pathogens in a tertiary care facility in Pakistan and to shed light on the magnitude of the radical problem of antimicrobial resistance faced by healthcare professionals.

Materials And Methods
This cross-sectional study was conducted at Holy Family Hospital, Rawalpindi, Pakistan, from July 2018 to January 2019. The study was approved by the Institutional Review Board of Rawalpindi Medical University (approval number: RSRS-2017-P-042).

Patients admitted to various inpatient wards who had undergone microbiological analysis of their clinical samples as part of their investigations during their hospital stay, were included in the study. Patients were included irrespective of their antibiotic status. Patients with polymicrobial infections or those with cultures positive for Gram-positive bacteria were excluded. Patient sampling was consecutive, and we included patients from the departments of medicine, surgery, gynecology, pediatrics, burn unit, and the intensive care unit (ICU). Samples collected from patients were blood, urine, pus, sputum, catheter tips, swab sticks, surgical, and burn wounds. These samples were sent to the microbiology laboratory of the hospital for microbial culture and sensitivity.

In the laboratory, specimens were cultured on suitable culture media, which include (but are not limited to) blood agar, MacConkey agar, and chocolate agar. After the identification of bacterial isolates, antibiotic sensitivity tests were performed by the disk diffusion method according to the Clinical Laboratory and Standard Institute criteria [9]. Bacteria were grown on Mueller Hinton agar, and their growth observed around antibiotic disks. Antibiotics used as the first-line for Gram-negative bacteria were ampicillin, gentamicin, cefazolin, tobramycin, co-trimoxazole, ceftazidime, and nitrofurantoin. Ampicillin-sulbactam, amoxicillin-clavulanate, amikacin, piperacillin-tazobactam, cefotaxime, cefepime, moxifloxacin, ciprofloxacin, imipenem, meropenem were used as second-line drugs. Aztreonam, tetracycline, and chloramphenicol were third-line drugs.

Extensively drug-resistant bacteria were defined according to the criteria described by the CDC [3]. Data were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (IBM Corp., Armonk, NY, USA). Frequencies and percentages of XDR bacteria were determined for all clinical isolates.

Results
A total of 673 isolates were included in this study. The general characteristics of the obtained samples including the patient sex, department of admission, and sample site are given in Table 1.
Sample Characteristics | Frequency | Percentage
--- | --- | ---
Patient sex | | 
Male | 366 | 54.4% |
Female | 307 | 45.6% |
Department | | 
Surgery | 280 | 41.6% |
Medicine | 197 | 29.3% |
Intensive care unit | 104 | 15.5% |
Burn center | 73 | 10.8% |
Gynecology and obstetrics | 11 | 1.6% |
Pediatrics | 8 | 1.2% |
Infectious sample site | | 
Infected wound | 241 | 35.8% |
Pus | 154 | 22.9% |
Catheter | 94 | 14.0% |
Urine | 80 | 11.9% |
Sputum | 25 | 3.7% |
Blood | 15 | 2.2% |
Others | 64 | 9.5% |

**TABLE 1: Basic characteristics of all clinical samples in the study (n = 673)**

Among all clinical samples, *P. aeruginosa* was the most common Gram-negative bacterium to be isolated followed by *Escherichia coli* and *Klebsiella pneumoniae*. This is represented in Table 2.

Organism Isolated | Total Frequency |  
--- | --- | ---
 | Frequency | Percentage
Pseudomonas aeruginosa | 257 | 38.2% |
Klebsiella pneumoniae | 196 | 29.1% |
Escherichia coli | 220 | 32.7% |

**TABLE 2: Frequency of various Gram-negative bacteria isolated (n = 673)**

Out of all 673 bacterial isolates, 431 (64.0%) were extensively drug-resistant. The rest 242 isolates were non-XDR (36.0%). Among the samples studied, *K. pneumoniae* had the highest rate of extensive drug resistance, with 68.4% (n = 134) of its isolates being XDR. It was followed by *P. aeruginosa* with 67.6% (n = 173) XDR isolates, and by *E. coli* with 56.1% (n = 124) of its isolates being extensively drug-resistant (Figure 1).
Figure 2 shows the proportions of Gram-negative XDR strains isolated from different clinical specialties. The highest percentage of XDR Gram-negative pathogens were isolated from the burn unit (n = 64, 87.7%), followed by ICU (n = 72, 69.2%) and the surgical unit (n = 193, 68.9%). 47.2% (n = 93) of isolates from the medical unit were XDR positive. From the department of gynecology and pediatrics, the rate of XDR Gram-negative bacteria was 63.6 and 25 percent, respectively.

