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ORIGINAL ARTICLE

Preanalytical criteria for collecting blood culture samples in the Microbiology area at Portoviejo General Hospital

Criterios preanalíticos en la toma de muestras de hemocultivo en el área de Microbiología del Hospital General Portoviejo

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Abstract The General Hospital of Portoviejo serves a high demand of patients with suspected infections, bacteremia, or sepsis, highlighting the importance of performing high-quality blood cultures. This study characterized errors in the preanalytical sample collection phase for the microbiological diagnosis of bacteremia through blood cultures. Existing laboratory protocols were analyzed and compared with national and international standards, focusing on asepsis, blood volume, biosafety, and techniques. Information was gathered through surveys conducted with healthcare personnel involved in sample collection, identifying key issues such as the need for more specific protocols, inadequate containers for sample transportation, and the presence of personnel in training. Although more than half of the respondents followed the correct sample collection and transport procedures, a significant proportion still needed to meet the standards. As a result, a standardized operational procedure was developed based on good laboratory practices in Ecuador and internationally to improve the quality of blood cultures in the hospital.

Keywords blood cultures, preanalytical phase, preanalytical errors, good clinical laboratory practices.

Resumen El Hospital General Portoviejo atiende una alta demanda de pacientes con sospecha de infecciones, bacteriemia o sepsis, lo que resalta la importancia de realizar hemocultivos de calidad. Este estudio caracterizó los errores en la fase preanalítica de la toma de muestras para el diagnóstico microbiológico de bacteriemias mediante hemocultivos. Se analizaron los protocolos existentes en el laboratorio y se compararon con estándares nacionales e internacionales, considerando aspectos como asepsia, volumen de sangre, bioseguridad y técnicas utilizadas. La información se recopiló mediante encuestas al personal de salud involucrado, identificando como principales problemas la falta de protocolos específicos, la carencia de recipientes adecuados para el transporte de muestras y la presencia de personal en formación. Aunque más de la mitad de los encuestados sigue correctamente los procedimientos de toma y envío, una proporción significativa no cumple con los estándares. Como resultado, se desarrolló un procedimiento operativo estandarizado basado en buenas prácticas de laboratorio ecuatorianas e internacionales para mejorar la calidad de los hemocultivos en el hospital.

Palabras clave hemocultivos, fase preanalítica, errores preanalíticos, buenas prácticas de laboratorio clínico.

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PUBLIS EDITORIAL

Introduction

Bloodstream infections represent a significant cause of morbidity and mortality, particularly in critically ill patients. Despite advancements in antimicrobial therapy and microbiological diagnostic techniques, the incidence of sepsis remains a major challenge in modern medicine (Duncan et al., 2021). Timely initiation of appropriate treatment is crucial for reducing associated mortality and improving patient prognosis, provided there is no antimicrobial resistance. When a pathogen invades the bloodstream, clinical manifestations can arise that may become fatal. Therefore, in addition to accurate clinical diagnosis, effective laboratory methods are required to rapidly identify the causative microorganism, determine its antimicrobial susceptibility, and optimize clinical management (Huemer et al., 2020).

Blood cultures are an essential tool in diagnosing bacteremia and sepsis. Their accurate execution depends on standardized processes and strict adherence to protocols at all stages, emphasizing the preanalytical phase (Lamy et al., 2016). The General Hospital of Portoviejo receives a high volume of local and referred patients suspected of severe infections, facing the challenge of ensuring the quality of performed blood cultures. To date, no prior research has been conducted to evaluate preanalytical criteria in this hospital, representing an opportunity to identify areas for improvement and enhance patient care.

The preanalytical phase is the stage of the process where most errors in clinical laboratories occur, with reported fre-quencies ranging from 17 to 84%, depending on the stu-died variables. These errors include issues related to sample quality, such as hemolysis, lipemia, insufficiency, or conta-mination, as well as failures in aseptic procedures, transpor-tation, and administrative records. Additionally, studies hi-ghlight the importance of having trained personnel and clear protocols to ensure compliance with good clinical laboratory practices (Alcantara et al., 2022).

Implementing quality management systems in clinical laboratories and using performance indicators enables tracking and evaluating each stage of the process, from the analysis request to the issuance of the result report (Chaudhry et al., 2023). This approach not only facilitates the identification of errors and delineation of responsibilities but also guides the implementation of improvements that directly impact clinical decision-making and, consequently, patient quality of life.

