Detecting In-Vitro Colistin Resistance- A Comparative Study Between Broth Microdilution Versus Vitek-2 For Colistin Susceptibility Testing

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ABSTRACT

Background: Susceptibility testing for polymyxins is a great challenge for a Clinical Microbiology laboratory. There are several methodological issues associated with MIC (Minimum Inhibitory Concentration) determination of colistin.

Methods: In our study, we have compared the results of colistin susceptibility testing by Automated system (Vitek-2, Biomerieux, France) with the reference Broth Microdilution method (BMD) to identify the type of discrepancies by Vitek-2 method and thus develop a practical and accurate approach for colistin susceptibility testing in a Clinical Microbiology laboratory. A total of 730 strains of Gram negative bacteria [Escherichia coli (325), Klebsiella sp.(346), Acinetobacter baumanii complex (37) and Pseudomonas aeruginosa (22)] from 485 patents were tested simultaneously by BMD and Vitek-2 method for colistin susceptibility testing.

Results: The Essential agreement (EA), Categorical agreement (CA), Very major error (VME) and Major error (ME) rates for Klebsiella sp.were 87.3%, 89.3%, 8% and 2.3% respectively, for Escherichia coli were 88.3%, 89.5%, 9.2% and 1.2% respectively, for Acinetobacter baumanii complex were 89.1%, 91.8%, 8.1% and 0% respectively, for Pseudomonas aeruginosa were 68.1%, 72.7%, 0% and 27.2% respectively.

Conclusions: Colistin susceptibility testing by Vitek-2 method is an easily adoptable method and the results of Vitek-2 with reference to BMD are acceptable to a great extent in Klebsiella sp., Escherichia coli and Acinetobacter baumanii complex. So, we believe that Vitek-2 method may be used for colistin susceptibility testing in low risk patients. However, BMD should be used in high risk immunosuppressed and immunocompromised patients who are admitted in critical care units. For Pseudomonas aeruginosa, BMD should be routinely used.

Keywords: Colistin, Susceptibility, Broth Microdilution, Vitek-2

Introduction

Polymyxins are a group of polypeptide antibiotics that were first isolated in 1947 from a spore-bearing soil bacillus (Bacillus polymyxa). There are several chemically different polymyxins which have been isolated from different strains of this bacillus and these are Polymyxin A to Polymyxin E. But, only polymyxin B and polymyxin E (colistin) have been used clinically. Polymyxin B and colistin differs from each other only by a single aminoacid in the peptide ring. The antibacterial spectrum of Polymyxins is mainly against Gram negative organisms viz Escherichia coli, Klebsiella sp., Enterobacter sp., Citrobacter sp., Acinetobacter sp. and Pseudomonas aeruginosa. Amongst Enterobacteriaceae, the members of Tribe Proteae (Proteus, Providencia, Morganella) and Serratia sp. are inherently resistant to Polymyxins.[1]

With the increase in multidrug resistance amongst Gram negative bacilli, Colistin has become the last resort for the treatment of infections caused by these microorganisms, particularly carbapenem-resistant Gram negative bacteria. Colistin is administered as an inactive prodrug, colistin methanesulfonate (colistimethate). In aqueous media and biological fluids, this prodrug is converted into colistin and several inactive methanesulfonated compounds.

Susceptibility testing for polymyxins is a great challenge for a clinical laboratory because of challenges in testing of polymyxins which include less diffusion of polymyxins into agar, inherent cationic properties of polymyxins, the occurrence of heteroresistance to polymyxins in many species, and lack of a reliable reference method that may allow reliable comparisons of commercial tests.[2] Because of several methodological issues associated with MIC testing of colistin, CLSI-EUCAST joint Polymyxin Breakpoints Working Group recommends use of Broth microdilution (BMD) method for susceptibility testing of colistin.[3] However, BMD method has not been adaptable
for a clinical Microbiology laboratory because it is manual and quite labor intensive.

