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COMPARATIVE EVALUATION OF AGAR DILUTION, BROTH MICRODILUTION, AND VITEK-2 METHODS FOR DETECTION OF COLISTIN RESISTANCE IN *ESCHERICHIA COLI* URINE ISOLATES.

Microbiology

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ABSTRACT

Introduction: Polymyxin (Colistin) is a novel class of antimicrobial that has a surface-active detergent-like action against most Gram-negative bacteria like *Escherichia coli*. The emergence of colistin-resistant bacteria, although reported infrequently to date, However, there is still no consensus regarding the breakpoints for outlining resistance to colistin. **Aim:** To compare the agar dilution, broth microdilution method, and Vitek-2 for the detection of colistin resistance in *E. coli* urine isolates. **Materials And Methods: A one-**year cross-sectional observational study was conducted in the Department of Microbiology at Maulana Azad Medical College, New Delhi. A total of 200 *E. coli* isolates were subjected to Agar dilution, Broth microdilution (BMD), and Vitek-2 methods for colistin. Using BMD as the gold standard, a comparative analysis between different methods was carried out. **Result:** Out of 200 *E. coli* isolates, Vitek-2 detected colistin resistance in six isolates. By Broth microdilution, two isolates were resistant to colistin. No isolates were resistant to colistin by the agar dilution method. Among the 198 isolates that were colistin-sensitive by the broth microdilution method, 4 were colistin-resistant by Vitek-2. **Conclusion:** The overall concordance rate was 1%, and the discordance rate was 99% between Colistin resistance from BMD and Vitek-2. Broth microdilution is the most sensitive, reliable, and gold standard method for colistin susceptibility testing.

KEYWORDS

Colistin, Broth microdilution, Vitek®2, Colistin agar dilution method.

INTRODUCTION:

The emergence of colistin-resistant bacteria, although reported infrequently to date, Considering the increasing use and demand for colistin. Increasing antibiotic resistance in gram-negative bacteria has recently renewed interest in colistin as a therapeutic option [1]. The polymyxins are a group of polypeptide antibiotics that were first isolated in 1947 from a spore-bearing soil bacillus (*Bacillus polymyxa*). Several chemically different polymyxins (A to E) could be isolated from different strains of this bacillus [2]. Only polymyxin B and polymyxin E (colistin) have been used clinically. Systemic use of colistin was restricted, mainly because of reports of serious nephrotoxicity and the emergence of alternative, less toxic antibiotics. Polymyxin B use has continued in the topical treatment of skin, ear, and ocular diseases. Increasing antibiotic resistance in gram-negative bacteria has recently renewed interest in colistin as an intravenous therapeutic option. Colistin is now increasingly being used for life-threatening infections with multidrug-resistant gram-negative bacteria [3] [4] [5]. In 2015, both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) joint working groups recommended the use of Broth microdilution (BMD) as the reference method [6]. In the last decade, frequent use of colistin against CRE has also led to the development of resistance to this drug. Colistin, a last-resort antibiotic, has witnessed renewed use. However, accurate susceptibility testing for colistin is challenging, with various methods available, leading to potential discrepancies. Ensuring reliable testing is crucial for effective patient treatment and antimicrobial stewardship. This study addresses the need to compare different colistin susceptibility testing methods, providing insights into their accuracy and relevance in clinical settings [7]. We aimed to evaluate the performance of three phenotypic susceptibility methods: agar dilution, Vitek-2 method against Broth microdilution, and confirmation by PCR to detect the presence of the plasmid-mediated colistin resistance (mcr) gene.

MATERIALS AND METHODS:

This prospective observational study was carried out at Department of Microbiology, Maulana Azad Medical College and Lok Nayak hospital, New Delhi, over a period of one year. Permission for the study was granted by the Institutional Ethical committee (IEC). The study was undertaken to compare various methods to detect Colistin susceptibility in urinary isolates. A total of 200 *E. coli* isolates isolated from urine samples were subjected to Agar dilution, BMD, and Vitek-2 methods for detection of colistin resistance.

Study Design: Observational cross-sectional study

Place Of Study: The present study was conducted in the Department of Microbiology, MAMC & LNH, New Delhi.

Period Of Study: One year

Study Population: The study population included all the patients admitted in the hospital.

