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Evaluation of blood cultures in a children's hospital located in Southeastern Anatolia

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Abstract

Aim: Bloodstream infections in hospitalized patients are one of the most important causes of morbidity and mortality despite antimicrobial therapy. Early diagnosis and treatment of these infections is crucial. The aim of this study was to evaluate the distribution and antibiotic susceptibility of bacteria isolated from blood cultures in a children's hospital in the Southeastern Anatolia during an 18-month period.

Material and Methods: 7 040 blood cultures which were sent from hospitalized patients in Gaziantep Children's Hospital between 01.07.2010 and 01.01.2012 were evaluated.

Results: A total of 7 040 blood cultures were evaluated in this study. Microbial growth was detected in 2075 (29.47%) blood cultures. The most frequently isolated bacteria were coagulase-negative staphylococci (%45.97) which were followed by *Salmonella spp.* (%7.8). 12.12% of enterococcal isolates were resistant to glycopeptide antibiotics. The most frequently isolated gram negative bacterium was *Salmonella spp.* 15.43% of *Salmonella spp.* showed decreased susceptibility against quinolones. The ESBL positivity rate of *E. coli* and *K. pneumoniae* strains was found to be 35.08% and 57.14%, respectively. The imipenem resistance rate of *P. aeruginosa* was found to be 33.33%. The most common nonfermentative bacterium was *S. maltophilia*.

Conclusions: The distribution of bacteria isolated from blood cultures and antibiotic resistance rates differ among different regions of Turkey. Different results obtained in our study may be related with regional tendencies to infections and patient population. Distribution of infectious agents and antibiotic resistance rates should be evaluated at regular intervals. This will lead to establishment of proper antibiotic usage policies in our country. (Turk Pediatri Ars 2015; 50: 102-7)

Keywords: Child, Southeastern Anatolia, blood culture

Introduction

Bloodstream infections in hospitalized patients are one of the most important causes of morbidity and mortality despite antimicrobial therapy. Early diagnosis and treatment of these infections is clinically significant. For the detection of bloodstream infections, providing suficient number of blood culture sets under appropriate conditions and at appropriate times constitute the clinician's side of the diagnosis, whereas accurate interpretation of the growths in blood culture sets delivered to the laboratory, definition of the bacteriae grown and studying antibiotic sensitivity tests constitute the microbiologist's side. Since definition of the causative agenst in the shortest time and performance of antibiotic sensitivity tests will provide early and accurate treatment, they are very important in terms of reducing the morbidity and mortality rates. Although there are various molecular methods which are studied directly from the sample or colony, the gold standard method with the highest sensitivity and reliability is still blood culture (1, 2).

Automated blood culture systems are widely used to define the agents in the blood in a rapid and accurate way (3) In our country, full automated blood culture systems including BACTEC and BACT/ALERT are used most commonly. The rich medium content of blood culture

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bottles in these systems enables growth of causative microorganisms and microorganisms under the effect of antibiotics can grow owing to the resins which bind antibiotics. However, the most important disadvantage of theses systems is high rates of contamination due to enriched medium in cases where antisepsis is not applied appropriately during obtaining samples (4). In this case, bacteriae which are found intensively on the skin flora emerge as growth in blood culture and make it difficult to make an agent-contamination interpretation.

It is very important for hospitals to determine the distributions of infectious agents and antibiotic resistance rates which can change at certain time intervals. In this way, the changes in infectious agents and antibiotic sensitivity rates are determined and antibiotic usage policies are shaped. In this study which was based on retrospective evaluation, we aimed to evaluate the distribution of the bacteriae grown in blood cultures and antibiotic sensitivities in a 18-month period in a children's hospital in the Southeastern Anatolia region.

