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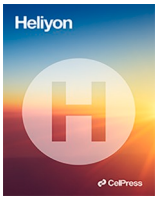

Research article

Microbiological performance and adherence in blood culture protocols: The role of a second anaerobic bottle

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ABSTRACT

Background: Bacteremia represents high rates of morbidity and mortality, especially in developing countries, highlighting the need for a diagnostic method that allows prompt and appropriate patient treatment. This study compared microbiological performance and adherence of two blood culture protocols for the diagnosis of bacteremia.
Methods: Quasi-experimental study conducted between June 2022 and February 2023. Two blood culture protocols were evaluated. Protocol 1 included two aerobic bottles and one anaerobic bottle. Protocol 2 included two aerobic and two anaerobic bottles. Protocols were analyzed in three phases: evaluation of protocol 1 (Phase 1); evaluation of protocol 1 plus educational activities for healthcare staff (Phase 2) and evaluation of protocol 2 (Phase 3).
Results: 342 patients and 1155 blood culture bottles (732 aerobic and 423 anaerobic) were included. Positivity was 17.6 %, 22.8 % and 19.4 % in phases 1, 2 and 3, respectively. Among patients with bacteremia, 84.5 % had positive anaerobic bottles, with 9.9 % showing growth only in this bottle. The contamination rates were 1.9 %, 0.3 %, and 0.8 % for each phase, mainly in aerobic bottles. Median positivity time was 11 h for both bottes aerobic and anaerobic. Overall nursing adherence increased from 13.1 % in Phase 1, 25.9 % in Phase 2, and 28.1 % in Phase 3 ($p = 0.009$).
Conclusions: The findings indicate that adding a second anaerobic bottle does not enhance blood culture positivity. Rather than increasing bottle quantity, staff training might be a more effective approach to optimize results.

1. Introduction

Bloodstream infections constitute a spectrum ranging from bacteremia to septic shock, representing high rates of morbidity and mortality (10 %–30 %), especially in developing countries [1]. This underscores the need for a diagnostic method that allows rapid management and appropriate selection of antimicrobials [2]. Despite significant progress in microbiology, blood cultures remain one of the most common laboratory tests and the gold standard, not only for establishing etiology and antimicrobial susceptibility but also

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for determining whether the isolated microorganisms are contaminants or true pathogens [3–5].

Internationally recognized institutions in the field of clinical microbiology have proposed a series of recommendations for the handling of cultured blood samples. These recommendations aim to improve the microbiological recovery percentage and reduce contamination by controlling preanalytical variables such as the inoculated sample volume, bottle types (aerobic and anaerobic), and aseptic techniques [2,3]. These guidelines serve as a foundation for constructing and implementing a blood culture protocol in each institution, with the goal of optimizing the sensitivity and specificity of laboratory tests, reducing contamination issues, and enhancing adherence by healthcare staff [6].

In 2017, a study was conducted in the city of Medellín, evaluating activities related to blood culture collection in fifteen healthcare institutions. Although all institutions had a written protocol, there were differences in sample preparation and collection, the definition of a contaminated sample, and the standardization of result indicators, leading to interinstitutional heterogeneity in terms of microbiological isolation rates and contamination [7].

Other studies have compared various variables that influence the collection and processing of blood samples. Among these variables, volume and bottle type have been identified as the most important variables for improving microbiological isolation [8,9]. Although traditionally three 10 ml bottles have been used, there is a growing trend towards the use of four bottles (with one or two anaerobic bottles) to increase the sample volume [2,3]. However, in practice the use of this quantity of bottles is not systematic; anaerobic bottles are often not used, and in most cases, the actual collected volume is unknown [7,8,10].

Considering the evidence on the evaluation of blood culture sample collection protocols, there is considerable variability in the applied procedures and obtained results, along with few studies comparing different protocols used within the same institution [11–13]. Therefore, the aim of this study was to compare microbiological performance and healthcare staff adherence of two blood culture protocols, including the addition of a second anaerobic bottle, in a tertiary healthcare institution in Medellín, Colombia.

2. Methods

Study design and population: A quasi-experimental before-and-after study was conducted at the Bolivarian University Clinic, a tertiary care institution located in Medellín, Colombia. The clinic has 200 beds and offers healthcare services to the pediatric, adult, and obstetric populations. The study included hospitalized patients over 18 years with a medical indication for blood culture due to suspected bacteremia, during the period from June 1, 2022, to February 28, 2023. The blood cultures that were for follow-up after the initial diagnosis were excluded. The study was approved by the Health Research Ethics Committee of the Pontifical Bolivarian University (Act No. 18 of 2022). Informed consent was not required as data collection was performed from secondary sources.

