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Research Article

Strain-Specific Transfer of Antibiotic Resistance from an Environmental Plasmid to Foodborne Pathogens

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Pathogens resistant to multiple antibiotics are rapidly emerging, entailing important consequences for human health. This study investigated if the broad-host-range multiresistance plasmid pB10, isolated from a wastewater treatment plant, harbouring amoxicillin, streptomycin, sulfonamide, and tetracycline resistance genes, was transferable to the foodborne pathogens *Salmonella* spp. or *E. coli* O157:H7 and how this transfer alters the phenotype of the recipients. The transfer ratio was determined by both plating and flow cytometry. Antibiotic resistance profiles were determined for both recipients and transconjugants using the disk diffusion method. For 14 of the 15 recipient strains, transconjugants were detected. Based on plating, transfer ratios were between 6.8×10^{-9} and 3.0×10^{-2} while using flow cytometry, transfer ratios were between $<1.0\times10^{-5}$ and 1.9×10^{-2}. With a few exceptions, the transconjugants showed phenotypically increased resistance, indicating that most of the transferred resistance genes were expressed. In summary, we showed that an environmental plasmid can be transferred into foodborne pathogenic bacteria at high transfer ratios. However, the transfer ratio seemed to be recipient strain dependent. Moreover, the newly acquired resistance genes could turn antibiotic susceptible strains into resistant ones, paving the way to compromise human health.

1. Introduction

The extensive use of antibiotics in human and veterinary medicine and its prophylactic and growth promoting use in agriculture and aquaculture have lead to a huge rise of antibiotic resistant bacteria [1–3] and an increase of antibiotic resistant genes in the horizontal gene pool.

Antibiotic resistance in bacteria can be intrinsic or acquired. In the case of intrinsic resistance, bacterial strains are inherently resistant to a certain compound and the resistance cannot be transferred horizontally [4]. Acquired resistance occurs by mutation and/or horizontal gene transfer events. The main mechanisms of horizontal gene transfer are conjugation (mobile genetic elements are being transferred from a donor to a recipient cell), transformation (uptake of naked DNA), and transduction (bacteriophages as transporters of genetic information). Conjugation is considered as the principal mode for antibiotic resistance transfer since many antibiotic resistance genes are situated on mobile elements, such as plasmids and conjugative transposons. Conjugation of broad-host-range plasmids enables DNA to be transferred over genus and species borders, whereas transformation and transduction are usually more limited to the same species [5]. When considering a medical point

Table 1: Overview of the recipient strains.

Strain	Species	Serovar/serotype	Origin
MB 1139	*Salmonella*	Enteritidis	Poultry
MB 1410	*Salmonella*	Enteritidis	Egg
MB 1561	*Salmonella*	Enteritidis	Poultry (transport)
MB 2264	*Salmonella*	Typhimurium	Human
MB 2265	*Salmonella*	Typhimurium	Human
MB 2272	*Salmonella*	Typhimurium	Human
MB 2292	*Salmonella*	Typhimurium	Human
MB 1641	*Salmonella*	Hadar	Poultry (cecal drop)
KS 1-1	*Salmonella*	Infantis	Poultry (house)
KS 87	*Salmonella*	Virchow	Poultry (house)
MB 3885	*Escherichia coli*	O157:H7	Beef (carpaccio)
MB 3890	*Escherichia coli*	O157:H7	Human
MB 4021	*Escherichia coli*	O157:H7	Bovine (carcass)
MB 4260	*Escherichia coli*	O157:H7	Nonhuman
LFMFP 476	*Escherichia coli*	O157:H7	Bovine (faeces)

of view, the transfer of antibiotic resistance determinants from environmental bacteria to pathogens is of utmost importance, and it is clear that environmental bacteria should not be seen as devoid of antibiotic resistance determinants because of the physical distance between these bacteria and clinical settings [6]. A recent study suggests that infected patients might enhance the spread of plasmid-encoded fitness, virulence and antibiotic resistance determinants as inflammation elicits concomitant *Salmonella* and *E. coli* blooms, which can strongly raise donor and acceptor densities in the gut, thereby boosting horizontal gene transfer [7].

