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Colistin resistance in Gram-negative bacteria analysed by five phenotypic assays and inference of the underlying genomic mechanisms

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INTRODUCTION

Colistin is a last resource antimicrobial against multi-drug resistant (MDR) pathogens, yet resistance emerges through plasmid-mediated genes (*mcr*) or chromosomal mutation of genes involved in LPS synthesis (i.e. *mgrB*, *phoPQ*, *pmrCAB*[1,2]. Detection of colistin resistance is critical, but phenotypic susceptibility testing is challenging due to poor diffusion of colistin in agar media, leading to an underestimation of resistance[3].

AIM

We aimed to determine the performance of different phenotypic assays to detect colistin resistance. Additionally, we aimed to infer the underlying mechanisms of resistance of a subset of resistant isolates and identify putative mechanisms related to high colistin resistance using next generation sequencing.

METHODS

A set of 97 gram-negative bacterial isolates were tested by four different methods:

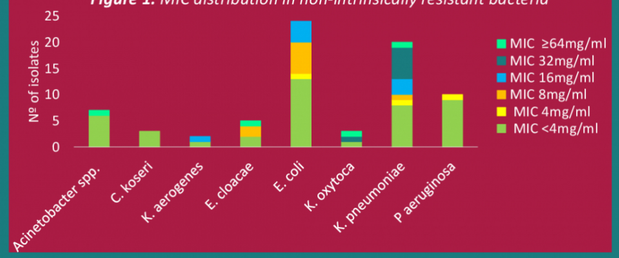
- Vitek 2® (bioMérieux, AST N242),
- Colistin MIC Test Strip (Liofilchem Diagnostics)
- UMIC Colistine kit (Biocentric)
- Rapid Polymyxin NP Test (ELITechGroup)

The standard broth microdilution method (BDM) was used as reference (conducted at the Swiss National Reference Center for Enteropathogenic Bacteria and Listeria, Institute for Food Safety and Hygiene). Susceptibility category (susceptible or resistant; EUCAST v10.0, 2020) and minimal inhibitory concentration (MIC) values were compared (**Figure 1**).

Whole genome sequences (WGS) were generated and analysed regarding the underlying resistance mechanisms, using NCBI and CARD databases, and alignment of implicated gene sequences.

Core genome multi locus sequencing typing (cgMLST) was used to describe the diversity of isolates.

Figure 1. *MIC distribution in non-intrinsically resistant bacteria*



RESULTS

% concordance	Gold Standard	Colistin MIC strip	VITEK2	UMIC	RP NP
Gold Standard (BDM)		96.9	95.6	97.9	98.8
Colistin MIC strip	96.9		98.9	99.0	97.6
VITEK2	95.6	98.9		97.8	96.3
UMIC	97.9	99.0	97.8		98.8
RP NP	98.8	97.6	96.3	98.8	

Table 1. *Categorical concordance of susceptibility or resistance*

% concordance	Vitek	UMIC	Colistin MIC strip
Acinetobacter spp.	100.0	100.0	85.7
C. koseri	100.0	66.7	66.7
E. cloacae	60.0	100.0	60.0
E. coli	70.8	79.2	54.2
Hafnia spp.	93.3	93.3	86.7
K. aerogenes	50.0	50.0	100.0
K. oxytoca	33.3	100.0	33.3
K. pneumoniae	84.2	85.0	65.0
P. aeruginosa	87.5	100.0	80.0
Total	72.5	80.4	62.9

Table 2. *MIC concordance between method and standard BDM in non-intrinsically resistant species (within 1 titer variation)*

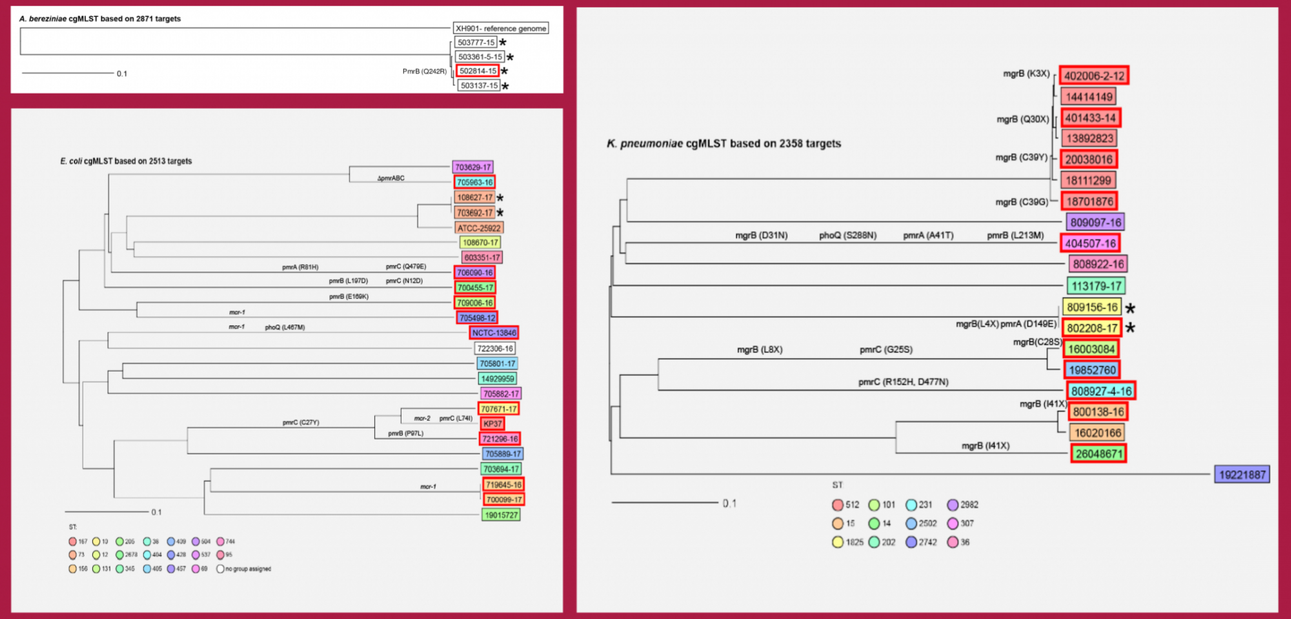


Figure 2. *Core genome MLST Neighbour Joining trees of E. coli, K. pneumoniae and A. bereziniae. The critical mutations found to most likely reflect the resistance mechanisms against colistin are indicated on the tree with the gene name and mutations found. (* indicates isolates from the same patient. Red boxes around isolate names indicate colistin resistance.)*

CONCLUSIONS

- The Rapid Polymyxin NP test showed the highest categorical (susceptible or resistant) concordance with BDM, however the UMIC test provided MIC values with higher concordance to our reference method.
- We found colistin resistance in diverse species predominantly mediated by spontaneous chromosomal mutations rather than plasmid acquisition.
- Mutations in the *mgrB* and *pmrB* genes were observed in isolates with higher MIC values (>16mg/ml) in *K. pneumoniae*, *E. coli* and *A. bereziniae*.

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