ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 2010, p. 4484–4486 Vol. 54, No. 10
0066-4804/10/$12.00 doi:10.1128/AAC.00460-10

IncI1 Plasmid Carrying Extended-Spectrum-β-Lactamase Gene $bla_{\text{CTX-M-1}}$ in *Salmonella enterica* Isolates from Poultry and Humans in France, 2003 to 2008[▽]

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Received 6 April 2010/Returned for modification 18 June 2010/Accepted 12 July 2010

We report the dissemination of a conjugative IncI1 plasmid carrying $bla_{\text{CTX-M-1}}$, conferring resistance to extended-spectrum cephalosporins, in *Salmonella enterica* isolates from poultry and humans in France from 2003 to 2008. By IncI1 plasmid subtyping, this plasmid was shown to be genetically related to that found in *Escherichia coli* isolates from healthy poultry in France.

Food-producing animals are the primary reservoir of zoonotic pathogens, and the prevalence of resistance to extended-spectrum cephalosporins (ESCs) in *Escherichia coli* and *Salmonella enterica* has increased in recent years. In Belgium and France, the emergence of resistance to ESCs, due to extended-spectrum β-lactamases (ESBLs), in *E. coli* and *S. enterica* from animal (mainly cattle and poultry) and human origins (1, 3, 6, 7, 10, 11, 14, 15, 17) has been reported. Resistance in these bacteria was reported to be conferred mainly by the ESBL genes $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-15}}$, and $bla_{\text{TEM-52}}$ (1, 3, 6, 11, 14, 15, 17). In addition, the occurrence of CTX-M-1 ESBL resistance in *E. coli* isolates recovered from food animals (cattle, poultry, and swine) in France was recently reported (7, 10, 11). The ESBL resistance genes were shown to reside on large conjugative plasmids of the IncI1 or IncHI2 incompatibility group (3, 5, 6).

Since 2003, a number of *S. enterica* strains showing resistance to ESCs by production of an ESBL not previously detected in *Salmonella* in France and with various additional resistances to other antibiotic families have been isolated from poultry ($n = 1$) and from humans ($n = 9$) in France (Table 1). The human isolates consisted of seven serovar Typhimurium (including a monophasic variant) strains, one serovar London strain, and one serovar Newport strain, and the avian isolate was of serovar Llandoff. The purpose of the present study was to identify the ESBL gene and to characterize the plasmid(s) carrying this gene. For this, in addition to other methods, we applied the recently described plasmid multilocus sequence typing method for IncI1 plasmids carrying ESBL genes (5).

The strains studied are shown in Table 1. Antibiotic susceptibility testing was done by the disc diffusion method, and MICs of ceftriaxone and ceftazidime were determined by Etest as described previously (1, 15, 17). Resistance to ESCs was transferred from all *Salmonella* strains to *E. coli* recipient strain J5 (resistant to rifampin) by conjugation as previously described (1, 15, 17). Depending on the strain, other resistances were transferred, mostly sulfonamide together with trimethoprim resistance (Table 1). The other resistances from multidrug-resistant strains were not transferred by conjugation. The levels of resistance to ESCs, as determined by the MIC, were lower in the transconjugant strains than in the parental strains. However, this was also observed in previous studies (3, 15). PCR assays to detect ESBL genes (TEM, SHV, and CTX-M) were performed on parental and transconjugant strains using previously described primers (1, 15, 17), and nucleotide sequencing of the amplicons identified the $bla_{\text{CTX-M-1}}$ resistance gene in all strains (Table 1). Plasmids extracted from the transconjugants were further characterized by PstI restriction analysis, which showed that they were similar, with a size of 100 kb as determined by S1 nuclease experiments (Fig. 1 and data not shown). Interestingly, the plasmid restriction profile was also similar to that from $bla_{\text{CTX-M-1}}$-carrying plasmids from *E. coli* isolated from healthy poultry in France (Fig. 1 and data not shown) (7) but distinct from $bla_{\text{CTX-M-1}}$-carrying plasmids from *E. coli* isolates from other animal sources in France (data not shown) (11). This suggested a possible common avian origin for this $bla_{\text{CTX-M-1}}$-carrying plasmid. Southern blot hybridization experiments using a $bla_{\text{CTX-M-1}}$ gene probe revealed one PstI fragment of 6 kb in all plasmids, which is the same size as that found in the *E. coli* plasmid of avian origin (Fig. 1). The $bla_{\text{CTX-M-1}}$ gene has been previously reported to be linked to the IS*Ecp1* insertion sequence (7, 11). PCR performed as described previously (11), using a forward primer in the IS*Ecp1* gene and a reverse primer in the $bla_{\text{CTX-M-1}}$ gene, was positive for all transconjugant plasmids (data not shown). The link with IS*Ecp1* was further confirmed by Southern blotting with an IS*Ecp1* probe, which revealed a fragment identical in size to that revealed by the $bla_{\text{CTX-M-1}}$ probe on the PstI-restricted plasmids (Fig. 1).

