In vitro bactericidal activity of cefepime and cefpirome against clinical isolates at Karachi

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Graphical abstract

Abstract: Antibiotics not only support to alleviate the infections but also facilitate to avert the multiplication of microbes. Due to the irrational use of antibiotics, the resistance of antibiotics has been augmented which results may increase in morbidity and mortality with the span of time. World renowned regulatory bodies like Food and Drug Administration (FDA), Center of Disease Control and Prevention (CDC), and World Health Organization (WHO) vigorously advocate the surveillance of the resistance of antibiotics. During the present study by Kirby-Bauer disk diffusion method 141 clinical isolates of Staphylococcus aureus (n=47, 33.34%), Escherichia coli (n=54, 38.3%), Proteus species (n=26, 18.4%), and Klebsiella pneumoniae (n=14, 9.92%) are evaluated against cefepime and cefpirome which comes of fourth generation cephalosporin. It has been found that cefpirome has better bactericidal activity than cefepime against E. coli and K. pneumoniae while cefepime has been possessed better antibacterial activity against S. aureus and Proteus species which were isolated from respiratory tract infections, blood stream infection, intra-abdominal and urinary tract infections, and skin and soft tissue infections. K. pneumoniae, E. coli, Proteus species, and S. aureus were 34.8%, 26.3%, 11.3%, and 37.7% resistance against cefepime respectively. S. aureus, E. coli, K. pneumoniae, Proteus species has shown 41.4%, 21.7%, 17.6%, and 8.9% resistance against cefpirome correspondingly.

Keywords: Cefepime, cefpirome, clinical isolates, in-vitro, susceptibility.

INTRODUCTION

Cephalosporins are belonging to beta-lactam antibiotics with 6-membered dihydrothiazine ring, thus forming the cephem nuclei. Cephalosporins have superior efficacy with tolerability as compared to other group of antibiotics. Persons are less hypersensitive with cephalosporins as compared to other beta-lactams like penicillin and its derivates. Cephalosporins are generally classified into four generations (Harrison and Denise, 2008). Two new fifth generation cephalosporin ceftaroline (PPI 0903, formerly TAK-599), and ceftobiprole (BAL 9141) is
under-trial with extended-spectrum cephalosporin activity against clinically important Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and *Enterococcus faecalis* (Noel et al., 2008; Saravolatz et al., 2011).

Bactericidal activity of antibiotics is mainly depends upon the concentration of drug in plasma and tissue. Microbes modify their traits to the antibiotics and convey their resistance qualities to their descendents (Craig, 1998). The resistance traits are developed due to suboptimal, irrational and extensive use (Knox et al., 2003), low dose antibiotics (Craig, 1998), long course of antibiotics (Seet-Tia and Chongsomchai, 2006), too early and too late administration (Sadique et al., 2009). It has been suggested by many workers that low concentration of antibiotics is one of the influential factor to enhance the resistance (Fantin et al., 1994; Mouton and den Hollander, 1994; Davies et al., 2000). The dosing interval for beta-lactams have been re-evaluated to keep plasma levels above distinct thresholds for prolong period of time (Crokaert, 2001). Moreover, since last two decades the use of broad spectrum antibiotics has been increased due to many factors like reduced drug expenditures, decreased drug interactions and risk for toxicity and other pharmacoeconomic benefits (Isais-Agdeppa and Bravo, 2005).

Due to good antibacterial activity even against resistant pathogens, mainly fourth generation zwitterionic cephalosporin, cefepime and cefpirome is indicated as empirical monotherapy for complicated urinary tract infections (UTIs), pneumonia, skin and soft tissue infections, intra-abdominal infections and febrile neutropenia (Endimiani et al., 2008).

Cefepime and cefpirome, fourth generation cephalosporins with certain benefits over the third generation cephalosporins and other penicillin derivatives like more resistant against beta-lactamase, extended spectrum of activity that includes many Gram-positive and Gram-negative bacteria, activity against multi-resistant bacteria and induction high potency, rapid penetration into the periplasmic space (Wynd and Paladino, 1996). Cefepime and cefpirome are the drugs of choice in life threatening nosocomial infections, febrile neutropenia, septicemia, and serious infections in patients of intensive care units (ICU) due to its high resistance against beta-lactamases. Cefepime and cefpirome have been described in some reports as less prone than other cephalosporins to hydrolysis by ESBLs and/or upregulation of efflux pumps (Wynd and Paladino, 1996; Angelescu and Apostol 2001). The pharmacokinetic profile of cefepime and cefpirome has been shown in table 1.

