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Original article

Direct Molecular Diagnosis of Bacterial Etiology of Sepsis in Children in Whole Blood Samples

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Abstract:

Background: Sepsis is one of the leading causes of morbidity and mortality in children worldwide, blood culture is an imperfect gold standard since it's insensitive and slow. A modern microbiological diagnostic work-up increases the sensitivity and enables to directly identify fastidious, slow-growing, unculturable, or nonviable bacteria. Objective: This study aimed to evaluate the performance of 23S rDNA PCR assays as a rapid detection method for diagnosis of sepsis in children with suspected bacteremia in comparison with automated blood culture. **Methods:** The study included 93 children (51 male and 42 female). They were under empirical antibiotic therapy, their ages ranged from 2 days to 6 years old, fulfilling sepsis - 3 criteria using QSOFA to identify suspect cases and SOFA to assess organ affection. Blood culture was positive in 63 samples and negative in 30 samples. PCR for 23S rRNA gene was done for 50 cases of them (20 positive blood culture and 30 negative blood culture) used as a study group. **Results:** The present study showed that, out of 20 blood culture positive samples, 20 (100%) were found positive by 23S rRNA gene PCR assays. Positive PCR results were obtained in 29 samples of the 30-blood culture negative samples. Accordingly, the resulting detection rate of 23SrRNA gene PCR (98%) was higher than blood culture (40%) in all fifty samples. **Conclusion:** The real time PCR of 23S rRNA gene provides rapid and reliable detection of bacterial pathogens directly from patient blood samples which helps to optimize antimicrobial therapy.

Keywords: Sepsis, Blood culture, 23S rRNA, real time PCR, children.

Introduction

Sepsis is a life-threatening organ dysfunction that results from the body's response to infection. It requires prompt recognition, appropriate antibiotics, careful hemodynamic support, and control of the source of infection [1]. The mortality rate from septic shock ranging from 25% to 50% [2].

According to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3), systemic inflammatory response syndrome (SIRS) criteria were replaced with clinical criteria based on the Sequential Organ Failure Assessment (SOFA) score (Table 1) that assigns points based on the magnitude of dysfunction in six organ systems [3,4].

Quick SOFA (qSOFA) (Respiratory rate ≥22 per minute, altered mentation: GCS ≤13 or ≤14 with new onset agitation, systolic blood pressure ≤100 mmHg) clinical criteria were proposed as an easy and faster bedside tool than SIRS (systemic inflammatory response syndrome) for rapid risk stratification of patients with suspected infection [5].

According to a large-scale point prevalence study involving PICU data from 26 countries, the prevalence of sepsis appears to be higher in developing countries [6].

The value of blood cultures for confirming the clinical diagnosis of sepsis, severe sepsis, and septic shock is suboptimal. Although most untreated patients with bacterial meningitis have positive blood cultures, only 30% of patients with bacterial pneumonia and intra-abdominal infections have positive cultures. Only 5% to 15% of all cultures were drawn for any reason, and only 50% of patients with septic shock are showing positive results [7].

In early sepsis, the concentration of bacteria is very low, and the time of diagnosis is very critical since mortality increases exponentially with every hour after infection. Common culture-based methods fail in fast bacteria determination [8].

The single most important factor for achieving a sensitivity of >95% is sampling sufficient volumes (i.e., at least two to three sets, filled with 10 ml per bottle), as >50% of the patients present with a bacteraemia of <1 colony-forming unit (CFU)/ml [9].

Routinely used blood culture methods are not an ideal gold standard, as the results often come too late, are incomplete or not sensitive enough, and can be misleading and relatively labor-intensive [10].

In fact, only 38–69% of septic patients are diagnosed by positive blood cultures. Current guidelines largely focus on initiating treatment within 60 min of presentation of sepsis, as outcomes is better than those with delayed treatment [11].

Failure to identify slow-growing or obligatory intracellular microorganisms and pathogens other than bacteria or yeast are the major drawbacks [12].

Direct rapid detection and identification of pathogen(s) is required for optimizing the therapeutic effect of antimicrobial therapy and for de-escalation of the started therapy to limit collateral damage, such as toxicity, side effects and selection of antimicrobial resistance [13].

The implementation of a rapid identification and antimicrobial susceptibility testing method, along with a well-established antimicrobial stewardship program, has the potential to decrease length of stay, broad-spectrum antibiotic use, and costs to the healthcare system [14].

Bacterial 70S ribosomes are macromolecular complexes consisting of two distinct subunits, the small 30S and the large 50S. The 30S subunit contains a single 16S rRNA and 21 r-proteins, while the 50S subunit is composed of two rRNAs (23S and 5S rRNA) and 33 r-proteins [15].

The 23S rRNA gene offers the same advantages as the 16S rRNA gene (e.g.,

universal distribution, conserved function, and invariant and variable regions), yet it includes additional diagnostic sequence stretches due to a greater length, characteristic insertions and/or deletions, and possibly better phylogenetic resolution because of greater sequence variation [16].

A novel real-time PCR method targeted on a conserved region of the 23S ribosomal DNA gene is optimal for detection and quantification of a wide range of human pathogenic bacteria in human blood and plasma samples. PCR assays are capable to detect as low as 10 plasmid copies per reaction of targeted bacterial 23S rDNA gene [17].

Table (1): Pediatric Sequential Organ Failure Assessment Score.

