Original Article

Increasing Antibiotic Resistant Pattern in Clinical Bacterial Isolates, From Tertiary Care Hospital, Hayatabad Medical Complex, Peshawar, Pakistan

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ABSTRACT

Bacterial infections are spreading worldwide especially in the developing countries. Most clinical pathogens have evolved mechanisms of resistance due to which most antibiotics are less or not effective to restrict their growth. Objective: To find the prevalence of antibiotics resistance in clinical isolates. Methods: Total (n=753) clinical specimens were collected, among them, total (n=105) bacteria were identified on the basis of standard culture characteristics and biochemical tests and their antibiotics resistance pattern were determined Results: Higher incidence of multidrug resistance bacteria were found in patients aged above 50 years and were prevalent in OPD, emergency and gynae wards. The dominant bacterial species were gram negative, Escherichia coli (29%), Staphylococcus aureus (19%), Pseudomonas aeruginosa (13.33%), Acinetobacter species (5.71%), whereas, gram negative isolates were Staphylococcus epidermidis (9.52%), Streptococcus specie (5.71%), and Enterococcus faecium. Antibiotics like amoxicillin/ clavulanic acid, cefuroxime and sulphamatoxazole/ trimethoprim were resistant to 64.61%, 63.07% and 61.53% of gram-negative bacteria respectively while ciprooxacin, doxycycline and fusidic acid were resistant to 70%, 52.5% and 52.5% gram positive bacteria respectively. The most susceptible antibiotics against gram negative were sulbactum/ cefoperazone and amikacin while to gram positive were linezolid, chloramphenicol and rifampicin Conclusion: Current study revealed increasing antibiotic resistance pattern that need intimate focus on surveillance of antibiotics resistance regularly and to ensure long lasting efficacy of antibiotics.

INTRODUCTION

Efforts intended at identifying new antibiotics were once a top research and development primacy among pharmaceutical industries. The powerful broad-spectrum drugs that appeared from these accomplishments provided extraordinary clinical efficiency but success has been compromised. Now we are facing a long list of microorganisms that have shown resistance for many classes of drugs and are no longer susceptible to most, if not all, antibiotics [1]. The development and antibiotics usage have been one of the key scientific triumphs of the 20th century. The bacterial infections were considered to be under control during the early period of antibiotic usage [2]. At that time there were no alarm of cuts and infection, and various bacterial diseases, such as cholera and syphilis were considered on their way to eradication [3]. However, extensive antibiotics usage has upheld the antibiotic-resistant pathogens. Resistance spreading promptly, predominantly in hospitals, where different bacteria may come in close contact with each other and providing the environment for distributing the resistant genes with other bacteria [4,5]. Bacterial infections due to both gram positive and negative bacteria have caused huge causalities. Most gram negative pathogens such as Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter spp. make hindrance in treatment by developing different strategies [6]. The use of antibiotics in any environment produces selection forces that favor the survival of antibiotic resistant pathogens [7].
**METHODS**

**Collection of samples and specimens:** In this study total 753 specimen samples were collected from patients who were admitted or visited tertiary care hospital Peshawar. Clinical history, informative details and demographic features like age and gender of each patient were noted. Different clinical culture and sensitivity test samples were taken from urine, wound (pus) swab, sputum samples, blood etc. and were transported on ice bag and glycerol stock to the Department of Microbiology, Kohat University of science and technology for further analysis. This research was conducted from May, 2017 to October, 2017.

**Bacteriological analysis:** The samples were streaked on culture media for isolation and growth. Then all the samples except urine samples were streaked on MacConkey to differentiate between gram positive and gram negative bacteria and also to examine lactose and non-lactose fermenting bacteria. Urine samples were streaked on Cystine Lactose Electrolyte-deficient (CLED) medium. Blood agar plates were used to differentiate fastidious bacteria especially streptococcus species. Hemolysis were observed, alpha hemolysis showed, Streptococcus aureus while beta and gamma hemolysis indicated Streptococcus pneumoniae. All the cultures were incubated for 24 hours [8]. For morphological identification the isolates were grown on agar media and their growth patterns, shape and color were observed. Gram staining was performed to further differentiate bacteria based on staining and shape.

