

Bacterial Isolates in Blood Culture of Children with Septicemia.

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Summary:

Background: Septicemia is a common condition in children with a resultant high morbidity and mortality. It's defined as the presence of bacteria in the blood and is often associated with severe infections, the alternative names (Blood poisoning, Bacteremia with sepsis, systemic inflammatory response syndrome).

Objective: To study the etiological agents and antibiotics susceptibility of septicemia in children admitted to Central Teaching Hospitals of Pediatric.

Patients and Methods: A total of 80 patients having signs and symptoms of Septicemia who were admitted to Central Teaching Hospital of Pediatric in Baghdad city. Samples were obtained during the period from the 1st January till the 30th of February 2011. Bacteriological specimen (blood) collection, isolation and Identification of causative microorganism by colonial morphology, Gram stains, Biochemical tests and other tests used for further identification (Catalase, Coagulase, Oxidase test) and determining susceptibility of bacteria to antibiotics and chemotherapeutic agents.

Results: The blood culture results showed that 20 (25.0 %) patients gave positive blood culture, 60 (75.0 %) patients gave negative blood culture. The microorganisms isolated from blood cultures of 20 patient gave positive blood culture with septicemia was *Staphylococcus aureus* 5 (25.0 %) *Escherichia coli* 5 (25.0 %), *Klebsiella pneumonia* 4 (20.0 %), *Enterobacter spp* 3 (15.0 %), *Serratia* 2 (10.0 %), *Pseudomonas spp* 1 (5.0 %), and the frequency of septicemia in 20 case according to gender represented male 11 (55.0 %), female 9 (45.0 %).

Conclusions: Positive blood cultures were obtained in 20 (25.0 %) of children. The commonest Gram positive bacterial isolate was *Staphylococcus aureus* and Gram negative bacterial isolate was *Escherichia coli* especially in males sex, in neonate age group who were delivered by cesarean section and preterm < 37 weeks gestational age. The antibiotics [Cefotaxime, Amoxicillin+ Clavulanic acid] are the most effective against *Staphylococcus aureus* and [Gentamycin, Amikacin, Imipenem] against Gram negative bacteria.

Key Word: Blood cultures, bacterial isolates, antibiotic sensitivity, children, septicemia.

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Introduction:

Septicemia is a common condition in children with a resultant high morbidity and mortality (1). It's defined as the presence of bacteria in the blood and is often associated with severe infections, the alternative names (Blood poisoning, Bacteremia with sepsis, systemic inflammatory response syndrome) (2). Bacterial pathogen of neonatal septicemia may vary from one country to another and within a country from one hospital or region to another (3). Microorganisms present in the circulating blood whether continuously or intermittently are threat to every organ in the body (4). Early-onset bacteremia (that occurring within 72 hours after birth) is currently caused by predominantly aerobic gram-positive organisms and late onset is due to predominantly aerobic gram-negative bacteria (5). Children with septicemia present with poor activity, fever, vomiting, chills, difficulty in breathing, tachycardia, malaise, refusal of feeds or lethargy hypothermia, respiratory

distress, abdominal distension, jaundice, toxicity and eventually the extreme form being-shock (6). Bacteria can be introduced in to the blood stream during tooth brushing (7). This form of bacteria almost never causes problems in normal individuals. However, bacteremia associated with certain dental procedures can cause bacterial infection of the heart valves known as endocarditis in high risk patients (8). The therapy of sepsis rests on antibiotics, surgical drainage of infected fluid collections, fluid replacement and appropriate support for organ dysfunction, this may include hemodialysis in kidney failure, mechanical ventilation in pulmonary dysfunction, transfusion of blood products and drug and fluid therapy for circulating failure. Ensuring adequate nutrition-preferably by central feeding, but if necessary by parenteral nutrition-is important during prolonged illness (9).

