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Diagnostic Value of 16S rRNA Gene by PCR for Rapid Laboratory Diagnosis of Sepsis in Children

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Authors' contributions

This work was carried out in collaboration between all authors. Author MESZ designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors NAEK and MM managed the analyses of the study. Author BED performed the statistical analysis and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Sepsis in children is a common health problem. Rapid laboratory diagnosis improves its prognosis by adequate therapy.
Aim: The objectives of the present study were to compare the use of blood culture with detection of 16S rRNA by PCR for detection of bacterial pathogens in children with clinically suspected sepsis below the age of 5 years and to correlate the laboratory findings of 16S rRNA by PCR with the risk factors for sepsis in those patients.
Materials and Methods: The study included 100 consecutive children below 5 years who were suspected to have sepsis on clinical basis. Blood samples were obtained from each child for blood culture by Bact/alert system, CRP and16S rRNA gene PCR amplification.
Results: In the present study all positive blood cultures samples were positive by 16S rRNA PCR. However, there was one negative sample by blood culture that was positive by PCR. The sensitivity,

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specificity and accuracy of 16S rRNA PCR compared to blood culture were 100%, 95.5% and 99% respectively.
Conclusion: The present study highlights the advantages of 16S rRNA PCR for rapid laboratory diagnosis of sepsis in children compared to blood culture. The association of positive results with risk factors of sepsis may be used as a guide for in rapid diagnosis in high risk patients.

Keywords: 16S rRNA PCR; sepsis; blood culture; children.

1. INTRODUCTION

Sepsis in children below 5 years represents a major health threat especially in limited resources countries. Sepsis and its complications like septic shock are the final pathways of infections with deregulations of the immune system resulting in end organ failure [1-3]. The core of treatment of such condition relies on accurate laboratory diagnosis for commencement of antibiotics therapy.

The principle of the diagnosis relies on laboratory techniques as the clinical diagnosis is hardened by non-characteristic signs [4]. The gold standard technique for diagnosis of sepsis is by detecting the pathogen by blood culture. However, blood culture has several limitations as it needs prolonged time for confirmation of the results even with automated blood culture systems and lacks sensitivity as it may yield negative results due to the low quantity of microbes in the blood [5]. Usually, empiric broad spectrum antibiotics are used in suspected conditions of sepsis which reduce the sensitivity of blood culture and results in emergence of antibiotics resistance [6]. Several biomarkers have been used as an adjuvant for the laboratory diagnosis of such condition such as C-reactive protein (CRP), interleukin (IL)-6, and procalcitonin (PCT) [7,8].

The advances of molecular techniques have made it possible for rapid laboratory diagnosis of sepsis within hours which decreases the use of empirical antibiotics [5,9,10]. Among molecular method, the detection of 16S rRNA for wide range of bacterial species by polymerase chain reaction (PCR) has been proved to be sensitive and valuable tool especially in newborn with empirical antibiotics intake and for non-cultivable pathogens [11,12].

Therefore the aims of the present study were i: to compare the use of blood culture with detection of 16S rRNA by PCR for detection of bacterial pathogens in children with clinically suspected sepsis below 5 years of old and to ii: correlate the laboratory findings of 16S rRNA by PCR with the risk factors of development of sepsis in those patients.

2. MATERIALS AND METHODS

The study was conducted on Mansoura University Children Hospital, Egypt. The study subjects included 100 consecutive children below 5 years who were suspected to have sepsis on clinical basis from January 2017 till May 2017. The study was approved by Mansoura faculty of medicine ethical committee and consents were obtained from the parents of each child. Complete clinical data for each child was reported.

Five milliliter blood samples were obtained from each child for blood culture by Bact/alert system (Biome ´rieux, Durham, NC, USA), CRP by turbid meter and 16S rRNA gene PCR amplification. Pediatric blood culture bottles were inoculated by 2 milliliter blood and the remaining three milliliter were divided into three tubes one for sera separation for CRP and one milliliter over EDTA for DNA extraction for molecular study and one over EDTA for total leucocytes count.

Blood cultures were processed according to the standard microbiological techniques [13]. Bacterial isolates were identified by gram stain and complete biochemical identifications processes.

2.1 16S rRNA Gene PCR

2.1.1 DNA extraction

DNA extraction was performed from EDTA blood samples by the use of Qiagen extraction kit for whole blood samples (Qiagen). The extracted DNA was kept frozen at -20ºC till time of the amplification.

2.1.2 Steps of 16S rRNA gene PCR

The amplification of 16S rRNA by PCR was performed by the use of two pairs of primers; their sequences and the amplifications products size were summarized in Table 1.

