Journal of Applied Microbiology

Journal of Applied Microbiology ISSN 1364-5072

ORIGINAL ARTICLE

In situ ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration

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Keywords
conjugation, continuous flow model, extended-spectrum β-lactams, transfer frequency.

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2010/1629: received 15 September 2010, revised 18 October 2010 and accepted 12 November 2010

doi:10.1111/j.1365-2672.2010.04907.x

Abstract

Aims: The behaviour of an *Escherichia coli* isolate of broiler origin harbouring a bla_{TEM-52}-carrying plasmid (lactose-negative mutant of B1-54, *IncII* group) was studied in an *in situ* continuous flow culture system, simulating the human caecum and the ascending colon during cefotaxime administration.
Methods and Results: Fresh faeces from a healthy volunteer, negative for cephalosporin-resistant *E. coli*, were selected to prepare inocula. The microbiota was monitored by plating on diverse selective media, and a shift in the populations of bacteria was examined by 16S rDNA PCR denaturing gradient gel electrophoresis. *Escherichia coli* transconjugants were verified by plasmid and pulsed-field gel electrophoresis profiles (PFGE). The avian extended-spectrum β-lactamase-positive *E. coli* was able to proliferate without selective pressure of cefotaxime, and *E. coli* transconjugants of human origin were detected 24 h after inoculation of the donor strain. Upon administration of cefotaxime to the fresh medium, an increase in the population size of *E. coli* B1-54 and the transconjugants was observed. PFGE and plasmid analysis revealed a limited number of human *E. coli* clones receptive for the bla_{TEM-52}-carrying plasmid.
Conclusions: These observations provide evidence of the maintenance of an *E. coli* strain of poultry origin and the horizontal gene transfer in the human commensal bowel microbiota even without antimicrobial treatment.
Significance and Impact of the Study: The fact that an *E. coli* strain of poultry origin might establish itself and transfer its *bla* gene to commensal human *E. coli* raises public health concerns.

Introduction

Escherichia coli, the best studied member of the bacterial family of *Enterobacteriaceae*, is a Gram-negative rod-shaped bacterium that mostly occurs as a normal commensal in the intestinal tract of animals and humans. Some strains are, however, important intestinal and extraintestinal pathogens. Human and animal pathogenic *E. coli* are able to cause a spectrum of illnesses ranging from self-limiting gastrointestinal infections to life-threatening bacteraemia.

In human and veterinary medicine, β-lactams are extensively used. The predominant cause of resistance to β-lactam antibiotics in Gram-negative bacteria is the production of β-lactamases. These enzymes can be located on the chromosome or on plasmids (Sheldon 2005).

Extended-spectrum β-lactamases (ESBLs) (e.g. TEM-52), capable of efficiently hydrolysing extended-spectrum

cephalosporins and associated with several treatment failures, disseminate through plasmid transmission among the family of the *Enterobacteriaceae*. These bacteria are part of the total microbiota of the digestive tract (Shah *et al.* 2004; Sheldon 2005; Smet *et al.* 2009).

The presence of cephalosporin-resistant *E. coli* in the intestinal tract of food-producing animals is extensively described in many reports (Hasman *et al.* 2005; Kojima *et al.* 2005; Riaño *et al.* 2006; Cloeckaert *et al.* 2007; Liu *et al.* 2007). There is recently a high concern among clinical microbiologists about ESBL-producing *E. coli* on poultry meat as a result of monitoring reports in the EU (European Union), e.g. in the Netherlands and Belgium, showing high prevalence levels (Kojima *et al.* 2005; Riaño *et al.* 2006; Liu *et al.* 2007; Smet *et al.* 2009). The diversity of ESBLs (e.g. TEM-52, CTX-M-1 and CTX-M-2) among these bacteria is high and they may act as a reservoir of ESBL genes for pathogens causing disease in humans. This raises a potential public health concern (Vollaard and Clasener 1994; Smet *et al.* 2008). The human digestive tract, colonized by a complex microbiota, has been proposed as a suitable environment for horizontal transfer of plasmids carrying antimicrobial resistance genes and contributes to the maintaining and the dissemination of resistance (Netherwood *et al.* 1999). In the case of ESBL genes, it is not exactly known how easy humans become carriers as the result of the consumption of poultry meat. The determination of the transfer frequency between *E. coli* populations of human and poultry origin is a first step in this study.

In vitro transfer of β-lactamase-encoding (*bla*) genes is relatively easy to demonstrate between individual strains and has been extensively studied (Smet *et al.* 2009). *In vivo* transfer of a *bla* gene in the intestine of mice has been shown to occur (Moubareck *et al.* 2003; Schjorring *et al.* 2008) as also indications of transfer of *bla* genes between commensal members of *Enterobacteriaceae* of human origin in clinical cases because of the treatment with a β-lactam antimicrobial agent (Bidet *et al.* 2005; Karami *et al.* 2007).