System/Score	0	1	2	3	4
Respiratory					
Pao2:Fio2	≥400	300-399	200-299	100-199 With respiratory support	<100 with respiratory support
Or					
SPO2: Fio2	≥292	264-291	221-264	148-220 with respiratory support	<148 with respiratory support
Coagulation	≥150	100-149	50-99	20- 49	<20
Platelet Count, x 10^3/UL					
Hepatic					
Bilirubin, mg/dl	< 1.2(20)	1.2- 1.9(20-32)	2- 5.9(33-101)	6-11.9(102 -204)	>12(204)
MAP by age group or vasoactive infusion, mm Hg or Ug/kg/ min					
<1 month	≥46	<46	Dopamine hydrochloride <5 or dobutamine hydrochloride (any)	Dopamine hydrochloride>5 or epinephrine bitartrate<0.1	Dopamine hydrochloride>15 or epinephrine >0.1 or norepinephrine bitartrate>0.1
1-11 month	≥55	<55			
12-23 month	≥60	<60			
24-59 month	≥62	<62			
60- 143 month	≥65	<65			
144- 216 month	≥67	<67			
>216 month	≥70	<70			
Neurologic					
Glascow Coma Score	15	13-14	10- 12	6- 9	<6
Renal Creatinine by age group, mg/dl					
<1 month	<0.8	0.8-0.9	1-1.1	1.2-1.5	≥1.6
1-11 month	<0.3	0.3-0.4	0.5-0.7	0.8-1.1	≥1.2
12-23 month	<0.4	0.4- 0.5	06-1	1.1-1.4	≥1.5
24-59 month	<0.6	0.6-0.8	0.9-1.5	1.6-2.2	≥2.3
60- 143 month	<0.7	0.7-1	1.1-1.7	1.8-2.5	≥2.6
144- 216month	<1	1-1.6	1.7-2.8	2.9-4.1	≥4.2
>216-month	<1.2	1.2-1.9	2-3.4	3.5-4.9	≥5

MAP; Mean arterial pressure, SPO2: Fio2; oxygen saturation to inspire oxygen fraction; Pao2; partial pressure of oxygen [3].

Methods

➢ Patients

Ethical approval and consent to participate.

The study was conducted according to the guidelines of the Helsinki Declaration [18] and approved by the Research Ethics Committee of Benha University Hospitals (MoHP No.: 0018122017/ Certificate No.: 1017). An informed consent was obtained from the parents before enrollment in the study.

- This cross-sectional study included 93 children.

➢ Inclusion criteria:

- All patients younger than 13 years of age.
- Children suspected of having sepsis, according to clinical criteria of the Third International Consensus definitions for Sepsis and Septic Shock (Sepsis-3).
- Children who were under empirical antibiotic therapy

➢ **Exclusion Criteria**:

- Age more than or equal to 13 years.

The study was conducted in the period between August 2020 to January 2021and from June 2021 to November 2021 in Pediatrics Department, Pediatric ICU and Neonatal ICU at Benha University Hospital. Blood culture (BC) was positive in 63 samples and negative in 30 samples (hematological, chemical, serological and microbiological investigations were done at Clinical and Chemical pathology department, faculty of medicine, Benha university). PCR for 23S rRNA gene was done for 50 cases of them (20 positive blood culture and 30 negative blood culture) used as a study group at molecular biology unit, medical biochemistry department faculty of medicine, Benha university.

➢ **Sample Collection**

Ten ml blood were taken from each subject under complete aseptic precaution and at the onset of fever and divided as follows; 3ml blood were inoculated in automated pediatric blood culture bottles, two ml blood were added in vacutainer ethylene diamine tetra-acetic salt (EDTA) (1.2 mg/ml) tube and stored at – 80 ^{o} C until use For PCR assay. Three ml blood were taken aseptically in vacutainer plain tubes, left to coagulate, centrifuged (at 1500 rpm for 15 minutes) to separate serum for CRP and chemical tests: alanine transaminase (ALT), lactate dehydrogenase (LDH)), creatinine and serum billirubin, two ml blood were taken aseptically in vacutainer EDTA tube for CBC.

- Microbiological tests:

- **Automated blood culture on BacT/Alert system** (BioMérieux, Craponne, France); after inoculating blood into automated blood culture bottles, they were incubated at 35°C for 7 days on Bact/Alert 3D (SN:3041384322). Positive bottles were studied with gram staining and then subcultured aerobically, and in CO2 on blood, chocolate and macConkey agar media. The identification and antimicrobial susceptibility tests of bacterial isolates from positive bottles were done using automated system (Vitek 2 compact) (BioMérieux, Craponne, France) (SN:vk2c18317).

- **Real time PCR Assays of 23S rRNA gene**

DNA extraction from the EDTA whole blood tube by QIAmp DNA Mini kit (Qiagen Gesellschaft mit beschränkter Haftung "GmbH", Germany) Ref (Mini61104). according to the manufacturer's protocol.

DNA amplification of the conserved 23SrRNA gene of the bacteria: Enzymatic amplification of the extracted DNA was performed on Applied Biosystem (Step One, Real- Time PCR system, Thermo Fisher Scientific, USA), (SN: 271003648); using HERA SYBR Green (willowfort.co.uk), specific primers (Invitrogen, Thermo Fisher Scientific, USA) (Forward primer, F(5′-TCGCTCAACGGATAAAAG-3´) (Number: Z3497B03), reverse primer, R (5′-GATGAn-CCGACATCGAGGTGC-3´) (Number: Z3497B04). Positive (ATCC QC stains of staphylococcus aureus and E. coli) and negative controls were included for each PCR reaction.

The PCR was performed in 20 µl reaction mix containing: 4µl template DNA, 1 µl of each primer and 4 µl DNase-free water was added to 10 µl HERA SYBR Green master Mix Kit (WF1030800X). After initial denaturation at 95 °C for 2min, then cycling for 35 cycles, denaturation at 95 °C for 15 Sec, then annealing at 60 °C for 15 Sec. Once the reaction is complete, Ct value data can be analyzed for gene expression. The melting temperature is shown in Figure (1).