This study aimed to characterize errors in the preanalytical phase of blood culture sample collection used in the microbiological diagnosis of bacteremia in the microbiology department of the General Hospital of Portoviejo.

Methodology

A qualitative, prospective, and cross-sectional study was conducted between June and September 2020 in the General Hospital of Portoviejo laboratory. Documents related to the quality management system of the hospital's microbiology department were included. A survey was administered to 16 professionals involved in blood culture sample collection. The survey addressed key aspects such as knowledge of procedures, aseptic criteria, blood volume per blood culture set, and biosafety measures. Data collection for the survey, applied via Google Forms, was conducted directly by the researchers. The data were processed using Microsoft Excel and analyzed using descriptive statistics, calculating frequencies and percentages.

Prior to the development of the research, approval was obtained from the Bioethics Committee of the Faculty of Health Sciences and the Presidency of the General Hospital of Portoviejo to review quality documents, collect blood culture data, and implement the survey with personnel involved in this procedure.

Results and discussion

The results of blood cultures performed between January and October 2020 were reviewed, and a total of 1,084 tests were observed. Table 1 shows that nearly 50% of the blood culture tests were performed on patients over 61 years old, with a greater focus on patients over 71 years old. Pediatric patients accounted for 22% of the blood cultures, a high percentage compared to the 13% reported by Rodríguez et al. (2017) in a similar study conducted at the San Francisco Hospital in Quito.

The blood culture was identified as the diagnostic method of choice in suspected bacteremia, being one of the most requested microbiological techniques in pediatrics. The detection of bacteremia was essential, as it was associated with high morbidity and mortality, especially in immunocompromised patients, those with intravascular catheters, and those receiving broad-spectrum antibiotics. This situation favored the emergence of bacteremias caused by microorganisms that were previously considered rare or contaminants. Furthermore, the growing frequency of antibiotic-resistant microorganisms was highlighted, a public health issue that has gained greater relevance today.

Similar results were reported by Rodríguez et al. (2017) for the predominance of male gender (Table 1). In that study,

60% of the patients who had blood cultures were male, and 13.48% of the blood cultures were positive for bacterial growth; this last value was similar to the 14.2% reported in the literature (Rodríguez et al., 2017).

Table 1. Distribution of age, gender, and blood culture results in the microbiology department of the General Hospital of Portoviejo

Indicator	Frequency	Percentage
Age (years)		
< 1	131	12.10
1 – 10	74	6.83
11 – 20	56	5.17
21 – 30	42	3.88
31 – 40	46	4.25
41 – 50	72	6.65
51 – 60	137	12.65
61 – 70	247	22.81
> 71	278	25.67
Gender		
Female	433	39.98
Male	651	60.02
Blood culture result		
Positive	146	13.48
Negative	938	86.52
Contamination rate		
Contaminated blood cultures	16	1.47

The staff responsible for conducting and analyzing the blood cultures indicated that, on occasion, results that could have been caused by germs proliferating due to contamination were reported as positive cases. Not all blood cultures performed during 2020 were recorded, which was related to atypical characteristics due to the COVID-19 pandemic, which may have influenced the reported data.

Table 2 shows that the most frequently isolated microorga-nism in blood cultures was *Klebsiella pneumoniae*, followed by *Escherichia coli*. Potential contaminant microorganisms such as Staphylococcus epidermidis, *Staphylococcus sapro-phyticus*, and *Enterococcus faecalis* were also identified. The frequency of appearance of potential contaminants in positi-ve blood cultures was 10.95%, with coagulase-negative S*taphylococcus* being the most common contaminant (7.53%), representing 68.75% of all contamination cases.

Rodríguez et al. (2017) reported an overall contamination rate of 7.0%, higher than the rate recommended by international standards. They also found that 49.8% of all positive blood cultures were contaminated, with coagulase-negative staphylococci being the predominant contaminant microorganisms, representing 92.4% of contamination cases.