More information is however needed on the transfer of specific antibiotic resistance genes from animal- or food-derived strains to human strains in the gut. A recent controlled experiment where healthy humans ingested a sulphonamide-susceptible recipient strain of human origin and a sulfonamide-resistant donor strain of animal origin showed the transfer of this *sul* gene to *E. coli* of human origin in the gut. It has to be noted that the volunteer from whom the transfer took place was already colonized with commensal sulfonamide-resistant *E. coli* before the start of the experiment. The reason that these volunteers also ingested an *E. coli* strain of animal origin was to shed light on the risk associated with antimicrobial drug-resistant *E. coli* in food. However, evidence of transfer of resistance genes from an animal-derived *E. coli* to a recipient of human origin was not provided in the human intestinal tract (Trobos *et al.* 2009). Another study reported the transfer of vancomycin resistance from an *Enterococcus faecium* isolate of animal origin to an *Ent. faecium* isolate from human origin in the intestines of humans (Lester *et al.* 2006).

These human-controlled experiments requiring volunteers are possibly not without risk and usually do not investigate the behaviour of the antibiotic-resistant strains (donors and transconjugants) with and without antibiotic treatment. Ethical and health constraints however hamper experimental studies in humans. Therefore, the objective of this study was to investigate whether an *E. coli* isolate of broiler origin could transfer its $bla_{\text{TEM-52}}$-carrying plasmid to *E. coli* of human origin in an *in situ* continuous flow culture system, simulating the human intestinal tract in different situations (disturbed or stabilized gut microbiota) and this is to highlight the risk for public health associated with ESBL-producing *E. coli* in food. The *in situ* continuous flow culture system underwent different culture scenarios that simultaneously allowed assessing the impact of a 4-day course of a third-generation cephalosporine (cefotaxime). This study also allowed us to measure to what extent the cephalosporin-resistant *E. coli* strains, both the donor from broiler origin and the human transconjugants, could colonize in this fermentation system in different situations (antibiotic treatment or not). Such data about transfer frequency and colonization rate are necessary to perform risk assessment of human intake of food containing antimicrobial drug-resistant bacteria.

Materials and methods

Strain preparation

Escherichia coli strain B1-54, isolated from a faecal sample of broilers during an earlier survey (Smet *et al.* 2009), was used as donor, after selection of a lactose-negative mutant to allow accurate enumeration of transconjugants. The B1-54 isolate carried a $bla_{\text{TEM-52}}$-carrying plasmid that belonged to the incompatibility group Inc*I1* and had a size of >100 kb. B1-54 also showed phenotypic resistance to nalidixic acid and tetracycline but these resistance genes are not located on the $bla_{\text{TEM-52}}$-carrying plasmid (Smet *et al.* 2009). The methodology was slightly modified from the one described by Varela *et al.* (1997). Briefly, mutants were isolated on minimal succinate (0·2%) plates containing 3 mmol l^{-1} β-thio-o-nitrophenyl-β-D-galactopyranoside (TONPG) (Sigma, Bornem, Belgium) plus 0·5 mmol l^{-1} isopropyl-β-D-thio-galactopyranoside (IPTG) (Sigma) and incubated at 37°C.

Mutants that grew on these plates were streaked on 1% (w/v) melibiose MacConkey agar plates (plus 0·5 mmol l^{-1} IPTG). Red colonies were picked and inoculated on 1% (w/v) lactose MacConkey agar plates. One white colony was selected for this study, and this lactose-negative mutant of *E. coli* B1-54 was grown overnight on a MacConkey agar plate supplemented with 2·5 mg l^{-1} cefotaxime.

The presence of red colonies (lactose positive) on the MacConkey agar plates (Oxoid Ltd, Basingstoke, Hampshire, UK) (with 2·5 mg ml^{-1} cefotaxime) refers to transconjugants (of human origin), while white colonies (lactose negative) refer to the lactose mutant of *E. coli* B1-54.

