Comparison of the Antibacterial Activities of Different Antibiotics Against Clinical Isolates

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**Abstract**

Antibiotic resistance is the capacity of certain strains of bacteria to develop a tolerance to specific antibiotics to which they were once sensitive. **Objective:** To evaluate the antibiotic resistance by measuring the zone of inhibition in terms of sensitivity S, and resistance R. **Methods:** A total of 1000 clinical isolates collected from different samples were obtained. Antibacterial activities were evaluated by performing antibiotic susceptibility pattern of all clinical isolates against 20 commercial antibiotic discs. **Results:** A total of 1000 clinical isolates had been identified where Staphylococcus Aureus showed the highest prevalence (41.0%), next to Pseudomonas species (14.4%), Escherichia coli (13.1%), Enterobacter (6.4%), and Klebsiella species (3.1%). Conclusions: It is needed to maintain the appropriate antibiotic usage, prescription by evaluating the antibiotic susceptibility pattern and proper hand washing recommended.

**Introduction**

The rise of antibiotic resistance in bacteria is a major public health problem as infections from resistant bacteria are becoming ever more difficult and expensive to treat [1-4]. Excessive use of antibiotics had led to emergence of bacteria that can escape themselves from antibiotics, the so called antibiotic resistant bacteria (ARB) which commonly appeared in developing countries where antibiotics were frequently used. Antibiotic resistance is a natural adaptation and represents an evolutionary response to the strong selective pressure resulting from exposure to the antibiotics [5,6]. Antibiotic resistance had resulted in morbidity, mortality and increased health care crisis. There was an exceptional variability no longer only among the bacteria causing various clinical infections in one geographic region however also over time in specific region. Asia Pacific region had the maximum levels of antibiotic resistance among the five global regions of the universe [7]. Antibiotic resistance is the significant reason of mortality in both developed and developing countries as the abuse of antibiotics is prompting an antibiotic resistance around the
world. Yearly 50,000 deaths was assessed in the USA and Europe alone from antibiotic resistance among pathogens [8]. A major source of morbidity and mortality in Pakistan is the occurrence of bacterial infections with most prevalence of nosocomial infections. Nosocomial infections or healthcare associated infections occur in patients under medical care. All efforts to decrease hospital acquired infections are dying out by the continual increase in antimicrobial resistance (AMR) within the country [9]. This study will help to find out the recent scenario of prevalence of antibiotics resistance in different clinical isolates in Lahore. This will also assist to devise the procedures to deal with the increasing levels of antibiotic resistance.

**M E T H O D S**

This study was Cross-sectional, carried out in the Pathology Department of Fatima Memorial Hospital, Lahore, Pakistan. A total of 1000 samples (sputum, swabs, blood, urine, pus and etc) were collected, consisting of almost all types of samples such as blood, pus, swabs, sputum, urine, fluids and semen etc. Each sample was collected in a sterile container. The sample container was labeled with the details of source, date and time of collection and transported to laboratory for analysis within one hour of collection. Patients with any type of infection, both genders, without prior treatment were included while the patients having antibiotic treatment, children and pregnant women and without signs and symptoms of infection were excluded.

**Isolation and storage of samples**

After sample collection, samples were cultured on selective media plates (Mannitol Salt agar, TCBS Agar, Eosin thiazine Agar, MSA agar, MacConkey Agar, enteric bacteria enteric bacteria Agar) from the sample container. Then the plates were incubated for twenty-four hours at 37°C. After incubation, isolated colonies were observed and CFU/ml was calculated for a few of the plates and a few showed large growth. Then the colonies were streaked on agar plates to induce pure cultures for storage.

**Identification by Colonial Morphology**

Colonial morphology of the isolates was identified by their growth on MacConkey agar, Cystine-Lactose-Electrolyte-Deficient (CLED) agar and blood agar base. Isolated colonies had been used to study colony characteristics. Standard identification and susceptibility techniques were further applied for the identification of these organisms. In case of E. coli small, dry lactose fermenting pink color colonies on MacConkey agar, yellow dry smooth colonies on CLED agar and mostly dry but sometime mucoid haemolytic colonies were observed on blood agar. The Klebsiella species showed large, mucoid, lactose fermenting colonies on MacConkey agar while yellow smooth, mucoid, raised colonies on CLED agar and large mucoid colonies on blood agar. Flat, large hemolytic colonies on blood agar and non-lactose fermenting colonies with yellow green pigment on MacConkey agar were produced by Pseudomonas aeruginosa.

**Gram stain results**

Dark Purple color organisms were observed in case of gram positive bacteria and pink color organisms were observed in case of gram negative bacteria in gram stained smear under the oil immersion lens of microscope.

