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Phylogeny of Salmonella Paratyphi B variant Java (ST-28) harbouring mcr-1

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Background: The polymyxin antibiotic colistin belongs to the last-resort antibiotics. Until recently, decreased susceptibility to colistin in Enterobacteriaceae was believed to be linked with chromosomal mutations and therefore not transferrable by horizontal gene transfer. However, in 2015 Liu et al. first described a plasmid-located colistin resistance gene (*mcr-1*) in *Escherichia coli* isolated from animals and patients in China. Since then, a number of publications reported the occurrence of *mcr-1* positive *E. coli* isolates from food, animals and humans- the first positive isolate dating back to the 1980s. However, also other Enterobacteriaceae like *Klebsiella* and *Salmonella* harbor the *mcr-1* gene. Among them, more and more *mcr-1* positive *S. enterica* subsp. *enterica* serovar Paratyphi B variant Java (*S.* Paratyphi B dT+) ST28 originating from animals and food imported from Europe were documented recently. *S.* Paratyphi B dT+ is a common cause of gastroenteritis worldwide. These zoonotic strains (belonging to the multidrug-resistant sequence type ST28) are frequently found in poultry.

Material/methods: A number of 396 *S.* Paratyphi B dT+ isolates originating from food producing animals and food products, received from 2006 to 2015 in the German National Salmonella Reference Laboratory were selected for PCR screening for the presence of the *mcr-1* gene. Whole-genome sequencing (WGS) using MiSeq technology was performed on *mcr-1* positive isolates representing different multidrug-resistance patterns. Negative isolates for *mcr-1* were included for plasmid comparison purposes. Raw reads were assembled *de novo* and the derived contigs were analysed using the bioinformatics tools provided by the Center for Genomic Epidemiology. In a second approach, raw reads were mapped to the full genome of strain 08-00436 (PacBio sequence of the earliest isolate identified) to carry out phylogenetic analysis. Furthermore S1-PFGE and Southern-

hybridization were used to investigate the diversity of *mcr-1* positive and negative plasmids in more detail.

Results: All together 68 of the 396 investigated *S.* Paratyphi B dT+ ST28 isolates were *mcr-1* positive. The *mcr-1* gene was located on plasmids belonging to the IncHI2 or IncX4 replicon group. Strains isolated from 2008 to 2011 tend to carry the *mcr-1* gene on large multidrug-resistant IncHI2 plasmids and build a cluster in the phylogenetic tree, whereas strains isolated after 2011 mainly carry *mcr-1* on IncX4 plasmids and show a higher phylogenetic diversity. Although all analysed strains were multidrug resistant to at least 3 classes of antibiotics, none of them was an ESBL producer.

Conclusions: Analysis of WGS data and S1-PFGE patterns show that *S*. Paratyphi B dT+ ST28 has evolved to colistin resistance through acquisition of the *mcr-1* gene in several independent events. Consistent numbers of *mcr-1* positive *S*. Paratyphi B isolates within the last five years underline the need for further monitoring and surveillance of this issue.