Fermentation system

Growth conditions for the caecum and ascending colon were mimicked in an *in situ* system, which operated as a continuous system. The growth medium for the human caecum and the ascending colon was prepared as described by MacFarlane *et al.* (2005). One 1·3-l glass fermentation vessel, operated under the control of a BioFlo 110 unit (New Brunswick Scientific, Edison, NJ, USA), was used in this study to simulate the microbial growth conditions of the caecum and ascending colon. Via a peristaltic pump, fresh sterilized medium was added at a constant rate of about 1·8 ml min^{-1}, and spent culture liquid was wasted at the same rate to maintain a constant working volume of 500 ml. Culture pH was maintained at 6·2, the temperature at 37°C and moderate agitation at 200 rev min^{-1}. Cultures were sparged by flushing the headspace of the vessel with a nitrogen carbon dioxide gas mixture (80 and 20%, respectively) at a flow rate of 20 ml min^{-1} to create anaerobic conditions. Fresh faeces from a healthy volunteer (adult), negative for cephalosporin-resistant *E. coli*, were used to prepare inocula. The stools were homogenized with a kitchen blender, divided in aliquots of 30 ml, supplemented with 15% glycerol and frozen at −80°C. After inoculation with 5-ml pooled stool content (corresponding to 1 g faeces), the fermentor was operated in batch mode for 24 h. Continuous culture was started by switching on the peristaltic pumps (Messens *et al.* 2009). The lactose mutant of *E. coli* B1-54 was grown overnight on a MacConkey agar plate (supplemented with 2·5 mg l^{-1} cefotaxime). Two loopfull of colonies were suspended in 10 ml of PBS up to a final concentration of approximately 2×10^8 CFU ml^{-1} and added to the fermentation system at the indicated times (Figs 1–3). Immediately after inoculation of the lactose mutant of *E. coli* B1-54 (the donor strain), a sample was taken to calculate the start number of the donor strain in the fermentation system.

Study design

Three experiments were carried out, and in all three cases, cefoxatime was supplied during four consecutive days. In a first negative control experiment (I), no ESBL+ donor strain (lactose mutant of *E. coli* B1-54) was added to evaluate the stability of the model.

A second experiment (II) consisted of adding the ESBL+ donor strain simultaneously with the intestinal content at the initiation of the batch mode, 24 h prior to the peristaltic simulation. During a final third experiment (III), the ESBL+ donor strain was added 24 h after the peristaltic simulation had started.

Bacterial population dynamics

The cultivatable microbiota was monitored and counted by dilution plating on diverse selective media (Oxoid

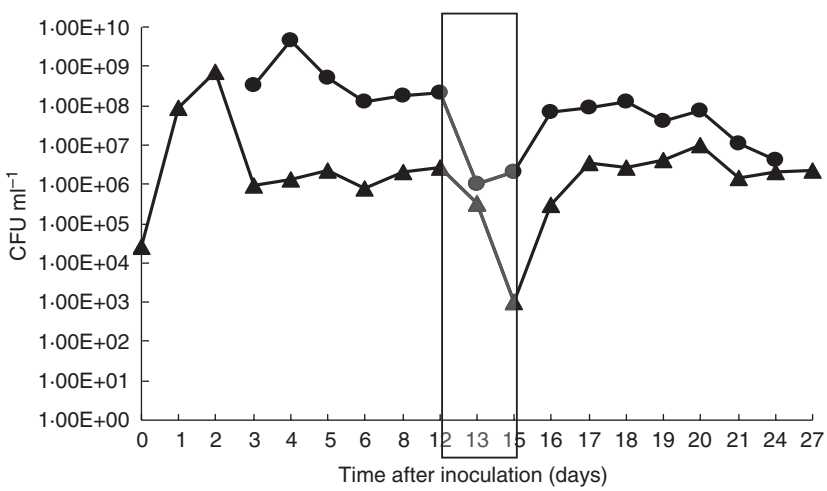

Figure 1 Influence of antimicrobial treatment on the microbial community of the human caecum and the ascending colon (blank experiment). Start of continuous culture at day 1. The start and end of the antimicrobial treatment (from day 12 to day 15) is indicated by the box. (–▲–) *Escherichia coli* (human) and (–●–) total aerobic bacteria.

LTD): Reinforced Clostridial medium for total anaerobes (37°C, 24 h, anaerobic conditions); de Man, Rogosa and Sharpe medium for lactic acid bacteria (37°C, 24 h, anaerobic conditions); Tryptone Soy Agar for total aerobes (37°C, 24 h, aerobic conditions); and MacConkey Agar (with and without 2·5 mg l^{-1} cefotaxime) for lactose-negative donor strains of *E. coli* (of poultry origin, white colonies), population of potential recipient *E. coli* (of human origin and cefotaxime susceptible, pink colonies) and *E. coli* transconjugants (of human origin and cefotaxime resistant, pink colonies) (37°C, 24 h, aerobic conditions).

A shift in bacterial populations, because of a continuous administration of cefotaxime (2·5 mg l^{-1}) during four consecutive days, was examined by 16S (V3 region) rDNA PCR denaturing gradient gel electrophoresis (DGGE), as described previously (Boon *et al.* 2000).

