Antimicrobial susceptibility patterns of the gram-negative bacteria isolated from septicemia in Children's Medical Center, Tehran, Iran

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Key words
Antimicrobial resistance • Antimicrobial resistant profile • Bloodstream infection

Summary
Introduction. The choice of antimicrobial treatment for septicemia is often empirical and based on the knowledge of local antimicrobial activity patterns of the most common bacteria causing such bloodstream infections. The current study aimed to study the prevalence of bacterial pathogens causing septicemia and their antimicrobial resistant profiles in hospital admitted patients.

Methods. This cross sectional study done at Children's Medical Center, Tehran, Iran. We examined 168 bacterial strains isolated from 186 clinically diagnosed septicemia cases refereed at Children's Medical Center, Tehran, Iran Over a period of twelve months from July 2010 to 2011 July. 11446 blood samples from patients of clinically suggestive septicemia were evaluated.

Results. Bacterial strains were isolated from 910 (7.95%) of blood cultures. Gram-negative bacteria identified were Pseudomonas species (20.5%), Pseudomonas aeruginosa (1.86%), Salmonella spp (1.09%), Acinetobacter naumannii (8.13%), Escherichia coli (4.06%), Klebsiella spp (5.16%). Gram-negative pathogens were more than gram positive in bloodstream infections. Antimicrobial susceptibility testing was done according to Clinical and Laboratory Standards Institute (CLSI, USA) guidelines against: amikacin, ampicillin, amoxicillin, amoxiclav, cefuroxime, cefotaxime, cefazidime, cefoperazone tetracycline, chloramphenicol, ciprofloxacin, gentamicin. Resistanc to different antibiotics in the most important isolated bacteria were: 32.1 %, 10.8%, 87.8%, 96%, 39.1%, 35.2, 49.4%, 69%, 80.02%, 22%, 59%, 30.1% respectively, for Pseudomonas spp, 32%, 3.7%, 84.2 %, 83.2%, 80.1%, 75.4%, 44.8%, 45.2%, 33.3%, 19%, 34.1, 11.5% respectively for Acinetobacter species.

Discussion. Resistant to majority of the antimicrobial agents for several pathogens implicated in bloodstream infections, particularly in Gram-negative bacteria, can make complication in treatment of infection cause by them.

Introduction
The increasing bloodstream infections mainly in developing countries is the one of most important health care systems concerne. Although the bloodstream infections mortality rate has reduced in latest two decades. Many studies worldwide have determined rising antibiotics resistance among causative bacteria for septicemia [1-3]. It can can lead to make a major challenge for the physicians for treatment of tham, because of they face to increase of antibiotics resistance in causative bacteria for septicemia [3-5]. Both Gram-negative and Gram-positive bacteria can cause bloodstream infections and that can be differs from different locality and/or different time [1, 2, 8]. Rapid and accurate diagnosis and select the appropriate antimicrobial treatment for patients with septicemia can help to decrease mortality and morbidity [7, 9]. Actually, for selection of empirical antibiotic therapy for bloodstream infections consider local antibiotic resistance paterns in frequent pathogens associated with septicemia is necessary and can be one of important point.

So, this study was performed to find the frequency of Gram-negative bacteria that causing septicemia in Children’s Medical Center hospital admitted patients and determined antimicrobial resistance profile till clinicians can select the best choice antibiotic therapy.

Methods
This study was performed at a Children’s Medical Center, Tehran, Iran, over a period of twelve months from July 2010 to 2011 July. During the current study, 803 blood samples from patients of clinically suggestive bloodstream infection were surveyed, out of 4162 patients admitted. An informed consent was achieved from the patients. Blood samples from neonates and pediatrics were collected by pediatricians. Blood samples were gathered after careful cleaning of the venous with 70% alcohol and consequently followed by povidone iodine. Under the aseptic conditions 5 ml of blood was drawn by venipuncture and transmitted into two culture bottles each containing 50 ml of 0.5% bile-broth and 50 ml of 0.5% glucose-broth. Both the bottles were incubated
at 37°C for 10 days aerobically. Routine subculturing was performed on MacConkey agar and 5% sheep blood agar after 24 hours, 48 hours, 5th day and 10th day. In between these time points, subculturing was performed until there was observable turbidity. Microorganisms that isolated from blood culture were recognized by cultural characters, morphology and variety of standard biochemical tests such as oxidase, TSI, SIM, citrate, OF, Lysine decarboxilase, gelatinase, MR/VP, etc. [10-15].

