In vitro conjugation kinetics of ESBL-producing *Escherichia coli* donors and various *Enterobacteriaceae* recipients

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Abstract

Background: Extended spectrum beta-lactamase (ESBL)-producing enterobacteria pose a major hazard to public health. Due to the possibility of genetic transfer, ESBL genes might spread to pathogenic enterobacterial strains. Thus, information on possible genetic transfer between enterobacteria is of high interest. It was therefore the aim of this *in vitro* study to screen the capacity of a wide range of *Enterobacteriaceae* for time dependent differences in conjugation with five ESBL-producing *Escherichia* (*E.) coli* strains.

Results: Conjugation frequencies for five potential *E. coli* donor strains, producing the enzymes CTX-M-1, CTX-M-15, SHV-12, TEM-1, TEM-52 and CMY-2, and six potential recipient strains (*E. coli, Serratia marcescens* subsp. *marcescens*, *Enterobacter cloacae, Salmonella* Typhimurium and *Proteus mirabilis*) were obtained. Hence, different combinations of donor and recipient strains were co-incubated for between 0 and 22 hours and spread on selective agar. Conjugation frequencies were calculated as transconjugants per donor.

Some of the donor and recipient strain combinations did not show plasmid transfer within 22 hours. Hence, the recipient *Proteus mirabilis* did not accept plasmids from any of the given donors and *E. coli* ESBL10716 was not able to transfer its plasmid to any recipient*. Enterobacter cloacae* only accepted the plasmids from the donors *E. coli* ESBL10708 and *E. coli* ESBL10716 while *E. coli* ESBL10708 did not transfer its plasmid to *Serratia marcescens* subsp. *marcescens*. *E. coli* IMT11716 on the other hand did not perform conjugation with the donor *E. coli* ESBL10689. The remaining mating pairs differed in conjugation frequency, ranging from log -5 to -8.5 transconjugants/donor. The earliest conjugation events were detected after 4 hours. However, some mating pairs turned positive only after 22 hours co-incubation.

Conclusion: The results of this study suggest that conjugation is a frequent event in the spread of ESBL genes among commensal and pathogen bacteria. This should be considered when addressing antibiotic resistance issues.

Background

Consequential to the global increase of multidrug resistant bacteria, severe economic and public health related costs have been predicted to rise significantly in the near future (1). In this context, extended-spectrum ß-lactamase (ESBL)-producing *Enterobacteriaceae* were identified as one of the antibiotic resistant bacterial groups currently posing the highest threat on public health (2). These bacteria have been detected in humans and animals equally. Within livestock, the highest prevalence of ESBL-producing bacteria was observed in poultry (3). ESBL-producing enterobacteria have been detected ubiquitous in poultry droppings and meat as well as the environment surrounding poultry (4, 5). CTX-M-1, SHV-12 and TEM-52 are the ESBL-types most commonly detected in European chicken with *Escherichia coli* and *Salmonella* spp. as the most common bacterial hosts. Often, these enterobacteria belong to

commensal bacterial populations in animals. As ESBL-producing enterobacteria are most often non-pathogenic, no clinical signs or impact on the performance are observed (3).

ESBL encoding genes are generally located on plasmids, which can be transferred between bacterial strains and species, including pathogenic strains (4, 6). Thereby, harmless, unnoticed colonization with ESBL-producing bacteria can lead to diseases, which are hard to cure with antibiotics, if the recipient happens to also carry pathogenic traits. It is known for some ESBL- carrying plasmids that they do not cause fitness costs to the bacterial host and can thus be passed on for generations, even in the absence of antibiotics (7-9). A transfer of ESBL-carrying bacteria from animal to human with a shared reservoir has been suggested (10-12). Antibiotic resistant bacteria may spread to humans by direct or indirect contact with animals, their food product, fecal matter or manure (4, 13, 14). The introduction of a TEM-52-carrying *E. coli* from poultry to the microbial community of a human stool sample resulted in the establishment of the strain as well as plasmid transfer to an *E. coli* of human origin. Both donor and transconjugants were present at a lower concentration than the human bacterial strains. A simulated treatment with a selective antibiotic substance (cefotaxime) shifted the balance to the benefit of the resistant strains, which remained at high concentrations, equal to the indigenous microbiota, even days after the termination of the treatment (15). This highlights a potential pathway for resistant bacteria from animal origin to persistently colonize the human gastrointestinal tract.

