Evaluation of Vitek®2 performance for colistin susceptibility testing for Gram-negative isolates

Surbhi Khurana1, Rajesh Malhotra2 and Purva Mathur1*

1Department of Laboratory of Medicine, JPNA Trauma Centre, All India Institute of Medical Sciences, New Delhi, India; 2Department of Orthopedics, JPNA Trauma Centre, All India Institute of Medical Sciences, New Delhi, India

*Corresponding author. E-mail: purvamathur@yahoo.co.in

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Background: The emerging resistance to the last-resort antimicrobial colistin is being reported globally. Underestimation of the burden of colistin resistance and misinterpretation of colistin susceptibility test results, using suboptimal testing methods, may be causing unexplained treatment failures and even mortality among critically ill patients. Thus, this study was conducted at an apex trauma centre to assess the performance of Vitek®2 for colistin susceptibility testing.

Methods: A total of 910 clinical isolates of Gram-negative bacteria (GNB), including Enterobacterales, Acinetobacter baumannii and Pseudomonas aeruginosa, were tested and analysed for colistin resistance using Vitek®2. Broth microdilution (BMD) was taken as the reference method. The essential (EA) and categorical (CA) agreements and very major error (VME) and major error (ME) rates were calculated. An MIC correlation was taken to be positive with EA>C21 90%, CA>C21 90%, VME<C20 1.5% and ME<C20 3.0% rates. Spearman’s coefficient was calculated and P<0.05 was considered statistically significant.

Results: A total of 64% of isolates were MDR. Overall, 196 (21.5%) and 110 (12%) of isolates were resistant to colistin by BMD and Vitek®2, respectively. The automated Vitek®2 method failed to detect the resistance in up to 48.5% of GNB tested. When comparing Vitek®2 colistin interpretive results with reference BMD for all 910 isolates, the CA was 88% (798/910) with 10% (95/910) VMEs and 1% (9/910) MEs.

Conclusions: The Vitek®2 method for colistin susceptibility testing, still in use in some settings; is a suboptimal and unreliable method.

Introduction

Polymyxins (polymyxin B and colistin), penta-cationic antibiotics that selectively bind to the LPS of Gram-negative bacteria (GNB), were introduced in the late 1950s. Resistance to these last-resort antimicrobials is facilitated by a cationic modification of LPS, impermeability and other efflux mechanisms. As well as the intrinsically resistant organisms such as Morganella spp., Proteus spp. and Providencia spp., acquired resistance to polymyxins can be both plasmid-mediated and chromosomal. While chromosomal resistance is attributable to the cross transmission of resistant isolates and to the previous use of colistin, plasmid-mediated resistance by the mcr gene was first described in China and it is now being reported all over the world.1

In 2016, CLSI and EUCAST jointly recommended only the broth microdilution (BMD) methodology to perform the colistin susceptibility tests.2,3 It is now well established that disc diffusion methodology is unreliable to detect colistin resistance.4–7 Error rates up to 41.5% have been reported for colistin Etests, which now are not recommended as a testing method.5–9

Automated systems have become the backbone of diagnostic microbiology labs even in developing countries. In smaller labs, it is difficult to ensure quality in disc diffusion and BMD. There is also a scarcity of trained technical staff; all of these drive the use of semi- or fully automated identification/antimicrobial susceptibility testing (ID/AST) systems. A very recent study reported that with a very major error (VME) rate of 36% for colistin testing, Vitek®2 may not be that reliable.10 This study mainly evaluated Gram-negative pathogens of family Enterobacterales. In recent times, non-fermenters such as Acinetobacter spp. and Pseudomonas spp. have become the most common pathogens causing healthcare-associated infections at many centres.11–13 The increasing use of
colistin for treatment of suspected sepsis in ICUs makes it necessary to evaluate the automated methods for testing and reporting colistin susceptibility, which is essential for any successful antimicrobial stewardship programme.16

Due to the lack of trained personnel in India, BMD is not an attractive option for AST. Automated methods such as Vitek®2 that provide more objective results and are less prone to operator error are preferred.

The aim of this study was to evaluate the performance of Vitek®2 to detect colistin resistance and to determine the prevalence of colistin resistance among GNB isolated from diagnostic specimens from January to August 2019.

Methods

This prospective study was conducted at the Microbiology Laboratory of the JPN Trauma Centre of the All India Institute of Medical Sciences, New Delhi, India. We tested and analysed a total of 910 sequential, non-duplicate GNB isolates collected from various clinical specimens of patients admitted to our centre from January to August 2019. The isolates were identified using the Vitek®2 automated system (Vitek®2 GN-card). The intrinsically colistin-resistant isolates—Morganella morgani, Proteus mirabilis, Proteus penneri, Proteus vulgaris, Providencia rettgeri, Providencia stuartii, Serratia marcescens and Burkholderia cepacia—were excluded from the study. Colistin MICs (range: 0.50–16 mg/L) were determined using the commercial Vitek®2 AST system (Vitek®2 AST-N280 for lactose fermenters) and AST-N281 (for non-lactose fermenters) (bioMérieux, Marcy-l’Etoile, France) as per the manufacturer’s instructions.