Antimicrobial resistant patterns of the bacterial isolates to several antimicrobials was detected by using the Kirby Bauer disc diffusion method by Clinical and Laboratory Standards Institute (CLSI) guidelines [11]. The antibiotic concentration for each disc was as follows: gentamicin (10μg), amikacin (30μg), ampicillin (10μg), amoxicillin (30μg), amoxiclav (20:10μg), cefotixin (30μg), cefotaxime (30μg), cefuroxime (30μg), cefazidime (30μg), cefoperazone (75μg), ciprofloxacin (5μg), tetracycline (30μg) and chloramphenicol (30μg) for all organisms. All the antibiotic discs were purchased from MAST company (UK).

**Results**

**Demographics**

Over a period of twelve months from July 2010 to 2011 July. 11446 blood samples from patients of clinically suggestive septicemia were evaluated. The causative organism could be identified in 910 (7.95%) bacterial strains. Gram-negative bacteria identified were Pseudomonas species 187 (20.5%), Acinetobacter baumannii 74 (8.13%), Klebsiella spp 47 (5.16%), Escherichia coli 37 (4.06%), Salmonella spp 10 (1.09%), Pseudomonas aeruginosa 17 (1.86%) (Tab. I).

The rates of resistance in Pseudomonas spp to various antimicrobials are given below: gentamicin (30.1%), amikacin (10.8%), ampicillin (87.8%), amoxicillin (96%), amoxiclav (80.02%), cefotaxime (69%), cefoxime (49.4%), cefazidime (35.2%), cefoperazone (39.1%), ciprofloxacin (22%), chloramphenicol (59%) and tetracycline (32.1%) (Tab. II).

The rates of resistance in Acinetobacter species to various antimicrobials are given below: gentamicin (11.5%), amikacin (3.7%), ampicillin (84.2%), amoxicillin (83.2%), amoxiclav (80.1%), cefotuxime (75.4%), cefotaxime (44.8%), ceftazidime (45.2%), cefoperazone (33.3%), ciprofloxacin (19%), chloramphenicol (34.1%) and tetracycline (32%) (table 2).

The rates of resistance in Salmonella spp to various antimicrobials are given below: cefuroxime (21%), cefotaxime (39%), ampicillin (46.4%), amoxicillin (27.3%), amoxiclav (15.4%), cefoperazone (32.5%), ciprofloxacin (6.3%), chloramphenicol (9.1%) and tetracycline (50%) (Tab. II).

The rates of resistance in Escherichia coli to various antimicrobials are given below: gentamicin (25%), amikacin (18%), ampicillin (92%), amoxicillin (90.9%), amoxiclav (73.9%), cefoxime (54.5%), cefotaxime (52.9%), cefoperazone (36.8%), ciprofloxacin (31.6%), chloramphenicol (39%) and tetracycline (33.7%) (Tab. II).

The rates of resistance in Pseudomonas aeruginosa to various antimicrobials are given below: gentamicin (42.2%), amikacin (30.8%), ampicillin (96.1%), amoxicillin (92.9%), amoxiclav (95.8%), cefotaxime (81%), cefotaxime (48.4%), cefazidime (34.2%), cefoperazone (47.3%), ciprofloxacin (22.4%), chloramphenicol (32%) and tetracycline (42.6%) (table 2).

The rates of resistance in Klebsiella spp to various antimicrobials are given below: gentamicin (54%), chloramphenicol (49%) cefuroxime (77.4%), cefotaxime (52%), ampicillin (98.7%), amoxicillin (71.4%), amoxiclav (52%), cefoperazone (32%) and tetracycline (62%). Klebsiella pneumoniae did not show resistance to amikacin and ciprofloxacin (Tab. II).