On the other hand, Paniagua et al. (1988) reviewed 3,227 blood cultures processed over eight months. They found 249

Table 2. Microorganisms in blood cultures performed in the microbiology department of the General Hospital of Portoviejo

	Microorganism	Frequency	Percentage
Most frequent	*Klebsiella pneumoniae*	32	21.92
	Escherichia coli	17	11.64
	Acinetobacter lwoffii	11	7.53
	Enterobacter aerogenes	10	6.85
	Pseudomonas aeroguinosa	10	6.85
	Klebsiella oxytoca	9	6.16
	Staphylococcus aureus	8	5.48
Contaminant	*Staphylococcus epidermitis*	6	4.11
	Streptococcus bovis	6	4.11
	Staphylococcus saprophyticus	5	3.42
	Enterococcus faecalis	5	3.42
	Serratia marcescens	5	3.42
	Klebsiella aerogenes	4	2.74
	Staphylococcus haemolyticus	4	2.74
	Proteus mirabilis	4	2.74
	Staphylococcus warneri	3	2.05
	Acinetobacter baumannii	3	2.05
	Enterobacter cloocae	1	0.68
	Pseudomona alcaligenes	1	0.68
	Achromobacter xylosoxidans	1	0.68

positive blood cultures for coagulase-negative *Staphylococcus* sp., of which 30% corresponded to septicemia and 70% were considered contaminants based on clinical criteria and alterations in the blood count. Most septicemia cases were in neonates. They observed that the probability of isolating coagulase-negative *Staphylococcus* sp. from a blood culture was 7.7%. Coagulase-negative staphylococci are generally harmless inhabitants of the skin but can become pathogenic under certain conditions.

The laboratory at the General Hospital of Portoviejo did not have a specific procedure for sample collection for this type of laboratory test, which is crucial in the treatment of sepsis. According to the Clinical Laboratory Operation Regulations approved by the Ministry of Public Health (MSP) of Ecuador in 2012, clinical laboratories must obtain an Annual Operating Permit, meeting the requirements of a Licensing Certificate, Quality and Biosafety Manuals, and certifications for waste management and staff training in the Technical Standard for Clinical Laboratories. ISO 15189 (2022), considered the Technical Standard for Clinical Laboratories, sets the requirements for the quality management system and technical aspects, including personnel, facilities, equipment, procedures, and quality assurance. This standard implies that laboratories must have specific procedures for their activities and define testing methods based on their characteristics and needs. Implementing ISO 15189 (2022) is necessary for laboratory accreditation and ensures quality and technical competence, benefiting patients by ensuring proper execution of tests and avoiding iatrogenic errors.

Regarding international protocols for blood culture sample collection, the most commonly used include the M47A document from the Clinical and Laboratory Standards Institute (Wilson et al., 2007) and the procedural manual from the Spanish Society of Infectious Diseases and Clinical Microbiology (Rodríguez et al., 2007). These protocols agree on several key points to reduce contamination and false positives, such as proper skin antisepsis with alcohol-based chlorhexidine, phlebotomist training, the use of sterile gloves, cleaning the blood culture bottle caps, and collecting samples during separate fever episodes (Tompkins et al., 2023).

Using a Vacutainer® system with a reflux prevention mechanism, adequate blood volume for children and adults, and collecting samples from multiple venipuncture sites are recommended (Ombelet & Rico, 2019). They emphasize the need for bottles with resin for patients on antibiotic therapy and the implementation of monitoring programs that include contamination and positivity indicators (Lamy et al., 2016).

The survey was conducted with staff dedicated to blood culture sample collection at the General Hospital of Portoviejo. It was observed that there were no differences between the number of men and women, but the sample size was small. In some countries in the region, 75.8% of those enrolled are women (Alarcón, 2019). According to MSP data, 60% of health professionals in Ecuador are women, with 19,014 women out of a total of 37,930 general physicians and 9,351 women out of 19,444 specialists. The nursing field is dominated by women (Pérez-Sánchez et al., 2021).

Half of the respondents were between 30 and 35 years old, 25% were between 36 and 40, and the remaining quarter were over 40 (Table 3). Age is considered an important factor in blood culture sample collection, as older individuals are believed to have more experience with the procedure. Pearse & Scott (2023) indicated that clinical laboratory graduates should have experience gained by performing various procedures. Thus, older individuals achieve better results, greater concentration ability, and higher skill in executing what they have learned.

Table 3. Sociodemographic characteristics of the surveyed healthcare staff

Indicator	Frequency	Percentage
Age (years)		
30 - 35	8	50
36 - 40	4	25
> 40	4	25
Gender		
Female	8	50
Male	8	50
Profession		
Bachelor's Degree in Clinical Laboratory	7	43.75
Bachelor's Degree in Nursing	9	56.25

The majority (56%) of the surveyed healthcare staff had a degree in nursing, and the remaining 44% had a degree in clinical laboratory science. This highlights the close relationship between nursing staff and patients and their collaboration with other healthcare team members in promoting user well-being.

