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Original Research Article **Laboratory**

Methods for Assessing in Vitro Susceptibility to Colistin: to what Extent could the Disk Diffusion Method be Considered Useful?

Imane Bentaher[1,3*], Abou Sessouma[2,3], Yassine Ben Lahlou[1,3], Elmostafa Benaissa[1,3], Mariama Chadli[1,3]

[1]Bacteriology Laboratory, Mohammed V Military Teaching Hospital, Rabat, Morocco
[2]Central Bacteriology Laboratory, Ibn Sina University Hospital, Rabat, Morocco
[3]Rabat Faculty of Medicine and Pharmacy, Rabat, Morocco

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***Corresponding author:** Imane Bentaher
Bacteriology Laboratory, Mohammed V Military Teaching Hospital, Rabat, Morocco

Abstract

Introduction: The worrying increase in multi-resistant Gram-negative bacilli (GNB) infections and the glaring lack of therapeutic alternatives has allowed colistin to regain a place in the antibiotic arsenal, often as a last resort. This study aimed to evaluate a method for testing sensitivity to colistin that is simple, inexpensive, and accessible: disk diffusion, compared to the gold standard method of liquid microdilution. ***Materials and methods:*** A comparative study was conducted on 124 strains of Gram-negative bacilli. Each strain was tested by agar diffusion (50 µg colistin disk) and broth microdilution (Sensititre® plate). ***Results:*** Our results showed that the diffusion method does not meet the performance criteria established by the CLSI, with a particularly high major error rate (45.8%) and a categorical agreement of 83.1%. ***Conclusion:*** Disk diffusion cannot be considered a reliable method for testing colistin susceptibility in the laboratory.

Keywords: colistin, Gram-negative bacillus, MIC, microdilution, resistance.

INTRODUCTION

The emergence of multidrug-resistant bacterial infections, particularly carbapenemase-producing Enterobacteriales (CPE), is now one of the major challenges facing public health [1]. These infections are associated with several conditions, such as pneumonia, meningitis, bacteremia, and urinary tract infections.

Their treatment is complicated by their high prevalence in hospitals and the rapid spread of resistant clones. The increase in their multidrug resistance, or even their total resistance, considerably reduces treatment options, often forcing the use of old and neglected antibiotics such as colistin (abandoned due to its nephrotoxicity and neurotoxicity). However, the growing emergence of strains resistant [2-4] to this antibiotic also compromises its effectiveness, making treatment even more difficult [1, 5].

Reliable and rapid detection of colistin susceptibility is therefore essential. Broth microdilution is currently considered the gold standard method, while agar diffusion methods are not recommended by EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Clinical and Laboratory Standards Institute) [1, 6]. However, its cost means that not all laboratories have access to it. Rapid tests are being developed to optimize management [6].

The aim of our study is to determine the place of the diffusion method in the study of the susceptibility of Gram-negative bacilli (GNB) to colistin.

MATERIALS AND METHODS

We conducted a prospective, single-center study in the bacteriology laboratory of the Mohamed V Military Teaching Hospital on 124 Gram-negative bacilli samples with heterogeneous resistance profiles in antibiograms performed on agar medium over a six-month period from December 2024 to May 2025.

We included all cultures positive for Gram-negative bacilli from diagnostic samples. Resistance phenotypes were characterized using conventional antibiograms on agar media, guided by the recommendations of the CA-SFM (Antibiogram Committee of the French Society of Microbiology). We

excluded duplicates. Species identification was performed by mass spectrometry (Maldi-Toff®).

1. **Determination of Gram-negative bacilli sensitivity to colistin by agar disk diffusion method:** A 0.5 McFarland bacterial suspension was prepared from each sample and the diffusion diameter of the 50 µg colistin disk on Muller-Hunton agar medium was measured according to the 2024 CA-SFM recommendations. (Figure 4, right)
2. **Determination of Gram-negative bacilli sensitivity to colistin using the microdilution method on Sensititre® plate:** We adopted a liquid dilution or microdilution method, the reference method recommended by EUCAST. We seeded a Sensititre® round-bottom polystyrene microplate containing decreasing concentrations of colistin in 11 wells or cups (from 0.125 to 128 µg/ml) from a 0.5 McFarland bacterial suspension, according to the 2024 CA-SFM recommendations. (Figure 4, left)
3. **Data analysis:** Categorical agreement (CA) for colistin was defined as the percentage of isolates classified in the same susceptibility category by the broth microdilution method (reference method) and disk diffusion according to CLSI [1,2,7]. A very major error (VME) is defined as an error indicating a false susceptibility result in the tested method and resistance in the microdilution reference method, which exposes the patient to the risk of treatment failure. Conversely, a major error (ME) indicates a false resistance result in the tested method and susceptibility in the reference method. Acceptable performance was evaluated according to the criteria established by the International Organization for Standardization: ≥ 90% for categorical agreement and ≤ 1.5% for VMEs and ≤ 3% for MEs [1,4].