Protocols: The study was divided into three phases, each with a duration of three months. In Phase 1 (baseline period), data were retrospectively collected on procedures performed for blood culture collection according to the current protocol at the clinic (protocol 1), which consisted of a set of 3 bottles, each containing 10 ml (two aerobic bottles and one anaerobic bottle). In Phase 2, information was prospectively collected after implementing educational activities directed at healthcare personnel related to the application of protocol 1. These activities were conducted by the institution as part of continuous improvement policies and consisted of workshops and lectures aimed at reinforcing knowledge of the current protocol. Finally, in Phase 3, a new protocol (protocol 2) was implemented, consisting of a set of 4 bottles, each containing 10 ml of sample (two aerobic and two anaerobic bottles), and educational activities were continued (Fig. 1S. Supplementary material). The inclusion of an additional anaerobic bottle was approved by the Infections Committee for implementation in the institution.

Blood culture collection and processing: Blood cultures were ordered by treating physicians on the basis of clinical criteria; only the first set of blood cultures taken from each patient was evaluated. In each phase, the number and type of bottles corresponding to the current institutional protocol were ordered; the reporting of this data in the medical record defined the adherence of the medical staff. Blood samples were taken by nursing staff, following each point of the institutional protocol: skin and bottle disinfection, sites and number of venipunctures, number of bottles indicated, and volume of blood inoculated in each bottle; the documentation of all these in the medical record defined the adherence of the nursing staff.

The volume of inoculated sample was measured using the marks on the side of the bottles with levels measuring every 5 ml, considering an optimal volume between 5 and 10 ml. According to institutional protocol, patients with bottles that do not meet this criterion should be requested to provide a new sample, whenever possible.

The BACT/ALERT FA Plus aerobic (containing 30 ml of complex medium and $\geq$1.6 g of adsorbent polymeric beads) and BACT/ALERT FN Plus anaerobic (containing 40 ml of medium and $\geq$1.6 g of adsorbent polymeric beads) bottles were used for blood cultures. They were set for a maximum time of 5 days on the BACT/ALERT 3D equipment from the commercial company Biomerieux.

Contamination of blood cultures was defined as the identification of one or more of the following organisms in only one of the bottles: coagulase-negative *Staphylococcus* spp., *Cutibacterium* (*Propionibacterium*) *acnes*, *Micrococcus* spp., viridans group streptococci, *Corynebacterium* spp., *Aerococcus* spp., or *Bacillus* spp.

Variables: The dependent variables included the appropriate volume of inoculated sample (between 5 and 10 ml), blood culture result and isolated microorganism, percentage and time to positivity, contamination percentage, and healthcare staff adherence to blood culture protocols. Each of these variables was compared according to the protocol applied in each phase of the study: Protocol 1 (Phase 1), Protocol 1 + education (Phase 2), and Protocol 2 (Phase 3). Other variables included gender, age, comorbidities, diagnosis, ordering specialty, use of empirical antibiotics, and the number and type of blood culture bottles ordered. Data collection was performed by researchers using a specifically designed form with validation and verification codes to prevent errors during data entry. The sources of information were the medical records of each patient and sample processing records from the clinical laboratory.

Statistical analysis: Descriptive statistics for qualitative variables were presented as absolute and relative frequencies, whereas

Table 1
Clinical and sociodemographic characteristics of the patients enrolled in the study. Bolivarian University Clinic, Medellín, Colombia. June 2022–February 2023.