**Biochemical tests:** For biochemical characterization tests like catalase, triple sugar ion (TSI), motility, indole, urease and oxidase were performed and according to Bergey's Manual of systematic Bacteriology, on the basis of morphological, physiological and biochemical features, characterization was done[10].

**Antibiotic susceptibility patterns:** By using Kirby-Bauer disc diffusion method [11], following the recommendation of the clinical and laboratory standard institute the antibiotic resistivity of bacterial isolates was assisted. With addition of 5% sheep blood, Mullen-Hinton agar was prepared. A suspension of bacteria was made and then streaked on agar plate. Antibiotic disks were applied and then incubated at 37°C for 24 to 48 hours. The following antibiotics were used for gram positive: cefoxitin (FOX), erythromycin (E), clindamycin (CL), gentamycin (CN), vancomycin (VA), lineozolid (LZD), chloramphenicol (C), teicoplanin (TEC), amoxicillin/clavulanic acid (AMC), penicillin (PEN), ciprofloxacin (CIP), rifampicin (RD), fusidic acid (FD), doxycycline (DO), polymyxin-B (PB), and sulbactum/cefoperazone (SCF), colistin sulfate (CST), meropenem (MEM), Imipenem (IPM), minocycline (MIN), amikacin (AK), tigecycline (TGC), ceftriaxone (CRO), cefuroxime (CE), nitrofurantoin and fosfomycin were used. All these antibiotic disks were purchased from Oxoid, UK. After 48 hours of incubation, the diameters of zone of inhibition around the antibiotic disks were measured by using graduated ruler and interpreted the results according to CLSI guidelines[20].

**RESULTS**

**Isolation and distribution of bacteria:** The specimen wise distribution of the isolates was 29.5% (n=31) from pus, 38% (n=40) from urine and 32.3% (n=34) from swab samples. Gender wise distribution of the isolates were 67.6% (n=71) from male and 32.4% (n=34) from female while age wise distribution was 8.5% (n=9), 13.3% (n=14), 47.6% (n=50) and 30.4% (n=32) from <10 years, teenagers, adults and senior citizens respectively as shown in Table 1. While Table 2 shows the frequency of patients that were admitted at different wards like OPD (n=42), ENT B (n=3), emergency (n=38), PEADS (n=1), gynae A (n=8), gynae C (n=2), gynae OPD (n=3), ENT OPD (n=1) and endocrinology (n=2) at Hayatabad Medical Complex Peshawar.

**Table 1:** Distribution of the bacterial isolates based on specimen,
gender and age.

Essential oil and standard drug were tested against Staphylococcus aureus (*S. aureus*), Bacillus subtilis (*B. subtilis*), Pseudomonas aeruginosa (*P. aeruginosa*) and Escherichia coli (*E. coli*). Cumin essential oil showed significant antibacterial activity against both gram-positive and gram-negative bacterial strains. Standard drug data showed that it was effective against *S. aureus* and least against *E. coli* which is a resistant gram negative strain.

### Biochemical Tests

| Isolates            | Biochemical Tests |
|---------------------|-------------------|
|                     | G. Staining  | Catalase | Coagulase | Oxidase | H2S | Motility | I | U |
| *E. coli*           | -             | -        | +        | -       | +   | -        | + | + |
| *P. aeruginosa*     | -             | -        | -        | -       | +   | -        | + | + |
| *S. aureus*         | +             | -        | -        | +       | -   | -        | + | + |
| *S. epidermidis*    | +             | -        | -        | -       | +   | -        | + | + |
| *Streptococcus species* | +     | -        | -        | -       | +   | -        | + | + |
| *E. faecium*        | +             | -        | -        | -       | +   | -        | + | + |
| Enterobacter species | -             | +        | +        | -       | +   | -        | + | + |
| *P. mirabilis*      | -             | +        | +        | -       | +   | -        | + | + |
| Acinetobacter       | -             | baccilus | +        | -       | +   | -        | + | + |
| Providencia species | -             | Straight rods | - | - | + | + | + | + |

