Detection of staphylococci and enterococci non-susceptible to daptomycin using the Neo-Sensitabs prediffusion method

Daptomycin, due to its large molecular weight, diffuses slowly into agar, resulting in small differences in the size of inhibition zones between susceptible and non-susceptible isolates.

Extending the diffusion time by a prediffusion method and eliminating the daptomycin depot after 2 hours, allow for better discrimination of non-susceptible isolates showing MICs near the susceptible breakpoint.

Data from a recent study (Katz et al 1) suggests that the 2+18 hours prediffusion method using Daptomycin (DAPCa) Neo-Sensitabs is a promising method for discriminating between daptomycin susceptible and non-susceptible isolates.

Principle

When using the 2+18/22 hours prediffusion, daptomycin has 4 times longer time to diffuse into the agar, before bacterial colonies are formed, than with the current diffusion method. This results in a much larger zone size difference between 2 consecutive MIC, as it can be seen in the enclosed regression line.

Another important feature is that with the prediffusion method the antimicrobial depot is eliminated after 2 hours, avoiding further antibiotic diffusion from the depot into the agar.

Procedure

1) One Daptomycin 30 µg Neo-Sensitabs (DAPCa) is placed on an uninoculated plate, containing plain MH agar.
2) After 2 hours at room temperature, the tablet is removed (knocking the plate against the table) and a short name (DAP) 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 with the strains to be tested using a McFarland 0.5 inoculum. Additional antimicrobial disks (Neo-Sensitabs) may be added now using a dispenser. Thereafter incubate at 35 degrees, overnight.
5) The zones of inhibition are measured and compared with the corresponding zone breakpoints.

Interpretation

a) Staphylococci

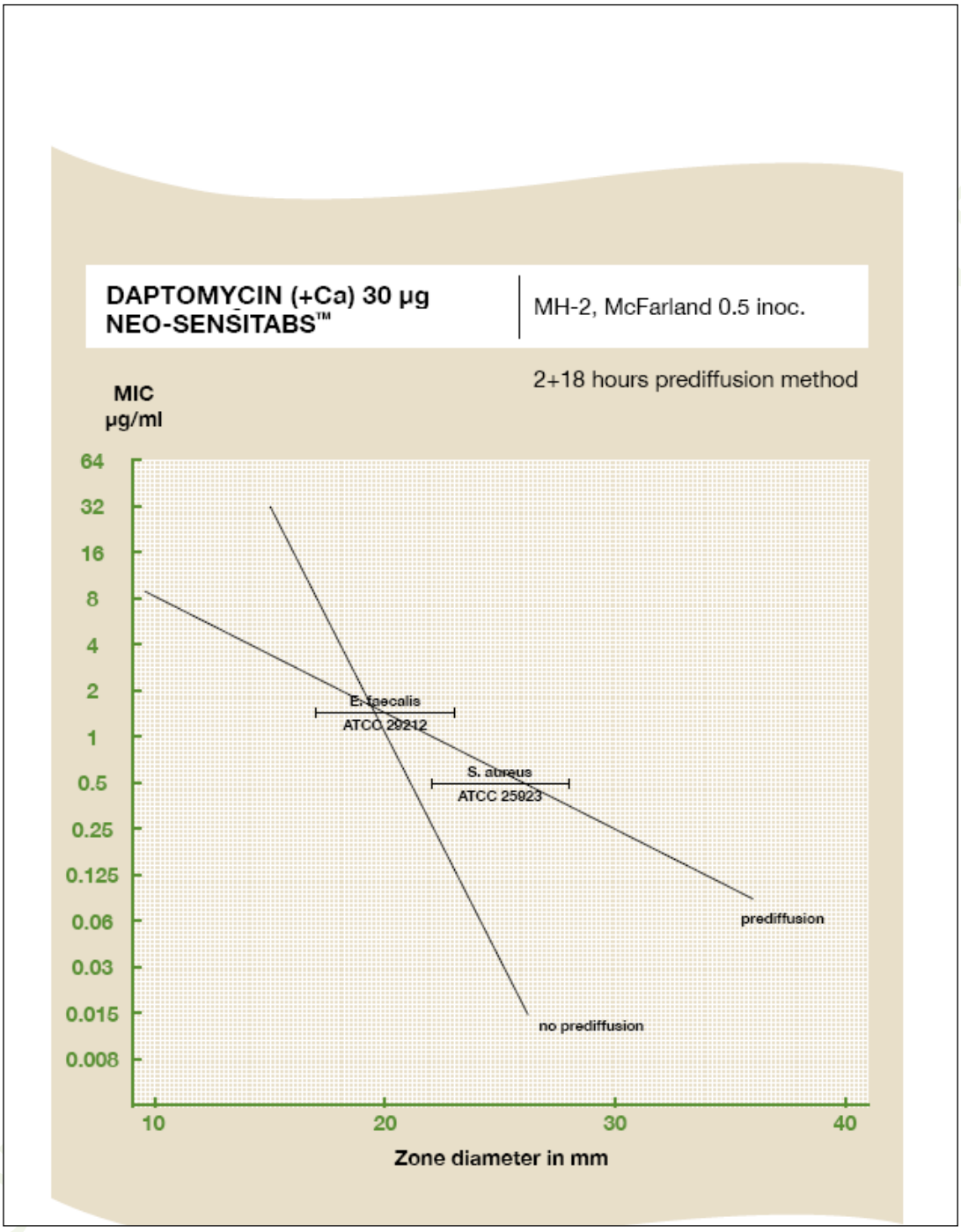
Susceptible:	zone of inhibition ≥ 22 mm (corresponding to MIC ≤ 1 µg/ml)
Non-susceptible:	zone of inhibition < 20 mm (corresponding to MIC ≥ 2 µg/ml)
Indeterminate:	zone 20-21 mm.

b) Enterococci

Susceptible:	zone of inhibition ≥ 12 mm (corresponding to MIC ≤ 4 µg/ml)
Resistant:	no zone of inhibition

S. aureus ATS 403, GISA strain, Daptomycin susceptible

S. aureus AST 408 GISA strain Daptomycin non-susceptible.


DAPTOMYCIN (+Ca) 30 µg
NEO-SENSITABS™
MH-2, McFarland 0.5 inoc.
2+18 hours prediffusion method
MIC
µg/ml
64
32
16
8
4
2
1
0.5
0.25
0.125
0.06
0.03
0.015
0.008
E. faecalis
ATCC 29212
S. aureus
ATCC 25923
prediffusion
no prediffusion
10
20
30
40
Zone diameter in mm

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