Diagnostic Microbiology and Infectious Disease 83 (2015) 93–98

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

An 11,000-isolate same plate/same day comparison of the 3 most widely used platforms for analyzing multidrug-resistant clinical pathogens☆

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ARTICLE INFO

Article history:
Received 9 February 2015
Received in revised form 22 May 2015
Accepted 24 May 2015
Available online 11 June 2015

Keywords:
Antibiotics
Carbapenemase
Vitek-2
Phoenix
MicroScan

ABSTRACT

Stewardship of the dwindling number of effective antibiotics relies on accurate phenotyping. We sought to conduct the first large-scale, same plate and day comparison of the 3 most widely used bacterial analyzers. A total of 11,020 multidrug-resistant clinical isolates corresponding to more than 485,000 data points were used to compare the 3 major identification and antibiotic susceptibility testing (AST) platforms. Bacterial suspensions, prepared from a single plate, were simultaneously tested on all platforms in the same laboratory. Discrepancies were derived from MIC values using 2014 interpretive guidelines. Molecular methods and manual microbroth dilution were reference standards. Most discrepancies were due to drug–organism–AST platform combination instead of individual factors. MicroScan misidentified *Acinetobacter baumannii* ($P < 0.001$) and underestimated carbapenem susceptibility in *Klebsiella pneumoniae*. Vitek-2 and Phoenix had higher discrepancies for bla_{KPC}-containing Enterobacteriaceae ($P < 0.05$) and reported false susceptibilities more often. While all platforms performed according to standards, each had strengths and weaknesses for organism identification, assaying specific drug–organism combinations and inferring carbapenemase production.

1. Introduction

Clinicians and infection preventionists rely on accurate laboratory results to direct therapy and support infection control or antibiotic stewardship (Bartlett et al., 2013; Boucher et al., 2009; Center for Disease Control, 2013a; Hoang et al., 2013; Pfeiffer and Beldavs, 2014; Talbot et al., 2006; WHO, 2014). Comparative effectiveness research is key to quality and cost in healthcare and considered a priority by the Institute of Medicine and the Agency on Healthcare Research and Quality (Sox and Greenfield, 2009; Agency for Healthcare Research and Quality, 2012). Furthermore, the College of American Pathologists (CAP) requires laboratories seeking accreditation to conduct comparison studies when they use multiple platforms for the same test (i.e., organism identification [ID] and antibiotic susceptibility testing [AST]). Earlier comparison studies indicated that the Phoenix (BD Diagnostics, Sparks, MD, USA) had the highest sensitivity for detecting extended-spectrum β-lactamases (ESBLs) and carbapenemase producers in Enterobacteriaceae relative to the Vitek-2 and MicroScan systems (Wiegand et al., 2007; Woodford et al., 2010). However, those studies looked at relatively small numbers of locally acquired isolates and relied on outside reference laboratories when comparing 2 or more platforms. This limits generalizability and introduces variance such as changes in inoculum densities, growth conditions, or sample handling (Bratu et al., 2005; Thomson and Moland, 2001).

To our knowledge, there are no large-scale studies that assessed the results of the 3 most widely used platforms after simultaneous testing and included over 200 confirmed carbapenemase producers. Such data would be useful for baseline accreditation efforts and future benchmarking.

In our study, the Phoenix, Vitek-2 (bioMerieux, Durham, NC, USA) and MicroScan (Seimens, Deerfield, IL, USA) platforms were evaluated for their ability to accurately characterize over 11,000 genetically diverse multidrug-resistant organisms (MDROs) including 1323 *Acinetobacter baumannii*, 547 *Klebsiella pneumoniae*, 678 *Pseudomonas aeruginosa*, 2072 *Escherichia coli*, and 6400 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates grown on the same plate, with the same set-up time on each platform by the same accredited laboratory. Furthermore, AST and identification discrepancy rates of >200 isolates confirmed to contain bla_{KPC}, bla_{NDM}, bla_{IMP}, or bla_{VIM} were compared to noncarbapenemase producers. Matrix-assisted laser desorption/

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http://dx.doi.org/10.1016/j.diagmicrobio.2015.05.018

ionization-time of flight (MALDI-TOF), sequencing, PCR, manual broth dilution, and/or the results of the submitting hospital laboratory were used as reference standards or to resolve discordances.

2. Methods

This study was undertaken as a quality improvement, infection control initiative authorized by policy memoranda 09-050, 11-035, and 13-016 and IRB protocol number HB-00050924-2.

2.1. Bacterial isolates

A. baumannii, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and MRSA isolates from medical treatment facilities were grown on blood agar plates (Difco, Detroit, MI, USA) for individual colonies. MDRO classification was based on previously published methods (Magiorakos et al., 2012). Isolates were collected from 2002 to 2014 from hospital laboratories across the United States, including Alaska and Hawaii, as well as Europe, Central and South America, Asia and the Middle East; they came from various anatomical sites, clinical settings (intensive care unit, ward, and outpatient clinics), and patient population representing both genders and all ages. Before submission to the central network laboratory where this study was conducted, isolates were characterized by the accredited laboratory of the submitting hospital (Waterman et al., 2012).