The frequency of isolation of XDR Gram-negative bacteria from clinical isolates of various tissue sites was determined (Figure 3). Of all sites, wound infections were observed to have the highest rate of XDR pathogens (n = 185, 76.8%). This was followed by catheter infections, which were caused by extensively drug-resistant pathogens in 76.6% of the cases (n = 72).
FIGURE 3: Percentages of Gram-negative XDR pathogens among various isolation sites

XDR: extensively drug-resistant

Eighty percent isolates of *P. aeruginosa* obtained from catheters, and 79.4% of isolates from wound sites were extensively drug-resistant. Similarly, 79.6% isolates of *K. pneumoniae* obtained from catheter sites were XDR. For *E. coli*, the highest percentage of XDR isolates was obtained from non-conventional sites (72.2%). This is shown in Table 3.

<table>
<thead>
<tr>
<th>Site</th>
<th>Eschericia coli</th>
<th>Klebsiella pneumoniae</th>
<th>Pseudomonas aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>XDR</td>
<td>Non-XDR</td>
<td>XDR %</td>
</tr>
<tr>
<td>Blood</td>
<td>1</td>
<td>0</td>
<td>100%</td>
</tr>
<tr>
<td>Urine</td>
<td>23</td>
<td>35</td>
<td>39.7%</td>
</tr>
<tr>
<td>Pus</td>
<td>27</td>
<td>29</td>
<td>48.2%</td>
</tr>
<tr>
<td>Sputum</td>
<td>5</td>
<td>4</td>
<td>55.6%</td>
</tr>
<tr>
<td>Catheter</td>
<td>21</td>
<td>9</td>
<td>70.0%</td>
</tr>
<tr>
<td>Wounds</td>
<td>34</td>
<td>15</td>
<td>69.4%</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
<td>5</td>
<td>72.2%</td>
</tr>
</tbody>
</table>

TABLE 3: Incidence of XDR Gram-negative pathogens in various isolation sites

XDR: extensively drug-resistant

Discussion

The phenomenon of antimicrobial drug resistance is growing at an alarming rate, with the frequency of MDR and XDR bacteria increasing daily. In our study, we found that 64% of Gram-negative bacteria isolated from hospital settings were extensively drug-resistant (XDR). *K. pneumoniae* was the most frequently resistant pathogen, with 68.4% of its isolates being XDR. The highest percentage of XDR Gram-negative pathogens was from the burn unit (87.7%), and catheters and wound infections were the most common sites of XDR bacteria.

The results of our study are remarkable in many aspects. Firstly, we found the percentage of extensive drug resistance to be much higher than other studies in the past. In a study from Eastern India, the XDR Gram-negative load was found to be 41.3% [10]. Another study from 2016, that reported the prevalence of XDR
There are a couple of limitations to our study. First, a study done in a single healthcare center may not speak and only contributes to the phenomenon of increased resistance resistant bacteria should not be used as empirical therapy. It has also been found by recent studies that (audits) should be undertaken to ensure compliance by healthcare professionals. All patients should undergo implementation of these guidelines. Physician awareness programs and quality improvement projects strict guidelines for antibiotics use by hospital administration and policymakers and ensuring the

Our personal recommendations for the management of this problem include the development of strict guidelines for antibiotics use by hospital administration and policymakers and ensuring the implementation of these guidelines. Physician awareness programs and quality improvement projects (audits) should be undertaken to ensure compliance by healthcare professionals. All patients should undergo culture and sensitivity testing before antibiotics administration, and the use of antibiotics targeting resistant bacteria should not be used as empirical therapy. It has also been found by recent studies that empirical therapy targeting resistant species has no mortality benefit over the use of standard antibiotics, and only contributes to the phenomenon of increased resistance.

There are a couple of limitations to our study. First, a study done in a single healthcare center may not speak
for the general prevalence of XDR pathogens in the hospital setting. A multi-center study with a larger cohort and a longer duration of study would be required to achieve generalizable results. Second, we included only three species of Gram-negative bacteria in our study and did not account for other important bacterial species such as *Acinetobacter baumannii*, which is a notable cause of nosocomial infections. Laboratory testing for Acinetobacter is costly, and therefore, not routinely carried out in the majority of laboratories in our countries.

**Conclusions**

The percentage of extensively drug-resistant Gram-negative bacteria was found to be 64%, which is alarmingly high. Strict surveillance and control measures should be undertaken to prevent the development of further resistance, which will decrease treatment options to a minimum. Judicious use of antibiotics after sensitivity testing should be practiced in all healthcare centers, and resistance patterns of Gram-negative bacteria should be studied on a cellular and genetic level to develop newer therapeutic options against these pathogens.

**Additional Information**

**Disclosures**

**Human subjects:** Consent was obtained by all participants in this study. Institutional Review Board, Rawalpindi Medical University issued approval RSRS-2017-P-042. It is stated that we have reviewed the research proposal titled ‘Frequency of Extensively Drug-Resistant Gram-Negative Pathogens in a Tertiary Care Hospital in Pakistan’ and have found it ethically acceptable. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

**References**


