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ORIGINAL ARTICLE

Colistin Susceptibility Testing Methods for Carbapenem Resistant *Acinetobacter baumannii,* A Comparative Study

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Acinetobacter baumanii, Colistin, Vitek, E-test

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ABSTRACT

Background: *Acinetobacter baumanii is an emerging nosocomial pathogen that is associated with several infections that are extremely difficult to treat. Extensive and pan-drug resistance to multiple antibiotic classes is rising leading to limited treatment options and making colistin a last resort. Practical and accurate susceptibility testing methods are a mandatory demand for rapid and reliable sensitivity reporting of colistin.* ***Objective:*** *The aim was to test colistin sensitivity of carbapenem-resistant Acinetobacter baumanii, using different commonly used testing methods in the routine microbiology laboratory as disc diffusion, broth microdilution, E-test and Vitek-2 system.* ***Results:*** *This research showed that disc diffusion and E-test failed to meet the CLSI criteria with high very major errors (100% and 33%). Comparative evaluation between the three studied antibiotic susceptibility methods to colistin against broth microdilution as a reference method, showed 100% categorical agreement with Vitek-2.* ***Conclusion:*** *We concluded that besides the reference broth microdilution method, Vitek-2 is an automated alternative reliable option to test the bacterial susceptibility to colistin in the laboratory.*

INTRODUCTION

Hospital acquired infections by multidrug resistant and extensive drug resistant bacterial pathogens are among the most challenging problems health care professionals are facing nowadays. Infections caused by *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Acinetobacter baumanii* represent the most challenging of them. *Acinetobacter baumanii* is an emerging nosocomial pathogen that is associated with several infections, as pneumonia, meningitis, bacteremia, and urinary tract infections, that are extremely difficult to treat. It has virulence properties that allow it to multiply on dry surfaces and endure disinfectants and so can survive in hospital environment[1,2].

Treatment of *Acinetobacter baumanii* is hindered by its high prevalence in the hospital environment and the rapidity of spread of the resistant clones specially in Egypt. Extremely and pan-drug resistance to multiple antibiotic classes are rising leading to limited treatment options and confinement to old and neglected antibiotics as colistin. It is considered as a last resort treatment although colistin resistant strains are also evolving further limiting treatment options[3,4].

Practical and accurate susceptibility testing methods are a mandatory demand for rapid and reliable sensitivity reporting of colistin. Disc diffusion methods although being the most implemented method in routine microbiology laboratories, remains deficient and unreliable regarding colistin testing. CLSI MIC breakpoints are a more accurate testing method for colistin sensitivity. Other methods as E-test and automated systems provide promising tools but with controversary sensitivity from different research[5-8].

In this research, we aim to test colistin sensitivity of carbapenem-resistant *Acinetobacter baumanii*, using different commonly applied susceptibility testing methods in the routine microbiology laboratory, to determine their reliability for better treatment outcome of this rapidly evolving pathogen.

METHODOLOGY

Clinical isolates:

Forty clinical isolates were collected from the Diagnostic Microbiology Laboratory of Alexandria Main University Hospital over a period of six months from January to June 2021. Approval of the Ethical Committee was obtained from the Faculty of Medicine, Alexandria University. These isolates belonged to samples from different sites of infection as urinary tract infections, respiratory tract infections and infected skin wounds. The isolated *Acinetobacter species* were identified by different biochemical reactions as oxidase, citrate, motility test and triple sugar iron as preliminary identification, followed by species level identification through Vitek-2 compact system (bioMérieux, Marcy l'Étoile, France)[9].

Characterization of Carbapenem non-susceptible *Acinetobacter baumanii* was performed using disc diffusion antibiotic sensitivity testing, guided by CLSI recommendations for interpretation of zone diameters.

Carbapenem non-susceptibility was further confirmed by Vitek-2 microdilution antibiotic sensitivity method[9,10].

Colistin Antibiotic sensitivity testing of *Acinetobacter baumanii:*

Disc Diffusion Antibiotic Sensitivity Testing

All *A. baumannii* isolates with a zone diameter ≤12 mm were considered resistant and those with a zone diameter ≥14 mm were considered susceptible[11,12]. Current guidelines of the CLSI and the EUCAST recommend that colistin testing should be performed by dilution methods, therefore colistin susceptibility disc diffusion results were compared against broth microdilution method[10,13].

Automated Vitek -2 Compact System

Colistin susceptibility testing using the GN222 AST Vitek-2 card (bioMérieux, Marcy l'Étoile, France) was performed, utilizing reference strain *A. baumanii* ATCC 19606 as a control. MIC ≤ 2 ug/ml was considered a sensitive interperative breakpoint while MIC ≥ 4 ug/ml was considered resistant according to manufacturer's instructions for Vitek-2 susceptibility testing system[9,10].

