Colistin Susceptibility testing

Recommendation:

*** Broth Micro Dilution ONLY should be used for susceptibility testing of colistin ***

Introduction

Colistin (Polymixin E) was first introduced in 1952 and is usually administered as the prodrug, Colistin Methanesulfonate (CMS). During the last 5 years colistin has become the drug of "last resort" in infections caused by Multi Drug Resistant Gram-negative bacilli, in particular those isolates harbouring carbapenemases.

Resistance

Colistin resistance can be intrinsic (*Proteus* spp, *Morganella morganii*, *Serratia* spp, *Burkholderia* spp and some group D Salmonella serovars) or acquired (*E. coli*, *Salmonella spp., Klebsiella spp., Pseudomonas aeruginosa*, *Acinetobacter spp).* Acquired resistance may include over expression of outer membrane proteins, Lipopolysaccharide modifications or efflux mechanisms. More worryingly, there have been recent reports of colistin resistance conferred by the mobile colistin resistance gene (mcr) and acquired on a plasmid.

Susceptibility testing

Antimicrobial susceptibility testing of colistin has been fraught with difficulties. Studies performed in collaboration with BSAC, EUCAST & CLSI have concluded that ONLY Broth Micro Dilution (BMD) can be used to susceptibility test colistin. Colistin BMD results are reliable for both susceptible and non-susceptible isolates. Results from many studies clearly show that disc diffusion tests cannot reliably discriminate between susceptible and resistant isolates, probably due to the poor diffusion of colistin. Currently available gradient tests underestimate colistin MIC values and undercall resistance, and should be avoided, even when quality control results are within range. Agar dilution tends to underestimate colistin MIC values and undercalls resistance. This method is not commonly used within diagnostic laboratories but should be avoided as a reference method. It should be noted that the failure of agar dilution tests would indicate that screening plates based on the inclusion of colistin in agar may fail to screen for colistin resistant organisms. Although no systematic evaluation of semi-automated systems has been performed, reports from the EUCAST network of collaborative laboratories and the results from NEQAS distributions show frequent occurrence of Very Major Errors. Users of semi-automated devices should apply rigorous QC (see below) and check with the manufacturer whether or not they are confident that their method for colistin AST gives correct results.

Recommendations

Use the following method for susceptibility testing colistin:

- MIC determination by Broth Micro Dilution (BMD)

Commercial BMD systems are available for colistin susceptibility testing and have been evaluated by EUCAST (see below). Alternatively isolates can be referred to Reference laboratories for a colistin MIC. However charges may apply.

Diagnostic laboratories which have implemented the BMD method for colistin susceptibility testing have experienced an increase in colistin resistance in Gram negative bacteria.

The following methods gave unacceptable levels of Major Errors (ME) and Very Major Errors (VME):

- Disc diffusion
- Gradient strips (All manufacturers)
- Agar dilution
- Breakpoint method
- Semi Automated methods (All manufacturers)

Quality Control

Colistin susceptibility testing must be performed with both:

- Colistin susceptible *E. coli* ATCC 25922 OR *P. aeruginosa* ATCC 27853
- Colistin resistant *E. coli* NCTC 13846 (*mcr*-1 positive) MIC range: target value 4 mg/L (and only occasionally 2 or 8 mg/L).

Summary of results for all commercial BMD methods

Data published on the EUCAST website: http://www.eucast.org/ast_of_bacteria/warnings/#c13111 (please check regularly for updates), and as a full article in CMI http://www.clinicalmicrobiologyandinfection.com/article/S1198-743X(17)30667-5/pdf.

75 susceptible and non-susceptible isolates tested.

Manufacturer	Essential Agreement (MIC within ±1 ref MIC)	Major Errors (False resistance)	Very Major Errors (False susceptibility)
Sensititre (Thermo Fisher Scientific)	**96%**	**4 (5.3%)**	**0 (0%)**
MICRONAUT-S (Merlin Diagnostika)	**96%**	**6 (8%)**	**2 (2.7%)**
MICRONAUT MIC-Strip (Merlin Diagnostika)	**99%**	**5 (6.7%)**	**2 (2.7%)**
SensiTest (Liofilchem)	**88%**	**7 (9.3%)**	**1 (1.3%)**
UMIC (Biocentric)	**82%**	**3 (4%)**	**3 (4%)**