This study was undertaken to investigate the time dependence of conjugation events as a first step to estimate the possible transfer rates of ESBL genes in the intestinal tract and in the environment. Conjugation kinetics of ESBL-carrying *Enterobacteriaceae* strains commonly detected in poultry were obtained *in vitro* within a 22-hour timeframe.

Methods

Strains and cultivation

A selection of *Enterobacteriaceae* strains were screened for conjugation frequencies of ESBL- carrying plasmids with ESBL-producing *E. coli* strains as potential donors (Table 1). The respective ESBL-types had previously been identified in another project (16) and comprised CTX-M-1, CTX-M-15, SHV-12, TEM-1 and TEM-52 as well as the ampC ß-lactamase CMY- 2 (Table 2). All donor strains were isolated from broilers samples. A total of 35 enterobacterial strains (Table 1) were screened as potential recipients. These species are commonly detected in the gastrointestinal tract of poultry (17).

The bacterial strains were stored in cryo stocks and cultivated aerobically overnight at 37 °C in Müller-Hinton broth (MHB) (Carl Roth GmbH + Co. KG, Germany) with or without antibiotic supplementation. MacConkey agar (Carl Roth GmbH + Co. KG, Germany) was used for all plates, except for an agar disc diffusion assay, where Müller Hinton agar (Carl Roth GmbH + Co. KG, Germany) was used, and incubated aerobically overnight at 37 C.

Table 1: Bacterial strains used for the screening

Name	Designation	Origin	Name	Designation	Origin
Actinetobacter baumanii	DSM 102929	DSMZ	*Escherichia coli*	IMT2358	IMT
Citrobacter freundii	DSM 15979	DSMZ	*Escherichia coli*	IMT9241	IMT
Enterobacter aerogenes	DSM 30053	DSMZ	*Escherichia coli*	PS 37	IAN
Enterobacter cloacae	IMT 20749/402	DSMZ	*Escherichia coli*	PS 79	IAN
Enterobacter cloacae	DSM 30060	IMT	*Escherichia coli*	PS 90	IAN
Erwinia billingiae	DSM 17872	DSMZ	*Escherichia coli*	IMT11716	IMT
Escherichia coli	ESBL 10682	RESET	*Klebsiella pneumoniae*	IMT 20750/402	IMT
Escherichia coli	ESBL 10689	RESET	*Klebsiella pneumoniae*	DSM 12059	DSMZ
Escherichia coli	ESBL 10708	RESET	*Klebsiella pneumoniae* subsp. *Pneumoniae*	DSM 30104	DSMZ
Escherichia coli	ESBL 10716	RESET	*Proteus mirabilis*	DSM 4479	DSMZ
Escherichia coli	ESBL 10717	RESET	*Salmonella enterica* subsp. *Enterica*	DSM 17420	DSMZ
Escherichia coli	DSM 2840	DSMZ	*Salmonella enterica subsp. enterica*	DSM 5569	DSMZ
Escherichia coli	IMT 13211/356	IMT	*Salmonella enterica* subsp. *enterica*	DSM 5569	DSMZ
Escherichia coli	IMT 20751/402	IMT	*Salmonella enteriditis*	LiCC-S664	Lisando
Escherichia coli Abbottstown	IMT 3910/123	IMT	*Salmonella livingstone*	S 145	BfR
Escherichia coli	F18		*Salmonella mbandaka*	S 100	BfR
Escherichia coli Abbottstown	IMT 203/7	IMT	*Salmonella* Typhimurium	L1219-R32	Lohmann; D. Taras
Escherichia coli	IMT15146	IMT	*Serratia marcescens* subsp. *marcescens*	DSM 30122	DSMZ
Escherichia coli	IMT11863	IMT	*Shigella flexneri*	DSM 4782	DSMZ
Escherichia coli	IMT2271	IMT	*Shigella sonnei*	DSM 5570	DSMZ

