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Original Research Article

Comparative Evaluation of Methods to Detect Minimum Inhibitory Concentration of Colistin among Multidrug Resistant Gram-Negative Bacilli in a Tertiary Care Hospital in Hyderabad

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Abstract:

Aim: The aim of this study is to assess the techniques used to determine the minimum inhibitory concentration of colistin in multidrug-resistant Gram-negative bacteria.

Methods: In a teaching hospital for tertiary care, a comparative study was conducted. Using the colistin agar dilution method and the E test method, 150 non-repetitive MDR-GNB were found and their colistin MIC was determined. The gold standard was the broth microdilution method.

Results: Eleven isolates out of 150 MDR-GNB isolates demonstrated colistin resistance using the BMD and Colistin agar dilution methods. In contrast, two of the BMD-resistant isolates had intermediate sensitivity, or a MIC of 2µg/dl by the E test, which led to a significant mistake.

Conclusions: Although the E test is simple to use, it has revealed VME in two isolates and is more expensive than agar dilution. Agar dilution demonstrated 100% categorical agreement, and with technician training, it can be used in laboratories with limited resources to evaluate several samples on a single plate.

Introduction

Globally, antimicrobial resistance (AMR) is acknowledged as a significant and expanding hazard to public health. According to the World Health Organization, AMR is among the top ten worldwide public health problems that humanity is now confronting.[1] Living in the "era of antibiotic resistance," doctors have been using "forgotten" antibiotics like Colistin again in the past ten years. Colistin is a polymyxin that has bactericidal activity and broad spectrum of activity against the majority of strains of Pseudomonas aeruginosa, Acinetobacter species, and Enterobacteriaceae.[2] A variety of nosocomial illnesses have been treated using colonist in. Gram-negative bacteria include Acinetobacter species, Pseudomonas aeruginosa, Escherichia coli, and Klebsiella species have developed colistin resistance. [3] Since there is currently a pressing need in clinical laboratories to ascertain the susceptibility of isolates to polymyxins, the scientific community has been motivated to create quick and accurate techniques in response to the recent advent of MDR Gram-negative bacteria and the ensuing rise in the usage of colistin. [4] The current study aims to compare the MIC of colistin sulphate using the reference method of broth microdilution and the E test and agar dilution. Over the course of 18 months, from April 2021 to October 2022, this cross-sectional study was carried out in the Department of Microbiology at the Niloufer Hospital for Women and Children in Hyderabad. The investigation comprised clinical samples that were obtained in the microbiology lab, including blood, urine, pus, sputum, and tracheal secretions. Ethical approval was given by the Institutional Ethical Committee (IEC). This study included multidrug-resistant Gram-negative bacteria (MDR-GNB) and eliminated species that were intrinsically resistant to colistin. In accordance with routine microbiolog-

ical protocol, samples were processed and identified down to the species level using conventional methods. [5, 6, 7, 8] In accordance with CLSI standards, the Kirby-Bauer disc diffusion method was used to screen for antibiotic susceptibility. [9] Using the disc diffusion method, bacterial isolates that demonstrated resistance to more than three antibiotic classes were classified as multidrug resistant. [9,11] The study contained 150 consecutive nonrepeating Gram-negative isolates that were resistant to multiple drugs. According to the CLSI guidance, the BMD technique was used to evaluate colistin resistance. [9] The colistin sulphate salt SIGMA (HI Media, India) was dissolved in Cation Adjusted Muller-Hinton broth to produce the minimum inhibitory concentration (MIC), which ranged from 0.5 µg/mL to 16 µg/mL. The CLSI MIC breakpoint for colistin is ≥4 µg/mL for resistance and ≤2 µg/mL for intermediate. [12] Mueller Hinton agar (MHA) was made for the Colistin agar test using colistin sulphate salt at concentrations ranging from 0 to 16 µg/mL in eight distinct petri plates. Each MHA plate was marked and separated into an equal number of sections or segments based on the number of tests that needed to be performed.

An inoculum containing 0.5 McFarland solution of bacterial colony was made in regular saline and diluted 1:10 in saline. After streaking 10 µL of the diluted solution onto a designated region, it was incubated for 24 hours at 37°C. The CLSI criteria were followed while interpreting the results. [9]

Data Analysis: The BMD method was utilised as a reference method for colistin resistance, and the data was recorded using Microsoft Excel. The tests' performance was assessed using the following metrics: sensitivity, specificity, negative and positive predictive values, essential and categorical agreement, major and very major mistakes, and so on.

Results:

150 MDR-GNB in all were examined. Urine made up 60% of the samples, followed by pus samples (20.7%), blood (13.3%), and miscellaneous samples (5%).

Table 1:

Sample (n=150)	Escherichia Coli (n=77)	Klebsiella Species (n=55)	Pseudomonas aeruginosa (n=10)	Acinetobacter baumannii (n=6)	Stenotrophomonas maltophilia (n=2)
Urine (n=90)	**55**	**34**	**1**	**-**	**-**
Blood (n=20)	**4**	**8**	**5**	**3**	**-**
Pus (n=31)	**13**	**12**	**3**	**2**	**1**
Stool (n=5)	**5**	**-**	**-**	**-**	**-**
Sputum (n=2)	**-**	**1**	**1**	**-**	**-**
Pleural Fluid (n=1)	**-**	**-**	**-**	**-**	**1**
ET-Aspirate (n=1)	**-**	**-**	**-**	**1**	**-**

All isolates were tested using the BMD technique, and their MICs were recorded in order to detect colistin resistance.