Transconjugant identification and horizontal gene transfer confirmation

A representative number of pink colonies were phenotypically identified as *E. coli* (Smet *et al.* 2008) after overnight aerobic incubation at 37°C on MacConkey agar with 2·5 mg l^{-1} cefotaxime. The selected *E. coli* transconjugants were genotyped using *Xba*I-pulsed-field gel electrophoresis (PFGE) (Bertrand *et al.* 2006). To demonstrate whether these transconjugants obtained the bla_{TEM-52}-carrying plasmid, plasmid DNA was obtained as described by Takahashi and Nagano (1984), the incompatibility (Inc) group was defined by the PCR-based replicon typing method (Carattoli *et al.* 2005), and PCR and sequencing were performed for the identification of the bla_{TEM-52} gene (Smet *et al.* 2009). These transconjugants were tested for susceptibility to: ampicillin, amoxicillin–clavulanic acid, cefoxitin, ceftazidim, cefepime, nalidixic acid, tetracycline, sulphonamides, trimethoprim, streptomycin, gentamicin and chloramphenicol as earlier described (Smet *et al.* 2008).

At a given sample point, the average transfer frequency was estimated by dividing the number of transconjugants per ml by the number of recipients (human *E. coli*) per ml.

Results

Experiment I: Caecal bacterial dynamics without ESBL+ donor strain

In a blank experiment (without administration of the lactose mutant of *E. coli* B1-54), simulating the human caecum and the ascending colon, *E. coli* of human origin (and cefotaxime susceptible) was able to stabilize at a population size of 6 ± 0·2 $\log_{10}$ CFU ml^{-1} for 12 days in continuous culture (Fig. 1). No spontaneous lactose-negative *E. coli* mutants were detected during the experiment. From day 12 onwards, the normal incubation medium was replaced by a stock containing 2·5 mg l^{-1} cefotaxime and maintained until day 15. Antimicrobial treatment gave an immediate decrease in the population size (from 6 $\log_{10}$ to 3 $\log_{10}$ CFU ml^{-1}) for *E. coli* of human origin. At day 16, the medium with 2·5 mg l^{-1} cefotaxime was replaced again by the normal incubation medium. After day 16, the population size of the cefotaxime-susceptible *E. coli* of human origin increased again and restored itself after discontinuation of the antimicrobial administration (Fig. 1).

Experiment II: Caecal bacterial dynamics with ESBL+ donor strain at the initiation of experiment

The aim of the second experiment was to investigate whether the lactose mutant of *E. coli* B1-54 of broiler origin was able to maintain itself and transfer its bla_{TEM-52}-carrying plasmid to *E. coli* strains of human origin as well as the behaviour of the human transconjugants, in a fermentation condition simulating a disturbance of the human microbial community.

The lactose mutant of *E. coli* B1-54 was added as donor strain together with the human stool sample in the fermentation system, which was operated in batch mode for 24 h. Continuous culture was then started by switching on the peristaltic pumps. Fluctuations in population size of several bacterial groups were seen during the first 5 days (Fig. 2a). The lactose mutant of *E. coli* B1-54 and the *E. coli* of human origin (and cefotaxime susceptible) could establish themselves after 5 days at a population size of 7 ± 0·1 $\log_{10}$ and 7 ± 0·2 $\log_{10}$ CFU ml^{-1}, respectively (Fig. 2a). Other average population sizes were 8 ± 1 $\log_{10}$, 9 ± 2 $\log_{10}$ and 9 ± 2 $\log_{10}$ CFU ml^{-1} for total aerobic bacteria, lactic acid bacteria and total anaerobes, respectively (Fig. 2b). Twenty-four hours after administration of the lactose mutant of *E. coli* B1-54, transconjugants of human origin, resistant to cefotaxime, were detected at a population size of 5 $\log_{10}$ CFU ml^{-1} (Fig. 2a). The average transfer frequency of ESBL resistance was $2{\cdot}5 \times 10^{-3}$ at day 2 of the fermentation experiment. From day 11 onwards, the normal incubation medium was replaced by a stock containing 2·5 mg l^{-1} cefotaxime. The antimicrobial treatment was maintained until day 14. During antimicrobial treatment (days 11–14), a decrease in the population size of the normal cefotaxime-susceptible human microbial community [*E. coli* (from 6 to 3 ± 0·04 $\log_{10}$) and total aerobic bacteria (from 6 to 3 ± 0·1 $\log_{10}$)] was seen. On the contrary, an increase in the population size of the lactose mutant of *E. coli* B1-54 (from 6 to 9 ± 0·3 $\log_{10}$) and of the

