P1017
Paper Poster Session
Culture-based diagnostic bacteriology

A universal screening culture medium for polymyxin-resistant Gram-negative bacteria

Patrice Nordmann[1], Aurélie Jayol[2], Laurent Poirel*[1]

[1]*University of Fribourg, Fribourg, Switzerland*

[2]*University of Fribourg, Laboratory of Microbiology, Department of Medecine, Fribourg, Switzerland*

Background: Multidrug resistant (MDR) Gram negative rods are emerging rapidly worldwide and therapeutic options are becoming limited. However those MDR remain mostly susceptible to polymyxins (colistin and polymyxin B) but emergence of polymyxin resistance was described worldwide.

We have developed a selective culture medium, the SuperPolymyxin medium, to detect any type of polymyxin-resistant gram negative bacteria.

Material/methods: The SuperPolymyxin medium was prepared using an Eosine Methylene Blue agar (EMB)-based culture medium wherein colistin or polymyxin B, daptomycin and amphotericin B were added.

Performance of the SuperPolymyxin medium was evaluated with a total of eighty-two Gram-negative strains from various enterobacterial and non-fermenters species. Seven strains were from intrinsically polymyxin-resistant species, 44 strains exhibited acquired resistance to polymyxins and 31 strains were polymyxin-susceptible. Some of those strains had well-characterized mechanisms of resistance, such as mutations in the *pmrA* or *pmrB* genes (*K. pneumoniae*, *A. baumannii*), alterations in the *mgrB* gene or its promoter sequences (*Klebsiella* spp.), or produced the plasmid-mediated MCR-1 colistin resistance determinant (*E. coli*).

The lowest limit of detection of the tested strains were determined and the sensitivity and specificity cut-off values were set at 1 x 10^3 CFU/ml.

MICs of polymyxins were determined using the broth microdilution method according to the Clinical Laboratory Standard Institute guidelines and results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing breakpoints.

Results: The basis of the SuperPolymyxin medium is the EMB medium that allows to distinguish between lactose fermenters giving colored colonies, and lactose non-fermenters giving colorless or light lavender colonies. Moreover, differentiation between lactose fermenters can be made since *E. coli* strains are dark blue-black colonies with a characteristic metallic green sheen, while *Enterobacter* spp. and *Klebsiella* spp. produce brown, dark-centered, mucoid colonies.

Polymyxin-resistant strains grew on the SuperPolymyxin medium in 24 h, except *P. aeruginosa* and *S. maltophilia* and the intrinsically polymyxin-resistant *Burkolderia* genus that grew in 24 to 48 h. The lowest limit of detection was below the cut-off value of 1 x 10^3 CFU/ml for all polymyxin-resistant strains, whereas the limit of detection of the polymyxin-susceptible strains was above 1 x 10^3 CFU/ml, being ≥ 1 x 10^6 CFU/ml. The sensitivity and specificity of the SuperPolymyxin medium for selecting polymyxin-resistant gram negatives were 100% in both cases regardless of the nature of the

polymyxin resistance mechanism (intrinsic, chromosomally or plasmid-encoded) and of its level (high or moderate).

Conclusions: The SuperPolymyxin medium is the first screening medium that is aimed to detect intrinsic and acquired polymyxin resistant Gram negative rods. Its usefulness applies for both human and veterinary medicine. In human medicine, it will contribute to an early identification of carriers of polymyxin-resistant strains, therefore preventing and containing outbreaks due to polymyxin-resistant isolates.