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IMPACT OF IMPLEMENTING MULTIPLEX ASSAYS AND SYNDROMIC TESTING PANELS ON IMPROVING DIAGNOSTIC EFFICIENCY: REVIEW

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Abstract

The emergence of commercial panel-based molecular testing has revolutionized clinical microbiology and practice by enabling quick pathogen diagnosis in positive blood culture containers, pulmonary samples, stool, and cerebrospinal fluid. This analysis specifically examines multiplex molecular panels that have been authorized or certified by the U.S. Food and Drug Administration (FDA). These panels are meant to diagnose infections in bloodstreams, respiratory tract, gastrointestinal system, or central nervous system, and they target more than five specific pathogens. Although panel-based assays offer the benefits of quick results and the ability to detect a wide range of microorganisms, they also come with challenges such as cost and determining the best strategies for test utilization and interpretation.

Keywords: panel-based molecular testing, pathogen diagnosis, pulmonary samples, blood culture containers, review.

1. Introduction

Advancements in diagnostic technology have brought about substantial transformations in the area of clinical microbiology, resulting in enhanced detection and diagnosis of infectious illnesses. These advancements include of commercial molecular tests that may detect and identify various pathogens linked to clinical syndromes, such as bloodstream, respiratory, gastrointestinal (GI), or central nervous system (CNS) infections, all at once. The multiplex tests are groundbreaking, since they allow healthcare practitioners to quickly detect specific illnesses. This enables them to make prompt choices about clinical management, such as hospital admission, isolation, and the use of antimicrobial medication. Occasionally, these technologies have also presented difficulties. Multiplex tests sometimes come with a high price tag, necessitating the creation of utilization management techniques to ensure their proper and cost-effective application. Existing clinical practice guidelines may not currently cover the use of these tests or provide information on how to interpret the findings. Some clinicians may lack

familiarity with certain organisms and/or resistance genes that are discovered, leading to uncertainty in clinical practice. This might result in improper medical care and unneeded follow-up laboratory tests, causing worry for both the healthcare professional and the patient.

The panel compositions may vary somewhat across manufacturers, but their overall fixed composition might provide difficulties in certain situations. The architecture of these multiplex platforms, even those that are promoted as closed systems, poses a potential danger of contamination that may be difficult to identify. Other obstacles include deciding how to include multiplex panels into laboratory procedures and h ow to ensure the correctness of data after deployment. While these tests do provide significant benefits, it is important to carefully incorporate multiplex assays into clinical practice. Moreover, the influence they have on public health laboratories should be taken into account.

It is expected that syndromic testing will become more prevalent in the future and will be conducted in settings other than clinical microbiology labs. Implementing these tests in point-of-care settings will need careful planning, including input from both clinical and laboratory experts. In this review, we examine the existing literature on multiplex molecular microbiology testing of positive blood culture bottles, lung specimens, feces, and cerebrospinal fluid (CSF). It is important to note that this subject is undergoing significant changes.

2. Expedited Analysis Of Blood Culture Bottles Showing Positive Results

Bacteremia and severe sepsis are significant contributors to death among patients who are admitted to the hospital (1). The frequency of hospitalizations for severe sepsis has risen in the previous decade, most likely because of an aging population with chronic medical conditions and a growing number of individuals with weakened immune systems (1, 2). Patients with septic shock who experience delays in receiving adequate antimicrobial treatment have higher fatality rates (3). Currently, the effectiveness of giving antimicrobial therapy early may be weakened due to a growing occurrence of bacterial resistance to drugs.

Despite the advancements made in the previous century with the use of automated, continuous-monitoring blood culture equipment, there are still delays in accurately identifying bacteria, detecting antibiotic resistance, and determining if a sample is contaminated. This may have a significant influence on the choices made about patient treatment, directly leading to illness and death, and possibly resulting in negative outcomes such as Clostridium difficile-associated diarrhea, the development of medication resistance, and greater expenditures for the patient. The extensive use of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has accelerated the identification of isolates in comparison to conventional biochemical approaches.

