

Detection of Colistin resistant P. aeruginosa, A. baumannii and Enterobacteriaceae

It is well-known the detection of P. aeruginosa showing colistin MICs of 4-16 ug/ml (R) cannot be accurately detected using the current disk diffusion method. Only high-level resistance (MIC \geq 128 ug/ml) will show no zone of inhibition around a 10 ug disk, and detected.

Testing of Acinetobacter, B. cepacia and Stenotrophomonas is unreliable by the current disk diffusion method.

Testing of one compound (colistin or polymyxin B) predict the results for the other. Colistin is preferred for testing, because it is likely to be used clinically and it is also slightly more sensitive in detecting resistance.

Siqueira et al (3) studied the importance of medium, calcium concentration and inoculum when testing polymyxin B against Acinetobacter spp and P. aeruginosa. Higher calcium content resulted in higher MICs

Lo-Ten-Foe (17), vann der Heijden (5) compared different methods: disc diffusion, E-test, broth microdilution, Vitek 2 and agar dilution for colistin susceptibility testing against P. aeruginosa, A baumannii, and heteroresistant E. cloacae.

The Vitek 2 displayed low sensitivity in the detection of heteroresistant subpopulations of E. cloacae. The current disk diffusion was an unreliable method for testing colistin.

Hiramatsu et al (6) compared disk diffusion, Etest and agar dilution for testing the susceptibility of metallo-beta-lactamases producing P. aeruginosa to polymyxins. The authors found poor concordance between disk diffusion and Etest methods compared to agar dilution

Goi-Barisic (7) and Reis (1) found that the current disk diffusion test with colistin was not adequate for routine testing of A. baumannii.

Ko et al (13) report the emergence of colistin-resistant K. pneumoniae isolates from blood in Korean hospitals.

Landman et al (2) report that citywide emergence of P. aeruginosa strains with reduced susceptibility to polymyxin B. 61 % of the isolates were susceptible to imipenem and 5% of the isolates had reduced susceptibility to polymyxin B (MIC 4 – 8 ug/ml).

Kitzis et al (8) report very high frequency of mutation to colistin resistance in gram negative bacteria, and resistance could not be detected by the current disc-diffusion for most resistant mutants.

Savini et al (15) report the isolation of a colistin-resistant Hafnia alvei, representing a serious clinical and microbiological concern.

Halaby et al (14) report the emergence of colistin resistance during use of selective decontamination of the digestive tract in an ICU, masked by the use of inappropriate laboratory methods. Colistin resistance among ESBL producing K. pneumoniae isolates emerged after the introduction of selective decontamination and the routinely used disc diffusion method failed to detect colistin resistance.

Rosco Diagnostica has developed the 2+18 (22) hours prediffusion method, permitting the detection of colistin resistant strains.

Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs™

Prediffusion method (2+18 or 2+22 hours) for antimicrobials diffusing poorly on agar

Page 2 / 5 - Document: 7.5.0

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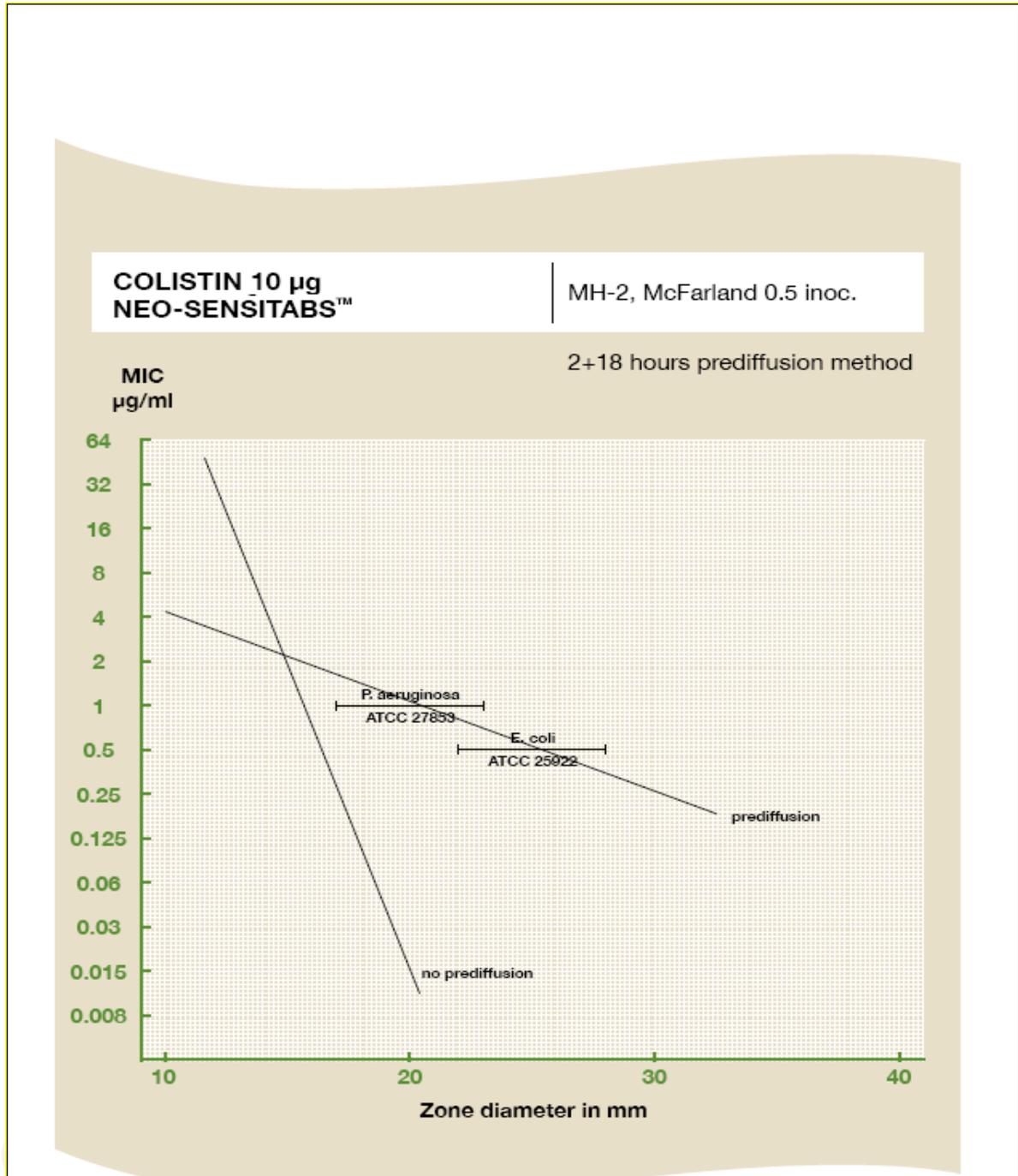
Procedure

