Comparison of multiplex PCR against blood cultures for the identification of microorganisms in a cohort of patients with bloodstream infections

Abstract

Bloodstream Infections (BSI), represent an important cause of both morbidity and mortality worldwide. However, the insufficient sensitivity of blood cultures whenever a patient has previously received antibiotic therapy, as well as the presence of slow growing and/or intracellular microorganisms, has generated the need of implementing new diagnostic methods. As a result, in 2012, Biofire Diagnostics, launched FilmArray®, a blood culture identification panel, which is an FDA, CE-IJD, and TGA certified multiplex PCR system that integrates sample preparation, amplification, detection and analysis.

Material and methods: the objective was to discern the sensitivity and specificity of the BioFire FilmArray® Blood Culture Identification (BCID) Panel compared to blood culture, for the identification of microorganisms causing bacteremia and the susceptibility profile in the National Institute of Respiratory Diseases. A total of 42 clinical records of patients with positive blood cultures, who also underwent a FilmArray® test were evaluated.

Results: The FilmArray® panel showed a sensitivity of 96.4% and a specificity of 50% when compared with the Gold Standard. The median in hours elapsed from sample reception to result reports by the clinical microbiology service was 31.31 (±19.35) with the FilmArray® versus the 123.42 hours (±71.28) median of the traditional blood culture method. This difference was statistically significant (p<0.001).

Conclusion: FilmArray® panel as an emerging diagnostic method, is of significant utility for pathogens identification even in comparison to the traditional method. By curtailing time to pathogen identification, FilmArray® contributes to an earlier establishing of targeted antimicrobial treatment.

Keywords: blood culture, PCR, microorganism, bloodstream infection

Abbreviations: BSI, blood stream infections; BCID, blood culture identification; INER, Instituto Nacional de Enfermedades Respiratorias

Introduction

Blood Stream Infections (BSI) represent an important cause of morbidity and mortality worldwide. Most treatment decisions in these cases are made taking into consideration the results of blood cultures, which has been the most important diagnostic procedure to identify the causal agent when there is a clinical suspicion of BSI. However, there is a significant delay in results when conventional methods like these are performed. This diagnostic method, based on the isolation of a microorganism and its identification and susceptibility test using standard biochemical techniques, is a process that can generally take from 48 to 72 hrs, and whose performance is variable. If 2 to 4 samples are obtained (40 to 80 ml of blood) before starting antimicrobial treatment, an etiological agent is detected in 80 to 96% of cases. In patients with bacteremia, which frequently causes sepsis and septic shock, an early and appropriate administration of antimicrobial treatments affects directly in the patient’s prognosis.

Another element to consider is that whenever a patient has received previous antibiotic treatment, blood cultures can present an insufficient sensitivity in pathogen identification, as well as when slow growth microorganisms and/or intracellular microorganisms are involved. These situations have generated the need of implementing new diagnostic methods that improve results in different instances of BSI. To this end, in 2012, BioFire Diagnostics launched its blood culture identification panel (BCID) FilmArray®, a qualitative in vitro diagnostic assay based in nucleic acids, that allows for a multiplex PCR analysis with automatic results readings directly from positive blood cultures in an hour for identification of bacterial pathogens and yeasts from positive blood cultures, detecting a total of 24 pathogens and 3 resistance genes that include: gram positive bacteria (Enterococcus, Listeria monocytogenes, Staphylococcus aureus, Streptococcus agalactiae, pyogenes y pneumoniae), gram negative (Acinetobacter baumannii, Haemophilus influenza, Neisseria meningitides, Pseudomonas aeruginosa, Enterobacter cloacae complex, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae,
Proteus, Serratia marcescens, yeasts (Candida albicans, glabrata, krusei, parapsilosis y tropicalis) and antibiotic resistance genes (mecA for meticillin resistance, vanA/B for vancomycin resistance and KPC for carbapenem resistance). This is the first PCR multiplex assay approved by the FDA. In BSI the use of multiplex PCR assays has been associated with reduced empiric broad spectrum antimicrobial therapy, which also reduces time to appropriate antimicrobial therapy.

Material and methods

This study’s objective was to discern the sensibility and specificity of BioFire FilmArray® BCID in relation to blood culture as the Gold Standard for identification of bacteremia causing microorganisms and to identify the profile of susceptibility at Mexico’s National Institute of Respiratory Diseases (INER) through an observational, retrospective, transversal study. The study included all clinical records from patients whose blood culture samples were received at the clinical microbiology laboratory at INER, that presented microorganisms growth and where the pathogen was identified through both blood culture and FilmArray® panel. This study was performed at the microbiology department of INER. All clinical records with samples processed after the acquisition of FilmArray® in INER were included, establishing a 12-month analysis period, from January 2017 to January 2018.