The most common adverse effects associated with these two cephalosporins are same like rash and diarrhea, pruritus, urticaria, nausea, vomiting oral candidiasis, colitis, headache, fever, erythema and vaginitis (Sagawa et al., 2000; Chapman and Perry, 2003). Moreover, elevated liver function, blood urea nitrogen, or partial thromboplastin and prothrombin times has also been observed. Similar to any parenteral beta-lactam drug, transient neutropenia or thrombocytopenia may also be occurred with cefepime (Wynd and Paladino, 1996).

In low socioeconomic countries like Pakistan, around 80% of population has not been facilitated to avail antibiotic susceptibility test. The physicians have been usually prescribed more than one antibiotic, leading cause of antibiotic resistance (Ullah et al., 2009).

**MATERIALS AND METHODS**

**Collection of specimens**

One hundred and eighty nine clinical isolates belonging to different genera like *Staphylococcus aureus*, *Escherichia coli*, and *Proteus* and *Klebsiella* species were collected on sterile swabs from different patients as shown in table 2 and different tertiary care hospitals and pathological laboratories at Karachi from May 2012 to March 2013. The isolates were identified based on their colony characteristics on different media and confirmed by biochemical reactions. The isolates were inoculated in case agar/ tryptic soya agar slants. These slants had been preserved at 4°C in the refrigerator. Antimicrobial resistance (AMR) has been determined by Clinical and Laboratory Standard Institute (CLSI, formally NCCLS) reference disk diffusion (Kirby-Bauer) method (Bauer et al., 1966; CLSI, 2011).

**Preparation of inoculums**

Muller-Hilton Broth (MHB) was used to prepare inoculums and matched with McFarland standard. All tubes were incubated at 37°C for few hours to develop the required turbidity as that of the McFarland standard. Muller- Hilton Agar (MHA) was used to determine the sensitivity of clinical isolates. Bauer, Kirby, Sherris and Tuck strongly suggested MHA for performing antibiotic
susceptibility tests using a single disk of high concentration (Bauer et al., 1966). This unsupplemented medium has been preferred by the Clinical and Laboratory Standard Institute for various reasons (CLSI, 2011).

**Inoculation of bacterial culture**
A sterile swab was dipped into a broth suspension of bacterial culture. Excess inoculum was removed by rotating the swab against the inside wall of the tube with slight pressure. The whole surface of MHA plate was then streaked uniformly in three directions approximately at 60° angle from each other. The lid was then replaced and the plates were allowed to dry for 10-15 min.

**Placement of antibiotic disc**
The appropriate antibiotic impregnated discs were placed on the agar surface with sterile forceps. Each disc was pressed down gently with the forcep to assure good contact with agar surface. The disc should be distributed such that each is at least 24 mm from center to center of its nearer neighbor and 12 mm from the edge of plate.

**Incubation**
The plates were overturned within 15 min of placing the disc on agar and incubated at 35-37°C for 24 hours. After incubation the diameter of the clear zones around the antibiotic disc were measured by using vernier caliper. All the bench work was carried out near a flame to create a zone of inhibition of invading bacteria and maintained the integrity.

**RESULTS**
In the present study, cefepime and cefpirome are belonging to fourth generation cephalosporin. These fourth generation cephalosporins were evaluated against the most common pathogens isolated in infections like *S. aureus*, *E. coli*, and *K. pneumoniae*. These clinical isolates has been mainly involved in various infections like respiratory tract infections, blood stream infection, intra-abdominal and urinary tract infections, burn infections, wound infection and surgical site infections (Wiseman and Lamb, 1997; Angelescu and Apostol et al., 2001; Witte et al., 2008).

One hundred and forty one clinical isolates were collected from different clinical laboratories of tertiary care hospitals at Karachi. Among these clinical isolates mostly *Escherichia coli* (38.3%) was collected, secondly its contender was *Staphylococcus aureus* (33.34%) while *Proteus* species (18.4%), and *Klebsiella pneumoniae* (9.92%) were also isolated.

The outcome susceptibility of clinical isolates has been shown in table 3 and graphical figure. Both cefepime and cefpirome discs contain 30µg content. By the present study, it has been found that *E. coli* and *K. pneumoniae* and *Proteus* species were more susceptible against cefpirome while cefepime has shown better bactericidal activity against *S. aureus*. *S. aureus*, *K. pneumoniae*, *E. coli*, and *Proteus* species were 37.7%, 34.8%, 26.3% and 11.3% resistance against cefepime respectively. *S. aureus*, *E. coli*, *K. pneumoniae*, *Proteus* species were 41.4%, 21.7%, 17.6%, and 8.9% resistant against cefpirome.

