23rd ECCMID Berlin, Germany 27–30 April 2013
ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

P 1585

Evaluation of two Etest methodologies for the detection of synergistic interactions of tigecycline combinations against *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*

V. Papoutsaki, D. Lekkakou, I. Galani, M. Souli, L. Galani, I. Karaiskos, N. Vakalis, H. Giamarellou
Hygeia General Hospital, Athens Greece

hygeia hospital

Abstract

Objective: To evaluate the Etest imprint and the Etest cross formation methods for testing antimicrobial combinations compared to the checkerboard methodology. **Methods:** Clonal relatedness of tested isolates was assessed by REP-PCR. Interactions between tigecycline and colistin, gentamicin, meropenem or fosfomycin were assayed using the checkerboard and two Etest methods: the imprint and the cross formation. The fractional inhibitory concentration (FIC) was calculated as: FIC index = MICA in combination/MICA alone + MICB in combination/ MICB alone where A and B the antimicrobials tested. Interactions were interpreted as synergy for FIC index <= 0.5, indifference for >0.5–4 and antagonism for >4. **Results:** A total of 30 non-duplicate *K.pneumoniae* isolates displaying different phenotypic profiles were evaluated. The MIC range of tigecycline, colistin, gentamicin and fosfomycin were 0.25-8, 0.125-512, 1->256 and 32->1024μg/ml with MIC50/90 of 2/4, 0.25/32, 2/>256 and 128/>1024μg/ml, respectively. Meropenem MICs ranged between 8->32μg/ml. *In vitro* interactions were evaluated in 120 isolate-antibiotic combinations. Tigecycline/fosfomycin combinations were evaluated only by Etest methods. The same test outcome by all methods was reported for 102 (85%) of isolate/antibiotic combinations. Agreement between the two Etest methods was observed in 92.5% while agreement between checkerboard and Etest imprint or Etest cross formation in 86.7% or 84.4%, respectively. Antagonism between tigecycline and other agents was rare, encountered in two isolates with tigecycline/meropenem (checkerboard) and one isolate with tigecycline/fosfomycin (Etest cross formation). Synergy was seen with colistin against 23.3% of the isolates (checkerboard) and against 16.7% of the isolates (Etest imprint). Synergy with gentamicin was seen against one isolate (3.3%) with both Etest methods. Synergy with meropenem was observed in two isolates with the imprint Etest. **Conclusions:** Checkerboard is a time-consuming and labour intensive method. Both Etest synergy methods appear to be a reliable alternative to checkerboard for testing antimicrobial combinations for routine use but It should be pointed out that the cross formation is simpler and more user-friendly.

Background

Carbapenemase producing (KPC) *Klebsiella pneumoniae* isolates have emerged as a major nosocomial pathogen in Greece constituting a real threat for hospitalized patients. Many of the circulating strains exhibit not only multi-drug (MDR) but some times extensively-drug (XDR) or even pan-drug resistance (PDR).
Tigecycline remains one of our last resorts and although is not currently approved for infections caused by MDR or XDR Gram-negatives, its off-label use is increasing globally because of the appealing in vitro spectrum of tigecycline and the lack of other active antimicrobial agents against those pathogens except for colistin.
The objective of this study was to evaluate the Etest imprint and the Etest cross formation methods for testing antimicrobial combinations compared to the checkerboard methodology.

References

- Giamarellou H, Poulakou G. Expert Opin Drug Metab Toxicol. 2011; 7(11): 1459-70.
- Giamarellou H. Int J Antimicrob Agents. 2010; 36 Suppl 2: S50-4.
- Pournaras et al. Int J Antimicrob Agents 2011;37:244-7.

Methods

- Clinical isolates were collected from Hygeia hospital between July 2011 and February 2012.
- VITEK 2 (bioMérieux) was used for identification and for initial susceptibility testing.
- Tigecycline, colistin, gentamicin, meropenem and fosfomycin (Sigma-Alldrich) MICs were measured by the broth microdilution method according to CLSI, using fresh cation-adjusted Muller-Hinton broth (Becton-Dickinson) and by Etest (bioMérieux) according to manufacturer instructions.
- Clonal relatedness was assessed by REP-PCR.
- Antibiotic interactions were assayed using the chequerboard MIC format (Figure 1) and two Etest methods: the imprint and the cross formation (Figure 2).
- Tigecycline/Fosfomycin combination was evaluated only by Etest methodology.
- The fractional inhibitory concentration (FIC) was calculated as: FIC index = MICA in combination/MICA alone + MICB in combination/ MICB alone where A and B were the antimicrobials tested. Interactions were interpreted as synergy for FIC index ≤ 0.5, indifference for FIC >0.5–4 and antagonism for FIC >4.
- Carbapenemase and ESBL genes were determined by PCR.

Results

- A total of 30 non-duplicate isolates displaying different phenotypic profiles were evaluated.
- The MIC range of Tigecycline, Colistin, Gentamicin, Meropenem and Fosfomycin are presented in Table 1.

