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ASSESING THE RELIABILITY OF GRADIENT DIFFUSION ASSAYS TO DETERMINE MEROPENEM MIC VALUE IN OXA-48 PRODUCING *Klebsiella pneumoniae.*

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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.

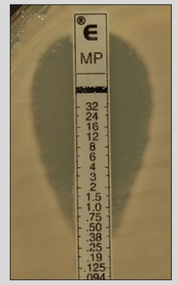
MATERIAL AND METHODS

- 92 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.
- 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 presence of scattered colonies in the inhibition zone was independent of the brand of the strips (p=1) and the clone (p=0.728)
- 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.

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)



Phenotype without intrahalo colonies

Phenotype with intrahalo colonies

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