- **Outcome measurement (follow up for 1 month long)**

QSOFA is used to predict PICU transfer and/or mortality in children in emergency department and quick Pediatric Logistic Organ Dysfunction (qPELOD-2)(Altered Mentation- Hypotension- Tachycardia) to predict length of stay in children in pediatric ICU [19].

➢ Statistical Analysis

The collected data was revised, coded, tabulated and analysed using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. The quantitative data were examined by Kolmogrov Smirnov test for normality. For comparison between two groups, student t-test, and Mann-Whitney test (for non-parametric data) were used. Probability (*p*) value: level of significance Values of $p \leq 0.05$ were considered significant [20].

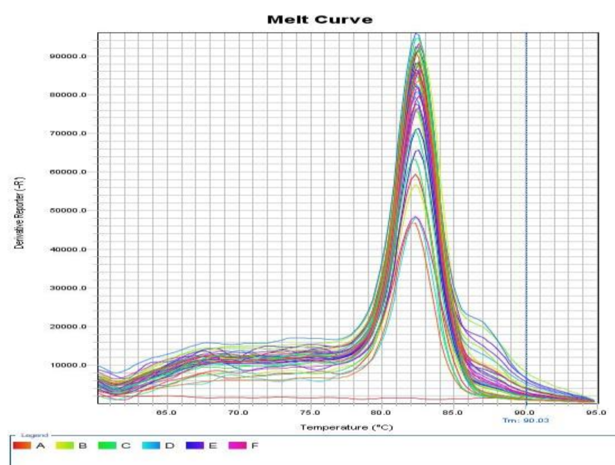

Figure (1): Showing high resolution melting analysis (HRMA) is a post PCR amplification method of analyzing DNA that does not require multiple expensive fluorescent probes.

Results

The study included 93 children (51 male and 42 female). They were under empirical antibiotic therapy, their ages ranged from 2 days to 6 years old, fulfilling sepsis - 3 criteria using QSOFA to identify suspect cases and SOFA to assess organ affection. Blood culture was positive in 63 samples and negative in 30 samples. PCR for 23S rRNA gene was done for 50 cases of them (20 positive blood culture and 30 negative blood culture) used as a study group as shown in Figure (2).

The fifty studied cases were divided into three groups including, 28 neonates (12 miles (42.9%) and 16 females (57.1)), their median age was 7.5 days (3 to 30 days), 9 infants [5 males (55.6%) and 4 females (57.1)], their median age was 5 months (1.25 to 24 months) in addition to 13 children [9 males (69.6%) and 4 females (30.8%)], their median age was 4, (2.5 to 12 years) as shown in table (2). Early onset sepsis (EOS) was in 39.3% of neonates and late onset sepsis (LOS) in 60.7 % of them. Among early onset sepsis neonates six cases were negative by blood culture and 4 isolates out of 5 positive blood culture cases, were *CoNS*, while in late onset sepsis neonates, thirteen cases were negative by blood culture and four cases were positive. No significant association was found regarding sex, delivery mode and sepsis onset with age groups.

The commonest symptoms were tachypnea in 100%, tachycardia in 86 % and altered conscious level in 75% of cases. The most frequent primary reason for admission in this study was respiratory diseases (38%), in the form of acute respiratory distress syndrome (ARDS) (20%) and pneumonia (18 %) as shown in table (3). No significant association was found regarding underlying disease with results of blood cultures or that of 23S rRNA gene by PCR.

Laboratory findings (CRP, LDH, WBC, ALT, creatinine, bilirubin) among the studied 50 cases were as shown in table (4).

Table (2): Demographic characters and age groups of the studied patients.

	Cases N=50	**Mean ± SD**	**Median (range)**
Age (months)		15.4 ± 26.8	0.75 (0.1-144)
		N (%)	**Median (range)**
	Neonate (Days)	28 (56%)	7.5 (3-30)
	Infant (months)	9 (18%)	5 (1.25-24)
	Child (years)	13 (26%)	4 (2.5-12)
Gender		**N**	**%**
	Males	26	52%
	females	24	48%
Mode of delivery in neonates (28 cases)		**N**	**%**
	CS	13	46.4 %
	NVD	15	53.6 %

Table (3): Comorbidities among the studied 50 cases.

		Cases N=50	
		N	**%**
Respiratory		19	38%
	ARDS	10	20%
	pneumonia	9	18%
Neurologic disorders		11	22%
	status epilepticus	1	2%
	meningitis	3	6%
	hydrocephalus	2	4%
	encephalitis	3	6%
	CP	2	4%
Sepsis without underlying disease		7	14 %
Cardiovascular		4	8%
	DVT	1	2%
	CHD	3	6%
Gastroenteritis		3	6%
CRF		3	6%
Polytrauma		2	4%
Osteomyelitis		1	2%

ARDS; acute respiratory distress syndrome, CP; cerebral palsy, CVS; cardiovascular system, CRF; chronic renal failure, DVT; deep venous thrombosis, CHD; congenital heart disease.

Table (4): Laboratory findings among the studied 50 cases.

	Cases N=50			
	mean	**SD**	**median**	**range**
CRP (mg/L)	43.6	33.3	30	12-130
LDH (U/L)	306.3	66.8	290	220-484
WBC ($X10^9/L$)	18.4	5.3	19	8-30
ALT (U/L)	72.6	16.1	70	44-130
Creatinine (mg/dL)	1.4	0.8	1.1	0.3-3.2
Bilirubin (mg/dL)	3.4	2.6	2.6	0.9-9

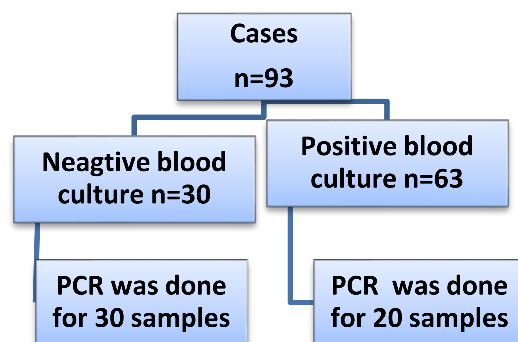

Figure (2): Study groups.