Characteristics	Total (n = 342) No. (%)	Phase 1 (n = 137) No. (%)	Phase 2 (n = 116) No. (%)	Phase 3 (n = 89) No. (%)	*p* value
Sex					0.817
Female	197 (57.6)	79 (57.7)	69 (59.5)	49 (55.1)	
Male	145 (42.4)	58 (42.3)	47 (40.5)	40 (44.9)	
Age median (IQR)	58 (34–75)	59 (31–76)	56 (34–73)	62 (36–74)	0.740
Comorbidities	272 (79.5)	106 (77.4)	96 (82.8)	70 (78.7)	0.555
Arterial hypertension	121 (35.4)	40 (29.2)	49 (42.2)	32 (36.0)	0.096
Diabetes	85 (24.9)	30 (21.9)	30 (25.9)	25 (28.1)	0.548
COPD	44 (12.9)	16 (11.7)	12 (10.3)	16 (8.0)	0.234
Chronic kidney disease	34 (9.9)	11 (8.0)	14 (12.1)	9 (10.1)	0.563
Ubication					0.257
Emergency room	184 (53.8)	70 (51.1)	57 (49.1)	57 (64.0)	
Hospitalization	120 (35.1)	50 (36.5)	45 (38.8)	25 (28.1)	
ICU	38 (11.1)	17 (12.4)	14 (12.1)	7 (7.9)	
Specialty ordering					
General Medicine	187 (54.7)	65 (47.5)	65 (56.0)	57 (64.0)	
Internal Medicine	66 (19.3)	30 (21.9)	26 (22.4)	10 (11.2)	
Intensivist	38 (11.1)	19 (13.9)	14 (12.1)	5 (5.6)	
Obstetrician-Gynecologist	24 (7.0)	10 (7.3)	6 (5.2)	8 (9.0)	
Emergency Medicine	8 (2.3)	5 (3.7)	0 (0.0)	3 (3.4)	
Primary focus					0.184
Unknown	102 (29.8)	53 (38.7)	35 (30.2)	14 (15.7)	
Respiratory	70 (20.5)	28 (20.4)	22 (19.0)	20 (22.5)	
Urinary	64 (18.7)	20 (14.6)	22 (19.0)	22 (24.7)	
Intra-abdominal	50 (14.6)	15 (11.0)	17 (14.7)	18 (20.2)	
Skin and tissues	39 (11.4)	13 (9.5)	15 (12.9)	11 (12.4)	
Intravascular	3 (0.9)	1 (0.7)	1 (0.9)	1 (1.1)	
Obstetric-Gynecological	13 (3.8)	6 (4.4)	4 (3.5)	3 (3.4)	
Central nervous system	1 (0.3)	1 (0.7)	0 (0.0)	0 (0.0)	
Previous use of antibiotics before BC	58 (17.0)	27 (19.7)	15 (12.9)	16 (18.0)	0.344
Empirical antibiotic					0.483
Aztreonam	11 (19.0)	5 (18.5)	1 (6.7)	5 (31.2)	
Ampicillin/sulbactam	9 (15.5)	3 (11.1)	4 (26.7)	2 (12.5)	
Ceftriaxone	7 (12.1)	4 (14.8)	0 (0.0)	3 (18.8)	
Piperacillin/tazobactam	6 (10.3)	3 (11.1)	2 (13.3)	1 (6.3)	
Vancomycin	6 (10.3)	3 (11.1)	1 (6.8)	2 (12.5)	
Meropenem	5 (8.6)	3 (11.1)	2 (13.3)	0 (0.0)	
Cefepime	2 (3.5)	1 (3.7)	1 (6.7)	0 (0.0)	
Cefazolin	2 (3.5)	2 (7.4)	0 (0.0)	0 (0.0)	
Duration of antibiotic use					
Greater than 48 h	32 (55.2)	11 (40.7)	10 (66.7)	11 (68.7)	0.118
Duration of use, median (IQR) (days)	2 (1–3)	1 (1–3)	3 (1–5)	2.5 (1–3)	0.317
Confirmed bacteremia	84 (24.5)	29 (21.2)	33 (28.4)	22 (24.7)	0.409
Number of bottles used					0.048
Only two bottles	4 (1.2)	4 (2.9)	0 (0.0)	0 (0.0)	
More than two bottles	338 (98.8)	133 (97.1)	116 (100.0)	89 (100.0)	
Number of aerobic bottles					0.066
One	3 (0.9)	3 (2.2)	0 (0.0)	0 (0.0)	
Two	312 (91.2)	125 (91.2)	107 (92.2)	80 (91.2)	
Three	11 (3.2)	2 (1.5)	2 (1.7)	7 (7.8)	
Four	10 (2.9)	5 (3.7)	4 (3.5)	1 (1.1)	
Five	6 (1.8)	2 (1.5)	3 (2.6)	1 (1.1)	
Number of anaerobic bottles					<0.001
Zero	1 (0.3)	1 (0.7)	0 (0.0)	0 (0.0)	
One	257 (75.1)	135 (98.5)	115 (99.1)	7 (7.9)	
Two	84 (24.7)	1 (0.7)	1 (0.9)	82 (92.1)	

Abbreviations: Chronic obstructive pulmonary disease (COPD); Intensive care unit (ICU); central nervous system (CNS); Interquartile range (IQR); Blood Cultures (BC).

quantitative variables were described using the median and interquartile range (IQR) due to did not meet the assumption of normality. The chi-square test was used to compare qualitative variables across the three study phases. For quantitative variables, the Kruskal Wallis test was applied. When comparisons were made between aerobic and anaerobic bottles, the chi-square test or Mann-Whitney *U* test was used for qualitative and quantitative variables, respectively. A p-value less than 0.05 was considered statistically significant. The analysis was conducted using the statistical program STATA® v15.0.