BfR: German Federal Institute for risk assessment; DSMZ: German Collection of Microorganisms and Cell Cultures, Leibnitz Institute, Braunschweig, Germany; IMT: Institute of Microbiology and Epizootics, Freie Universität Berlin, Berlin, Germany; IAN: Institute of Animal Nutrition, Freie Universität Berlin, Berlin,

Antibiotic resistance screening

To perform conjugation trials, potential donors and recipients were chosen based on miss matching antibiotic resistance profiles. Hence, resistance and sensitivity to 20 different antibiotic substances were determined for the potential recipients (n=35) and the potential donors (n=5) by agar disc diffusion tests. To qualify as recipients, strains had to show an inhibition zone around the 5 µg/mL cefotaxime disc (CTX) and be resistant to an additional antibiotic substance, which inhibited the growth of at least one potential donor. This allowed the detection of transconjugants on double antibiotic MacConkey agar. Recipient and donor strains for the conjugation screening were chosen by non-overlapping antibiotic resistances.

Specification of antibiotic resistance and susceptibility

Suitable antibiotic dosages for the inhibition of the strains were determined by examination of growth kinetics in broth microdilution tests for 24 hours at 37°C.

In short, strains were pre-cultured from cryo stocks overnight in MHB without antibiotics and subsequently washed twice in phosphate-buffered saline (PBS, Sigma-Aldrich, Chemie GmbH, Germany). The cells were re-suspended in MHB without antibiotics and diluted to 105 cells/mL. Minimal inhibitory concentrations (MIC) were obtained for the relevant antibiotics by broth microdilution in duplicates. Turbidity was measured at 690 nm every 10 minutes for 24 hours in a microtiter plate reader (Name, Tecan Austria GmbH, Austria). Non- inoculated media served as negative controls, while inoculated MHB without antibiotics provided the positive control. The MIC was defined as the concentration, at which no growth was observed within the 24-hours (h) period of measurement. According to the MIC and growth curves, antibiotic concentrations for agar plates for the conjugation trials were chosen.

Screening for conjugation

Potential donor and recipient strains were co-cultivated for 22 h to identify positive conjugation pairs (supplementary data). The screening was implemented in duplicates. To obtain viable and antibiotic resistant bacterial cells in their log-phase, bacteria strains were pre-cultured twice in MHB with antibiotics (same antibiotic concentration as in agar plates), which could not inhibit the growth of the respective strain and once in MHB without antibiotics. Thereafter they were washed twice in PBS and diluted in MHB without antibiotics to 106 cells/mL. Equal volumes (100 µL) of the donor and recipient suspensions, were added to 800 µL MHB without antibiotics. Single strain donor and recipient dilutions served as control. All samples were incubated for 22 hours and subsequently spread on double antibiotic agar plates at different dilutions. The antibiotic combinations for the different donor and recipient strains are shown in the supplementary data. Positive and negative controls were obtained by spreading the control suspensions on double (negative) and single (positive) antibiotic agar plates. All plates were incubated overnight, and conjugation events were identified as colony growth on double antibiotic agar plates. Colony forming units (cfu)/mL were obtained to estimate useful dilution levels for the 22-h kinetic assay.

Kinetic assay

The conjugation events identified during the screening were further investigated in 22 h kinetics (supplementary data). Duplicates of the donor and recipient strains (Table 3) were precultured (MHB with antibiotics), washed and diluted to 106 cells/mL as described above. One mL of donor and recipient suspensions, respectively were inoculated in 8 mL MHB without antibiotics, mixed thoroughly, dispensed in 1.4 mL aliquots and incubated for 0, 2, 4, 6, 8 and 22 h, respectively. Inocula (1 mL, 105 cells/mL) with only one bacterial strain (donor or recipient) served as controls. Immediately after the inoculation, 300 µL

of the suspension were plated on two double antibiotic agar plates to identify transconjugants present at hour 0. Simultaneously, dilution series were spread on MacConkey agar plates without antibiotics to obtain the total cell count. This procedure was repeated after 2, 4, 6, 8 and 22 h with suitable dilutions. The single-strain suspensions were plated on the corresponding double and single antibiotic agars for negative and positive controls respectively. The plates were incubated overnight and the conjugation frequency (CF) was calculated as transconjugants/donor (18).

