A Six-Month Survey of the Frequency of Extensively Drug-resistant Gram-Negative Bacteria by VITEK 2 System in 2020

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ABSTRACT

Background and Aim: Increasing of resistant bacteria is a major concern globally. The emergence of XDR gram-negative bacteria is a more serious problem due to treatment limitations. This study aimed to evaluate the frequency of extensively drug-resistant (XDR) gram-negative bacterial isolates in different clinical samples from Payvand Clinical and Specialty Laboratory, Tehran, Iran, for 6 months by VITEK 2 system.

Materials and Methods: During March 2020-September 2020, different clinical samples were collected from patients referred to Payvand Clinical and Specialty Laboratory. Bacterial identification and antimicrobial susceptibility test (AST) were performed applying an automated VITEK 2 system. The frequency of identified bacteria, their resistance to common antibiotics and also XDR bacteria were calculated and reported, respectively.

Results: Overall, 4125 urine specimens, 34 sputum samples, and 1 tracheal aspirate tube were submitted to Payvand Laboratory during 6 months. Of them, 486 urine, 32 sputum, and a tracheal aspirate tube samples were culture positive. Gram-negative isolated bacteria were included in this study. Based on AST, 63.3% of the isolated Klebsiella pneumoniae, 100% of Pseudomonas aeruginosa, and all Acinetobacter baumannii isolates were susceptible to amikacin and colistin. Totally, 31 XDR gram-negative bacteria, including: K. pneumonia (ssp. pneumonia (n=20), and ozaeae (n=2), Escherichia coli (n=3), P. aeruginosa (n=5), and A. baumannii (n=1) were identified from 18 urine samples, 12 sputum specimens, and a tracheal aspirate tube.

Conclusion: The rate of XDR bacteria was high in the investigated laboratory in this study. Therefore, accurate screening and antimicrobial stewardship is recommended in different medical centers of Iran.

Keywords: Automation, Drug resistance, Gram negative bacteria, antibiotics

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1. Introduction

Many pathogenic bacteria, such as Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus spp., and many members of Enterobacteriaceae, namely Escherichia coli, Klebsiella pneumoniae, and Proteus spp. are increasingly developing resistance (1, 2). Because of the importance of drug resistance, the World Health Organization named the year 2011 "Combat Antimicrobial Resistance" to warn about the increase in resistant bacteria worldwide. Such global challenge is elevating rapidly and is life-threatening (3-5). Therefore, different terms are used to categorize these bacteria, such as multiple drug-resistant (MDR), extensively drug-resistant (XDR), and pan-drug resistant (PDR) bacteria (6).

According to the Centers for Disease Control and Prevention and European Center for Disease Prevention and Control, MDR bacteria are resistant to the member of three or more antibiotic families. In addition, XDR is defined as the bacteria resistant to the...
members of all antibiotic families except one or two families, which are usually old antibiotics. PDR is usually referred to as *Mycobacterium tuberculosis*, which is resistant to all existing antibiotic families (1, 7). Various studies showed that the early detection of gram-negative infections is crucial because of their life-threatening role and the importance of starting rapid suitable antimicrobial treatment (8, 9).

**VITEK 2** is a rapidly fully automated system for bacterial identification and antimicrobial susceptibility test (AST). This system uses a fluorogenic method for bacterial identification and a turbidimetric technique for susceptibility testing using a 64-well card (10). The present study aimed to evaluate the frequency of XDR gram-negative bacteria isolated from different clinical samples in Payvand Clinical and Specialty Laboratory, Tehran, Iran in 2020.

### 2. Materials and Methods

#### Sample Collection

All samples, which were referred to Payvand Clinical and Specialty Laboratory for culture during March 2020-September 2020 (6 months), were included in the present study. The demographic data were submitted during sample collection. The information of all patients was kept private during data analysis and manuscript preparation. All the isolated microorganisms, including gram-positive and gram-negative bacteria, as well as isolated yeasts, were stored in a -70°C freezer in trypticase soy broth (TSB) with 15% glycerol. But based on the aims of this study only gram negative bacteria were included for further study and the remained were role out.