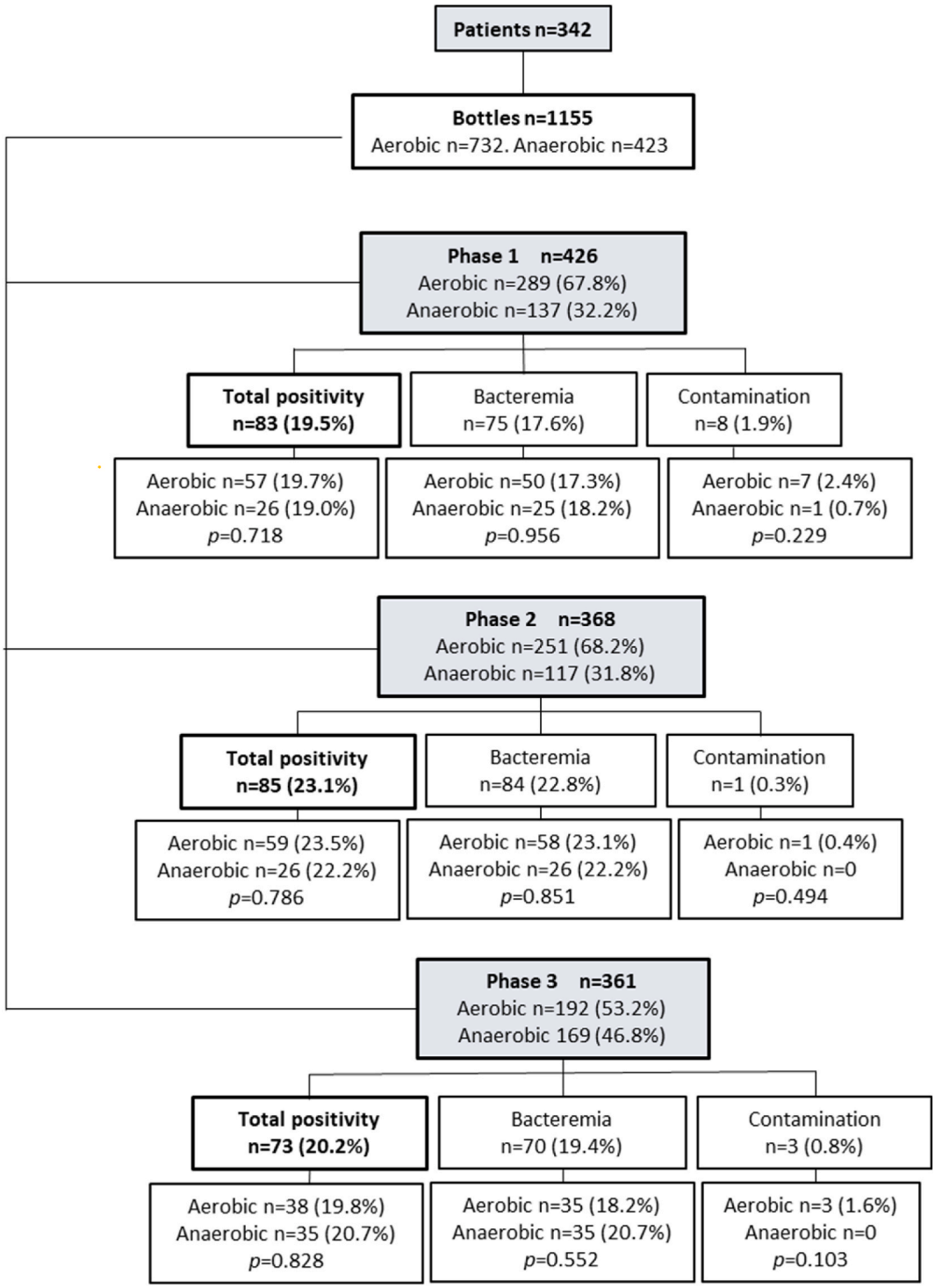

Fig. 1. Positivity and contamination according to the study phases.

3. Results

During the study period, 342 patients were admitted, with 57.6 % (n = 197) being female, and a median age of 58 years (IQR: 34–75). The most common comorbidity was hypertension (35.4 %), followed by diabetes (24.9 %) and chronic obstructive pulmonary disease (12.9 %). Regarding the service, most patients (53.8 %) had blood cultures ordered from the emergency department, with general medicine contributing the highest number of orders (54.7 %). The main sources of infection were respiratory (20.5 %) and urinary (18.7 %). The overall percentage of patients with confirmed bacteremia was 24.6 %. In general, the clinical and socio-demographic characteristics of the patients were comparable across each phase (Table 1).

Volume: A total of 1155 blood culture bottles were collected. The overall percentage of bottles with inadequate volume during sample collection was 8.9 % (n = 103), and when comparing this parameter between aerobic and anaerobic bottles, the percentage was very similar, 9.2 % vs. 8.5 % ($p = 0.084$).

