Studies on the Antimicrobial Resistance Pattern of Bacterial Pathogens isolated from Cancer Patients

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ABSTRACT

The present study deals with the antimicrobial resistance pattern of different antimicrobials being used to treat infections in cancer patients. The isolated strains were tested against antibiotics belonging to cephalosporin and aminoglycoside groups. The activity of these drugs was evaluated against 50 bacterial strains isolated from cancer patients undergoing anticancer therapy. The susceptibility was determined by broth dilution method according to British Standard Antimicrobial Chemother (BSAC, 2003) USA guidelines.

The overall Minimum Inhibitory Concentration (MIC) was determined at which 50 and 90 percent of bacterial isolates were inhibited. The resistance of Gram positive isolates against Cefoperazone, Ceftrixone and Ampicillin was 0%, 30% and 68% respectively. Whereas Pseudomonas aeruginosa among Gram negative bacteria showed resistance against Cefoperazone, Ceftriaxone and Ampicillin which was 0%, 27% and 64% respectively. For Enterobacteriaceae, it was recorded as 0%, 10% and 91% respectively. The order of activity against Gram-negative and Gram positive strains was Cefoperazone > Ceftriaxone > Ampicillin. Overall frequency of isolation of Gram positive bacteria and Gram-negative bacteria was 56% and 44% respectively. The results are useful with reference to the current resistance and susceptibility pattern among isolates against cephalosporin and aminoglycosides.

Keywords: Antibiotics, Resistance, Sensitivity, Cancer, Chemotherapy, Infections and Immune system.

INTRODUCTION

A frequent occurrence of infection is a persistent problem in cancer patients. They are more susceptible to variety of infections due to suppression of immune system as a result of chemotherapeutic agents being used for treatment. Cancer patients are at high risk for a wide variety of bacterial, viral, and fungal infections throughout the phases of immune recovery. These infections can be life threatening and are responsible for the high morbidity and mortality rate among the cancer patients (Pittet et al., 1997; Weinstein et al., 1997). Moreover the frequency of infection is related to the type of underlying neoplastic disease (Saif & Shannon, 2005). Such infections are generally the cause of death in a substantial number of patients (Hsueh et al., 2004).

Studies on Antimicrobial resistance in common bacterial pathogens have revealed that the Frequency of infections by Gram positive bacteria is increasing as compared to that of Gram negative bacteria during the past decade (Koll & Brown, 1993; Viscoli et al., 1988). This increase is mainly caused by the use of catheters i.e., central venous, peripheral and arterial resulting in a parallel increase in catheter-related infections (Butt et al., 2004). Amongst the Gram negative infections P. aeruginosa is a leading infectious agent in the immuno-compromised patients associated with significant morbidity and mortality (Yetkin et al., 2006). P. aeruginosa was responsible for 35% of fatal infections in patients with acute leukemia (Mazzalai, 2009).

The knowledge of antimicrobial resistance pattern is of particular concern in cancer patients where changing spectrum in the incidence and epidemiology of infecting organism has resulted in an increase in resistance to many antibiotic compounds (Cruciani et al., 2000). The emergence of resistance to beta-lactam antimicrobial agents as a result of the production of type 1 and extended-spectrum beta-lactamases (ESBL) is of great concern (Borg et al., 2006). Rolston (1998) suggested that the widespread use of fluoroquinolone prophylaxis has also resulted in the development of resistance among E. coli and other Enterobacteriaceae.

Amongst the Gram positive infectious bacteria, Staphylococcus is the major cause. A
slight decline in infections caused by *S. aureus* but a considerable increase in the incidence of infections caused by coagulase negative staphylococci has been reported (Koll & Brown, 1993; Viscoli et al., 1988 and Rolston, 1998). The predominant species is *S. epidermidis*, although *S. hominis* and, *S. haemolyticus* are also often isolated.

Antimicrobial agents act on bacteria in different ways, either by killing bacteria due to inhibition of vital activities or inhibition of protein production leading to arrest in bacterial growth and thereby preventing bacteria from reproduction (Cheesbrough, 2000).

In many cancer related infections, the determination of antimicrobial susceptibility of a clinical isolate is often crucial for the optimal antimicrobial therapy of infected patients. The emergence of multidrug-resistant microorganisms has intensified the problem (Fluit et al., 1999; 2000). Assessment is required to monitor the spread of resistant pathogens throughout the hospital and community. Standard procedures and breakpoints have been defined to predict therapeutic outcome both in time and at different geographic locations.