**Bacterial Identification and AST**

Bacterial identification and AST were carried out using an automated VITEK 2 system (BioMerieux, France). In this system, pure cultures are needed for bacterial inoculation preparation. Consequently, different media were used for isolation based on the clinical samples. Sheep blood agar and MacConkey agar (QUELAB, UK) were utilized for urine samples, and blood agar, MacConkey agar, chocolate agar, and Sabouraud dextrose agar (QUELAB, UK) were applied for sputum specimens. In addition, all the four mentioned media and TSB were used for tracheal tube culture (11).

All inoculated plates were incubated at 37°C for 24 h. Afterward, gram staining was performed, and using two or three pure colonies, a bacterial suspension was prepared by special PBS of the VITEK 2 system from each bacterial sample with turbidity equal to standard 0.5 McFarland (1.5×10⁸ CFU/mL). The OD of bacterial and fungal suspensions by VITEK 2 spectrophotometer must be in the ranges of 0.5-0.63 and 1.8-2.2, respectively. Further dilution was executed for AST based on the manufacturer’s protocol. Moreover, especial GP, GN, N240, GN76, AST-STO3, and AST-GP75 cards were used for microbial isolation and AST. According to the protocol, all the bacterial suspensions must be used 20 min after preparation. For quality control, standard ATCC bacteria, namely *E. coli* 25922, *K. pneumoniae* ATCC700603, and *P. aeruginosa* ATCC27853 were injected into the VITEK 2 system simultaneously with the clinical samples.

**Confirmation of ESBLs Production by Disk Synergy Test**

Flagged samples as extended-spectrum betalactamase (ESBLs) producers by VITEK 2 system were confirmed by manual double-disk synergy test (DDST). In order to complete the DDST, a bacterial suspension with 0.5 McFarland turbidity (1.5×10⁸ CFU/mL) was prepared and inoculated to Mueller-Hinton agar. A ceftazidime disk alone and a ceftazidime-clavulanic acid disk were placed by sterile forceps at a distance of 20 mm from the center. All plates were incubated for 24 h at 37°C. A difference of ≥ 5 mm between the diameter of the zone of inhibition around ceftazidime-clavulanic acid disk versus ceftazidime disk alone was reported as ESBL producer (12-14).

**Statistical Analysis**

The frequency of isolated XDR bacteria, as well as resistant and susceptible bacteria, was entered in an excel file and was reported after percentage calculation.

### 3. Results

In the current study, 4125 urine, 34 sputum, and 1 tracheal tube samples were submitted to Payvand Clinical and Specialty Laboratory. We found that 486 urine, 32 sputum, and 1 tracheal tube specimens were positive in culture. The isolated gram-positive and gram-negative bacteria, as well as *Candida* species are shown in Table 1. We included the gram-negative bacteria for further analysis in this study.

Two different microorganisms were isolated from eight sputum, four urine, and one tracheal tube samples. The isolated bacteria were *K. pneumoniae ssp. pneumonia* and *E. coli* from three of four urine samples, in addition to *E. coli* and *P. aeruginosa* from the remaining urine specimens. Furthermore, two isolated bacteria from eight sputum samples included *P. aeruginosa* and *C. glabrata* from sample one, *Stenotrophomonas maltophilia* and *K. pneumonia* from sample two, *C. krusei* and *K. pneumoniae* from sample three, *P. aeruginosa* and *K. pneumoniae* (ESBL+) from sample four, *P. aeruginosa* and *K. pneumoniae* from sample five, *E. coli* and *C. glabrata* from sample six, *K. pneumoniae* and *C. albicans* from sample seven, and *P. aeruginosa* and *K. pneumonia* from sample eight. *K. pneumoniae* and *P. aeruginosa* were isolated from the only tracheal tube. The isolated bacteria are listed in Table 1. The results of AST for all gram-negative isolates are demonstrated in Table 2.
Based on the results of VITEK 2 and manual DODT, 123/265 (46.1%) E. coli and 10/78 (12.82%) K. pneumoniae isolates were ESBL-positive, respectively. Moreover, we observed that 31 (7.43%) of gram-negative isolates were XDR, namely (n=18) K. pneumoniae subspecies, (n=3) K. pneumoniae ozaenae, (n=3) E. coli, 6 P. aeruginosa, and (n=1) A. baumannii. The results of AST are shown in Table 2.