The aim of this study was to investigate if an environmental multiresistance plasmid can be transferred to two model Gram-negative foodborne pathogens, that are, *Salmonella* spp. and *Escherichia coli* O157:H7. It is generally agreed that Gram-negative bacteria pose the greatest risk to public health as the increase in resistance of Gram-negative bacteria is faster than in Gram-positive bacteria and as there are fewer new and developmental antibiotics active against Gram-negative bacteria [8].

To determine the transfer ratio, the transconjugants were analysed by both plating and flow cytometry (*gfp* as the reporter gene) [9–11]. Furthermore, the extent to which their phenotype was influenced was analysed by determining the antibiotic resistance profiles against five antibiotics for the recipients and the transconjugants.

2. Material and Methods

2.1. Bacterial Strains, Plasmid, and Growth Conditions. The plasmid donor strain was *Pseudomonas putida* strain SM1443, a KT2442 (SM1315) strain with the mini-Tn*5*-*lacI*^{q} cassette inserted into the chromosome [12]. The *lacI*^{q} repressor cassette prevented the expression of the *gfp* gene in the donor.

The plasmid used in this study was the broad-host-range plasmid pB10. This plasmid, belonging to the IncP-1β subgroup, was isolated from a wastewater treatment plant and contains resistance to the antibiotic agents amoxicillin, streptomycin, sulfonamides, and tetracycline and to inorganic mercury ions [13]. To mark the plasmid with a *gfp* gene and a kanamycin resistance gene (Km), insertion of the mini-Tn*5*-Km-$P_{A1\text{-}04/03}$::*gfp* cassette was performed in two steps. First, a triparental mating was performed in which the helper plasmid RK600 [14], present in *Escherichia coli* HB101, mobilised the delivery plasmid pJBA120, containing the mini-Tn*5* cassette, from the donor *E. coli* MV1190(λ-*pir*) [15], into the rifampicin-resistant recipient *Pseudomonas putida* UWC1 harbouring pB10. *P. putida* UWC1 derivatives with the mini-Tn*5*-Km-$P_{A1\text{-}04/03}$::*gfp* cassette inserted either in the chromosome or in pB10 were obtained by selection in Luria Bertani (LB) broth (10 g tryptone, 5 g yeast extract, and 5 g NaCl per litre) with 10 μg tetracycline mL^{-1}, 50 μg kanamycin mL^{-1}, and 100 μg rifampicin mL^{-1}. In the second step, *gfp*-marked plasmids were obtained by mating the *P. putida* UWC1 derivatives with *Ralstonia eutropha* JMP228n [16]. Selection on LB agar plates with 10 μg tetracycline mL^{-1}, 50 μg kanamycin mL^{-1}, and 100 μg nalidixic acid mL^{-1} resulted in JMP228n clones carrying pB10 containing a randomly inserted mini-Tn*5*-Km-$P_{A1\text{-}04/03:}$::*gfp* cassette. Subsequently, one clone, designated JMP228n (pB10::*gfp*), was mated with *E. coli* K12 to obtain *E. coli* K12 (pB10::*gfp*) after selection on LB agar plates with 10 μg tetracycline mL^{-1} and 50 μg kanamycin mL^{-1} at 43°C. Ultimately, this strain was mated with *P. putida* SM1443 to obtain the donor strain for the experiments, *P. putida* SM1443 (pB10::*gfp*), after selection on LB agar plates with 10 μg tetracycline mL^{-1}, 100 μg rifampicin mL^{-1}, and 50 μg kanamycin mL^{-1} at 28°C.

The recipient strains were 10 *Salmonella* spp. and five *E. coli* O157:H7 strains (Table 1). The tested *Salmonella* serovars belong to the most frequently occurring *Salmonella* serotypes in human salmonellosis in Europe, with *Salmonella*

Enteritidis and *Salmonella* Typhimurium being the most frequent [17]. None of the five *E. coli* O157:H7 strains carried *stx*1 and *stx*2 genes. For one strain (LFMFP 476), no additional information on the presence of other virulence genes was available, but the four other strains all carried the *eae* and *ehx* genes (data not shown).

The recipient strains were first tested on their inability to grow on kanamycin (50 μg mL^{-1}) containing plates as this antibiotic was used as selective marker to detect transconjugants harbouring pB10::*gfp*.