The aims and objectives of this study are (I) to study the spectrum of bacterial isolates in clinical specimen of cancer patient; (II) to study the antimicrobial resistance pattern of different antimicrobials that are used for treating infections in cancer patients; and (III) to assess the use of new potent antibiotic against infection in cancer patients to reduce the mortality and morbidity due to these infections.

**MATERIALS AND METHODS**

The study was carried out at Institute of Nuclear Medicine and Oncology Lahore (INMOL). Total 50 hospitalized cancer patients undergoing anticancer therapy with suspected blood stream infections were studied. No discrimination was made on the basis of age or gender.

**Bacterial strains and culture conditions**

Bacterial strains were isolated by adding 5 ml blood obtained from peripheral veins of the patients to culture bottles containing brain heart infusion (BHI) broth (Oxoid, Hampshire, UK). The blood culture bottles were incubated at 37°C and regular subculture were made. Single isolated colonies were obtained by streaking the culture on blood agar and MacConkey agar plates by incubating at 37°C for 24 hours. The plates containing purified strains were stored at 4°C till further use for sensitivity testing. The microorganisms were characterized into Gram positive and Gram negative strains by Gram staining method. Each bacterial isolate was further identified by standard biochemical tests according to the manual of clinical microbiology (Cheesbrough, 2000; Greenwood et al., 1992).

**Preparation of antibiotic stock solution**

Three antibiotics Ceftriaxone (Roche Fontenay, France) Cefoperazone + sulbactum (High-Q-international) and Ampicillin (sigma, UK) were obtained from local manufacturer. All the stock solutions of antibiotics were prepared in sterilized distilled water as specified by the manufacturer. Different dilutions were prepared for minimum inhibitory concentration (MIC) determination.

**Preparation of inoculum**

Inoculum was prepared by inoculating the single purified colonies from blood agar plates into Mueller-Hinton broth (Oxoid, UK) and incubated at 37°C for 24 hrs. Optical density of inoculum was measured with a Spectrophotometer (Roche, Germany) at 546 nm. The density of the inoculum was adjusted to 10^5 CFU/ml and was used in MIC determination.

**MIC Determination**

Antibiotic sensitivity against cephalosporins (Cefoperazone, Ceftriaxone) and aminoglycosides (Ampicillin) was determined in Gram negative and Gram positive bacterial isolates. MIC was determined in duplicate in Mueller-Highton as outlined by the British Standard Antimicrobial Chemother (2003). Different interpretive resistance and sensitive breakpoints are used for ceftriaxone, BSAC breakpoints of ≤ 1 μg/ml (susceptible) and ≥ 2 g/ml (resistant) were respectively applied for Gram negative and positive bacteria. For cefoperazone BSAC breakpoints of ≤ 4μg/ml (susceptible) and ≥ 8 μg/ml (resistant) was applied. For ampicillin, BSAC breakpoint of 8μg/ml (susceptible) and ≥ 16μg/ml (resistant) were used Gram negative and positive bacteria respectively (Andrews, 2001).

**RESULTS**

During the study period a total of 50 bacterial isolates were collected from blood cultures of cancer patients. Overall frequency of isolation was 56% Gram positive bacteria and 44% Gram negative bacteria (Fig. 1). Among the Gram positive isolates *Staphylococcus aureus* (38%) was most common isolate followed by *Streptococci* (18%). Among Gram negative bacteria *P. aeruginosa* (20%) was the most frequent isolate followed by *E. coli*. 

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coli (10%), Proteus (6%), Klebsiella (4%), Shigella (2%) and Citrobacter (2%) (Fig:2).

The overall susceptibility results of cefoperazone, ceftriaxone and ampicillin against bacterial isolates from blood stream infections of cancer patients are shown in table 1 and 2. In Gram negative bacteria highest in vitro activity against P. aeruginosa strain was observed for cefoperazone. The MIC\textsubscript{50} and MIC\textsubscript{90} of cefoperazone was 0.125 \(\mu\text{g/ml}\) and 0.25 \(\mu\text{g/ml}\) respectively and ranged from 0.125 - 64 \(\mu\text{g/ml}\). Ceftriaxone had three times less in vitro activity against P. aeruginosa isolates than cefoperazone (Table: 1) whereas in vitro activity of ampicillin was least with MIC\textsubscript{50} and MIC\textsubscript{90} of 4 \(\mu\text{g/ml}\) and 64 \(\mu\text{g/ml}\) respectively. In case of Enterobacteriaceae, cefoperazone was found more effective than ceftriaxone and ampicillin (Table.1). Therefore, the order of activity of the antimicrobials in Gram negative isolates was found in the order of cefoperazone> ceftriaxone> ampicillin.

