P0834
Paper Poster Session
Problems in antimicrobial susceptibility testing

Colistin stability and MIC testing in agar dilution in comparison to E-test, micro- and macrobroth

Agata Turlej-Rogacka*[1], Xavier Basil Britto[1], Lore Janssens[1], Christine Lammens[1], Olympia Zarkotou[2], Youri Glupczynski[3], Herman Goossens[1], Surbhi Malhotra-Kumar[4]

[1]*University of Antwerp, Laboratory of Medical Microbiology, Wilrijk, Belgium*

[2]*Tzaneio General Hospital of Piraeus, Department of Microbiology, Piraeus, Greece*

[3]*Centre National de Référence de la Résistance Aux Antibiotiques, Chu Dinant-Godinne Ucl Namur, Yvoir, Belgium*

[4]*University of Antwerp, Laboratory of Medical Microbiology, Wilrijk, Antwerpen, Belgium*

Background: The emergence and spread of multidrug resistant Gram-negative bacteria has led to the reintroduction of old antibiotics, such as colistin, into clinical practice. However, its unique physical properties challenge antibiotic susceptibility testing (AST) with no standard method defined to date. Additionally, heteroresistant cell population further complicates colistin AST. Here, we compared microbroth, macrobroth, E-test and agar dilution methods for colistin MIC testing.

Material/methods: Colistin susceptibility testing was performed according to the CLSI guidelines with colistin sulphate salt (Lot#SLBD8306V, Sigma) and set of six strains (four clinical isolates and two controls) (Table). Agar dilutions were performed twice, whereas other methods were repeated at three different time-points. Additionally, the shelf life of colistin agar plates was tested over one week, and MIC reproducibility and distribution of colistin in agar was evaluated by testing in triplicates at each time point. The obtained MICs were read independently by two researchers. To investigate solubility and distribution of colistin in agar plates, strains were spotted on different regions of the plate (Figure A). In order to control the material influence, we tested microbroth in both 96-well polystyrene plate and in 16-well glass bottom plates. For heteroresistance detection, growth of CS-1 was monitored for 24h at 37°C using a Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific) with readings every 15 minutes.

Results: MIC readings were highly comparable between the investigators with Cohen's kappa coefficient of 0.948. The summary of the obtained MICs can be found in the table. The overview of the mean values of $\log_2$MICs of all tested methods is presented in figure B. For agar dilutions we obtained very reproducible results irrespective of the spot position and the batch. We also found that colistin is stable in agar plates over one week (Figure C). When investigating heteroresistance, the growth of CS-1 was detected even in medium supplemented with 8 µg/ml of colistin (Figure D).

Conclusions: Both broth dilution methods showed variability that might be explained by the presence of heteroresistant subpopulations, which we confirmed for the CS-1 strain. E-test always underestimated the MIC value observed with the other methods. In conclusion, we found that agar dilution method performed very well in our hands and it provided the most reliable results out of all methods.

Table. Obtained MIC values.

Strain	Species	Isolation site	Agar (µg/ml)	Macrobroth (µg/ml)	Microbroth (µg/ml) polystyrene plate	Microbroth (µg/ml) glass-bottom plate	E-test (µg/ml)
CS-1	*Klebsiella pneumoniae*	Bronchial-aspirate	0.25-0.5	0.25-32	0.5-4	0.5-16	0.125-0.25
CS-2	*Klebsiella pneumonia*	Blood	0.25-0.5	0.5-32	0.5-64	0.25-4	0.125-0.25
CR-1	*Klebsiella pneumoniae*	Infection site	128-256	32-64	64-128	64	4-8
CR-2	*Klebsiella pneumoniae*	Infection site	128	32-64	64-128	32	4-8
ATCC25922	*Escherichia coli*	Not applicable	0.25	0.25-2	0.5-4	0.25	0.125
ATCC27853	*Pseudomonas aeruginosa*	Not applicable	2	0.5-1	1-2	0.5	0.5-1

