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In Vitro Antimicrobial Combinations for Pan-Drug Resistant Acinetobacter Species

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ABSTRACT

Background: Pandrug resistant Gram-negative organisms (PDRGNs) have emerged, as a major threat to hospitalized patients. They have been associated with mortality rates ranging from 30 to 70%. Because of the high morbidity and mortality rates of severe pandrug resistant acinetobacter spp infections, combination therapies, as opposed to monotherapy, are suggested. A synergistic effect may be developed when antibiotics are used in combination. Through this synergistic effect, treatment efficacy can be improved and resistance can be prevented.

Aim of the work: To investigate the use of in vitro antibiotic synergy test (checkerboard) for pandrug resistant acinetobacter species with a clinical feedback on the most synergistic antimicrobial combination.

Materials and Methods: During this study, one hundred isolate of drug resistant acinetobacter species identified by routine culture and sensitivity using disc diffusion susceptibility test, were collected from critically ill patients admitted to Ain Shams University Internal Medicine Intensive Care Units. The isolates were subjected to: (i) Determination of MIC using Vitek 2 automated system to confirm resistance of acinetobacter species to all commercially available antibiotics, (ii) Broth micro-dilution method (BMD) for determination of tigecycline susceptibility, and (iii) Determination of antimicrobial synergy by broth micodilution (Checkerboard method).

Results: Vitek 2 system results showed that, all of the 100 isolates were resistant to all antibiotics included in the study. On the other hand, 100% of the isolates were sensitive (S) to Colistin. As regards the results by Broth microdilution antibiotic susceptibility method, all 100 isolates (100%) were resistant to ampicillin/sulbactam, meropenem and ciprofloxacin, whereas 95 isolates (95%) were resistant to amikacin, whereas all 100 isolates (100%) tested were sensitive to tigecycline. The results of the antibiotic combinations were as follows; the activity of ampicillin/sulbactam in combination with amikacin showed synergy in (48%), addition in (42%) and indifference in (10%). The activity of ampicillin/sulbactam in combination with ciprofloxacin showed, synergy in (36%), addition in (52%) and indifference in (12%). The activity of meropenem in combination with amikacin showed, synergy in (26%), addition in (53%) and indifference in (21%). No antagonistic activity was detected between any of the antibiotic combinations used.

Conclusion: The prevalence of XDR/PDR resistant Acinetobacter spp. was highest in blood samples (43%) followed by sputum samples (35%) recovered from critically ill patients admitted to Ain Shams University Internal Medicine Intensive Care Units. Vitek 2 system showed that, all of the 100 isolates were resistant to all antibiotics included in the study. On the other hand, 100% of the isolates were sensitive (S) to colistin. Broth microdilution antibiotic susceptibility method showed that, all 100 isolates (100%) were resistant to ampicillin/sulbactam, meropenem and ciprofloxacin, whereas 95 isolates (95%) were resistant to amikacin, whereas all 100 isolates (100%) tested were sensitive to tigecycline, indicating that acinetobacter spp. did not attain resistance to tigecycline yet. The broth microdilution antibiotic synergy test (Checkerboard method), being the reference method for assessing antimicrobial synergy, showed that the highest synergic activity belongs to ampicillin/sulbactam and amkacin (48%), and the lowest synergic activity belongs to meropenem and amikacin (26%).

Keywords: PDRGNs, Antimicrobial combinations, Checkerboard broth microdilution method.

INTRODUCTION

Acinetobacter spp. have emerged as one of the most important pathogens involved in health care associated infections in recent decades, characterized by their ability to accumulate different mechanisms of antimicrobial resistance, often showing a multidrug-resistant phenotype [(1)].

Due to the high morbidity and mortality rates of severe drug resistant acinetobacter spp infections, combination therapies, as opposed to monotherapy, are suggested. A synergistic effect may be developed when antibiotics are used in combination. Through this synergistic effect, treatment efficacy can be improved and resistance can be prevented [(2)].

Pandrug resistant bacteria employ several mechanisms in attaining resistance that include; enzymatic deactivation of antibiotics, decreased cell wall permeability to antibiotics, altered target sites of antibiotic, efflux mechanisms and increased mutation rate [(3)].

