Journal of Antimicrobial Chemotherapy (2009) **63**, 1286–1300
doi:10.1093/jac/dkp101
Advance Access publication 18 March, 2009

JAC

Comparative analysis of extended-spectrum-β-lactamase-carrying plasmids from different members of Enterobacteriaceae isolated from poultry, pigs and humans: evidence for a shared β-lactam resistance gene pool?

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Keywords: RFLP, ESBLs, conjugation

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Sir,

β-Lactam antibiotics are extensively used in human and veterinary medicine. The detection rate of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae isolated from humans has increased rapidly worldwide.[1] In addition, ESBLs have been increasingly described in bacterial populations circulating in animals.[2,3] Recently, a high diversity of ESBLs in *Escherichia coli* was reported in Belgian poultry farms. In that instance, CTX-M enzymes were the predominant ESBL family.[4] CTX-M-2-producing *Salmonella enterica* serovar Virchow strains and TEM-52-producing *S. enterica* serovar Infantis strains have also been isolated from Belgian poultry.[2,3] This raises a potential public health concern. Moreover, the presence of ESBLs in the microbiota of food-producing animals may pose a human health hazard since these bacteria may represent a reservoir of resistance genes for pathogens causing disease in humans and animals.[4] Therefore, to demonstrate whether a common ESBL gene pool exists among isolates in different hosts, we characterized the plasmids and determined the location and transfer possibilities of the ESBLs $bla_{\text{TEM-52}}$, $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-15}}$ that were present in different members of Enterobacteriaceae isolated from humans, broilers and pigs.

Fourteen $bla_{\text{TEM-52}}$-, $bla_{\text{CTX-M-2}}$- or $bla_{\text{CTX-M-15}}$-carrying clonally unrelated strains were used in this study (Table 1). These strains were isolated in Belgium from humans, pigs and broilers. The human *E. coli* strains were isolated from patients hospitalized at the Ghent University Hospital. All isolates from poultry were obtained from the faeces of healthy broilers. The *E. coli* and *Klebsiella pneumoniae* isolates were obtained as described previously.[4] The *S. enterica* isolates from poultry were collected in the framework of mandatory *Salmonella* monitoring programmes in Belgium. The two porcine *E. coli* isolates originated from pigs with diarrhoea. The ESBL gene of each isolate was characterized as described previously by isoelectric focusing, PCR and sequencing.[4] Plasmid transfer experiments were carried out as described previously.[2] The antimicrobial susceptibility of the parental strains and their *E. coli* transconjugants was determined by the Kirby–Bauer disc diffusion test (Neo-Sensitabs, Rosco Diagnostica, Taastrup, Denmark) (Table 1).[4] For the parental strains and their *E. coli* transconjugants, plasmid profiles were determined and the size of each ESBL-carrying plasmid was estimated.[2] The incompatibility (Inc) group of each ESBL-carrying plasmid was defined by the PCR-based replicon typing method.[5] Restriction fragment length polymorphism (RFLP) fingerprint analysis and Southern blot hybridization were performed as described previously.[2]

In order to better understand the spread and persistence of mobile β-lactam resistance plasmids among different members of Enterobacteriaceae isolated from different reservoirs, a closer look at the pool of conjugative plasmids was appropriate and timely.

All isolates tested here contained high-molecular-weight ESBL-carrying plasmids (~150 kb) and, for all these isolates, *E. coli* transconjugants were obtained. The $bla_{\text{CTX-M-2}}$-, $bla_{\text{TEM-52}}$- and $bla_{\text{CTX-M-15}}$-carrying plasmids belonged to IncHI2, IncI1 and IncI1, respectively (Table 1), as has already been demonstrated in previous reports.[2,3,6] RFLP analysis of plasmid DNA from the transconjugants revealed, in most cases, closely related fingerprints for plasmids carrying the same ESBL gene. All $bla_{\text{TEM-52}}$-carrying plasmids showed the same fingerprint pattern analysis, suggesting that this is a rather stable plasmid circulating in different members of the Enterobacteriaceae, present in different animal reservoirs and in humans. Southern blot hybridization with a $bla_{\text{TEM-52}}$ probe revealed two *Pst*I fragments of 2.75 and 2.9 kb, as has already been shown in a previous report.[3] The spread of a $bla_{\text{CTX-M-2}}$-carrying multiresistant plasmid among *E. coli* and

Table 1. Characteristics of the parental strains and the ESBL-carrying plasmids analysed in this study

ESBL/parental strain[a]	Species	Source	Year of isolation	Non-β-lactam resistance (parental strains)[b]	Co-transferred resistance	Transfer frequency	Replicon typing
TEM-52							
B1-54	*E. coli*	broiler	2007	TET, NAL	none	1.27×10^{-3}	I1
B8-6	*E. coli*	broiler	2007	TET, SULPH, TMP, KAN, CHL, STR, NAL	none	1.11×10^{-3}	I1
B7-9	*K. pneumoniae*	broiler	2007	TET, KAN, NEO	none	1.2×10^{-3}	I1
But 31	*E. coli*	human	2006	none	none	1.35×10^{-3}	I1
10101-1	*S. enterica* serovar Infantis	broiler	2004	none	none	1.4×10^{-3}	I1
CTX-M-2							
B4-25	*E. coli*	broiler	2007	TET, SULPH, TMP, STR	TET, SULPH, TMP, STR	0.89×10^{-3}	HI2
BUT 10	*E. coli*	human	2006	TET, SULPH, TMP, STR	TET, SULPH, TMP, STR	0.99×10^{-3}	HI2
138P	*E. coli*	pig	2006	TET, SULPH, TMP, STR	TET, SULPH, TMP, STR	1.1×10^{-3}	HI2
CODA-1	*S. enterica* serovar Virchow	broiler	2004	TET, SULPH, TMP, STR, NAL	TET, SULPH, TMP, STR	0.8×10^{-3}	HI2
142-1	*S. enterica* serovar Virchow	broiler	2001	TET, SULPH, TMP, STR, NAL	TET, SULPH, TMP, STR	0.76×10^{-3}	HI2
CTX-M-15							
B4-75	*E. coli*	broiler	2007	none	none	6.9×10^{-3}	I1
BUT 11	*E. coli*	human	2006	TET, SULPH, TMP, STR NAL, ENR, NEO, KAN, GEN	none	5.4×10^{-3}	I1
135P	*E. coli*	pig	2006	SULPH, TMP, ENR, NEO, GEN, FFC, NAL	none	6.3×10^{-3}	I1
CODA-2	*S. enterica* serovar Typhimurium	broiler	2007	none	none	5.7×10^{-3}	I1