Results

Antibiotic resistance screening of recipient strains

The results obtained from the agar disc diffusion tests (supplementary data) identified six potential recipients, 5 potential donors and 24 donor-recipient combinations for the screening assay (supplementary data). Suitable antibiotic concentrations for the preparation of the double antibiotic agars were obtained from the broth dilution. The levels of antibiotic supplementation inhibited the growth of the donor, while the recipients' growth was not affected and vice versa. This led to the usage of 2 or 8 µg CTX/mL agar, 25 µg colistin (CT)/mL agar, 25 or 100 µg chloramphenicol (C)/mL agar, 25 µg sulfamethoxazole/trimethoprim (SXT)/mL agar and 30 µg nitrofurantoin (F)/mL agar.

Screening for suitable mating pairs

The results from the screening for conjugation are presented in Table 2. *Proteus mirabilis* DSM 4479, who did not show conjugation with any of the given donors, was excluded from further trials. The same applied to the potential donor *E. coli* 10716, who did not transfer plasmids to three potential recipients *Serratia marcescens* subsp. *marcescens* DSM 5570, *Enterobacter cloacae* DSM 30060 and *Proteus mirabilis* DSM 4479. As the remaining four donors and five recipients proved the ability to produce transconjugants, they were further studied for the kinetic study.

Table 2 Screening for conjugation

Recipient	Donor *E. coli* ESBL10682 (CTX-M-1)	*E. coli* ESBL10689 (TEM-52)	*E. coli* ESBL10708 (SHV-12)	*E. coli* ESBL10716 (CTX-M-15)	*E. coli* ESBL10717 (CMY-2, TEM-1)
***E. coli* IMT 20751/402**	+	+	nd	nd	+
***E. coli* IMT11716 (APEC)**	+	-	nd	nd	+
***Salmonella* Typhimurium L1219-R32**	+	nd	+	nd	+
***Serratia marcescens* subsp. *marcescens* DSM 5570**	+	+	-	-	nd
***Enterobacter cloacae* DSM 30060**	-	-	+	-	+
***Proteus mirabilis* DSM 4479**	-	-	-	-	-

Nd = not determined, + = bacterial growth, - = no growth/<5 colonies

Kinetic assay

Varying time depending conjugation frequencies were observed for different donor and recipient pairs within the 22 h incubation period (Table 3). The earliest conjugation events were observed after 4 hours for the donors *E. coli* ESBL10682 with the recipients *E. coli* IMT 20751/402, *Salmonella* Typhimurium DSM30122 and *Serratia marcescens* subsp. *marcescens* DSM 30122 and the mating pair and *E. coli* ESBL10689/*E. coli* IMT 20751/402. The highest conjugation frequency was detected after 4 hours incubation of donor *E. coli* 10682 and the recipient strain *Salmonella* Typhimurium L1219-R32. *Serratia marcescens* subsp. *marcescens* DSM 5570 also accepted ESBL-carrying plasmids from *E. coli* 10682 after 4 h co-cultivation. The conjugation frequency was reduced after 6, 8 and 22 hours coincubation for both recipients. The same donor also transferred ESBL-carrying plasmids to the recipient strains *E. coli* IMT 20751/402 and *E. coli* IMT11716 after 4 and 22 hours respectively. Here, a lower conjugation frequency was observed initially.

The donor strain *E. coli* ESBL 10689 induced conjugation after 4 and 8 hours coincubation, respectively, when *E. coli* IMT 20751/402 and *Serratia marcescens* subsp. *marcescens* DSM 5570 served as

recipients. For *E. coli* IMT 20751/402, conjugation frequency decrease between 4 and 8 h, but increased again after 22 h. On the other hand, conjugation frequency for the *Serratia marcescens* subsp. *marcescens* DSM 5570 recipient remained rather stable. No conjugation was observed between *E. coli* ESBL 10689 and *E. coli* IMT 20751/402.

