AN EVALUATION OF THE RESISTANCE PATTERN OF DIFFERENT CLINICAL ISOLATES OF BACTERIA AGAINST CEPHRADINE AND CEFOTAXIME

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ABSTRACT
During the present study an effort has been made to determine the sensitivity of clinical isolates to accomplish this task, ninety clinical isolates of Escherichia coli (n=41), Klebsiella spp. (n=12), Proteus spp. (n=6), Pseudomonas aeruginosa (n=11), and Staphylococcus aureus (n=20) were collected from different pathological laboratories and medical center in Karachi.
An in vitro study of these clinical isolates were carried out by ICLS reference Disk diffusion (Kirby-Bauer) method, using Cephradine and Cefotaxime.
An attempt has been made to measured the Zone of inhibition produced by Cephradine and Cefotaxime against these clinical isolates.
Results indicated that both Cephradine and Cefotaxime has excellent in-vitro antibacterial activity, but Cefotaxime had relatively broad spectrum of activity against most of clinical isolates tested its mean that Cefotaxime is more effective against Gram-positive and Gram-negative bacteria.
On the basis of present study it can be evaluated that Cephradine and Cefotaxime were two very good antibacterial agent in the field of antimicrobial Chemotherapy but different clinical isolates had started to develop resistance to these antibiotics, which is shocking for prescriber because these two cephalosporins are massively prescribe for most of infections in our country.

Keywords: Cephradine and Cefotaxime

INTRODUCTION
Antibiotics active against bacteria are bacteriostatic or bactericidal, that is they either inhibit the growth of Susceptible organisms or destroy, harmful micro organisms, they are derived from special micro organisms or other living system and are produced on an industrial scale using a fermentation process, Although the principles of antibiotic action were not discovered until the twentieth century, the first known use of antibiotics was by the Chinese over 2,500 years ago, today, over 10,000 antibiotic substances have been reported.

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Antibiotics differ chemically so it is understandable that they also differ in the types of infections they cure and the ways in which they cure them.

On the basis of their mechanism of action, antibiotics are classified as:

1. Those that affect bacterial cell-wall biosynthesis, causing loss of viability and often cell lysis (penicillins and cephalosporins, bacitracin, cycloserine, vancomycin).
2. Those that act directly on the cell membrane, affecting its barrier function
and leading to leakage of intracellular components (polymyxin).

3. Those that interfere with protein biosynthesis (chloramphenicol, tetracyclines, erythromycin, spectinomycin, streptomycin, gentamycin).

4. Those that affect nucleic acid biosynthesis (rifampicin, novobiocin, quinolones)

5. Those that block specific steps in intermediary metabolism (sulfonamides, trimethoprim).

**Penicillins**

The penicillins are the oldest class of antibiotics, and have a common chemical structure which they share with the cephalosporins. The two groups are classed as the beta-lactam antibiotics, and are generally bactericidal that is, they kill bacteria rather than inhibiting growth.

**Cephalosporin**

The cephalosporins like the penicillins contain a beta-lactam chemical structure (i.e., a large family of broad-spectrum beta-lactam antimicrobial drugs). Since their introduction for clinical use in the 1960s, they have become widely used. In comparison with many older agents, the cephalosporins demonstrate low rates of drug-associated toxicity and favorable pharmacokinetic profiles. Cephalosporins are now used as first-line agents in the treatment of many infections, including pneumonia, meningitis, and gonorrhea. The "cepha" drugs are the most diverse classes of antibiotics and are themselves subgrouped in to 1st, 2nd, 3rd and 4th, Generations.

**Cephradine**

Cephradine is an oral 1st generation cephalosporin antibiotic. It inhibits the third and final stage of bacterial cell wall synthesis by preferentially binding to specific penicillin-binding proteins (PBPS) located inside the bacterial cell wall, so the bacteria die. Cephradine is used to treat bacterial infections of the respiratory tract, urinary to treat, skin, bone and joint.

The in-vitro antibacterial activity of cephradine includes excellent gram-positive but limit gram-negative coverage.

