Impact of a Rapid Molecular Diagnostic Test for the Identification of Bloodstream Infections in Intensive Care Units: Experience from a Developing Country

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Abstract

Background Rapid diagnostics have been demonstrated to be a crucial component of antimicrobial stewardship programs. However, most of the studies have been conducted in developed countries where health-care facilities have 24/7 microbiology laboratories. Colombia is an example of a developing country with limited resources in which hospitals are not able to implement a 24/7 model and samples are usually processed once a day. Here, we compared time to pathogen identification by QuickFISH® with conventional cultures and its effect on decrease duration of therapy in critical patients. Methods A multicenter, ambispective cohort study was conducted in four high-complexity ICU hospitals between 2016-2017. Adult patients admitted to the ICU with positive blood cultures and signs of systemic inflammatory response syndrome were included in the study. Patients with bloodstream infections identified by either QuickFISH® or PNA FISH® were observed prospectively and compared with those patients with bloodstream infections identified by conventional blood cultures alone who were analyzed retrospectively. Duration of treatment, time to final reports and survival rate were compared between the two groups. Additionally, the performance of the molecular test was compared with the conventional blood culture. Results A total of 153 patients were included in the study. Among them, 72 (47%) were in the QuickFISH® / PNA FISH® group and 81 (53%) in the conventional blood culture group. 87% of the patients had a bacteria identified (n=133) and 13% (n=20) a candida. QuickFISH® / PNA FISH® had 96% (89%-100%) concordance with blood culture. The microbiological identification report was 26 hours faster in the QuickFISH® group than in blood culture group (29 hours vs. 55 hours; p = 0.0001). The duration of antimicrobial therapy was 3.2 days shorter in the QuickFISH® group compared to the BC group (13.7 days vs. 16.9 days; p = 0.026). Conclusions Molecular diagnostic methods such as QuickFISH® reduce the time to final reports as well as the duration of therapy in ICU patients with bloodstream infections. Despite having more impact in 24/7 laboratories, QuickFISH® methods may be a promising diagnostic tool in developing countries if incorporated with antimicrobial stewardship programs.

Background

Sepsis is the condition most frequently diagnosed in the Intensive Care Unit (ICU) and the main cause of death in critically ill patients.[1] Up to 20% of these infections are primary bloodstream infections (BSIs) with up to 30% of mortality reported in these patients.[2] In this context, the rapid identification of the causative microorganisms is extremely important as any delay in the administration of appropriate antimicrobials increases mortality in 8% per hour.[3]

Blood cultures (BC), followed by sub-culturing on to solid media, is still considered the gold standard for diagnosing BSIs. However, conventional BC procedures can take ≥72 hours to provide identification of the microorganism, which may result in poor clinical outcomes, increased medical costs and emergence of antimicrobial resistance.[4] In contrast, molecular methods such as *in situ* hybridization-based methods (PNA FISH® / *Quick*FISH®, OpGen®, Maryland, USA) identify the microorganisms directly from the positive

BC bottle, improving the level of information for the decision making process, reducing as well ,the time to identification.[5-7] These methods have proven to have sensitivity and specificity between 90% and 100%.[6, 8] *Quick*FISH® is a rapid and simple slide-based assay that provides pathogen identification, directly from a positive blood culture, in less than 30 minutes.[6]

In recent years, several new technologies have entered to clinical microbiology laboratories such as accelerated phenotypic methods, molecular techniques, MALDI-ToF and whole genome sequencing among others. Despite the evidence that they optimize workflows within the lab, increase diagnostic resolution and decreased time-to-result, few Latin American microbiology laboratories have access to these new technologies because of the high costs and, in some cases, specialized human resources needed to use the instrumentation. Additionally, the vast majority of the studies evaluating the new rapid molecular tests have been conducted in developed countries where microbiology laboratories have 24/7 availability to perform and read these tests. Unfortunately, in Latin America few laboratories can have this continuous workflow and most of them are only able to do so once per day.