The colistin MICs were also determined using the reference BMD method (MIC range: 0.25–16 mg/L) colistin sulfate salt (Sigma, St. Louis, MO, USA) dissolved in CAMHB (BD, Franklin Lakes, NJ, USA), and according to CLSI recommendations in untreated 96-well polystyrene microplates (Greiner, Frickenhausen, Germany).15

EUCAST MIC breakpoints of > 2 mg/L for resistance and ≤ 2 mg/L for susceptibility were used for Enterobacteriales, Pseudomonas aeruginosa and Acinetobacter baumannii.2 The same breakpoints were used for all the other organisms (including Moraxella group, Bordetella hinzii, Comamonas testosteroni, Myroides spp., Sphingomonas paucimobilis) tested in this study, since currently no CLSI and EUCAST MIC-interpretation criteria for defining susceptibility are available.

The MICs for mcr-1-positive Escherichia coli NCTC 13846 (range: 2–8 mg/L), E. coli ATCC® 25922 (range: 0.25–2 mg/L) and P. aeruginosa ATCC® 27853 (range: 0.5–4 mg/L) and a clinical isolate of P. mirabilis (colistin MIC >16 mg/L) were used as quality-control strains for each run of the colistin MIC tests. Each isolate was tested in duplicate, and for all discordant results repeat testing was performed. All resistant strains were tested twice by both the methods. Sensitivity, specificity and positive and negative predictive values (PPV and NPV) were determined, since the prevalence of colistin resistance impacts the PPV and NPV of tests. To assess performance of Vitek®2 as compared with BMD for MIC testing of colistin, essential agreement (EA) and categorical agreement (CA) were evaluated. EA was defined as the percentage of Vitek®2 MIC results that were within ± 1 log₂ dilution of reference BMD MIC results. CA was the percentage of Vitek®2 interpretive results (susceptible or resistant) that agreed with reference BMD interpretive results.

Categorical disagreements were classified as VMEs and major errors (MEs). A VME for Vitek®2 was defined as a colistin-susceptible isolate determined using BMD interpreted as a colistin-resistant isolate (false susceptibility result). VME rates were calculated using the number of isolates resistant by BMD as the denominator. An ME for Vitek®2 was defined as a colistin-resistant isolate determined using BMD interpreted as a colistin-susceptible isolate (false resistant result). ME rates were calculated using the number of isolates susceptible by BMD as the denominator.

Acceptable agreement for Vitek®2 compared with BMD was defined as EA ≥ 90%, CA ≥ 90%, VME ≤ 1.5% and ME ≤ 3% as described by CLSI.16 Spearman’s coefficient was calculated to determine the concordance of Vitek®2 MICs with those of BMD. A P value < 0.05 was considered statistically significant. Since the clinical isolates included in the study were sent for routine AST testing to the laboratory, no ethical clearance was obtained for this study.

Results

The clinical isolates (n = 910) belonging to order Enterobacterales included Klebsiella pneumoniae (n = 245), E. coli (n = 158), Enterobacter cloaceae (n = 42), Citrobacter freundii (n = 8), Salmonella Typhi (n = 7), Raoultella planticola (n = 4), Cronobacter spp. (n = 2) and Pantoea spp. (n = 2). The non-Enterobacterales isolates included A. baumannii (n = 273), P. aeruginosa (n = 139), Aeromonas hydrophila (n = 10), Stenotrophomonas maltophilia (n = 9), S. paucimobilis (n = 5), B. hinzii (n = 2), Moraxella group (n = 2), C. testosteroni (n = 1) and Myroides spp. (n = 1).

Pus and wound swab (n = 322, 35%) were the most common source of isolates, followed by endotracheal aspirates (n = 119, 13%), blood (n = 183, 20%), bronchoalveolar lavage fluid (n = 89, 10%), sterile body fluids (n = 60, 7%), tissue (n = 52, 6%), urine (n = 49, 5%), pleural fluid (n = 27, 3%), and miscellaneous samples (n = 9, 1%).

The sensitivity of Vitek®2 compared with BMD ranged from 12.5% to 72%, and specificity was ≥ 94% (Table 1). The PPV ranged from 83% to 100% while NPV ranged from 10% to 95.5%. Despite 100% specificity of Vitek®2 for isolates for which no colistin breakpoints are available (A. hydrophila, S. maltophilia and other non-fermenters), the sensitivity was very low (0%–33%).