Material and Methods

7040 blood cultures sent with Bactec Ped Plus blood culture bottles to the microbiology laboratory from hospitalized patients in Gaziantep Children's Hospital between 07.01.2010 and 01.01.2012 were studied with the BACTEC 9 120 (Becton Dickinson and Company, Sparks, MD, USA) automated blood culture system. The samples with no growth on the seventh day were considered negative and the samples sent with a prediagnosis of brucellosis were incubated for 21 days. The blood cultures which gave positive growth were gram stained and planted in 5% sheep blood agar, eosine methylene blue (EMB) agar, chocolate agar and Sabouraud dextrose-agar (SDA) media. They were evaluated after 24-hour incubation at 35 °C. If the samples with no bacteria on gram staining and with no growth in culture were processed again in the same way and no growth was observed again, they were considered as false positive. When diphteroids and bacillus sp. were not grown in two different bottles, this was considered as contamination. The definition of the bacteriae grown was made using VITEK2 Compact (bioMerieux, Marcy l'Etoile, France) full automated diagnostic system as well as conventional methods and antibiotic sensitivities were determined using VITEK2 and Kirby-Bauer disc diffusion method and Mueller-Hinton agar according to the cirteria of the Clinical and Laboratory Standards Institute (CLSI). The germ tube test was performed for the grown *Candida* species and *albicans, non-albicans Candida* differentiation was made. Extended-spectrum beta lactamase (ESBL) growth was investigated using combined disc method in accordance with the CLSI recommendations. With this objective, ceftazidim (30 µg), ceftazidim/clavunalic acid (30/10µg) and cefotaxim (30µg), cefotaxim/clavulanic acis (30/10µg) discs were placed in Mueller-Hinton medium with at least 25 mm intervals between and zone diameters were measured after 16-18-hour incubation. In the evaluation of presence of extended-spectrum beta-lactamase, *Klebsiella pneumoniae* ATCC 700603 was used as a positive control strain and *Escherichia coli* ATCC 25922 was used as negative control strain. Meticillin resistance in staphylococcus strains was determined with cefoxitin disc (30µg). The strains the antibiotic sensitivities were found to be moderate were considered resistant.

Statistical analysis

Descriptive statistical method was used for statistical analysis. The categorical variables were expressed as percentage (%) values.

Results

No growth was found in 4 965 (70.53%) of a total of 7 040 blood cultures examined. In the isolatesin which growth was isolated (n=2 075), 1 350 (65.06%) 630 (30.36%) and 55(4,58) of isolates showed gram (+) bacteria, gram (-) bacteria and yeast like fungi growth respectively. The most commonly isolated bacteriae was coagulase negative staphylococci (CNS) (n=954, 45.97%) which was followed by *Salmonella spp*. (n=162, 7.8%). The rates of the isolated bacteriae are shown in Table 1 and Table 2. Among the grown *Candida* species 52 (54.74%) were defined to be *Candida albicans* and 43 (45.26%) were defined to be *non-albicans Candida*.

Antibiotic sensitivities of gram positive bacteriae

Meticillin resistance was found with a higher rate (73.69%) in CNSs compared *to Staphylococcus aureus.* Meticillin resistance was observed in 65 (43.92%) of 140 *S. aureus* isolates and glycopeptide resistance was not found. Resistance to trimetoprim-sulphametoxasol and gentamycine was found with a higher rate in meticillin resistant *S. aureus* isolates (MRSA) compared to meticillin sensitive *S. aureus* isolates (MSSA). Penisilin resistance was found only in one of 5 *Streptococcus pneumoniae* strains. Among all enterococci, resistance to glycopeptide antibiotics was found in 12 (12.12%) strains.

