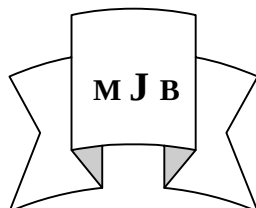


Combination of C-Reactive Protein, Erythro sedimentation Rate and White blood cell for the Detection of Gram Negative Bacteremia in Children under 9 Years Old.

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Abstract

Bacteremia caused by gram negative bacteria isolated randomly from 80 children aging 0-9 years was studied from first of February/ 2008- end of June/ 2008 for their susceptibility profile. The various isolates were *Escherichia coli* (39 isolates, 48.75%), *Pseudomonas aeruginosa* (23 isolates, 28.75%) and *Proteus spp* (18 isolates, 22.5%). Males were more infected (70%) than females. Antibiotics susceptibility pattern of these isolates against 28 different antibiotics revealed that norfloxacin, ofloxacin, tobramycin and amikacin were effective for treatment of gram negative bacteremia. Azithromycin, bacitracin, erythromycin, kanamycin, oxytetracyclin and rifamycin were found to be non effective against isolated organisms. The level of CRP in sera of 71.2% patients was more than 24 mg/dL and this concentration revealed to bacteremia while the value of WBC was > 15000 / mm in 82.5% of patients and ESR was found to be >35mm/h in 100% of patients. In this study we found that the addition of CRP testing to that of WBC and ESR ensure the detecting of bacteremia.

مساهمة بروتين اعادة التنشيط - C ومعدل ترسيب كريات الدم الحمر وعدد خلايا الدم البيض في التحري عن تجرثم الدم المتسبب عن البكتريا السالبة لصبغة غرام عند الاطفال تحت سن التاسعة

الخلاصة

تمت دراسة تجرثم الدم المتسبب عن العزلات السالبة لصبغة كرام المأخوذة عشوائيا من 80 طفلا دون سن التاسعة للفترة من اول شهر شباط / 2008 الى نهاية حزيران / 2008 وتمت دراسة حساسيتها تجاه المضادات الحيوية. وتم الحصول على 39 عزلة (48.75%) من بكتريا *Escherichia coli* و 23 عزلة (28.75%) من بكتريا *Pseudomonas aeruginosa* و 18 عزلة (22.5%) من بكتريا *Proteus spp*. وكانت بكتريا *Escherichia coli* الاكثر شيوعا في احداث التجرثم (وجد ان الذكور اكثر عرضة للاصابة من الاناث بمعدل 70%). اظهرت دراسة حساسية 28 مضاد حيوي تجاه العزلات البكتيرية بان المضادات الحيوية norfloxacin و ofloxacin و tobramycin و amikacin فعالة في علاج تجرثم الدم المتسبب عن البكتريا السالبة لصبغة كرام في حين وجد ان المضادات الحيوية erythromycin و bacitracin و azithromycin و oxytetracyclin و rifamycin غير فعالة تجاه العزلات البكتيرية. كما وجد ان مستوى بروتين اعادة التنشيط C في امصال 71.2% من المرضى كان اكبر من 24 mg/dL وهذا التركيز يشير الى وجود التجرثم الدموي في ان عدد خلايا الدم البيض كان اكبر من 15000/mm في 82.5% من المرضى ومعدل ترسيب كريات الدم الحمر اكبر من 35mm/h في 100% من المرضى. وبهذه الدراسة وجد ان اجراء اختبار بروتين اعادة التنشيط يؤكد الاصابة بتجرثم الدم.

