Comparative analysis of traditional and rapid diagnostic methods in infectious diseases.

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ANNOTATION

Our study addresses a significant issue in the medical and scientific community—the delayed administration of appropriate antimicrobial treatments due to the time-consuming process of phenotypic susceptibility data collection in gram-negative bloodstream infections. Our research indicates that a multiplex PCR rapid diagnostic test (RDT) significantly outperformed two clinical scoring tools in predicting ceftriaxone susceptibility. Multiplex PCR also led to reduced instances of undertreatment with ceftriaxone and minimized overtreatment with carbapenems. Furthermore, multiplex PCR demonstrated high sensitivity and specificity in predicting ceftriaxone susceptibility. The results of our study underscore the potential RDTs to reduce the time to appropriate antimicrobial therapy, leading to improved patient outcomes and reduced healthcare costs.

Keywords: *bloodstream infections, extended-spectrum β-lactamase (esbl), antimicrobial resistance, pathogen identification; delayed appropriate therapy, risk factors, rapid diagnostics.*

Introduction.

An estimated 2 million bloodstream infections (BSI) occur in North America and Europe annually and are associated with 250,000 deaths .With antimicrobial resistance on the rise, extended-spectrum beta-lactamase-producing *Enterobacterales* (ESBL-E) have been identified by the CDC and WHO as organisms of concern . From 2013 to 2019, cases of ESBL-E infections were estimated to have increased by 50%. Infections of ESBL-E are associated with increased cost, morbidity, and mortality when compared

with non-ESBL-producing bacteria; they are also associated with delayed administration of appropriate antimicrobial therapy .Susceptibility data from blood cultures can take up to 72 hours to result, contributing to these delays and, consequently, poorer clinical outcomes .The risk of undertreatment of resistant organisms must be continually weighed against the repercussions of overtreatment with carbapenems, the treatment of choice for invasive infections by ESBL-E.

Several scoring tools utilizing risk factors for ESBL-E BSI have been developed to aid clinicians predict their presence and determine the need for empiric treatment with carbapenems. Augustine and colleagues created a weighted scoring system utilizing three independent risk factors for ESBL-E BSI: (i) recent outpatient gastrointestinal/genitourinary procedure, (ii) recent course of beta-lactams or fluoroquinolone, and (iii) prior infection/colonization with ESBL-E. They concluded that patients with scores ≥3 or critically ill patients with a score of 1–2 (Approach 2) should receive carbapenem therapy (8). Additionally, Lee and colleagues developed a scoring tool that identified nursing home residence, recent antimicrobial therapy, recent invasive procedures, and frequent emergency department (ED) visits as risk factors for ESBL-E, and recommended empiric carbapenem therapy with a score ≥2. Both of these methods were found to have high negative predictive values (NPV, 97%–99%), although a limitation of these studies is that they were conducted in populations with a relatively low incidence of ESBL-E. Furthermore, these scoring tools had poor positive predictive values (PPV, 33%–40%), which could lead to overtreatment with carbapenem therapy.

In April 2021, the University of Utah Health began utilizing the BCID2 platform for rapid identification of organisms in blood cultures. This new RDT detects the most common gene conferring ESBL resistance, (CTX-M). Despite this, providers frequently select meropenem out of concern for other mechanisms of ceftriaxone resistance. The use of a similar RDT by Verigene at the Detroit Medical Center and the University of Maryland Medical Center was found to be effective in predicting ceftriaxone susceptibility of select *Enterobacterales*. The purpose of this study was to validate the

usage of the BCID2 platform in predicting ceftriaxone susceptibility at the University of Utah Health and compare its discrimination performance against two ESBL diagnostic scoring tools.

MATERIALS AND METHODS

This was a retrospective observational study evaluating adult patients with BSI by select *Enterobacterales* species across the University of Utah Health system. Patients were included if they had at least one positive blood culture with *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus* spp., or *Salmonella* spp., which are all species of *Enterobacterales* detected by the BCID2 panel. While BCID2 can also detect *Enterobacter cloacae*, *Klebsiella aerogenes*, and *Serratia marcescens*, patients with BSI by these organisms were excluded due to the possibility of AmpC beta-lactamase production. Patients were excluded if carbapenemases were detected. Patients who had more than one species of *Enterobacterales* identified were excluded. However, detection or isolation of non-*Enterobacterales* organisms (i.e., *Staphylococcus* spp., *Bacteroides*, etc.) did not preclude patients from this study. If a patient had multiple episodes of BSI by *Enterobacterales*, only the first episode within the study period was included. Pregnant and incarcerated patients were excluded.