Positivity and contamination: Of the total bottles cultured, 20.8 % (n = 241) tested positive, with 19.8 % (n = 229) classified as confirmed bacteremia and 1.0 % (n = 12) as contamination. When comparing the percentage of confirmed bacteremia and contamination in each phase of the study, positivity was higher in phase 2 than in the other phases (22.8 % in phase 2 vs. 17.6 % in phase 1 and 19.4 % in phase 3). In phase 2, a lower percentage of contaminated bottles was also observed (0.3 % in phase 2 vs. 1.9 % in phase 1 and 0.8 % in phase 3) (Fig. 1). When contrasting positivity between aerobic and anaerobic bottles, no statistically significant differences were observed (19.5 % vs. 20.3 %, respectively; $p = 0.818$). In the case of adding the second anaerobic bottle in phase 3, the percentage of confirmed bacteremia increased by 2.5 % (18.2 % in aerobes and 20.7 % in anaerobes; difference = 2.5 %). Regarding contamination, a higher percentage of contaminated aerobic bottles was reported compared with anaerobic bottles in all phases of the study (Fig. 1). Of the 84 patients with confirmed bacteremia, 71 (84.5 %) had positive anaerobic bottles, with 64 (90.3 %) showing the same microorganism as the aerobic bottle. In the remaining 7 patients, growth was obtained only in the anaerobic bottle (Fig. 2S. Supplementary material).

In relation to the overall positivity time (evaluating only bottles classified as confirmed bacteremia), a median of 11 h was found in both cases (IQR: 12.0–16.6 in aerobic and IQR: 12.0–16.0 in anaerobic) ($p = 0.794$). Fig. 2 shows the median positivity times in each phase according to the evaluated bottle, where no statistically significant differences were observed. Comparing the positivity time between aerobic and anaerobic bottles for the major pathogenic microorganisms, similar values were observed, with *E. coli, K. pneumoniae*, and *S. pneumoniae* taking approximately 11 h. For *Salmonella* spp., the median was 16.8 h in aerobic bottles and 14.6 h in anaerobic bottles. For *S. aureus*, the median positivity time was longer in both types of bottles, with 18 h (Table 1S. Supplementary material).

Microorganisms isolated from cultures: The distribution of species by phase and bottle is presented in Table 2. In total, 251 isolates were isolated including pathogens and contaminants, distributed across 25 species. Of these, 239 microorganisms were classified as true pathogens, with 150 growing in aerobic bottles and 89 in anaerobic bottles. The main microorganisms isolated in confirmed bacteremia, both in aerobic and anaerobic bottles, were *Escherichia coli*, accounting for 47.3 % (n = 113), *Klebsiella pneumoniae* 13.8 % (n = 33), and *Staphylococcus aureus* 10.9 % (n = 26). Ten bottles exhibited polymicrobial growth with 2 or more different microorganisms. Twelve isolates considered as contaminants belonged to five different species: *Staphylococcus epidermidis* (n = 5), *Staphylococcus hominis* (n = 3), *Staphylococcus capitis* (n = 2), *Streptococcus mitis* (n = 1) and *Bacillus subtilis* (n = 1).

When comparing the isolated microorganisms in anaerobic bottles per patient, it was found that in 90.3 % (n = 64) of the patients, the same microorganism was isolated in aerobic bottles, and in 9.9 % (n = 7), the microorganism was isolated only in the anaerobic bottle. Regarding the latter, two were reported in phase 1 (*E. coli* n = 1 and *Bifidobacterium dentium* n = 1); four in phase 2 (*E. coli* n = 1, *K. pneumoniae* n = 1, *Streptococcus pneumoniae* n = 1 and *Bacteroides fragilis* n = 1) and one in phase 3 (*Enterococcus faecalis*).

Adherence of healthcare staff: Overall nursing adherence (compliance with all protocol points) was 13.1 % in Phase 1, 25.9 % in Phase 2, and 28.1 % in Phase 3 ($p = 0.009$). Volume recording was the variable with the lowest adherence, with percentages of 11.7 %, 26.7 %, and 29.2 % in Phases 1, 2, and 3, respectively (Fig. 3). Regarding medical staff adherence, the number of total ordered bottles

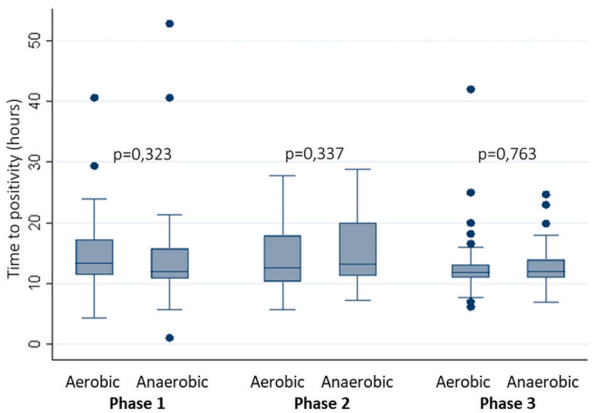

Fig. 2. Comparison of positivity time in bottles with pathogenic microorganisms (only confirmed bacteremia).