METHODOLOGY:

Clean midstream urinary samples were collected from the patients. Samples were transported immediately after collection to the Microbiology laboratory and processed immediately. Sample processing and identification up to species level was done by conventional methods as per laboratory SOP [8].

Culture-

All the samples were inoculated on 5% Sheep blood agar and MacConkey agar using 0.001 ml loop and were incubated for 18-24 hours at 37°C. The next day growth was examined as lactose fermenting flat colonies were processed by isolating them on a MacConkey agar. Identification of organism was done by a standard battery of biochemical tests- Citrate test, Triple sugar Iron (TSI), Urease test, Indole test, Methyl Red Test. The colony was inoculated in peptone water for 2 hours at 37ºC in incubator. After which it was adjusted according to 0.5 Mc Farland and then inoculated in various biochemical media. Sugar fermentation tests included glucose (with Durham's tube), lactose, mannitol and sucrose. They were inoculated using a wire loop and incubated at 37ºC for 18-24 hours. The results were read the next day according to the color change of the indicator and controls were setup simultaneously. All the *Escherichia coli* isolates were included in the study and their antimicrobial susceptibility results were noted. These samples were then further subjected to following tests-

Colistin Resistance Detection Test

Colistin agar test (CAT)

Broth microdilution test (BMD)

Vitek®2 Compact system

Genotypic Detection of Mcr gene using Polymerase chain reaction.

Phenotypic Detection Of Colistin Resistance

Colistin Agar Dilution Test (CAD):

The colistin agar dilution test is used to determine the minimum inhibitory concentration and the susceptibility of microorganisms to colistin by looking at their ability to grow on Mueller-Hinton agar plates containing serial dilutions of colistin. Incubate the colistin agar plates for 18–24 hours at 35ºC. The MIC was read as the lowest concentration that completely inhibited the growth of the test isolate. The MIC was interpreted as follows: MIC ≤ 2 µg/ml = intermediate sensitivity, and MIC ≥ 4 µg/ml = resistant [9].

Broth Microdilution Test (BMD):

The conventional broth microdilution method is the gold standard for polymyxin susceptibility testing for clinical isolates. The minimum inhibitory concentration (MIC) of the drug is measured by inoculating the drug in serial two-fold dilutions along with the isolate in microquantities. The control strains [*Escherichia coli* ATCC 25922 (Negative control) and *Escherichia col*i NCTC 13846 (Positive control)] were used. The MIC was interpreted, and results were noted [10].

Vitek®2 Compact System:

It is an automated method for bacterial identification and antimicrobial susceptibility testing of microorganisms. A GN ID card (GNR 280) was used. There are 64 wells, each of which can have separate test beds. Detection was done using a vacuum device integrated with a microbial suspension. The inoculated cards pass through a mechanism that cuts the transfer tube to seal the cards, before being placed in a carousel incubator that holds up to 30 or 60 cards. The results were noted the next day [11].

RESULT:

Out of 200 *E. coli* isolates, Vitek-2 detected colistin resistance in six isolates. By Broth microdilution, two cases of colistin resistance were detected. By Agar dilution, no isolates were resistant to colistin. The two isolates that were colistin-resistant by broth microdilution were also colistin-resistant by Vitek-2. Among the 198 isolates that were colistin-sensitive by the broth Microdilution method, 4 were colistin-resistant by Vitek-2.

Table 1: E. Coli Isolates Resistant To Colistin By BMD, CAD And Vitek-2.

Colistin Resistance	BMD	CAD	Vitek-2	PCR
E. coli (n=200)	2	Nil	6	20

BMD: Broth Microdilution Method,
CAD: Colistin Agar Dilution Test.

Poor agreement exists between colistin resistance by Broth microdilution and Vitek-2 (kappa = 0.010) [Table 2]. Among the two isolates that were colistin-resistant by Broth microdilution, both were colistin-resistant by Vitek-2 as well. Among the 198 isolates that were colistin-sensitive by the broth microdilution method, 4 patients were colistin-resistant by Vitek-2. Overall, the concordance rate was 1% and the discordance rate was 99% between Colistin resistance by the broth microdilution method and Vitek-2, so Vitek-2 is not to be preferred over BMD for colistin.