To better clarify the genetic relatedness of the plasmids, the

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▽ Published ahead of print on 19 July 2010.

TABLE 1. Characteristics of the *Salmonella* strains and their transconjugants producing CTX-M-1 used in this study[a]

Strain[b]	Serovar	PFGE type	Origin	Yr	MIC (μg/ml) of:		Coresistance markers[c]	IncI1 pMLST[d]	SGI1
					Cro	Caz			
03-8748	Newport	NDc	Human	2003	>256	8	Su, Tm		−
03-8748TC1					256	2	Su, Tm	3	
05-9280	Typhimurium	XTYM-1	Human	2005	>256	32	Cm, Spt, Str, Su, Tc, Tm		+
05-9280TC1					>256	32	Su, Tm	3	
06CEB6542SAL	Llandoff	ND	Poultry	2006	64	4	Su, Tc		−
06CEB6542SALTC1					8	0.5	Su, Tc	3	
06-6550	Typhimurium	XTYM-115	Human	2006	256	16	Su, Tc, Tm		−
06-6550TC1					64	4	Su, Tm	3	
07-819	London	ND	Human	2007	>256	8	Su, Tm		−
07-819TC1					256	4	Su, Tm	3	
08-843	Typhimurium	XTYM-1	Human	2008	256	16	Cm, Spt, Str, Su, Tc, Tm		+
08-843TC1					256	8	Su, Tm	3	
08-1537	Typhimurium	XTYM-117	Human	2008	>256	64	Su, Tm		−
08-1537TC1					128	8	Su, Tm	3	
08-1745	Typhimurium	XTYM-117	Human	2008	>256	16	Su, Tm		−
08-2211	Typhimurium	XTYM-117	Human	2008	256	8	Su, Tm		−
08-2712	4,5,12:i:−	XTYM-30	Human	2008	256	16	Su, Tc, Tm		−
08-2712TC1					64	8	Su, Tm	3	

[a] Abbreviations: Caz, ceftazidime; Cm, chloramphenicol; Cro, ceftriaxone; Spt, spectinomycine; Str, streptomycin; Su, sulfonamide; Tc, tetracycline; Tm, trimethoprim; ND, not determined.
[b] Strains ending in "TC1" are *E. coli* transconjugant strains.
[c] Antibiotics other than β-lactams.
[d] IncI1 plasmid multilocus sequence type (pMLST), according to Garcia-Fernandez et al. (5), of the CTX-M-1 plasmids extracted from transconjugants.

bla$_{CTX-M-1}$-positive plasmids were typed by the PCR-based replicon typing as previously described (2), demonstrating that they all belong to the IncI1 incompatibility group. IncI1 plasmids were recently observed in *E. coli* and different serovars of *Salmonella* isolated in Belgium, France, Germany, Spain, and the United Kingdom and were found to be associated with relevant β-lactamases such as CMY-2, CMY-7, CTX-M-1, CTX-M-15, and TEM-52, suggesting a high prevalence of this kind of plasmid in Europe (3, 5, 8, 9, 13). In addition, further characterization using the recently described pMLST method for the characterization of IncI1 plasmids showed that all *Salmonella* *bla*$_{CTX-M-1}$-carrying plasmids in this study were of sequence type 3, as reported for *E. coli* isolates from poultry in France (data not shown) (5). This was also confirmed for the *E. coli* *bla*$_{CTX-M-1}$-carrying control plasmids of poultry origin in this study. Thus, like restriction typing, replicon typing and pMLST indicated a possible common avian origin for the *bla*$_{CTX-M-1}$-carrying plasmid emerging in *E. coli* and *Salmonella* in France.

