16S Ribosomal Ribonucleic Acid Gene Polymerase Chain Reaction in the Diagnosis of Bloodstream Infections: A Systematic Review and Meta-Analysis

Guoming Su¹,*, Zhuqing Fu², Liren Hu³, Yueying Wang¹, Zugo Zhao², Weiqing Yang¹

¹ Guangdong Provincial Key Laboratory of Medical Molecular Diagnostics, Guangdong Medical College, Dongguan, China, ² Department of Microbiology and Immunology, Guangdong Medical College, Zhanjiang, China, ³ Department of Epidemiology and Health Statistics, School of Public Health, Guangdong Medical College, Zhanjiang, China

☯ These authors contributed equally to this work.

* yangysu@126.com

Abstract

Objective
We aim to evaluate the accuracy of the 16S ribosomal ribonucleic acid (rRNA) gene polymerase chain reaction (PCR) test in the diagnosis of bloodstream infections through a systematic review and meta-analysis.

Methods
A computerized literature search was conducted to identify studies that assessed the diagnostic value of 16S rRNA gene PCR test for bloodstream infections. Study quality was assessed using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. We calculated the sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) and their 95% confidence intervals (95% CI) for each study. Summary receiver operating characteristic (SROC) curve was used to summarize overall test performance. Statistical analysis was performed in Meta-DiSc 1.4 and Stata/SE 12.0 software.

Results
Twenty-eight studies were included in our meta-analysis. Using random-effect model analysis, the pooled sensitivity, specificity, PLR, NLR, and DOR were 0.87 (95% CI, 0.85–0.89), 0.94 (95% CI, 0.93–0.95), 12.65 (95% CI, 8.04–19.90), 0.14 (95% CI, 0.08–0.24), and 116.76 (95% CI, 52.02–262.05), respectively. The SROC curve indicated that the area under the curve (AUC) was 0.9690 and the maximum joint sensitivity and specificity (Q*) was 0.9183. In addition, heterogeneity was statistically significant but was not caused by the threshold effect.
Conclusion
Existing data suggest that 16S rRNA gene PCR test is a practical tool for the rapid screening of sepsis. Further prospective studies are needed to assess the diagnostic value of PCR amplification and DNA microarray hybridization of 16S rRNA gene in the future.

Introduction
Bloodstream infections (BSIs) remain a major cause of morbidity and mortality especially in the Intensive Care Unit [1–5]. Moreover, inadequate antibiotic therapy is associated with higher mortality rates. Early microbiological diagnosis is of paramount importance for appropriate antibiotic treatment which increases the survival rate of patients [4]. Therefore, it is evident that a rapid, sensitive and specific diagnosis of BSIs is urgently needed.

Conventional identification methods have several limitations such as lack of rapidity and sensitivity. Blood cultures followed by conventional identification methods are currently the reference method for the detection of pathogens in blood. This well-established method can detect a wide range of microorganisms. However, disadvantages do exist, as the time to detection is often too long. After the detection of bacteria by conventional blood culture, identification and assessment of antibiotic sensitivity take at least a further 24 h [6, 7]. The sensitivity is also unacceptably low in the detection of pathogenic bacteria in cases of low-grade bacteremia, in cases where blood cultures are inoculated without adequate sample volume, and in cases where antibiotics are used before blood samples are taken [8, 9]. If blood cultures were negative, repetition of sampling would then be required, while positive cases need further identification of the isolated microorganism using different culture media and biochemical tests [9]. For these reasons, development of detection methods that provide more rapid results and higher sensitivity is expected to optimize use of antibiotics.

An ideal diagnostic tool for BSIs should be rapid, sensitive and unaffected by antibiotic therapy. In recent years, polymerase chain reaction (PCR) assay using the 16S ribosomal ribonucleic acid (rRNA) gene has been used as a diagnostic tool in many setting [1, 9, 10]. This test is based on the rationale that 16S rRNA gene of bacteria comprises both conserved and variable regions [11]—the conserved regions are targeted by universal primers for identification of bacterial infection and the variable regions by genus or species-specific assays [2]. Amplified target regions may then be subjected to downstream applications such as sequence analysis and microarray hybridization [5, 9, 11, 12]. In addition, the PCR test has the advantages of amplifying minute amounts of DNA, even from nonviable bacteria [13], and costing less money than blood cultures in both negative and positive cases [9].

However, the results of these studies were variable although inspiring. Some studies revealed the diagnosis of BSIs by 16S rRNA gene PCR test with no less than 95% sensitivity and 95% specificity [1, 14, 15], whereas others reported low sensitivity values ranging from 41% to 90% [9, 16–18] and low specificity values ranging from 32% to 90% [2, 3, 19, 20]. Therefore, we performed a systematic review and meta-analysis to evaluate the accuracy of 16S rRNA gene PCR test compared with conventional blood culture in the diagnosis of BSIs.

