SDRP JOURNAL OF FOOD SCIENCE & TECHNOLOGY 2016
ISSN:2473-6419

RESEARCH

Assessment of the occurrence of MCR producing Enterobacteriaceae in Swiss and imported poultry meat

DOI: 10.15436/JFST.1.4.5

Katrin Zurfluh, Simone Buess, Roger Stephan* and Magdalena Nüesch-Inderbinen
Institute for Food Safety and Hygiene, Vetsuisse Faculty University of Zurich, CH-8057 Zurich, Switzerland.

RECEIVED DATE: 24-11-2016; ACCEPTED DATE: 13-12-2016; PUBLISHED DATE: 21-12-2016

CORRESPONDENCE AUTHOR: Roger Stephan
E-MAIL: stephanr@fsafety.uzh.ch

CONFLICTS OF INTEREST
THERE ARE NO CONFLICTS OF INTEREST FOR ANY OF THE AUTHORS.

ABSTRACT:
To determine whether *mcr*-positive Enterobacteriaceae are present in poultry meat at retail level, 128 fresh meat samples from Switzerland (n=40), Germany (n=69), Hungary (n=6), Denmark (n=6), Austria (n=2), and Italy (n=5), obtained from retail stores in Switzerland and Southern Germany were analyzed. The plasmid-mediated colistin resistance gene *mcr-1* was detected by PCR in 33 (25.8%) meat samples (5 samples originating from Italy and 28 samples originating from Germany). Thereof, 12 *mcr-1* harbouring *E. coli* were obtained from 12 samples (10 from Germany and two from Italy) by subculturing for further characterization. Despite repeated subculturing, the remaining 21 samples were discarded due to mixed growth of *E. coli* with *Proteus* spp., which possess intrinsic resistance to polymyxins. The isolates belonged to various sequence types (ST) and clonal complexes (CC), including *E. coli* of the endemic avian and human pathogenic CC10, CC23, and CC155. Further STs included ST156, ST1431 and ST3519, which are associated with humans and animals. Our data highlight the importance of surveillance of retail poultry meat from different countries in context of the global dissemination of *mcr*-positive *E. coli*.

KEYWORDS: Colistin, Resistance, *Escherichia coli,* Meat

INTRODUCTION

Following their initial recognition in 2015 *(12)*, it has become apparent that Enterobacteriaceae harboring the plasmid-mediated colistin resistance gene *mcr-1* represent an increasing threat to human and animal health. In human medicine, colistin has so far rarely been used due to nephrotoxicity but has now become a critically important antibiotic for the treatment of infections due to multidrug resistant (MDR) bacteria *(15)*. By contrast, in veterinary medicine, colistin is widely used for the treatment of diarrhea in food-producing animals, indicating that the emergence and global spread of *mcr-1* is associated with the livestock sector *(14)*. There is growing concern that animal derived food may represent a reservoir for colistin resistant bacteria, since several retrospective analyses of stored cultures have identified *mcr-1* in *E. coli* isolated from meat products in many countries worldwide *(15)*. The presence of *mcr*-harboring *E. coli* in food is not desirable, since it is not only one of the most important opportunistic pathogens for humans, but may also become established in the human gut as a commensal, where the resistance gene could be transferred to pathogenic bacteria. Recent studies indicate that the international animal and food trade plays an important role regarding the epidemiology of *mcr-1 (6)*, however, more data are urgently required in order to assess the prevalence of colistin resistant *E. coli* in different food matrices from different countries of origin. The aim of the present study was therefore to assess the occurrence of *mcr-1* positive *E. coli* in chicken and turkey meat originating from several European countries, and to characterize the isolates with respect to their phylogenetic groups, multilocus sequence types and antimicrobial susceptibility profiles.

MATERIALS AND METHODS

Sampling. In August and September 2016, 128 different poultry meat products (108 from chicken and 20 from turkeys) were obtained from retail stores in Switzerland and Southern Germany. Products consisted of poultry breast, poultry legs, chicken wings or minced poultry meat. Countries of meat origin included Germany (chicken meat n=50 and turkey meat n=19, total amount n=69), Switzerland (chicken meat n=40), Denmark (chicken meat n=6), Hungary (chicken meat n=6), Italy (chicken meat n=4 and turkey meat n=1, total amount n=5), Austria (chicken meat n=2).