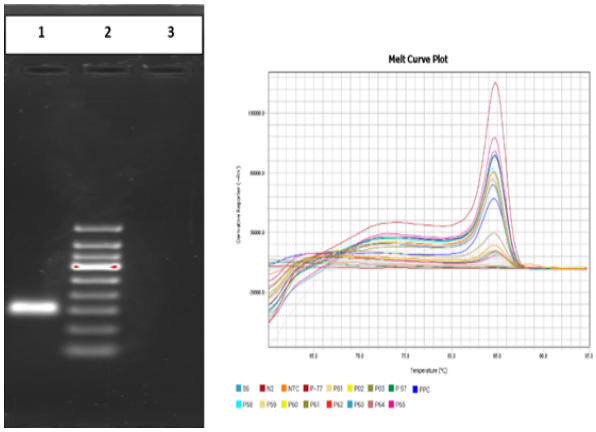

Figure 1: PCR assay for the detection of mcr gene (TM: 82ºC), Lane: 1 – Positive control, 2 – Marker 145 bp DNA ladder, 3 –Negative Control.

Table 2: Inter-kappa Agreement Between Colistin Resistance By The Broth Microdilution Method And Vitek-2 In E. coli (n = 200)

Colistin resistance (Vitek-2)	Colistin resistance (BMD)		Total	Kappa
	Resistant (n=2)	Sensitive (n=198)		
Sensitive	0	194	194	0.010
Resistant	2	4	6	
Total	2	198	200	

Molecular Detection Of Colistin Resistance

All the isolates were subjected to PCR using primers targeting *the mcr* gene to compare the resistance pattern of colistin in *E. coli* isolates. Out of 200 *E. coli* isolates, 20 had the *mcr* gene. It was found to be statistically significant (p-value = 0.018) [Figure 1].

DISCUSSION:

At present, CLSI-EUCAST joint recommendations call for using Broth microdilution (BMD) as the gold standard method to assess colistin susceptibility. Among 198 patients who were colistin-sensitive by the broth microdilution method, 4 patients were colistin-resistant by Vitek-2. Overall, the concordance rate was 1% and the discordance rate was 99% between collagen resistance by the broth microdilution method and Vitek-2. Another study done at AIIMS New Delhi showed 21.5% and 12% of isolates were resistant to colistin by BMD and Vitek-2, respectively [12]. In the present study, out of 200 isolates, there were 4 isolates resistant to Vitek-2 but sensitive to agar dilution and BMD. In a similar Jaipur-based study done by Ayushi et al in 2019, 6.27% of isolates were found to be colistin-resistant by the reference method BMD. Rates of essential agreement (EA) between Vitek-2 and BMD were moderate (85%) [13]. In another study done by Arroyo et al, 22 (19.1%) strains were resistant to colistin and 93 (80.8%) strains were susceptible according to the reference Broth microdilution method. They found 98.2% perfect matches with only 2 very serious errors (1.7%) [14]. In another statistically significant study done by Poonam et al Out of 32 isolates tested by BMD, 65.6% were found to be susceptible to Colistin and 34.3% were resistant [15]. Our study highlights that Vitek-2 is not reliable for colistin susceptibility testing. Out of 200 *E. coli* isolates, 20 isolates exhibited the mcr gene with a statistically significant p-value (0.018) as compared to BMD, which showed 2 isolates resistant to colistin. In our study, it was found that the number of isolates in which mcr was detected was greater than the phenotypic expression. Certain genes that were expressed genotypically were not expressed phenotypically. This indicates that the molecular method is preferred over the phenotypic method.

CONCLUSION:

The overall concordance rate was 1%, and the discordance rate was 99% between Colistin resistance from BMD and Vitek-2. This study demonstrates that Colistin remains part of the last-line drugs used to treat multidrug-resistant Gram-Negative bacilli, like carbapenem-resistant GNB. Broth microdilution is the gold standard method to assess colistin susceptibility. It was observed that Vitek-2 is over-reporting and giving false positive resistance to colistin; therefore, it is not to be preferred over BMD for detecting colistin resistance. Therefore, the use of molecular methods (PCR) needs to be increased for better detection of resistance marker genes in routine laboratory settings.

Declaration Of Interests:

No declaration of interest

Ethical Approval Letter-

Number: F.1/1EC/MAMC/(82/10/2020/No.101
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