**DISCUSSION**
*In vitro* bactericidal action has been commonly performed, because provide an idea of resistance of antibiotics against pathogens inside the body (Jones and Preston, 1983). Bacteria has been transferred their resistant traits to their new generations, which is not a hidden iceberg for health-associated professional but also for the pharmaceutical companies. Microorganisms are present everywhere either in soil, water, food, air and even in and on human (Chong et al., 2010; Arsalan et al., 2010; Arsalan et al., 2013a; Arsalan et al., 2013b; Arsalan et al., 2013c). The unreasonable use of antibiotics has been one of the major factor, amplified the probability of resistance in human beings (Arsalan et al., 2014).

Cephalosporins, belonging to β-lactam antibiotics, are one of the most frequently prescribed antibiotics. Still four generation of cephalosporins are marketed by pharmaceutical companies in Pakistan. Due to its better tolerability, durability, and excellent pharmacokinetic profile, it is one of the most trusted class of antibiotics. Several pathogens have been produced Extended-Spectrum Beta-Lactamase (ESBL) enzyme which aid in the resistance against bactericidal activity of cephalosporins. Ceftaroline and ceftobiprole are the novel broad spectrum cephalosporins, have been soon introduced in market due to the trustworthy profile of cephalosporins (Lemaire et al., 2009; Biek et al., 2010).

Due to illogical use of antibiotics, resistance has been increased. Microorganisms have been transferred their resistance traits to their next generation. Globally, it has been observed that Gram-positive are the main concerned in infections, the infections caused by pathogens can also be reduced by preventive measurements (Arsalan et al., 2010; Arsalan et al., 2014). It has been noted 60-70% reported infections have been related to Gram-positive. However, Gram-negative pathogens prominently *E. coli* and *K. pneumoniae* and *Proteus* species have been caused several life threatening infections (Isais-Agdeppa and Bravo, 2005). Antibiotics resistance of pathogens has been increased by long-term care, and hospital settings, which has been possessed severe problem in the choice of an appropriate antibiotic for proper treatment (Witte et al., 2008; Gaynes and Edwards 2005).
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Table 1: Pharmacokinetic Profile of Cefepime and Cefpirome

<table>
<thead>
<tr>
<th>Drug</th>
<th>Half-Life</th>
<th>Steady State C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Volume of Distribution</th>
<th>Serum Protein Binding</th>
<th>Mean Renal Clearance (ClR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>1.3 to 1.9 hours (Blumer et al., 2001)</td>
<td>50-mg/kg intravenous regimen was 177 mg/l for every 12 h and 188 mg/l for every 8 h. (Blumer et al., 2001)</td>
<td>18.0 ± 2.0I (Blumer et al., 2001)</td>
<td>Approx. 20% (Barbhaiya et al., 1992)</td>
<td>105 ml/ min (Barbhaiya et al., 1992) 85% excreted unchanged in urine (Wynd and Paladino, 1996)</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>1.7 to 2.3 hours (dexa medica, 2013)</td>
<td>Value of C&lt;sub&gt;max&lt;/sub&gt; between 86.7 and 97.4 mg / L after administration of a single IV injection at a dose of cefpirome 1 g and between 23.2 and 30.6 mg / L 2 hours after administration of IM with the same doses (cefpirome 1 g) (dexa medica, 2013)</td>
<td>Volume of distribution at steady state is between 15.3 and 21.3 L (dexa medica, 2013)</td>
<td>Approx. 8-12% (dexa medica, 2013)</td>
<td>Total renal clearance is approximately 6.6 to 10.6 liters / h and renal clearance rate ranged from 4.9 to 6.7 liters / hour (dexa medica, 2013)</td>
</tr>
</tbody>
</table>

Table 2: Summary of clinical isolates

<table>
<thead>
<tr>
<th>Clinical Isolates</th>
<th>Source of isolates</th>
<th>Number of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Surgical, burn and accidental wound pus (skin and soft tissue infections), blood (blood stream infection), sputum (respiratory tract infections)</td>
<td>47</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Stool and urine (intra-abdominal and urinary tract infections), blood (blood stream infection), surgical, burn and accidental wound pus (skin and soft tissue infections)</td>
<td>54</td>
</tr>
<tr>
<td><em>Proteus species</em></td>
<td>Wounds and urine (urinary tract and skin and soft tissue infections)</td>
<td>26</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Sputum (respiratory tract infections), blood (blood stream infection)</td>
<td>14</td>
</tr>
</tbody>
</table>