Among all studied cases (n=93), sixty-three cases had positive blood culture, the most frequent isolates were *CoNS* (50.6 %). 14.2% of isolates were *Klebsiella pneumoniae*, as shown in table (5).

Time to positivity (TTP) of the twenty cases who had positive blood culture and who were selected for PCR, was significantly shorter when associated with ARDS (mean 11.8 ± 2 SD) and *pseudomonas aeroginosa* with *staphylococcus haemolyticus* (10 hours) and significantly longer when associated with neurologic diseases (mean 18 ± 2-3 SD) and *klebsiella pneumoniae* (21 hours). Otherwise, no significant association was found regarding co morbidities, onset of sepsis, improvement, type of organisms, outcome and other studied parameters with TTP.

The antibiogram of the thirty two isolates of *CoNS* by blood culture in the 63 positive blood culture samples was that, Vancomycin was the most sensitive [32 (100%)], followed by Tigecycline [30 (93.8 %)] then Linezolid [27 (84.8 %)], and Rifampicin [24 (75 %)], and all were Methicillin resistant. The antibiogram of nine isolates of *Klebsiella pneumoniae* were most sensitive to Levofloxacin [7 (77.8%)], followed by Ciprofloxacin [6 (66.7%)] and Tigecycline [5 (55.6%)] and all were carbapenemase producing.

Results of 23SrRNA gene PCR in blood culture positive samples were as follows: of 20 blood culture positive samples, 20 (100%) were found positive by 23SrRNA gene PCR assays, including, (10 Coagulase negative staphylococci, 3 cases of mixed infections which were (1 *Pseudomonas aeruginosa* + *S. haemolyticus*, *Klebsiella pneumoniae* + *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* + *S. hominis*), 2 *Acinetobacter baumannii*, 2 *Klebsiella pneumoniae,* 1 *Staphylococcus aureus*, 1 *Escherichia coli*, 1 *Enterobacter clocae*).

The incubation time of blood culture until positive detection in the automated BacT/Alert system was evaluated in 20 blood culture bottles. The mean time to positivity was 14.85 hours.

Results of 23SrRNA gene PCR in blood culture negative samples were as follows: of the 30-blood culture negative samples, positive PCR results were obtained in 29 samples as shown in table (6) and Figure (3). Accordingly, the resulting detection rate of 23SrRNA gene PCR (98%) was higher than blood culture (40%) in all fifty samples.

The outcome of cases in one month among the studied 50 cases was as shown in table (7), 27/ 50 (54%) of cases improved. No significant association was found regarding improvement or outcome with results of blood cultures or that of 23srRNA by PCR.

Table (5): Results of organisms isolated by BC in 63 cases (positive blood culture).

Organism		Cases N=63	
		N	**%**
CoNS		32	50.6%
	S.epidermidis	16	25.4%
	S. hominis	6	9.5%
	S. haemolyticus	7	11.1%
	S.warneri	2	3.1%
	S.simulans	1	1.5%
Klebsiella pneumoniae		9	14.2%
Pseudomonas aeruginosa		3	4.8%
Streptococcus agalactiae		2	3.2%
Acinetobacter baumannii		3	4.8%
E.coli		1	1.6%
S .aureus.		1	1.6%
Stenotrophomonas maltophilia		1	1.6%
Enterobacter cloacae		1	1.6%
Rhizobium radiobacter		1	1.6%
Candida parapsilosis		3	4.8%
Positive alarm on BacT /Alert and no growth on subculture on routine media		3	4.8%
Kocuria and polymicrobial more than 2organisms (contamination)		3	4.8%

Table (6): Results of 23SrRNA gene PCR and blood culture.

		Culture	
		negative	**positive**
23SrRNA	**negative**	1	0
	positive	29	20

Table (7): Outcome among the studied 50 cases (for one month after diagnosis).

	Cases N=50	
	N	**%**
Improved	27	54%
Not improved (there were ≥ 2criteria of the following, hypotension- Altered Mentation -tachycardia (quick PELOD-2))	16	32%
Died	7	14%

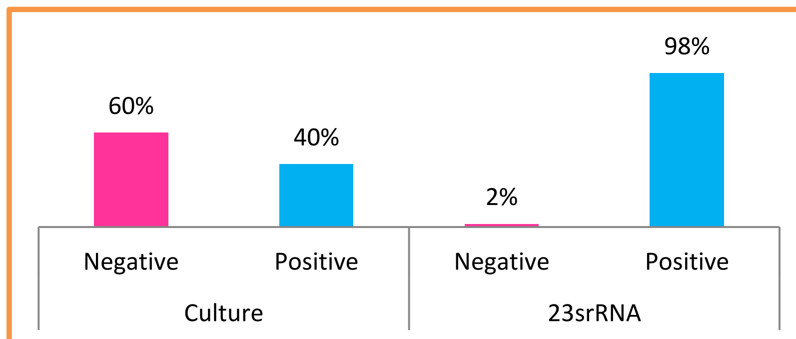

Figure (3): Results of 23SrDNA PCR among selected cases.

Discussion

In 2017, the World Health Organization (WHO) recognized sepsis as a global health problem and started demanding the member countries measures for prevention, diagnosis, and treatment [21].