Eleven (7.3%) of the 150 MDR-GNB isolates that were found were colistin-resistant. Five of the eleven isolates were Klebsiella species, followed by four isolates of Escherichia coli and two isolates of Acinetobacter baumannii. The BMD method (gold standard), the colistin Agar test, and the E test method were used to assess the colistin resistance of all MDR-GNB.

Eleven (7.30%) colistin-resistant organisms were found using the BMD and Agar dilution methods, however the E test method only found colistin resistance in nine isolates and missed two organisms, producing a false-negative result. The agar dilution test revealed that every isolate that was resistant to BMD was also resistant.

Table 2: Comparison of colistin susceptibility

S.No	Method	Intermediate	Resistant
1	**Broth microdilution**	**139**	**11**
2	**Agar dilution**	**139**	**11**
3	**E test**	**141**	**9**

Only The Intermediate And Resistant Colistin Categories Are Available In CLSI.

Sensitivity, specificity, positive and negative predictive values, and essential agreement (EA), which is the proportion of isolates with MIC values within a single two-fold dilution of the reference standard, were used to evaluate the diagnostic efficacy of the Agar dilution and E test method with the BMD method (gold standard). The percentage of isolates having results in the same Intermediate/Resistant category as the reference standard is known as categorical agreement (CA). When an isolate is resistant by the test technique displaying false re-

sistance but intermediate by the reference method, this is known as a major error (ME).

When an isolate is intermediate by the test technique but resistant by the reference method, this is known as a very large error (VME). A significant association between the approaches was implied by the significant p value of <0.05 for the Agar dilution MIC compared to the BMD MIC. There was no association in the MIC values, as indicated by the p value of >0.05 for the E test MIC compared to the BMD MIC.

While the E test revealed 98% categorical agreement, the Agar dilution demonstrated 100% categorical agreement. The E test and agar dilution had essential agreement of 41.7% and 84.7%, respectively. For two isolates, the E test revealed a very significant inaccuracy.

Table 3:

Accuracy	Essential Agreement(EA)	Categorical Agreement(CA)	Major Error(ME)	Very Major Error(VME)
AGAR Dilution MIC	84.7% (127)	100%	-	-
E Test MIC	41.3% (62)	98.7%	-	1.3%(n=2)

Multidrug-resistant (MDR) Gram-negative bacteria (GNB) have caused a rise in nosocomial infections globally during the past ten years, particularly in intensive care units. [13] Similar to research conducted by Punyatoya Kar et al. in 2021, when urine samples accounted for 51% of MDR isolates, the bulk of MDR isolates in this investigation came from urine samples. [14] Escherichia coli was the most common bacteria isolated in this investigation. Since there were the most pee samples in this investigation, Escherichia coli was the most often isolated pathogen.

The best metric currently available to show how well an antibiotic works against bacterial strains is the MIC value. The efficiency of antibiotic therapy can be greatly increased by using the MIC value in treatment, which is based on specifically set PK parameters. [15] 76.2% of isolates in this investigation had a MIC of less than 0.5 µg/ml, which is comparable to the findings of other studies conducted by Shubham Chauhan et al. (2022) and Gupta et al. (2020). [16,17]

In the current investigation there was 100% categorical agreement between Agar dilution and BMD. Other research revealed categorical agreement of 90.5% by Punyatoya Kar et al. in 2021 [14], 78.5% by L. Singhal et al. in 2018, and 92% by Janet et al. in 2013 [18]. [18] Agata Turlej-Rogacka et al.'s study also revealed that the agar dilution approach outperformed the currently advised broth dilution method in terms of reproducibility, robustness, and usability [20].

Similar to prior investigations conducted by LA Arroyo et al., Gupta et al., and Punyatoya Kar et al., which showed 98.2%, 97%, and 95% categorical agreement, the current study's E test approach demonstrated 98.7% categorical agreement [14,16,21]. Although the E test is simple to conduct, two bacterial isolates in the current investigation displayed VME. In 2021, Surojit Das et al. and Punyatoya Kar et al. reported VMEs of 23.3% and 37%, respectively, whereas investigations by LA Arroyo et al., Janet A. Hindler et al., and Gupta et al. reported VMEs of 1.7%, 1%, and 2%, respectively [14,22].

Conclusion

7.3% of the study's multidrug-resistant clinical isolates had colistin resistance, according to the reference BMD technique. Colistin is a last-resort medication that should be kept and used sparingly following antibiotic stewardship and antibiotic susceptibility testing because there are currently no new antibiotics being developed to combat Gram-negative pathogens.

Since colistin has a limited therapeutic window, neurotoxicity and nephrotoxicity are the main side effects associated with its parenteral use, and it falls into the reserve category of antibiotics according to the AWaRe classification, the treating clinician must fill out a justification form before beginning antibiotic therapy. Since even GNBs that do not fall within the MDR category exhibit colistin resistance, it is necessary to monitor colistin resistance in all Gram-negative bacteria. 100% CA and 84.7% EA without ME or VME were observed in the agar dilution. Multiple isolates can be evaluated on a single plate, and minimal training is needed. Despite its ease of use, the E test is more expensive than the BMD and AD tests. Additionally, it displays a very significant inaccuracy of 1.3%; hence, BMD should validate the E test result. Thus, with technician training, the Agar dilution method can be implemented in labs with little resources.

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