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Validation of a novel Chromatic Agar Selective against Colistin-resistant Enterobacteriaceae

E. Widlake[1], R. Marrollo[3], F. Brovarone[3], F. Demetrio[2], D. Vitagliano[2], F. Brocco[2], TR Walsh[1], E. Carretto[3], JM. Tyrrell[1]

[1]Department of Medical Microbiology & Infectious Diseases, Cardiff University, Heath Park, Cardiff, Wales, UK. [2]AScientific Division, Liofilchem s.r.l, Roseto degli Abruzzi, Italy. [3]Clinical Microbiology Laboratory, IRCCS Arcispedale S. Maria Nuova, Reggio Emilia, Italy

School of Medicine
Ysgol Meddygaeth

Introduction

In recent years colistin (COL) has remerged as a last line treatment to combat the multi-drug (MDR) and extensively-drug (XDR) resistant Enterobacteriaceae that are disseminating globally. However, mobile colistin resistance (*mcr*) is threatening the efficacy of this legacy therapeutic. Often present in pre-existing MDR/XDR phenotypes, mcr is resulting in pan-drug resistant infections worldwide. As a result, it is pertinent to develop rapid, accurate, reliable screening for COL resistance (COL-R), particularly given the difficulties surrounding antimicrobial susceptibility testing (AST) of cationic polymyxins. In this work, a 3-centre validation of a novel chromatic agar, 'Chromatic-COL (Liofilchem®) is presented, indicative of key Gram-negative species whilst selecting for a COL-R phenotype.

Methods

COL-R (n=134, 66 of which were *mcr-1*-positive) and colistin sensitive (COL-S, n=79) strains were tested across 3 sites, (1) Cardiff University, Wales, UK, (2) IRCSS Arcispedale S. Maria Nuova (Reggio Emilia, Italy), (3) Liofilchem (Roseto, Italy). The test sample consisted of Enterobacteriaceae (n=206) and *P. aeruginosa* (n=7).

COL MICs of all strains were determined by CLSI/EUCAST guidelines using agar dilution and the ComASP™ Colistin – (Liofilchem®, Italy). For each strain, 10 µl of 0.5 MacFarland was streaked on to Chromatic-COL plates

Plates were incubated overnight at 37°C and analysed for levels of growth.

Results

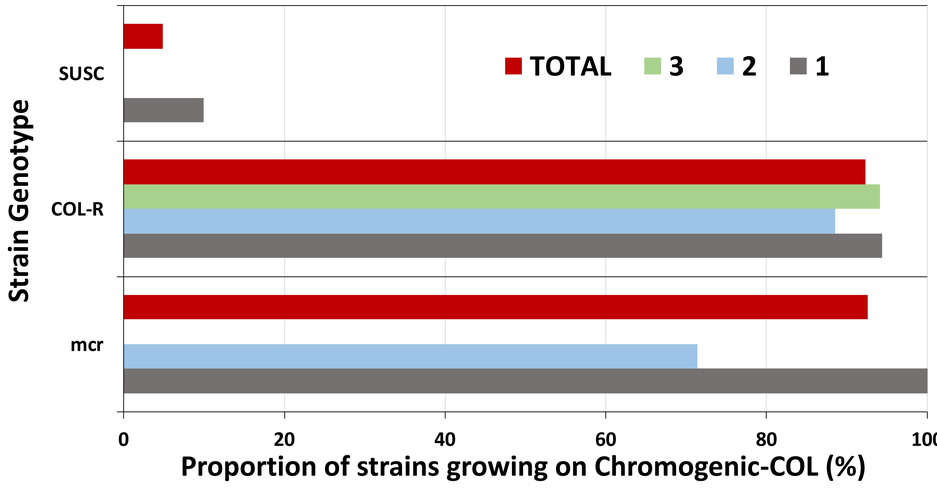

Figure (1): Proportion of strains (%) from each location growing on the Chromatic-COL plates, by category of *mcr*-positive, intrinsic/unidentified mechanism of colistin resistance (COL-R) and COL-S.

Chromatic-COL plates were sensitive in the detection of COL-R phenotype, with an overall COL-R sensitivity of 93.3% (125/134 resistant isolates), correctly selecting for 91% (61/68) of COL-R strains, either intrinsically resistant or with undefined resistance mechanisms, and 97% (64/66) of *mcr*-positive strains. In particular, all *Proteus mirabilis* (n=18) were correctly identified. COL-R/*mcr*-positive strains that were not correctly selected for upon the Chromatic-COL were primarily *E. coli*. Of the COL-S strains, only 4 false-positive results (5%) were obtained, all of which were *K. pneumoniae* strains. All *P. aeruginosa* were identified as COL-S and showed no growth on the Chromatic-COL plates. Limited inoculum effect was seen from the streaking procedure.

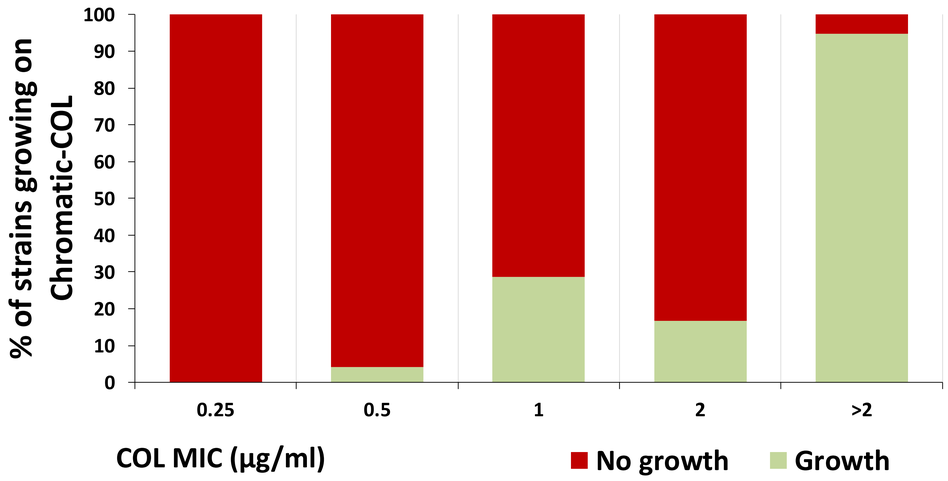

Figure (2): Proportion of strains (%) growing on Chromatic-COL, by their corresponding, pre-determined COL MICs.

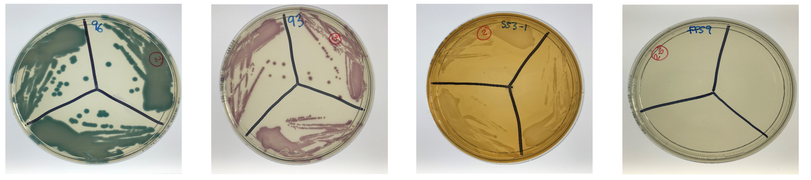

Figure (3): Examples of Chromatic-COL plates showing (left to right); COL-R *K. pneumoniae*, COL-R *E. coli*, COL-R *P. mirabilis,* and a COL-S *K. pneumoniae.*

Conclusions

- Novel Chromatic-COL agar plates are a rapid, accurate and likely cost-effective screening method for detection of COL-R Gram-negative pathogens.
- The plates show 97% sensitivity against the threat of *mcr*-positive isolates.
- Future work will include real-life validation of these plates in a clinical setting.

Contacts- tyrrelljm@cardiff.ac.uk; Edoardo.Carretto@ausl.re.it