The co-cultivation of donor strain *E. coli* ESBL 10708 with *Enterobacter cloacae* DSM 30060 or *Salmonella* Typhimurium L1219-R32 resulted in conjugation only after 22 h. The same applied for donor *E. coli* ESBL 10717, which showed transconjugants for *E. coli* IMT 20751/402, *E. coli* IMT 11716 or *E. cloacae*.

In summary, observed conjugation frequencies were within the range of 10-9 – 10-5 transconjugants/donor. The highest conjugation frequency was observed for the *Salmonella* Typhimurium recipient strain and a CTX-M-1 carrying plasmid. For the majority of the investigated strains, no transconjugants were observed after 8 h. Four of the mating pairs led to transconjugants above detection level within 4 h of co-cultivation. Differences in conjugation frequency and incubation time were observed depending on bacteria genera, species and strain.

Table 3: Conjugation frequencies of selected donor- and recipient strains [log_{10}transconjugants/donor]

Donor	***E. coli* ESBL10682**				***E. coli* ESBL10689**		***E. coli* ESBL10708**		***E. coli* ESBL10717**		
Recipient	***E. coli* IMT 20751/402**	***E. coli* IMT11716**	***Salmonella* Typhimurium L1219-R32**	***Serratia marcescens* subsp. *marcescens* DSM 5570**	***E. coli* IMT 20751/402**	***Serratia marcescens* subsp. *marcescens* DSM 5570**	***Enterobacter cloacae* DSM 30060**	***Salmonella* Typhimurium L1219-R32**	***E. coli* IMT 20751/402**	***E. coli* IMT 11716**	***Enterobacter cloacae* DSM 30060**
0 h	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
2 h	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
4 h	-7.00	NT	-4.98	-5.74	-7.26	NT	NT	NT	NT	NT	NT
6 h	-6.62	NT	-6.27	-7.05	-7.30	NT	NT	NT	NT	NT	NT
8 h	-6.70	NT	-5.91	-6.71	-8.48	-6.91	NT	NT	NT	NT	NT
22 h	-6.78	-6.52	-5.92	-7.26	-6.45	-6.98	-5.86	-5.70	-6.80	-6.25	-6.49

NT = no transconjugants

Discussion

This *in vitro* study investigated the conjugation kinetics between ESBL-producing *E. coli* donors and various *Enterobacteriaceae* recipients. In this study we used relatively high concentrations of cefotaxime in the agar. This was due to the results from the initial resistance screening, where 8 µg ctx/mL did not affect the growth of the donors negatively in the broth microdilution assay. Surprisingly, these donors were inhibited by 30 µg CTX discs in the agar diffusion trial. The Clinical Laboratory Standards Institute (CLSI) suggests this disc type for screening for ESBL- producing bacteria and 1 µg ctx/mL for broth

microdilution (19). Hence, the donors would have failed to be identified in the recommended agar disc diffusion test but easily be recognized as ESBL-producers in the broth microdilution test.

