AUCTORES
Globalize your Research

Journal of Pharmaceutics and Pharmacology Research
Humaira Zafar *

Open Access

Review Article

Proportionate Exploration to Distinguish Microbial Genetics and Their Resistant Genes via Multiplex Polymerase Chain Reaction (PCR) with Conformist Diagnostic Approaches

Humaira Zafar [1]*, Irfan Ali Mirza [2], Muhammad Fayyaz [3]

[1] Professor of Pathology, Microbiologist, Al Nafees Medical College & Hospital, Islamabad, PhD Scholar-NUMS, Pakistan

[2] Professor of Pathology, Consultant Microbiologist, National University of Medical Sciences, Rawalpindi, Pakistan

[3] Assist Prof of Microbiology, Consultant Microbiologist, National University of Medical Sciences, Rawalpindi, Pakistan

***Corresponding Author:** Humaira Zafar, Prof of Pathology, (Microbiologist), Department of Pathology Al Nafees Medical College & Hospital, Pakistan.

Received date: November 13, 2023; **Accepted date:** December 04, 2023; **Published date:** January 24, 2024

Citation: Humaira Zafar, Irfan Ali Mirza, Muhammad Fayyaz, (2024), Proportionate Exploration to Distinguish Microbial Genetics and Their Resistant Genes via Multiplex Polymerase Chain Reaction (PCR) with Conformist Diagnostic Approaches. *J. Pharmaceutics and Pharmacology Research*, 7(2); **DOI:10.31579/2688-7517/156**

Abstract

The increased emergence of anti-microbial resistance (AMR) to commonly used anti-microbial has become a solemn Global health concern. The researchers, scientists and health care providers are whole-heartedly devoted to solve this issue. To combat this challenge, besides no compromise on quality, due concern for cost effective approach was kept in mind.

The availability of direly required good microbiology diagnostic facilities are limited in many areas around the Globe. The culture and sensitivity are referred as a gold standard for diagnosing infections. But its time-consuming proceedings adds up to the misery of patients specially to start accurate and timely management. The newly added approach i.e multiplex PCR via biofire filmarray holds a promising methodology to contribute positive in such scenarios. Various syndromic panels of biofire filmarray are used to diagnose the bugs and their AMR genes for different site infections.

Therefore, the objectives of current review article were to identify evidence regarding opting multiplex PCR via biofire filmarray for rapid and accurate genetic level identification of AMR cases. After a thorough literature review, the results supported the utility of multiplex PCR, by justifying it as a rapid, cost effective, and accurate diagnostic tool in microbiology. Hence, it is concluded from the results of current review article, that multiplex PCR via biofire filmarray holds a great utility to accurately diagnose and manage the cases of AMR. Thus, timely and accurate management can reduce the morbidity and mortality rates in such cases.

Key words: amr; conventional techniques; culture and sensitivity; film array; intensive care settings; multiplex pcr; patient's outcome

Introduction

The term Bacterial antimicrobial resistance (AMR) implies to changing in bacterial genetic characteristics and ultimately making them less effective. AMR is acquainted as leading health threats for 21st century challenges. A report by world health organization (WHO) disclosed that mortality rate from AMR by the year 2050 might reach to 10 million people annually. Which will become true especially if same current statistics be continuing. Thus, a consequence is necessitating an urgent and vigilant attention at Global level to formulate a strong action plan in view to combat AMR [1].

Around the Globe, management of AMR cases is becoming a challenge for the clinicians. Every day, it is bringing up a new delinquent, adding upto the severity of situation. The highlighted ones include delayed recovery, high costs for managements, prolong hospital stays, increased morbidity and mortality rates [2].

A combination of AMR along with sepsis in intensive care units (ICUs) is further worsening the scenario due to high mortality. Sepsis in ICUs imparts a high rate of morbidity and mortality amongst all age brackets. Only available option to reduce this high incidence is provision of timely and accurate anti-microbial, hence to improve patient's survival. Therefore, the appropriate diagnosis, based upon clinical and laboratory assessment are the only way outs to improve patient's survival rate. [3]

Sepsis is defined as a condition having dysregulated host response to infection, ultimately causing life- threatening organ dysfunction.[4] In 2016 a combine effort by Society of Critical Care Medicine and European Society of Intensive Care Medicine was made to finalize definitions of sepsis and septic shock. Hence, spawned the Third International Consensus Definitions for Sepsis and Septic Shock, which are called as Sepsis-3 guidelines It has replaced systemic inflammatory response syndrome i.e SIRS.[5] They are still in use due to their good sensitivity and specificity. Comparatively with advancement in science and technology, new efforts were made by establishing sequential organ failure assessment (SOFA), quick Sequential Organ Failure Assessment (qSOFA) scoring. But it's successful usage to confirm sepsis is still a debate and remained a reason for controversy. Therefore, the accuracy of sepsis -3 guidelines still ranks superior.[6]