Among Gram positive bacterial isolates cefoperazone is most effective with MIC\textsubscript{50} of 0.125 \(\mu\text{g/ml}\) as compared to Ceftriaxone (Table.2). It inhibited 50% of isolates at 1 \(\mu\text{g/ml}\) concentration. For ampicillin 50% and 90% isolates had MIC of 16\(\mu\text{g/ml}\) and 64 \(\mu\text{g/ml}\), respectively. Therefore, the order of activity of cephalosporins and aminoglycosides in Gram positive bacterial isolates was cefoperazone> ceftriaxone> ampicillin.

The percentage sensitivity and resistance of Enterobacteriaceae, P. aeruginosa and Gram positive bacteria against Ampicillin, Ceftriaxone and Cefoperazone was compared. High resistance was observed in Enterobacteriaceae against ampicillin that showed 91% resistance whereas only 9 % strains were susceptible. The sensitivity against ceftriaxone and cefoperazone was 90% and 100%, respectively whereas only 10% strains were resistant to ceftriaxone. No resistance strains were found against cefoperazone (Fig., 3). In P. aeruginosa resistance against ampicillin, ceftriaxone and cefoperazone was 64%, 21% and 0% respectively. Higher susceptibility rates 79% and 100% were observed against ceftriaxone and cefoperazone respectively than against ampicillin where susceptible strains were only 36% (Fig., 4). Similar trend was observed in Gram positive bacteria where 68%, 30% and no resistance was observed against ampicillin, ceftriaxone and cefoperazone while 32%, 70% and 100% sensitivity for ampicillin, ceftriaxone and cefoperazone was observed, respectively (Fig.5).
Fig. 3: Comparison of sensitivity and resistance of Enterobacteriaceae against Ampicillin, Ceftriaxone and Cefoperazone
Abbreviations: S= sensitive, R=resistant Amp=Ampicillin, Ctx=Ceftriaxone, Cfz=Cefoperazone

Fig. 4: Comparison of sensitivity and resistance of P. aeruginosa against Ampicillin, Ceftriaxone and Cefoperazone according to BSAC sceptibility and resistance breakpoints (BSAC, 2003).
Abbreviations: S= sensitive, R=resistant Amp=Ampicillin, Ctx=Ceftriaxone, Cfz=Cefoperazone
Fig. 5: Comparison of sensitivity and resistance of Gram positive bacteria to Ampicillin, Ceftriaxone and Cefoperazone according to BSAC susceptibility and resistance breakpoints (BSAC, 2003).
Abbreviations: S= sensitive, R= resistant Amp=Ampicillin, Ctx=Ceftriaxone, Cfz=Cefoperazone

Table 1: Antimicrobial activity of cephalosporins and aminoglycosides against Gram negative bacterial pathogens isolated from cancer patients.

<table>
<thead>
<tr>
<th>Antimicrobial class and agent tested</th>
<th>Activity* against \textit{a} \textit{P. aeruginosa}</th>
<th>Activity* against \textit{a} \textit{Enterobacteriaceae}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity</td>
<td>MIC (_{50})</td>
</tr>
<tr>
<td></td>
<td>MIC (_{\text{Range , \mu g/ml}})</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefoperazone</td>
<td>0.125-64</td>
<td>0.125</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.125-64</td>
<td>1</td>
</tr>
<tr>
<td>\textbf{Aminoglycosides}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.125-64</td>
<td>4</td>
</tr>
</tbody>
</table>

*\textbf{MIC}_{50} \text{ and } \textbf{MIC}_{90}; \text{MICs at which 50\% and 90\% of the isolates, respectively, were inhibited. The unit for all MICs are microgram per milliliter. } \% \text{ S, } \% \text{ R ; percent of isolates susceptible and resistant per BSAC criteria (2003).}
Table 2: Antimicrobial activity of Cephalosporin and Aminoglycosides against Gram positive bacterial pathogens isolated from cancer patients.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>MIC range µg/ml</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoperazone</td>
<td>0.125-64</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.125-64</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.125-64</td>
<td>16</td>
<td>64</td>
</tr>
</tbody>
</table>

MIC<sub>50</sub> and MIC<sub>90</sub>, MICs at which 50% and 90% of the isolates, respectively, were inhibited. The unit for all MICs are microgram per milliliter. %S; %R; percent of isolates susceptible and resistant per BSAC criteria (BSAC, 2003).