Out of 18 isolated K. pneumoniae ssp. pneumonia, 11 and 7 were from urine and sputum samples, respectively. All 3 K. pneumoniae ssp. ozaenae were isolated from urine. In addition, 2 and 1 E. coli isolates were from urine and sputum specimens, respectively. It was found that 2 and 6 P. aeruginosa isolates were from urine and sputum samples, respectively. The only A. baumannii isolate was isolated from urine.

Table 1. Frequency of isolated bacteria in this study

<table>
<thead>
<tr>
<th>Isolated organisms/number</th>
<th>Frequency of gram positive bacteria</th>
<th>Frequency of gram negative Enterobacteriaceae</th>
<th>Gram negative non fermented bacteria</th>
<th>Candida spp.</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoNS</td>
<td>MRSA</td>
<td>MSSF</td>
<td>MR-CoNS</td>
<td>E. coli</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>9</td>
<td>11</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>E. coli</td>
<td>2</td>
<td>44</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>K. pneumoniae</td>
<td>5</td>
<td>82</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K. pneumoniae ozaenae</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>K. pneumoniae pneumonia</td>
<td>11</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
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<tr>
<td>K. pneumoniae ozaenae</td>
<td>7</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>E. coli</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>VRE</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>GBS</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>S. dysgalacteae</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td></td>
<td>369</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. The results of antimicrobial susceptibility test among XDR isolated bacteria

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>K. pneumonia ssp pneumonia</th>
<th>K. pneumonia ssp ozaenae</th>
<th>P. aeruginosa</th>
<th>E. coli</th>
<th>Acinetobacter spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>R</td>
<td>I</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Piperacillin/Tazobactam</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ceftriazone</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Cefepime</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Imipenem</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Meropenem</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
<tr>
<td>Sulfamethoxazole/trimethoprim</td>
<td>100%</td>
<td>-</td>
<td>-</td>
<td>100%</td>
<td>-</td>
</tr>
</tbody>
</table>
4. Discussion

The length of hospitalization and the rising cost of care during infection with resistant organisms, especially MDR organisms, is a global challenge (1,15). The severity of gram-negative infections is usually higher than gram-positive infections, such as bacteremia (16-18). In such situations, an immediate antimicrobial prescription is needed. However, the chance of empirical therapy, which covers most cases, is decreasing because of antimicrobial limitations (19).

In the present study, 4125 urine, 34 sputum, and 1 tracheal aspiration tube samples were submitted to Payvand Clinical and Specialty Laboratory for direct investigation. The AST was performed simultaneously with isolation using VITEK 2 system and special cards suggested antibiotics in the CLSI protocol should be tested. Similarly, in the current study, all CLSI suggested antibiotics for each isolated bacteria were tested. Likewise, in the current study, all CLSI suggested antibiotics for each isolated bacteria were tested. Moreover, they recommended improving the prevention criteria to inhibit the spreading of XDR bacteria.

Finally, they recommended improving the prevention criteria to inhibit the spreading of XDR bacteria.

It should be noted that sampling in both studies was performed only in one center. The frequency and variation of microbial isolates in the present study were higher than the mentioned research. According to the findings of AST, 52% and 100% of P. aeruginosa isolates were imipenem-resistant in the study conducted by Mirzaei et al. and the current study, respectively. In addition, 62.7% and 100% of A. baumannii isolates were MDR and XDR, respectively. Most samples were from the Burn Intensive Care Unit (ICU) (60.5%) and Burn Wards (20.4%). Moreover, they reported that 16.5% and 15.53% of P. aeruginosa isolates and 74.75% and 73.13% of A. baumannii isolates were MDR and XDR, respectively.

Based on the AST results, 46% of E. coli isolates and 12.82% of K. pneumoniae isolates were ESBL-positive. Furthermore, 31 isolates of gram-negative bacteria were confirmed as XDR, while no PDR was detected. Zhou et al. in 2019 reported that E. coli and K. pneumoniae might be the main gram-negative XDR bacilli (20). Similarly, E. coli and K. pneumoniae were the most frequently isolated gram-negative bacteria in the present investigation. The MDR and XDR gram-negative prosthetic joint infections were evaluated by Papadoulous et al. (21, 22). In their study, the prevalent gram bacilli were E. coli, P. aeruginosa, K. pneumoniae, and Enterobacter spp. However, in the current research, the detected gram-negative bacteria were more variable than the latter study. E. coli, K. pneumoniae, P. aeruginosa, and Enterobacter spp. were similarly reported as the most frequent isolated bacteria from different clinical samples.