Introduction

Fever is a common presenting symptom in pediatric outpatient's practices and emergency rooms practically in

children < 3 years of age [1]. Childhood bacteremia is defined as the presence of bacteria in the blood stream of a febrile child who was previously healthy; the child does not

clinically appear to be ill and has no apparent focus of infection (occult bacteremia OB) [2,3]. A percentage of children with bacteremia will go on to develop serious bacterial infection [4]. Although information derived from the history and physical examinations are helpful, they don't provide highly sensitive screen for the detection of true bacteremia in children [4]. Although antibiotic treatment is necessary for children with serious bacterial infection (SBI), it is also important to limit therapy to those children at greatest risk. Because the majority of febrile young children do not have SBI, laboratory tests and expectant antibiotic therapy of these children adds to cost, time, discomfort, and parental anxiety and may contribute to antibiotic resistance [5]. Because it is clinically difficult to identify children with occult SBI, a number of diagnostic and management strategies have been suggested and the topic remains a source of considerable debate [5]. Blood cultures remain the gold standard in detecting OB, however, the average time for detection of positive culture is 15-16 hours and may be as long as 24-48 hours increasing the risk of complications [6]. A laboratory predictors identify to date include the white blood cell count (WBC), C-reactive protein (CRP), procalcitonin, erythrocyte sedimentation rate (ESR), and other inflammatory mediators [7]. CRP is an acute phase protein with a well known association with infection and other inflammatory conditions and plays an important role in vivo [8]. Many studies have addressed the value of CRP in differentiating bacterial from viral etiology [9]. The study of CRP levels in patients with true bacteremia or contaminated blood culture were compared [7, 10]. Bacteremia caused by gram negative bacilli remains common world wide, in

large measure this is due to increasing occurrence of antimicrobial resistance [11]. We sought to prospectively study the diagnostic properties of quantitative CRP in comparison with WBC and ESR predictors of bacteremia in children with fever without apparent source of infection and study the causative agents of bacteremia by gram negative bacteria and their susceptibility against antibiotics.

Material and Methods

This prospective study was performed between the first of February/ 2008 until the end of June/2008. Eighty blood samples obtained randomly from children aging 0-9 years presented to Babylon Maternity and Children Hospital with a temperature $>38^{\circ}\text{C}$ were evaluated by residents in Pediatric Emergency House officers. Those children who, after careful history and physical examination, had clinically undetectable source for the fever were enrolled in the study. Demographic information, age and sex, temperature, duration of fever, total WBC, erythrocyte sedimentation rate (ESR), Grams stain and semi-quantitative CRP concentration were recorded. All patients were studied by a blood culture done using standard media and techniques. Blood samples were cultured on aerobic and anaerobic media using nutrient agar and MacConkey agar. Specimens have been analyzed with hemocytometer for WBC counts simultaneously. C-reactive protein test was done by using latex kit from HUMATEX CRP (GERMANY) for qualitative and semi-quantitative determination. In this study we used 28 different antibiotics to estimate the sensitivity of bacteremia causative agents, using disk diffusion agar (Muller Hinton agar). For hemocytometer WBC counts, blood specimens were examined

microscopically on a Neubauer chamber (Neubauer hemocytometer) by the same technician. Bacterial isolates were identified to the level of species using cultural characteristic and conversional biochemical tests [12,13].

Results and Discussion

Eighty children were enrolled in this study ranging in age from 0-9 years. *E. coli* was the causative organism of bacteremia in 39 cases (48.75%) and *Pseudomonas aeruginosa* was the organism recovered from 23 cases (28.75%) of bacteremia while 18 cases

(22.5%) only were caused by *Proteus* species (Figure 1) and the result is compatible with the results of other study which found that *E. coli* was the causative agent of bacteremia in 32.1% and 7.1% for *Pseudomonas aeruginosa* [7]. *E. coli* is usually the most common blood culture isolate in community acquired infections [14]. High case fatality rates have characterized cases associated with *Pseudomonas aeruginosa* [15]. A French study found that 69% of intensive care units (ICU) associated bacteremias were due to Gram negative bacilli [16].