Table 1. Gram positive bacteriae isolated in blood cultures

Gram positive bacteriae	n	%
Coagulase Negative staphylococcus	954	70.67
Streptococcus spp.	87	6.44
Enterococcus faecium	69	5.11
Staphylococcus aureus	148	10.96
Enterococcus faecalis	21	1.56
Enterococcus spp.	9	0.67
Streptococcus pneumoniae	5	0.37
Other	57	4.22
TOTAL	1 350	100

Table 2. Gram negative bacteriae isolated in blood cultures

Gram Negative bacteriae	n	%
Salmonella spp.	162	25.72
Klebsiella pneumoniae	77	12.22
Serratia spp.	60	9.53
Escherichia coli	57	9.04
Brucella melitensis	51	8.10
Pseudomonas aeruginosa	39	6.20
Stenotrophomonas maltophilia	40	6.35
Proteus spp., Enterobacter spp., Morganella spp., Citrobacter spp.	28	4.44
Acinetobacter baumanii	27	4.28
Sphingomonas paucimobilis	12	1.90
Other	77	12.22
TOTAL	630	100

Antibiotic sensitivity of gram negative bacteriae

Decreased sensitivity to quinolones was found with a rate of 15.43% in the isolates of *Salmonella spp*. which were the most commonly isolated gram negative bacteriae. The most sensitive antibiotics for *E. coli* and *K. pneumoniae* strains were found to be aminoglycosides. The ESBL positivity rates were observed to be 35.08% and 57.14%, respectively. A decrease in the rates of sensitivity especially to ampicillin was found in *E. coli* isolates (17.54%). The isolates of *Pseudomonas aeruginosa* isolated were found to be resistant to most of the antibiotics studied including ceftazidim (64.10%). The most efficient antibiotics for *P. aeruginosa* isolates were found to be sulbactam cefoperazon (92.31) and piperacillin tazobactam (92.31%) and 13 isolates (33.33%) were found to be resistant to imipenem.

Discussion

Bloodstream infections constitute clinical pictures which carry severe morbidity and mortality risks and early diagnosis and treatment can reduce the morbidity and mortality rates. Changes have been observed in the distribution of the microorganisms which lead to these infections and a shift from gram negative bacteriae to gram positive agents has occured (5-7).

In studies, gram positive bacteriae constitute the majority because of CNSs found in the skin flora. In Inönü University, the rate of gram positive bacteriae was reported to be 68.5% and the rate of gram negative bacteriae was reported to be 3.5% (8). In our study, the rate of growth was found to be 65.06% for gram positive bacteriae and 30,36% for gram negative bacteriae. Studies have not shown great differences between gram positive and gram negative rates in adult and pediatric patients. In a study preformed by Gülmez et al. (9) in which blood culture growths in a pediatric patient groups were evaluated, gram positive bacteriae constituted 68.8% of the growths. In another study involving pediatric patients, coagulase negative staphylococci, *Micrococcus spp., Bacillus spp.* and diphteroid bacilli were excluded from the evaluation as contamination, gram negative bacteriae were found to be the agent with a rate of 74% (10). In our study, the rate of gram positive bacteriae was found to be high because agent-contamination evaluation could not be made for the blood culture growths especially for CNSs. The rate of growth was 29,47% in all blood cultures and gram positive bacteriae were isolated with a higher rate (65.06%) compared to gram negative bacteriae. In most studies, agent-contamination differentiation is not made and the same situation is observed. There are multiple variables in relation with gram positive bacteriae being causative agents. Pantient's clinical course and the technique samples had been taken should be considered when evaluating agent-contamination (4).

In recent years, *Candida* yeast growth rates have increased significantly because of neutropenia, preterm delivery, surgical interventions and intensive intravenous catheter usage. This rate was found to be 12.5% in Fırat University and 10.08% in another study in pediatric patients (7, 9). In years, the isolation rates of non-albicans *Candida* have increased. The rate of growth of non-*albicans Candida* was found to be 52.7% by Gülmez et al. (9) and 54.07% by Durmaz et al. (11). In our study, *Candida* spp. were grown in 4.58% of the blood

cultures. Of the *Candida* types isolated 54,7% were defined to be *C. albicans* and 45.3% were defined to be non-*albicans Candida*.