Patients & Methods:

Patients: The retrospective study include 80 patient having signs and symptoms of septicemia who were admitted to Central Teaching Hospital of Pediatric in Baghdad city. Samples were obtained during the period from the 1st January till the 30th of February

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2011. Data were obtained from the patients including age group; A (neonates), B (> 28 days to 1 years), C (1 years to < 12 years), sex, and etiological agent, gestational age and mode of delivery.

Methods: Thus microbiologic blood culture results continue to be considered the gold standard for diagnosis of bacterial sepsis. The skin at the venepuncture site must be prepared using a bacterial disinfectant 2 % solution of tincture iodine should be allowed to evaporate on the skin surface at least 1 minute before blood was withdrawn. The tincture iodine was wiped off with 70% alcohol to avoid possible skin irritation; tourniquet was applied. From peripheral vein (before antibiotic therapy) 2 ml blood was aspirated for aerobic blood culture bottle (brain heart infusion) then mixed with 10 times its volume of broth (1 ml of blood in 10 ml broth). The rubber cover of the bottles was cleaned with iodine before inoculation (10). Blood was cultivated into bottle containing brain heart infusion broth (BHI) contain sodium polyanethol sulphonate (SPS) in a final concentration of 0.05%. The bottles were incubated at 35°C for 7 successive days and the bottles were examined at least daily to follow any changing such as turbidity, gas bubbles, haemolysis, or appearance of small aggregates of bacterial growth in the broth (11). Blind subcultures were carried out after 1, 3, 7 days on blood agar plate was incubated aerobically and chocolate agar plate incubated under 5-8 % CO₂ atmosphere in the candle jar for 24 hrs at 37°C. MacConkey agar plate was also sub cultured and incubated for 24 hrs at 37°C. Vandebitte *et.al.* reported the primary media bottles that showed negative culture results should not be discarded before 7 days of incubation (12).

Identification of causative microorganism: Colonial morphology or any change exhibited on the media (11, 13, 14). Gram stains method (13, 15) Biochemical tests. Other tests used for further identification: Catalase test (13, 14,15). Coagulase test (13). Oxidase test (16,17).

Antibiotic sensitivity test: use the disk diffusion method (Kirby-Bauer method) for determining susceptibility of bacteria to antibiotics and chemotherapeutic agents according to Vandepitte and Bauer (10,18). Antibiotic sensitivity was tested for Gram negative bacteria and Gram positive

bacteria (Amikacin (AK), piperacillin (PRL), Ceftriaxone (CI), Cefotaxime (CTX), Imipenem (IPM), Amoxicillin+ Clavulanic acid (AM+CL), Gentamycin (G), Cephalothin (CF), Oxacillin (OX), Clindamycin (CD), Penicillin (P), Cephalexin (CL)).

Statistical Analysis:

The suitable statistical methods were used in order to analyze and assess the results, they include the followings:

Descriptive statistics: Statistical tables including observed frequencies with their percentages.

Inferential statistics: These were used to accept or reject the statistical hypotheses, they include the followings: -Chi-Square (χ^2).

P value > 0.05 level of significance was considered statistically non significant difference.

Results:

The blood culture results showed that 20 (25.0 %) patients gave positive blood culture, 60 (75.0 %) patients gave negative blood culture. The microorganisms isolated from blood cultures of 20 patient gave positive blood culture with septicemia was *Staphylococcus aureus* 5 (25.0 %), *Escherichia coli* 5 (25.05 %), *Klebsiella pneumoniae* 4 (20.0 %), *Enterobacter spp* 3 (15.0 %), *Serratia* 2 (10.0 %), *Pseudomonas spp* 1 (5.0 %), and the frequency of septicemia in 20 case according to gender represented male 11 (55.0 %), female 9 (45.0 %) Table (1).