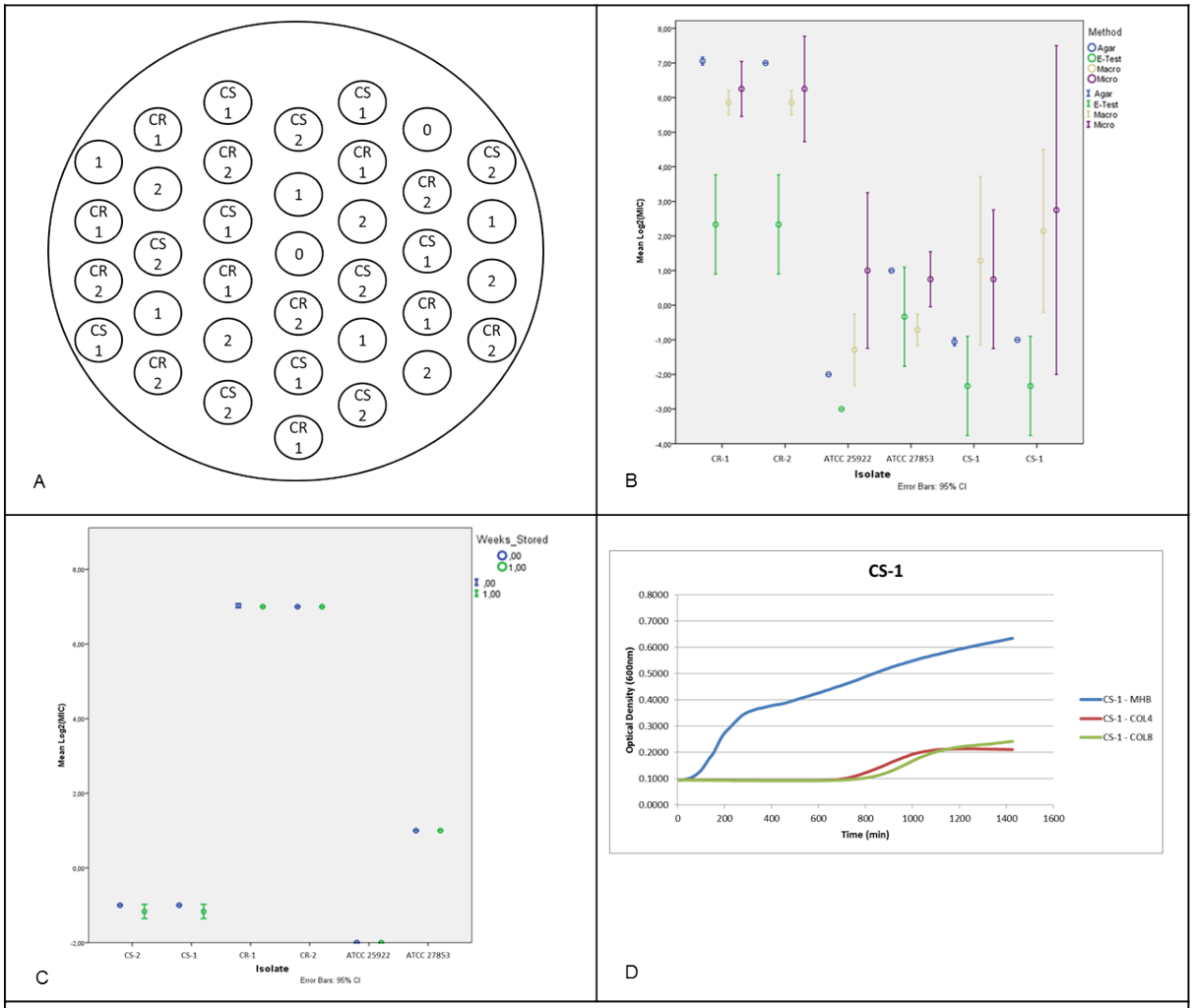

Figure A: Strain distribution scheme for agar dilution, 0 – blank, 1 – ATCC25922, 2 – ATCC27853; **B:** Overview of the mean values of log_2MICs of all the tested methods; **C:** Plates storage influence on log_2MIC value; **D:** Growth assays utilizing CS-1 without colistin (MHB) and with colistin (COL4 – 4 µg/ml, COL8 – 8 µg/ml).