Many currently available microbiology diagnostic techniques have pros and cons. Traditional or conventional microbiology testing is one at the same things. They generally include culture, microscopy, antigen detection, serology and latest techniques.[7]

Culture & Sensitivity: The principal means of conventional testing is culture followed by biochemical analysis and antimicrobial susceptibility testing. In the case of bacteria and fungi, clinical specimens are inoculated on a range ofmedia and incubated. Use of selective and differential media may allow enhanced identification and suggest a presumptive diagnosis. Biochemical testing helps identification of species. Anti-microbial susceptibility is than applied to get the spectrum of sensitivity and resistant pattern. The positive cultures represent a 'gold standard' diagnosis. [7]

Direct Microscopy: The microscopic assessment is used for direct clinical specimens for rapid detection of bacterial, fungal and protozoal diseases. However, further confirmation will definitely be required. [7]

Serological Testing: The serological testing is based on the detection of a host humoral response to infection. In some cases, this is very specific like western blot for HIV confirmation. But in some cases, cross reactivity and nonspecific antibodies results in false positive results. The antigen and antibody techniques are mostly monoclonal antibodies directed against specific antigens are used for the diagnosis. Commonly use modalities include enzyme linked immunosorbent assay (ELISA) and tagged immune-microscopy. They are quite specific and positivity indicates a diagnosis. While sensitivity is often low and a negative result is frequently clinically unhelpful. [7] *Latest Techniques*: Next in sequence are the latest techniques with good sensitivity and specificity index. The molecular identification comes under the heading of latest techniques. Therefore, they rank superior to conventional techniques. Common examples includes polymerization chain reaction (PCR), Ligase chain reaction, Nucleic acid sequence based amplification, Transcription mediated amplification, Branched DNA, Strand displacement amplification, Hybridization techniques, Multiplex PCR, Microarrays are the advancements in microbiological lab diagnostics**.** [7]

In this context, recent multiplex PCR-based or array-based multi-pathogen detection tests, as well as, in certain cases, antibiotic resistance gene detection assays, aid in the diagnosis of bacterial infections subsequent to or concurrent with SARS-CoV-2 infection in patients with COVID-19. [7-8]

A published report from HonKong revealed that sepsis imparts high morbidity and mortality in critical care setting and ranges between 30% – 80% of all mortalities. However, 30% - 40% of all cases are usually form blood stream infections (BSIs). [9] The frequently observed six resistant bacteria for such scenario were Escherichia coli, followed by Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa. Analyzing the drug wise incidence of mortality, methicillin-resistant Staphylococcus aureus (MRSA), caused 100,000 deaths in the year 2019. In the same year next in sequence were 50,000 to 100,000 deaths due to multidrug-resistant tuberculosis. This was followed by third-generation cephalosporin-resistant Escherichia coli, carbapenem-resistant Acinetobacter baumannii, fluoroquinolone-resistant Escherichia coli, carbapenem- resistant Klebsiella pneumoniae, and lastly third-generation cephalosporin-resistant Klebsiella pneumoniae. [2]

Besides blood septicemia, other common etiologies of sepsis in ICUs could be respiratory sepsis, meningococcemia or GIT septicemia due to biliary causes or acute cholecystitis. [6] Global censuses for the year 2022, revealed that amongst all of the lower respiratory tract infections (LRTIs) a sub category i.e ventilator associated pneumonia (VAP), is the leading cause of mortality in ICUs. The estimated mortality can be even upto 70%. [10] Besides FilmArray blood identification panels, diagnostic accuracy for FilmArray respiratory, Meningitis/Encephalitis (FA/ME) and GIT panel also ranges between 89.5% sensitive and 97.4% specific. [11] Another study results also reported the same sensitivity of 90% and a specificity of 97%, for identifying any microorganism in the CSF. [12]

