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Short Communication

Time-Critical Management of Sepsis from Days to Hours in A Race of Survival Via Real-Time Pcr to Rapidly Detect Colistin and Carbapenam Resistant Enterobacterale Genes

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Abstract

Septicemia remains a major cause of morbidity and mortality in intensive care settings especially of low- and middle-income countries (LMICs). The delayed diagnosis and increasing antimicrobial resistance (AMR) worsens the scenario for high mortality. The emergence of colistin and carbapenem resistant Enterobacterales, further limits the management options. The conventional culture and antimicrobial susceptibility testing (AST), requires several days to yield results and ultimately cannot detect the resistance mechanisms, particularly hetero-resistance. Here comes a hope by using real-time polymerase chain reaction (RT-PCR), to rapidly detect AMR genes in cost effective manner. Thus, patient's outcome can be improved in terms of mortality especially in LMICs. Based upon available evidence, it is concluded that due to fast turnaround time, high throughput, increased sensitivity and specificity, along with an ability for linear limit of detection, utility of real time PCR is strongly sanctioned for LMICs. The strong diagnostic evidence suggests good outcomes for microbiological diagnostics especially in septicemic patients, having colistin and carbapenem resistant Enterobacter ales infections.

Key Words: antimicrobial resistance; antimicrobial susceptibility testing; enterobacterales

Introduction

Septicemia has emerged as a leading cause of morbidity and mortality worldwide especially in under developed and low middle income countries. This came to be especially true for patients with serious underlying etiologies or debilitated pathologies. Thus, necessitating availability of rapid diagnostics modalities to proceed for précised therapeutic interventions. The emergence and global spreading of multidrug-resistant (MDR) bugs i.e. colistin and carbapenem-resistant Enterobacter ales (CRE), have pointedly worsened management of sepsis by minimizing the options. These Enterobacter ales possess an assorted range of resistance genes like carbapenems genes i.e. blaKPC, blaNDM, blaVIM, blaIMP, and blaOXA-48. Similarly, plasmid-mediated mobilized colistin resistance genes i.e. mcr-1 to mcr-10, is also bringing an added challenge to effective antimicrobial therapy. [1,2]

The traditional culture based phenotypic expression and antimicrobial susceptibility testing (AST) methods, though the gold standards by are inherently time-consuming. Approximate time of 3 days for routine sample reaching up to 7 days for blood culture proceedings for definitive results. In the milieu of septicemia, such adjournments are clinically deplorable. As the delay in diagnosis is directly linked with inappropriate antimicrobial therapy usage ultimately causing increased mortality. Besides that, phenotypic methods might miss detecting certain resistance genes. This came to be especially true for Heter resistance of any of the mobilized colistin resistance genes i.e mcr1 to mcr10. Thus, it contributes to therapeutic failure. [3]

In this situation, real-time polymerase chain reaction (RT-PCR) has arisen as a powerful molecular diagnostic modality. Comparable to other diagnostic modalities in microbiology, it a rapid, highly sensitive, and specific test for detecting universal or bacterial resistance genes. Cherry on top is having an ability to directly detect them from clinical samples besides pure growths. Further advancement of RT-PCR to multiplex RT-PCR enable synchronized recognition of multiple carbapenemase and mcr genes within a single assay, meaningfully enhancing diagnostic effectiveness. Recent studies have confirmed that these assays attain the sensitivity and specificity of approximate100% for carbapenems gene detection, with exceptional concordance with sequencing-based methods. [4,5]

Further strengthening the noteworthy recompences of RT-PCR is its rapid turnaround time, often providing results within 1–4 hours. This permits clinicians to pledge or adjust targeted antimicrobial therapy at an early stage of infection. Thereby improving patient outcomes by limiting unnecessary broad-spectrum antibiotic usage. Premature detection of CRE and colistin resistance genes also enables rapid execution of infection prevention and control measures, limiting the nosocomial transmission, predominantly in intensive care units (ICUs). [6]

Additionally, RT-PCR validates high analytical sensitivity, with a low limit of detection to 10^2–10^3 copies of genes per milliliter of sample. This makes it principally more appreciated for low bacterial loads or the ones already receiving empirical therapy, where culture yield might had compromised. Notably, molecular assays can spot resistance genes even in cases where phenotypic expression is not obvious, thereby diagnosing "quiet reservoirs" of resistance that may otherwise go unobserved. [5,7]