Table 1. The sequences of the used primers and their bp products

	Sequence	bp
Primer Pair 1	PSL(f):5′-AGGATT AGA TAC CCT GGT AGT CCA-′3	630 bp
	UN1(r): 5′-AGG CCC GGG AAC GTA TTC AC-′3	
Primer Pair 2	P11P(f): -5′GAG GAA GGT GGG GAT GACGT-′3	216 bp
	P13P(r): 5′-AGG CCC GGG AAC GTA TTC AC-′3	

All DNA extracts were examined by amplification of the 16S rRNA gene using two pairs of universal primers [14]. The amplification mixtures (Qiagen) were performed in total volume 50 microns with 2 microns of the extracted DNA. Two rounds of PCR were carried for each pair of the primer with similar steps. The amplifications steps were denaturing step for 5 minutes at 94ºC, followed by 30 cycles of 1 minute at 9ºC, 1 minute at 55ºC, and 1 minute at 72ºC; a final extension cycle of 72ºC for 10 min. Sterile water, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923 were used as the negative and positive controls, respectively.

Separate dedicated pipetting devices with filter-sealed tips were used for each procedure. PCR results were considered positive when visible PCR products of the correct size were found simultaneously in two reactions.

2.2 Statistical Analysis

Data were collected, revised, coded and entered to the statistical package for social sciences (SPSS) version 24. The quantitative data were presented as mean, standard deviations and ranges. The comparison between the studied groups was done by using One Way Analysis of Variance (ANOVA). Area under the ROC curve (AUC) was used as an accuracy index for evaluating the diagnostic performance of CRP and total leucocytes counts compared to blood culture.

3. RESULTS

There were 100 children with clinical evidence of sepsis with mean age± SD 4.1±0.9 years, 54 males and 46 females. The outcome was discharge of 70% and death in 30% of them. Blood cultures were positive in 79% and 16S rRNA PCR was positive in 80%, Table 2.

The most common isolated bacterial species were *Staphylococcus* species and *Klebsiella pneumonia* for 32.9% for each followed by *E. coli* (18.9%) and *Enterobacter* spp. (15.2%). The most common *Staphylococcus* species was *S. aureus* (13.9%), Table 3.

Table 2. Demographic, clinical and laboratory findings in patients

Age –years (mean± SD)	4.1± 0.9
Gender	
Male	54 (54%)
Female	46 (64%)
Outcome	
Death	30 (30%)
Blood culture	79 (79%)
16S rRNA PCR	80 (80%)
CRP (mg/L)	
Minimum	6.0
Maximum	96.0
median	12.00
Total leucocytic countsx10^3/L (mean± SD)	13.6± 6.7

Table 3. Bacterial isolates from positive blood cultures

	N0.	%
Staphylococcus species	26	32.9%
S. aureus	11	13.9%
S. haemolyticus	5	6.3%
S.capitis	3	3.1%
S. lugdunensis	4	5.1%
S.scuiri	3	3.1%
Klebsiella pneumonia	26	32.9%
Enterobacter spp.	12	15.2%
E.coli	15	18.9%
Total	79	100%

The findings of the present study was that all positive blood cultures samples were positive by 16S rRNA PCR. However, there was one negative sample by blood culture that was positive by PCR, Table 4.

The sensitivity, specificity and accuracy of 16S rRNA PCR compared to blood culture results were 100%, 95.5% and 99% respectively, Table 5.

Table 4. Comparison of 16S rRNA PCR with blood cultures

		Culture				Total
		Negative		Positive		
		No.	%	No.	%	
16S rRNA PCR	Negative	20	100%	0	0%	20 100%
	Positive	1	1.3%	79	98.7%	80 100%
Total		21	100%	79	100%	100 100%
		P=0.0001				

Table 5. Diagnostic evaluation of 16S rRNA PCR compared to blood culture

	Sensitivity	Specificity	Positive predicative value	Negative predictive value	Accurracy
16S rRNA PCR	100%	95.5%	98.8%	100%	99%

Table 6. Comparison of some demographic and risk factors with 16S rRNA PCR results

	16S rRNA PCR				P
	Positive		Negative		
	No.	%	No.	%	
Gender					P=0.3
Male	45	45.0%	9	9.0%	
Female	34	34.0%	12	12.0%	
Age	3.7± 1.2		3.6± 0.4		P=0.4
Blood transfusions	9	9.0%	12	12.0%	P=0.5
Immunocompromized conditions	59	59.0%	21	21.0%	P=0.002
Intravenous cannula	62	62.0%	21	21.0%	P=0.0001
Urinary Catheter	66	66.0%	0	0%	P=0.0001

The positive results of 16S rRNA PCR were associated with presence of risk factors for sepsis in children such as the presence of immunocompromized conditions (59.0%, P=0.002), the use of intravenous cannula (62.0%, P=0.0001) and the presence of urinary catheter (66.0%, P=0.0001), Table 6.

Receiver operative curve was plotted for CRP and TLC compared to blood culture results. Cut of value of CRP was 14.5 mg/L with sensitivity 78.5% and specificity 67.7%. Total leucocytes counts $11.5x10^3$/L had sensitivity 53.2% and specificity 62%, Table 7 and Fig. 1.