Laboratory results were obtained from the BioFire FilmArray® log in the clinical microbiology lab and clinical data was collected from clinical records of patients diagnosed with bacteremia through blood culture, including antimicrobial treatment at the moment of sample procurement, results for both FilmArray® and blood culture as well as and time elapsed between sample delivery at microbiology lab and results reports. All clinical variables were registered in data collection formats, descriptive statistics was performed through median, frequencies and percentages were estimated. Sensibility and specificity were calculated by comparing the positive and negative results by both methods and comparative statistical analysis was performed using Chi square test for dichotomous qualitative variables and a t of Student test to compare medians of quantitative variables, considering as statistical significance a probability (p) of <0.05. All statistical analysis was performed using SPSS 21 statistical package.

Ethical aspects

This study follows the ethical guidelines established for the use of patient information and has been approved by INER’s ethical committee.

Results

42 clinical records were evaluated. All patients had a positive blood culture to which a FilmArray® assay was additionally performed (Table 1).

A higher frequency of bacteremia was observed amongst male patients 64% (27/42), age median was 41 years of age with a predominance of patients 60 years or older 26% (11/42). A comorbidity was reported in 65% (27/42) of evaluated patients, with HIV (Human Immunodeficiency Virus) infection being the most frequent, and Type II Diabetes (DM). 24% of all patients were admitted to the Intense Care Unit (ICU). Blood cultures identified 42 microorganisms through the VITEK® 2 system and the FilmArray® system identified a total of 34 microorganisms (Table II), the pathogens not identified by FilmArray® were microorganisms that are not currently included in the system’s identification spectrum; Stenotrophomonas maltophilia, Ochrobactrum anthropi, Pseudomonas putida, Acinetobacter haemolyticus, Aeromonas hydrophila/caviae, Cryptococcus neoformans and Aspergillus sp.

A general sensibility of 71.1% was found for the FilmArray® system and a specificity of 50% in comparison with traditional gold standard. Additionally, when analysis was performed excluding those microorganisms not included in the FilmArray® panel, a sensibility and specificity of 96.4% and 50% respectively were estimated. No mutations were identified in any sample included in the present study.

Median time lapsed in hours from sample reception and results from the clinical microbiology laboratory was 31.31 (±19.35) for the FilmArray® system and 123.42 (±71.28) for the conventional method, the reduced time to results with FilmArray® in comparison with blood culture, was statistically significant (p<0.001) (Table 2).

A 38% (16/42) of patients included in this study died. In 81% (13/16) of these cases, the causal agent of the bacteremia was identified with FilmArray®. No statistical significant difference was found between patients who died with a pathogen detected by FilmArray® and those that were undetected by FilmArray® (P=0.17) (Table 3).

Table 1 Demographic characteristics of patients diagnosed with bacteremia thorough FilmArray® and blood culture

| Basal demographic characteristics | n=42 |
|----------------------------------|------|
| Average age (years±SD)           | 40±14.4 |
| 0 to 10 years (%)                | 3 (7) |
| 11 to 20 years (%)               | 3 (7) |
| 21 to 30 years (%)               | 8 (19) |
| 31 to 40 years (%)               | 7 (17) |
| 41 to 50 years (%)               | 4 (10) |
| 50 to 60 years (%)               | 6 (14) |
| >60 years                        | 11 (26) |
| Gender                           |      |
| Female (n, %)                    | 15 (36) |
| Male (n, %)                      | 27 (64) |
| Service                          |      |
| Emergency Room                   | 11 (26) |
| HIV clinic                       | 9 (21) |
| Tuberculosis clinic              | 7 (17) |
| COPD clinic                      | 4 (10) |
| ICU                              | 4 (10) |
| Pediatric pneumonia              | 3 (7%) |
| Others                           | 4 (10) |
| Comorbidities                    |      |
| HIV infection                    | 14 (33) |
| Type II Diabetes                 | 11 (26) |
| Systemic Hypertension            | 6 (14) |
| Cancer                           | 3 (10) |

SD, standard deviation; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit; HIV, human immunodeficiency virus.
Table 2 Comparison of detected microorganisms by gold standard method (blood culture) versus FilmArray® panel