As reported by several publications,[9-11] *Quick*FISH® requires minimal sample preparation as cells do not need to be lysed to isolate genetic material required for PCR-based techniques, and generates visual results that match Gram-stain morphology, allowing pathogen identification to be obtained in less than 30 minutes,.[6] Considering that the *Quick*FISH® technique is ideal for a clinical microbiology laboratory because is simple to perform, quick and less expensive than other methods, the aim of this study was to compare the impact in terms of time to pathogen identification, and duration of the therapy between conventional blood culture procedures and *Quick*FISH®n patients with bacteremia and candidemia in Colombia. To our knowledge this is the first study in Latin America.

Methods

A multicenter, ambispective cohort study was conducted in four high-complexity ICU hospitals between 2016-2017. Adult patients admitted to the ICU with positive BC and signs of systemic inflammatory response syndrome were included in the study. Exclusion criteria were patients with incomplete medical records or death within the first 72 hours after blood cultures were drawn. The following kits of *Quick*FISH® or PNA FISH® kits were used based on the Gram stain result once it was available; *Staphylococcus Quick*FISH® (*Staphylococcus aureus*, coagulase-negative staphylococci [CoNS]), *Enterococcus Quick*FISH® (*Enterococcus faecalis,* other enterococci), Gram Negative *Quick*FISH® (*Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa*), Yeast Traffic Light® PNA-FISH® (*Candida albicans/C. parapsilosis*, *C. tropicalis, C. glabrata/C. krusei*). Patients with microorganisms identified by *Quick*FISH® or PNA FISH were observed prospectively and patients with pathogens identified and reported by conventional BC procedures were identified retrospectively. Times were calculated from the time of blood sample arrival at the microbiology laboratory to the time of pathogen identification report to clinicians. Laboratory and medical staff, as well as a study coordinator at each hospital, were trained to ensure that the *Quick*FISH® and BC results were reported effectively to the primary physician.

Due to hospital policies, all BCs from the exposed group had to be identified by traditional culture techniques despite the *Quick*FISH® result, allowing us to evaluate any discrepancy and/or detect microorganisms not included in the *Quick*FISH® panel. In both groups, the final therapeutic decision was responsibility of the treating physician.

This study had the approval of the ethics committees of the 4 participating institutions, the authorization of the Reviewer Commission Specialized in Medical Devices and Other Technologies (INVIMA) and due to the Resolution 8430/1993 of the Ministry of Health of Colombia, this investigation was classified as minimum risk.

For the statistical analysis, proportions were used to describe categorical variables and averages for numerical variables. Concordance percentages were established between the microbiological reports of the QuickFISH® and conventional BC groups. Values of $p < 0.05$ were considered statistically significant.

Results

A total of 153 patients were included in the study. Of these patients, 72 (47%) were in the *Quick*FISH® / PNA FISH® group and 81 (53%) in the conventional BC group. A comparison of clinical variables between the groups are displayed in **Table 1**. The mean patient age in years was 61(SD +/- 14 years) and 54% of patients were male. There were no significant differences between the study groups in terms of preexisting medical conditions or clinical variables**.** From the 153 patients included, 87% had a bacteria identified (n=133) and 13% (n=20) a candida. *Quick*FISH® / PNA FISH® had 96% (89%-100%) concordance with BC (**Table 2)**.

The mean time for the Gram report was 24 hours in both groups ($p = 0.257$). In contrast, the microbiological identification report was 26 hours faster in the *Quick*FISH® group than in BC group (29 hours vs. 55 hours; $p = 0.0001$).

Furthermore, the duration of antimicrobial therapy was 3.2 days shorter in the *Quick*FISH® group compared to the BC group (13.7 days vs. 16.9 days; $p = 0.026$).

Discussion

This is the first study in Latin America to evaluate the implementation of hybridization-based methods (*Quick*FISH®/PNA FISH®) for the diagnosis of BSIs
in laboratories that lack 24/7 availability. Our results are in accordance to many other studies that have also reported that FISH methods provide faster results compared to conventional cultures.[6, 9, 12, 13] Ly and colleagues'[11] also found a reduction in the duration of antibiotic treatment (-2.0 days; $p = 0.01$), as well as Forrest et al which evaluated the clinical utility of enterococcal PNA FISH®, showing less time to effective therapy (1.3 vs. 3.1 days).[9]

