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

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Introduction

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[1,2]. In addition, heteroresistant cell populations further complicate colistin AST. Here, we compared microbroth, macrobroth, E-test and agar dilution methods for colistin MIC testing.

Material and Methods

Colistin susceptibility testing was performed using colistin sulphate salt (Lot#SLBD8306V, Sigma) and a 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. MIC cut-offs were set according to the EUCAST, since there are no colistin breakpoints for Enterobacteriaceae in CLSI guidelines[1,2]. 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 triplicate 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)**. 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.

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

Table. Overview of the strains used in the study and the obtained MIC values.

Results and Discussion

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 for over one week **(Figure C)**. On investigating heteroresistance, the growth of CS-1 was detected even in medium supplemented with 8 µg/ml of colistin **(Figure D)**.

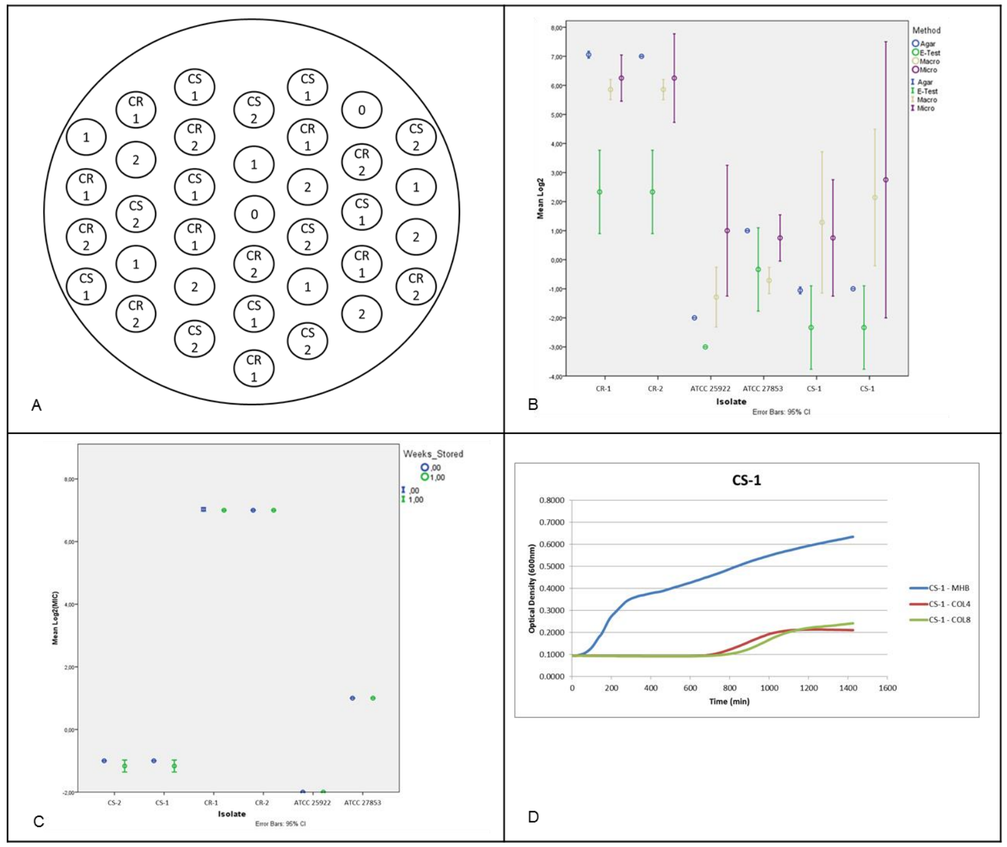

Figure. A: Strain distribution scheme for agar dilution, 0 – blank, 1 – ATCC25922, 2 – ATCC27853; **B:** Overview of the mean values of $\log_2$MICs obtained with all tested methods; **C:** Influence of agar plate storage on $\log_2$MIC values; **D:** Growth assays utilizing CS-1 without colistin (MHB) and with colistin (COL4 – 4 µg/ml, COL8 – 8 µg/ml).

Both broth dilution methods showed variability that might be explained by the presence of heteroresistant subpopulations, which we confirmed for the CS-1 strain. MIC values for CR strains obtained with E-test were significantly lower than with the other methods (e.g. 16-folds compared to the agar dilution). Such high discrepances were not obsereved for colistin sensitive strains. In conclusion, we found that agar dilution performed very well in our hands and provided the most reliable results of all methods tested.

References

(1) The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0, 2016. http://www.eucast.org.
(2) CLSI. *Performance Standards for Antimicrobial Susceptibility Testing.* 26th ed. CLSI supplement M100S. Wayne, PA: Clinical and Laboratory Standards Institute; 2016

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