Microbiological analysis and screening for *mcr-1*. Of each meat sample, 10 g were taken at random using gloves, sterile forceps and scalpels. The units were aseptically and accurately weighed and placed in a sterile Stomacher® bag. Samples were homogenized using a Stomacher® sample blender and incubated overnight at 37°C in 100 ml Enterobacteriaceae Enrichment (EE) broth (BD, Franklin Lakes, USA) for selective enrichment. Thereafter, one loopful of each of the cultures was streak-plated on in-house produced LB agar plates containing 4mg/l colistin, 10mg/L vancomycin and 5mg/l amphotericin B for selection and incubated at 37°C for 24 h under aerobic conditions. For preliminary screening for *mcr-1* genes, DNA was obtained from a loopful of bacteria growing on selective plates and primers and PCR conditions were used as described previously *(11, 12)*. Positive cultures were subcultured on LB agar plates containing 4mg/l colistin, 10 mg/L vancomycin and 5mg/l amphotericin B. Purification of mixed cultures was performed by the replicate streak-plate method using selective plates as described above. Resulting colonies were analyzed for the presence of *mcr-1* by PCR as described above. Custom-sequencing was performed by Microsynth (Balgach, Switzerland) and the nucleotide- and translated protein-sequences were analyzed with CLC Main Workbench 7.0.2 (CLC bio, Aarhus, Denmark). For database searches the BLASTN program of NCBI (http://www.ncbi.nlm.nih.gov/blast/) was used.

Colonies were identified using API ID 32 E (bioMérieux, Marcyl'Etoile, France). Isolates were subjected to susceptibility testing against 13 antimicrobial agents by the disc diffusion method according to CLSI protocols and evaluated according to CLSI criteria *(4)*. The panel included ampicillin, amoxicillin-clavulanic acid, cephalothin, cefotaxime, nalidixic acid, ciprofloxacin, gentamicin, kanamycin, streptomycin, sulfamethoxazole, trimethoprim, tetracycline, and chloramphenicol (Becton, Dickinson, Heidelberg, Germany). Susceptibility to colistin was tested by the microbroth dilution method and interpreted according to the guidelines of EUCAST (*7*). Multidrug resistance (MDR) was defined as resistance to three or more classes of antimicrobials, including polymyxins, and counting ß-lactams as one class.

Phylogenetic classification and multilocus sequence typing

E. coli isolates were assigned to one of the four phylogenetic groups A, B1, B2 or D using triplex PCR targeting the *chuA* gene which is absent in groups A and B1but present in groups B2 and D , the *yjaA* gene which is present in group B2, and an unspecified DNA fragment termed TspE4.C2, detectable in group B1, as described by Clermont et al. *(3)*. Group A and B1 typically contain commensal *E. coli* strains while groups B2 and D consist of virulent extra-intestinal strains *(10)*.

For multilocus sequence typing, internal fragments of the seven housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA*, and *recA*) were amplified by PCR, as described by Wirth et al. *(18)*. Sequencing of the amplification products was performed by Microsynth (Balgach). Sequences were imported into the *E. coli* MLST database (http://mlst.ucc.ie/mlst/dbs/Ecoli) website to determine each isolate's sequence type (ST) and association with clonal complexes (CC).

RESULTS AND DISCUSSION

Screening by PCR targeting the *mcr-1* genes in the bacteria growing on the initial selective medium gave rise to 33 (25.8%) *mcr-1*-positive mixed cultures (28 of the 69 meat samples originating from Germany and all of the 5 meat samples originating from Italy). Thereof, 12 pure cultures harbouring *mcr-1* were obtained by subculturing for further analysis. They included seven (6.5%) of the 108 chicken meat samples and five (25%) of the 20 turkey meat samples. Despite repeated subculturing, the remaining 21 were discarded due to mixed growth with *Proteus* spp., which possess intrinsic resistance to polymyxins. None of the meat samples from Switzerland, Denmark, Austria or Hungary tested positive for *mcr* genes. Sequencing of the *mcr* genes amplified by PCR from the 12 isolates revealed for all amplicons 100% nucleotide homology to *mcr-1 (12)*.

An overview of the meat samples from which *mcr-1*-harboring Enterobacteriaceae were isolated, the genetic characteristics and antimicrobial resistance profiles of the isolates is shown in Table 1. All except one of the isolates were MDR (Table 1).