Table 3: Resistance Pattern of Clinical Isolates Involved in Different Infections

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Proteus species</em></th>
<th><em>Klebsiella pneumoniae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefepime</td>
<td>37.7%</td>
<td>26.3%</td>
<td>11.3%</td>
<td>34.8%</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>41.4%</td>
<td>21.7%</td>
<td>8.9%</td>
<td>17.6%</td>
</tr>
</tbody>
</table>

Fig. 1: Resistance Pattern of Clinical isolates against Cefepime and Cefpirome
S. aureus is one of the most frequent reported clinical isolate in present study. Mainly, infections are associated with Gram-positive pathogens alone or in mixed culture of Gram-negative and Gram-positive bacteria. S. aureus has been commonly isolated from surgical, nosocomial, burn, and blood stream infections (Yoshikawa and Bradley 2002; Zorgani et al., 2010). It has been found that S. aureus is most resistant against cefepime and cefpirome among all clinical isolates. The present study has shown 37.7% resistant to cefepime. Nasiri et al. (2013) supported the present study by 30% S. aureus resistance against cefepime. However, 41.4% S. aureus were resistant to cefpirome in current study. Chaudhury et al. (2003) have been observed 56.8% resistance of S. aureus against cefpirome. In contrast, of present study 20.4% bacteriacidal activity of S. aureus has been reported in Mexico (Santos et al., 2000).

E. coli is one the most frequent isolated pathogen in intra-abdominal and urinary tract infections (Sader et al., 2001). The present study revealed 26.3% E. coli resistance against cefepime supported by Liao and co-workers found 23% resistance (Liao et al., 2006). Moreover, 21.7% E. coli was resistance against cefpirome in present study. E. coli against cefpirome has shown 13% and 33.4% resistance by Hafeez and co-workers (2000) and Gupta et al. (2006) respectively.

Klebsiella pneumoniae has been involved in respiratory tract infections, blood stream infection, and urinary tract infections. K. pneumoniae is difficult to treat because fewer antibiotics are effective against K. pneumoniae. The outcome of current study has shown 34.8% and 17.6% K. pneumoniae resistance against cefpieme and cefpirome respectively. Bedenic and co-workers (2001) have found 23.8% K. pneumoniae resistance against cefpieme. 84% susceptibility has been reported by Hafeez et al. (2000). Moreover, 73.4% and 64.7% susceptibility of K. pneumoniae against cefpirome has been reported by Liao and mates (2006) and da Silva Nogueira et al. (2011) correspondingly.

Proteus species have been ubiquitously found. It is present in intravenous solutions, on the skin of human-being food, and in contaminated water. Proteus species were resistant to in general by used antibiotics leading to a higher prevalence of resistant bacteria (Feglo et al., 2010). In present study, Proteus species have been shown 11.3% and 8.9% resistance against cefpieme and cefpirome respectively. There is huge diversity in the result of Proteus species susceptibility against cefpieme, Gupta and his co-workers (2006) has shown 100% sensitivity, similarly, 97% and 85% antibacterial activity of cefpirom against Proteus species by Hafeez and colleagues (2000) and Sader et al. (2005) respectively.

It has been concluded from the present study that cefpirome has been possessed better susceptibility against E. coli, K. pneumoniae and Proteus species, while cefpirome has shown better antibacterial activity against S. aureus.

**CONCLUSION**

Resistance of antibiotics is now become curse to humankind. Globally prominent regulatory authorities like FDA, infection prevention society (IPS), CDC, WHO and local bodies in even local bodies for prevention of infection. In Pakistan, like Infection Control Society of Pakistan (ICSP) and Infectious Disease Society of Pakistan (IDSP) has been strongly discouraged the unjustified use of antibiotics in Pakistan. For irrational use of antibiotics, awareness program should be initiated. Health associated professionals should always try to check the in-vitro susceptibility of pathogens for proper treatment. In the mean while, it should be preferred to use the empirical therapy for infection control and preventions. The present study has been strongly recommended to prepare local guidelines at least for empirical therapy for the use of antibiotics in particular infections.

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