Table 1: Susceptibility of the studied isolates to the antibiotics tested

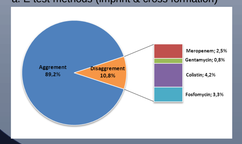
	Antimicrobial Agent				
	Meropenem	**Tigecycline**	**Colistin**	**Gentamycin**	**Fosfomycin**
MIC range (mg/L)	8->32	0,25-8	0,125-512	1- >256	32- >1024
MIC_{50} (mg/L)	>32	2	0,25	2	128
MIC_{90} (mg/L)	>32	4	32	>256	>1024
Susceptibility (%)*	0	40	60	70	10

*According to EUCAST breakpoints

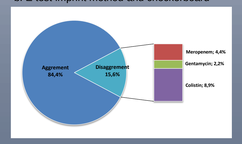
- *In vitro* interactions were evaluated in 120 isolate-antibiotic combinations.
- The same test outcome by all methods was reported for 102 (85%) of isolate/antibiotic combinations.
- Agreement between the two Etest methods was observed in 92.5% while agreement between checkerboard and Etest imprint or Etest cross formation in 84.4% or 86.7%, respectively (Figure 1).
- Antagonism between tigecycline and other agents was rare, encountered in two isolates with tigecycline/meropenem (checkerboard) and one isolate with tigecycline/fosfomycin (Etest cross formation).

Figure 1. Agreement between synergy detection methods

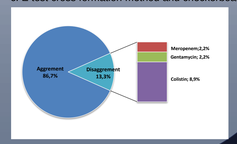
a. E test methods (imprint & cross formation)



b. E test imprint method and checkerboard



c. E test cross formation method and checkerboard

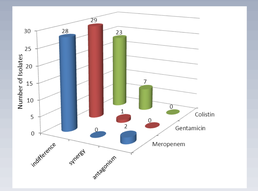


Results

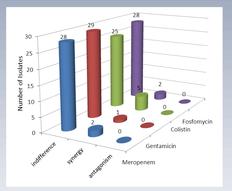
- Synergy was seen with colistin against 23.3% of the isolates (checkerboard) and against 16.7% of the isolates (Etest imprint). (Figure 2, Table 2).
- Synergy with gentamicin was seen against one isolate (3.3%) with both Etest methods (Figure 2).
- Synergy with meropenem was observed in two isolates with the imprint Etest. (Figure 2).

Figure 2. Results of synergy testing

a. Checkerboard method



b. E test imprint method



c. E test cross formation method

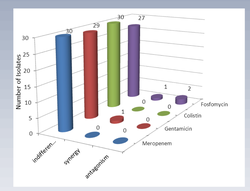


Table 2. Fractional inhibitory concentration for Tigecycline/Colistin combination

			MIC (mg/L)		FIC		
Isolate	**Isolate Resistance Marker**	**Clonal type**	**Tigecycline**	**Colistin**	**Checkerboard**	**Etest Imprint**	**Etest Cross formation**
34	KPC	A1	1	0,125	2	1	1
37	KPC	A2	1	0,125	0,98	2	2
39	KPC	K1	1	0,125	0,98	2	2
40	KPC, CTX-M-15	K2	1	0,125	0,98	2	2
41	KPC, CTX-M-15	L	1	0,125	1,96	2	2
38	KPC, SHV-12	J	2	0,125	0,51	2	2
24	KPC	A2	2	0,125	1,46	2	2
5	KPC	D	4	0,125	0,73	2	2
2	KPC, SHV-12	D	0,5	0,25	2	3	2
6	KPC, SHV-12	A1	1	0,25	2	3	2
35	KPC, SHV-12	A2	1	0,25	0,51	2	2
4	KPC, SHV-12	B	1	0,25	2	3	2
28	KPC, SHV-12	A3	2	0,25	0,73	2	2
27	KPC, SHV-12	A1	2	0,25	2	2	2
31	KPC, SHV-12	A1	8	0,25	0,54	1,5	2
17	KPC, SHV-12	A1	1	0,5	0,53	1	1,5
33	KPC, SHV-12	A3	4	0,5	0,56	2	1,5
29	KPC, SHV-12	A4	1	2	0,38	1	1,5
44	KPC	ND	4	4	0,5	2	2
30	KPC, SHV-12	H	1	8	0,75	2	1,5
12	KPC, SHV-12	A1	1	16	0,38	1	1
3	KPC, SHV-12	A1	1	16	0,5	0,75	0,75
36	KPC, VIM	C	1	16	0,5	2	2
16	KPC	A1	2	16	0,38	0,38	0,75
23	KPC, SHV-12	B	2	16	2	1	1
1	KPC, CMY	F	2	16	0,25	0,38	0,625
22	KPC	C	16	16	2	0,41	0,53
11	KPC, VIM, SHV-12	E	2	32	0,51	1,5	1,5
25	KPC	G	2	32	0,56	0,25	1
42	KPC, SHV-12	M	4	64	0,53	0,5	1

Conclusion

- Combinations of Tigecycline with other antimicrobials produce primarily an indifferent effect.
- The most promising combination was that with Colistin.
- Both Etest synergy methods appear to be a reliable alternative to checkerboard for testing antimicrobial combinations for routine use but it should be pointed out that the cross formation is simpler and more user-friendly.

Contact Information: Lamprini Galani, Hygeia General Hospital, 4 Erythrou Stavrou Street. & Kifisias Avenue, 151 23 Marousi, Athens Greece, Tel : ++32106867946, Fax : ++32106867377, E mail: lgalani@gmail.com