Table 2
Isolated microorganisms in confirmed bacteremia according to the study phase and bottle used.

Pathogen isolates per bottle and phase (confirmed bacteremia)							Total isolates		Isolates with growth only under anaerobic conditions		
Microorganisms	Aerobic bottles			Anaerobic bottles							
	Phase 1	Phase 2	Phase 3	Phase 1	Phase 2	Phase 3	Aerobic	Anaerobic	Phase 1	Phase 2	Phase 3
Gram-negative bacilli	**42**	**39**	**29**	**20**	**17**	**27**	**110**	**64**	**1**	**2**	**0**
Escherichia coli	24	26	21	13	11	18	71	42	1	1	0
Klebsiella pneumoniae	10	8	4	4	3	4	22	11	0	1	0
Grupo *Salmonella*	1	2	1	1	1	1	4	3	0	0	0
Klebsiella oxytoca	0	0	2	0	0	2	2	2	0	0	0
Stenotrophomonas maltophilia	3	0	0	0	0	0	3	0	0	0	0
Others[a]	4	3	1	2	2	2	8	6	0	0	0
Gram-positive cocci	**13**	**19**	**8**	**5**	**9**	**9**	**40**	**23**	**0**	**1**	**1**
Staphylococcus aureus	7	11	0	4	4	0	18	8	0	0	0
Streptococcus pneumoniae	1	2	2	0	2	2	5	4	0	1	0
CoNS[b]	2	2	1	0	1	1	5	2	0	0	0
Enterococcus faecalis	0	0	0	0	0	1	0	1	0	0	1
Others[c]	3	4	5	1	2	5	12	8	0	0	0
Strict anaerobes	**0**	**0**	**0**	**1**	**1**	**0**	**0**	**2**	**1**	**1**	**0**
Bifidobacterium dentium	0	0	0	1	0	0	0	1	1	0	0
Bacterioides fragilis	0	0	0	0	1	0	0	1	0	1	0
Total	**55**	**58**	**37**	**26**	**27**	**36**	**150**	**89**	**2**	**4**	**1**

[a] Others Gram-negative Bacilli*: Serratia marcescens, Enterobacter cloacae, Morganella morganii, and Proteus mirabilis*.
[b] CoNS: *Staphylococcus epidermidis, Staphylococcus haemolyticus*.
[c] Others Gram-positive Cocci*: Streptococcus anginosus, Streptococcus viridans, Streptococcus pyogenes, Streptococcus sanguinis, and Streptococcus salivarius*.

was considered. In Phases 1 and 2, three bottles were ordered in 89.8 % and 90.5 % of cases, respectively. In Phase 3, four bottles were ordered in 95.5 % of cases.

4. Discussion

Blood cultures are among the most widely used clinical laboratory tests and serve as the diagnostic reference for bloodstream infections. Sample volume, type, and number of bottles used have been identified as key variables for improving their performance, and in this study, they are evaluated to determine the percentages of microbiological recovery for both aerobes and anaerobes, contamination rates, and healthcare staff adherence.

The overall utility of anaerobic bottles is also evident, identifying 9.9 % more bacteremia cases that would not have been detected if only aerobic bottles were used. This finding is consistent with reports by Lafaurie et al. and Guajardo et al., who observed the utility of anaerobic bottles in diagnosing bacteremia by 15.8 % and 15.6 %, respectively [14,15]. This is significant because this undetected bacteremia would otherwise not be adequately treated.

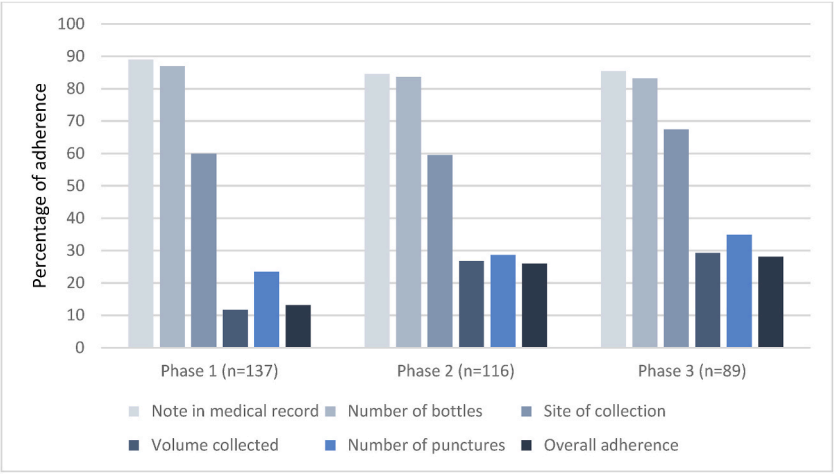

Fig. 3. Nursing staff adherence to protocols in each phase of the study.