Materials and Methods
Search strategy
This systematic review was performed according to the PRISMA Statement [21] (S1 PRISMA Checklist) and Cochrane Collaboration guidelines (http://handbook.cochrane.org/). A
A systematic literature search was performed for studies that assessed the diagnostic value of 16S rRNA gene PCR test for BSIs. We searched PubMed, Embase, the Cochrane Library, ClinicalTrials.gov (www.clinicaltrials.gov) and the World Health Organization International Trials Registry Platform search portal (http://apps.who.int/trialsearch/Default.aspx) up to March, 2015. The search terms were “RNA, Ribosomal, 16S”, “16S ribosomal ribonucleic acid gene”, “16S rRNA gene”, “16S rDNA”, “sepsis”, “bloodstream infections”, “bacteremia”, and “septicaemia”. We gave the detailed search strategies in S1 Table. Our searches were not limited by publication date, country or language. The databases search was conducted independently by two authors (Guoming Su and Yueying Wang). To ensure comprehensive acquisition of literature, we also manually searched for any additional studies in the reference lists of retrieved studies and recent reviews.

**Inclusion and exclusion criteria**

Studies were then included if they met the following inclusion criteria: (1) studies assessed diagnostic value of 16S rRNA gene PCR test for BSIs such as neonatal sepsis and bacteremia; (2) Blood cultures followed by conventional identification methods were used as the reference standard; (3) each study contained no less than ten specimens; and (4) Studies provided sufficient data to allow construction of two-by-two tables. If the same experimental results were repeatedly or multiply published, only the most informative publication was included. Relevant articles were excluded if they were review articles, meta-analysis, commentaries, letters, or case reports. Two reviewers (Guoming Su and Zhuqing Fu) independently screened studies according to eligibility criteria. Disagreements were resolved by consensus.

**Data extraction and quality assessment**

Two reviewers (Guoming Su and Zhuqing Fu) independently extracted information from eligible studies using a predefined data extraction form, and then another reviewer (Yueying Wang) verified them. Disagreements between reviewers were resolved through discussion. The following information was subtracted from the studies: the first author, publication year, country, disease type, participant characteristics, specimen type, test methods, cut-off of index test, and data for a two-by-two table (true positive, false positive, false negative, and true negative information), respectively.

The quality of each study was assessed using the revised Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool (http://www.bris.ac.uk/quadas/). This tool comprises 4 domains that discuss patient selection, index test, reference standard, and flow and timing [22]. Each domain is assessed in terms of risk of bias by using signaling questions which are answered with “yes,” “no,” or “unclear”. And risk of bias is judged as “low”, “high”, or “unclear”. In addition, the first 3 domains are simultaneously assessed in terms of concerns regarding applicability which are also rated as “low”, “high”, or “unclear” with the similar criteria.

**Definitions of amplification methods**

Amplification methods are defined as following: (1) PCR is defined as a conventional PCR amplification strategy that targets 16S rRNA gene in microorganisms. (2) Real-time PCR is defined that is an amplification method with a real-time monitoring system. (3) PCR-hybridization is defined as an amplification method followed by reverse hybridization or DNA microarray hybridization. (4) PCR-sequencing is defined that is an amplification method followed by sequence analysis. (5) RT-qPCR is a reverse transcription-quantitative PCR method for the determination of copy number of PCR templates such as RNA or cDNA in a PCR reaction. (6) FQ-PCR is a fluorescent quantitative PCR method for the determination of copy number of amplification cycles.
Data analysis

All analyses were undertaken using the Meta-DiSc 1.4 [23] and Stata/SE 12.0 software [24]. The Spearman model was applied to assess heterogeneity caused by different cut-off threshold effects, while the heterogeneity that was caused by other factors was checked using Cochran-Q value and I² test [25, 26] for diagnostic odds ratio (DOR). If heterogeneity (p < 0.05 or I² > 50%) was statistically significant among studies, the random-effect model [27] was performed for the meta-analysis; otherwise, the fixed-effect model [28] was chosen. For each study, the following indexes of test accuracy were calculated: sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and their 95% confidence intervals (95% CI). Summary receiver operating characteristic (SROC) curve was used to summarize overall test performance [29]. The area under the curve (AUC) and Q point value (Q') were also counted to assess the overall performance of the diagnostic test accuracy [30]. In addition, subgroup and meta-regression analysis [31, 32] were conducted to explore the possible sources of heterogeneity among studies. Publication bias was inspected using Deeks' funnel plot asymmetry test [33]. A two-sided p-value of < 0.05 was considered to be statistically significant.

Results

Search results

A total of 1,715 titles and abstracts were found from initial searches of the electronic database. Four records were identified through reviewing the references of the other meta-analysis and reviews. Firstly, 367 records were excluded using EndNote X6 due to duplication. We applied the inclusion and exclusion criteria to filter out 1,286 records, because they were considered as review articles, meta-analysis, commentaries, letters, case reports, or records about apparently irrelevant to study question. Leaving 66 articles were eligible for further full-text review. Subsequently, additional 38 articles were further excluded after a full-text review. The list of full-text excluded articles, along with detailed reasons for exclusion, is presented in the supporting information (S2 Table). Finally, a total of 28 studies that met inclusion criteria were included in the present meta-analysis [1–5, 9, 10, 12, 14–20, 34–46]. The details of study selection flow are summarized in Fig 1.

Characteristics and quality of the studies

The main characteristics of the included studies are shown in Table 1. Our meta-analysis included 28 studies which were published between 1997 and 2014. A total of 7,378 specimens were taken from infants to adults. Of 7,378 specimens, 219 were venous access ports [10], 10 were cerebrospinal fluids [38, 40, 41], and the rest were blood samples. All specimens were confirmed by conventional identification methods which were currently the reference standard in the diagnosis of bacterial infections.