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The clinical relevance of in vitro synergy test findings is uncertain. However, in vitro models can be used to perform screening for synergistic combinations to be further explored in prospective clinical studies. In addition, in situations where there are no evidence-based treatment options, in vitro data can be useful to support therapeutic decisions for severe infections with pandrug resistant Acinetobacter spp [(4)].

In vitro synergy tests include the checkerboard broth microdilution and time-kill curve methods which are the most widely used techniques to assess synergy and are considered to be the gold standard but are time-consuming and labor-intensive [(5)].

On the other hand, E-test used to investigate the effects of antibiotic combinations is relatively new compared to the standard methods. However, E-test is much more expensive than the standard one's [(6)].

As regard in vitro effective combination therapy for suspected Gram-negative sepsis and severe infections with pandrug resistant acinetobater spp., it typically includes a broad-spectrum beta-lactam, an aminoglycoside, ampicillin/ sulbactam, a carbapenem, colistin, or rifampin. In addition, these combinations have been proven to be successful against pandrug resistant Acinetobacter spp [(7)].

MATERIALS AND METHODS

During this study, one hundred isolate of drug resistant acinetobacter species identified by routine culture and sensitivity using disc diffusion susceptibility test, were collected from critically ill patients admitted to Ain Shams University Internal Medicine Intensive Care Units. The isolates were subjected to: (i) Determination of MIC using Vitek 2 automated system to confirm resistance of acinetobacter species to all commercially available antibiotics, (ii) Broth micro-dilution method (BMD) for determination of tigecycline susceptibility, and (iii) Determination of antimicrobial synergy by broth micodilution (Checkerboard method). **The study was approved by the Ethics Board of Ain Shams University.**

Statistical Methodology:

The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20, IBM Corp., USA, 2016). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

i. Descriptive statistics:

Frequency and percentage of non-numerical data.

ii. Analytical statistics:

McNemar test: used to assess the statistical significance of the difference between a qualitative variable measured twice for the same study group.

RESULTS

Regarding demographic data as shown in Table (1), out of 100 drug resistant acinetobacter spp. isolates, 54% were isolated from males and 46% isolates were isolated from females, the mean of patient's age was 46.3 years with a SD of 21.4 years. Concerning association between frequency of studied isolates and patients' demographic data (gender, age), no statistically significant difference (NS) was found as P Value was (> 0.05).

The most frequent clinical presentation found among our patients as shown in Table (2), was sepsis 51% (51/100) followed by pneumonia 33% (33/100). The least clinical presentation was empyema 2% (2/100).

As regards the activity of different antibiotic combinations using checkerboard Broth micro-dilution method which is considered to be the gold standard reference method, table 3 showed that the activity of ampicillin/sulbactam in combination with amikacin was synergic in 48% of isolates (48/100), additive in 42% of isolates (42/100) and indifferent in 10% of isolates (10/100). Whereas the activity of ampicillin/sulbactam in combination with ciprofloxacin was synergic in 36% of isolates (36/100), additive in 52% of isolates (52/100) and indifferent in 12% of isolates (12/100). Finally the activity of meropenem in combination with amikacin was synergic in 26% of isolates (26/100), additive in 53% of isolates (52/100) and indifferent in 21% of isolates (12/100). No Antagonistic activity was detected in our results.

Table (1): Frequency of studied isolates as regards gender and age.

		N	%	P Value
Sex	Female	46	46.0%	0.169
	Male	54	54.0%	
Age (years)	**Mean**	**SD**		0.826
	46.3	21.4		

Table (2): Distribution of clinical presentation among our patients.

Clinical presentation	Number	%
Sepsis	51	51.0%
Pneumonia	33	33.0%
Surgical site infection	7	7.0%
Meningitis	4	4.0%
Diabitic foot	3	3.0%
Empyema	2	2.0%

Table (3): Activity of different antibiotic combinations using checkerboard Broth micro-dilution method.