Molecular diagnostics for infection hold the promise to greatly accelerate diagnosis, they are currently challenged when the abundance of a pathogen is very low, and even more so when host cell background is high. For example, in bacteremias where pathogen burden can be as low as 10–100/ml [22].

This study aimed to evaluate the performance of 23S rDNA PCR assays as a rapid detection method for diagnosis of sepsis in children with suspected bacteremia in comparison with automated blood culture.

The study included 93 children (51 male and 42 female). They were under empirical antibiotic therapy, their ages ranged from 2 days to 6 years old, fulfilling sepsis - 3 criteria using QSOFA to identify suspect cases and SOFA to assess organ affection. Blood culture was positive in 63 samples and negative in 30 samples. PCR for 23S rRNA gene was done for 50 cases of them (20 positive blood culture and 30 negative blood culture) used as a study group.

The fifty studied cases were divided into three groups including, where group I included 28 neonates [12 males (42.9%) and 16 females (57.1)], their median age was 7.5 days (3 to 30 days). Group II included 9 infants [5 males (55.6%) and 4 females (57.1)], their median age was 5 months (1.25 to 24 months) in addition to group III which included 13 children [9 male (69.6%) and 4 female (30.8%)], their median age was 4, (2.5 to 12 years). Early onset sepsis (EOS) was in 39.3% of neonates and late onset sepsis (LOS) in 60.7 % of them.

Our study revealed that the most frequent primary reason for admission in this study was respiratory diseases (38%), in the form of ARDS (20%) and pneumonia. (18%), followed by neurologic disorders (22%).

Our findings were in agreement with a study which concluded that the most common infections in children aged less than 7 years were infection of the lower (27.4%) and upper (11.4%) respiratory tract, the gastrointestinal system (22.7%) and skin (5.6%) [23].

The present study showed that laboratory diagnostic criteria such as, CRP (range: 12 to 130 mg/L), LDH (range: 220 to 484 U/L), WBC (range: 8 to 30 X 10^9/L), ALT (range: 44 to 130 IU/L) and creatinine (range: 0.3 to 3.2 mg/dl) were significantly high in patients with sepsis.

Our results were in line with a study which showed that serial CRP measurement can be used as a diagnostic tool for finding clinical infections, monitoring effects of treatment, outcome, and early detection of relapse of the disease [24], and were close to another study that revealed the value of CRP in predication of patients with suspected sepsis especially who present with the systemic inflammatory response syndrome (SIRS) manifestation [25]. Our laboratory findings were in concordance with a study concluded that the serum LDH level was significantly higher in septic than control (p=0.000) and in non-survivor than survivor group (p=0.000) [26].

The present study showed that among all studied cases (n=93), sixty-three cases had positive blood culture, 50.6 % of isolates were *CoNS* and 14.2% of isolates were *Klebsiella pneumoniae*. While, in a different study, they showed that the nine bacterial isolates from sepsis and suspected blood stream infection patients were, *S. pneumoniae, S. haemolyticus, E. faecalis, A. baumannii, Acinetobacter parvus, E. coli, Burkholderia cepacia, Chryseobacterium indolegenes* and one was uncultured bacterium [27] and another study revealed that 27 out of 265 were blood culture positive samples with the following isolates (9 *coagulase-negative*

staphylococci, 6 *Staphylococcus aureus*, 5 *Escherichia coli*, 3 Enterococcus spp. 3 *Acinetobacter baumannii*, and 1 *Klebsiella pneumoniae*) [28]. The difference of bacterial isolates from blood in these studies and ours may be due to different epidemiological distribution and seasonality of infection.

Our study showed that the antibiogram of thirty-two isolates of *CoNS* by blood culture in the 63 positive blood culture samples was that Vancomycin was the most sensitive (32 (100%). This was in agreement with a study that revealed the antimicrobial susceptibility pattern of *CoNS* isolated from neonates was highest to Vancomycin (100%), Linezolid (100%), Netilmicin (100%) [29]. The nine isolates of *Klebsiella pneumoniae* in the present study were most sensitive to Levofloxacin 7 (77.8%). While, in different study, eighty-four isolates of *K. pneumoniae* in blood culture samples showed highest sensitivity to Imipenam and least sensitivity to Ampicillin [30], The difference in antibiogram of *K. pneumoniae* may be explained by difference in antibiotic prescribing and selective pressure.

The present study showed that the mean time to positivity in the twenty cases who had positive blood culture and who were selected for PCR, was 14.85 hours. Time to positivity (TTP) was significantly shorter when associated with ARDS and *pseudomonas aeroginosa* with *staphylococcus haemolyticus*, this may be explained by higher microbial load and significantly longer when associated with neurologic diseases and *klebsiella pneumoniae* and this may be explained by lower microbial load. Our findings were close to a study that showed that the median TTP was 15.7 hours. TTP was below 24 hours in 85.3%, longer than 48 hours in 3.8% and longer than 72 hours in 2.0% of episodes. TTP was long in bacteremia caused by *Proteus mirabilis* (median 18.6 hr) [31].

Our study revealed that results of 23SrRNA gene PCR in positive blood culture samples were as follows: of 20 blood culture positive samples, 20 (100%) were found positive by 23SrRNA gene PCR assays, including, (10 *Coagulase negative staphylococci*, 3 cases of mixed infections which are (1 *Pseudomonas aeruginosa* + *S. haemolyticus*, *Klebsiella pneumoniae* + *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* + *S. hominis*), 2 *Acinetobacter baumannii*, 2 *Klebsiella pneumoniae*, 1 *Staphylococcus aureus*, 1 *Escherichia coli*, 1 *Enterobacter clocae*).

Our study revealed that results of 23SrRNA gene PCR in negative blood culture samples were as follows: of the 30-blood culture negative samples, positive PCR results were obtained in 29 samples. Accordingly, the resulting detection rate of 23SrRNA gene PCR (98%) was higher than blood culture (40%) in all fifty samples.