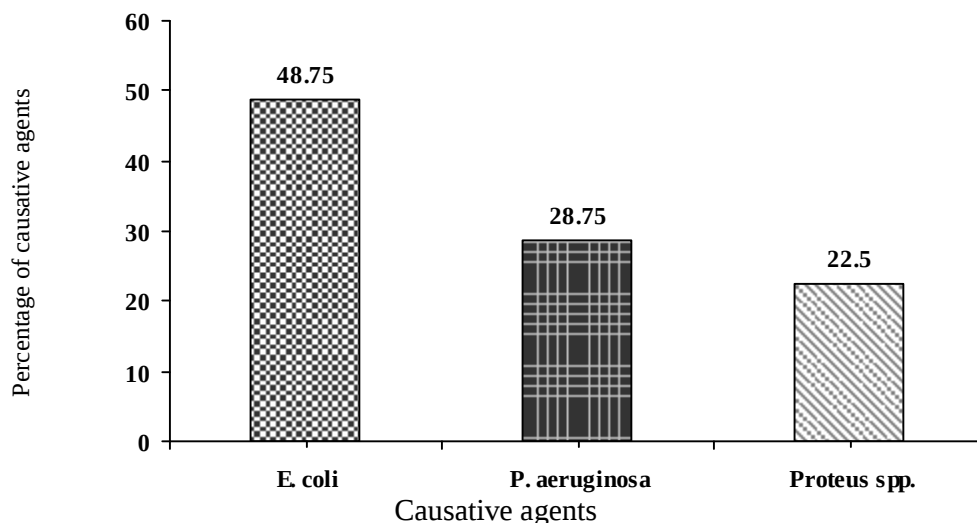


Figure 1 Percentage of Etiological agents causing bacteremia in children (0-9)years old.

Table 1 shows the distribution of bacteremia infection according to age and gender and frequency of bacteremia causes, the results showed that bacteremia were more in children < 1 year than in other age group. The study showed that bacteremia was

more in males children than females children and there was a highly significant relation between *E. coli* bacteremia and age factor ($P < 0.01$) while was not significant the relation between age factor and *P. aeruginosa* and *Proteus spp* infection.

Table 1 Age and gender-wise distribution and frequency of bacteremia causes isolated from children under 9 years old ($p < 0.01$).

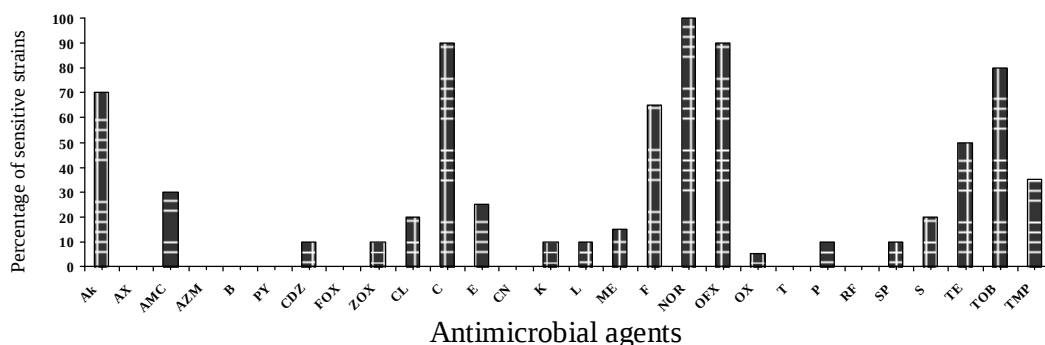
Age group (Year)							Total
	<i>Escherichia coli</i>		<i>Pseudomonas aeruginosa</i>		<i>Proteus species</i>		
	Male	Female	Males	Females	Males	Females	
<1	12	5	12	3	2	5	39
1-3	4	-	-	2	4	-	10
4-6	3	3	3	-	-	-	9
7-9	12	-	1	2	3	4	22
Total	31	8	16	7	9	9	80
	39		23		18		

There are no known sex-based differences existing in the prevalence or course of bacteremia [17]. In a study on bacteremia in children found that it was more prevalent among male patients than female (64.9% versus 35.1%) [18]. The age as a predictor of bacteremia and the incidence of bacteremia proportionally increases with the process of age, children age 1-36 months were found to be at an increased risk of clinically undetectable serious bacterial infection, Variations in age-based risk are dependent on the infecting organism [5].