Table 3: Biochemical and Morphological characteristics of resistant bacterial isolates TSI, triple sugar iron, M, Motility, I, Indole, U, Urease

Among the total of 105 bacterial isolates, 61.90% (n=65) were gram negative and 38.1% (n=40) were gram positive. The biochemical tests are given in Table 3 used for the identification process. *E. coli* was the most frequently found gram negative bacteria whereas *S. aureus* was most frequently found gram positive bacteria. Overall frequency of *E. coli* (29%) was high, followed by *S. aureus* (19.4%), and *P. aeruginosa* (13.3%). Among less frequently found bacteria, the Acinetobacter specie (5.71%), Enterobacter, Coliform and Proteus mirabilis (3.80%) and Providencia species (2.85%) were gram negative whereas, *S. epidermidis* (9.52%), Streptococcus species (5.71%), and Enterococcus faecium (3.80%) were gram positive as shown in Table 4.

### Antibiotic susceptibility of Gram negative Bacteria

| Antibiotic | Sensitive % | Intermediate % | Resistance % |
|------------|-------------|----------------|--------------|
| CTX        | 32.3        | 0              | 58.46        |
| TZP        | 69.23       | 9.23           | 21.53        |
| CAZ        | 41.53       | 1.53           | 56.92        |
| CN         | 50.76       | 0              | 49.23        |
| SCF        | 70.67       | 10.76          | 18.64        |
| CE         | 36.92       | 0              | 63.07        |
| CT         | 50.76       | 24.61          | 24.61        |
| MEM        | 67.69       | 7.69           | 24.61        |
| AMC        | 27.69       | 7.69           | 64.61        |
| IPM        | 63.07       | 9.23           | 27.69        |

Table 4: Prevalence of Gram positive and Gram negative Resistant Bacterial isolates

**Antibiotics resistance profile of Bacterial isolates:**

Antibiotic susceptibility of gram negative and gram positive bacteria are shown in Figure 1 and Figure 2 respectively. The AK (73.84%) followed by SCF (70.6%) are most sensitive and then TZB (69.2%) and MEM (67.6%) were found effective antibiotics against Gram negative isolates shown in Table 5 and LZD (87.5%), C (85%) and RD (82.5%) were found effective antibiotics against gram positive isolates as shown in Table 4. The most resistant antibiotics against gram negative isolates were CTX (58.46%), CAZ (56.92%), SXT (61.53%), CE (63.07%), and AMC (64.61%) while against gram positive were SXT (47.5%), E (50%), DD (52.5%), CIP (70%) and FD (52.5%) as shown in Table 5 and 6 respectively.

![Figure 1](https://doi.org/10.54393/pbmj.v5i3.177)

**Figure 1:** Antibiotic susceptibility of gram negative isolates

**Figure 2:** Antibiotic susceptibility of gram positive isolates

| Antibiotics discs | Sensitive % | Intermediate % | Resistance % |
|-------------------|-------------|----------------|--------------|
| CTX               | 32.3        | 0              | 58.46        |
| TZP               | 69.23       | 9.23           | 21.53        |
| CAZ               | 41.53       | 1.53           | 56.92        |
| CN                | 50.76       | 0              | 49.23        |
| SCF               | 70.67       | 10.76          | 18.64        |
| CE                | 36.92       | 0              | 63.07        |
| CT                | 50.76       | 24.61          | 24.61        |
| MEM               | 67.69       | 7.69           | 24.61        |
| AMC               | 27.69       | 7.69           | 64.61        |
| IPM               | 63.07       | 9.23           | 27.69        |
**DISCUSSION**