Donor and recipient strains were all grown in LB broth. For all solid media, 1.5% agar was added. *P. putida* was incubated at 28°C, *Salmonella* spp. and *E. coli* at 37°C. To maintain the plasmid in the donor and the transconjugants 50 μg kanamycin mL^{-1} was added to the medium.

2.2. Filter Mating. Mating experiments were conducted in triplicate on 0.22 μm polycarbonate filters (25 mm diameter) (Whatman, UK). The donor and recipient cultures were grown overnight and washed twice with sterile saline (0.85% NaCl) to remove antibiotics. The $OD_{610\,nm}$ was adjusted to 0.25–0.35 (approximately 10^8 cells mL^{-1}) for both donor and recipient strains. Seventy-five μL of both donor and recipient was diluted in 2 mL of sterile saline and distributed evenly over the filter using a Swinnex device (Millipore, USA). The filters were transferred to LB agar plates and incubated overnight at 28°C. Afterwards, the filters were submerged in 5 mL sterile saline and vortexed twice for 1 min. The suspended bacteria were analysed by plate counting and by flow cytometry. For the plate counting, LB plates, which contained kanamycin, were incubated at 42°C. The presence of the antibiotic counter selected for the recipient strain, while the high temperature counter selected for the donor strain. The transfer ratio was determined as the number of transconjugant CFU per total cell count (donor, recipient and transconjugant cells), as determined by flow cytometry.

2.3. Flow Cytometry Analysis. Diluted bacteria were detected and quantified with a Cyan ADP Flow Cytometer (Dako, Denmark), using the 488 nm laser. The dilution factor ranged from 1000 to 2500. Dilutions were made with filter sterilized Evian water. Each sample consisted of 980 μL of the diluted sample, 10 μL Na_2EDTA (500 mM, pH8), and 10 μL Dako Cytocount beads. These beads were used to determine the cell concentration. FL1 fluorescence emission was collected with a photomultiplier tube using a 530/40 emission filter, for FL2 fluorescence a 575/25 emission filter was used and side scatter light (SSC) was collected using a 488/10 emission filter. The sheath fluid consisted of Milli-Q water. The threshold trigger was set to SSC. The analysis of a sample was done by collecting data for 100 000 events in threefold. Summit v4.3 software was used to process the results. Pure cultures of donor, recipient, and transconjugant were analysed by flow cytometry to set the gates that distinguish between the transconjugant population and the donor and recipient population on a FL1 versus FL2 plot. When the transconjugants of a specific filter mating sample could not clearly be visually detected on the plot, their number was considered to be below the detection limit ($<1 \times 10^{-5}$ transconjugants per total cells). The transfer ratio was determined as the number of transconjugant cells per total cell count.

2.4. Antibiotic Susceptibility Screening. The antibiotic susceptibility of the recipients and transconjugants was determined by using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for five antibiotics (amoxicillin, kanamycin, streptomycin, sulfonamides, and tetracycline) [18]. The visual turbidity of the bacterial isolates was adjusted to a 0.5 McFarland standard in sterile saline. The suspension was plated on a Mueller-Hinton agar plate (Oxoid, UK) and antibiotic disks (Oxoid) were applied on the plate. Inhibition zone diameters were measured after incubating the plates during 16–18 h at 37°C. Classification as "susceptible", "intermediate resistant" or "resistant" was based on the inhibition zone diameters according to CLSI guidelines. *E. coli* ATCC 25922 was used as quality control strain to monitor the performance of the susceptibility testing.

2.5. Molecular Confirmation of Plasmid Transfer. Transfer of the plasmid pB10::*gfp* was confirmed by PCR. DNA from the recipient and transconjugant strains was obtained by an alkaline lysis method. For each strain, a few bacterial colonies were suspended in 1 mL Ringer solution. After centrifuging the sample for two minutes at 14000 *g*, 100 μL sterile water was added to the pellet. The samples were incubated for 15 minutes at 90°C and subsequently centrifuged for one minute at 14000 *g*. Fifty μL of the supernatant was kept at −20°C.

