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Bacterial Etiology of Occult Bacteremia in Febrile Children (3–36 Months) in Southwest Iran Using Blood Culture and PCR

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Abstract

Background and Objective: The aim of this study was to investigate the common causes of bacterial fever in children aged 3 to 36 months suspected of occult bacteremia using blood culture and PCR methods.

Methods: We analyzed 120 children referred to Aboozar Hospital of Ahvaz, Iran. Blood samples were subjected to culture and PCR, and demographic and laboratory data (WBC, ESR, CRP) were recorded.

Findings: Blood culture result was positive in two patients (1.7%). The number of patients with definite positive PCR was 20 (16.66%). There was no significant difference between the mean values of WBC and neutrophil in positive and negative bacteremia. Mean values of ESR in bacterial positive cases were significantly higher compared with the negative ones. The percentage of patients with a history of antibiotic use was higher in the positive bacteremia group (55%) compared to the negative bacteremia group (46%), but no significant difference was observed.

Conclusion: While laboratory markers like WBC and neutrophil counts have limited accuracy in predicting bacteremia, the PCR method offers a more reliable alternative for diagnosing causative organisms. This is particularly relevant given the clinical presentation of patients, where traditional lab values may not be sufficient for diagnosis.

Keywords*:* Occult bacteremia, PCR, Blood culture, FWLS

1. Introduction

Fever without Localizing Sign (FWLS), is a diagnostic challenge in children less than 36 months. When a child has a fever for less than a week, the history and clinical examination do not identify the cause, and the central temperature is the sole outward sign, known as FWLS (1). Although viral infections are the most common cause in this age group, in some circumstances, rapid diagnosis of severe bacterial infections (SBIs), such as sepsis, bacterial meningitis, pneumonia, urinary tract infections, bacterial gastroenteritis, and septic arthritis, should be taken into consideration, as these conditions require immediate treatment with antibiotics (2). Children with FWLS are typically classified into three age groups: 0–1 month, 1-3 months, and 3–36 months (3). Occult bacteremia (OB) is defined as positive blood culture in a child with FWLS (4). The prognosis for newborns with FWLS is favorable, and many cases are self-limited (5). However, approximately 1% to 30% of patients will experience a serious bacterial illness (6). Rapid identification of bacteremia in FWLS children is crucial for reducing pediatric mortality because no treatment for bacteremia can prevent serious issues and severe complications (7).

Given that most febrile diseases in children aged 3 to 36 months are viral in origin, empiric antibiotic therapy is not warranted in cases of suspected bacteremia until blood culture results are available (8).

Streptococcus pneumonia (S pneumoniae), Haemophilus influenza (H. influenza), Neisseria meningitides (N. meningitides) and *Salmonella* species are common causes of bacterial pathogens responsible for occult bacteremia (9). Some studies have also identified *Staphylococcus aureus* as a significant pathogen in children under three years of age (10). Against this background, the aim of this study was to evaluate the most prevalent bacterial causes of fever in FWLS children aged 3 to 36 months with suspected bacteremia using culture and PCR method.

2. Material and Methods:

This cross-sectional study was conducted between July 2018 and July 2019 at Abuzar Children's Hospital, the sole pediatric referral hospital in Ahvaz, Khuzestan. The study aimed to identify the bacterial cause of occult bacteremia in infants between 3 and 36 months of age who were suspected of having a fever without a localizing source. Infants were included in the study if they had been hospitalized with a diagnosis of occult bacteremia from a specialist doctor and had a temperature above 38°C. Additionally, they could not have any localized abnormalities on examination and had to have a white blood cell count over 15,000, a positive C-reactive protein test, and an erythrocyte sedimentation rate above 20. Patient demographic and clinical data were collected from parent questionnaires and medical records. The information gathered included age, gender, the child's general health, fever severity, nonspecific symptoms like anorexia, fever duration, and prior antibiotic use. Laboratory values for white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) levels were also obtained.

2.1. Ethical statement

The study design was approved by the Research Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Iran (IR.AJUMS.REC.1395.252). Written informed consent was also obtained from the parents of all the included children.

2.2. Culture

Before starting antibiotics, 10 to 15 ml of venous blood was drawn from the study subjects, and the blood was then placed into bottles containing blood culture media. The samples were incubated at 37°C for 48 hours. Subsequently, 0.5 ml of the culture media was inoculated onto blood agar, MacConkey agar, and chocolate agar plates. Chocolate agar plates were incubated in a candle jar at 37°C, while blood agar and MacConkey agar plates were incubated under standard aerobic conditions for 24 hours (11). After 24 hours, the plates were examined, and in case of bacterial growth, gram staining was done to evaluate the morphology of the bacteria. Finally, tests such as catalase and coagulase, along with biochemical tests were conducted to identify the genus and species of bacteria. Cultures without visible growth were re-incubated for up to 7 days and re-examined if turbidity appeared (11).