In our study, CNSs constituted 70.67% of the gram positive bacteriae isolated in blood cultures with positive growth and 45.97% of all blood cultures. *S. aureus* is among the important pathogens isolated in blood cultures. Specifying meticillin resistance especially in pediatric patients is essential for an accurate guidance of treatment in these patients. Gülmez et al. (9) reported that in 5 years mean ratio of *S. aureus* isolation in blood cultures in pediatric patients was approximately 7.06%, meticillin resistance decreased in years and ratio was 0% in 2011. Duman et al (8) isolated *S. aureus* in 7% and 30.8% of these were resistant to methicillin. This rate was found to be 58,3% in the study performed by Kaya et al. (12) and 48.2% in the study performed by Tuncer et al. (13). In our study, *S. aureus* constituted 10.96% of all gram positive bacteriae and meticillin resistance was found in 43.92% of the isolates.

Enterococci are the third most common bacteriae isolated in nasocomial infections in USA and in bloodstream infections in developed countries and a gradual increase in resistance especially to glycopeptides has become an important problem in recent years (14, 15). Bar et al. (16) reported the vancomycin resistance in enterococcus strains isolated in blood cultures to be 34%. In our study, resistance to glycopeptide antibiotics was found in 12 (12.12%) of 99 enterococcus strains isolated.

Enteric fever is the most important cause of community aquired septisemia in southeastern Anatolia in our country and in developing countries found in the same region. The definite diagnosis is made by isolation of bacteriae from blood, urine and stool. The rate of bacterial isolation is not high because of inititation of antibiotic treatment before the culture samples were obtained and low levels of bacteriae in the blood (17). In our study, the most common gram negative bacteria isolated was *Salmonella spp.* with a rate of 25.72% and decreased sensitivity to quinolones was observed with a rate of 15.43 in the isolates. No study with similar results has been found in our country excluding one study conducted in the province of Gaziantep in which *Salmonella spp.* were found with a rate of 32.92% in blood cultures (18). In a study conducted in Cambodia in which blood culture growths were evaluated in a pediatric patient group, it was observed that the most common bacteria was *Salmonella spp.* with a rate of 29.8% after contamination evaluation was made (19). In this study, it was concluded that 90.5% of all growths were community aquired septicemia. The high rate observed in our study was thought to be related with reflection of community aquired infection which is already endemic in the region to blood cultures before antibiotherapy.

It has been reported that the most common gram negative bacteria isolated in blood cultures is *E. coli*. the rate of growth of *E. coli* was reported to be 11.1% in a study which included pediatric patients and 14.5 in a study conducted in Malatya in adult patients (8, 12). In our study, *E. coli* (9.04%) was in the fouth order among the gram negative bacteriae isolated following *Salmonella spp.* (25.72%), *K. pneumoniae* (12.22%) and *Serratia spp.* (9.53%).

Extended spectrum beta lactamase producing *Enterobacteriaceae species* are increasing day by day in our country as well as in the whole world and this increases the mortality rates. In a study in which ESBL rates were evaluated in *E. coli* isolates, ESBL was found in 35.5% of the isolates (20). Similarly, ESBL positivity was found with a rate of 32, 8% in *E. coli strai*ns and 15.4% in *K. pneumoniae* strains in Inönü University (8). The rates of ESBL varies from country to country, from hospital to hospital and even from between clinics. The ESBL rates in *E. coli* and *K. pneumoniae* isolates were found to be 11% and 13%, respectively in the study of Ho et al. (21), 6.7% and 47.3%, respectively in blood cultures in the SENTRY program in Latin America and 20% and 24%, respectively in Korea (5, 22). In one study conducted with pediatric patients, ESBL positivity was found with a rate of 17,9% in *E.coli*'s isolated in blood cultures and 52,9% in *K. pneumoniae* isolates (23). This rates were found to be 35,08% and 57,14, respectively in our study.