2.2. Strain evaluation

At the central laboratory, all isolates undergo concurrent testing on the 3 AST platforms according to CLSI guidelines and CAP standards as previously described (Lesho et al., 2014). Characterization by pulsed-field gel electrophoresis, multilocus sequence typing, PCR, and whole genome sequencing (WGS) are performed as described previously (Lesho et al., 2014; McGann et al., 2014). Suspected isolates carrying a carbapenemase gene were confirmed by the Carba NP assay, Real Time-Polymerase Chain Reaction (RT-PCR), or WGS (Lesho et al., 2013; McGann et al., 2013; Milillo et al., 2013). A large number (>300) of distinct clades of *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, *E. coli*, and MRSA were included (data not shown).

2.3. Identification and antibiotic susceptibility testing

The following were used as controls: ATCC strain *K. pneumoniae* 700603, *E. coli* 35218 and 25922, *P. aeruginosa* 27853, *Proteus vulgaris* 49132, and *Providencia stuartii* 49809. Before testing, all analyzer panels were prevalidated according to CAP guidelines. All platforms were simultaneously inoculated from a single culture plate and analyzed using Phoenix panels NC44 or NC47 (Siemens, Deerfield, IL, USA), Vitek-2 cards GN30, GN59, or GN ID (bioMerieux, Durham, NC, USA), and MicroScan Walk Away panels NMIC/ID133 (BD Diagnostics). Technicians rotated between the AST analyzers to mitigate operator bias.

Raw MIC results were converted to their respective sensitive (S), intermediate (I), and resistant (R) categorical calls according to 2014 CLSI guidelines using a Perl script (CLSI, 2014). Only antibiotics reported by all platforms were considered. When derived categorical calls differed, these disagreements were classified into 3 groups: a minor discrepancy (mD) is an I call from 1 analyzer contrasted against 2 S or R calls from the other platforms; a major discrepancy (MD) is an R call contrasted against 2 S calls; and a very major discrepancy (VMD) is an S call contrasted against 2 R calls.

Analogous to the minor, major, and very major error lexicon, we used the term discrepancy for this comparison study as it is not feasible to determine the MIC on such a large number of organism–antibiotic combinations using manual broth or agar dilution methods. Hence, the analyzer results themselves were used for discrepancy calls with discrepancy types between instruments attributed to the platform reporting the outlier categorical call. In rare cases where the derived calls were R, I, and S, the MIC values were determined based on manual microbroth dilutions (MBDs), per CLSI guidelines (CLSI, 2014), or the Sensititer AIM and Trek (Thermo Fisher Scientific, Waltham, MA, USA) system using plate GN2F. Controls for manual MBDs included at least 2 isolates from each species, one being sensitive and the other, resistant. When discordant organism identification was seen, the isolate was retested on each platform, and final adjudications were based on MALDI-TOF, 16S sequencing, or WGS as previously described (Carbonnelle et al., 2011; Center for Disease Control, 2013b). In addition to the reference standards described above, we could also refer to the ID and AST results of the submitting hospital laboratory (also CAP accredited) for further adjudication.

All statistics were calculated using Fisher's exact ($P < 0.05$) or Yate's χ^2 tests using the R software package (R Developmental Core Team, 2010). A *P*-value of less than 0.01 or 0.05 was considered significant for data analyzed by the Yate's χ^2 test or Fisher's exact, respectively.

3. Results

3.1. Organism identification

Organism identifications among the 3 platforms agreed at the species level for more than 99% of the 11,020 samples tested. MicroScan and the Phoenix misidentified 52 out of 11,149 organism identified, while the Vitek-2 misidentified only 5 (Table 1).

MicroScan misidentified *A. baumannii* significantly more often than Vitek-2 or Phoenix ($P < 0.001$, Yate's corrected χ^2), mainly due to reporting *Shigella* species in 16 of 1363 (1.2%) of cases. Vendor contacts were unable to either resolve or explain this occurrence. Likewise, Phoenix misidentified significantly more *A. baumannii* than did Vitek-2 but did not favor misidentification of any one genus The Phoenix and MicroScan instruments misidentified 9 isolates of *P. aeruginosa*. Overall misidentification of *E. coli* and *K. pneumoniae* were less than 2% on any platform. MRSA was the least likely to be misidentified by any platform, but when discrepancies occurred, they were identified as other *Staphylococcus* species. In all cases of discrepant identifications, MALDI-TOF, sequencing, and/or results of the submitting laboratory agreed with the majority decision, further supporting our conclusion that the outlier instrument is incorrect. Overall, Vitek-2 has the highest identification accuracy rate among all MDROs tested.

3.2. Antimicrobial susceptibility

Conflicting AST results among platforms were classified into mD, MD, or VMD (defined in Methods). Consistent with other reports, regardless of organism or drug, all instruments produced a significantly higher proportion of mDs than any other type (data not shown) (Kiyosuke et al., 2010; Markelz et al., 2012; Rybak et al., 2013). Overall, MicroScan had the highest number of discrepancies due to frequently reporting a 2-fold higher MIC yielding a categorical call of I or R relative to S or I on the other platforms, respectively. Full antibiograms or organisms with their respective RIS combinations can be found as supplemental files.

3.3. Gram-negative organisms

Occurrences of MD and VMD for *A. baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli* MDRO isolates were summed (Table S1) and tallied by specific organism and drug (Table 2). All instrument/drug/organism combinations performed at or better than the manufacturer's specified error rate with exception to 2 notable combinations. The first exception was *E. coli* tested on the MicroScan against azteronam, which resulted in 101/1322 isolates reporting an MIC corresponding to resistance compared to a sensitive MIC interpretation on the other platforms (Table 2). To ensure the discrepancy rate was above 5%, samples were repeated, and an overall error rate of 7.95% was calculated.