2.3. Polymerase Chain Reaction (PCR)

For molecular detection, 0.5 ml of blood was collected into EDTA tubes (12). Following the manufacturer's instructions, DNA was extracted using a commercial kit (QIAamp DNA Mini Kit, Qiagen, Hilden, Germany). The acquired DNA was evaluated immediately or put into storage at -80 °C until needed. For the PCR process, 5µl of template DNA was used. Primers targeting *S. pneumoniae* and *H. influenza* were selected based on Park et al. (13). Primers for *N.meningitides*, *Salmonella* and *S. aureus* were used as described in other studies (14-16). In addition, *S. aureus* (ATCC 29213) and

Salmonella enterica (ATCC 35640), *S. pneumoniae (*ATCC 6301), *H. influenzae* (ATCC 35056) and *N. meningitidis* (ATCC 1037) were used as positive control.

2.4. Statistical analysis

The obtained data were analyzed by descriptive statistics including frequency, mean, and standard deviation along with graphs and statistical tables. The normality of the data was evaluated by Kolmogorov-Smirnov test and the homogeneity of variances was evaluated by Leven test. T-test was used to assess the significance of the differences. A P-value < 0.05 was considered statistically significant. SPSS version 22 was used for statistical analysis.

3. Results

2.5. Demographic characteristics

A total of 120 blood samples from children aged 3 months and 3 years with suspected bacteremia were collected. The mean age was 19.1 to 10.4 months. The age distribution was as follows: 30.8% of patients were under 3 years old, 31.6% were between 13 and 24 years old, and 37% were between 25 and 36 years old. The patient population was 50.8% male and 49.2% female. Although a higher proportion of patients with a history of antibiotic use had positive bacteremia, this difference was not statistically significant ($p > 0.05$). Demographic, clinical and laboratory characteristics of patients are shown in Table 1.

Table 1. Demographic, clinical, and laboratory characteristics of patients

Parameter	Total Sample (N=120)	PCR Positive group (N=20)	PCR Negative group (N=100)	P value
Gender (Male/Female) (n)	61/59			
Median age (months)	19.1± 17.41			
History of Antibiotic use		11 (55%)	46 (46%)	0.52
Clinical symptoms		N (%)	N (%)	
Body Temperature (C)		38.99±0.63C	38.83±0.6C	0.27
Duration of fever (hours).		24.00±5.51	31.44±4.55	0.303
WBC Count		20047.50±5594.77	19176.50±4106.16	0.42
ESR		56.55±23.16	39.36±12.36	0.0001
Neutrophil Count		80.1±8.22	82.67±10.72	0.21
CRP1+		4(20%)	71(71%)	0.012
CRP3+		9(45%)	5(5%)	0.01

2.6. Blood Culture Findings

Only two individuals (1.6%) had positive blood culture results, both yielding *S. aureus*. There was a significant difference in the number of patients with positive and negative blood culture results, with the latter group having a disproportionately higher number of patients (P 0.05).

2.7. PCR Detection of Bacteremia

According to analysis, 20 (16.6 %) of the blood samples were PCR positive. The difference between PCR-positive and PCR-negative groups was statistically significant ($p<0.05$). Among the five pathogens screened, only two bacteria—*Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumonia* (*S. pneumoniae*)—were identified as causes of bacteremia in PCR-positive cases (Figure1).

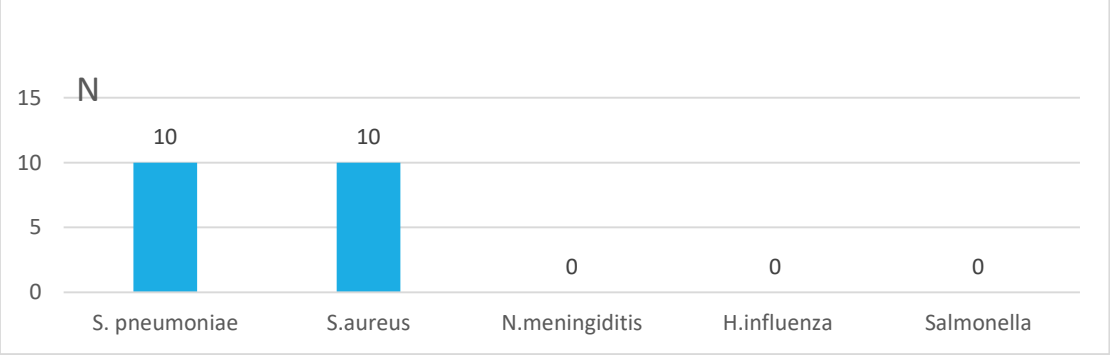

Figure 1. Frequency of children suspected of occult bacteremia according to their PCR results

2.8. Clinical Findings:

Temperature: According to our findings, the mean body temperature did not significantly differ between bacteremia-positive and bacteremia-negative patients. However, fever >39°C was more prevalent in PCR-positive cases than in PCR-negative cases (Table 2).