RESULTS

The study involved 124 patients with an average age of 45.6 years, ranging from 8 days to 86 years, and a predominance of males with a male-to-female ratio of 2.35.

1. Distribution according to the type of sample:

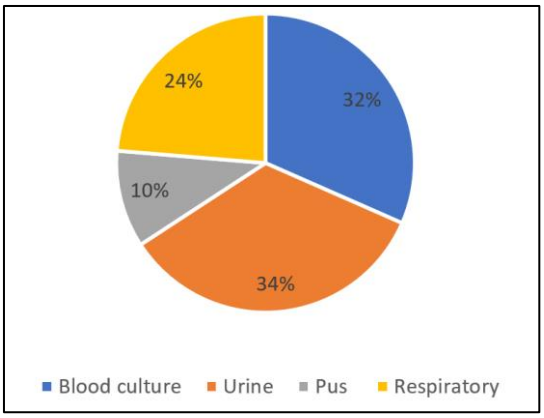

Figure 1: Distribution of samples according to type

2. Distribution according to the prescribing department:

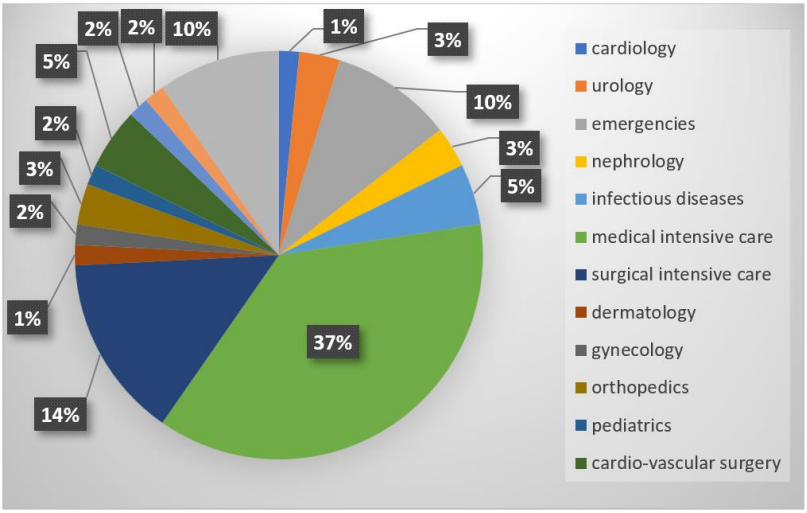

Figure 2: Distribution of samples according to the prescribing department

3. Distribution according to the beta-lactam resistance phenotype in the antibiogram:

There is a predominance of carbapenemase-producing Gram-negative bacilli.

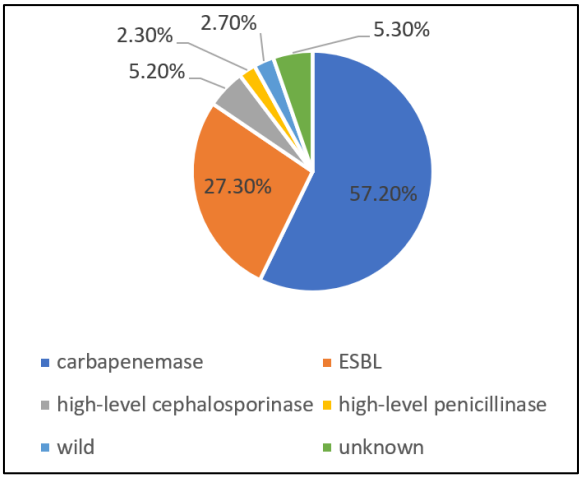

Figure 3: Distribution of samples according to the resistance profile in the antibiogram

After 24 hours of incubation, the sensitivity results for each method were as follows:

1. **Disk diffusion method:** We interpreted colistin sensitivity in relation to its latest critical diameter adopted in 2013 by the CA-SFM: 15 millimeters. [8] of 124 samples, 111 (89.5%) were sensitive and 13 (10.5%) were resistant.
2. **Sensititre® microdilution method:** According to CA-SFM 2024, the critical MIC (minimum inhibitory concentration) for BGNs is set at 2 mg/L. Out of 124 samples, 100 (80.6%) were sensitive and 24 (19.4%) were resistant.

