P0835
Paper Poster Session
Problems in antimicrobial susceptibility testing

Assesing the reliability of gradient diffusion assays to determine meropenem MIC value in oxa-48 producing Klebsiella pneumoniae

Paula Pescador-Martín[1], Maria Rosa Gómez-Gil[1], Guillermo Ruiz-Carrascosa[2], Maria Pilar Romero-Gómez[1], Julio Garcia Rodriguez[3], José Ramón Pano-Pardo*[4]

[1]*Hospital Universitario La Paz, Madrid, Spain*

[2]*Hospital Universitario La Paz, Microbiología Y Parasitología, Madrid, Spain*

[3]*Hospital Universitario La Paz-Carlos III-Cantoblanco, Madrid, Spain*

[4]*Hospital Clínico Lozano Blesa, Zaragoza, Spain*

Background: Accurate meropenem MIC determination is essential to guide antibiotic therapy especially in infections caused by carbapenemase-producing *Enterobacteriaceae* (CPE) as it remains being first line therapy when susceptible. We have evaluated the agreement between the MIC values for meropenem obtained by microdilution and gradient diffusion assays in OXA-48 producing *Enterobacteriaceae* isolated from blood cultures.

Material/methods: Ninety-two clinical OXA-48-CPE isolates collected from blood cultures from December 2010 to January 2015 in Hospital La Paz, Madrid, were included.

MIC testing was conducted by panel 44 Microscan® (Siemmens) microdilution and 2 different brands of gradient diffusion assays (bioMérieux Etest® and Liofilchem® MIC Test Strips). All the assays were read by two independent observers.

Among the strains that presented colonies in the inhibition zone, two MIC values were read: one, considering the intra-halo colonies near the edge of the strip according to manufacturer instructions, and another not considering them.

The results obtained by gradient diffusion assays were compared with those from microdilution assays and agreement was assessed based on MICs and EUCAST interpretative criteria (Clinical breakpoints v 5.0). Agreement was defined as identical MIC results or MIC results agreeing within ±1 log_2 dilution by both methods.

Clonality was studied among 61 of the *Klebsiella pneumoniae* strains, using DiversiLab® system (bioMérieux) and ST405/ST11 homemade clone specific PCR.

Categorical variables were compared using a chi-squared test.

Results: The 92 clinical isolates were identified as *K. pneumoniae* (n=80), *Escherichia coli* (n=7), *Serratia marcescens* (n=3), *Enterobacter cloacae* (n=1) and *Enterobacter aerogenes* (n=1). In reference to the clonality study for *K. pneumoniae*, 36% belonged to ST405 and 51% to ST11.

The distribution of meropenem MIC values determined by microdilution and E-test ranged from ≤ 1 to ≥ 8 μg/mL.

Gradient diffusion interpretation by two independent observers found 100% of agreement and the agreement between Etest® and Liofilchem® assays was 97%.

Forty-five strains out of ninety-two presented colonies in the E-test inhibition zone. Forty-two of them were *K. pneumoniae*, one *E. coli*, one *E. aerogenes* and one *S. marcescens.*

The rates of agreement between E-test and microdilution, as well as the error rates, for the *K.pneumoniae* strains with or without colonies in the inhibition zone are shown in the table.

The presence of scattered colonies in the inhibition zone was independent of the brand of the strips (p=1) and the clone (p=0.728).

Type of *K.pneumoniae* strain in reference to E-test phenotype		% Agreement			No. (%) of errors		
		Same	±1	±2	Very major	Major	Minor
Without intra-halo colonies (n=38)		79.31	89.47	94.73	0 (0)	0 (0)	5 (13)
With intra-halo colonies (n=42)	Not considering the colonies	30.95	78.57	90.47	3 (75)	0 (0)	7 (17)
	Considering the intra-halo colonies	19.04	30.95	59.52	1 (25)	11 (35)	22 (52)

Conclusions: Gradient diffusion has a good agreement (89.5%) with microdilution for OXA-48-producing *K.pneumoniae* isolates that do not present intra-halo colonies.

For OXA-48-producing *K.pneumoniae* isolates that present intra-halo colonies, gradient diffusion is not reliable (agreement<80%) although agreement is better when not considering the intra-halo colonies to determine the MIC.

The presence of colonies in the inhibition zone is neither clone-dependent nor strip brand-dependent.