Besides many advantages, RT-PCR there are some limitations as well. A key downside is its dependance on predefined genetic targets. This confines to recognition of known or targeted resistance genes only. Emerging or scarce resistance mechanisms may be missed, imposing balancing methods such as whole genome sequencing (WGS) for wide-range surveillance. Furthermore, the existence of a resistance gene does not always relate with phenotypic resistance, this is true as a gene expression can be prejudiced by regulatory mechanisms. Therefore, RT-PCR should ideally be used in combination with phenotypic AST to safeguard precise quantifiable construal. [3,8] The initial cost for setup and infrastructure requirements also carries a noteworthy challenge, especially for resource-limited settings, having a high burden of antimicrobial resistance. The need for specific equipment, skilled staffs, and quality assurance systems may limit extensive adoption. [6] Ranking superior to all those limitations, mentioned above, owing the immense benefits i.e. reduced hospital stay, enhanced antimicrobial stewardship, and reducing spread of MDR pathogens, may counterbalance preliminary hoards. [6]

Literature review:

Septicemia accounts for more than 20% global mortality ultimately producing a high healthcare cost. Misdiagnosis, delayed diagnosis or initiation of wrong treatment are the main contributors for high mortality. The biggest challenge for confirming the diagnosis via blood culture and sensitivity is the low concentration of pathogens in blood stream. Thus, necessitating initiation of broadspectrum antibiotics usage, which itself came to be the major etiological factor for the development of AMR. 9

Tracing back to the published evidence from WHO, in June 2023, AMR was notified as global research agenda in human health. The report emphasized theme to diagnose blood stream pathogens and their AST directly from positive blood culture bottles. 10 Another article narrated various diagnostic approaches other than routine biochemical approaches, which have the ability for detecting lower limit level of a pathogen. They include matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), polymerase chain reaction (PCR), and fluorescence in situ hybridization (FISH). But all of them all of them have certain limitations, regarding the rapid identification of pathogens along with AMR genes detection. [11]

Then comes a ray of hope to use nanopore sequencing in clinical microbiology to detect a pathogen and its anti-microbial resistant genes (ARGs). 12 A published study for the year 2024, had shown a comparative analysis of shorter blood culture incubation times by using BD BACTEC™ bottles and real-time nanopore sequencing along with data analysis. The results of study demonstrated the significant results of using nanopore sequencing in view of having lower limit of detection for 102–104 CFU/mL, in appx 2 hours of incubation and 40 minutes of nanopore sequencing. However, the AMR genes were also identified at a lower limit of detection for 103–107 CFU/mL. Which is achieved appx after 5 hrs. of incubation and only 10 min to 3 hrs. of nanopore sequencing. Thus, summarizing an appx time of 7 hrs. to 9 hrs. starting from the sample collection till the decision for accurate antibiotics. These results contribute for better management of sepsis compared with traditional culture-based proceedings. [13]

The Minion small pocketable device is a commonly used one for nanopore sequencing. The final sequencing data will then be analyzed in real-time. [14,15] The biggest advantage of nanopore sequencing is the availability of variety of kits for targeted samples and long sequencing reads.[16]

Current developments in molecular diagnostics and availability of multiplex syndromic panels like blood culture identification systems via film array, have supplemented integration of RT-PCR into routine clinical roadmaps. This helps in simultaneous detection of anti-microbial resistant gene and the specific microbe in one processing time, ultimately had bridged a gap between molecular diagnostics and management policymaking. [7]

Bringing out the summary from entire available evidence, real-time PCR embodies a rebellion in the microbiological diagnostics for septicemia especially colistin and carbapenem resistant Enterobacter ales. High throughput, fast turnaround time, increased sensitivity and specificity, along with great ability for linear limit of detection, had valued its utility in microbiology. Still superseding certain limitations of cost, inability to detect novel resistance genes, technical support even in low middle income countries.

The increased burden of AMR Globally and in low middle income countries, it is urged from the Global policy makers to facilitate provision of extra funds to such countries. This will bring an aiding scenario for them to combat AMR for MDR infections, along with high morbidity and mortality rate in a comfortable manner.

Conclusion:

The fast turnaround time, high throughput, increased sensitivity and specificity, along with an ability for linear limit of detection, had treasured the utility of real time PCR in microbiology. The literature review had endorsed its usage for microbiological diagnostics for septicemic patients, especially colistin and carbapenem resistant Enterobacter ales.

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