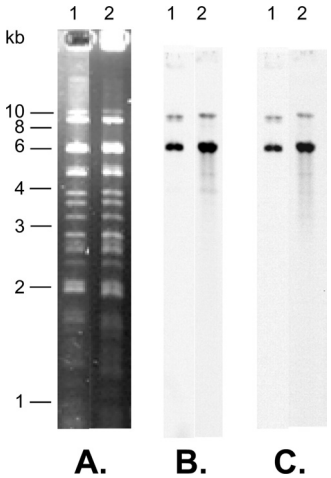

FIG. 1. Restriction analysis (PstI) (A) and Southern blot hybridization with a *bla*$_{CTX-M-1}$ probe (B) or with an IS*Ecp1* probe (C) of plasmid DNAs isolated from *E. coli* transconjugants with *E. coli* isolates from poultry origin in France (lanes 1) and the *Salmonella* isolates in this study (lanes 2) as parental strains.

Among the ESC-resistant *Salmonella* strains studied, two serovar Typhimurium isolates showed an additional multidrug resistance profile with resistances to chloramphenicol, streptomycin, spectinomycin, sulfonamide, tetracycline, and trimethoprim (Table 1). This multidrug resistance profile is characteristic of the *Salmonella* genomic island 1 (SGI1) antibiotic resistance gene cluster commonly found in the serovar Typhimurium DT104 epidemic clone (12). Identification of SGI1 and mapping of its antibiotic resistance gene cluster performed as described previously (4) confirmed that the two isolates possessed SGI1, with its classical antibiotic resistance gene cluster consisting of a complex class 1 integron (12). This combination of SGI1-mediated multidrug resistance in *Salmonella* strains associated with ESC resistance has rarely been reported, and further surveillance of such strains is thus warranted (3).

To assess the genetic diversity of the serovar Typhimurium strains, PulseNet standard pulsed-field gel electrophoresis (PFGE) of XbaI-digested chromosomal DNA was carried out (16). These strains showed four different PFGE profiles (Fig. 2 and Table 1). The XTYM-1 profile exhibited by both SGI1-carrying isolates is the most prevalent profile for DT104 strains in France (16).

In conclusion, this study showed the spread of an IncI1 plasmid carrying the *bla*$_{CTX-M-1}$ gene among *S. enterica* sero-

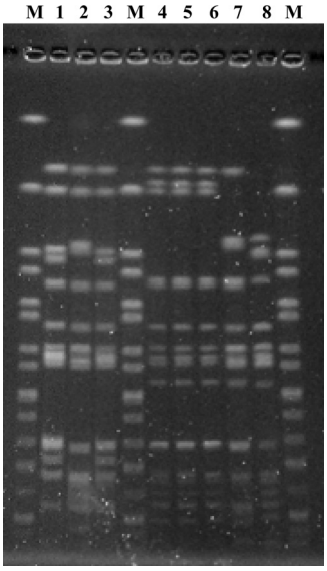

FIG. 2. XbaI-PFGE profiles of the *S. enterica* serovar Typhimurium (and monophasic variant) strains studied. M, XbaI-digested DNA from *S. enterica* serovar Braenderup strain H9812; lane 1, strain 05-9280; lane 2, strain 06-6550; lane 3, strain 08-843; lane 4, strain 08-1537; lane 5, strain 08-1745; lane 6, strain 08-2211; lane 7, strain 08-2712; lane 8, strain 09-1581 (unrelated to the study).

vars Llandoff, London, Newport, and Typhimurium of animal and human origin. According to our plasmid analyses, this plasmid is likely the same as that found in *E. coli* from poultry in France. We thus suspect horizontal transfer events that can contribute to its spread between bacterial populations from animals and humans as well (14). The further spread of such plasmids in multidrug-resistant strains carrying SGI1 is of concern.

We thank Alessandra Carattoli for her kind assistance and suggestions in the characterization of the plasmids of this study. We are also grateful to Laurent Poirel, Jean-Yves Madec, and Danièle Meunier for providing the *E. coli* control plasmids of this study.

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