Table (1): Distribution of the Microorganisms causing Septicemia (Positive blood culture) in children According to the Gender

Microorganisms	Positive blood culture (No. 20)			P-value
	Male %	Female %	Total %	
<i>Staphylococcus aureus</i>	4(36.3)	1(11.1)	5 (25.0)	N.S.
<i>Escherichia coli</i>	3 (27.3)	2(22.2)	5 (25.0)	N.S.
<i>Klebsiella pneumoniae</i>	2(18.2)	2(22.2)	4 (20.0)	N.S.
<i>Enterobacter spp</i>	2 (18.2)	1(11.1)	3 (15.0)	N.S.
<i>Serratia</i>	0 (0.0)	2(22.2)	2 (10.0)	N.S.
<i>Pseudomonas spp</i>	0 (0.0)	1(11.1)	1 (5.0)	N.S.
Total	11(55.0)	9(45.0)	20 (100.0)	N.S.

N.S. : P > 0.05

Table (2): Distribution of blood culture bacterial isolates in three age groups.

Microorganisms	Age Group			Total %	P-value
	Group A Neonates %	Group B > 28days to < 1 Years%	Group C 1 Years to 12Years %		
<i>Staphylococcus aureus</i>	2 (25.0)	2 (33.3)	1 (16.6)	5 (25.0)	N.S.
<i>Escherichia coli</i>	3 (37.5)	1 (12.5)	1 (16.6)	5 (25.0)	N.S.
<i>Klebsiella pneumoniae</i>	0 (0.0)	2 (33.3)	2 (33.3)	4 (20.0)	N.S.
<i>Enterobacter spp</i>	1 (16.6)	1 (12.5)	1 (16.6)	3 (15.0)	N.S.
<i>Serratia</i>	2 (33.3)	0 (0.0)	0 (0.0)	2 (10.0)	N.S.
<i>Pseudomonas spp</i>	0 (0.0)	0 (0.0)	1 (16.6)	1 (5.0)	N.S.
Total Positive Culture	8 (40.0)	6 (30.0)	6 (30.0)	20(100.0)	N.S.
Sterile Negative Cultures	15 (25.0)	17 (28.4)	28 (46.6)	60 (100.0)	N.S.
Total Culture	23 (28.8)	23 (28.8)	34 (42.4)	80 (100.0)	N.S.

N.S. : P > 0.05

Table (3): Demographic and clinical features at the time of presentation of neonatal Sepsis.

Characteristic	Total No. of patients (N= 80) %	Percent of blood culture positive patients (N=20) %	P-value
Gestational Age - Term (> 37 weeks) - Preterm (< 37 weeks)	31(38.75) 49 (61.25)	3 (15.0) 5 (25.0)	N.S.
Route of Delivery - Normal Vaginal delivery - Cesarean Section	45(56.25) 35 (43.75)	6 (30.0) 2 (10.0)	N.S.

N.S. : P > 0.05

Table (4): Bacterial Isolates and antibiotic Sensitivity pattern for Gram Negative Bacteria in children with Septicemia.

Antibiotic for Gram (-) bacteria	Bacterial Isolates				
	<i>Escherichia coli</i> N=5 %	<i>Klebsiella pneumoniae</i> N=4 %	<i>Pseudomonas</i> N=1 %	<i>Enterobacter spp</i> N=3 %	<i>Serratia</i> N=2 %
Amikacin	2 (40.0)	3 (75.0)	R	3 (100.0)	1 (50.0)
Pipracillin	2 (40.0)	1 (25.0)	1 (100.0)	R	R
Ceftriaxone	3 (60.0)	1 (25.0)	R	1 (33.3)	1 (50.0)
Cefotaxime	3 (60.0)	R	1 (100.0)	1 (33.3)	1 (50.0)
Imipenem	1 (20.0)	2 (50.0)	R	2 (66.6)	2 (100.0)
Amoxicillin + Clavulanic acid	1 (20.0)	1 (25.0)	1 (100.0)	R	1 (50.0)
Gentamycin	3 (60.0)	3 (75.0)	1 (100.0)	2 (66.6)	1 (50.0)

Table (5): Bacterial Isolates and antibiotic Sensitivity pattern for *Staphylococcus aureus* in children with Septicemia