Activity	Synergy	Additive	Indifferent
Ampicillin/ sulbactam +Amikacin	48 (48%)	42 (42%)	10 (10%)
Ampicillin/ sulbactam+Cip	36 (36%)	52 (52%)	12 (12%)
Meropenom+Amikacin	26 (26%)	53 (53%)	21 (21%)

DISCUSSION

In this study among the 100 isolates of drug resistant Acinetobacter species, 43% were isolated from blood samples, 35% from sputum samples, 10% from wound specimens, 6% from central line samples, 4% from CSF samples and 2% from pleural fluid. This was in accordance with the Turkish study of ***Kurutepe & Gazi*** [8] where the most common sites of isolation of drug resistant acinetobacter spp. among 50 specimens were from blood samples 78%, respiratory samples 12% or various clinical samples 10%.

However, in a study by ***Cortivo et al.*** [9], they recovered higher drug resistant acinetobacter isolates than our study from respiratory samples (56%), lower samples from blood (23%), nearly same number from wound samples 8%, (4%) from other body fluids, and (2%) from catheter tips.

This study was in agreement with several studies ***Savov et al.*** [10]; ***Teo et al.*** [11], where synergy between ampicillin/ sulbactam and amikacin was found using the broth microdilution checkerboard methods with synergy percentage of (40%), (55%) and (45%) respectively. In a study by ***Pranita et al.*** [12] on 12 clinical isolates, synergic activity of β-lactam–aminoglycoside and β-lactam–fluoroquinolone combinations, were (79%) and (58%) respectively.

In contrast in another study by ***Temocin et al.***[13], antibiotic combination between ampicillin/ sulbactam and amikacin showed synergy in (17%), addition in (33%) and indifference in (50%), while most synergy was found between sulbactam and meropenem (43%).

In the present study, by disc diffusion method all of the 100 (100%) isolates tested were resistant by disc diffusion agar susceptibility method to both ampicillin/sulbactam and amikacin. Whereas intermediate resistance to ampicillin/ sulbactam was reported in 5 (5%) of isolates and 95 (95%) showed resistance. Moreover 5 (5%) of the isolates reported sensitivity to amikacin.

Disc diffusion method is one of the most frequently used techniques in microbiology laboratories. However, the high rates of errors with some antimicrobial agents of this study demonstrated that this method isn't reliable compared to the broth microdilution method, once this last one is considered the gold standard by CLSI [14].

On the other hand, according to ***Mahon et al.*** [15]; ***Matthew*** [16], discrepancy between disc diffusion agar susceptibility method and BMD method could be explained as the disc diffusion method offers the ability to view growth on the plate rather than growth in a tube or well, inner colonies can be visualized within the zones of inhibition. These inner colonies are believed to be subpopulations of the original strain that exhibit increased antibiotic resistance, thus allowing them to grow closer to the disc i.e. where the antibiotic concentrations are higher.

The variations in the susceptibility to various antimicrobial agents can be attributed to geographic variations which may greatly differ in antibiotic prescribing attitudes of physicians, infection control practices and underlying resistance mechanisms [17].

CONCLUSION

The prevalence of XDR/PDR resistant Acinetobacter spp. was highest in blood samples (43%) followed by Sputum samples (35%) recovered from critically ill patients admitted to Ain Shams University Internal medicine intensive care units. Vitek 2 system showed that, all of the 100 isolates were resistant to all antibiotics included in the study. On the other hand, (100%) of the isolates were sensitive (S) to Colistin. Broth microdilution antibiotic susceptibility method showed that, all 100 isolates (100%) were resistant to ampicillin/sulbactam, Meropenem and Ciprofloxacin, whereas 95 isolates (95%) were resistant to amikacin, whereas all 100 isolates (100%) tested sensitive to Tigecycline, indicating

that Acinetobacter spp. Strains recovered from Internal medicine ICUs did not attain resistance to Tigecycline yet. The broth microdilution antibiotic synergy test (Checkerboard method), being the reference method for assessing antimicrobial synery, showed that the highest synergic activity belongs to Ampicillin/sulbactam and Amkacin (48%), and the lowest synergic activity belongs to Meropenem and Amikacin (26%).

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