**DISCUSSION**

The potential for antimicrobial resistance is an important concern for clinicians treating patients with confirmed or suspected bacterial infections as they are often resistant to a broad range of antimicrobial agents. Detection of microorganisms in blood cultures is considered as indicator of infection and has been shown to be a valid marker for surveillance of Blood stream infection (Pittet et al., 1997). It is noted that they cause extensive changes in the microbiology, epidemiology, clinical and prognostic significance of positive blood cultures over a period of 20 year (Zinner, 1999; Saif & Shannon, 2005; Schimanski et al., 2006). During last thirty years most of the infections in cancer patients were reported to be caused by aerobic Gram negative bacilli. A shift in the bacterial spectrum towards Gram positive cocci has been reported in the western countries over the last twenty years. Although the exact cause of this shift is not known, long-dwelling intravascular devices, fluoroquinolone prophylaxis and chemotherapy-induced mucositis have been considered as important factors (Donowitz et al., 2001). This trend however is not prominent in the developing world (Pizzio, 1993).

In our study although Gram negative bacilli (44%) were the frequent isolates but more than half (56%) of the patients were infected with Gram positive cocci and S. aureus was the most common (38 %). Among the isolates a definite shift towards Gram positive microorganisms has been observed in our study. Similar shift has also been noted by Karamat et al., 1993.

In the United States and Europe, S. aureus and E. coli were identified as the two most common blood culture isolates from hospitalized cancer patients (Fritsche et al., 2003; Sader et al., 2002). Similar results have been reported by SENTRY for laboratories in the United States, Canada, Latin America, and Europe. Among the Gram negative bacteria, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumonieae are the common pathogens (Diekema, 1999). These pathogens were also common isolates with P. aeruginosa as the predominant isolate in our study. The results of the present study indicated the in vitro activities of cephalosporins and aminoglycosides against blood culture isolates of Gram positive and negative bacteria. It is clear that among cephalosporins, cefoperazone has greater in vitro activities against Gram negative bacterial isolates than ceftriaxone where 100% and 79% strains were susceptible to cefoperazone and ceftriaxone in case of P. aeruginosa and 100% and 90% sensitive strains in case of bacterial isolates belonging to Enterobacteriaceae (Fig., 3).

Increasing antimicrobial resistance has been reported for Klebsiella pneumonieae and Enterobacter by the Canadian Antimicrobial Resistance Study Group (Toye, 1993). Cefoperazone and ceftriaxone (third generation of cephalosporins) are susceptible to both Gram positive and Gram negative bacteria. Cefoprazone are highly active against bacterial pathogens isolated from cancer patients. High rate of sensitivity to Ceftriaxone and Cefoperazone (third generation of Cephalosprins) in Gram positive bacteria was also reported by (Viscoli et al., 1988).

For aminoglycosides (Ampicillin) used in this study, high resistance rates were observed in both Gram negative and positive strains. The resistance against P. aeruginosa and other gram negative bacteria was 64% and 91% respectively. In Gram positive bacteria 68% resistance was observed. High resistance to ampicillin in Gram
positive bacteria was also reported by Brinkmann et al. (2005); Zinner (1999). Ampicillin is thus not found suitable for infection treatment in cancer patients due to its high resistance rates. High rate of extended spectrum B lactamases by most of strains were reported to be major cause of high resistance.

The present study provides important information on the current resistance pattern among bacterial isolates against cephalosporins and aminoglycosides. Resistance against cephalosporins and fluoroquinolones appears to be increasing more rapidly with an increase in their use for treatment. Antimicrobial resistance rates require vigilance with respect to both the appropriate use of antimicrobial agents and continued surveillance for changes in rates of resistance among bacterium infections.

**REFERENCES**