The detailed quality information of the included studies is shown in S3 Table. According to QUADAS-2 tool, 24 (85.7%) studies were at low risk of patient selection bias. A similar situation was observed in the flow and timing. As for index test and reference standard, the overwhelming majority (82.1% and 92.9%, respectively) studies were at high or unclear risk due to insufficient information to judge whether their test results were interpreted blind. Only 6 studies reported blinded interpretation of index test [1, 10, 12, 18, 19, 36], and 2 studies reported the blinded interpretation of reference standard [35, 37]. From an overall perspective, the qualities of the reported studies all turned out to be moderate to high concerns about applicability. Risk of bias and applicability concerns graph is presented in Fig 2. Risk of bias and applicability concerns summary is presented in Fig 3.
Heterogeneity analysis and diagnostic accuracy

Spearman correlation coefficient of sensitivity and 1-specificity was found to be -0.177 with a ρ value of 0.367, indicating that there was no heterogeneity caused by the threshold effect. As was depicted in the Fig 4, statistically significant heterogeneity was observed when we pooled DOR of included studies. The result suggested that there should be other factors rather than threshold effect resulting in variations in accuracy estimates. We performed the univariable meta-regression analysis based on the publication year, sample size, disease type (sepsis or bacteremia), population characteristics (neonates or adult), and PCR test (qualitative or quantitative). Meta-regression analysis indicated that disease type and PCR test were significantly (p < 0.05) associated with specificity and that population characteristics were significantly (p < 0.05) related to the sensitivity. The detailed results of meta-regression analysis are presented in S4 Table and S1 Fig. Therefore, we decided that a random-effects model was used to eliminate some heterogeneity.

After analysis using the random effect model, our meta-analysis showed that sensitivity and specificity were 0.87 (95% CI, 0.85–0.89) and 0.94 (95% CI, 0.93–0.95), respectively (Fig 5A and 5B). The results suggested that 16S rRNA gene PCR test had a higher specificity than sensitivity in the diagnosis of BSIs. The overall PLR was 12.65 (95% CI, 8.04–19.90) (Fig 5C), the overall NLR was 0.14 (95% CI, 0.08–0.24) (Fig 5D), and the pooled DOR was 116.76 (95% CI, 52.02–262.05) (Fig 4). The SROC curve for the included studies was shown in Fig 6. The pooled AUC and Q* of SROC curve were 0.9690 and 0.9183, respectively.

Subgroup analysis

We also performed subgroup analyses according to population characteristics, disease type and test methods (Table 2). For the diagnostic accuracy of 16S rRNA gene PCR test for neonates, the sensitivity was 0.85 (95% CI, 0.81–0.88), the specificity was 0.96 (95% CI, 0.95–0.96), the PLR was 13.19 (95% CI, 7.08–24.57), and the NLR was 0.14 (95% CI, 0.06–0.29). Additionally, the AUC and Q* of SROC curve were 0.9714 and 0.9221. However, for adult patients, the
Table 1. Characteristics of the studies included in the meta-analysis.