**Tab. I. Number and percentage of isolated Species of Bacterial.**

| Species of isolated Bacterial | Number of isolated bacteria | Percentage of isolated bacteria |
|------------------------------|-----------------------------|--------------------------------|
| Pseudomonas species          | 187                         | 20.5%                          |
| Acinetobacter baumannii      | 74                          | 8.13                           |
| Klebsiella spp               | 47                          | 5.16%                          |
| Escherichia coli             | 57                          | 4.06%                          |
| Pseudomonas aeruginosa       | 17                          | 1.86%                          |
| Salmonella spp               | 10                          | 1.09%                          |

**Tab. II. Frequency of antibiotics resistance.**

|                | gentamicin | amikacin | amoxicillin | amoxiclav | cefotaxime | cefuroxime | cefazidime | cefoperazone | ciprofloxacin | chloramphenicol | tetracycline |
|----------------|------------|----------|-------------|-----------|------------|------------|------------|--------------|--------------|----------------|-------------|
| Pseudomonas spp| 30.1%      | 39.6%    | 87.8%       | 96%       | 69%        | 49.4%      | 35.2%      | 59.1%        | 22%          | 59%            | 52.1%       |
| Acinetobacter species| 11.5%    | 3.7%     | 84.2%       | 85.2%     | 80.1%      | 75.4%      | 44.8%      | 45.2%        | 33.5%        | 19%            | 34.1%       |
| Salmonella spp    | 21%        | 39.6%    | 46.4%       | 27.3%     | 15.4%      | -          | 32.5%      | 6.3%         | 9.1%         | 50%            | -           |
| Escherichia coli  | 25%        | 18%      | 92%         | 90.9%     | 73.9%      | 54.5%      | 52.9%      | -            | 56.8%        | 31.6%          | 39%         |
| Pseudomonas aeruginosa| 42.2% | 30.8%    | 96.1%       | 92.9%     | 95.8%      | 81%        | 48.4%      | 34.2%        | 47.3%        | 22.4%          | 52%         |
| Klebsiella spp    | 54%        | 0%       | 98.7%       | 52%       | 52%        | 77.4%      | 52%        | -            | 52%          | 0%             | 49%         |

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Antimicrobial Susceptibility Patterns of the Gram-negative Bacteria

Discussion

Bacterial antibiotics resistant is remaining as an alarming problem in the therapy of bloodstream infections [16, 17]. Bacterial bloodstream infection mostly caused by strains that are resistant to a wide range of antimicrobial agents [18]. The current study investigated the antimicrobial resistance profile of 372 Gram-negative bacteria isolated from bloodstream infections. The data demonstrated the frequency of antimicrobial resistance among bacterial pathogens isolated from bloodstream infections. Gram-negative bacteria is the most cause of bloodstream infections in many counties [1, 2, 7, 18]. Also, this type of bacteria have been the most common contributing pathogens of bloodstream infections in the present study. This is considerable that, different etiological agents of bloodstream infections can be related to the varying demography of bloodstream infections in developing countries because of different geographical area. In some studies demonstrated that Acinetobacter species, Salmonella typhi and Escherichia coli were the most common Gram-negative bacteria that involve in that bloodstream infections [19-21]. These results are approximately similar to our results in current study. Gram-negative bacteria is more resistance to antibiotics than Gram-positive. Acinetobacter species demonstrated very high levels of resistance to beta-lactam antibiotics in some reports [22, 23], that it is similar to the results of current study. Acinetobacter species showed the lower levels of resistance to aminoglycoside and low level resistance rates to quinolones. Similarly, Escherichia coli demonstrated distinctly higher levels of resistance to beta-lactam antibiotics than other classes of antimicrobials. The organism demonstrated maximum resistance to ampicillin and minimum to amikacin [22, 23]. These results can support the finding of this study, because of our results of antibiotic susceptibility testing are similar to them.

Conclusion

Extensive frequency of antimicrobial resistance levels were distinguished in our study, is similar to another studies [17, 18]. The high frequency of antibiotic resistance rates in Children’s Medical Center might be because of indifferentiation and excess of use of treatment in our country because of their easy availability [19]. The other cause might be frequency of ESBL production among Gram-negative isolates from neonatal bloodstream infection [20]. Also, other cause could be the altering patterns of antibiotic utilize and varies in lifestyle. In the light of over findings there continues an increasing requirement for new agents, though a recent study reported polymyxin are being restored to as potential choices for the therapy [21] Proper antimicrobial treatment for bloodstream infections is important in declining morbidity and mortality among patients with bloodstream infections caused by bacteria. Therefore, accurate microbiological diagnosis and their antimicrobial resistance profile can be very important for the rapid initiation of sufficient treatment for bloodstream infections [23].

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