The other methods for susceptibility testing of polymyxins include Disk diffusion method, E-test method, and automated methods (Vitek-2, Phoenix, Microscan etc). Although both Disk diffusion method and E-test method are less labor intensive and easy to perform but, both of them are associated with false susceptibility results when compared with BMD method.[4,5] Vitek-2 method has also been found to have low sensitivity in detecting colistin resistance in Gram negative organisms.[5,6] The studies pertaining to evaluation of other automated systems like Phoenix, Microscan and Sensititre systems with respect to BMD method for colistin susceptibility are scarce.[5]

We at our tertiary health care set-up are using Vitek-2 for antimicrobial susceptibility testing. So, keeping the pros and cons of automated systems especially with respect to colistin susceptibility testing in mind, we planned to compare the results of colistin susceptibility testing by Vitek-2 method with the reference Broth microdilution method to establish a practical and accurate approach for colistin susceptibility testing in a Clinical Microbiology laboratory.

**Material and Methods**

This study was done on 730 clinically significant strains of Gram-negative bacteria isolated from clinical specimens of the patients in a tertiary health care set-up between August 2018 to December 2018. The clinical specimens included Blood, Pus/Tissue, Body fluids, Respiratory specimens and Urine. These isolates were derived from both in-patients and out-patients. These patients comprised of mixed population of immunocompetent, immunosuppressed/immunocompromised, critically ill patients. The Gram negative bacteria included *Escherichia coli* (325), *Klebsiella* sp. (346), *Acinetobacter baumanii* complex (37) and *Pseudomonas aeruginosa* (22). These 730 strains were the non-repeat isolates from the same sample of 485 patients. The determination of MIC value of colistin by Vitek-2 method was done as per the manufacturer instructions.

A. BMD procedure was done as per CLSI guidelines.[7,8] Stock solution of colistin in concentration of 5120 μg/ml was prepared with every batch of test and Broth Microdilution plates were prepared with different concentrations of colistin solution. Bacterial suspension was prepared in the concentration of 5 x 10⁵ CFU/ml and inoculated in microbroth plate. The last two wells of each row of microbroth plate acted as Growth control and Sterility control. Growth control well contained only adjusted bacterial suspension and Sterility control well contained only Cation adjusted Mueller Hinton Broth (CAMHB) which was used to prepare various dilutions. In every batch Quality control strains were used as control. The controls used were *Escherichia coli* ATCC35218, *Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27853. The microbroth plate was then incubated at 37°C for 16-18 hrs. Reading of the test strains was taken only after satisfactory reading of the control strains. The minimum concentration of colistin which inhibits the visible growth of the bacteria was taken as its MIC. Interpretation of the results was done as per CLSI guidelines 2018. Based on epidemiological cut-off value for *Enterobacteriaceae*, *Klebsiella* sp. and *Escherichia coli* were considered as Sensitive if MIC value was <=2 μg/ml and as Resistant if MIC value was >=4 μg/ml. For *Pseudomonas aeruginosa* and *Acinetobacter* sp. MIC value of <=2 μg/ml was interpreted as Sensitive and MIC value of >=4 μg/ml was interpreted as Resistant.[7]

B. Comparison between Vitek-2 and BMD results: BMD was considered as gold standard and the MIC values obtained by Vitek-2 was compared with BMD Essential Agreement (EA), Categorical Agreement (CA), Very Major Error rate (VME) and Major Error rates (ME) were calculated. If the MIC of the isolates by Vitek-2 were within +/- one doubling dilution in comparison to BMD, then the two methods were considered to be in essential agreement for that isolate. Those isolates which fall in the same category of interpretation were considered to be in Categorical agreement. If the isolate was resistant by BMD and susceptible by Vitek-2, it was considered as very major error. If the isolate was susceptible by BMD but resistant by Vitek-2 it was considered as Major error. EA, CA, VME rate and ME rate were calculated as percentage.[9]

Acceptable performance between the two methods was evaluated according to criteria established by the International Organization for Standardization: >90% for essential or category agreement and <3% for VME or ME.[10]
C. Re-confirmation of discrepant results: Re-confirmation of the discrepant results between Vitek-2 and BMD method (Both VME and ME) was done by repeat testing.