Table 2. Relationship between body temperature and positive and negative cases of bacteremia

PCR	*Temp.*	N (%)	P-value
Positive (n= 20)	***<39***	7 (35%)	**<0.0001***
	≥ 39	13 (65%)	
Negative (n= 100)	***<39***	51 (51%)	
	≥ 39	49 (49%)	

WBC Count: The mean white blood cell (WBC) counts in patients with positive and negative PCR did not differ significantly.

ESR levels: The mean ESR values in PCR-positive patients were substantially higher than those in PCR-negative cases (Table 3).

Table 3. Correlation between laboratory parameters (WBC and ESR) and positive cases of bacteremia

Variables	PCR	Mean±SE	P-value
WBC	Positive	20047.50±5594.77	0.42
	Negative	19176.50±4106.16	
ESR	Positive	56.55±23.16	<0.0001*
	Negative	39.36±12.36	

CRP Analysis: According to the findings in Table 4, all participants had elevated C-reactive protein (CRP) levels. In positive PCR cases, CRP +3 was most frequent (45%) and in negative PCR cases, CRP +1 was most frequent (71%). The distribution of patients based on CRP +3, +2, +1 in the two groups with positive and negative PCR also showed a significant difference (Table 4).

Table 4. Correlation between CRP and positive cases of bacteremia

PCR	CRP	N (%)	P-value
Positive	1+	4 (20%)	**0.012***
	2+	7 (35%)	
	3+	9 (45%)	
Negative	1+	71 (71%)	
	2+	24 (24%)	
	3+	5 (5%)	

3. Discussion

Before the development of conjugate vaccines, occult bacteremia was present in 3 to 5% of children aged 3 to 36 months without an identifiable source of infection (fever without localizing signs or FWLS) (17). *S. pneumoniae* was responsible for the vast majority (80%) of occult bacteremia that occurred before standard conjugate immunization. *H. influenzae* type b caused a smaller percentage (10%), while *N. meningitidis* was responsible for an even smaller percentage (5%) (18).

Occult bacteremia is a serious clinical problem since 5 - 10% of the affected children may develop severe bacterial infections (SBIs) including septic arthritis, osteomyelitis, sepsis, meningitis, and urinary tract infections (UTI). Early detection and treatment of bacteremia could reduce these infections (19). Currently, conjugated polysaccharide vaccinations against *S.pneumoniae* are not routinely administered to newborns in Iran; however, over the past few years, *H. influenzae* has been added to the list of baby injectable vaccines.

In our investigation, only two patients (1.7%) had positive blood culture results, and both were identified as *S. aureus.* Our results are in accordance with those of Gomez et al (9), who reported positive blood culture in only 1.5% of the febrile children younger than 5 years with suspected occult bacteremia. In a different investigation, blood cultures performed on 28% of febrile kids with unexplained causes yielded positive results (7). Importantly, in our study, there was a notable difference between the results of blood cultures and PCR. While blood cultures identified positive results in just two cases (1.7%), PCR results showed bacteremia in 20 (34.2%) of the patients. This stark difference highlights both the potential limitations of blood culture—such as sampling errors—and the higher sensitivity of PCR for microbial detection.

S. pneumoniae and *S. aureus* were the two most frequently found isolated microbes (8.3 %). The widespread immunization against *H. influenzae* in Iran in recent years may partially explain the absence of *H. influenzae* in our current study's samples.

The percentage of patients with positive PCR results (34.2%) was substantially lower than that of patients with negative PCR results (65.8%) (P <

0.05), indicating that viral causes are more likely to be the primary cause of the incidence.

In a study conducted in Kuwait by Elhassanien et al, occult bacteremia was found in 19.2% of patients. *Klebsiella pneumoniae, S. pneumoniae, Serratia marcescens*, and *Citrobacter freundii* were identified as the causative agents of occult bacteremia (18). All participants in our study had received only the basic immunizations included in Iran's national immunization schedule according to their age. It should be noted that vaccination with pneumococcal vaccine and *N. meningitides* has not yet been included in the national vaccination program for children in Iran. However, the risk of bacteremia from other non-vaccine-preventable pathogens persists, including S. aureus, E. coli, Acinetobacter spp., and non-typhoidal Salmonella spp. The impact of vaccination is clear, as the inclusion of the H. influenzae type B vaccine in Iran's childhood and adolescent vaccination schedule has led to a documented reduction in cases of bacteremia and meningitis caused by this bacterium (20). As mentioned in the results, none of the samples showed this bacterium.