Although in our study a difference in mortality of 22% among the groups was observed (QuickFISH® 13% versus BC 35%), it is not possible to establish a statistical association between the implementation of the QuickFISH® and its direct impact on mortality; the study sample is a limitation as well as its design ; furthermore, part of difference in mortality between the groups could be explained in part to the higher proportion of patients with candidemia in the control group which have been shown to have significantly higher mortality compared to bacterial infections.[14-17]

In contrast to our results, other studies have not shown impact on duration of treatment with the use of PNA FISH®.[18, 19] This difference may be attributed to the absence of an antimicrobial stewardship (AMS) program and timely response to the laboratory report. Indeed, most of the studies that have favorable outcomes from implementing PNA FISH® or *Quick*FISH®, incorporated an AMS program.[12, 19] In our study, all of the participating hospitals had active AMS programs as demonstrated by previous studies done by our group (data not published).

In conclusion, molecular diagnostic methods such as *Quick*FISH® reduce the time to final reports as well as the duration of therapy in ICU patients with BSIs. *Quick*FISH® methods may be a promising diagnostic tool in developing countries if incorporated with AMS programs.

Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of the International Center for Training and Medical Research (CIDEIM, per its abbreviation in Spanish) and the participating institutions. CIDEIM deemed that informed consent was not necessary for the study given that its design entailed no risk for subjects.

MVV has received consulting honorarium and/or research grant support from Pfizer, MSD, AstraZeneca, Zambon, West Colombia and Abbott. CP has been consultant for Merck, MSD, Pfizer, Novartis, AstraZeneca, and Amarey. CH has been consultant for MSD and Merck. SV has received consulting honorarium from MSD and Pfizer. The other authors declare that they have no competing interests.

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Authors' contributions

SR helped design the study, was involved in data collection process statistical analysis and was responsible for manuscript preparation. CH designed the protocol, helped in data collection, had a major contribution in educating all the participating hospitals and helped in manuscript preparation. CP was involved in the study design, data collection and statistical analysis as well as manuscript preparation. SS, SV and KO helped collect information from the participating institutions and actively participated in data analysis and manuscript preparation. AC was a major contributor in study design, was the person in charge of laboratory training and helped significantly in manuscript preparation. MVV was the principal investigator of the study and had a main role in designing the study, supervised the study development, and her expertise was fundamental for manuscript preparation. All authors read and approved the final manuscript.

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Tables

Table 1 Comparison of clinical variables between the *Quick*FISH® group and the Conventional blood culture group.

Variable	*Quick*FISH® group	Blood culture group	*p* value
	n=72 (47%)	n=81 (53%)	
Age, years (median [SD])	62 (+/-16)	60 (+/-19)	0.09
Sex			0.89
Male	39 (54%)	44 (54%)	
Female	33 (46%)	37 (46%)	
Preexisting medical conditions	65 (90%)	72 (89%)	0.87
Serum lactic acid (mean [SD])	1.93 (+/-0.97)	2.52 (+/-2.1)	0.29
Mechanical ventilation	30 (42%)	43 (53%)	0.14
Renal failure	25 (35%)	29 (36%)	0.98
Inotropes and vasopressors	23 (32%)	28 (34%)	0.83
Type of infection			0.09
Bacteremia	66 (92%)	66 (83%)	
Candidemia	6 (8%)	14 (17%)	
Mean time to Gram report (hours)	24	24	0.257
Mean time to final report (hours)	29	55	0.0001
Mean duration of therapy (days)	13,6	16,8	0.026
Survival rate	54 (75%)	38 (47%)	0.009

SD: Standard Deviation

Table 2 Performance of the *Quick*FISH® method compared to conventional blood culture for 53 positive blood culture bottles.

Microorganism	No. of positive cultures	No. of positive *Quick*FISH®	% of concordance
S. aureus	9	8	89%
CoNS	13	13	100%
E. faecalis	3	3	100%
Other Enterococci	1	1	100%
E. coli	16	15	94%
K. pneumoniae	5	5	100%
P. aeruginosa	1	1	100%
C. albicans/C. parapsilosis	4	4	100%
C. tropicalis	1	1	100%
Total	53	51	96%

CoNS: Coagulase-negative staphylococci