Results

A total of 730 Gram negative bacteria isolated from various clinical specimens were studied for colistin susceptibility by Broth microdilution method and Vitek-2 method.

Out of 730 Gram negative bacteria, 346 strains were *Klebsiella pneumoniae*, 325 strains were *Escherichia coli*, 22 strains were *Pseudomonas aeruginosa* and 37 strains of *Acinetobacter baumannii* complex.

**Colistin Resistance:** The overall resistance to Colistin amongst Gram negative bacilli was found to be 19.17% (140/730) by gold standard BMD method. For *Enterobacteriaceae* (Escherichia coli and *Klebsiella* sp.), the resistance to colistin was found to be 19.5% (131/671) and for Non fermenters (*Acinetobacter* sp. and *Pseudomonas aeruginosa*), the resistance to colistin was found to be 15.2% (09/59).

The detailed results of these isolates are presented below:

**Escherichia coli** The total number of *Escherichia coli* strains which were sensitive to colistin by Vitek-2 method was 315 and by BMD were 288. Out of 10 strains which were found to be resistant by Vitek-2 method, six strains were resistant by BMD method also, but four of these resistant strains were found to be sensitive by BMD. Out of 35 strains detected resistant by MBD, six were also detected resistant by Vitek-2 method. Vitek-2 method failed to detect resistance in 29 strains of *Escherichia coli*.

**Klebsiella sp.:** The total number of *Klebsiella* sp. strains which were sensitive to colistin by Vitek-2 method was 273 and by BMD were 252. Out of 73 strains which were found to be resistant by Vitek-2 method, 66 were found to be resistant by BMD method also i.e. seven strains which were detected as resistant by Vitek-2 method were found to be sensitive by MBD method. Out of 94 strains detected as resistant by BMD method, 66 were also detected by Vitek-2 method i.e. Vitek-2 failed to detect resistance in 28 strains of *Klebsiella* sp..

**Acinetobacter sp.**

The total number of *Acinetobacter* strains which were found to be sensitive to colistin by Vitek-2 method was 34 and by BMD was 31. The three strains detected as resistant by Vitek-2 method were also detected as resistant by BMD method. In comparison to BMD method, Vitek-2 method failed to detect colistin resistance in three cases.

**Pseudomonas aeruginosa**

The total number of *Pseudomonas aeruginosa* strains which were found to be sensitive to colistin by Vitek-2 method was 13 and by BMD method were 19. However, six strains detected resistant by Vitek-2 method were found to be sensitive by BMD method.

The performance of Vitek-2 method with respect to BMD method is shown in table-1.

Discussion

Colistin is the mainstay of treatment in patients with Carbapenem resistant Gram negative bacteria infections. However, there are controversies in susceptibility test results using different methods. In the present study, a comparison between automated and user friendly Vitek-2 method and gold standard BMD method for colistin have been done in a clinical microbiology laboratory. Atol of 730 Gram negative bacteria routinely isolated from various clinical specimens were tested for colistin susceptibility by Microbroth dilution method and Vitek-2 methods. A discrepancy between two test methods was found to be 8.0%, 9.5%, 8.1%, 27.27% for *Klebsiella* sp., *Escherichia coli*, *Acinetobacter* sp. and *Pseudomonas aeruginosa* respectively. The discrepancy for *Pseudomonas aeruginosa* is apparently more because of the lower number of the isolates considered in the study. But, the important thing to note for *Pseudomonas aeruginosa* is that MBD detected *Pseudomonas aeruginosa* strains as colistin sensitive, however, these strains were detected as colistin resistant by Vitek-2 method. This is in contrast to *Klebsiella* sp., *Escherichia coli* and *Acinetobacter* sp. which were more detected as colistin resistant by BMD method than Vitek-2 method.