Our results were in agreement with a study that showed that the novel 23S rDNA-targeted PCR assay was able to specifically detect all of the 47 bacterial isolates included in the study that represent an estimated 90% of the reported causes of blood stream infections [17].

The results of the present study were in concordance with a study which showed that out of 27 blood culture positive samples, 24 (88.9%) were found positive by 23S rRNA PCR assays whereas 3 blood culture positive samples were PCR negative which were identified as coagulase-negative staphylococci [28].

The findings of the present study were in concordance with a study of 19 patients that compared the conventional culture analysis of material aspirated from disc in cases of spondyloarthritis that identified 14 of 19 patients (74%) with broad-range (16s rRNA gene) PCR assay that identified 19 of 19 patients (100%) with five more pathogens (*Staphylococcus simulans*, *Staphylococcus sciuri*, Brucella spp, *Actinomyces israelii* and *M .tuberculosis* complex) were identified with the help of this method [32].

Similarly, a higher bacterial detection by 23S rDNA before and after antibiotic treatment was confirmed by a study of 134 continuous ambulatory peritoneal dialysis (CAPD) peritonitis cases. Samples were collected before antibiotic therapy. Ninety-five cases of them were positive by culture 70.9% (95/134) and thirty-nine cases were negative by culture. By 23S bacterial ribosomal RNA sequencing assay, the same microorganisms were confirmed in 75 of the culture-positive samples and discrepant results were in 30 of the 39 culture-negative samples. Of the 32 samples from patients who developed CAPD peritonitis during antibiotic treatment, 17 (53.1%) were positive by PCR assay, and 5 (15.6%) were positive by culture [33].

Our findings agreed with a study reported a novel droplet digital polymerase chain reaction (ddPCR) assay that can
detect *A. baumannii* and *K. pneumoniae* in blood samples within 4 h, with a specificity of 100% for each strain, clinical validation of 170 patients with suspected blood stream infections tested by blood cultures that detected four (2.4%) *A. baumannii* cases and seven (4.1%) K. *pneumoniae* cases and ddPCR that detected 23 (13. 5%) *A. baumannii* cases, 26 (15.3%) K. *pneumoniae* cases, and four (2.4%) co-infection cases, including the 11 cases detected via blood culture [34].

While, in other studies the bacterial detection rate of culture-negative samples was lower than our study but, still higher when compared with blood culture. In one study, broad range 16S rDNA PCR showed positive results for 23 out of 300 culture-negative different human samples [35] and in another study 16 out of 475 cases of infection, 9 false-negative PCR results and 5 false positive PCR results due to contamination were obtained [36]. Also, in another study in which a new commercial PCR test (SepsiTest; Molzym) was evaluated for rapid diagnosis of bacteremia by using a universal PCR from the 16S rRNA gene, of the 342 samples, BC was positive in 54 samples (15.8%) and 47 were also PCR positive. Among the 288 BC-negative samples, a positive PCR result was obtained in 41 the resulting detection rate of PCR (25.7%) was higher than BC (15.8%). The seven samples that were positive by BC but negative by PCR included four *Staphylococcus epidermidis*, two *Escherichia coli* and one *Streptococcus oralis* and *E. coli*) [37].

The bacterial detection by 23S rDNA in our study was higher than 16S rDNA in a study which concluded that 16S rRNA PCR/ Sanger sequencing identified a probable pathogen in 10% culture-negative different specimens. In cardiovascular specimens, bacterial cultures were positive in 21% and 16S rRNA PCR/sequencing was positive in 17% of patients [38]. Another study found that 36 (58.06%) out of 62 of the patients presenting with pyrexia of unknown origin (PUO) were meeting the clinical criteria for Sepsis-3 guidelines and were 14–80 years of age using the broad range 16S rRNA PCR assay were positive, the same results were obtained with 16 S – 23 S rRNA inter spacer region PCR assay [27].

Also, in a study in which blood samples from fifty neonates with suspected sepsis were tested by 16S rDNA PCR assay and compared with blood culture; twenty-eight (56%) neonates gave positive bacterial blood culture, while 35 (70%) neonates gave positive PCR results [39]. The different bacterial detection rates between different studies can be explained by appropriate selection of septic cases according to proper clinical and laboratory criteria, higher sensitivity of 23S rDNA, higher levels of bacteremia in samples or lack of inhibitors of PCR reaction. The sensitivity of 23S rDNA was confirmed by a study that quantied *P. aeruginosa* directly from clinical samples using 23S rRNA- targeted qRT- PCR system with high sensitivity (blood, 1 cell/ml; stool, 100 cells/g) without cross-reaction (within 6 h) [40].

The present study showed that the outcome in one month among the studied 50 cases was as follows, 27/ 50 (54%) of cases improved, while 16/50 (32%) of them didn't improve (according to quick PELOD-2 creiteria; there were ≥ 2criteria of the following, hypotension, altered mentation and tachycardia), 7/50 (14%) of them died.

Our findings were close to a study which found that survival rate was 48/62 (77.41%) and 14/16 (87.5%) among sepsis and suspected blood stream infection (BSI) patients respectively (*P*=1.00), death was recorded in only 14 (22.58%) patients in sepsis group and 2 (12.5%) in suspected BSI group [23].

Conclusion:

This study suggests that the real time polymerase chain reaction of 23S rRNA gene has been demonstrated to be a laboratory useful, rapid and important test for the diagnostics of infections in patients with culture-negative results directly from whole blood sample in the early phase of bloodstream infection in which diagnostic uncertainty is common and results in delayed treatment or the overuse of antimicrobials and help in therapeutic decision making. Also, blood culture alone is not ideal diagnostic standard test for sepsis, but it complements PCR and clinical diagnosis.

