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Genome-based characterization of colistin-resistant *Escherichia coli* isolates from human patients and livestock products in Germany

Wiebke Rackwitz[1], Bernd Neumann[1,2], Niels Pfennigwerth[3], Klaus-Peter Hunfeld[4], Michael Kresken[5,6], Rasmus Leistner[7], Michael Probst-Kepper[8], Stephan Fuchs[9], Guido Werner[1], Yvonne Pfeifer[1]*

[1] Robert Koch Institute, FG 13 Nosocomial Pathogens and Antibiotic Resistance, Wernigerode, Germany; [2] Institute for Hygiene, Medical Microbiology and Clinical Infectiology, University Institute of the Paracelsus Medical Private University, Hospital Nürnberg, Nuremberg, Germany; [3] National Reference Centre for Multidrug-resistant Gram-negative Bacteria, Department for Medical Microbiology, Ruhr University Bochum, Bochum, Germany; [4] Krankenhaus Nordwest, Zentralinstitut für Labormedizin, Mikrobiologie & Krankenhaushygiene, Frankfurt (Main), Germany; [5] Antiinfektives Intelligence GmbH, Rheinbach, Germany; [6] University of Applied Sciences, Cologne, Germany; [7] Charité Universitätsmedizin Berlin, Institute of Hygiene and Environmental Medicine, Berlin, Germany; [8] MVZ Dianostic GmbH, Bielefeld, Germany; [9] Robert Koch Institute, MF1 Bioinformatics, Berlin, Germany

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INTRODUCTION

Colistin is one of the few remaining antimicrobial substances for the treatment of infections with multidrug-resistant Gram-negative pathogens. In November 2015 a very high prevalence of the plasmid-mediated colistin resistance gene ***mcr-1*** in *Escherichia coli* from livestock and several human cases was reported from China [1]. An intensive screening of strain collections started worldwide to assess the extent of ***mcr-1*** distribution. This study characterized 144 colistin-resistant *E. coli* isolates from different sources in Germany.

MATERIAL & METHODS

Colistin susceptibility testing was performed by broth microdilution. The ***mcr-1***-screening by PCR included colistin-resistant *E. coli* isolates from human patients, collected from German laboratories between January 2016 and July 2019. For whole genome sequencing (WGS; Illumina MiSeq) and further comparative analyses the following isolates were selected:

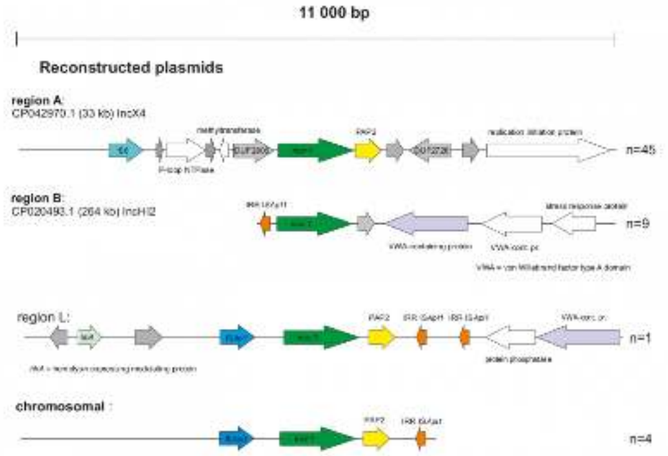
- all detected ***mcr-1***-positive *E. coli* from human patients (2016-2019) **n = 60**
- colistin-resistant *E. coli* without ***mcr*** genes from human patients (2016-2019) **n = 63**
- genome sequences of ***mcr-1***-positive *E. coli* from livestock products (2011-2015) **n = 21**

The *E. coli* phylogeny was analyzed by multilocus sequence typing and core genome (cg)MLST; and the genetic environment of ***mcr-1*** was compared. Further resistance genes were extracted from WGS data by the ResFinder tool (CGE).

RESULTS

Analysis of the genetic context of ***mcr-1*** revealed its presence on very similar IncX4 plasmids of ca. 33kb size in 45/81 *E. coli* isolates from humans and food products (region A; **FIG 1**/**FIG 2**).