| Study            | Country | Disease type | Participant characteristics | Specimen type | Test methods | Cut-off       | TP  | FP | FN | TN |
|------------------|---------|--------------|----------------------------|---------------|--------------|---------------|-----|----|----|----|
| Liu CL, 2014[1]  | China   | Sepsis       | Neonates                   | Blood         | PCR          | 630 and 216 bp| 95  | 28 | 0  | 583|
| Hassan RM, 2014[9]| Egypt   | Bloodstream infections | All age groups             | Blood         | PCR-sequencing | Sequence similarity≥97% | 58  | 21 | 11 | 198|
| Guembe M, 2013[10]| Spain   | Bloodstream infections | Adult patients            | Venous access ports | PCR-sequencing | Sequence similarity≥99% | 12  | 53 | 3  | 151|
| Shaat SS, 2013[2] | Egypt   | Sepsis       | Neonates                   | Blood         | PCR          | 1100 bp       | 17  | 7  | 0  | 26 |
| Negoro E, 2013[12]| Japan   |             | NR                         | Blood         | PCR-hybridization | Fluorescent signal | 38  | 2  | 3  | 292|
| Matsuda K, 2011[35]| USA     | Bloodstream infections | Pediatric patients         | Blood         | PCR-hybridization | Fluorescent pattern | 122 | 11 | 1  | 94 |
| Vallee Jr DL, 2010[4]| Philippines | Bacteremia | Adult patients             | Blood         | PCR          | 400 bp        | 45  | 0  | 3  | 66 |
| Chen LH, 2009[38]| China   | Sepsis; meningitis | Children                  | Blood; CSF    | FQ-PCR       | C<sub>T</sub> values <35 cycles | 15  | 10 | 0  | 170|
| Handschur M, 2009[57]| Austria | Bloodstream infections | NR                        | Blood         | PCR-sequencing | Sequence similarity>99.8% | 7   | 0  | 2  | 13|
| Wellhausen N, 2009[20]| Germany | Bacteremia | Adults and children        | Blood         | PCR          | 450 bp        | 47  | 41 | 7  | 247|
| Dutta S, 2009[14]| India   | Sepsis       | Neonates                   | Blood         | PCR          | 380 bp        | 50  | 7  | 2  | 183|
| Ohlin A, 2008[18]| Sweden  | Bacteremia   | Neonates                   | Blood         | real-time PCR| CP value with a range of 19–29.8 | 21  | 12 | 29 | 233|
| Wu YD, 2007[39]| China   | Sepsis       | Neonates                   | Blood         | FQ-PCR       | C<sub>T</sub> values <35 cycles | 20  | 23 | 0  | 787|
| Jordan JA, 2006[16]| USA     | Sepsis       | Neonates                   | Blood         | PCR          | 380 bp        | 7   | 30 | 10 | 1186|
| Shang S, 2005[15]| China   | Sepsis; meningitis | Neonates                  | Blood         | PCR          | 371 bp        | 8   | 9  | 0  | 155|
| Tong MQ, 2004[40]| China   | Sepsis       | Neonates                   | Blood; CSF    | PCR          | 371 bp        | 8   | 9  | 0  | 268|
| Shang S, 2001[41]| China   | Sepsis       | Neonates                   | Blood; CSF    | PCR-hybridization | 371 bp        | 26  | 0  | 0  | 30 |
| Sleigh J, 2001[19]| New Zealand | Bacteremia | Adult patients             | Blood         | PCR          | NR            | 15  | 48 | 13 | 121|
| Jordan JA, 2000[42]| USA     | Bacteremia   | Infants                    | Blood         | PCR          | 380 bp        | 24  | 3  | 1  | 520|
| Draz NI, 2013[3]| Egypt   | Sepsis       | Neonates                   | Blood         | PCR          | 1100 bp       | 20  | 15 | 8  | 7  |
| Ohlin A, 2012[34]| Sweden  | Sepsis       | Neonates                   | Blood         | real-time PCR| NR            | 44  | 31 | 12 | 281|
| Esparcia O, 2011[17]| Spain   | Sepsis       | Neonates                   | Blood         | RT-qPCR      | CT: between cycles 30 and 32 | 3   | 3  | 4  | 73 |
| Fujimori M, 2010[5]| Japan   | Sepsis       | Neonates                   | Blood         | RT-qPCR      | NR            | 6   | 9  | 0  | 24 |
| Yadav AK, 2005[44]| India   | Sepsis       | Neonates                   | Blood         | RT-qPCR      | NR            | 6   | 9  | 0  | 24 |
| Makhoul IR, 2005[45]| Israel  | late-onset sepsis | Neonates                  | Blood         | PCR          | 10 CFU/ml of blood | 9   | 0  | 4  | 202|
| Jordan JA, 2005[46]| USA     | Sepsis       | Neonates                   | Blood         | real-time PCR| CT value >1.0 | 51  | 0  | 2  | 32 |
| Laforgia N, 1997[43]| Italy   | Sepsis       | Neonates                   | Blood         | PCR          | 861 bp        | 4   | 2  | 0  | 27 |
| Reier-Nilsen T, 2009[36]| Norway | Sepsis       | Infants                    | Blood         | PCR          | 1500 bp, 1100 bp or 500 bp | 4   | 6  | 2  | 36 |

TP, true positive; FP, false positive; FN, false negative; TN, true negative; CSF, cerebrospinal fluid; PCR, polymerase chain reaction; FQ-PCR, fluorescent quantitative polymerase chain reaction; RT-qPCR, reverse transcription-quantitative PCR; CP, Crossing Point; C<sub>T</sub>, cycle threshold; bp, base pairs; NR, no report.

doi:10.1371/journal.pone.0127195.001
sensitivity was 0.79 (95% CI, 0.69–0.87), the specificity was 0.77 (95% CI, 0.73–0.81), the PLR was 4.37 (95% CI, 1.17–16.34), and the NLR was 0.24 (95% CI, 0.05–1.14). Additionally, the AUC and Q of SROC curve were 0.7668 and 0.7074, indicating a lower accuracy compared with neonates. As for the types of disease, the sensitivity of sepsis was significantly higher than bacteremia. Interestingly, compared with other amplification methods, PCR-hybridization showed a higher level of overall accuracy. After including 3 studies, the pooled sensitivity, specificity, PLR, and NLR (95% CI) increased to 0.98 (95% CI, 0.95–0.99), 0.97 (95% CI, 0.95–0.98), 37.98 (95% CI, 4.78–301.89), and 0.03 (95% CI, 0.01–0.13), respectively. Additionally, the AUC and Q of SROC curve were 0.9958 and 0.9751.

Publication bias
The Deeks’ test did not indicate any strong statistical evidence of publication bias, with p-value of 0.24 for the overall analysis. The shape of the funnel plot of the pooled DOR of 16S rRNA gene PCR in the diagnosis of BSIs also did not show any evidence of obvious asymmetry (Fig 7), indicating that there was no potential publication bias.

Discussion
A previous meta-analysis had demonstrated that 16S rRNA gene PCR test had excellent sensitivity and specificity in the diagnosis of bacterial meningitis [47]. However, we did not know whether the test had a similar effect to BSIs. Studies focusing on the diagnostic value of 16S rRNA gene PCR test have been conducted in recent years. Thus, in this study we evaluated the accuracy of 16S rRNA gene PCR test in the diagnosis of BSIs.