Figure 1 shows the most common errors in blood culture sample collection reported by the respondents. The absence of protocols in the laboratory room (43.75%) was the most frequently cited error, followed by the lack of proper containers for sample transportation (25%), too many staff members in the learning phase (18.75%), and 12.5% mentioned

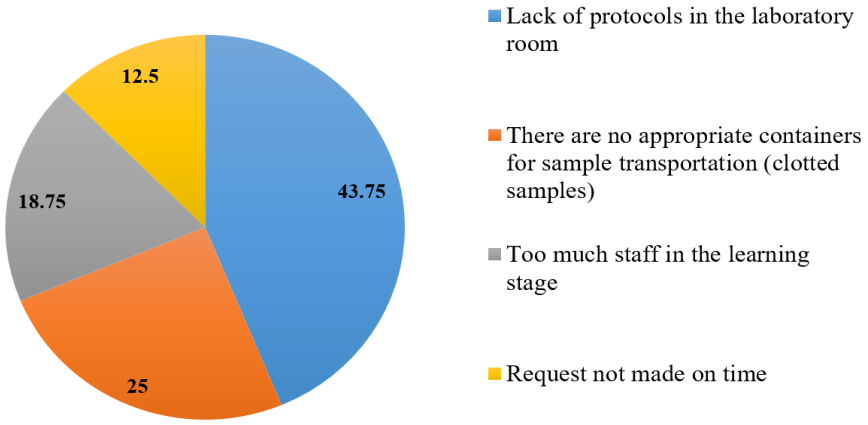

Figure 1. Most common errors made in blood culture sample collection.

that orders were not made on time. Iqbal et al. (2023) suggested that the quality of the analytical phase and its results largely depended on the preanalytical phase, but it was not given the attention it deserved. Particularly in high-demand laboratories, students were not adequately trained during their internships. The economic cost of preanalytical errors was 10% of the total cost of obtaining and sending samples, and in public hospitals, these errors cost the country as a whole (Iqbal et al., 2023). Respondents reported a lack of preparation at the beginning of their clinical practice activities and the absence of protocols.

In Table 4, it is observed that 62.5% of the staff surveyed indicated that blood cultures are taken based on clinical suspicion of sepsis, while 37.5% reported that they were taken prior to systemic antimicrobial therapy, which is corroborated by the literature of Alados et al. (2014) in their Manual on Clinical Microbiology Procedures, where they stated that blood cultures remain the primary diagnostic method for determining the etiology of bacteremia. Its easy execution makes it accessible to any center, and it is the only method that allows the isolation of viable microorganisms necessary for determining their antibiotic sensitivity. Its usefulness is highly associated with its exclusive use in patients with a clinical presentation compatible with bacteremia. Performing it under other circumstances increases healthcare costs and does not provide clinically useful information.

Table 4. Survey results on practices and protocol compliance in blood culture collection at the General Hospital of Portoviejo

Indicator	Frequency	Percentage
Reason for performing blood cultures		
Indicated before systemic antimicrobial therapy	6	37.5
Clinical suspicion of sepsis	10	62.5
Time of sample collection		
Near the fever peak	10	62.5
Far from the fever peak	6	37.5
Compliance with the blood culture collection protocol		
Yes	11	68.75
No	5	31.25
Strict compliance with biosafety standards		
Yes	10	62.5
No	6	37.5
Technique for performing blood culture		
Manual	10	62.5
Automatic	6	37.5
Compliance with training		
Yes	6	37.5
No	10	62.5

Delays hinder the practical value of diagnosis in obtaining results because it is not favorable in all patients, with its lowest performance being in patients on antibiotic treatment or those with fungal infections, slow-growing bacteria, or those requiring special growth conditions. Another limiting factor is the high proportion of contaminated blood

cultures by microorganisms belonging to the skin microbiota; this process generates diagnostic errors and inadequate treatments and incurs high economic costs for the healthcare system. The sensitivity of blood cultures is related mainly to the sample volume, the timing of collection, and the absence of prior antibiotic treatment (Cohen et al., 2015).