The results of susceptibility test of antibiotics showed that bacteremia isolates were sensitive to norfloxacin, Amikacin, ofloxacin and tobramycin, while most isolates were more resistant

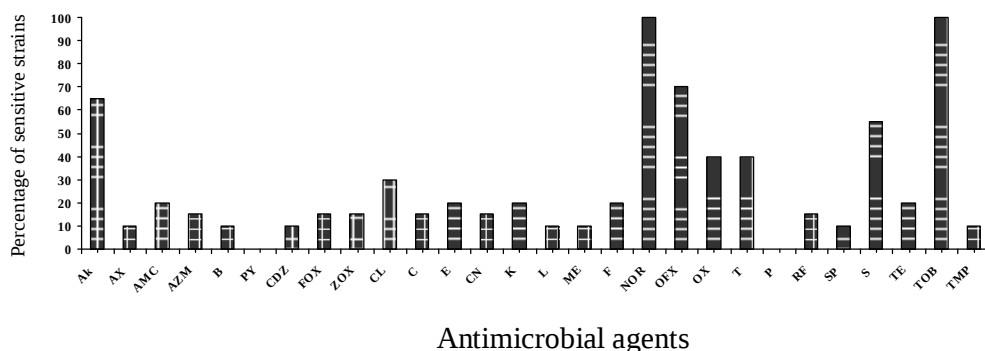
to azithromycin, The remaining antibiotics showed different degrees of susceptibility Figure 2,3,4. *E. coli* was found to be resistant to amoxillin, azithromycin, bacitracin, carpenicillin, cefoxitin, gentamycin and oxytetracyclin Figure 2. *Pseudomonas aeruginosa* showed high degree of resistance to carpenicillin (100% of strains) Figure 3.

Proteus spp. isolate was found to be sensitive to almost all antibiotics except azithromycin and lincomycin (Figure 4). Other study by Gupta *et al.*, 2002 antibiotics susceptibility pattern of these isolates revealed were affected by cephalosporin, norfloxacin, nitrofurantion and ciprofloxacin while trimethoprim was found to be not effective [21].



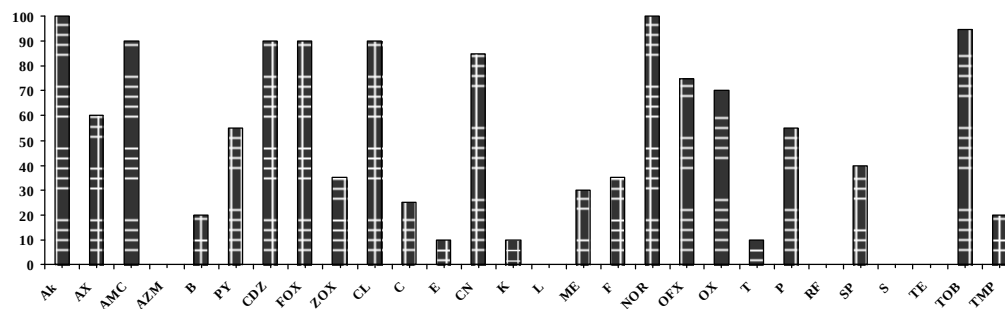
AK \Amikacin, AX \Amoxillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol E \Erythromycin, CN\Gentamycin, K\Kanamycin, L\Lincomycin, ME\Methicillin, F\Nitrofurantoin, NOR\Norfloxacin, OFX \Ofloxacin, OX\Oxacillin, T\Oxytetracyclin, P\Penicillin G, RF \Rifamycin, SP\Spiramycin, S\Streptomycin, TE\Tetracycline, TOB\Tobramycin, TMP\Trimethoprim.

Figure 2 Antimicrobial potency and spectrum for 28 selected antimicrobial agents tested against *E. coli*



AK \Amikacin, AX \Amoxillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol E \Erythromycin, CN\Gentamycin, K\Kanamycin, L\Lincomycin, ME\Methicillin, F\Nitrofurantoin, NOR\Norfloxacin, OFX \Ofloxacin, OX\Oxacillin, T\Oxytetracyclin, P\Penicillin G, RF \Rifamycin, SP\Spiramycin, S\Streptomycin, TE\Tetracycline, TOB\Tobramycin, TMP\Trimethoprim.