In the study performed by Mirzaei et al., 3248 clinical samples were collected from the Burns Center of the Northeast of Iran. They observed that 309 cases were culture-positive, with 75 and 234 specimens being positive for P. aeruginosa and A. baumannii, respectively. Most samples were from the Burn Intensive Care Unit (ICU) (60.5%) and Burn Wards (20.4%). Moreover, they reported that 16.5% and 15.53% of P. aeruginosa isolates and 74.75% and 73.13% of A. baumannii isolates were MDR and XDR, respectively. It should be noted that sampling in both studies was performed only in one center. The frequency and variation of microbial isolates in the present study were higher than the mentioned research. According to the findings of AST, 52% and 100% of P. aeruginosa isolates were imipenem-resistant in the study conducted by Mirzaei et al. and the current study, respectively. In addition, 62.7% and 100% of A. baumannii isolates were MDR and XDR, respectively. Finally, they recommended improving the prevention criteria to inhibit the spreading of XDR bacteria.

According to Magiorakos A-P et al. study to investigate MDR and PDR bacteria, all or almost all suggested antibiotics in the CLSI protocol should be tested. Similarly, in the current study, all CLSI suggested antibiotics for each isolated bacteria were tested but no PDR (pan drug resistant) bacteria was detected.

Different studies showed that the rate of infections with gram-negative bacteria, including Enterobacteriaceae, A. baumannii, P. aeruginosa, and S. maltophilia is increasing in China and other countries (24-27). Furthermore, gram-negative bacteria were the most frequent bacteria in the ICU, neonatal ICU.
(NICU), and Cardiac Care Unit of Saudi Arabia hospitals. It was shown that A. baumannii was the most prevalent isolated gram-negative bacteria in this region followed by K. pneumonia, E. coli, and S. maltophilia (25-28). The emergence of XDR bacteria is a global challenge because of limitations in the treatment of these pathogens (25-31). However, E. coli and K. pneumoniae were the most frequently isolated resistant bacteria from a teaching hospital in Sri Lanka and a tertiary care hospital in Nepal (29, 31).

Finally, to understand the accurate frequency of XDR and PDR organisms, multicenter sampling is recommended in future studies. Sampling from only one clinical laboratory was the main limitation of this study. Moreover, we investigated XDR bacteria only among gram-negative bacteria. As a result, a similar evaluation of gram-positive resistant bacteria is also recommended. Such local assessments may determine whether any modifications to treatment guidelines are necessary.

5. Conclusion
The rate of XDR bacteria was high in the investigated laboratory in this study. Therefore, accurate screening based on a standard protocol, antimicrobial stewardship, and surveillance is recommended in different medical centers of Iran. In addition, to decrease antimicrobial resistance, the monitoring of MDR and XDR organisms in all clinical laboratories is recommended.

Acknowledgment
None.

Ethic approval
None.

Conflict of interest
There is no any conflict to declare.

Authors contribution
This study was done in Payvand clinical and Specialty laboratory (a private clinical laboratory), Tehran-Iran, under scientific supervision and management of Dr. Behzad Poopak. The idea and study design was done by Dr. Mojdeh Hakemi-Vala (Ph.D In medical bacteriology), Dr. Hadi Rezaei (Ph.D In medical bacteriology) and Dr. Behzad Poopak (Ph.D in hematology, Doctorate in Clinical Laboratory Sciences). Routine sampling was done based on the physician’s request and standard protocols. The practical parts including: bacterial isolation, identification and processing of Vitek 2 system was done by MS. Sepideh Ghasemshahi and Mr. Mohammad Ahmadpour under supervision of Dr. Hadi Rezaei, head of department of microbiology, Payvand Clinical and Speciality laboratory. All demographic and practical data registration including age, gender, background diseases and bacterial reports was done by Mrs. Aazam Booskabadi. Data analysis and draft preparation of the recent paper was done by Dr. Mojdeh Hakemi-Vala.

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Reference