Of the possible six recipient and 5 donor strains, only six suitable mating pairs were found that could be used in kinetic mating experiments. Successful conjugation was observed in 92 – 100 % of the donor/recipient combinations (51 donors, 1 recipient and 48 donors and 1 recipient) investigated (20, 21) compared to 52.2 % in this present study (5 donors, 6 recipients). This rate is affected by chance and a higher number of recipient strains may have altered the outcome. Also, this study used different bacterial species while only *E. coli* donors and recipients were used in the study by Franiczek and Krzyzanowska (21). The results of this study suggest that the propensity of ESBL-producing donors for gene transfer differ significantly between strains from the same species. *E. coli* ESBL10682 and *E. coli* ESBL10717 transferred their plasmids to 4 of the possible 6 and 5 potential recipients respectively. On the contrary, *E. coli* ESBL10716 did not transfer its plasmid to any of the recipients provided. This suggests that the *bla* genes were located on the chromosome or a non-conjugative plasmid (20) in this donor. *E. coli* ESBL10689 and *E. coli* ESBL10717 were able to perform conjugation with 2 out of 4 or 3 potential recipients, respectively. A plausible reason for the cases were the recipient did not accept the plasmid is that the recipients may already harbor plasmids with the same replicon (22). Also, the incubation time might have been too short, or the initial concentrations of donor and recipients were too low (23). The latter is rather unlikely, as the initial concentration of 105 cfu/mL is quite high and the long incubation time of 22 hours in media increases cell concentrations even further. Furthermore, the transconjugants could have been under detection limit. Here, the detection limit was 3 cfu transconjugants/mL, which makes this option rather unlikely at the given bacterial concentrations and incubation time. Finally, the recipient may have specific endonucleases which destroy the plasmids after uptake and thereby prevent the formation of transconjugants (24, 25). This may be the case for the *Proteus mirabilis* recipient, which did not mate with any of the given donors.

When co-cultivated, the recipient strains *S. marcescens* and *Salmonella* Typhimurium showed lower growth rates than the donor strains (supplementary data). Hence, the donor/recipient ratio and subsequent conjugation frequencies was shifted towards the donor. This effect should be considered when evaluating conjugation events as conjugation frequencies per recipient cfu would have been higher than conjugation rates per donor cfu. Thus, calculation of conjugation events per donor may be biased and other methods of calculation may give different results (26, 27). However, as the present study was designed to find model strains to study conjugation kinetics in detail, this was not the focus of the research. Hence, the calculation of transconjugants/donor was sufficient to compare the different mating pairs.

Conjugation frequencies differed between various donor and recipient strains in the employed *in vitro* assay. Genera and strain depending variations in conjugation frequency have been described previously (18, 20). In some studies, it was suggested that conjugation occurs more regularly with donor and recipients from different genera. Donor strains belonging to *Enterobacter cloacae*, *E. sakazakii, E. agglomerans, Serratia marcescens, Citrobacter freundii* and *E. coli* showed conjugation frequencies

between 10-7 and 10-1 transconjugants per donor with the recipient *E. coli* K12C600 (20, 21). Correspondingly, conjugation rates were rather low in the present study, when *E. coli* strains served as recipients (10-9-10-7 transconjugants/donor). On the contrary, Yamaichi et. al., (9) described higher conjugation frequencies for mating pairs of the same species than interspecies donor/recipient combinations. This corresponds with our findings for the *E. coli* ESBL10689 donor. Thus, this study cannot confirm a general statement to either direction, but rather suggests strain specific differences. These relatively high conjugation rates reported in the literature compared to the frequencies shown in the present study may depend on different strains used. While some studies used different strains as donors and a consistent *E. coli* recipient (20, 21), the present study used varying donor and recipient strains. In the mentioned studies, especially *Citrobacter freundii*, a strain not investigated in the present study, showed high conjugation rates, while *S. marcescens* donors showed rather low conjugation frequencies, comparable to the results of this study. In another study, *Enterobacter* spp. donors reached an average of 105 transconjugants/donor when co-cultivated with *E. coli* recipient strains (28) compared to 10-6 transconjugants/donor when used as a recipient for *E. coli* donors in the present study. Unfortunately, no information on incubation time or cell concentrations was provided. In this study, the highest conjugation frequency of 1.04 × 10-5 transconjugants/donor occurred when *E. coli* 10682 was co-incubated for 4 hours with the pathogen *Salmonella* Typhimurium L1219- R32. This frequency corresponds to results obtained from conjugation trials with *Klebsiella* spp. donors and *Salmonella* spp. recipients with 24 hours co-incubation (18).

Also, higher initial concentrations of donors and recipients used in studies such as Franiczek et al. (20) or Franiczek and Krzyzanowska (21) (109 cells/mL compared to 105 cells/mL in this study) can explain the differences in conjugation frequencies. The reason for the relatively low initial bacterial concentrations in this study was that cell numbers were chosen according to realistic amounts present in the gastrointestinal tract (17, 29, 30). Therefore, the detection limit must be considered when evaluating the time for the first observed conjugation event. The impact of initial concentrations of the mating pair on the number of transconjugants after a given time of coincubation and thereby the detection limit of conjugation was previously described in a study by Handel et al. (23).