In our study 60.95% of the gram negative bacteriae isolated were *Enterobactericeae* and 18.73% were nonfermentative bacteriae. The agent of brucellosis which is endemic in the region (*B. melitensis*) was found with a rate of 8,10%. The rate of growth of *Brucella* spp. was found to be 13.5% in adult patients in Fırat University, 2.9% in Atatürk Education and Research Hospital and 10.6% in Ankara Education and Research Hospital (7, 24, 25).

Non-fermentative bacteriae constituted 18.73 % of the total isolates, in which *Stenotrophomonas, maltophilia,*

P aeruginosa, Acinetobacter baumannii, Sphingomonas paucimobilis constituted 6.35%, 6.29%, 4.28%, 1.90% respectively. When compared with the other studies, the fact that the most common nonfermentative bacteria isolated was *S. maltophilia* was a different finding. In Fırat University, *S. maltophilia* was found with a rate of 0.9% and the most commonly isolated nonfermentative bacteria was *A. baumannii* (10.4%) (7). In the study performed in Pamukkale University, *S. maltophilia* was not isolated at all and the most commonly isolated nonfermentative bacteria was *P. aeruginosa* (6.5%) (26).

Stenotrophomonas maltophilia is observed more commonly in patients who have been hospitalized for long term, who receive wide spectrum antibiotic treatment, who are neutropenic and have an underlying severe condition and uncontrolled use of carbapenems in intensive care units causes to an increase in isolation of this microorganis (27). In a study conducted by Çelebi et al. (28), the most common risk factors evaluated for S. *maltophilia* in pediatric patients included prolonged hospitalization, use of wide spectrum antibiotics and total parenteral nutrition and it was observed that 85.7% of the patients received mechanical ventilation and central venous catheter was used in 78.5%. The most common predisposing factor was premature delivery (53.6%). It was thought that the high rate in our study was related with the fact that carbapenems were used intensively in the hospital.

In our study, the most efficicent antibiotics for *P. aeruginosa* isolates were found to be sulbactam cefoperazon (2.31%) and piperacillin tazobactam (92.31%) and 13 isolates (33.33%) were found to be resistant to imipenem. Esel et al. (29) found that the most sensitive antibiotic for *P. aeruginosa* was ceftazidim (81.2%) and they found imipenem resistance to be 37.5% similar to our study.

Actinetobacter baumannii has multi-resistance against antibiotics and leads to infection especially inpatients with immunosupression and in patients with underlying severe condition receiving wide spectrum antibiotic treatment (19). In studies evaluatimng the bacteriae grown in blood cultures, the rates of *A. baumannii* were found to be 10.4%, 11.7%, 5%, respectively. In our study, this rate was found to be 4.28% (7, 8, 25).

In our study, the rates of isolation of *Serratia spp.* (9.53%) and *S. paucimobilis* (1.90%) were found to be higher compared to the other studies; this was found to be related with periodical epidemics which occured in the hospital during the process of the study.

Conclusively, it is known that the bacteriae isolated from blood cultures and antibiotic resistance rates vary by time and geographical regions as well as the service type of the hospital, size of the hospital, antibiotic treatment protocols administered and the differentiation of nasocomial/community-aquired infection. Since our study was conducted in a large regional children's hospital, the population was composed of pediatric patients. The different findings we obtained may be related with the patient population and regional tendencies to certain infections. Periodical evaluation of the results will enable precautions including coplying with antisepsis rules during obtaining samples and obtaining sufficient number of samples at the appropriate time by predicting contamination rates. Considering that administration of treatment in accordance with culture antibiogram results instead of empirical treatment will decrease the mortality rates and antibiotic resistance, we think that determination of variable infectious agent distributions and antibiotic resistance rates by hospitals according to the samples obtained from patients will enlighten the establishment of antibiotic usage policies.

Ethics Committee Approval: Ethics committee approval was not received due to the retrospective nature of this study.

Informed Consent: Written informed consent was not obtained due to retrospective nature of this study.

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