Table 1: Comparative between Vitek-2 and BMD method results for Colistin susceptibility testing in major Gram negative bacteria isolated from clinical specimens.

| Gram negative bacteria          | Number of strains tested | EA    | CA   | VME | ME  |
|--------------------------------|--------------------------|-------|------|-----|-----|
| *Klebsiella* sp.               | 346                      | 87.3% | 89.3%| 8%  | 2.3%|
| *Escherichia coli*             | 325                      | 88.3% | 89.5%| 9.2%| 1.2%|
| *Acinetobacter baumannii complex* | 37                      | 89.1% | 91.8%| 8.1%| 0%  |
| *Pseudomonas aeruginosa*       | 22                       | 68.1% | 72.7%| 0%  | 27.2%|

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In our study, the categorical agreement of Vitek-2 for Acinetobacter baumannii complex was acceptable and that of Escherichia coli and Klebsiella pneumoniae was marginally acceptable. However, Vitek-2 was not in CA agreement with BMD in case of Pseudomonas aeruginosa. The Categorical and Essential disagreement in Pseudomonas aeruginosa was because of major errors and not due to very major errors. This disagreement in Pseudomonas aeruginosa may be because of the lesser number of Pseudomonas aeruginosa strains considered in our study. In our study, for Acinetobacter baumannii complex, EA/CA was found to be 89.1%/91.8% and VME/ME rates were 8.1%/0%. Vouli S et al in their study found the EA/CA between Vitek-2 and BMD to be 88.9%/89.7% in carbapenem resistant Acinetobacter baumannii clinical isolates.[11] In a study by Bakthavatchalam YD et al, the EA, CA, VME rate and ME rate was found to be 69%, 93%, 8% and 0% respectively for Klebsiella pneumoniae and 81%, 97%, 3% and 0% respectively for Acinetobacter baumannii.[12] Dafopoulou K et al found 75.6% EA and 100% CA with nil ME and VME rates in Klebsiella pneumoniae isolates. The EA, CA, VME, ME rates were found to be 85%, 90%, 0% and 10% respectively in Acinetobacter baumannii. The isolates in their study were mainly colistin resistant.[13]

Colistin gradient diffusion tests (E-tests and MIC strip tests) have also not been found to be suitable for the measurement of colistin MIC in clinical isolates in various studies.[12,13,14]

It is also important to test all the MDR isolates by BMD method of Colistin susceptibility testing because false sensitive or false resistant would put the patient on inappropriate antibiotics. We have considered both carbapenem susceptible (non MDR) and non-susceptible (MDR) bacterial strains in our study and colistin susceptibility testing by BMD was performed simultaneously with Vitek-2 method on the same day of isolation as a routine susceptibility testing method for colistin. This is in contrast to other studies where the authors have done the susceptibility testing on carbapenem non susceptible (MDR) stocked strains which were revived at the time of testing whereby changes can occur due to subcultures and further population diversity.[13]

Conclusions
In our study, colistin susceptibility testing was done simultaneously with Vitek-2 and BMD method and the agreement between Vitek-2 results and BMD was marginally acceptable for Escherichia coli and Klebsiella pneumoniae and acceptable in Acinetobacter baumannii complex. In a tertiary health care facility, colistin is used both empirically and therapeutically because of the type of patient population who are generally referred cases from primary/secondary health care facilities and already on high-end antibiotics or immunosuppressed or post-transplant or malignancy patients who are on antimicrobial prophylaxis or treatment. In these patients there are chances of isolation of multidrug resistant bacteria where colistin is the only drug of choice therapeutically. In immunosuppressed or immunocompromised patients, colistin susceptibility testing should be carried out and interpreted routinely using gold standard microbroth dilution method for deciding the optimum choice of drug for all the indicated organisms except Pseudomonas aeruginosa which requires further large scale testing. Based on the acceptable agreement between Vitek-2 and BMD method in our study, colistin susceptibility testing done by Vitek-2 method in low risk patients is acceptable.

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