The time until detection of transconjugants differed significantly between donors with the same recipients. Conjugation kinetics for ESBL-carrying plasmids have previously been studied, but mainly with longer time intervals (15). It was also shown in the present study that both time as well as number of conjugation events differed between different strains. Some strains showed a higher CF early during incubation (*Salmonella* spp./*E. coli* ESBL 10682, *S. marcescens* subsp*. marcescens* DSM 5570/*E. coli* ESBL 10682) with declining conjugation frequencies, while other strains increased CF at later time points (*E. coli* IMT 20751/402/*E. coli* ESBL 10682, *E. coli* IMT 20751/402/*E. coli* ESBL 10689). These results suggest that the most severe differences occur within the first day and therefore short time intervals should be chosen when investigating conjugation kinetics.

The aim of this study was to identify mating pairs fitted for future *in vivo* studies in poultry. These mating pairs should comprise a donor producing an ESBL type with high prevalence in broilers (3) and perform

conjugation at bacterial concentrations commonly observed in the hindgut (17, 30). The most fitted mating pairs were *E. coli* ESBL10682/*E. coli* IMT 20751/402, *E. coli* ESBL10682/*Salmonella* Typhimurium DSM30122, *E. coli* ESBL10682/*Serratia marcescens* subsp. *marcescens and E. coli* ESBL10689/*E. coli* IMT 20751/402 due to their formation of transconjugants after a relative short incubation period. This will ensure that the passage time of the ingesta, and thereby the time a donor or recipient resides in the intestinal tract if not established there, will be sufficient for conjugation to be detected. To enhance the chance to detect the conjugation events, high transfer rates are preferred (23). Hence, the mating pair *E. coli* ESBL10682/*Salmonella* Typhimurium DSM30122 revealed best fitted. Also, *Salmonella* Typhimurium is a common pathogen of importance for public health. Thus, the chosen mating pair could be used to address research questions focusing on this topic as well. Conjugation frequencies are commonly obtained from *in vitro* trials. To understand the impact of the complex system in the intestinal tract *ex vivo* and *in vivo* trials should follow these studies.

Conclusion

Different ESBL-carrying plasmids were transferred to recipients of the *Enterobacteriaceae* family at frequencies of 10-9 – 10-5 transconjugants/donor within 22 h with earliest events after 4 h of coincubation. This suggests that genetic transfer occurs within a short time period and may be quite frequent *in vivo*. Thus, measures to control ESBL-producing bacteria should comprise methods inhibiting conjugation. Donor strains also differed in their capacity to transfer ESBL- plasmids.

Abbreviations

APEC	Avian pathogenic *E. coli*
BfR	German Federal Institute for risk assessment
C	Chloramphenicol
CF	conjugation frequency
cfu	colony forming units
CLSI	Clinical Laboratory Standards Institute
CT	colistin
CTX	cefotaxime
E. coli	*Escherichia coli*
ESBL	extended-spectrum beta-lactamas
DSMZ	German Collection of Microorganisms and Cell Cultures, Leibnitz Institute

F	nitrofurantoin
h	hours
IAN	Institute of Animal Nutrition, Freie Universität Berlin
IMT	Institute of Microbiology and Epizootics, Freie Universität Berlin
MHB	Mueller Hinton Broth
MIC	minimal inhibitory concentrations
Nd	not determined
NT	no transconjugants
PBS	Phosphor buffered saline
S. marcescens	*Serratia marcescens*
SXT	sulfamethoxazole/trimethoprim
WHO	World Health Organization

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors contributions

W.V: and E.S. planned the experiment, which was carried out by E.S. who also wrote the manuscript with input from all authors. J.Z. helped supervise the project. W.V. and J.Z. conceived the original idea. W.V. supervised the project. All authors discussed the results and commented on the manuscript

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