**R E S U L T S**

**Antibiotics Sensitivity**

The antibiotic sensitivity pattern indicated the effective antibiotics to control bacterial infection caused by clinical isolates. Antibiotics such as: Vancomycin (77.9%), Klaricid (58.0%), Fusidic acid (52.0%), Vibramycin (42.0%), Erythromycin (39.7%), Linezolid (32.8%), Oxicillin (17.2%), Meteronidazole (12.1%), Tazocin (10.0%), Nalidixic acid (7.1%), Ofloxacin (5.6%), Gentamycin (3.8%), Levofloxacin (3.6%). Norfloxacin (2.2%) and Gatifloxacin (1.1%) were most sensitive for gram positive isolates as illustrated in Figure 1. Antibiotics such as: Tazocin (75.1%), Meteronidazole (71.2%), Ciprooxacin (38.4%), Ooxacin (35.7%), Gentamycin (33.3%), Klaricid (26.4%), Levofloxacin (22.1%), Gatifloxacin (11.4), Vibramycin (6.7%), Vancomycin (6.0%), Erythromycin (4.0%), Fusidic acid (3.8%), and Linezolid (3.6%) were most sensitive for gram negative isolates as shown in Figure 2.

**Antibiotics Resistance**

Antibiotic resistance pattern revealed that the majority of the clinical isolates were resistant to several antibiotics. Various degree of resistance to Amoxicillin (46.2%), Cefotaxime (12.7%), Ciprofloxacin (4.0%) and Ampicillin (3.1%) was noted for gram positive isolates as shown in Figure 3. Widespread resistance to various antibiotics for gram negative isolates was noted as shown in . Various degree of resistance to Cefotaxime (21.2%), Oxicillin (14.2), Nalidixic acid (13.0%), Norfloxacin (12.3%) and Amoxicillin (4.5%) was seen for gram negative isolates.
Sensitivity and Resistance pattern of clinical isolates to antibiotics. The number of clinical isolates was 1000 out of 1000 samples with an infection rates 100%. This was quite higher compared to a other study by Mehta A et al which showed an infection rate of 20% [10]. Gender wise distribution of clinical isolates of positive cultures showed that the clinical isolates obtained from the male patients 553(55.3%) were more from the female patients 447(44.7%). In 2014 Khan et al, had also reported the greater percentage of clinical isolates in males (58%) than in females (42%) in Peshawar [11]. Similar finding were found in other study(). In Riyadh, Saudi Arabia, Baddour et al., had also reported the greater percentage of gram positive isolates in males (64.4%) than in females (35.6%) [12]. Out of 1000 clinical isolates, 450(45%) were gram positive isolates and 550(55%) were gram negative isolates. Gram negative bacteria dominated gram positive bacteria in our study. On evaluating the clinical isolates, staphylococcus aureus (41.0%) was found to be the commonest among gram positive isolates. Other gram positive isolates obtained from the samples tested were haemolytic streptococci (0.6%) and Actinobacteria species (0.6%). Among gram negative isolates obtained from the samples, Escherichia coli (13.1%) and pseudomonas species (14.4%) were found to be the most common. Other gram negative isolates were Klebsiella specie (3.1%), proteus species (0.9%) and Citrobacter species (0.2%). Similar findings were also found in other study [13]. Antibiotics which retained their effectiveness and showed high sensitivity to gram positive isolates in our research were Tazocin (75.1%), Meteronidazole (71.2%), Ciprooxacin (38.4%), Ooxacin (35.7%), Gentamycin (33.3%), Klaricid (26.4%), Levofloxacin (22.1%), Gatifloxacin (11.4), Vibramycin (6.7%), Vancomycin (6.0%), Erythromycin (4.0), Fusidic acid (3.8%), and Linezolid (3.6%). These results were similar to the findings of study conducted against gram negative bacteria [14]. Levofloxacin also had improved activity against gram negative bacteria [15]. According to a research done by khan et al [16] tazocin and meteronidazole were still effective against gram negative bacterial infections. In another study conducted by Saghir et al [17], Imipenem was the most effective antibiotic against gram negative bacteria. This is also in line with the study done by Sood et al [18]. The antibiotic resistance pattern had revealed that Cefotaxime (21.2%), Oxicillin (14.2) and Nalidixic acid (13.0%) were highly resistant to gram negative isolates. Similarly Norfloxacin (12.3%) and Amoxicillin (4.5%) were less resistant to gram negative isolates in our study. Most gram negative bacteria were resistant to Oxicillin and nalidixic acid which was due to beta lactamase activity by the bacteria. This was similar to the study done by Vlahovic et al[19] and Onwubiko.
et al., [20] who had denoted that cefotaxime and cephalosporin were the most misused antibiotics.

**Conclusions**

From the results of the current study it is concluded with no hesitation that there is alarming increase in resistance to nearly all the antibiotics with a very few exception of antibiotics used in this study.

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