The PCR reaction was performed with the primers trfA_fw and trfA_rev to amplify a 281 bp fragment of the replication initiation gene *trfA*, encoded by the plasmid, as previously described [19]. These primers are specific for plasmids belonging to the IncP-1α, β, ε subgroups. The PCR amplification products were detected by electrophoresis on a 1% agarose gel in TAE buffer and visualised by ethidium bromide staining.

3. Results

3.1. Characterization of the Recipient Strains. Before starting the conjugation experiments, the antibiotic susceptibility profiles of the recipient strains and presence of IncP-1α, β, ε plasmids were determined (Table 2). The three *Salmonella* Enteritidis strains were susceptible to the tested antibiotics, except one (MB 1139), which displayed an intermediate resistance to kanamycin. There was much more variation in the antibiotic susceptibility profiles of the *Salmonella* Typhimurium strains. The *Salmonella* Typhimurium strain MB 2264 was resistant to the four antibiotics which are indigenous to the plasmid but susceptible to kanamycin, while *Salmonella* Typhimurium strain MB 2265 was susceptible to all the antibiotics. The two other *Salmonella* Typhimurium strains (MB 2272 and MB 2292) showed resistance to, respectively, one (amoxicillin) and two antibiotics

Table 2: Inhibition zone diameters (mm) of the recipients (R) and the transconjugants (T).

Strain	Kanamycin		Amoxicillin		Streptomycin		Sulfonamides		Tetracycline	
	R	T	R	T	R	T	R	T	R	T
Salmonella Enteritidis (MB 1139)	*17*	*	28	*	15	*	20	*	23	*
Salmonella Enteritidis (MB 1410)	20	<**7**	20	**7**	17	*13*	20	<**7**	21	<**7**
Salmonella Enteritidis (MB 1561)	21	<**7**	27	**7**	18	*13*	22	<**7**	22	<**7**
Salmonella Typhimurium (MB 2264)	19	<**7**	<**7**	<**7**	**8**	**8**	<**7**	<**7**	**9**	<**7**
Salmonella Typhimurium (MB 2265)	20	<**7**	26	**7**	15	*14*	20	<**7**	21	<**7**
Salmonella Typhimurium (MB 2272)	20	<**7**	<**7**	<**7**	15	*12*	25	<**7**	21	<**7**
Salmonella Typhimurium (MB 2292)	19	<**7**	<**7**	<**7**	15	*13*	<**7**	<**7**	20	<**7**
Salmonella Hadar (MB 1641)	18	*	<**7**	*	**9**	*	21	*	<**7**	*
Salmonella Infantis (KS 1-1)	19	<**7**	25	**7**	15	*12*	19	<**7**	19	<**7**
Salmonella Virchow (KS 87)	19	<**7**	26	**7**	15	*12*	21	<**7**	20	<**7**
E. coli O157:H7 (MB 3885)	21	<**7**	22	**7**	16	*13*	24	<**7**	22	<**7**
E. coli O157:H7 (MB 3890)	18	<**7**	21	**7**	*14*	*14*	24	<**7**	20	<**7**
E. coli O157:H7 (MB 4021)	21	<**7**	21	**7**	18	15	24	<**7**	21	<**7**
E. coli O157:H7 (MB 4260)	19	<**7**	20	**7**	15	*13*	24	<**7**	20	<**7**
E. coli O157:H7 (LFMFP 476)	19	<**7**	22	**11**	15	*14*	24	<**7**	21	<**7**

Bold: considered as resistant according to CLSI guidelines.
Italic: considered as intermediate resistant according to CLSI guidelines.
*: no transconjugants obtained.

(amoxicillin and sulfonamides). The *Salmonella* Hadar strain MB 1641 was susceptible to kanamycin and sulfonamides. The strains of *Salmonella* Infantis KS 1-1 and *Salmonella* Virchow KS 87 were susceptible to all five antibiotics. All the recipient *E. coli* strains were susceptible to the antibiotics tested, except strain MB 3890 which was intermediate resistant to streptomycin.

The absence of IncP-1α, β, ε plasmids in the recipient strains was confirmed by PCR as in none of the 15 recipient strains a PCR fragment of 281 bp, specific for IncP-1α, β, ε plasmids, was detected (data not shown).