The correlation with reference MICs was poor for Vitek®2 and a 45-degree correlation could not be obtained. Vitek®2 tended to underestimate MICs for resistant isolates (Figure 1).

The performance of Vitek®2 and reference BMD to determine colistin MICs is presented in Table 2. Generally, for all the isolates tested, the BMD MIC values were higher than those obtained by Vitek®2. Figure 1 shows the correlation between both the tests for all Gram-negative bacterial isolates studied (n = 910), Enterobacterales (n = 468), A. baumannii and P. aeruginosa (n = 139).

Overall, 714 (78.5%) and 800 (88%) of isolates were colistin susceptible by BMD and Vitek®2, respectively. The EA was found to be highest in E. coli (89%) followed by A. baumannii (88%). This could be because there were only 8158 resistant isolates of E. coli. Poor CA (≤ 90%) was found in all the isolates except for E. coli (among Enterobacterales), A. baumannii and P. aeruginosa (non-Enterobacterales). Very high VME (28%–100%) and up to 6% ME were observed among all the tested isolates. Except for K. pneumoniae, A. hydrophila and S. maltophilia, strong MIC correlation (Spearman’s ρ > 0.8) was not seen for any of the isolates.

Discussion

As the burden of acquired resistance to colistin is rising, accurate detection and reporting is essential to roll out a diagnostic stewardship programme, especially for counties like India.17

The semi-automated Vitek®2 has been reported as a reliable colistin testing method.5,6,8,18 However, we found that the
Table 1. Sensitivity, specificity, and predictive values of detecting colistin resistance using Vitek®2 with BMD as reference method

| Organism (n)                        | Vitek®2 | BMD | Sensitivity (false S) | Specificity (false R) | PPV | NPV |
|-------------------------------------|---------|-----|-----------------------|-----------------------|-----|-----|
| Enterobacterales (n = 468)          |         |     |                       |                       |     |     |
| *K. pneumoniae* R 73 5             | 72%     | 96.5% | 94% | 83% |
| S 29 138                           |         |     |                       |                       |     |     |
| *E. coli* R 1 0                    | 12.5%   | 100% | 100% | 95.5% |
| S 7 150                            |         |     |                       |                       |     |     |
| *E. cloacae* R 6 0                  | 35%     | 100% | 100% | 69% |
| S 11 25                            |         |     |                       |                       |     |     |
| others<sup>a</sup> R 0 1           | 0%      | 94%  | 0                | 77% |
| S 5 17                             |         |     |                       |                       |     |     |
| Non-Enterobacterales (n = 442)     |         |     |                       |                       |     |     |
| *A. baumannii* R 15 3              | 47%     | 99%  | 83% | 93% |
| S 17 238                           |         |     |                       |                       |     |     |
| *P. aeruginosa* R 5 0               | 42%     | 100% | 100% | 95% |
| S 7 127                            |         |     |                       |                       |     |     |
| *A. hydrophila* R 0 0               | 0%      | 100% | NA             | 10% |
| S 9 1                              |         |     |                       |                       |     |     |
| *S. maltophilia* R 0 0              | 0%      | 100% | NA            | 10% |
| S 8 1                              |         |     |                       |                       |     |     |
| others<sup>b</sup> R 1 0           | 33%     | 100% | 100% | 80% |
| S 2 8                              |         |     |                       |                       |     |     |

R, resistant; S, susceptible; NA, not applicable.

<sup>a</sup>Citrobacter spp. (n = 8), Cronobacter spp. (n = 2), Pantoea spp. (n = 2), *R. planticola* (n = 4), *Salmonella Typhi* (n = 7).

<sup>b</sup>*B. hinzii* (n = 2), *C. testosteroni* (n = 1), *Moraxella* group (n = 2), *Myroides* spp. (n = 1), *S. paucimobilis* (n = 5).

Figure 1. Correlation between Vitek®2 and reference BMD for (a) all Gram-negative bacterial isolates studied (n = 910), (b) Enterobacterales (n = 468), (c) *A. baumannii* (n = 273) and (d) *P. aeruginosa* (n = 139). MICs within EA (within ± 1 dilution of reference MICs) are shaded and MICs identical to reference MICs are within boxes. EUCAST breakpoints (resistant > 2 mg/L) are shown as lines.
Table 2. Performance characteristics of the reference BMD method and Vitek®2