Broth Microdilution

Stock solutions of colistin from colistin sulphate powder (Sigma-Aldrich, St. Louis, MO) were reconstituted before use in sterile distilled water according to the manufacturer's instructions. Dilution methods were performed according to CLSI procedure. A concentration of 0.5 MacFarland of the inoculum was prepared in Brain heart infusion broth and colistin was incorporated in the media in concentration range 0.25- 8 ug/ml in a double fold dilution range[10,13].

Colistin MIC E-test

Colistin MIC was determined using E-test following the manufacturer's recommendation of Colistin Ezy MICTM Strip (CL) (0.016-256 mcg/ml) (EM020, HiMedia Laboratories, India). MIC readings were recorded where the ellipse intersects the MIC scale on the strip. Interpretive criteria as sensitive ≤ 2 ug/ml and resistant ≥ 4 ug/ml were considered, as recommended by the manufacturer's instructions, while using *A. baumanii* ATCC 19606 as a control. Isolated colonies, microcolonies and hazes appearing in the zones of inhibiton were considered as heteroresistant subpopulations in the growth and MIC reading was recorded at a point on the scale above which no resistant colonies were observed close to the MIC strip[14].

Data Analysis

Colistin Categorical agreement (CA) was defined as the percentage of isolates classified in the same susceptibility category by broth microdilution method and the disc diffusion, Vitek or Etest according to the CLSI. Very major errors (VMEs) denoted a false-susceptible result, and major errors (MEs) denoted a false-resistant result, while minor errors (MinEs) were intermediate zone diameters that had susceptible or resistant MIC, or intermediate MIC with a susceptible or resistant zone diameter. Acceptable performance was evaluated according to criteria established by the International Organization for Standardization: ≥90% for category agreement and ≤1.5 % for VMEs or MEs[15].

RESULTS

Source of *Acinetobacter baumanii* isolates

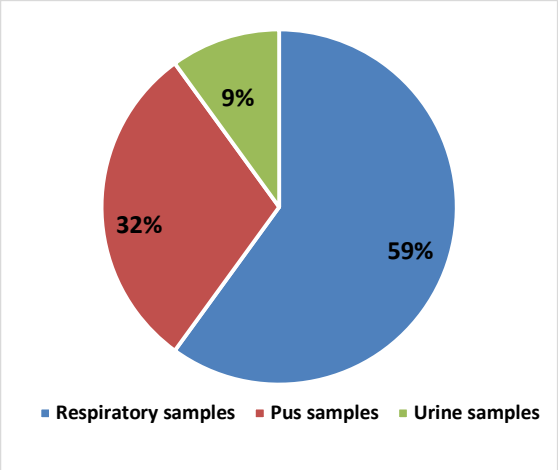

Fig. 1: Source of Isolates

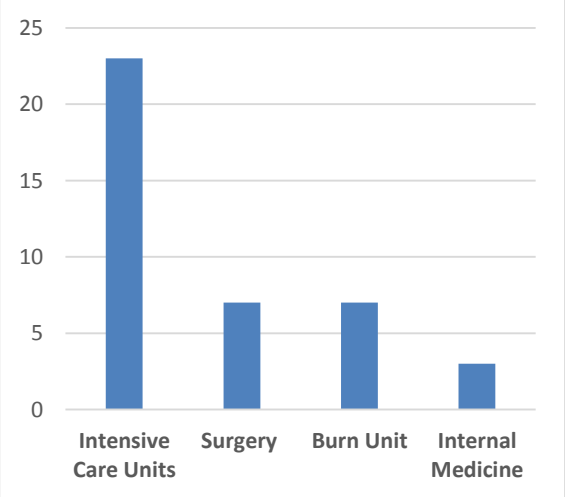

Fig. 2: Departments

Most of the isolated *Acinetobacter baumanii* were obtained from respiratory samples; sputum, bronchoalveolar lavage and MiniBAL, urine and wound swabs were also encountered. (Figure 1) Intensive Care Units were the main source of these samples, followed by Surgical Departments, Burn Unit, and Internal Medicine Departments of the Main University Hospital of Alexandria University. (Figure 2) All samples were sent and processed in the Diagnostic Microbiology Laboratory of Alexandria Main University Hospital.