3.2. Plasmid Transfer Analysed by Plating. Suspensions, obtained after filter mating, were plated on LB plates supplemented with kanamycin and incubated at 42°C. Transconjugants were obtained for 13 of the 15 tested strains (Figure 1). The strains that did not yield transconjugants were *Salmonella* Enteritidis MB 1139 and *Salmonella* Hadar MB 1641. Repetition of the conjugation experiments confirmed these results (data not shown). The other *Salmonella* spp. strains resulted in transfer ratios ranging from 3.7×10^{-7} to 3.0×10^{-2} transconjugants per total cell count. The highest transfer ratios were found for the two remaining *Salmonella* Enteritidis strains (MB 1561: 3.0×10^{-2}; MB 1410: 9.1×10^{-4}), followed by *Salmonella* Virchow KS 87 (7.2×10^{-4}) and *Salmonella* Infantis KS 1-1 (9.2×10^{-5}), while the lowest transfer ratios were observed for the *Salmonella* Typhimurium strains, with transfer ratios in the order of 10^{-7}. For MB 2265 a transfer ratio of 1.9×10^{-5} was observed, which was the fifth highest transfer ratio found for the *Salmonella* spp. strains tested. One of the *E. coli* strains (MB 3890) had a similar transfer ratio as some *Salmonella* spp. strains (2.2×10^{-5}), while the other four *E. coli* strains had much lower transfer ratios (10^{-8}–10^{-9}).

3.3. Plasmid Transfer Analysed by Flow Cytometry. The conjugation efficiency was also assessed by flow cytometry, because this method allowed a rapid and culture-independent screening of the individual transconjugant and parental cells. Using the same mating mixtures as described above, transconjugants could be detected for only 5 of the 15 tested strains, due to the rather poor detection limit (Figure 1). These strains were all *Salmonella* spp., more specifically *Salmonella* Enteritidis (MB 1561: 1.9×10^{-2}; MB 1139: 2.5×10^{-4}, MB 1410: 1.9×10^{-4}), *Salmonella* Virchow (1.5×10^{-4}), and *Salmonella* Infantis (1.2×10^{-4}). No transconjugants could be obtained by plating for *Salmonella* Enteritidis MB 1139, while the four other strains showed the highest transfer ratio determined by plating. For the 10 other strains the transfer ratio was below the detection limit ($<1 \times 10^{-5}$ transconjugants per total cell count). This is consistent with the low transfer ratios obtained by plating (10^{-5}–10^{-9}).

3.4. Characterization of the Transconjugants. To confirm that plasmid transfer had occurred and to analyse which effect this transfer had on the phenotype, the presence of the plasmid in the transconjugants and the antibiotic resistance profiles of the transconjugants were examined.

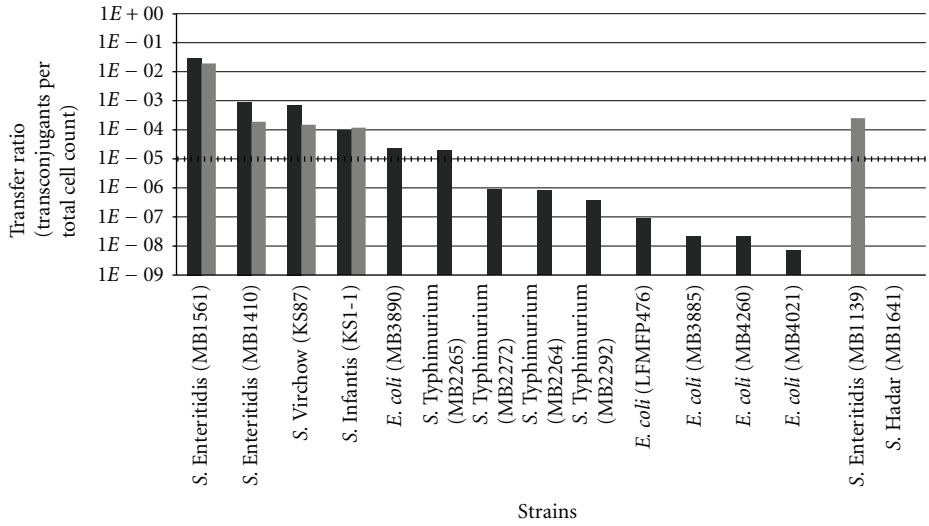

Figure 1: Transfer ratio, expressed as number of transconjugants per total cell count, determined by plating (black bars) and by flow cytometry (grey bars) for the 15 recipient strains. The dashed line represents the detection limit of flow cytometry.