In this study, the total positivity rate of bottles was 19.8 % (n = 229), excluding contaminations, a value above the percentages reported in the literature ranging between 5 % and 15 % [16]. When comparing phases, there was a 5.2 % increase between Phase 1 and Phase 2, reaching 22.8 %. These results may be associated with the initiation of educational activities that promote adherence to protocols and, consequently, improvement in sample collection.

In terms of contamination, it decreased from 1.9 % at the beginning of the study to 0.3 % in Phase 2 and ended at 0.8 % in Phase 3, remaining below the recommended 1 % in the latest update of the Clinical & Laboratory Standards Institute (CLSI) M47 manual [3]. Several studies have demonstrated that staff education and clear protocols with well-established preanalytical variables for blood culture collection reduce contamination rates [17,18]. In 2015, Park et al., in a study where education was provided to a group of students responsible for blood collection for blood cultures, found a reduction in the contamination rate from 1.3 % to 1.0 %, $p < 0.001$ [19]. Regarding anaerobic bottles, the results demonstrate that they do not contribute to the overall contamination percentage, suggesting that both types of bottles were handled with the same aseptic techniques and that increasing the number of bottles does not increase the contamination rate.

Indeed, an important point to consider is the sample volume. Guidelines argue that a greater volume improves the sensitivity of blood cultures; however, the literature search did not identify randomized controlled trials to support this claim [2,3,16]. Although quantitative measurement of the exact volume was not possible in this study, the addition of an extra bottle in protocol 2 suggests that during phase 3, the sample volume is greater; hence, the positivity rate should be higher. However, this does not correlate with the results obtained in this study, where it was evidenced that the addition of a second anaerobic bottle did not improve the positivity of blood cultures.

In relation to the isolated pathogenic microorganisms, the majority corresponded to Gram-negative bacilli (GNB), both in aerobic and anaerobic bottles, with *E. coli* and *K. pneumoniae* being the primary ones. This is associated with the most frequent primary infection sources, the respiratory and urinary tracts. Gram-positive cocci (GPC) show a predominance of *S. aureus* growth. This contrasts with data obtained by Jaimes et al. in another tertiary institution in Medellín, where the growth of *S. aureus* doubled that of *E. coli* and *K. pneumoniae*, possibly explained by their more frequent primary sources, skin and soft tissue [20]. However, a global study of a 20-year follow-up on the occurrence of bacteremia and causative microorganisms showed that during this period, these three microorganisms consistently ranked high in isolation frequency in blood cultures, with the recent trend towards the predominance of GNB bacteremia mainly *E. coli*, especially in Europe and Asia-Pacific [21]. It is worth noting that in these lists of microorganism frequencies, *Pseudomonas aeruginosa* always ranks fourth or fifth. However, during this study, no isolates of this microorganism were obtained.

In this study, the use of anaerobic bottles solely for detecting strict anaerobes had a low yield, accounting for 2.4 % of bacteremia, isolating only *B. fragilis* and *B. dentium*. This finding is similar to that reported by Guajardo, who reported that anaerobic blood cultures have low utility for detecting bacteremia caused by these microorganisms, detecting only 2.2 % of this type [15]. However, in this study, not using anaerobic bottles would have prevented the detection of other pathogens such as *E. coli*, *K. pneumoniae*, and *E. faecalis*, which account for a significant proportion of undetected bacteremia (9.9 %), leading to inadequate antibiotic therapy. This aligns with Lafaurie's findings, where he found that 7.7 % of bacteremia episodes diagnosed with anaerobic bottles were predominantly due to facultative anaerobes, with less frequent obligate anaerobic pathogens [14]. In 2022, Ransom and Burnham demonstrated higher recovery of *S. aureus* in the anaerobic bottle and that microorganisms such as *P. aeruginosa* and *E. coli* would have been missed in 3 % and 14 % of cases, respectively, if not for the use of this bottle [22]. The evidence observed in these studies strengthens the case for the importance of including at least one anaerobic bottle in blood culture protocols. Likewise, the growth of *Stenotrophomonas maltophilia* is noteworthy as the sole strict aerobic microorganism, which is uncommon in bloodstream infections [23]. This highlights the need to use both types of bottles to improve the overall blood culture yield. The absence of isolation of *Candida* spp. throughout the study period is noted. However, although its detection is relevant for patient treatment, this microorganism does not rank among the top ten causative agents of bloodstream infections [21]. Patient characteristics, the complexity of their illness, and the time of inoculation, culture medium, and bottle atmosphere influence the recovery of this microorganism, considering that it only grows in aerobic bottles [24].