This study closely followed a methodology previously described .Briefly, we assessed the validity of three methods for determining whether a ceftriaxone or a carbapenem would have been indicated in gram-negative BSI: (i) the CTX-M result from the BCID2 panel, (ii) the ESBL prediction score by Augustine and colleagues, and (iii) the ESBL prediction score by Lee and colleagues. Time 0 was defined as the time blood cultures were drawn, at which point all patients were hypothetically started on empiric ceftriaxone. Blood cultures were presumed positive at 24 hours, and patients were managed hypothetically by each method. A patient was switched to a carbapenem if (i) CTX-M was detected on the BioFire BCID2 panel (suggesting ESBL phenotypic resistance prior to finalized susceptibilities), (ii) the ESBL prediction score by Augustine and colleagues was ≥3, or (iii) the ESBL prediction score by Lee and colleagues was ≥2.

Otherwise, the patient was continued on ceftriaxone. Definitive antibiotic therapy was determined at 72 hours when phenotypic antibiotic susceptibility reports were available.

For each diagnostic method, the sensitivity, specificity, PPV, and NPV of each method were determined for predicting ceftriaxone susceptibility. Additionally, the theoretical number of patients who unnecessarily escalated to a carbapenem or undertreated with ceftriaxone was calculated. A theoretical number of carbapenem days were determined.

Accurate and timely diagnosis of infectious diseases is critical for effective treatment, outbreak control, and antimicrobial stewardship. Diagnostic methods can be broadly divided into traditional (culture-based and serological) and rapid (molecular and point-of-care) approaches. Each has distinct advantages and limitations,

Accurate and timely diagnosis of infectious diseases is critical for effective treatment, outbreak control, and antimicrobial stewardship. Diagnostic methods can be broadly divided into traditional and rapid approaches, each with its own advantages, limitations, and clinical applications. Traditional methods, often considered the gold standard for confirming infections, include microbiological culture, microscopy, serological tests, and biochemical identification. Bacterial cultures allow growth on selective media, enabling identification of pathogens and determination of antibiotic susceptibility, while fungal cultures use specialized media such as Sabouraud agar. These methods are highly specific and support epidemiological studies, but they are time-consuming, often taking from 24 hours to several weeks, require skilled personnel, and may fail for fastidious or slow-growing pathogens.

Microscopy techniques, including Gram staining, Ziehl–Neelsen staining for Mycobacterium tuberculosis, and Giemsa staining for malaria parasites, provide rapid and inexpensive visualization of pathogens but have lower sensitivity, especially when pathogen load is low, and cannot determine antibiotic susceptibility. Serological methods such as ELISA, agglutination tests, complement fixation, and Western blot detect host immune responses or pathogen antigens. While useful, they may not

distinguish between active and past infections and can produce false-negative results in early stages before antibody production. Biochemical and phenotypic identification methods, including API strips and automated systems like VITEK, allow species-level identification and metabolic profiling but are time-consuming and may misidentify rare or novel pathogens.

Rapid diagnostic methods are designed to provide speed, accessibility, and early detection. Molecular diagnostics, such as PCR and RT-PCR, detect pathogen DNA or RNA with high sensitivity and specificity, while isothermal amplification techniques like LAMP and RPA allow nucleic acid amplification without thermal cycling, making them suitable for low-resource settings. Rapid immunoassays, including lateral flow tests and rapid ELISA kits, detect antigens or antibodies quickly and are suitable for point-of-care use, although they are generally less sensitive than molecular methods and can produce false-positive results due to cross-reactivity. Biosensor-based diagnostics, including electrochemical, optical, and nanomaterial sensors, provide real-time detection and are portable, but they remain largely experimental and expensive to implement. Microfluidic platforms and lab-on-a-chip systems enable the processing of small samples and simultaneous detection of multiple pathogens, offering rapid and multiplexed diagnostics, though they are technically complex and costly. Next-generation sequencing (NGS) can identify all genetic material in a sample, allowing detection of both known and novel pathogens with high resolution, but it is expensive, requires bioinformatics expertise, and is not practical for routine clinical use.

In modern clinical practice, a combined approach is often employed, using rapid tests for initial screening and triage, followed by traditional culture or molecular methods for confirmation, antimicrobial susceptibility testing, and epidemiological surveillance. Future development in infectious disease diagnostics aims to create low-cost, high-sensitivity multiplex point-of-care platforms integrated with digital health systems and artificial intelligence, as well as portable molecular diagnostics that provide both rapid detection and antimicrobial resistance profiling. While traditional diagnostic methods remain essential for pathogen characterization and epidemiological studies,

rapid diagnostics provide speed, accessibility, and early detection, and a combined approach leveraging the strengths of both ensures accurate diagnosis, effective treatment, and better global health outcomes.

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