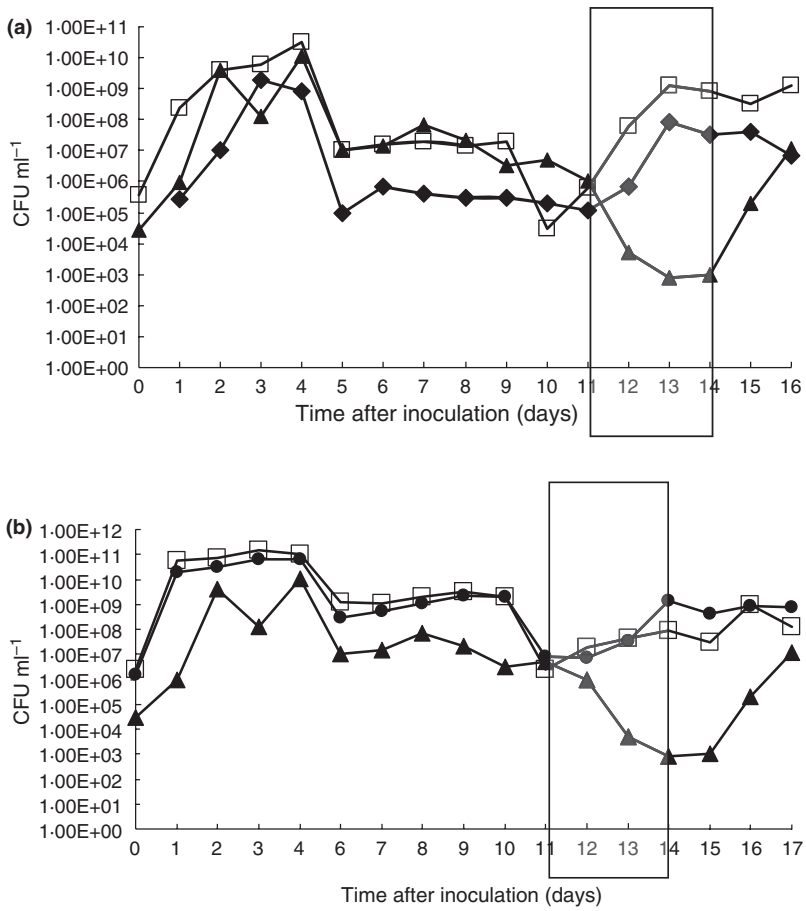

Figure 2 Administration of *Escherichia coli* B1-54 before the microbial community of the human ceacum and the ascending colon reached a steady state. Start of continuous culture at day 1. The start and end of the antimicrobial treatment (from day 11 to day 14) is indicated by the box. (a) (–▲–) *E. coli*; (–▫–) B1-54 and (–◆–) transconjugants. (b) (–●–) Lactic acid bacteria; (–▫–) anaerobes and (–▲–) total aerobic bacteria.

human transconjugants (from 5 to 8 ± 0·2 $\log_{10}$), both resistant to cefotaxime, was noted (Fig. 2a,b). After stopping the antimicrobial treatment, the population size of the human *E. coli* community, susceptible to cefotaxime, restored itself to the same level as before cefotaxime administration, while the population size of the cefotaxime-resistant lactose mutant of *E. coli* B1-54 and the human transconjugants was maintained at the same high level as during antimicrobial treatment (Fig. 2a).

The microbiota was disturbed as indicated by a different DGGE pattern compared to the fermentation condition without antibiotic (data not shown). At the start of antimicrobial treatment, one band disappeared, but reappeared during antimicrobial treatment. Also, a few bands appeared more strongly during antimicrobial treatment. After stopping the antimicrobial treatment, the DGGE pattern of the microbiota returned to the initial condition, but the few more prominent bands that appeared during antimicrobial treatment remained in the pattern. Pure cultures of the lactose mutant of *E. coli* B1-54, of a human transconjugant and of a human cefotaxime-susceptible *E. coli*, showed similar DGGE patterns (data not shown).

Experiment III: Caecal bacterial dynamics with ESBL+ donor strain 24 h after the initiation of experiment

The aim of this third experiment was to verify whether the lactose mutant of *E. coli* B1-54 of broiler origin was able to maintain itself and to transfer its $bla_{\text{TEM-52}}$-carrying plasmid to *E. coli* strains of human origin as well as the behaviour of the human transconjugants in a fermentation condition in which the human microbial community was already dominantly present.