| Organism (n)                      | Method | R    | S    | EA   | CA   | VME  | ME      | Spearman’s coefficient |
|----------------------------------|--------|------|------|------|------|------|---------|------------------------|
| Enterobacterales (n = 468)       |        |      |      |      |      |      |         |                        |
| K. pneumoniae (n = 245)          | BMD    | 102  | 143  | 187  | 211  | 29   | 5 (3.5) | p = 0.69596 (P = 0.000)* |
|                                 | Vitek®2| 78   | 167  | 138  | 151  | 7    | 0       |                        |
| E. coli (n = 158)                | BMD    | 8    | 150  | 141  | 151  | 7    | 0       | p = 0.15382 (P = 0.054)  |
|                                 | Vitek®2| 1    | 157  | 154  | 151  | 7    | 0       |                        |
| E. cloacae (n = 42)              | BMD    | 17   | 25   | 29   | 31   | 11   | 0       | p = 0.48858 (P = 0.001)* |
|                                 | Vitek®2| 6    | 36   | 29   | 32   | 9    | 0       |                        |
| othersa (n = 23)                 | BMD    | 5    | 18   | 14   | 9    | 5    | 1 (6)   | p = -0.01672 (P = 0.100) |
|                                 | Vitek®2| 1    | 22   | 9    | 5    | 5    | 0       |                        |
| Non-Enterobacterales (n = 442)   |        |      |      |      |      |      |         |                        |
| A. baumannii (n = 273)           | BMD    | 32   | 241  | 239  | 253  | 17   | 3 (1)   | p = 0.36258 (P = 0.000)* |
|                                 | Vitek®2| 18   | 255  | 239  | 253  | 17   | 3 (1)   |                        |
| P. aeruginosa (n = 139)          | BMD    | 12   | 127  | 109  | 132  | 7    | 0       | p = 0.28633 (P = 0.007)* |
|                                 | Vitek®2| 5    | 134  | 109  | 132  | 7    | 0       |                        |
| A. hydrophila (n = 10)           | BMD    | 9    | 1    | 1    | 1    | 9    | 0       | p = 0.66667 (P = 0.035)  |
|                                 | Vitek®2| 0    | 10   | 1    | 1    | 9    | 0       |                        |
| S. maltophilia (n = 9)           | BMD    | 8    | 1    | 0    | 1    | 8    | 0       | p = 0.7333 (P = 0.023)*  |
|                                 | Vitek®2| 0    | 9    | 0    | 1    | 8    | 0       |                        |
| othersb (n = 11)                 | BMD    | 3    | 8    | 8    | 9    | 2    | 0       | p = 0.45707 (P = 0.158)  |
|                                 | Vitek®2| 1    | 10   | 8    | 9    | 2    | 0       |                        |

Significant differences are highlighted in bold (*P < 0.05).
R, resistant; S, susceptible.
aCitrobacter spp. (n = 8), Cronobacter spp. (n = 2), Pantoea spp. (n = 2), R. planticola (n = 4), Salmonella Typhi (n = 7).
bB. hinzii (n = 2), C. testosteroni (n = 1), Moraxella group (n = 2), Myroides spp. (n = 1), S. paucimobilis (n = 5).

automated Vitek®2 method failed to detect the resistance in 87.5% (n = 7) E. coli, 65% (n = 11) E. cloacae, 58% (n = 7) P. aeruginosa, 53% (n = 17) A. baumannii and 28% (n = 29) K. pneumoniae of the isolates. The acceptable EA ≥ 90% was observed in none of the isolates. Although an acceptable CA (> 90%) among E. coli, A. baumannii and P. aeruginosa was observed, VME rates of 86%, 53% and 58% were also observed, respectively. Despite the strong positive MIC correlation among K. pneumoniae, A. hydrophila, and S. maltophilia, the VME rates were found to be well in excess of the 1.5% rate recommended by the CLSI.

In a recent study, colistin MICs determined by Vitek®2 were reported to be unreliable, especially for E. cloacae and A. baumannii complex isolates. Another study showed that semi-automated systems including Vitek®2 performed poorly, with 31 VMEs. Our study highlights that Vitek®2 is not reliable for colistin susceptibility testing, especially for Enterobacteriales, A. baumannii and P. aeruginosa, for which CLSI and EUCAST MIC interpretation criteria for defining susceptibility have been published.

However, the lower numbers of isolates (and resistant isolates) for some species is a limitation of this study. This often happens when clinical isolates routinely tested are studied, rather than picking specific resistant isolates to study.

It is noteworthy to mention that misinterpreting colistin susceptibility test results may lead to inexplicable treatment failures and even mortality, as isolates identified as susceptible may rather resist antibiotic therapy owing to colistin heteroresistance.17,21–23

Although performing AST methods such as BMD for clinical testing is technically demanding, laboratories need to train their staff to perform BMD and overcome common difficulties including making initial dilutions, multiple skipped wells, contamination, or other quality control problems, none of which are involved in automated systems. Further detection of mcr genes amongst these bacteria would also provide molecular epidemiological data.

Most studies on colistin resistance have included one or a few species of Enterobacterales. This is one of few studies that report colistin resistance amongst a large collection of GNB, for many of which a breakpoint is also not available.

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Transparency declarations
None to declare.
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