A published report for the year 2021, by United State (US) Food and Drug Administration (FDA) had approved the usage of blood culture identification (BCID) panel of BioFire FilmArray. It was labelled as a rapid, multiplex polymerase chain reaction for the identification of microbial genetics along with AMR genes. Though the proceedings were carried out on positive blood cultures, but still it is considered to save the processing time. A published study from Honkong, for the year 2021, had correlated septic shock with increased mortality in critically ill patients. It was clarified that delay in diagnosis and hence delayed management of condition are the main contributors for said condition. Hence, rapid diagnosis and accurate management are the only things to improve patient's outcome. Upon comparison with the results of conventional methods, the rapid provision of accurate results by biofire FilmArray are considered to provide an aid for modifying empirical antimicrobial therapy in approximately 32% of patients. Simultaneously it is of good utility in infection prevention and control practices especially for patients with multidrug resistant pathogens. Thus, also contributing one to reduce AMR.[9]

So far, despite a thorough literature search, in the year 2021, only one published case series report, from Pakistan was identified. The study setting involved Agha Khan University hospital, Karachi. It was carried out in monsoon, where five consecutive cases of meningitis presented in emergency department. The report highlighted the utility of meningitis/ encephalitis syndromic panel of FilmArray for confirming Enteroviral (EV) meningitis. The diagnosis was done by directly analyzing presence of Enteroviral RNA in cerebrospinal fluid (CSF). The patients age ranged between 18 to 35 years age and did not have any prior co-morbidities. Upon confirmation redundant antibiotics were immediately bunged. All of them were managed well and discharged for home with no neurologic complications. The evidence was also there in the report, supported by a study from California, that incidence of Enteroviral meningitis is more comparable with the Enteroviral encephalitis. The study results came to be in line with the available literature that sensitivity of reverse transcriptase-PCR (RT-PCR) for confirming EVs in CSF is 100% and specificity of 96.2%, when compared with viral cultures.[13]

Literatur Ereview

Sepsis in intensive care units (ICUs) imparts a high rate of morbidity and mortality amongst all age brackets. The situation is worsened by the presence of AMR bugs along with an intensifying threat of multidrug resistant (MDR) and extensively drug resistant (XDR) bacteria. Only available option to reduce this high incidence is provision of timely and accurate anti-microbial, hence to improve patient's survival. Time consuming culture proceedings compels the clinicians to start empirical therapy, based upon the available local statistics form antibiogram.

In view of that various new technologies have been introduced to address the issue. Multiplex PCR via FilmArray i.e BioFire, helps in simultaneous identification of microbial genetics along with AMR genes. It has many identification panels. Amongst them, blood culture identification panel (BCID) is notorious for rapid diagnosis of blood stream infections from positive blood cultures. The equipment is fully automated, requires minimal sample and a rapid turnaround time of about 1 hour approximate. [14]

For the year 2022, Lancet published a systematic review highlighting the severity of situation by analyzing Global statistics for the burden of AMR. The data was gathered by reviewing statistics of 204 countries and estimating deaths and disability-adjusted life-years (DALYs) linked with bacterial AMR. The statistical analysis using a predictive statistical models showed 4·95 million deaths due to AMR in 2019, especially 1·27 million deaths from bacterial AMR. This was recognized as top most cause of mortalities amongst intensive care settings in hospitals. It was further analyzed that AMR in Lower respiratory tract infections (LRTIs), ranked amongst top of all infections attributing to mortality in critical care settings. [2]

The increasing incidence of AMR has taken a serious concern amongst the global health issues. This is due to its serious outcomes like high morbidity rate, high mortality rates, financial burden on the families and health care systems. So far despite a thorough search only one Egyptian study for the year 2022 was found which had focused the utility of filmarray in pediatric population. In the study, three filmarray syndromic panels were used i.e pneumonia, blood and meningitis/encephalitis. The aim of study was to assess the performance of point-of-care multiplex PCR in diagnosis of pathogens and their antibiotic resistance genes in in a pediatric intensive care unit (PICU). The results strongly supported its utility in confirming the rapid diagnosis. It was estimated that turnaround time (TAT) of FilmArray panels was 1–1.5 hrs, which was found to be much less than in standard-of-care microbiology i.e 48–72 hrs. Moreover, identification of resistant genes favored the initiation of accurate management, hence improved patients' outcomes were observed.[15] The study results were further strengthened by another study of Switzerland in 2017, supporting substantial decline of AMR in ICU settings due to accurate diagnosis and hence management [16]

Multiplex PCR via FilmArray will be considered as gold standard and its comparative analysis in terms of its diagnostic accuracy will be identified by calculating sensitivity and specificity. The biofire respiratory panels helps to identify 21 targets. *Viruses* includes Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza an Influenza A/H1 Influenza A/H1-2009 Influenza A/H3 Influenza B Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus. *Bacteria* icnludes Bordetella parapertussis* Bordetella pertussis Chlamydia (Chlamydophila) pneumoniae Mycoplasma pneumonia. [17-18]