| Blood culture                  | FilmArray®                  | Gram negative | Gram positive |
|-------------------------------|-----------------------------|---------------|--------------|
| E. coli (12)                  | E. coli (12)                |               |              |
| Pseudomonas aeruginosa (7)    | Pseudomonas aeruginosa (7)  |               |              |
| Klebsiella pneumoniae (3)     | Klebsiella pneumoniae (3)   |               |              |
| Enterobacter cloacae complex (1) | Enterobacter cloacae complex (1) |       |              |
| E. cloacae (1)                | Enterobacter cloacae complex (1) |       |              |
| Acinetobacter baumannii (1)   | Acinetobacter baumannii (1) |               |              |
| Citrobacter freundii (1)      | Enterobacteriaceae (1)      |               |              |
| Haemophilus influenzae (1)    | Haemophilus influenzae (1)   |               |              |
| Enterococcus avium (1)        | Enterococcus (1)            |               |              |
| Staphylococcus aureus (1)     | Staphylococcus aureus (1)   |               |              |
| No growth                     | Streptococcus agalactiae (1)|               |              |
| Estafilococcus epidermidis (1)| Not Detected                |               |              |
| No growth (0)                 | Candida parapsilosis (1)    | Yeasts        |              |
| Candida albicans (2)          | Candida albicans (3)        |               |              |
| Aspergillus sp (1)            | Not Detected                |               |              |
| Ochrobactrum anthropi l       | Not Detected                |               |              |
| Pseudomonas putida (1)        | Not Detected                |               |              |
| Acinetobacter haemolyticus (2)| Not Detected                |               |              |
| Aeromonas hydrophila/caviae (1)| Not Detected                |               |              |
| Gram negative bacillus (1)    | Not Detected                |               |              |
| Cryptococcus neoformans (2)   | Not Detected                |               |              |
| Stenotrophomonas maltophilia (1) | Not Detected              |               |              |

Table 3 Difference in time lapse (hours) between sample reception and results with FilmArray® vs blood culture and endpoint (mortality) in patients with microorganisms detected by FilmArray® vs not detected with FilmArray® (p<0.05)

|                      | Diagnosis by FilmArray® 8 (n=34) | Conventional diagnosis (Culture) (n=42) | (p)           |
|----------------------|----------------------------------|----------------------------------------|---------------|
| Median in hours      | 31.31 (± 19.35)                  | 123.42 (± 71.28)                       | p<0.001       |
| Deceases patients    | Detected by FilmArray® (n=13)    | Not Detected by FilmArray® (n=3)       | P=0.17        |
| before 30 days       | 81% (13/16)                      | 19% (3/16)                             |               |
| (16/42).             |                                  |                                        |               |

Discussion

The importance of developing new identification methods for microorganisms different to cultures, originates from the need of curtailing the time to etiological diagnosis and therefore, shorten the time lapse to an effective and directed treatment, avoiding the unnecessary use of antibiotics or its delay and all related risks. The present study compared cultures; the Gold Standard of microbiologic diagnosis, against the FilmArray® identification panel, finding as a focal point, the reduction of the time elapsed between sample reception and a microorganism identification result, which was statistically significant (p<0.001) where FilmArray® had a median of 31.31 hours vs 123.42 hours that had to elapse for a blood culture to report results.

A higher prevalence of bacteremia was observed in male patients (64%). Clinical records revealed a median age of 41 years, with a higher prevalence in patients 60 years or older. A prospective study by Pazos et al. (2001) that analyzed a total of 320 blood cultures reported a median age of 66.9 years (CI 95%: 65-69) and a male predominance (59%). Several authors have revealed similar distributions. This could be attributed amongst other things, to qualitative and quantitative deficiencies of the immune system associated with age, as well as the increase of comorbidities that associates with older age groups. It is known that bacteremia increases the risk of death 14 times higher, with advanced age being a variable related to a poor prognosis.

An HIV infection was one of the main reported comorbidities in patients with suspected bacteremia in 33% of cases, followed by Type II Diabetes with 26%, both of which are associated with a higher risk of bacteremia. Despite a reduction in opportunistic infections rate as a consequence of the introduction of highly active antiretroviral therapy, HIV infection continues to be the main risk factor for
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was the most frequently involved yeast, supposes the faster and more accurate targeted antimicrobial therapy compared to reduce the time for an accurate diagnosis and, therefore, install a and specific method for the detection of infectious etiologies, as well analyzed, the FilmArray® multiplex PCR method demonstrated to be and, therefore, prevent severe infection cases and even development strategies in order to have a quick and adequate antimicrobial therapy in Mexico are required.

Conclusion

On a clinical horizon, where the development of resistance by specific microorganisms to the drugs so far available, undermines daily medical practice, arises the need to propose new diagnostic strategies in order to have a quick and adequate antimicrobial therapy and, therefore, prevent severe infection cases and even development of greater resistance. Once the results of this scientific research were analyzed, the FilmArray® multiplex PCR method demonstrated to be an excellent diagnostic tool, which provides with by a fast, sensitive and specific method for the detection of infectious etiologies, as well to reduce the time for an accurate diagnosis and, therefore, install a faster and more accurate targeted antimicrobial therapy compared to the blood culture.

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Conflicts of interest

Authors declare that there is no conflicts of interest.

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