4. DISCUSSION

Blood culture for the diagnosis of sepsis is considered a gold standard. Moreover, the bacterial growth is used for determination antibiotics susceptibility. However, it has some drawbacks for its use as the need for extended duration of culture, it detects only organisms that can be cultured and need high volume and to be repeated. On the other hand, molecular techniques such as PCR take short time to detect sepsis even after empirical antibiotic start [15].

Staphylococci species, *Klebsiella pneumonia, E.coli and Enterobacter spp* were the isolated bacteria from blood culture. The bacterial pathogens responsible for sepsis in children in previous studies were more or less similar to these finding [16,17]. Thus, the main bacterial etiology of sepsis in the present study can be detected by the used 16SrRNA PCR.

The comparison between 16S rRNA PCR and blood culture in children with sepsis were factorable. The sensitivity, specificity and accuracy of 16S rRNA PCR compared to blood culture results were 100%, 95.5% and 99% respectively. This is in line with previous study by Liu et al. The excellent negative predictive value of 16SrRNA PCR suggests that it can be a rapid diagnostic technique with confirmed results within 4-8 hours for eliminating diagnosis of

Table 7. Area under ROC curve for CRP and TAC

Area under the curve					
Test result variable(s)	Area	Std. error[a]	Asymptotic sig.[b]	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
CRP	.839	.049	.000	.743	.934
TAC	.538	.062	.597	.416	.659

The test result variable(s): CRP, TAC has at least one tie between the positive actual state group and the negative actual state group. Statistics may be biased.
a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

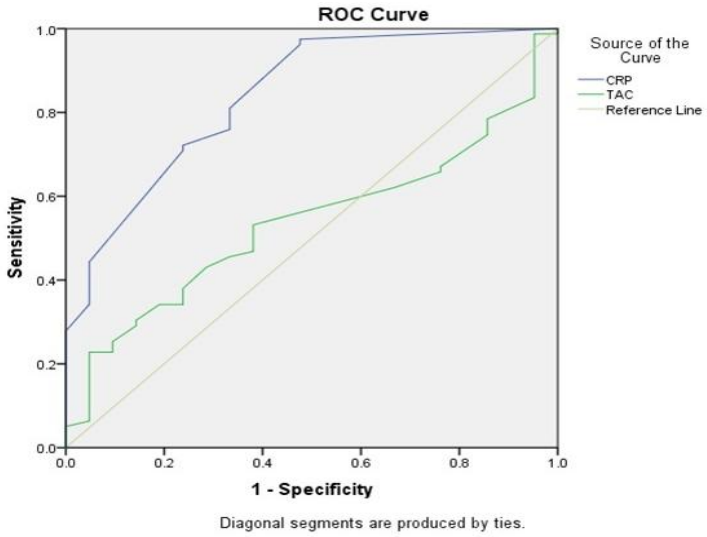

Fig. 1. Receiver operative curve for CRP and TLC compared with culture

bacterial sepsis [18,19] thus avoiding the unnecessary antibiotics use. The single positive sample by 16SrRNA PCR that was negative by blood culture can be explained by detection of fastidious bacterial species such as *S. pneumonia* which provide a diagnostic challenge that can result in accurate diagnosis of sepsis [18].

Assumption has been made those total leucocytes counts may be used for rapid diagnosis of sepsis in children. However, in the present study there was reduced sensitivity (53.2%) and specificity (62%) for total leucocytes counts $11.5x10^3$/L for diagnosis of sepsis when compared with blood culture. Similar finding was reported previously [20].

Another biomarker for diagnosis of sepsis is CRP. In the present study cut of value of CRP was 14.5 mg/L with sensitivity 78.5% and specificity 67.7%. CRP is the rapid and commonly used acute phase protein that is used for diagnosis of sepsis. The sensitivity of CRP increases after 24 hours of development of sepsis. The sensitivity of CRP varies from 47% to 100% in previous studies [21,22]. CRP was reported to be a good single indicator for laboratory diagnosis of sepsis [23]. However, its sensitivity depends upon the time of sampling after development of sepsis. The cut off value of CRP for detection of infection was similar to previous report by Beceiro Mosquera et al. who reported CRP value >13.5 mg/L as a marker of sepsis.

The sensitivity and specificity of 16SrRNA PCR for sepsis detection depends upon the presence of clinically suspected sepsis which in turn depends upon the presence of underlying risk factors.

There was statistically significant association between the presence of risk factors such as immunocompromized conditions, the use of intravenous cannula and the presence of urinary catheter and positive 16SrRNA PCR. These findings may indicate the usefulness of this

diagnostic method as a rapid laboratory diagnosis in the presence of risk factors requiring rapid diagnosis especially in countries with limited resources where routine application of molecular methods may be restricted by the cost.

5. CONCLUSION

The present study highlights the advantage of 16SrRNA PCR for rapid diagnosis of sepsis in children compared to blood culture. The association of positive results with risk factors of sepsis may be used as a guide for rapid diagnosis in high risk patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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