Figure 3 Antimicrobial potency and spectrum for 28 selected antimicrobial agents tested against *P. aeruginosa*



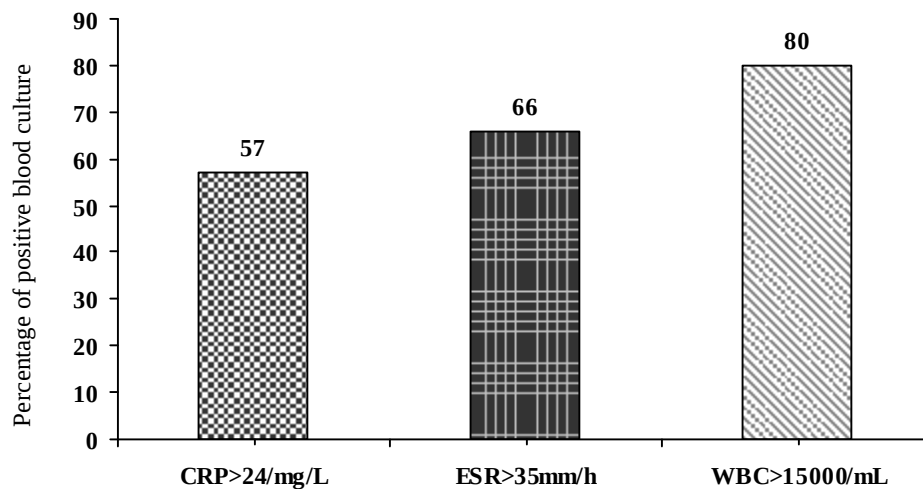
Antimicrobial agents

AK \Amikacin, AX \Amoxillin, AMC \Amoxicillin + Clavulanic acid, AZM \Azithromycin, B \Bacitracin, PY \Carbenicillin, CDZ \Cefodizime, FOX \Cefoxitin, ZOX \Ceftizoxime, CL \Cephalexin, C \Chloromphenicol E \Erythromycin, CN\Gentamycin, K\Kanamycin, L\Lincomycin, ME\Methicillin, F\Nitrofurantoin, NOR\Norfloxacin, OFX \Ofloxacin, OX\Oxacillin, T\Oxytetracyclin, P\Penicillin G, RF \Rifamycin, SP\Spiramycin, S\Streptomycin, TE\Tetracycline, TOB\Tobramycin, TMP\Trimethoprim.

Figure 4 Antimicrobial potency and spectrum for 28 selected antimicrobial agents tested against *Proteus spp.*

Figure 5 shows the value of diagnostic test in which the CRP level was more than 24 mg/dL in 57 patients (71%) of all patients' studies, and ESR was more

than 35 mm/h in 66 patients (80%) while WBC was found to be more than 15000/ mL in each patient with positive blood culture.



Diagnostic test

Figure 5 The percentage of diagnostic tests in presence of a positive blood culture

This result agrees with another study which found the value of CRP above 30 mg/L for acute gastroenteritis and acute otitis media compared with the value of WBC which was found more than 15000mm³/L [7]. In another study used WBC, ESR and CRP to distinguish a SBI in infants showed that non of these parameters alone commonly used by physicians is a reliable diagnostic tool to rule out SBI in infant with fever without source of infection and according to this study the value of WBC > 15000, ESR> 20 mm/h and CRP= 2 mg/dL [21]. The value of CRP in this study appears to be higher in most children and the other who has low level of CRP may be treated with antibiotic before the study and this led to decreased level of CRP in the sera of patients[24]. Because of level of CRP remain elevated with on going inflammation and tissue destruction, but with resolution they decline rapidly because of relatively short half-life of 4-7 hours [22].

Conclusion

In this study we have found that the elevation of CRP concentrations is non completely sensitive alone for detecting infection in patients with bacteremia.

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