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Short Communication

Replicon typing of plasmids carrying $bla_{CTX\text{-}M\text{-}15}$ among *Enterobacteriaceae* isolated at the environment, livestock and human interface

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HIGHLIGHTS

- IncF and IncI1 are major replicon types involved in $bla_{CTX\text{-}M\text{-}15}$ harbouring plasmids
- No specific plasmid multi-locus sequence type was found to be predominant
- Two novel IncI1 plasmid multi-locus sequence types are described

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ABSTRACT

One of the currently most important antibiotic resistance mechanisms in *Enterobacteriaceae* is based on the production of ESBL enzymes that inactivate β-lactam antibiotics including cephalosporins and monobactams by hydrolyzing their β-lactam ring.

In humans, the most prevalent ESBL enzyme type is encoded by $bla_{CTX\text{-}M\text{-}15}$. CTX-M-15 producing enterobacterial strains were also frequently isolated from environmental samples including surface water and freshwater fish. Plasmids, which can be grouped in different plasmid incompatibility types, play a key role in the horizontal spread of these multidrug resistance genes.

The purpose of this study was to investigate the diversity of plasmids that carry $bla_{CTX\text{-}M\text{-}15}$ genes among *Enterobacteriaceae* isolated at the environment, livestock and human interface.

In total, 81 $bla_{CTX\text{-}M\text{-}15}$-harboring isolates collected between 2009 and 2014 were tested for its ability to transfer $bla_{CTX\text{-}M\text{-}15}$ by conjugation. These plasmids were further typed. Transfer of a single $bla_{CTX\text{-}M\text{-}15}$-harboring plasmid was observed in 32 (39.5%) of the isolates. The most frequent replicon types detected among these plasmids are IncF-type plasmids (n = 12) (mostly multi replicon plasmids with a combination of following replicons: IncFII, IncFIA and IncFIB), followed by IncI1 (n = 8), IncK (n = 3) and IncR (n = 1). A noticeable number of plasmids (n = 8) could not be assigned to any of the tested replicon types.

Knowledge about the plasmid types circulating in bacterial populations is indispensable for understanding epidemiological dynamics and for establishing intervention strategies to stop further dissemination of particular plasmids.

1. Introduction

Extended-spectrum β-lactamases (ESBLs) are mainly plasmid-encoded enzymes that are able to inactivate a variety of β-lactam antibiotics, including penicillins, 2nd-, 3rd- and 4th-generation cephalosporins and monobactams (e.g. aztreonam). The inactivation occurs by the hydrolysis of the β-lactam ring. In recent years, ESBL-producing *Enterobacteriaceae* are no longer restricted to nosocomial infection (Paterson and Bonomo, 2005) and have been reported in asymptomatic human carriers (Geser et al., 2012b; Nüesch-Inderbinen et al., 2013). Furthermore, they have been reported in healthy food-producing animals (Carattoli, 2008; Geser et al., 2012a) as well as in feral animals (e.g. birds and fish) (Abgottspon et al., 2014; Guenther et al., 2011; Zurfluh et al., 2013b) and in the environment, e.g., in aquatic systems such as rivers and lakes (Zurfluh et al., 2013a).

After the shift of ESBL variants from the SHV and TEM groups towards CTX-M enzymes in the early 2000s, currently CTX-M-15 is the most widespread ESBL-enzyme in Europe (Coque et al., 2008a). CTX-M-15 derived from CTX-M-3 by a single amino acid substitution at position 240 (D240→G) (Poirel et al., 2002). The predominance of

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CTX-M-15 is associated, among others, with the successful expansion of the pandemic and highly virulent *Escherichia coli* B2:O25:H4-ST131 strain (Coque et al., 2008a; Naseer and Sundsfjord, 2011).

The wide dissemination of resistance genes is most often linked to horizontal gene transfer via plasmids. Thereby bacterial conjugation can also occur among different species. The replicon types most frequently detected in antibiotic resistance genes harboring plasmids in *Enterobacteriaceae* include the incompatibility (Inc) groups F, A/C, L/M, I1, HI2, and N (Carattoli, 2009). To better understand and to follow the transmission of antimicrobial resistance genes from different environments, plasmid typing by PCR-based replicon typing and plasmid multi locus sequence typing (pMLST) have been proven to be useful techniques (Carattoli et al., 2005; García-Fernández et al., 2011; Villa et al., 2010).

The purpose of this study was to type plasmids encoding CTX-M-15 originating from *Enterobacteriaceae* isolated from the environment, livestock and human interface in Switzerland. Plasmids harboring $bla_{\text{CTX-M-15}}$ were transferred by conjugation or transformation experiments into a plasmid-free *E. coli* acceptor strain and were further typed by PCR-based plasmid replicon typing (PBRT) as described previously (Carattoli et al., 2005; Villa et al., 2010). IncI1 plasmids were further characterized by using plasmid multi-locus sequence typing (pMLST) (García-Fernández et al., 2011).

2. Materials and methods

2.1. Strain collection

2.1.1. Environmental strains

46 $bla_{\text{CTX-M-15}}$-harboring strains obtained from rivers and lakes in Switzerland (Zurfluh et al., 2013a) were included in this study for further characterization.

2.1.2. Strains from fish of two lakes in Switzerland

Strains obtained from guts of freshwater fish were investigated for the presence of *bla* genes in a recent study (Abgottspon et al., 2014). 12 $bla_{\text{CTX-M-15}}$-harboring isolates were included in this study.