FIG 1: Examples of genetic context of *mcr-1* detected in *E. coli*



RESULTS

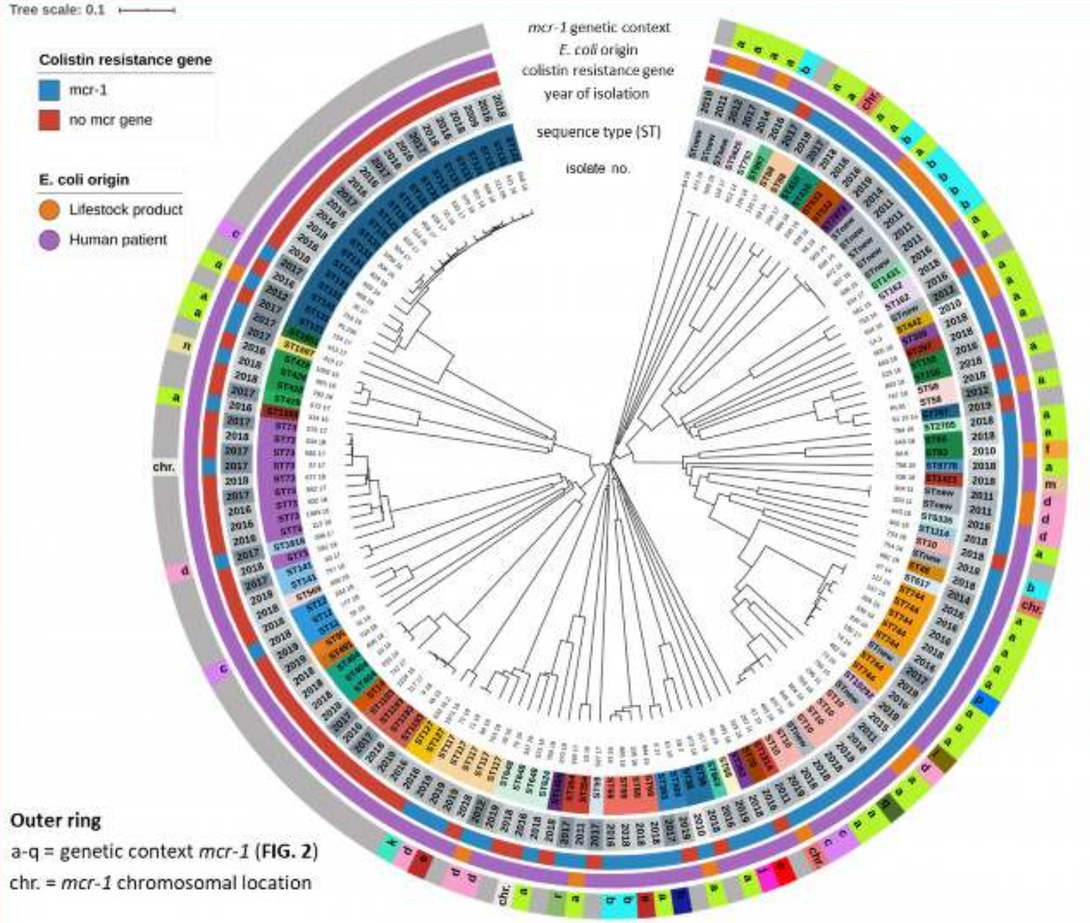


FIG 2: Phylogeny of 144 colistin-resistant *E. coli* isolates from human patients and livestock products

The majority (80%) of the 123 patients with colistin-resistant *E. coli* suffered from urinary tract infections. MLST/cgMLST showed that colistin resistance gene ***mcr-1*** occurred in *E. coli* of very different sequence types (STs; **FIG 2**). However, frequent STs (ST131, ST73 and ST1193) represented colistin-resistant *E. coli* without ***mcr*** genes. Clonal transfer was only detected for single patients in the same hospital, and the ***mcr-1***-positive *E. coli* from livestock products were not closely related to isolates from human patients

Further resistance genes in ***mcr-1***-positive *E. coli* from human patients encoded mainly beta-lactamases (TEM-1); in a few strains extended-spectrum beta-lactamases (ESBL, e.g. CTX-M) and carbapenemases (NDM, OXA-48) were detected. In contrast, the majority of *E. coli* from livestock products carried ESBL or AmpC-beta-lactamases (CMY-2) and was resistant to third-generation cephalosporins (**Table 1**).

Table 1: Antibiotic susceptibilities of colistin-resistant *E. coli*

Resistances	*mcr-1 E. coli* n=60 Human patients	non-*mcr-1 E. coli* n=63 Human patients	*mcr-1 E. coli* n=21 Livestock products
Colistin*	**100%**	**100%**	**100%**
Ampicillin	**70%**	30%	**86%**
Cefotaxime	25%	16%	**86%**
Ceftazidime	20%	16%	**71%**
Meropenem*	5%	0%	5%
Ciprofloxacin	**72%**	37%	48%
Amikacin	10%	5%	5%
SXT	**78%**	22%	43%

Broth microdilution, EUCAST v11.0; * MICs ≥ 2mg/L for colistin and ≥8 mg/L for meropenem were used because several isolates with *mcr-1* and/or carbapenemase genes showed MICs of 2 mg/L and 8 mg/L, respectively.

SUMMARY / CONCLUSION

Our data show the presence of *mcr-1* in *E. coli* from human patients in Germany and highlight the importance of horizontal transfer of this colistin resistance gene by similar IncX4 plasmids in *E. coli* strains from humans and livestock.

Non-*mcr*-mediated Colistin-resistance is wide-spread in *E. coli* from human patients and further investigations are required to resolve the underlying mechanisms.

REFERENCES / CONTACT INFORMATION

[1] Liu Y.Y. *et al.* Lancet Infect Dis. 2016, 16:161-168.

* Corresponding author: Dr. Yvonne Pfeifer, Robert Koch Institute, FG13 Nosocomial Pathogens and Antibiotic Resistance, Burgstr. 37, 38855 Wernigerode, Germany, E-mail: pfeifery@rki.de