Gram-positive coverage includes non-pencillinase and pencillinase-producing *Staphylococci* (e.g., *S. aureus*) and *Streptococci* (except *Enterococci*). The gram positive spectrum is limited to some strains *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. Cephradine is inactive against *Enterococci* (e.g., *Enterococcus faecalis*), methicillin-resistant *Staphylococci*, *Bacteroides fragilis*, *Citrobacter spp.*, *Enterobacter spp.*, *Listeria monoytogenes*, *Proteus spp.*, other than *P. mirabilis*, *Providencia spp.*, *Pseudomonas spp.*, and *Serratia Spp.*

**Cefotaxime**

Cefotaxime is a member of the cephalosporin antibiotic class of drugs that has a rather wide spectrum of activity and is also useful as a pre-surgery antibiotic.

This medicine is used to treat serious infections caused by susceptible strains of microorganisms in lower respiratory infections, genitourinary infections, genitourinary infections, gynecologic infection, skin infection and central nervous system infections.

Cephalosporin antibiotics work by inhibiting bacterial cell wall biosynthesis and are active against a wide range of both Gram-positive and Gram-negative bacteria. A positive feature of these drugs is that they display a resistance to penicillinases and are useful to treat infections that are resistant to penicillin derivatives.

**MATERIALS AND METHOD**

To determine the resistance pattern of different clinical isolates of bacteria against Cephradine and Cefotaximine a total of ninety clinical isolates of different samples infection e.g., Ear Swab, Urine, Pus, Sputum and Blood
containing pathogen namely as *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were collected from different pathological laboratories and hospitals in Karachi. (Which were further identified by morphological and cultural methods and sensitivity was carried out). The present study was conducted with Cephradine and Cefotaximine disk.

Zone of inhabitation produced by antimicrobials against clinical isolates were determined by CLSI (formally NCCLS) reference disk diffusion (Kirby-Bauer) method.

**Preparation of inoculum**

The inoculum was prepared by touching the top of the colonies of the isolates with sterile wire loop and suspending in a tube containing broth with different isolates collected from pathological labs. All work was carried out near flame. Same procedure applied with all isolates. Then incubates these test tubes in incubator at 37 degrees centigrade for few hours until turbidity appears equal to McFarland turbidity standard.

**Preparation of media plates**

Nutrient agar was prepared and sterilized as instructed by manufacturer. Pour this media into sterile Petri dish about 20-25 ml per plate. Care must be taken to pour on the plates on a level surface so that the depth of medium is uniform. The plates are then set aside on a flat surface and allowed to solidify.

**Inoculation of cultures**

A sterile swab was used for this purpose. Sterile swab was dipped into a broth suspension of organism. Remove excess fluid by pressing and rotating the swab evenly over the surface of medium in three directions, rotating the plates approximately 60 degrees to ensure even distribution. After inoculation allow the surface of agar to dry.

**Placement of antibiotic disc**

By using sterile forceps, the appropriate antimicrobial disc of Cephradine and Cefotaxime were placed on the agar surface. Each disc should be lightly pressed down to ensure its contacts with agar. It should be moved once in place. The disc should be about 15mm from edge of plate and no closer to each other then 25mm from disc to disc.

**Incubation of plates**

Within 30min. of applying the disc, invert the plates and incubate at 37°C for 24 hours.

**Examination of plates**

After 24hrs.of incubation the plates were examined to ensure the growth. Measure the diameter of each zone of inhibition in mm.

**RESULTS AND DISCUSSION**

The purpose of the present study was to evaluate the resistant pattern of different infection causing organism including *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, and *Staphylococcus aureus* against Cephradine and Cefotaxime.

Resistance is a major factor limiting the long-term successful use of antimicrobial agent. In the preantibiotic era, many people died because of infection caused by such pathogens. Antibiotic have reduced the mortality from infectious disease but not the prevalence of these disease. It was not long after the clinical introduction of the first antibiotics in the 1950s that the first report of bacterial resistance began to appear. Use and often abuse or misuse of antimicrobial agents has encouraged the evaluation of bacteria toward resistance resulting often in therapeutic failure (Staut.).

Gram-positive cocci such as *Staphylococcus aureus* predominate as cause of nosocomial and community acquired infections. These organisms frequently reveal a high natural, intrinsic resistance to
antimicrobials. These bacteria are able to acquire resistance to frequently used drugs rapidly through selective pressure of the environment and via the genetic evolution of bacteria. *Staphylococcus aureus* are the most commonly isolates bacteria causing nosocomial infections, gram-negative bacteria such as *Escherichia coli* are most often resistant to antibiotics as a result of the acquisition of resistant genes or gene mutation (Siu LK).