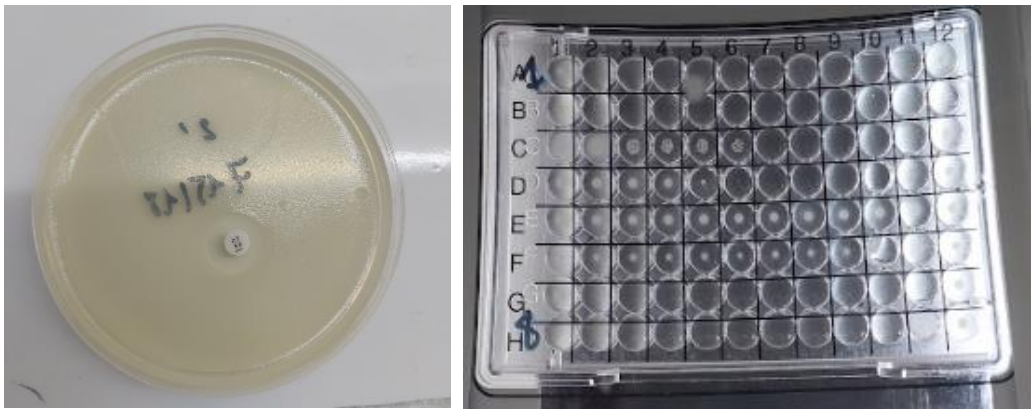

Figure 4: Sensititre® microdilution plate result for colistin left) vs. disk diffusion result with 50 µg colistin disk (right)

3. **Comparison of the two methods:**

Sensitivity tests showed that most of the isolates tested (80.6%) were sensitive to colistin by broth microdilution, and only 19.4% were resistant. Discrepancies were found in the sensitivity results obtained by disk diffusion. Disk diffusion did not allow resistant or sensitive isolates to be determined (Table 1).

The comparative evaluation of the diffusion method against broth microdilution as the reference method showed a categorical agreement of 83.1% (103 isolates), with a very major error of 45.8% (11 isolates) and a major error of 5% (5 isolates) (Table 2).

Table 1: Relationship between susceptibility test results between disk diffusion and the reference method of broth microdilution

Disk diffusion		**Broth microdilution µg/ml**									
	< 0.125	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**
Sensitive	14 (11.7%)	21 (16.8%)	39 (31.2%)	13 (10.4%)	10 (7.8%)	1 (1.3%)	7 (5.2%)	3 (2.6%)	7 (5.2%)	0	0
Resistant	0	1 (1.3%)	1 (1.3%)	0	0	1 (1.3%)	0	0	1 (1.3%)	0	3 (2.6%)

Table 2: Comparison between the results of disk diffusion and liquid microdilution susceptibility tests by categorical agreement

	Broth microdilution		**Disk diffusion**	
	Susceptible 100	Resistant 24	Susceptible 111	Resistant 13
Very Major Error (VME)			11 (45.8%)	
Major Error (ME)			5 (5%)	
Categorical Agreement (CA)			103 (83.1%)	

- Very major error: Number of false sensitives (sensitive to diffusion but resistant to the reference method) / Total number of strains resistant to the reference method x 100%

- Major error: Number of false resistants (resistant to diffusion but sensitive to the reference method) / Total number of strains sensitive to the reference method x 100%

- Categorical agreement: Total number of similar results / Total number of isolates tested x 100%

DISCUSSION

Colistin, also known as polymyxin E, is an older antibiotic with notable in vitro activity against certain bacterial species, for which it is sometimes the only effective treatment option. As a result, its use has seen renewed interest in recent years, particularly in the treatment of severe infections caused by resistant Gram-negative bacilli. This use is tending to increase in a context where highly resistant pathogens remain a clinical concern and therapeutic alternatives remain limited [2,4,9,10,11].

Although long considered rare, colistin resistance is steadily increasing, particularly in multidrug-resistant Gram-negative bacilli such as Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii [1, 6, 8]. This emergence is concerning, particularly due to the spread of the plasmid gene mcr-1 [6].

Accurate determination of colistin susceptibility is essential to guide treatment and monitor the evolution of resistance [12,13].

In our study, the majority of GBS tested were susceptible, but 19.4% were resistant to colistin, reflecting the widespread use of this antibiotic in Moroccan hospitals. Indeed, numerous studies highlight an increase in Gram-negative bacill resistance to colistin worldwide.

We found that the disk diffusion method was unreliable, as it did not detect all resistant or susceptible isolates, with a categorical agreement of 83.1%, a very major error of 45.8%, and a major error of 5%. This result is consistent with many previous studies [1-4].

This unreliability of the diffusion method for colistin can be explained by the cationic nature of its structure, its high affinity for certain components of agar, and its relatively high mass, which prevent it from diffusing properly in agar [6,12,16]. Sometimes, characteristics specific to certain bacterial strains (such as the production of natural polymyxins) can distort sensitivity results [16,17].

CONCLUSION

Therefore, based on our results and those reported in the literature [2,18], we conclude that the disk diffusion test is not suitable for use alone to assess colistin susceptibility, as this may falsely reduce the available treatment options or lead to inappropriate treatment. Therefore, susceptibility results must be determined by measuring the MIC using the gold standard method: broth microdilution.

Declaration of Conflicts of Interest: The authors declare that they have no conflicts of interest.

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