Table 1: *mcr-1* positive *E. coli* isolated from poultry meat from Germany and Italy

Sample ID	Origin	Species	Phylogenetic group	MLST/ CC	*mcr-1*	MIC colistin [mg/L]	Additional antibiotic resistance profile
chicken meat							
PF 11	Germany	*E. coli*	B1	156/156	+	8	AM, CF, NA, CIP, TE
PF 61	Germany	*E. coli*	A	3519	+	8	AM, S
PF 65	Germany	*E. coli*	A	10/10	+	8	AM, NA, S, SMZ, TMP
PF 75	Germany	*E. coli*	B1	156/156	+	8	AM, CF, NA, CIP, TE,
PF 94	Italy	*E. coli*	A	650/23	+	4	AM, NA, TE, S, C, SMZ, TMP
PF 100	Germany	*E. coli*	B1	new[a]	+	4	AM, CF, NA, CIP, TE
PC11	Germany	*E. coli*	A	1251/10	+	8	AM, CF
Turkey meat							
PF 52	Germany	*E. coli*	B1	58/155	+	4	AM, SMZ
PF 56	Germany	*E. coli*	B1	1431	+	4	AM, TE, C, SMZ
PF 63	Germany	*E. coli*	B2	355/73	+	8	AM, CF, NA,TE, S, SMZ, TMP
PF 88	Italy	*E. coli*	A	744	+	8	AM, NA, CIP, TE, K, S, C, SMZ, TMP
PF 91	Germany	*E. coli*	B1	1431	+	8	AM, TE, C, SMZ,

Abbreviations: MLST, multilocus sequence type; CC, clonal complex; AM, ampicillin; CF, cephalothin; CIP, ciprofloxacin; NA, nalidixic acid; K, kanamycin; S, streptomycin; SMZ, sulfamethoxazole; TMP, trimethoprim; TE, tetracycline; C, chloramphenicol.

[a] new allelic combination: *adk* 6, *fumC* 29, *gyrB* 32, *icd* 16, *mdh* 20, *purA* 8, and *recA* 44.

Our data are supportive of a previous report whereby the prevalence of *mcr-1* in poultry meat from Germany is higher than that of other EU countries *(9)*. Together with the detection of *mcr-1*-positive *E. coli* in meat from Italy, these results coincide with the fact that sales of polymyxin for the use in livestock are higher in Germany and Italy compared to other EU countries and highlight the impact of national agricultural practices *(9)*. Concurrently, data from the SENTRY Antimicrobial Surveillance Program 2014-2015 show that while the overall prevalence of *mcr*-harboring clinical *E. coli* isolates is still low (0.1%), the highest percentage of the collected isolates originated from Germany (26.3%), followed by Italy (21%) *(2)*. These findings are suggestive of a link between antibiotic consumption in food-producing animals and resistance genes in isolates from humans. Food as the source of extra-intestinal pathogenic *E. coli* (ExPEC) infections in human is discussed controversially *(16)*. Nevertheless, among the isolates analyzed in this study, multilocus sequence typing showed that six (50%) belong to CC10, 23, 73 or CC155 (Table 1), all of which have been described as avian pathogenic and/or human ExPEC *(5, 13, 8)*. Further, two of the isolates from turkey meat belonged to ST1431 and one from chicken meat to ST3519, both sequence types previously reported as associated with the dissemination of the extended-spectrum ß-lactamase (ESBL) CTX-M-1 and the carbapenemase OXA-48 in humans, companion animals and the poultry-farming environment *(17, 1)*. Two further isolates (from meat originating from the same retail chain) belonged to ST156, a clone very recently emerged in fowl in China,

and found to coproduce MCR and the metallo-ß-lactamase NDM-5 *(19)*. For one isolate, a new allelic combination was observed (Table 1). Our data highlight the establishment of the *mcr* gene within globally successful *E. coli* clones that may enhance the spread of colistin resistance throughout the food chain.

Conclusively, this study allowed a comparison of poultry meat from different countries with regard to the occurrence of *mcr-1*. Furthermore, MDR *E. coli* clones associated with *mcr-1* in poultry meat were identified. Major clonal complexes (CC10 and CC23), which are shared between human and avian isolates, as well as ST typically found in avian strains, were observed. The occurrence of *mcr*-positive clones associated with risk of extra-intestinal diseases in humans in retail meat is a potential threat to public health. Increased surveillance of the dissemination of the *mcr* gene throughout the international food market is a keystone of addressing the emergence of *mcr*-positive bacteria.

ACKNOWLEDGMENT/ FUNDING

This work was partly supported by the Swiss Federal Office of Public Health, Division Communicable Diseases. Samples were mainly collected by the consumer protection organization “Gesundheitstipp”.