Transconjugants were obtained for 13 of the 15 tested strains by plating (Table 2). As expected, the transconjugants were all resistant to kanamycin (inhibition zone diameter <7 mm). The inhibition zone diameter of sulfonamides and tetracycline was less than 7 mm for all the transconjugants, meaning that they were all completely resistant to these compounds. For amoxicillin the inhibition zone diameter was 7 mm or less, except for *E. coli* LFMFP 476 for which the inhibition zone diameter was 11 mm. This value is still considered as resistant according to CLSI guidelines. The decrease in inhibition zone diameter was less pronounced for streptomycin. According to the CLSI guidelines 11 of the transconjugant strains are considered to be intermediate resistant to streptomycin, one *E. coli* strain (MB 4021) remained susceptible. *Salmonella* Typhimurium MB 2264 was already resistant to amoxicillin, streptomycin, sulfonamides, and tetracycline before conjugation. Phenotypically, this strain gained only the resistance to kanamycin upon conjugation.

The presence of the pB10 plasmid in the transconjugants was confirmed by PCR. While none of the recipient strains contained the fragment (see above), the transconjugant strains all showed a clear band of the expected size after gel electrophoresis (data not shown).

4. Discussion

This study demonstrated that the broad-host-range plasmid pB10, carrying multiple resistance genes, could be transferred to foodborne pathogens under laboratory conditions and that this event made the recipient strains antibiotic resistant. The results show that the antibiotic resistance genes present in the general horizontal gene pool can be transferred from environmental strains to pathogenic organisms, but that the transfer ratio is dependent on the recipient strain. The role of natural environments in the evolution of resistance traits in pathogenic bacteria has recently been reviewed [20]. Other studies examined the conjugation between food related (pathogenic) bacteria [21–24], but to our knowledge there are fewer studies describing the transfer from environmental strains to (foodborne) pathogens [25–27].

In this study high transfer ratios were encountered with the highest transfer ratio in the *Salmonella* Enteritidis strain MB 1561 (order of magnitude of 10^{-2}). The plasmid used in this study, pB10, is a broad-host-range plasmid that could be transferred between laboratory strains of *Pseudomonas* and *E. coli*, and from *Pseudomonas* to *Sinorhizobium meliloti* at high transfer ratios with an order of magnitude of 10^{-1} transconjugants per recipient cells [13]. Four out of five *E. coli* 0157:H7 recipient strains showed lower transfer ratios than those observed for the *Salmonella* spp. strains. Recently, a study was published describing the dissemination of NDM-1-positive bacteria in the New Delhi environment and its implications for human health [27]. NDM-1-positive isolates containing the $bla_{\text{NDM-1}}$ gene were circulating in New Delhi as early as 2006 and plasmids carrying the gene can have up to 14 other antibiotic resistance determinants. These authors found the presence of the $bla_{\text{NDM-1}}$ gene in nonfermentative Gram-negative bacteria, like *P. putida*, which were not previously reported to carry this gene. The transfer of $bla_{\text{NDM-1}}$ was examined from bacteria, isolated from waste seepage, to the nonpathogenic *E. coli* J53 and to clinical strains of *Salmonella* Enteritidis and *Shigella sonnei*. Transfer into the *Salmonella* Enteritidis and *Shigella sonnei* recipients was 10 to 1000 times less efficient than into the *E. coli* J53 lab strain. In our study, transfer was more efficient in the *Salmonella* spp. strains than in the *E. coli* strains. It has been demonstrated that the donor affects the host range of pB10 in an activated-sludge microbial community [16], and it has been posed that in general all conditions influencing the host, including the genetic background of the host, might also

influence the frequency of plasmid transfer by conjugation [28].

For all strains, except for *Salmonella* Hadar, transconjugants could be detected by plating and/or by flow cytometry. Other studies also showed that *Salmonella* Hadar is less receptive for mobile genetic elements than other *Salmonella* serovars [29, 30]. It could be that in *Salmonella* Hadar a yet unexplained mechanism blocks the acquisition of plasmid DNA by conjugation [30].