Antibiotic for <i>Staphylococcus aureus</i>	Bacterial Isolates	
	<i>Staphylococcus aureus</i> N=5 %	
Cefotaxime	3 (60.0)	
Amoxicillin + Clavulanic acid	3 (60.0)	
Imipenem	2 (40.0)	
Cephalothin	2 (40.0)	
Oxacillin	2 (40.0)	
Clindamycin	2 (40.0)	
Ceftriaxone	1 (20.0)	
Cephalexin	1 (20.0)	
Penicillin	1 (20.0)	
Pipracillin	1 (20.0)	

Discussion:

A total of 80 blood culture samples were collected among which 20 (25.0 %) patients gave positive blood culture, 60 (75.0 %) patients gave negative blood culture. The commonest bacterial isolates were *Staphylococcus aureus* 5 (25.0 %), *Escherichia coli* 5 (25.0 %), and *Klebsiella pneumoniae* 4 (20.0 %). In most of the studies, gram negative bacteria have taken over the gram positive bacteria especially in the hospital settings (4). Gram negative and gram positive bacteria were in a ratio of about 2:1. These results agreed with Nwadioha et al (1) in Nigerian Tertiary Hospital. From this 20 positive blood culture 11 (55.0 %) of 44 male and 9 (45.0 %) of 36 female had positive blood cultures. These results agreed with Tinuade et al (19) in Nigeria and Al-Bayaa (20).

The most common microorganisms causing septicemia in males were shown in Table (1), *Staphylococcus aureus* 4 (36.3 %), *Escherichia coli* 3 (27.3). The study results showed no significant difference (P>0.05). In Table (2) Bacterial isolates according to age group; A (neonates), B (> 28 days to 1 years), C (1 years to < 12 years) were 40.0 %, 30.0 % and 30.0 % respectively. *Staphylococcus aureus* predominated in all the three age groups (A, B and C) 5 (25.0 %). this was followed by *Escherichia coli* 5 (25.0%) These results agreed with Nwadioha et al (1), the higher incidence of septicemia in neonates compared to other pediatric age groups may be related to immaturity of the immune system (21). The study results showed no significant difference (P > 0.05) Table (2).

The infection rate was inversely related to gestational age (GA) 5 (25.0 %) of 49 neonates who were born < 37 weeks of gestation (preterm) septicemia. This declined to 3 (15.0 %) of 31 neonates who were born > 37 weeks of gestation (term). 6 (30.0 %) of 45 neonates who were born by normal vaginal delivery and 2 (10.0 %) of 35 neonates who were born by cesarean section had proven septicemia. These results showed no significant difference (P > 0.05) show in Table (3) and agreed with Karambinand Zarkesh (3).

When cultures are positive and sensitivities available, treatment should be modified to reflect the susceptibility of the isolated organism, the duration of therapy depends on the culture results as well as the initial response to therapy. The antimicrobial sensitivity pattern differs in different studies as well as at different times in the same hospital. This is because of emergence of resistant strains as a result of indiscriminate use of antibiotics (22).

The bacterial isolates and antibiotic sensitivity pattern in children with septicemia in the present study revealed that [Gentamycin, Amikacin, Imipenem] respectively are the most effective against Gram negative bacteria these results agreed with Nwadioha et al (1) in Nigerian Tertiary Hospital and Al-Bayaa (20) show in Table (4) and [Cefotaxime, Amoxicillin+ Clavulanic acid] are the most effective against *Staphylococcus aureus*,

Cefotaxime a third-generation cephalosporin is generally very well tolerated in children these results agreed with Nwadioha et al (1) show in Table (5).

Conclusions: Positive blood cultures were obtained in 20 (25.0 %) of children. The commonest Gram positive bacterial isolate was *Staphylococcus aureus* and Gram negative bacterial isolate was *Escherichia coli* especially in males sex, in neonate age group who were delivered by cesarean section and preterm < 37 weeks gestational age. The antibiotics [Cefotaxime, Amoxicillin+ Clavulanic acid] are the most effective against *Staphylococcus aureus* and [Gentamycin, Amikacin, Imipenem] against Gram negative bacteria.

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