Infectious diseases are the result of host invasion with a pathogen. The detection, treatment and prevention of human diseases are the challenges physicians, pharmacists and microbiologists are facing. In our total studied clinical samples 38% urine, 32% wound and 30% swabs samples were collected from patients. Similar specimen samples were also studied by Khurshid et al., (2002) [13] at Ayub Medical College, Pakistan. Gram negative pathogens were more prevalent in our study which is correspondence with a study conducted at tertiary general hospital China where 59% gram negative isolates were observed [14]. In our study the majority of infections are due to E. coli 29% followed by S. aureus 19.04%. In another study it is reported that S. aureusas 18.5% and E. coli as 16.7% isolated from nosocomial infection patients [15]. The most susceptible antibiotic against gram negative bacteria were AK and SCF while against gram positive were LZD, C and RIF. Similar studies on antibiotic susceptibility profiling were conducted by Sekhar, et al (2014) [16] in India where all gram positive isolates were sensitive to DO while gram negative to AK, SCF, and MEM and George et al (2018) [17] who reported IMP as most effective antibiotic against gram negative while VA and CL were effective against gram positive. Antibiotics are notorious to put selective pressure on antibiotic susceptible bacteria and boost the development of antibiotics resistant. It is therefore a possible reason of little disagreement with other studies [18]. Moreover, high antibiotic resistance rates were observed in gram negative bacteria against CE, AMC and SXT while in gram positive against CIP and DO. In a previous pilot study resistance against AMC was observed in gram negative isolates while against PEN to gram positive isolates [19]. The changes in bacterial resistance profile may be due to the close relationship of bacteria with each other and their easy acquisition of genes in different environments [20].

**CONCLUSIONS**

It is concluded that the most prevalent pathogens in our study were E. coli (29%), S. aureus (19.4%) and P. aeruginosa (13.3%) and higher incidence were observed in urine specimens, male and old age people. The AK and SCF in gram negative while LZD, C and RIF were found most susceptible while CE, AMC and SXT in gram negative and CIP and DO in gram positive were found most resistant antibiotics. So knowledge of the clinical, bacteriological finding as well as antibiotic susceptibility profiles are essential for choice of appropriate antibiotic with maximum effectiveness in correct management of the patient and to reduce the risk of complications.

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**Table 5:** Prevalence of gram negative antibiotic susceptible isolates

| Antibiotics | Sensitive % | Intermediate % | Resistance % |
|-------------|-------------|----------------|--------------|
| FOX         | 60          | 5              | 40           |
| E           | 45          | 5              | 50           |
| DO          | 40          | 7.5            | 52.5         |
| CN          | 55          | 2.5            | 42.5         |
| VA          | 77          | 12.5           | 10           |
| LZD         | 87.5        | 0              | 12.5         |
| C           | 85          | 5              | 10           |
| TEC         | 52.5        | 35             | 12.5         |
| AMC         | 55          | 2.5            | 42.5         |
| P           | 52.5        | 0              | 47.5         |
| CIP         | 27.5        | 2.5            | 70           |
| RD          | 82.5        | 0              | 17.5         |
| FD          | 47.5        | 0              | 52.5         |
| DA          | 60          | 10             | 30           |
| PB          | 47.5        | 2.5            | 5            |
| SXT         | 50          | 2.5            | 47.5         |

**Table 6:** Prevalence of gram positive antibiotic susceptible isolates

| Antibiotics | Sensitive % | Intermediate % | Resistance % |
|-------------|-------------|----------------|--------------|
| CIP         | 47.69       | 3.07           | 49.23        |
| MH          | 56.92       | 1.53           | 41.53        |
| AK          | 73.84       | 4.61           | 21.53        |
| TGC         | 56.38       | 4.61           | 40           |
| PB          | 66.15       | 4.61           | 29.23        |
| SXT         | 33.84       | 4.61           | 61.53        |
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