- 1) One Colistin 10 ug Neo-sensitabs is placed on an uninoculated plate, containing plain MH agar.
- 2) After 2 hours at room temperature, the tablet is removed (by knocking the plate against the table) and the short name COL is written on the back of the plate, for identification purposes.
- 3) The plate is maintained at room temperature for further 18-22 hours (overnight).
- 4) The plate is now inoculated by the strain to be tested, using Mc Farland 0.5 inoculum and additional antimicrobial disks (Neo-sensitabs) may be added using a dispenser. Thereafter incubate at 35 degrees overnight.
- 5) The zones of inhibition are measured and compared with the corresponding zone breakpoints.

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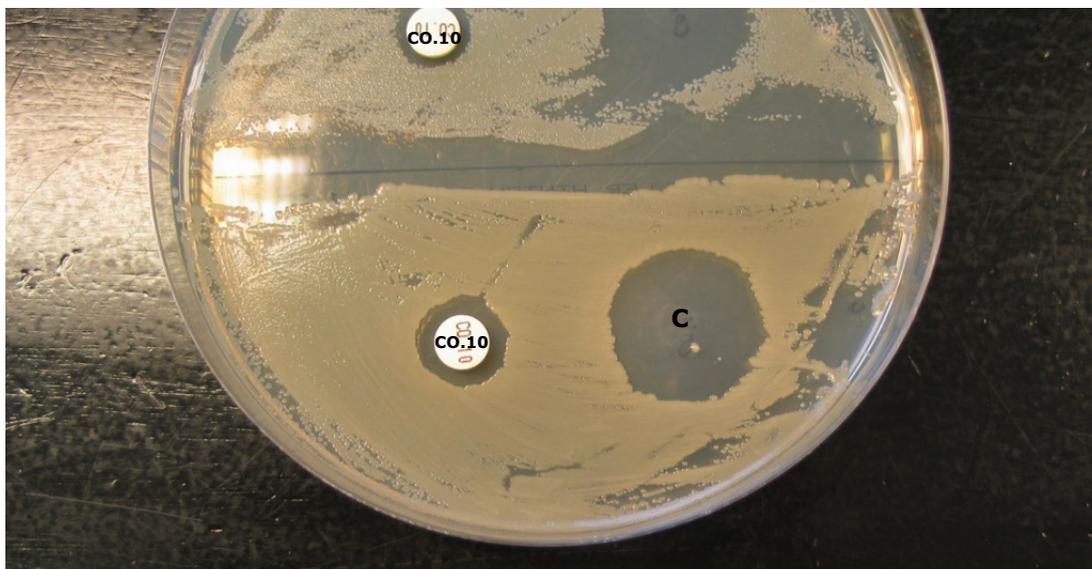
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Colistin susceptible *E. coli* ATCC 25922

C=Colistin

Interpretation

Heteroresistant strains will currently show colonies inside the inhibition zone.

Susceptible strains: inhibition zone ≥ 15 mm (MIC ≤ 2 ug/ml).
Intermediate (indeterminate): 14- 11 mm
Resistant: ≤ 10 mm (MIC > 4 ug/ml)

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