Recommendations:

Results could be strengthened by detecting genus and species-specific genes in variable regions of 23S rRNA gene by using real time PCR or Sequencing for identification and differentiating pathogenic from contaminant. Studies of larger groups and other related genes are needed. Adapt 23S rRNA gene real time PCR as rapid test for detection of bacterial infection. This will be of promising value in timely treating septic patients to avoid overuse of non-indicated antimicrobial combinations, in hospitalized high-risk patients. Antimicrobial Stewardship is easy to be applied in presence of rapid and direct method of diagnosis, this is the corner stone in prevention and control of super bugs. Both blood culture and molecular based diagnostic strategies are complementary to each other, to make the maximal diagnostic performance and efficiency. Appropriate strict aseptic collection procedures of blood samples is prerequisite for relevant diagnosing infection in sepsis.

References

1- [1] Hon KL, Leung KKY, Oberender F, Leung AKC. Paediatrics: how to manage septic shock. Drugs in Context 2021; 10: 1-5.

2- [2] Weiss SL, Peters MJ, Alhazzani W, Agus MSD, Flori HR, Inwald DP, et al. Surviving sepsis campaign international guidelines for the management of septic shock and sepsis-associated organ dysfunction in children. Intensive Care Med 2020 ;46(1):10–67.

3- [3] Matics TJ, Sanchez-Pinto LN. Adaptation and Validation of a Pediatric Sequential Organ Failure Assessment Score and Evaluation of the Sepsis-3 Definitions in Critically Ill Children. JAMA Pediatr. 2017; 2;171(10): 172352.

4- [4] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer MS, et al. The Third International Consensus Definitions for Sepsis and Septic Shock. JAMA 2016; 315 (8):801-810.

5- [5] Fernando SM, Tran A, Taljaard M, Cheng W, Rochwerg B, Seely AJE, et al. Prognostic accuracy of the quick sequential organ failure assessment for mortality in patients with suspected infection: a systematic review and meta-analysis. Ann Intern Med. 2018;168(4): 266-275.

6- [6] Souza DC, Oliveira CF, Lanziotti VS. Pediatric sepsis research in low- and middle-income countries. Rev Bras Ter Intensiva 2021;33(3):341-345.

7- [7] Murray PR, Masur H. Current approaches to the diagnosis of bacterial and fungal bloodstream infections in the intensive care unit. Critical Care Medicine 2012; 40, 3277–3282.

8- [8] Iyengar SN, Dietvorst J, Ferrer-Vilanova A, Guirado G, Muñoz-Berbel X, Russom A. Toward Rapid Detection of Viable Bacteria in Whole Blood for Early Sepsis Diagnostics and Susceptibility Testing. ACS Sens. 2021; 6: 3357−3366.

9- [9] Towns ML, Jarvis WR, Hsueh PR. Guidelines on blood cultures. J Microbiol Immunol Infect 2010; 43(4):347-349.

10- [10] Sinha M, Jupe J, Mack H, Coleman TP, Lawrence SM, Fraley SI, et al. Emerging Technologies for Molecular Diagnosis of Sepsis, Clinical Microbiology Reviews 2018; 31(2): e00089-17.

11- [11] Sweeney TE, Liesenfeld O, May L. Diagnosis of bacterial sepsis: why are tests for bacteremia not sufficient? EXPERT REVIEW OF MOLECULAR DIAGNOSTICS 2019; 19(11):959–962.

12- [12] Dubourg G, Raoult D. Emerging methodologies for pathogen identification in positive blood culture testing. Expert Rev Mol Diagn 2016;16(1):97-111.

13- [13] Opota O, Jaton K, Greub G. Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Eur Soc Clin Microbiol Infect Dis. 2015;21(4):323-331.

14- [14] Roth F, Leedahl ND, Leedahl DD, Guerrero DM. Clinical and Financial Impact of Rapid Antimicrobial Susceptibility Testing in Blood Cultures Antibiotics 2022, 11(122):1-5

15- [15] Kaiser G. Ribosomes In: Microbiology. Available at: https://bio.libretexts.org/Bookshelves/Microbiology/Microbiology_(Kaiser)/Unit_1%3A_Introduction_to_Microbiology_and_Prokaryotic_Cell_Anatomy/2%3A_The_Prokaryotic_Cell__Bacteria/2.4%3A_Cellular_Components_within_the_Cytoplasm/2.4D%3A_Ribosomes. Accessed on December 20, 2022.

16- [16] Hunt DE, Klepac-Ceraj V, Acinas SG, Gautier C, Bertilsson S, Polz MF. Evaluation of 23S rRNA PCR Primers for Use in Phylogenetic Studies of Bacterial Diversity ASM Journals Applied and Environmental Microbiology 2006; 72 (3): 2221–2225.

17- [17] Gaibani P, Mariconti M, Bua G, Bonora S, Sassera D, Landini MP, et al. Development of a Broad-Range 23S rDNA Real-Time PCR Assay for the Detection and Quantification of Pathogenic Bacteria in Human Whole Blood and Plasma Specimens. Biomed Res Int. 2013; 2013: 264651.

18- [18] World Medical Association. Declaration of Helsinki-Ethical Principles for Medical Research Involving Human Subjects, the 59th WMA General Assembly, 2008; Seoul, South Korea.

19- [19] Van Nassau SC, Van Beek RH, Driessen GJ, Jan A Hazelzet JA, Herbert M, Boeddha NP, et al. Translating Sepsis-3 Criteria in Children: Prognostic Accuracy of Age-Adjusted Quick SOFA Score in Children Visiting the Emergency Department with Suspected Bacterial Infection. Front Pediatr. 2018; 6: 266.