Our results showed that the pooled sensitivity of the 16S rRNA gene PCR test was 0.87 and the pooled specificity was 0.94. This test may be a valid tool for confirming the diagnosis of BSIs, although not perfect. To illustrate the overall performance of 16S rRNA gene PCR test, we also counted the AUC and Q of the SROC curve. A SROC curve is usually used to summarize overall test performance, while the AUC under the SROC curve is a measure of the overall performance of a diagnostic test to accurately differentiate those with and those without the condition of interest [30]. Q is defined by the intercept of the SROC, which is closest to the ideal top-left corner of the SROC space and which corresponds to the highest value of sensitivity and specificity for the test [30, 48]. In present meta-analysis, the data showed that the AUC and Q were 0.9690 and 0.9183, indicating very good ability to diagnose BSIs. The DOR reflects the relationship between the result of the diagnostic test and the disease, the value of which ranges from 0 to infinity—higher values indicating better discriminatory test performance [49]. Our meta-analysis showed that the pooled DOR was 116.76, suggesting a high level of overall accuracy. Compared with the DOR and SROC curve, the likelihood ratio (PLR and NLR) is considered to be more clinically meaningful for our measures of diagnostic accuracy [50]. The PLR represents the value by which the odds of the disease increase when a test is
Fig 3. Risk of bias and applicability concerns summary. Review authors' judgments about each domain for each included study.

doi:10.1371/journal.pone.0127195.g003
positive. Whereas NLR shows the value by which the odds of the disease decrease when a test is negative. The PLR value was 12.65 in the overall analysis, which suggested that patients with a positive PCR result had about a 13-fold chance of being diagnosed with BSIs rather than non-BSIs. On the other hand, the NLR was 0.14, which suggested that if a PCR result was negative, the probability rate of the individual having BSIs was 14% in theory.

**Fig 4. Diagnostic Odds Ratio with Cochran-Q value.**
doi:10.1371/journal.pone.0127195.g004

**Fig 5. Forest plots for the diagnostic accuracy of 16S rRNA gene PCR.** A. Sensitivity; B. Specificity; C. Positive likelihood ratio; D. Negative likelihood ratio.
doi:10.1371/journal.pone.0127195.g005
Heterogeneity is a potential problem in interpreting the results of any meta-analysis. The threshold effect arises when differences in sensitivities and specificities occur due to different cut-offs or thresholds used in different studies to define a positive or negative test result [23]. We took the threshold effect as the first factor in our meta-analysis. We used the Spearman correlation coefficient to analyze the threshold effect. The result showed that Spearman value was found to be -0.177 ($\rho = 0.367$) using Meta-Disc analysis, suggesting that the heterogeneity was not caused by the threshold effect. However, the Cochran-Q value and $I^2$ test showed that the heterogeneity among studies was too obvious to be ignored. To find the possible reasons

![Fig 6. Summary receiver operating characteristic curve of 16S rRNA gene PCR diagnostic value in bloodstream infections.](image)

doi:10.1371/journal.pone.0127195.g006

Table 2. Summary of subgroup analysis of the included studies by different study characteristics.

| Subgroups          | No. of Studies | Sensitivity (95% CI) | Specificity (95% CI) | PLR (95% CI) | NLR (95% CI) | DOR (95% CI) | AUC   | Q*     |
|--------------------|----------------|----------------------|----------------------|--------------|--------------|--------------|-------|--------|
| Overall            | 28             | 0.87 (0.85–0.89)     | 0.94 (0.93–0.95)     | 12.65 (8.04–19.90) | 0.14 (0.08–0.24) | 116.76 (52.02–262.05) | 0.9690 | 0.9183 |
| **Population**     |                |                      |                      |              |              |              |       |        |
| characteristics    |                |                      |                      |              |              |              |       |        |
| Neonates           | 17             | 0.85 (0.81–0.88)     | 0.96 (0.95–0.96)     | 13.19 (7.08–24.57) | 0.14 (0.06–0.29) | 121.17 (41.97–349.79) | 0.9714 | 0.9221 |
| Adult patients     | 3              | 0.79 (0.69–0.87)     | 0.77 (0.73–0.81)     | 4.37 (1.17–16.34)   | 0.24 (0.05–1.14)  | 25.86 (1.69–395.53)   | 0.7668 | 0.7074 |
| **Disease type**   |                |                      |                      |              |              |              |       |        |
| Sepsis             | 15             | 0.90 (0.87–0.93)     | 0.95 (0.95–0.96)     | 11.36 (5.76–22.41) | 0.12 (0.05–0.29)  | 108.89 (33.22–356.94) | 0.9681 | 0.9168 |
| Bacteremia         | 7              | 0.77 (0.71–0.82)     | 0.94 (0.93–0.95)     | 25.97 (7.54–89.42)  | 0.20 (0.08–0.47)  | 158.82 (24.81–1016.59) | 0.9304 | 0.8656 |
| **Test method**    |                |                      |                      |              |              |              |       |        |
| PCR                | 15             | 0.88 (0.84–0.91)     | 0.95 (0.94–0.95)     | 13.06 (6.42–26.58) | 0.14 (0.06–0.32)  | 120.94 (31.82–459.64) | 0.9653 | 0.9124 |
| PCR-sequencing     | 3              | 0.83 (0.74–0.90)     | 0.83 (0.79–0.86)     | 6.17 (1.96–19.45)   | 0.20 (0.13–0.32)  | 30.61 (9.76–95.99)    | 0.8946 | 0.8255 |
| PCR-hybridization  | 3              | 0.96 (0.95–0.99)     | 0.97 (0.95–0.98)     | 37.98 (4.78–301.89) | 0.03 (0.01–0.13)  | 1568.82 (431.48–5703.98) | 0.9958 | 0.9751 |
| Real-time PCR      | 3              | 0.73 (0.65–0.80)     | 0.93 (0.90–0.95)     | 9.06 (4.77–17.21)   | 0.21 (0.06–0.76)  | 40.16 (9.68–166.52)   | 0.9458 | 0.8849 |