2.1.3. Samples from healthy cattle

Four $bla_{\text{CTX-M-15}}$-positive *Enterobacteriaceae* strains isolated previously from healthy cattle (Geser et al., 2012a) were selected for this study.

2.1.4. Samples from healthy humans

14 and five $bla_{\text{CTX-M-15}}$ harboring strains isolated in two previous studies on the fecal carriage rates of ESBL-producing *Enterobacteriaceae* in healthy humans and in primary care patients in Switzerland were also included in this study.

2.2. Conjugation mating experiments

Conjugation experiments were performed with the plasmid-free recipient strain *E. coli* HK225 (Strep^r, Rif^r) (Kayser et al., 1982). Briefly, single colonies of the donor and recipient were inoculated in LB broth (Difco Laboratories) and grown overnight at 37 °C. Subsequently, equal volumes of the donor and recipient cultures were mixed and incubated overnight at 37 °C without shaking. Serial dilutions were then plated on LB agar (Difco Laboratories) selection plates supplemented with a combination of 600 μg/ml streptomycin (Sigma-Aldrich, Buchs, Switzerland) 100 μg/ml rifampicin (Sigma-Aldrich) and 10 μg/ml cefotaxime (Sigma-Aldrich).

For selected strains the conjugation frequency per donor was determined by plating serial dilutions of the mating on selective plates on which the donor strain and the conjugant can grow (LB-agar supplemented with 10 μg/ml cefotaxime) as well as on plates on which only the transconjugants can grow (LB-agar supplemented with 600 μg/ml streptomycin, 100 μg/ml rifampicin and 10 μg/ml cefotaxime). The transfer frequency was calculated by the quotient of the number of transconjugants over the number of transconjugants plus donor. For all transconjugation experiments (including determination of conjugation frequency) the following controls were included: donor strain alone and acceptor strain alone to ensure the effectiveness of the selective plates used.

2.3. PCR-based replicon typing (PBRT)

Plasmid incompatibility (Inc) types were determined by PCR-based replicon typing (Carattoli et al., 2005; Villa et al., 2010).

2.4. Antibiotic susceptibility testing of transconjugants/transformants

Susceptibility testing was performed by agar diffusion methods with antibiotic disks (BD, Sparks, MD) according to the manufacturer's protocols. Results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2013).

3. Results and discussion

In total, 81 $bla_{\text{CTX-M-15}}$-harboring strains were tested for its ability to transfer the cefotaximase-phenotype by conjugation. Transfer of a single $bla_{\text{CTX-M-15}}$-harboring plasmid to the susceptible *E. coli* HK225 recipient was observed for 31 (38.3%) of the isolates by the conjugation experiments and one plasmid was transferred by transformation (Table 1). The transfer rates (transconjugants per donor cell) ranged from 3.62×10^{-2} (*E. coli* strain fish140b) and 1.63×10^{-4} (*Klebsiella pneumoniae* OW66E1) to 9.39×10^{-6} (*E. coli* strain fish63) (Table 2). Thereby, IncF plasmids showed the lowest transfer rates regardless of the origin of the isolate. Nevertheless, IncF plasmids are the most important plasmids in the dissemination of $bla_{\text{CTX-M-15}}$ in the clinical field (Coque et al., 2008a, 2008b). The IncI1 plasmids selected in this study showed variable transfer rates (Table 2). However, IncI1 plasmids are known to be easily transferable and play a key role in the dissemination of ESBL resistance determinants (Zurfluh et al, 2014).

In a study, where the location of $bla_{\text{CTX-M-15}}$ in *E. coli* isolates from different countries was investigated, in only 37% of the isolates the $bla_{\text{CTX-M-15}}$ was transferable by conjugation or transformation which correlates well with the transfer ability described in our study (Coque et al., 2008b). Possible explanation for transconjugation failure could be either plasmid integration or transposition of mobile elements (likely IS*Ecp1*-associated) into the chromosome (Coque et al., 2008b; Poirel et al., 2005).

The replicon types detected among the 31 transconjugants and one transformant are summarized in Table 1. The most frequently detected replicon types among $bla_{\text{CTX-M-15}}$-carrying plasmids are IncF-type plasmids ($n = 12$) (mostly as multi replicon plasmids with a combination of following replicons: IncFII, IncFIA and IncFIB), followed by IncI1 ($n = 8$), IncK ($n = 3$) and IncR ($n = 1$). Notably, a number of plasmids ($n = 8$) could not be assigned to any of the tested replicon types.

IncF plasmids were identified in strains from all origins (environmental, livestock and human). Moreover, $bla_{\text{CTX-M-15}}$ harboring plasmids of the IncI1 type also seem to be widely distributed since they were found in strains from all origins except cattle. IncF plasmids represent a narrow host range replicon type. Nevertheless, they are a heterogeneous and largely diffused plasmid family (Coque et al., 2008b). The most likely driving force in the evolution of IncF plasmids are recombination events which were also responsible for multi-replicon plasmids. In plasmids with multiple replicons, one is usually highly conserved whereas the other is free to divergence (Osborn et al., 2000).

In the dissemination of bla_{ESBL} genes, IncI1-type plasmids play a central role (Carattoli, 2009; Johnson et al., 2011; Rodrigues et al., 2013; Zurfluh et al., 2014). All IncI1 plasmids share in their plasmid backbone structure a gene cluster encoding the type IV pili, which are supposed to