However, this method usually requires culturing, which might cause possible delays. Because bacterial infections have a significant impact on both national and global health, there are several initiatives aimed at addressing antimicrobial resistance. These initiatives emphasize the

importance of innovative diagnostic tests that can quickly identify bacteria and detect resistance. One such test involves directly testing positive blood culture bottles using MALDI-TOF MS, which provides accurate identification. However, this approach requires processing the contents of the blood culture bottle, is not approved or cleared by the Food and Drug Administration (FDA), and has a lower success rate compared to testing colony isolates. Many labs, including ours, have embraced the use of MALDI-TOF MS as an alternate method for identifying bacterial isolates from positive blood cultures. This method involves incubating high-inoculum subcultures on solid medium for a short period of time (2 to 6 hours) before using MALDI-TOF MS for identification (7-9).

As an illustration, we employ this method to examine positive blood culture bottles that exhibit Gram-negative bacilli when subjected to Gram staining. In our experiments, this approach has demonstrated outstanding efficacy, allowing us to identify organisms in 92% (45/49) of blood culture bottles that tested positive for Gram-negative bacilli after a 4-hour incubation period (based on our unpublished data). This provides a method to decrease the time it takes to complete the testing process compared to traditional methods, without incurring significant expenses. This is possible since MALDI-TOF MS is currently widely accessible in clinical microbiology labs and has a cheap cost per test. The limitations of this strategy include the lack of consideration for antibiotic resistance and its inapplicability in labs without access to MALDI-TOF MS technology.

3. FDA-Approved/Cleared Assays

Currently, there are three multiplex assays that have been approved by the FDA to simultaneously detect various microorganisms and specific resistance genes directly from positive blood culture bottles. These assays include the FilmArray Blood Culture Identification (BCID) panel by BioFire Diagnostics, LLC (approved in 2013), and the Verigene Gram-positive blood culture (BC-GP) and Gram-negative blood culture (BC-GN) tests by Luminex Corporation (approved in 2012 and 2014 respectively) (10).

4. Evaluation of Assay Performance

Ward et al. (11) conducted a comparison of the accuracies and turnaround times of multiplex tests and traditional, culture-based techniques (mostly MALDI-TOF MS-based colony identification) utilizing positive blood culture samples (n = 173). The Verigene and BCID tests decreased the time it takes to complete by 27.9 and 29.1 hours, respectively, in comparison to traditional procedures. The Verigene and BCID tests accurately identified 90.6% and 87.2% of samples, respectively, in comparison to traditional approaches. The Verigene assay produced 6 incorrect positive findings, including 2 instances when viridans group streptococcal isolates were mistakenly recognized as Streptococcus pneumoniae. In contrast, the BCID test produced 25 incorrect positive results. Further examination revealed that the inaccurate positive outcomes of the BCID test were probably caused by the presence of Pseudomonas aeruginosa DNA in the BacT/Alert standard anaerobic bottles (bioMérieux) owing to contamination (11).

This advisory serves as a reminder that meticulous attention must be given to the creation of quality control measures when using multiplex molecular panels, particularly when testing includes substances (such as the contents of blood culture bottles) that are not inherently included in the assays. Bhatti et al. conducted a study comparing the BCID and Verigene assays to conventional culture-based methods using the Vitek MS Ruo system (bioMérieux). The study found that the BCID assay correctly identified 95% of identifiable isolates in monomicrobial cultures (n = 118), while the Verigene assay correctly identified 99% of identifiable isolates. Both tests exhibited reduced identification times compared to traditional procedures (1.15 to 2.5 hours vs 25.6 hours). The BCID panel identified the presence of mecA in four staphylococcal isolates. Among them, three were Staphylococcus aureus and one was a coagulase-negative *Staphylococcus* species (CoNS). All four isolates were shown to be sensitive to methicillin. The discrepancy in the mecA findings of these isolates was attributed to the presence of a modified staphylococcal cassette chromosomal mec element (12).