Colistin susceptibilities determined by disc diffusion (DD), Vitek-2 system and E-test in relation to broth microdilution (BMD)

Antibiotic susceptibility testing showed that most of the tested *Acinetobacter baumanii* isolates (92.5%) were sensitive to colistin by broth microdilution and only 3 isolates were resistant. The same results were obtained by Vitek-2 system. Discrepancies were found in the susceptibility results by disk diffusion and E-test. Disk diffusion failed to determine the resistant isolates and all tested were sensitive, while E-test determined only 2 resistant isolates. (Table 1)

Table 1: Relation of susceptibility test results between disk diffusion, Vitek-2 and E-test to broth microdilution, as a reference test method, regarding number of tested isolates

Disk Diffusion (Total 40)	**Broth Microdilution ug/ml**					
	< 0.25	**0.5**	**1**	**2**	**4**	**8**
Sensitive	5 (12.5%)	11(27%)	13(32%)	8 (20%)	2 (5%)	1 (2.5%)
Intermediate						
Resistant						
Vitek-2 (Total 40)	**Broth Microdilution ug/ml**					
	< 0.25	**0.5**	**1**	**2**	**4**	**8**
Sensitive	5 (12.5%)	11(27%)	13(32%)	8 (20%)		
Intermediate					2 (12.5%)	1 (2.5%)
Resistant						
E-Test (Total 40)	**Broth Microdilution ug/ml**					
	< 0.25	**0.5**	**1**	**2**	**4**	**8**
Sensitive	5 (12.5%)	11(27%)	13(32%)	8 (20%)	1 (2.5%)	
Intermediate					1 (2.5%)	1 (2.5%)
Resistant						

Comparison between the susceptibility testing methods through Categorical Agreement (CA)

Comparative evaluation between the three studied antibiotic susceptibility methods to colistin against broth microdilution as a reference method, showed 100% categorical agreement with Vitek-2 system. One very major error and three very major errors were detected in results of E-test and disk diffusion respectively. This refers to 97.5% and 92.5% categorical agreement to E-test and disk diffusion respectively. (Table 2)

Table 2: Comparison between results of disk diffusion, Vitek-2 and E-test in relation to results of broth microdilution as regards categorical agreement

	Broth Microdilution		**Disk Diffusion**		**VITEK-2**		**E-test**	
	Susc.	Resis.	Susc.	Resis.	Susc.	Resis.	Susc.	Resis.
	37	3	40	0	37	3	38	2
Very Major Errors (VME)			3 (100%)		zero		1 (33.3%)	
Categorical Agreement (CA)			92.5%		100%		97.5%	

Categorical agreement: Total number of similar results/ Total number of tested isolates x 100%
Very major errors: Very major errors based on interpretation/Total resistant strains x100%

DISCUSSION

Infection caused by multidrug resistant and extensive drug resistant Gram-negative bacteria such as *Acinetobacter baumanii* are increasing globally. This increasing antimicrobial resistance has narrowed the options available for the treatment of such infections. Polymyxins such as colistin and polymyxin B are now considered a last resort to treat many of these infections. For this reason, reliable and accurate methods to detect susceptibility to these antibiotics should be available in the microbiology laboratory[1,11].

In this study, we tested three antibiotic susceptibility methods (disk diffusion, Vitek2, E-test) on 40 *Acinetobacter baumanii* clinical isolates, and the results were compared to the broth microdilution method BMD (as the reference method)[13].

Most of *Acinetobacter* isolates were susceptible to colistin despite its overuse in our hospital ICUs. Only 7.5% of the isolates were resistant. Many studies

detected a higher level of resistance to colistin. For example, Dafopoulou et al[5] from Greece, detected 90% resistance to colistin among 20 Acinetobacter isolates. Meanwhile, Vourli et al[16] detected 24.8% resistance rate.

We observed that Vitek-2 showed the highest CA with BMD (100%) with no VME. This is in agreement with Dafopoulou et al[5] who reported 90% CA of Vitek2, they however detected 10% major errors (ME).

Unlike our study, alarming rate of VME in Vitek-2 was detected in the study by Vourli et al[16] (37.9%) with CA of 89.7%. Another study by Chew et al[17] also agreed with our study in the CA of Vitek-2 (>90%) and disagreed in its VMEs (36%).

As regards E-test, we found a CA of 97.5% with BMD and 33% VMEs. This agrees with Dafopoulou et al[5] who found 35% of VMEs and 65% of CA with BMD. Chew et al[17] also showed 92% CA and 12% of isolates exhibiting VMEs. It appears that E-test in our study and many others[5,8,17] failed to meet the recommendations of CLSI as regards VMEs (not exceeding 1.5%)[15] and for that reason, it is not advised to adopt this commercial method to detect susceptibility to colistin in the laboratory.

The disk diffusion method was not a reliable option as it failed to detect all resistant isolates when compared to BMD (VMEs 100%). This finding comes in agreement with previous studies[18-20].

CONCLUSION

Therefore, based on the results of this research, we conclude that besides the reference BMD method, Vitek-2 is an automated alternative reliable option to test the bacterial susceptibility to colistin in the laboratory.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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