[a]All strains were isolated from faeces, with the exception of BUT 10 that was isolated from the human throat. The RFLP fingerprint pattern of the ESBL-carrying plasmid of the 10101-1 isolate and the 142-1 isolate have been described previously[2,3] and were taken into account for RFLP fingerprint analysis to compare with the ESBL-carrying plasmids of the isolates used in this study.
[b]Antimicrobial drugs used were the following: chloramphenicol (CHL), enrofloxacin (ENR), florfenicol (FFC), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), neomycin (NEO), tetracycline (TET), trimethoprim (TMP), streptomycin (STR), sulphonamides (SULPH).

S. enterica isolates from pigs and broilers was demonstrated. Only the plasmid from the human *E. coli* isolate differed in RFLP fingerprint pattern from the other $bla_{CTX\text{-}M\text{-}2}$-carrying plasmids. Southern blot hybridization with a $bla_{CTX\text{-}M\text{-}2}$ probe revealed a >10 kb *Eco*RI fragment in the plasmids from the porcine and broiler *E. coli* isolates and from the *Salmonella* Virchow CODA-1 isolate. Two *Eco*RI fragments of 6 and 10 kb were found in the plasmid from the human *E. coli* isolate and in the plasmid from the *Salmonella* Virchow 142-1 isolate.[2] For the $bla_{CTX\text{-}M\text{-}15}$-carrying plasmids, results of RFLP analysis were identical except for the plasmid from the human *E. coli* strain. For the animal strains, Southern blot hybridization with a $bla_{CTX\text{-}M\text{-}15}$ probe revealed two *Eco*RI fragments of 6.5 and 7 kb and one 5 kb *Pst*I fragment. The plasmid from the human *E. coli* strain showed 5 and >10 kb *Eco*RI fragments and a 5.5 kb *Pst*I fragment. The Southern blot results suggest the existence of two copies of the tested ESBLs on their ~150 kb plasmids.

The differences seen in the RFLP analyses for the $bla_{CTX\text{-}M\text{-}2}$- and $bla_{CTX\text{-}M\text{-}15}$-carrying plasmids may possibly reflect the rapid evolution of these plasmids as they were exposed to different environmental stresses. The human, porcine and poultry environments may be experienced by bacteria in different ways.

In summary, ESBL resistance plasmids appear to move readily between different microorganisms and different ecosystems. TEM-52-carrying cephalosporin-resistant organisms may have been transmitted from food animals to humans, or vice versa.[3]

For the CTX-M-2- or CTX-M-15-carrying cephalosporin-resistant organisms, exchange between food animals and humans, however, remains unclear, mainly due to the unknown plasticity and evolutionary speed of the plasmids carrying them.

Acknowledgements

We are grateful to A. Carattoli for providing the plasmid incompatibility group controls. We thank Hanne Vereecke and Danielle Vandergheynst for their skilled technical assistance.

Funding

This work was supported by a grant from the Federal Public Service of Health, Food Chain Safety and Environment (grant number RT 06/3 ABRISK).

Transparency declarations

None to declare.

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Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkp133
Advance Access publication 15 April 2009

Molecular characterization of resistance to fluoroquinolones in *Bartonella henselae* and *Bartonella quintana*

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Keywords: DNA gyrase, Etest diffusion assay, disc diffusion assay, *in vitro* selection

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Sir,

Bartonella species are Gram-negative bacilli that belong to the alpha 2 subgroup of *Proteobacteria*. Due to the fastidious nature of these bacteria and the limited number of available isolates worldwide, there are few data on *in vitro* susceptibility to antibiotics. There are anecdotal reports of the use of fluoroquinolones in the treatment of infections due to *Bartonella henselae* or *Bartonella quintana*.[1] However, failures or relapses have been observed with these compounds suggesting that antibiotic resistance may develop during treatment.[1,2] Thus, deciphering the molecular mechanism of resistance for these bacteria remains important since *in vivo* data do not correlate well with *in vitro* findings. Bacterial resistance to fluoroquinolones can essentially develop through two main mechanisms, namely a decrease in the intra-bacterial concentration of the drug, or alterations in the drug's target enzymes.[3] Target site alteration results from mutations in the chromosomal genes encoding the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase IV.[3] We have recently reported that fluoroquinolone-resistant mutants can be easily obtained *in vitro* for *Bartonella bacilliformis* suggesting that these antibiotics should not be used to treat infectious due to this bacterium, i.e. Carrion's disease.[4] Moreover, we have demonstrated that an intrinsic mutation in the QRDR region of DNA gyrase confers heterogeneity of susceptibility to fluoroquinolones and a low