CI, confidence interval; PLR, positive likelihood ratio; NLR, negative likelihood ratio; DOR, diagnostic odds ratio; AUC area under the curve; Q*, Q point value.

doi:10.1371/journal.pone.0127195.t002
for heterogeneity, we undertook a univariable meta-regression analysis based on the publication year, sample size, disease type (sepsis or bacteremia), population characteristics (neonates or adult), and PCR test (qualitative or quantitative). Unexpectedly, we found that disease type, population characteristics and PCR test were attributable to the sources of heterogeneity. Thus, we performed the subgroup analyses according to population characteristics, disease type and test methods. Compared with the previous meta-analysis conducted by Pammi et al [51], we found that there was similar specificity (0.96, 95% CI: 0.94–0.97) and sensitivity (0.90, 95% CI: 0.78–0.95) to our subgroup analysis based on sepsis. It was noteworthy that PCR-hybridization test was more accurate in distinguishing patients with BSIs from non-BSIs people than other amplification methods, whereas the result should be interpreted with caution due to limited data and heterogeneity. But we did not conduct subgroup analyses based on the fluorescent quantitative PCR (FQ-PCR) and reverse transcription-quantitative PCR (RT-qPCR) owing to limited original data.

A previous study conducted by Loonen et al [52] suggested that deoxyribose nucleic acid (DNA) isolation methods could possibly affect BSIs diagnostics. In our meta-analysis, a reliable estimate of the amount of nucleic acid isolation was not provided in included studies, and extraction processes varied from boiling techniques [14, 15, 38–42] to differently commercial DNA extraction kits[1–4, 10, 17, 18, 20, 36, 37, 45], even RNA extraction kits [5]. Therefore, it was overwhelmingly difficult to compare the success of each of these methods.

Similar to other meta-analyses, several limitations should be acknowledged. Firstly, While the methodological quality of studies was assessed according to the QUADAS-2 tool, most studies were at unclear risk bias in index test and reference standard due to lacking of blinding results interpreted. Secondly, only published English and Chinese language studies were included in this meta-analysis, so the language bias might influence the results. Thirdly, the cut-off values varied widely, which made it difficult to determine the optimized cut-off value. Finally, Only 902 (12.2%) of 7,378 specimens had positive blood culture results, which could lead to broad variance about sensitivity.

In conclusion, our study is the first comprehensive meta-analysis to date that has assessed the accuracy of 16S rRNA gene PCR test in the diagnosis of BSIs. Despite the limitations mentioned above, the current evidence suggests that the 16S rRNA gene PCR test is a rapid, practical and valid tool for confirming the diagnosis of BSIs, especially sepsis. However, there is
insufficient data to fully confirm diagnostic accuracy of PCR-hybridization test. Further meta-analysis involving more prospective studies with analysis of subgroups by amplification methods should be performed in the future.

Supporting Information

S1 Fig. Univariable meta-regression & Subgroup analysis.
(TIF)
S1 PRISMA Checklist. PRISMA checklist.
(DOC)
S1 Table. Search strategy.
(DOC)
S2 Table. Articles excluded along with the reasons for exclusion.
(DOC)
S3 Table. The detailed quality information of the included studies.
(XLS)
S4 Table. Meta-regression analyses of potential source of heterogeneity.
(DOC)

Acknowledgments

We thank all authors of primary studies included in our meta-analyses.

Author Contributions

Conceived and designed the experiments: GS WY. Performed the experiments: GS ZF YW ZZ. Analyzed the data: GS ZF YW. Contributed reagents/materials/analysis tools: ZF ZZ. Wrote the paper: GS ZF. Critical revision of the article: LH.

References

1. Liu CL, Ai HW, Wang WP, Chen L, Hu HB, Ye T, et al. Comparison of 16S rRNA gene PCR and blood culture for diagnosis of neonatal sepsis. Archives de pediatrie: organe officiel de la Societe francaise de pediatrie. 2014; 21(2):162–9. Epub 2014/01/07. doi:10.1016/j.arcped.2013.11.015 PMID:24388336.

2. Shaat SS, El Shazly SA, Badr Eldin MM, Barakat SS, Hashish MH. Role of polymerase chain reaction as an early diagnostic tool for neonatal bacterial sepsis. The Journal of the Egyptian Public Health Association. 2013; 88(3):160–4. Epub 2014/01/01. doi:10.1097/01.EPX.0000441294.14692.4c PMID:24374951.

3. Draz NI, Taha SE, Abou Shady NM, Abdel Ghany YS. Comparison of broad range 16S rDNA PCR to conventional blood culture for diagnosis of sepsis in the newborn. Egyptian Journal of Medical Human Genetics. 2013; 14(4):403–11. doi:10.1016/j.ejmhg.2013.05.004

4. Valle DL Jr, Andrade JI, Cabrera EC, Rivera WL. Evaluation of buffy coat 16S rRNA PCR, buffy coat culture and whole blood PCR for detection of bacteraemia. Memorias do Instituto Oswaldo Cruz. 2010; 105(2):117–22. Epub 2010/04/30. PMID:20428667.