The pneumonia panel helps to identify 33 targets. *Bacteria* includes Semi-Quantitative Bacteria Acinetobacter calcoaceticusbaumannii complex Enterobacter cloacae complex Escherichia coli Haemophilus influenzae Klebsiella aerogenes Klebsiella oxytoca Klebsiella pneumoniae group Moraxella catarrhalis Proteus spp. Pseudomonas aeruginosa Serratia marcescens Staphylococcus aureus Streptococcus agalactiae Streptococcus pneumoniae Streptococcus pyogenes. *Atypical Bacteria* includes Qualitative Bacteria Chlamydia pneumoniae Legionella pneumophila Mycoplasma pneumonia. List of Viruses include, Adenovirus Coronavirus Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza An Influenza B Parainfluenza Virus Respiratory Syncytial Virus. The AMR genes includes, Methicillin Resistance mec A/C and MREJ, Carbapenemases i.e imipenam (IMP), Klebsiella pneumonia carbapenemases (KPC), new Deli metallo-betalactamases (NDM), oxacillinases (OXA-48), Verona integron-encoded metallo-β-lactamase (VIM), Extended spectrum beta lactamase (ESBL), and cefotaxime (CTX-M). [17,18]

The meningitis/ encephalitis panel helps to identify 14 targets. *Bacteria* includes Escherichia coli K1 Haemophilus influenzae Listeria monocytogenes Neisseria meningitidis Streptococcus agalactiae Streptococcus pneumonia. List of *Viruses* includes Cytomegalovirus (CMV) Enterovirus Herpes Simplex Virus 1 (HSV-1) Herpes Simplex Virus 2 (HSV-2) Human Herpesvirus 6 (HHV-6) Human Parechovirus Varicella Zoster Virus (VZV). *Yeast* include Cryptococcus neoformans/gattii. [17]

The biofire blood panel helps to identify 27 targets. *Gram-Positive Bacteria* i.e Enterococcus Listeria monocytogenes Staphylococcus Staphylococcus aureus Streptococcus Streptococcus agalactiae Streptococcus pneumoniae Streptococcus pyogenes. *Gram-Negative Bacteria* i.e Acinetobacter baumannii Enterobacteriaceae Enterobacter cloacae complex Escherichia coli Klebsiella oxytoca Klebsiella pneumoniae Proteus Serratia marcescens Haemophilus influenzae Neisseria meningitidis Pseudomonas aeruginosa Sample Type: Positive Blood Culture FDA-cleared and CE-marked. Yeast i.e Candida albicans Candida glabrata Candida krusei Candida parapsilosis Candida tropicalis. The AMR genes includes mecA – methicillin resistance KPC – carbapenem resistance vanA/B – vancomycin resistance. [17]

The biofire GIT panel helps to identify 22 targets. *Bacteria* i.e Campylobacter (jejuni, coli, and upsaliensis) Clostridium difficile (toxin A/B) Plesiomonas shigelloides Salmonella Vibrio (parahaemolyticus, vulnificus, and cholerae) Vibrio cholerae Yersinia enterocolitica Diarrheagenic E.coli/Shigella: Enteroaggregative E.coli (EAEC) Enteropathogenic E.coli (EPEC) Enterotoxigenic E.coli (ETEC) lt/st Shiga-like toxin-producing E.coli (STEC) stx1/stx2 E.coli O157 Shigella/Enteroinvasive E.coli (EIEC). *Viruses* i.e Adenovirus F40/41 Astrovirus Norovirus GI/GII Rotavirus A Sapovirus (I, II, IV, and V) and *Parasites* i.e Cryptosporidium Cyclospora cayetanensis Entamoeba histolytica Giardia lamblia. [17]

The available literature supports that conventional techniques used to have delayed turnaround time, which prevents early treatment optimization.

Therefore, on same time breakthrough in microbiological diagnostics i.e multiplex PCR via biofire filmarray holds a promising approach for reducing time from pathogen identification to its resistant markers. Therefore, the objectives of current review article were to identify evidence regarding opting multiplex PCR via biofire filmarray for rapid and accurate genetic level identification of AMR cases. The second objective is focused on its simultaneous comparison with conventional diagnostic techniques.

Conclusion

Multiplex PCR via biofire filmarray holds a great utility to accurately diagnose and manage the cases of AMR. Thus, timely and accurate management can reduce the morbidity and mortality rates in such cases.