20- [20] Landis JR, Kock GG. The measurement of observer agreement for categorical data. Biometrics 1977;33(1): 159-174.

21- [21] Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD. Recognizing Sepsis as a Global Health Priority - A WHO Resolution. N Engl Med J 2017;377(5):414-417.

22- [22] Houa HW, Bhattacharyya RP, Hung DT, Han J. Direct detection and drug-resistance profiling of bacteremias using inertial microfluidics. Lab Chip 2015; 15(10): 2297–2307.

23- [23] Dossetor PJ, Martiniuk ALC, Fitzpatrick JP, Oscar J, Carter M, Watkins R et al. Pediatric hospital admissions in Indigenous children: a population-based study in remote Australia. BMC Pediatr. 2017; 17 (1): 195.

24- [24] McWilliam S, Riordan A. How to use: C-reactive protein. Archives of Disease in Childhood - Education and Practice 2010; 95(2):55-58.

25- [25] Pradhan S, Ghimire A, Bhattarai B, Khanal B, Pokhare K, Lamsal M, et al. The role of C-reactive protein as a diagnostic predictor of sepsis in a multidisciplinary Intensive Care Unit of a tertiary care center in Nepal. Indian J Crit. Care Med. 2016; 20 (7): 417–420.

26- [26] Mohamed NA, Youness ER. Clinical Role of Serum Lactic Dehydrogenase Assessment in Critically Ill Pediatric Patients with Sepsis. Med. J. Cairo Univ. 2021; 89(5): 1917-1927.

27- [27] Mishra D, Satpathy G, Wig N, Fazal F, Ahmed NH, Panda SK. Evaluation of 16S rRNA broad range PCR assay for microbial detection in serum specimens in sepsis patients. Journal of Infection and Public Health 2020; 13(7): 998-1002.

28- [28] Firoozeh F, Shiralinezhad A, Momen-Heravi M, Aghadavod E, Zibaei M. Rapid Detection of Pathogenic Bacteria in Whole Blood Samples Using 23S rRNA PCR Assays. The Open Microbiology Journal 2019; 13:101-105.

29- [29] Ansari F, Banerjee T, Kumar A, Anupurba S. Coagulase - Negative staphylococci in Neonatal Blood: How

Concerning? Journal of Laboratory Physicians 2023; 15(1):126-130.

30- [30] Pushkar, Aashana, Madhu, Sharma M, Yadav A. Antibiogram of *klebsiella pneumoniae* recovered from blood stream infection at tertiary care hospital, Rohtak, Haryana. Pravara Med Rev 2022; 14 (03): 40 -44.

31- [31] Lambregts MMC, Bernards AT, van der Beek MT, Visser LG, Boer MG. Time to positivity of blood cultures supports early reevaluation of empiric broad-spectrum antimicrobial therapy. PLOS ONE 2019; 1- 12.

32- [32] Lecouvet F, Irenge L, Vandercam B, Nzeusseu A, Hamels S, Gala JL. The etiologic diagnosis of infectious discitis is improved by amplification-based DNA analysis. Arthritis Rheum 2004; 50(9):2985-2994.

33- [33] Yoo TH, Chang KH, Ryu DR, Kim JS, Choi HY, Park HC, et al. Usefulness of 23S rRNA Amplification by PCR in the Detection of Bacteria in CAPD Peritonitis American Journal of Nephrology 2006; 26(2):115-120.

34- [34] Zheng Y, Jin J, Shao Z, Liu J, Zhang R, Sun R, et al. Development and clinical validation of a droplet digital PCR assay for detecting *Acinetobacter baumannii* and *Klebsiella pneumoniae* in patients with suspected bloodstream infections. Microbiology open 2021; 10(6): e1247.

35- [35] Aguilera Arreola MG, Martínez-Peña MD, Hernández-Martínez F, Enriques SRJ, Verdín BR, Majalca-Martínez C, et al. Cultivation independent approach for the direct detection of bacteria in human clinical specimens as a tool for analysing culture negative samples: a prospective study. Springer Plus 2016; 15(5):332.

36- [36] Fenollar F, Roux V, Stein A, Drancourt M, Raoult D. Analysis of 525 Samples To Determine the Usefulness of PCR Amplification and Sequencing of the 16S rRNA Gene for Diagnosis of Bone and Joint Infections. Journal of Clinical Microbiology 2006; 44(3):1018-1028.

37- [37] Wellinghausen N, Kochem A, Disqué C, Mühl H, Gebert S, Winter J, et al. Diagnosis of Bacteremia in Whole-Blood Samples by Use of a Commercial Universal 16S rRNA Gene-Based PCR and Sequence Analysis. Journal of clinical microbiology 2009;47(9): 2759–2765.

38- [38] Fida M, Khalil S, Abu Saleh O, Challener DW, Sohail MR, Yang JN, et al. Diagnostic Value of 16S Ribosomal RNA Gene Polymerase Chain Reaction/Sanger Sequencing in Clinical Practice. Clin. Infect Dis. 2021;73(6):961-968.

39- [39] Draz NI, Taha SE, Abou Shadyet NM, Abdel Ghany YS. Comparison of broad range 16S rDNA PCR to conventional blood culture for diagnosis of sepsis in the newborn. The Egyptian Journal of Medical Human Genetics 2013; 14(4) :403- 411.

40- [40] Niikura M, Atobe S, Takahashi A, Kado Y, Sugimoto T, Tsuji H, et al. Clinical Significance of Rapid and Sensitive Quantification of Pseudomonas aeruginosa by Quantitative Reverse Transcription PCR Targeting of rRNA Molecules. researchsquare.com 2020; https://doi.org/10.21203/rs.3.rs-61576/v1.

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