REFERENCES

1. Blaak, H., Hamidjaja, R. A., van Hoek, A. H., de Heer, L., de Roda Husman, A. M. and Schets, F. M. 2014. Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. *Appl. Environ. Microbiol.* 80: 239-246.
2. Castanheira, M., Griffin, M. A., Deshpande, L. M., Mendes, R. E., Jones, R. N. and Flamm, R. K. 2016. Detection of *mcr-1* among *Escherichia coli* clinical isolates collected worldwide as part of the SENTRY Antimicrobial Surveillance Program in 2014 and 2015. *Antimicrob. Agents Chemother.* 60: 5623-5624.
3. Clermont O., S. Bonacorsi, and E. Bingen. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. *Appl. Environ. Microbiol.* 66: 4555-4558.
4. Clinical and Laboratory Standards Institute. 2011. M100-S21. Performance standards for antimicrobial susceptibility testing, 21st informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
5. Giufrè, M., Graziani, C., Accogli, M., Luzzi, I., Busani, L., Cerquetti, M. and the *Escherichia coli* Study Group. 2012. *Escherichia coli* of human and avian origin: detection of clonal groups associated with fluoroquinolone and multidrug resistance in Italy. *J. Antimicrob. Chemother.* 67: 860-867.
6. Grami, R., Mansour, W., Mehri, W., Bouallègue, O., Boujaâfar, N., Madec, J. Y. and Haenni, M. 2016. Impact of food animal trade on the spread of *mcr-1*-mediated colistin resistance, Tunisia, July 2015. *Euro. Surveill.* 21: pii=30144. doi: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.8.30144.
7. EUCAST. Clinical Breakpoints. http://www.eucast.org/clinical_ breakpoints.
8. Hasman, H., Hammerum, A. M., Hansen, F., Hendriksen, R. S., Olesen, B., Agersø, Y., Zankari, E., Leekitcharoenphon, P., Stegger, M., Kaas, R. S., Cavaco, L. M., Hansen, D. S., Aarestrup, F. M. and Skov, R. L. 2015. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro. Surveill.* 20: pii=30085. doi: 10.2807/1560-7917.ES.2015.20.49.30085.
9. Irrgang, A., Roschanski, N., Tenhagen, B. A. and Grobbel, M., Skladnikiewicz-Ziemer T,, Thomas K., et al. 2016. Prevalence of *mcr-1* in *E. coli* from livestock and food in Germany, 2010–2015. *PloS one.* doi:10.1371/journal.pone.0159863.
10. Johnson, J. R., Delavari, P., Kuskowski, M. and Stell, A. L. 2001. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. *J. Infect. Dis.* 183: 78-88.
11. Liassine, N., Assouvie, L., Descombes, M. C., Tendon, V. D., Kieffer, N., Poirel, L. and Nordmann, P. 2016. Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract Enterobacteriaceae in Switzerland. *Int. J. Infect. Dis.* 51: 4-5.
12. Liu, Y. Y., Wang, Y., Walsh, T. R., Yi, L. X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L. F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J. H. and Shen, J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect. Dis.* 16: 161-168.
13. 13 Maluta, R. P., Logue, C. M., Casas, M. R. T., Meng, T., Guastalli, E. A. L., Rojas, T. C. G., Montelli, A. C., Sadatsune, T., de Carvalho Ramos, M., Nolan, L. K. and da Silveira, W. D. 2014. Overlapped sequence types (STs) and serogroups of avian pathogenic (APEC) and human extra-intestinal pathogenic (ExPEC) *Escherichia coli* isolated in Brazil. *PLoS One* 9, e105016.

14. Poirel, L. and Nordmann, P. 2016. Emerging plasmid-encoded colistin resistance: the animal world as the culprit. *J. Antimicrob. Chemother.* 71: 2326-2327.
15. Schwarz, S. and Johnson, A. P. 2016. Transferable resistance to colistin: a new but old threat. *Antimicrob. Chemother.* 71: 2066–2070.
16. 16 Singer, R. S. 2015. Urinary tract infections attributed to diverse ExPEC strains in food animals: evidence and data gaps. *Front. Microbiol. 6*: 28. doi: 10.3389/fmicb.2015.00028.
17. Stolle, I., Prenger-Berninghoff, E., Stamm, I., Scheufen, S., Hassdenteufel, E., Guenther, S., Bethe, A., Pfeifer, Y. and Ewers, C. 2013. Emergence of OXA-48 carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* in dogs. *J. Antimicrob. Chemother.* 68: 2802-2808.
18. Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L. H., Karch, H., Reeves, P. R., Maiden, M. C. J., Ochman, H. and Achtman, M. 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol. Microbiol.* 60: 1136-1151.
19. 19 Yang, R. S., Feng, Y., Lv, X. Y., Duan, J. H., Chen, J., Fang, L. X., Xia, J., Liao, X. P., Sun, J. and Liu, Y. H. 2016. Emergence of NDM-5 and MCR-1-Producing *Escherichia coli* clone ST648 and ST156 from a single muscovy duck (*Cairina moschata*). *Antimicrob. Agents Chemother.* doi:10.1128/AAC.01365-16.