Altun et al. conducted a study to assess the clinical effectiveness of the BCID panel for both monomicrobial and polymicrobial growth in blood culture bottles. The BCID test demonstrated sensitivities of 91.6% (153/167) and 71% (17/24) for monomicrobial and polymicrobial cultures, respectively, when compared to traditional techniques such as panel of desktop spot tests and Vitek2 XL- and MALDI-TOF MS-based colony identification. It is worth mentioning that 7.8% (13/167) of the species in the monomicrobial category were not included in the BCID panel. Within the group of specimens including many types of microorganisms, the panel was unable to identify 2 instances of Enterococcus faecalis, as well as 1 instance each of Escherichia coli and alpha-hemolytic Streptococcus species. However, it is important to note that most of the unidentified organisms were not included in the panel. The BCID panel was unable to reliably determine the methicillin susceptibility of S. aureus in a polymicrobial sample that included both S. aureus and CoNS, where mecA was found. This was due to the methicillin resistance of the CoNS isolate and the methicillin susceptibility of the S. aureus isolate (13).

5. Effects on both patient health outcomes and financial costs

Due to the expensive nature of these tests, several studies have evaluated their clinical and economic effects. In general, these investigations demonstrate a reduction in the duration required for identifying the organism and typically indicate a decrease in the time needed to optimize antibiotic treatment. Nevertheless, the effects of these panels on death rates and periods of hospitalization have not been definitively determined, and the interpretation of the data is confounded by inadequate research design in most instances. Specifically, the data obtained from studies conducted before and after an intervention is challenging to interpret because of the modifications that occur beyond the scope of the research throughout the course of time.

The therapeutic effect of quick molecular tests for evaluating positive blood culture bottles is likely influenced by institution-specific characteristics, such as unique patient groups and local resistance rates, as well as the presence of antibiotic stewardship initiatives. The effectiveness of

these panels is maximized when the findings are promptly communicated and properly responded to by healthcare practitioners responsible for the patient's care. Antibiotic deescalation is best achieved by delivering the results to an antimicrobial stewardship expert, such as an infectious diseases physician, infectious diseases pharmacist, or clinical microbiologist with a doctoral degree. These experts can then offer personalized and prompt guidance to the healthcare providers responsible for the patient's care (14).

6. Direct Pathogen Detection from Blood

The effectiveness of blood culture-based diagnoses is reduced when antimicrobial medication is started before the culture. Furthermore, meticulous or nonculturable microorganisms (such as *Coxiella burnetii*, *Tropheryma whipplei*, and *Rickettsia* species) do not proliferate in standard blood cultures, often eluding identification. Additionally, presently used methods have intrinsic delays in the time it takes to identify viruses, which is directly linked to the time it takes for them to develop (31). There is a need to quickly identify infections directly from blood without the time delay caused by culture-based approaches. The current constraints of multiplex molecular assays for this particular purpose consist of their moderate sensitivity, capability to identify only a restricted range of microbial targets, absence of standardization, susceptibility to inhibition by human genomic DNA, and contamination of reagents. Currently, there are no assays approved or cleared by the FDA specifically for directly detecting bacteria in blood samples. However, the T2Candida panel developed by T2 Biosystems is an in vitro diagnostic assay that can directly detect *Candida* species from whole-blood specimens. This assay has shown to be highly sensitive in comparison to traditional blood culture methods (32).

7. Summary

The use of multiplex molecular tests, which can detect many pathogens directly from clinical specimens, has caused a significant change in the way infectious illnesses are diagnosed. Instead of requesting many separate tests for each particular pathogen, healthcare practitioners now have the choice to order a single test that may identify several species linked to an infectious condition. Syndromic multiplex panels are innovative and effective techniques that may help diagnose infectious illnesses promptly and impact choices related to patient care, such as antimicrobial treatment, antimicrobial stewardship, and infection prevention and control. It is expected that syndromic testing will be more used in the future. Having a comprehensive understanding of the performance attributes and constraints is crucial when constructing multiplex assays.

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