In Table 4, it is observed that 62.5% of the staff indicated that the samples were taken close to the fever peak, while 37.5% reported that the samples were taken far from the fever peak and symptoms. This practice does not comply with the guidelines from the Murcia Health Clinical Practice Guide, which states that for blood cultures, the sample should be collected as soon as possible after a fever peak to avoid affecting the isolation of causative microorganisms. However, a study (Hernández-Bou et al., 2016) found no significant differences in isolation rates if blood was drawn during afebrile intervals or simultaneously with the fever peak.

It has been recommended that the optimal time for sample collection be as soon as possible after the appearance of clinical symptoms, although blood can be sampled at any time. The blood collection during or immediately after a fever peak is considered optimal, except in cases of endocarditis. The detection of bacteremia through blood cultures established that the presence of fever at the time of blood culture collection was neither sensitive nor specific for the presence of bacteremia. Hernández-Bou et al. (2016) evaluated the timing of blood culture collection in relation to temperature elevation in more than 1,400 patients with bacteremia and fungemia. They found no correlation between the timing of sample collection and the likelihood of a positive blood culture.

Most of the healthcare staff (68.75%) followed the steps for properly collecting and delivering blood culture samples in chronological order, while 31.25% did not. According to Alados et al. (2014), the correct methodology for blood culture extraction includes using gloves and a mask; cleaning the vials' caps with chlorhexidine; selecting the blood collection site (avoiding blood extraction via catheter); disinfecting the skin with chlorhexidine, letting the disinfectant act; performing the puncture without touching the patient's skin with the hand; avoiding contact between the needle and cotton; extracting the necessary amount of blood (10 ml per bottle in adults and between 1 and 5 ml in children); inoculating the anaerobic bottle first, followed by the aerobic one (without adding air), and other tubes if necessary; gently shaking the bottles, and urgent transport to the microbiology service or, if not possible, maintaining at room temperature. If done correctly, sensitivity and specificity are high, but incorrect execution can lead to erroneous results, so the procedure should not be performed unless optimal conditions are met (Cuervo et al., 2001).

A total of 62.5% of the staff strictly followed laboratory safety rules, whereas 37.5% did not. Similar results were reported by Alados et al. (2014) in their microbiology manual, where they emphasized that the blood inoculated in the vials may contain, in addition to bacteria or fungi, other viable microorganisms such as hepatitis or HIV viruses, which represent an infection risk for those handling the samples. Knowing and strictly adhering to universal prevention measures for handling blood is crucial, particularly in preventing accidental needle sticks during blood extraction or processing positive vials.

The replacement of glass vials with plastic vials has helped reduce accidents caused by breakage. Some companies have recently developed blood collection and puncture systems that eliminate the risk of accidental needle sticks while processing positive vials. Cuervo et al. (2001) highlighted the importance of staff strictly following laboratory biosecurity rules to minimize these risks.

One respondent indicated that blood cultures are taken by medical or nursing staff, not by laboratory personnel; however, laboratory technicians must have all the necessary knowledge to perform this procedure and its subsequent analysis, as they are the ones professionally trained to collect, process, and interpret biological samples. Techniques used for blood culture collection indicated that 62.5% were performed using the manual method, while 37.5% used the automatic method (Suk-Fong et al., 2018).

A majority of the staff, 62.5%, reported not having received training on blood culture collection in the past two years, compared to 37.5% who had received it. Training on any topic within the healthcare team is fundamental for professional growth and is recommended by all international standards to ensure quality care for users. The research findings highlighted the need to update the knowledge of professionals involved in blood culture sample collection to avoid false positives and contamination—factors that could compromise results, treatment, and, consequently, the patient's life.

Conclusions

No specific procedure for blood culture collection was identified in the laboratory despite its importance in treating sepsis. The main errors detected include the absence of protocols, the lack of suitable containers for sample transport,

and the involvement of trainees, although some staff follow international standards. In response to this issue, a standardized operating procedure was designed based on Ecuadorian Good Clinical Laboratory Practices and international protocols, which will be presented for implementation in the Microbiology Laboratory of the Portoviejo General Hospital.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Conceptualization: Carlos E. Mera, Jean P. Sanclemente. **Research:** Carlos E. Mera, Jean P. Sanclemente, Ivón Howland. **Methodology:** Ivón Howland. **Supervision:** Ivón Howland. **Validation:** Ivón Howland. **Writing the original draft:** Carlos E. Mera, Jean P. Sanclemente, Ivón Howland. **Writing, review and editing:** Carlos E. Mera, Jean P. Sanclemente, Ivón Howland.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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