Interestingly, while average fever duration and temperature did not significantly differ between patients with and without bacteremia. However, fevers ≥39°C were more common in bacteremia-positive cases ($P < 0.05$). This supports previous findings that high-grade fever may be a predictor of occult bacteremia(10). Regarding laboratory results, the mean WBC counts in positive and negative cases of bacteremia did not significantly differ. However, the mean ESR values in positive cases of bacteremia were significantly higher compared with negative cases ($P<0.05$). The findings of the present investigation were in agreement with those of Gomez et al showing that WBC is not a reliable predictor of the occurrence of bacteremia (21).

Considering that all patients in the study had a positive CRP test, the primary analysis compared the distribution of patients across three categories of CRP levels (+3, +2, and +1) between the PCR-positive and PCR-negative groups. This comparison was conducted to examine if there was a correlation between the degree of CRP elevation and a patient's PCR status. In positive PCR cases, patients with +3 CRP (45%) had the highest frequency compared to others, and in negative PCR cases, the highest frequency was related to patients with +1 CRP (71%). Also, there was a significant difference between the distribution of patients based on CRP +3, +2, +1 in two groups with positive and negative PCR ($P<0.05$).

Although the proportion of patients with a history of antibiotic use was higher in bacteremia-positive cases than in negative cases, the difference was not statistically significant ($P > 0.05$).

The level of neutrophils in positive cases of bacteremia was lower than that in negative cases, but this difference was not significant either ($P<0.05$). This is consistent with research by Gomez et al. which concluded that neutrophil count is not a good predictive factor for the presence of bacteremia (21).

Based on the PCR results in our study, *S. pneumoniae* and *S. aureus* were the two causes of positive bacteremia cases, and other common microorganisms such as *H. influenzae* and *N.meningitidis* were not among the common causes. Notably, empirical treatment for occult bacteremia often involves third-generation cephalosporin which may not adequately cover these organisms. Our findings suggest that including antibiotics with better *Staphylococcus aureus* coverage, such as first-generation cephalosporins, might improve outcomes in some cases.

A key finding of this study is the superiority of PCR over blood culture for diagnosing bacteremia. The blood culture method identified only two positive cases, whereas the PCR method detected 20 positive cases. This significant difference demonstrates
the higher sensitivity of PCR in identifying microorganisms.

Based on these findings, since conventional laboratory markers like WBC, neutrophil count, and CRP (as measured qualitatively) were not effective predictors of bacteremia, the PCR method appears to be a superior alternative for identifying the causative organisms. Our findings suggest that diagnosing bacteremia should integrate PCR data with the patient's clinical history and disease progression. We suggest that an anti-Staphylococcus aureus antibiotic be added to the empirical treatment regimen for suspected cases of occult bacteremia, especially when patients do not show a positive clinical response to ceftriaxone. We also recommend further research in this area. Determining more accurate factors to predict occult bacteremia and measure its relationship with responsible bacterial microorganisms needs more studies. One of the main limitations of this study was the small sample size, which may have affected the statistical power of the findings. In addition, the absence of PCR gel electrophoresis images limited the ability to visually validate the amplification results.

4. Conclusion

Although blood culture is the standard method for detecting occult bacteremia, our findings suggest that the PCR method is a superior alternative for identifying the causative organisms, as it requires less specialized laboratory skill. Given the microorganisms identified in this study—including *S. pneumoniae* and *S. aureus*—the use of antibiotics with better anti-staphylococcal coverage appears to be a more effective treatment strategy. When examining laboratory factors, ESR and CRP were significantly higher in patients with positive bacteremia compared to those with negative bacteremia. However, other conventional markers like elevated WBC and neutrophilia did not effectively predict bacteremia. This suggests that using WBC count or neutrophilia as a sole criterion for initiating empirical antibiotic treatment in cases of fever without a source or occult bacteremia may not be appropriate. Further research is needed to identify more accurate predictive markers to optimize empirical treatment strategies for occult bacteremia.

Footnotes

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Conflict of Interests Statement

The authors declare no conflict of interest. Data Availability: All data generated or analyzed during this study will be available from the corresponding author on reasonable request.

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All data generated or analyzed in this study are available from the corresponding author upon reasonable request.

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Ethical Approval

The study was approved by the Ethical Committee of Ahvaz Jundishapur University of Medical Sciences, Iran. IR.AJUMS.REC.1395.252).

Authors' Contribution

A. Sh. And R.N. developed the study concept and design. Z.R. acquired the data. B.Ch., A. Kh and M.A analyzed and interpreted the data, and wrote the first draft of the manuscript. All authors contributed to the intellectual content, manuscript editing and read and approved the final manuscript. M.A. and A.Sh. provided administrative support.

Informed Consent

Informed consent was obtained from the participants.

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