P1009
24th ECCMID
Barcelona

Determination of the sensitivity and specificity of the MIC Test Strips (MTS) mechanisms of detection against in-house methods for 644 multi-drug resistant (MDR) strains: a European multi-centre study

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Introduction. ESBL positive and Carbapenemases positive Enterobacteriaceae (CPE) have now become a global concern. CPE are often express MDR and XDR phenotypes with increasing reports of pan-resistance. The spread of ESBLs is regions of low resistance and KPC, MBLs and OXA-48/181 in regions where ESBLs are established impact on therapeutic failure and increase morbidity.

Therefore, the implementation of a rapid and accurate assessment to detect antibiotic resistance mechanisms in Gram-negative pathogens is essential. To evaluate various Liofilchem® MTS in detecting extended-spectrum β-lactamases (ESBLs) and carbapenemases [metallo-β-lactamases (MBLs) and OXA-48 (and OXA-181)]. Five centres across Europe and one in Russia tested the MTS against in-house methods.

Methods. In total, 644 MDR Gram-negative isolates (103 from Greece; 104 from Poland; 66 from Italy; 125 from the UK; 110 from Spain and 136 from Russia) (Table) were tested. In-house methods for phenotype included agar dilution, microbroth dilution, double-disk synergy and modified Hodge test. In-house genotype testing included PCR, PCR fragment restriction digestion and sequencing. Those isolates containing only ESBLs, MBLs, OXA-48 and AmpC numbered 285, 86, 30 and 21, respectively. Of the double mechanisms of resistance ESBL plus MBL and ESBL plus OXA-48 were the most numerous with 61 and 21, respectively. In-house control ATCC and NCTC strains were used to validate all data. In addition we also tested 10 strains with defined phenotypes under double-blind conditions – see Table 1

Table 1. Results of accuracy of MTS for detecting β-lactamases with defined genotypes across 5 countries

		relevant genotype	ESBL MTS			MBL MTS		KPC (MER)	KPC (ERT)
Ref.	Species		CTX	CTZ	FEP	IMI	MER		
C1	E. coli		6/6	6/6	6/6	6/6	6/6	6/6	5/6
C2	P. stuarti	TEM-1, CTX-M-15	6/6	6/6	6/6	6/6	6/6	6/6	6/6
C3	E. coli	TEM-1, OXA-1, CTX-M-15	6/6	6/6	6/6	6/6	6/6	6/6	6/6
C4	Klebsiella	SHV-1, OXA-48	6/6	6/6	6/6	6/6	6/6	4/6	4/6
C5	Klebsiella	TEM-1, DHV-1, CTX-M-15, OXA-1,	6/6	6/6	6/6	6/6	6/6	6/6	5/6
C6	E. coli	DHA-1, NDM-1	6/6	6/6	6/6	6/6	6/6	4/6	4/6
C7	Enterobacter	CMY-2	6/6	6/6	6/6	6/6	6/6	6/6	6/6
C8	Klebsiella	TEM-1, SHV-1, CTX-M-15, OXA-1, CMY-7	6/6	6/6	6/6	6/6	6/6	6/6	6/6
C9	Klebsiella	SHV-1, KPC	6/6	6/6	6/6	6/6	6/6	6/6	6/6
C10	Klebsiella	TEM-1, SHV-1, CTX-M-15, OXA-1, DHA-1, NDM-	6/6	6/6	6/6	6/6	6/6	2/6	2/6

Results. In Table 2, the numbers denote the correct result according to the in-house phenotype. The sensitivity and specificity for the ESBLs ranged from 84-100% and 78-100%, respectively; and for MBLs were 85-100% and all 100%, respectively. The overall sensitivity and specificity for ESBLs and MBLs were 95 and 93%, and 95 and 100%, respectively. Those strains containing both types of enzymes had an overall sensitivity of 98%. Detection of OXA-48 was based on carbapenem resistance but non-MBL and non-KPC (data not shown) and had an overall sensitivity of 26/30 (87%).

Table 2. MTS Sensitivity and Specificity against in-house genetic methods in 6 centres (ND; not detected)

		ESBLs	MBLs	OXA-48	AmpC	ESBL + MBL	ESBL + OXA-48	ESBL + AmpC	MBL + OXA-48
Site 1	Sensitivity	19/20* (95)	21/24**(88)	2/2# (100)	6/8 (75)	8/8 (100)	3/4 (75)	3/4 (75)	/0
	Specificity	80/83 (96)	79/79 (100)						
		* 1 ND	** 1 NEG	# none of			3 ESBL POS	1 negative	
			** 2 ND	MBL and KPC			1 ND		
Site 2	Sensitivity	49/52* (94)	25/26**(96)	0/1# (0)	/0	/0	/0	/0	/0
	Specificity	41/52 (80)##	78/78 (100)						
		* 3 ND	** 1 NEG	# KPC POS					
Site 3	Sensitivity	22/23* (96)	9/14**(64)	0/2# (0)	5/6 (83) ##	/0	/0	/0	/0
	Specificity	43/43 (100)	44/44 (100)						
		* 1 NEG	** 2 NEG	# POS for KPC	## POS for KPC				
			** 3 ND	MBL and KPC					
Site 4	Sensitivity	42/42 (100)	11/11 (100)	/0	/0	35/36 (97)	/0	4/4 (100)	2/6
	Specificity	80/83 (96)	79/79 (100)						4 ND
Site 5	Sensitivity	42/50 (84)*	11/11 (100)	24/24 (100)	5/5 (100)	/0	4/4(100)	1/1 (100)	/0
	Specificity	47/60 (78)	99/99 (100)						
Site 6	Sensitivity	98/98 (100)	/0	0/1 (0)	/0	17/17 (100%)	13/13 (100)	/0	/0
	Specificity	38/38 (100)	118/119 (99%)						
	TOTAL Specificity	272/285 (95)	82/86 (95)						
	TOTAL Sensitivity	333/359 (93)	497/498 (100)						

Conclusions. This is the first European multi-centre study evaluating MTS detecting mechanisms of β-lactam resistance against in-house methods. The overall sensitivity and specificity for ESBLs and MBLs were very high and based on elimination of other carbapenem resistance (MBLs and KPC), detection of OXA-type carbapenems was acceptable. Therefore, for most types of β-lactam resistance mechanisms, MTS is a good alternative to routine methods .

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