5. Fujimori M, Hisata K, Nagata S, Matsunaga N, Komatsu M, Shoji H, et al. Efficacy of bacterial ribosomal RNA-targeted reverse transcription-quantitative PCR for detecting neonatal sepsis: a case control study. BMC pediatrics. 2010; 10:53. Epub 2010/07/30. doi:10.1186/1471-2431-10-53 PMID:20667142; PubMed Central PMCID: PMCPMC2922101.

6. Beekmann SE, Diekema DJ, Chapin KC, Doern GV. Effects of rapid detection of bloodstream infections on length of hospitalization and hospital charges. Journal of clinical microbiology. 2003; 41(7):3119–25. Epub 2003/07/05. PMID:12843951; PubMed Central PMCID: PMCPMC165359.
7. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. The Lancet infectious diseases. 2004; 4(12):751–60. Epub 2004/11/30. doi: 10.1016/s1473-3099(04)01205-8 PMID: 15567125.

8. Isaacman DJ, Karasic RB, Reynolds EA, Kost SI. Effect of number of blood cultures and volume of blood on detection of bacteremia in children. The Journal of pediatrics. 1996; 128(2):190–5. Epub 1996/02/01. PMID: 8636810.

9. Hassan RM, El Enany MG, Rizk HH. Evaluation of broad-range 16S rRNA PCR for the diagnosis of bloodstream infections: two years of experience. Journal of infection in developing countries. 2014; 8(10):1252–8. Epub 2014/10/15. doi: 10.3855/jijd.4687 PMID: 25313600.

10. Venkatesh M, Flores A, Luna RA, Versalovic J. Molecular microbiological methods in the diagnosis of bloodstream infections. The Journal of molecular diagnostics: JMD. 2006; 8(3):357–63. Epub 2006/07/11. doi: 10.2353/jmoldx.2006.050138 PMID: 16825509; PubMed Central PMCID: PMCPMC1867603.

11. Otterbein LE, Hunfeld KP, Enrich T, Haberhauen G, Wissing H, Hoeft A, et al. A multiplex real-time PCR assay for rapid detection and differentiation of 25 bacterial and fungal pathogens from whole blood samples. Medical microbiology and immunology. 2008; 197(3):313–24. Epub 2007/11/17. doi: 10.1007/s00430-007-0063-0 PMID: 18008085.

12. Dutta S, Narang A, Chakraborty A, Ray P. Diagnosis of neonatal sepsis using universal primer polymerase chain reaction before and after starting antibiotic drug therapy. Archives of pediatrics & adolescent medicine. 2009; 163(1):6–11. Epub 2009/01/07. doi: 10.1001/archpediatrics.2008.513 PMID: 19124696.

13. Ohlin A, Backman A, Bjorkqvist M, Ohlsson N, Schollin J. Real-time PCR of the 16S rRNA gene in the diagnosis of neonatal sepsis. Expert review of anti-infective therapy. 2010; 8(9):1037–48. doi: 10.1586/eri.10.89 PMID: 20818947; PubMed Central PMCID: PMC2956278.

14. Wellinghausen N, Kochen AJ, Dische Q, Muhl H, Gebert S, Winter J, et al. Diagnosis of bacterial meningitis, early-onset neonatal sepsis, and spontaneous bacterial peritonitis. Diagnostic microbiology and infectious disease. 2011; 69(2):153–60. Epub 2011/01/22. doi: 10.1016/j.diagmicrobio.2010.10.022 PMID: 21251558.

15. Ohlin A, Backman A, Bjorkqvist M, Molling P, Jurstrand M, Schollin J. Real-time PCR of the 16S rRNA gene in the diagnosis of neonatal bacteraemia. Acta paediatrica (Oslo, Norway: 1992). 2008; 97(10):1376–80. Epub 2008/07/16. doi: 10.1111/j.1651-2227.2008.00924.x PMID: 18624992.

16. Zimolzak BW, Anaccheri D, Columbus LA, Hsueh PR, O’Keane V, et al. Diagnosis of bloodstream infections: two years of experience. Journal of infection in developing countries. 2014; 8(1):80. Epub 2014/07/02. doi: 10.3855/jijd.4819 PMID: 24962320.

17. Wellinghausen N, Kochen AJ, Dische Q, Muhl 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):2759–65. Epub 2009/07/03. doi: 10.1128/jcm.00567-09 PMID: 19571030; PubMed Central PMCID: PMCPMC2738079.

18. Moher D, Liberati A, Tetzlaff J, Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. 2009; 6(7):e1000097. Epub 2009/07/03. doi: 10.1371/journal.pmed.1000097 PMID: 19571030; PubMed Central PMCID: PMCPMC2738079.

19. Blumberg PD, Barriere S, Steigbigel RT. Molecular diagnostic methods for bloodstream infections. Expert review of anti-infective therapy. 2009; 8(9):1017–27. Epub 2009/07/09. doi: 10.1586/eri.09.89 PMID: 19571030; PubMed Central PMCID: PMCPMC2738079.