Two methods were used in this study for the detection of transconjugants: a cultivation-dependent (plating) and a cultivation-independent method (flow cytometry). The most important advantages of flow cytometry are that it provides a rapid screening of bacterial cultures, takes into account the nonculturable fraction of the bacteria, and is less labour intensive than plating. Other studies used flow cytometry in combination with evolutionary algorithms to determine the optimal parameters for transconjugant formation [9] or in combination with automated cell sorting of green fluorescent transconjugant cells [31]. This approach allowed them to identify the transconjugants [31]. However, in our study the detection limit was rather high, so rare events could not be observed. For five of the 15 analysed strains transconjugants could be detected by flow cytometry. With plating, transconjugants were detected for 13 of the 15 analysed strains. There was one strain (*Salmonella* Enteritidis MB 1139) for which transconjugants only could be detected by flow cytometry and not by plating, even after repeated conjugation experiments. In some cases transconjugants cannot be detected by cultivation because the cells enter into a viable-but-nonculturable (VBNC) state [9]. In a previous study, a strain-dependent influence of temperature on the VBNC state was found [32]. These authors found a different temperature influence for plasmid-bearing cells and plasmid-free cells of two *Pseudomonas* strains, which was not seen in an *E. coli* strain. Whenever no transconjugants were detected by flow cytometry in our study, the transfer ratios determined by plating were lower than or just around 10^{-5}. These findings indicate that although flow cytometry offers many advantages, it is not always the method of choice due to its high detection limit.

In the last step of this study, the antibiotic resistance profiles of the transconjugants were determined to verify whether the recipient phenotype was altered by receiving the plasmid. Transconjugants were obtained for 13 of the 15 analysed strains. All these transconjugants showed a decrease in inhibition zone diameter for kanamycin, indicating that they all expressed the kanamycin resistance gene. For the plasmid-encoded antibiotic resistances, the strains showed complete resistance against amoxicillin, sulfonamides, and tetracycline. For streptomycin, only slight or no decreases in inhibition zone diameter were observed, resulting in intermediate resistant strains. *E. coli* MB 4021 remained susceptible according to CLSI guidelines although there was a decrease in inhibition zone diameter. Even though there can be a fair to almost perfect agreement between the measurement of minimum inhibitory concentration (MIC) values and the assessment of resistance genes, situations occur in which susceptible isolates carry the corresponding resistance genes [33]. These resistance genes may not be expressed if they are distant from the promoter or if they are associated with a weak promoter in an integron. The same occurs with free gene cassettes which are not incorporated into an integron and lack the integron promoter which is required for expression [33]. An alternative explanation could be a low MIC test sensitivity as is known with *aadA* genes and streptomycin resistance [33, 34]. A poor agreement was found between genotypes and phenotypes for streptomycin (66% agreement) in a previous study [35]. In the majority of cases, this disagreement was due to the presence of an *aadA* gene in isolates classified as susceptible to streptomycin. The streptomycin resistance in pB10 is situated on a truncated Tn*5393*c streptomycin resistance transposon. This transposon contains the *strA* and *strB* genes, which encode the two different streptomycin-resistance proteins aminoglycoside-3′-phosphotransferase and aminoglycoside-6-phosphotransferase [36]. The association of *strA* and *strB* normally leads to high-level expression of streptomycin resistance [35, 37]. At the moment, it is not clear to us why the streptomycin resistance was not fully expressed.

5. Conclusion

In this paper, we demonstrate that an environmental plasmid was transferred to foodborne pathogens (*Salmonella* spp. and *E. coli* O157:H7) under laboratory conditions. The detection of transconjugants was done by flow cytometry and by plating. Not only does this transfer occur at rather high transfer ratios (order of magnitude 10^{-2}), but the acquisition of the plasmid also makes the pathogens resistant to multiple antibiotics. In worst case scenarios, infections with these plasmid-mediated antibiotic resistant pathogens can lead to exacerbation of the patient's condition, treatment failure and thus compromise human health. Therefore, it is important to know if these plasmids can be transferred to potential pathogens and if these antibiotic resistance genes can be expressed in the new hosts.

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