The cephalosporins have a wide range of activity against many important Gram-positive and Gram-negative bacteria and are therefore of value for the treatment of serious infections and for the initial empirical therapy of undiagnosed infection in the current era, they have been developed over the post 46 years. The first generation agents developed in the 1960s have generally been replaced by the newer compounds, but still Cephradine have major share of market as this salt is ranked top 10 products in the last quarter of 2006 according to IMS in Pakistan. So, in the present study it was compared the antibacterial spectrum of this first generation Cephradine against third generation Cefotaxime.

A total of ninety clinical isolates of different infection's samples e.g., Ear swab, urine, pus, sputum and blood were collected from different pathological laboratories. Containing pathogen namely as *Escherichia coli*, *Klebsiella Spp.*, *Proteus Spp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* which are the most dominating cause of serious infection in our country.

They were identified and marked with code number. The antibacterial activity was carried out by disk diffusion method and the result were presented in table.

Out of ninety samples containing *Escherichia coli*, in this group the 13 samples showed resistant to Cephradine while only 4 samples showed resistant to Cefotaxime. The average zone of inhibition of Cephradine is 9.97mm, while average zone of inhibition of Cefotaxime is 17.78 mm. This shows remarkable antibacterial activity of Cefotaxime against Gram-negative *Escherichia coli* as compare to Cephradine.

Out of ninety containing *Klebsiella Spp.*, in this group the 5 samples showed resistant to Cephradine and 4 samples showed resistant to Cefotaxime. The average zone of inhibition of Cephradine is 12.27mm, while average zone of inhibition of Cefotaxime is 17.63mm. This shows both of these salts are active against *Klebsiella Spp.*, while Cefotaxime have broad spectrum as compare to Cephradine.

Out of ninety containing *Proteus Spp.*, in this group the only 1 sample showed resistant to Cephradine while none showed resistant to Cefotaxime, the average zone of inhibition of Cephradine is 11.5mm, while average zone of inhibition of Cefotaxime is 19mm.

<table>
<thead>
<tr>
<th>Name of organisms</th>
<th>Total no. of used clinical isolate</th>
<th>No of clinical isolate resistant to</th>
<th>Average diameter of zone of inhibition (mm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Cephradine</td>
<td>Cefotaxime</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>41</td>
<td>13</td>
<td>4</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>12</td>
<td>5</td>
<td>4</td>
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<tr>
<td><em>Proteus spp.</em></td>
<td>6</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>11</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><em>Staphylococcus aureus.</em></td>
<td>20</td>
<td>8</td>
<td>6</td>
</tr>
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</table>
inhibition of Cefotaxime is 21.8mm. which show both of these antibiotics have remarkable antibacterial spectrum against Proteus Spp., while zone of inhibition of Cefotaxime is tremendously high as compare to Cephradine.

Out of ninety containing Pseudomonas aeruginosa, in this group four samples of Cephradine showed resistant, while only one sample showed resistant to Cefotaxime. The average zone of inhibition of Cephradine is 14mm, while the average zone of inhibition of Cefotaxime is 19 mm. which show both of these antibiotics have good antibacterial spectrum against Pseudomonas aeruginosa and no remarkable difference in zone of inhibition of Cephradine and Cefotaxime was noted.

Out of ninety containing Staphylococcus aureus, in these group eight samples of Cephradine showed resistant, while six samples showed resistant to Cefotaxime. The average zone of inhibition of Cephradine is 12.65mm, while the average zone of inhibition of Cefotaxime is 15.12mm. Which show both of these antibiotics have almost same antibacterial spectrum against Staphylococcus aureus.

The results of present study are in confirmation with the work of Ahmed M et al has reported Cefotaxime is highly effective against Gram-positive and Gram-negative bacteria among the Cephalosporins. Cefotaxime was active against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa.

Hence the microorganisms are developing resistance to Cefotaxime and Cephradine and thus resistance is increasing which is shocking for prescribe because these two Cephalosporins are massively prescribe for most of infections in our country. While in the illumination of present study it is concluded that Cefotaxime have excellent spectrum against Gram-positive and Gram-negative bacteria, while Cephradine is good choice of drug among the antibiotics for Gram-positive infections but careful when clearly diagnose as Gram-negative infection in the patient.

Hence this study will be very useful for the Pharmacist and Physicians for prescribing.

REFERENCES