Recommendations:

1. The results of current review article had provided a road map to carry out researches at National & International level to identify the utility and significance of multiplex PCR using a biofire film array.
2. Literature review has provided the evidence that biofire filmarray should be used as an accurate, rapid and cost effective microbiological diagnostic options to combat AMR.
3. The wide variety of microbial etiology and their management options, helps in identification for statistics of rare diseases. Thus, it's incorporation in microbiological diagnostics will be helpful for infection prevention and control strategies.
4. Provision of Evidence for allocation of increased funds by government to improve microbiological laboratory services in our country to combat AMR.

References

1. Temkin E, Fallach N, Almagor J, Gladstone BP, Tacconelli E, Carmeli Y, etal. (2018). Estimating the number of infections caused by antibiotic-resistant Escherichia coli and Klebsiella pneumonia in 2014: a modelling study. Lancet Glob Health 6:969–979.
2. Muray CJ. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 399: 629–655.
3. Lindner HA, Schamoni S, Kirschning T, Worm C, Hahn B, Centner FS, etal. (2022). Ground truth labels challenge the validity of sepsis consensus definitions in critical illness. J Trans Med. 20:27.
4. Zhang Y, Luo H, Wang H, Zheng Z, Ooi OC. (2020). Validation of prognostic accuracy of the SOFA score, SIRS criteria, and qSOFA score for in-hospital mortality among cardiac-, thoracic-, and vascular-surgery patients admitted to a cardiothoracic intensive care unit. J Card Surg. 35(1):118-127.
5. Fernando SM, Rochwerg B, Seely AJE. (2018). Clinical implications of the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) CMAJ.; 190(36): 1058-1059.
6. Han M, Mak W, Low JK, Junnarkar SP, Huey TCW, Shelat VG. (2019). A prospective validation of Sepsis-3 guidelines in acute hepatobiliary sepsis: qSOFA lacks sensitivity and SIRS criteria lacks specificity (Cohort Study). Int J Surg. 72: 71-77.
7. Laupland KB, Valiquette L. The changing culture of the microbiology laboratory. Can J Infect Dis Med Microbiol. 2013; 24(3): 125–128.
8. Bogdan I, Citu C, Bratosin F, Malita D, Romosan I, Gurban CV, etal. (2022). The Impact of Multiplex PCR in Diagnosing and Managing Bacterial Infections in COVID-19 Patients Self-Medicated with Antibiotics. Antibiotics (Basel). 11(4):437.
9. Rule R, Paruk F, Becker P, Neuhoff M, Chausse J, Said M, etal. (2021). Clinical utility of the BioFire FilmArray Blood Culture Identification panel in the adjustment of empiric antimicrobial therapy in the critically ill septic patient. PLoS One. 16(7): 0254389.
10. Kamel NA, Alshahrani MY, Aboshanab KM, Borhamy MIE. (2022). Evaluation of the BioFire FilmArray Pneumonia Panel Plus to the Conventional Diagnostic Methods in Determining the Microbiological Etiology of Hospital-Acquired Pneumonia. Biology (Basel). 11(3):377.
11. Tansarli GS, Chapin KC. (2020). Diagnostic test accuracy of the BioFire® FilmArray® meningitis/encephalitis panel: a systematic review and meta-analysis. Clin Microbiol Infect. 26(3):281-290.
12. Gómez JT, Tsokani S, Ferreira CA, Muñoz SA, Villegas MJJ, Tabares CS, etal. (2022). Biofire FilmArray Meningitis/Encephalitis panel for the aetiological diagnosis of central nervous system infections: A systematic review and diagnostic test accuracy meta-analysis. E Clinical Medicine. 44:101275.
13. Jafri L, Farooq Khan A, Arshad T. (2021). A Case Series on Enteroviral Meningitis in Pakistan. Cureus. 13(10): 19048.
14. Peri AM, Bauer MJ, Bergh H, Butkiewicz D, Paterson DL, Harris PN, etal. (2022). Performance of the BioFire Blood Culture Identification 2 panel for the diagnosis of bloodstream infections. Heliyon. 8: 09983.
15. Nawawy AAE, Antonios MA, Tawfik ME, Meheissen MA. (2022). Comparison of a Point-of-Care FilmArray Test to Standard-of-Care Microbiology Test in Diagnosis of Healthcare Associated Infections in a Tertiary Care Pediatric Intensive Care Unit. Antibiotics (Basel). 11(4): 453.
16. Mayer LM, Kahlert C, Rassouli F, Vernazza P, Albrich WC. (2017). Impact of viral multiplex real-time PCR on management of respiratory tract infection: a retrospective cohort study. Pneumonia. 9-4.
17. Biofire FilmArray system.
18. FilmArray Respiratory panel quick guide.

DOI: 10.31579/2688-7517/156