The human stool sample was added in the fermentation system and operated in batch mode for 24 h so that the human microbial community was predominantly present in the fermentation system. The lactose mutant of *E. coli* B1-54 was supplemented after the switch to continuous culture (after 1 day). The lactose mutant of *E. coli* B1-54 and the cefotaxime-susceptible *E. coli* of human origin could establish themselves at a population size of 3 ± 1 $\log_{10}$ and 7 ± 1 $\log_{10}$ CFU ml^{-1}, respectively (Fig 3a). Other average population sizes were 7 ± 0·1 $\log_{10}$ CFU ml^{-1} for total aerobic bacteria, lactic acid bacteria and total anaerobes (Fig. 3b). As seen in experiment II, 24 h after administration of the lactose mutant of

E. coli B1-54, transconjugants of human origin were detected. The population size (1 $\log_{10}$ CFU ml^{-1}) of the transconjugants was remarkably lower in comparison with the second experiment. The average transfer frequency was $2{\cdot}5 \times 10^{-5}$ at day 3. After 11 days of normal operation, the switch was made to medium stock with 2·5 mg l^{-1} cefotaxime. Gradual replacement of the regular medium in the fermentor by medium supplemented with 2·5 mg l^{-1} cefotaxime resulted in a decrease in the *E. coli* of human origin (and cefotaxime susceptible) (from 7 to $5 \pm 0{\cdot}04$ $\log_{10}$) and an increase in the cefotaxime-resistant lactose mutant of *E. coli* B1-54 (from 3 to $5 \pm 0{\cdot}1$ $\log_{10}$ CFU ml^{-1}) and the transconjugants of human origin (from 2 to $5 \pm 0{\cdot}1$ $\log_{10}$ CFU ml^{-1}) (Fig. 3a,b). At day 14, the feed was switched back to the medium without 2·5 mg l^{-1} cefotaxime. After day 14, the population size of the cefotaxime-resistant lactose mutant of *E. coli* B1-54 and the transconjugants were maintained at the same high level as during antimicrobial treatment, and the population size of the cefotaxime-susceptible human *E. coli* community gradually restored (Fig. 3a,b).

Denaturing gradient gel electrophoresis observations and characterization showed highly comparable results with experiment II.

Verification of the *Escherichia coli* transconjugants

A total of 60 *E. coli* transconjugants were retrieved from the MacConkey agar plates (supplemented with 2·5 mg l^{-1} cefotaxime) during fermentation experiments II and III and were verified by plasmid analysis (data not shown) and PFGE profiles. All isolated transconjugants contained a large plasmid of >100 kb from incompatibility group Inc*I1*, and PCR and sequencing revealed the presence of a $bla_{\text{TEM-52}}$ gene on this plasmid (data not shown). Antimicrobial susceptibility revealed that the *E. coli* transconjugants, besides showing an ESBL phenotype (i.e. a marked synergistic effect between resistance to