20. Jordan JA, Durso MB, Butchko AR, Jones JG, Brozanski BS. Evaluating the near-term infant for early onset sepsis: progress and challenges to consider with 16S rDNA polymerase chain reaction testing. The Journal of molecular diagnostics: JMD. 2006; 8(3):357–63. Epub 2006/07/11. doi: 10.2353/jmoldx.2006.050138 PMID: 16825509; PubMed Central PMCID: PMCPMC1867603.

21. Dutta S, Narang A, Chakraborty A, Ray P. Diagnosis of neonatal sepsis using universal primer polymerase chain reaction before and after starting antibiotic drug therapy. Archives of pediatrics & adolescent medicine. 2009; 163(1):6–11. Epub 2009/01/07. doi: 10.1001/archpediatrics.2008.513 PMID: 19124696.

22. Negoro E, Iwasaki H, Tai K, Ikegaya S, Takagi K, Kishi S, et al. Utility of PCR amplification and DNA microarray hybridization of 16S rDNA for rapid diagnosis of bacteremia associated with hemotological diseases. International journal of infectious diseases: IJID: official publication of the International Society for Infectious Diseases. 2013; 17(4):e271–6. Epub 2012/12/12. doi: 10.1016/j.ijid.2012.10.010 PMID: 23226627.

23. Dutta S, Narang A, Chakraborty A, Ray P. Diagnosis of neonatal sepsis using universal primer polymerase chain reaction before and after starting antibiotic drug therapy. Archives of pediatrics & adolescent medicine. 2009; 163(1):6–11. Epub 2009/01/07. doi: 10.1001/archpediatrics.2008.513 PMID: 19124696.

24. Schellinger S, Gambini C, Heintz A, Mertens P, Hossfeld D, et al. Comparative study of multiplex real-time PCR systems for microbiological diagnosis of bloodstream infections. Journal of clinical microbiology. 2009; 47(9):2759–65. Epub 2009/07/03. doi: 10.1128/jcm.00567-09 PMID: 19571030; PubMed Central PMCID: PMCPMC2738079.

25. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. The Lancet infectious diseases. 2004; 4(12):751–60. Epub 2004/11/30. doi: 10.1016/s1473-3099(04)01205-8 PMID: 15567125.

26. Isaacman DJ, Karasic RB, Reynolds EA, Kost SI. Effect of number of blood cultures and volume of blood on detection of bacteremia in children. The Journal of pediatrics. 1996; 128(2):190–5. Epub 1996/02/01. PMID: 8636810.
44. Yadav AK, Wilson CG, Prasad PL, Menon PK. Polymerase chain reaction in rapid diagnosis of neonatal sepsis. Indian pediatrics. 2005; 42(7):681–5. Epub 2005/08/09. PMID: 16085969.

45. Makhoul IR, Smolkin T, Sujov P, Kassis I, Tamir A, Shalginov R, et al. PCR-based diagnosis of neonatal staphylococcal bacteremias. Journal of clinical microbiology. 2005; 43(9):4823–5. Epub 2005/09/08. doi: 10.1128/jcm.43.9.4823-4825.2005 PMID: 16145149; PubMed Central PMCID: PMCPMC1234095.

46. Jordan JA, Durso MB. Real-time polymerase chain reaction for detecting bacterial DNA directly from blood of neonates being evaluated for sepsis. The Journal of molecular diagnostics: JMD. 2005; 7(5):575–81. Epub 2005/11/01. doi: 10.1016/s1525-1578(10)60590-9 PMID: 16258155; PubMed Central PMCID: PMCPMC1867550.

47. Srinivasan L, Pisapia JM, Shah SS, Halpern CH, Harris MC. Can broad-range 16S ribosomal ribonucleic acid gene polymerase chain reactions improve the diagnosis of bacterial meningitis? A systematic review and meta-analysis. Annals of emergency medicine. 2012; 60(5):609–20 e2. doi: 10.1016/j.annemergmed.2012.05.040 PMID: 22883680.

48. Jones CM, Athanasiou T. Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. The Annals of thoracic surgery. 2005; 79(1):16–20. Epub 2004/12/29. doi: 10.1016/j.athoracsur.2004.09.040 PMID: 15620907.

49. Glas AS, Lijmer JG, Prins MH, Bonsel GJ, Bossuyt PM. The diagnostic odds ratio: a single indicator of test performance. Journal of clinical epidemiology. 2003; 56(11):1129–35. Epub 2003/11/15. PMID: 14615004.

50. Gallagher EJ. Clinical utility of likelihood ratios. Annals of emergency medicine. 1998; 31(3):391–7. Epub 1998/03/20. PMID: 9506499.

51. Pammi M, Flores A, Leeflang M, Versalovic J. Molecular assays in the diagnosis of neonatal sepsis: a systematic review and meta-analysis. Pediatrics. 2011; 128(4):e973–85. Epub 2011/09/29. doi: 10.1542/peds.2011-1208 PMID: 21949139.

52. Loonen AJ, Bos MP, van Meerbergen B, Neerken S, Catsburg A, Dobbelaer I, et al. Comparison of pathogen DNA isolation methods from large volumes of whole blood to improve molecular diagnosis of bloodstream infections. PloS one. 2013; 8(8):e72349. doi: 10.1371/journal.pone.0072349 PMID: 23977288; PubMed Central PMCID: PMCPMC3744477.