Regarding the positivity time, it is described in the literature that 85–90 % of blood cultures are positive within 48 h [25]. In this study, positivity time for the most frequent microorganisms tended to be similar between aerobic and anaerobic bottles, except *Salmonella* spp. This contrasts with the time difference described in other reports, in which anaerobic bottles show faster positivity [26]. A likely explanation for this finding could be the concentration of the inoculum, with a higher concentration of the microorganism resulting in faster blood culture positivity. When comparing GNB with GPC, the positivity times of *S. aureus* stand out beyond the median, taking almost 6 h longer to grow. Several articles agree with these results, as *E. coli and K. pneumoniae* grow faster than Gram-positive microorganisms such as S. *aureus*, for which average positivity times of 21 h ± 1 have been reported [27].

The clinical and sociodemographic characteristics of patients in each phase did not show significant differences in terms of sex, age, and comorbidities, facilitating comparisons between groups. The population is evenly distributed between women and men (57.6 % and 42.4 %), mostly around 60 years old, and a large percentage have chronic comorbidities, with hypertension, diabetes, and chronic kidney disease (ERC) being the most relevant. In addition, the primary indications for blood culture requests were related to an unknown focus and respiratory and urinary issues. This population resembles the one described in 2018 by Chávez-Vivas in the city of Cali, Colombia, when studying the epidemiological characteristics of patients with sepsis and septic shock. In that study, diabetes and hypertension were described as the most prevalent comorbidities, and the frequent primary foci were the pulmonary and urinary tracts [28].

Regarding the use of antibiotics prior to sample collection, approximately 17 % of patients received empirical therapy, and half of them used it for more than 48 h. It is worth emphasizing that all bottles used were equipped with antibiotic inhibition resins. In 2019,

Cheng et al. published a study evaluating the sensitivity of blood cultures before and after a period of more than 2 h of antibiotic use, resulting in a 12 % decrease in positivity [29]. These data are relevant and comparable to those obtained in the current study, as Phase 2 was the period with the least use of empirical antibiotics, although without statistical significance, and at the same time, it showed the highest microbiological recovery. This may indicate that combined with other factors such as education, avoiding the use of antibiotics before sample collection could improve the performance of blood cultures.

In relation to the adherence of nursing staff, there was an improvement from the beginning of the educational activities (Phase 2). This improvement is evident when comparing overall adherence in Phase 1 (13.1 %) with Phase 2 (25.9 %), which is maintained in Phase 3 (28.1 %). This change can be related to the improvement in information recording in medical records. These results are comparable to those obtained in the study conducted by Steiner et al. who demonstrated that educational intervention influences the percentage of bottles with an adequate volume by up to 43 % ($p < 0.001$), increasing the likelihood of test positivity [30].

The main strength of this study was to evaluate the application of different protocols for blood culture sample collection and processing at different time points, all within the same institution and with a similar population. This facilitates comparison and decision-making regarding best practices for blood culture studies. In addition, it serves as a foundation for other institutions with similar characteristics when making decisions and standardizing their processes based on evidence. The limitations include the fact that exact volume of inoculated sample was not recorded on the laboratory worksheet, as only the guide on the side of the bottle was used as a visual reference to determine if it was within the appropriate range (between 5 and 10 ml). Additionally, the institution's blood culture equipment lacks an automatic sensor to measure bottle volume, which could have facilitated the collection of this information. From the findings of this study and considering experiences from other institutions, there is a lack of a standardized recommendation for the number and type of bottles to be used in bacteremia studies. Defining a blood culture protocol should be done in accordance with each institution's context. Therefore, further research is needed with different populations, such as neonates, pediatric patients, and institutions with different complexities, such as cardiovascular and transplant centers, so that each center can define its own guidelines.

In conclusion, the results highlight that retraining healthcare staff has a positive impact, increasing positivity of blood cultures, reducing contamination, and improving adherence. The importance of including anaerobic bottles in the blood culture set to identify microorganisms that may not be detected in aerobic bottles is emphasized, without increasing the contamination rate. However, the use of two anaerobic bottles did not increase positivity. These findings suggest that instead of increasing the number of bottles in the blood culture set, staff training may be a more effective strategy to optimize test results.

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Ethical approval statement

The study was approved by the Health Research Ethics Committee of the Pontifical Bolivarian University (Act No. 18 of 2022). Informed consent was not required as data collection was performed from secondary sources.

Data availability statement

Has data associated with your study been deposited into a publicly available repository?
No, data included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Paula C. Vergara: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Cristian García:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Data curation, Conceptualization. **Melissa Accini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Conceptualization. **Liliana Franco:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. **Johanna M. Vanegas:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Santiago Atehortúa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35615.

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