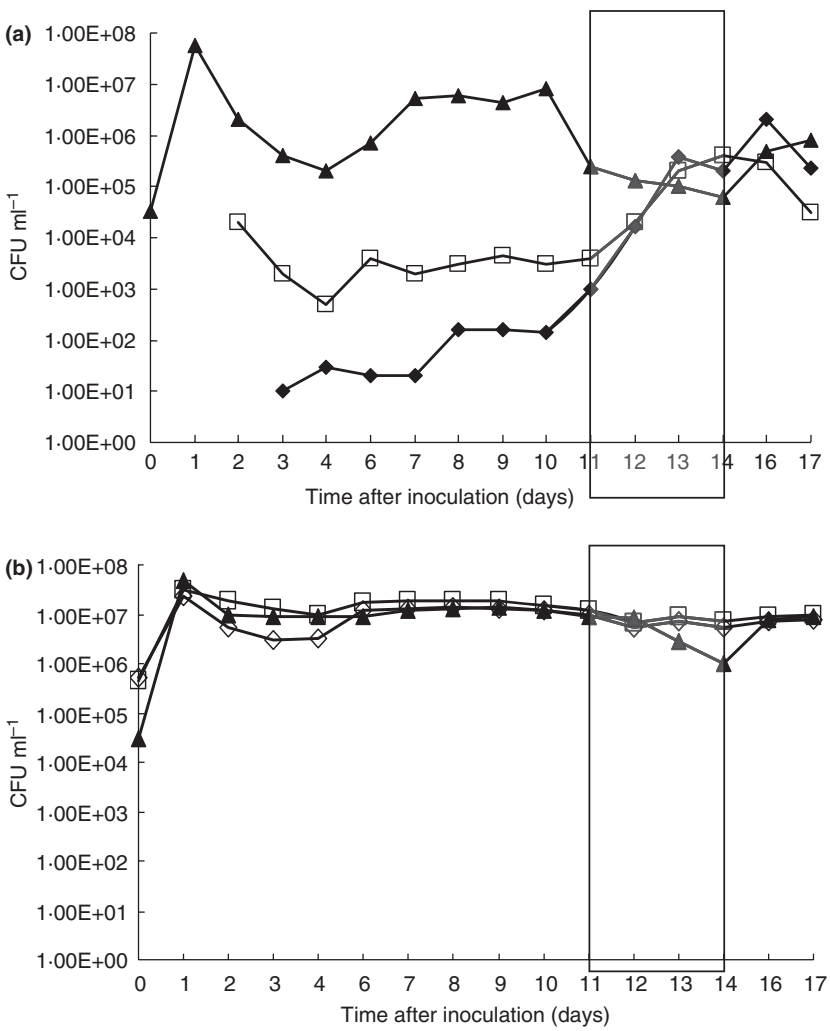

Figure 3 Administration of *Escherichia coli* B1-54 after the microbial community of the human ceacum and the ascending colon reached a steady state. Start of continuous culture at day 1. The start and end of the antimicrobial treatment (from day 11 to day 14) is indicated by the box. (a) (–▲–) *E. coli*; (–□–) B1-54 and (–◆–) transconjugants. (b) (–◇–) Lactic acid bacteria; (–□–) anaerobes and (–▲–) total aerobic bacteria.

extended-spectrum cephalosporins and amoxicillin with clavulanic acid), were susceptible to all other tested non-β-lactam antimicrobial agents. PFGE revealed only two different fingerprint patterns among the isolated *E. coli* transconjugants of human origin (Fig. 4). The most predominant fingerprint pattern was obtained for 55 of the 60 isolates (91·7%) (lane 3 and 5 in Fig. 4), with a distinct fingerprint pattern obtained for the other isolates. PFGE was also performed for a total of 20 random cefotaxime-susceptible *E. coli* strains isolated from the stool sample. Surprisingly, only one PFGE pattern was seen and it was interesting to notice that this fingerprint pattern was identical to the predominant fingerprint pattern found among the transconjugants.

Discussion

In this study, it was experimentally shown that a lactose mutant of ESBL-producing *E. coli* B1-54, isolated from a faecal sample of broilers, was able to proliferate in the fermentation system simulating a part of the human gastrointestinal tract. The results suggest that the population size of the donor strain depends on ongoing microbial competitions during the experiments that might suffer from standardization. Previous reports suggested that in order for antibiotic resistance transfer to occur, high numbers ($>10^6$ CFU g^{-1}) of both donor and recipient (compatible) strains should coincide at the same time in the gut (Lester *et al.* 2006). In our study, *E. coli* transconjugants of human origin were detected 24 h after inoculation of the donor strain B1-54 in both experiments, even with the low concentration of the donor strain (3 log CFU ml^{-1}) in experiment 3. Nevertheless, a substantial difference in transfer frequency (10^{-5} *vs* 10^{-3}) was calculated in the two fermentation conditions. Thus, attention should be given to assess the reproducibility of the fermentation conditions to allow accurate comparison of different scenarios. Other limitations of the system are the fact that only the latter part of the digestive tract is simulated while dynamics in resistance throughout the digestive tract (Catry *et al.* 2007) can have an influence on transfer frequencies. Also factors like age, immunity, disease conditions cannot be evaluated in the presented model (Levy 2002; Graffunder *et al.* 2005). The normal human bacterial microbiota, dominantly present and acting as barrier for other bacteria, gives this *in situ* model nevertheless advantages in mimicking the human gastrointestinal tract in comparison with animal models. Also, the possibility to measure the transfer frequency of antimicrobial resistance determinants and to monitor the behaviour of the donor, recipient and transconjugants are important advantages of this *in situ* model in comparison with animal models (Schjorring *et al.* 2008). Despite the continuous input and output of the growth medium, mimicking gastrointestinal transit, it has to be noted that conjugation of the $bla_{\text{TEM-52}}$-carrying plasmid still appeared even without selective pressure of an antimicrobial agent. This easy transfer of the $bla_{\text{TEM-52}}$-carrying plasmid to another recipient was not so surprising as the transfer of this plasmid has also been investigated by *in vitro* liquid mating experiments between individual strains in a previous report, for which the transfer frequency was $1{\cdot}27 \times 10^{-3}$ (Smet *et al.* 2009). A comparison between these transfer frequencies reveals that the transfer of $bla_{\text{TEM-52}}$-encoded resistance between *E. coli* strains can be influenced by the human gut microbiota under stabilized conditions. Probably, the lower transfer frequency of 10^{-5} is a more realistic estimation of the normal situation.

Because of antimicrobial cefotaxime administration, both the lactose mutant of *E. coli* B1-54 of broiler origin and the human *E. coli* transconjugants were able to proliferate quickly. In both simulated conditions (disturbed and stabilized gut microbiota), an increase by 2–3 log of both resistant *E. coli* populations was observed. After antimicrobial administration, the population size of the normal microbial community, susceptible to cephalosporins, increased again to previously obtained levels, while the cephalosporin-resistant lactose mutant of *E. coli* B1-54

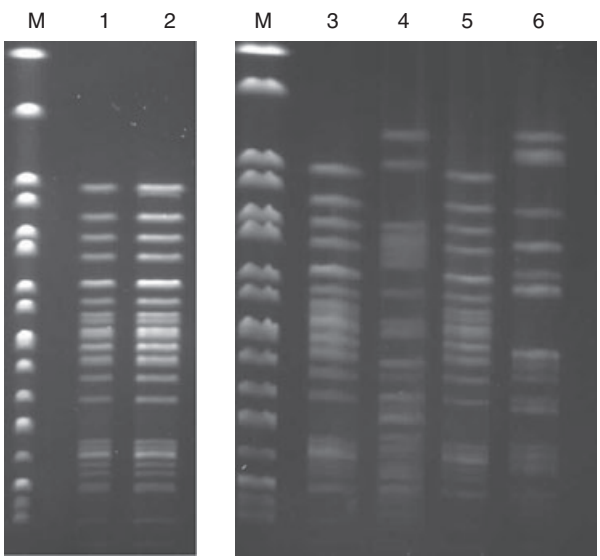

Figure 4 Pulsed-field gel electrophoresis profiles (PFGE) profiles of the donor, recipient and the *Escherichia coli* transconjugants (experiment 2). M: marker; 1 and 2: *E. coli* of human origin (susceptible to cefotaxime; recipient); 3: *E. coli* transconjugant (dominant PFGE fingerprint pattern, experiment 2 before antimicrobial treatment); 4: *E. coli* transconjugant; 5: *E. coli* transconjugant (dominant PFGE fingerprint pattern, experiment 3, before antimicrobial treatment); 6: *Escherichia coli* B1-54 (donor).

and the *E. coli* transconjugants were able to maintain at the increased levels in the fermentation system. Nevertheless, our experiments clearly support other reports (Cloeckaert *et al.* 2007; Persoons *et al.* 2010) in which third-generation cephalosporins substantially enhance the proliferation of ESBL-producing bacteria in the digestive tract. From the molecular cultivation-independent DGGE approach, it was also evident that after antimicrobial administration, some bacterial groups, other than the *E. coli* population and probably cefotaxime (inherently or acquired) resistant, had increased in population size. These results indicate that antimicrobial agents may not only select for resistant bacteria, but may also increase the potential of these resistant bacteria as donors of resistance genes by increasing their population size.

The human *E. coli* transconjugants were genotyped using PFGE. As *E. coli* has a polyclonal population structure, it would be expected to find several PFGE patterns. However, only two PFGE patterns were found, indicating that only two human *E. coli* clones received the $bla_{\text{TEM-52}}$-carrying plasmid. Besides a dominant transconjugant clone that was genetically identical to the dominant clone in the faecal *E. coli* population of the human donor, also a minor transconjugant clone was selected with a genotype that was not picked up in the random selection of the faecal *E. coli* isolates. The reason behind this phenomenon is unknown and needs further investigation.

To our knowledge, this is the first time that an *in situ* system simulating the human caecum and the ascending colon was used to study the behaviour of an *E. coli* isolate of broiler origin as well as of the human *E. coli* transconjugants after the transfer of an ESBL gene from a poultry-derived *E. coli* to (a) recipient(s) of human origin. These observations provide evidence of the maintenance of an *E. coli* strain of poultry origin and the horizontal gene transfer in the human commensal bowel microbiota even without antimicrobial treatment.

In conclusion, the ability of an ESBL-producing *E. coli* strain of animal origin to be maintained under normal or disturbed simulated physiological conditions in the human gut and to transfer the ESBL resistance gene residing in a large plasmid to commensal bacteria of human origin without antimicrobial treatment raises public health concerns. In addition, cephalosporin treatment increases potential health risk by increasing the population size of the cephalosporin-resistant microbial population. Further research is necessary to determine the risk of carriage in the human gut of ESBL-producing microbiota from consumption of food products such as poultry with a high prevalence of ESBL-producing *E. coli* and the potential risk of long-term carriage of a high population level of this resistant microbiota in the human gut after antimicrobial cephalosporin treatment.

Acknowledgements

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

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