Letters in Applied Microbiology

Letters in Applied Microbiology ISSN 0266-8254

ORIGINAL ARTICLE

Residual concentrations of antimicrobial growth promoters in poultry litter favour plasmid conjugation among *Escherichia coli*

M. M. S. Saraiva[1,2], N. M. V. Silva[1,3], V. A. Ferreira[2], A. L. B. Moreira Filho[4], P. E. N. Givisiez[1], O. C. Freitas Neto[5], A. Berchieri Júnior[2], W. A. Gebreyes[6,7] and C. J. B. de Oliveira[1,7]

1 Department of Animal Science, Center for Agricultural Sciences, Federal University of Paraiba (CCA/UFPB), Areia, PB, Brazil
2 Department of Pathology, Theriogenology and One Health, Laboratory of Ornithopathology, São Paulo State University (FCAV/Unesp), Jaboticabal, SP, Brazil
3 Instituto Federal do Sertão Pernambucano - Campus Petrolina, Petrolina, Brazil
4 Department of Animal Science, Center for Human, Social and Agricultural Sciences, Federal University of Paraiba (CCHSA/UFPB), Bananeiras, PB, Brazil
5 Department of Preventive Veterinary Medicine, Veterinary School, Federal University of Minas Gerais (UFMG), Belo Horizonte, MG, Brazil
6 Department of Preventive Veterinary Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH, USA
7 Global One Health Initiative (GOHi), The Ohio State University, Columbus, OH, USA

Significance and Impact of the Study: Although antimicrobial resistance represents an increasing threat to public health globally, information about the emergence and dissemination of antimicrobial resistant bacteria at the animal–environment interface is scarce. Conjugation is a process in which bacteria can transfer genes conferring resistance to other bacteria. This is an important phenomenon because genes are transferred through plasmids that may confer resistance to many antimicrobials at the same time. Our findings revealed that low concentrations of certain drugs in poultry litter increase conjugation rates of plasmids containing antimicrobial-resistant genes among *Escherichia coli*, supporting the importance of drug stewardship practices for the control of antimicrobial transfer.

Keywords
antimicrobial residues, antimicrobial resistance, HGT, horizontal gene transfer, mobile resistance genes, plasmid conjugation, sugarcane bagasse, wood shavings.

Correspondence
Celso José Bruno de Oliveira, Department of Animal Science, Center for Agricultural Sciences, Federal University of Paraiba, 12 Road PB079, Areia, PB 58397-000, Brazil.
E-mail: celso.oliveira@academico.ufpb.br; celso.bruno.oliveira@gmail.com

2021/0069: received 22 July 2021, revised 1 February 2022 and accepted 4 February 2022

doi:10.1111/lam.13671

Abstract

Considering that plasmid conjugation is a major driver for the dissemination of antimicrobial resistance in bacteria, this study aimed to investigate the effects of residual concentrations of antimicrobial growth promoters (AGPs) in poultry litter on the frequencies of IncFII-FIB plasmid conjugation among *Escherichia coli* organisms. A 2 × 5 factorial trial was performed *in vitro*, using two types of litter materials (sugarcane bagasse and wood shavings) and five treatments of litter: non-treated (CON), herbal alkaloid sanguinarine (SANG), AGPs monensin (MON), lincomycin (LCM) and virginiamycin (VIR). *E. coli* H2332 and *E. coli* J62 were used as donor and recipient strains, respectively. The presence of residues of monensin, lincomycin and virginiamycin increased the frequency of plasmid conjugation among *E. coli* in both types of litter materials. On the contrary, sanguinarine significantly reduced the frequency of conjugation among *E. coli* in sugarcane bagasse litter. The conjugation frequencies were significantly higher in wood shavings compared with sugarcane bagasse only in the presence of AGPs. Considering that the presence of AGPs in the litter can increase the conjugation of IncFII-FIB plasmids carrying antimicrobial resistance genes, the real impact of this phenomenon on the dissemination of antimicrobial resistant bacteria in the poultry production chain must be investigated.

Introduction

The emergence of antimicrobial resistant bacteria has been considered a major threat to the human civilization (WHO 2017). The epidemiology of antimicrobial resistance is very complex due to the dissemination of resistant bacteria and also antimicrobial resistance genes (ARGs) in a given environment and among animals and humans that cohabit this environment (Woolhouse *et al.* 2015). In this aspect, horizontal mechanisms of gene transfer among bacteria play a key role in the dissemination of genes conferring resistance to drugs of various classes, including highest priority critically important antimicrobials, such as certain extended spectrum β-lactams (ESBL) (Fischer *et al.* 2014; Saliu *et al.* 2020), carbapenems (Pulss *et al.* 2018) and polymixin (Yin *et al.* 2017).

Antimicrobial growth promoters (AGPs) are compounds added to the animal feed at subinhibitory concentrations in order to enhance animal performance. They have been used for several decades in animal production systems, mainly in swine and poultry. However, the positive association between the use of certain AGPs and the increase of resistance in human pathogenic bacteria to the same drug classes has led to restrictions in the use of AGPs (Dibner and Richards 2005; Aarestrup 2012). While the use of these compounds has been completely banned in the European Community since 2006 through Authority Regulation No. 1831/2003 (Castanon 2007; Millet and Maertens 2011), certain drugs are stil allowed to be used as AGPs in some countries, such as United States and Brazil.

A significant amount of AGPs in the feed is not absorbed in the gut of broiler chickens and are thus eliminated to the environment through excreta (Sarmah *et al.* 2006; Heuer *et al.* 2011; de Souza *et al.* 2016). Therefore, litter is expected to have residual amounts of these antibiotics together high bacterial populations, mainly enterobacteria such as *Escherichia coli*, a natural inhabitant of the animal gut.

Plasmid conjugation is a very common phenomenon in *E. coli* (Lopatkin *et al.* 2017) and primarily serves as a mechanism accelerating the spread of ARGs in enterobacteria (Leungtongkam *et al.* 2018). For instance, the spread of ESBL-encoding genes contributes to increased survival rates of enterobacteria in the gut of infected animals (Blaak *et al.* 2015; Dame-Korevaar 2017; Borges *et al.* 2019), and increased contamination levels of poultry litter (Heuer *et al.* 2011).

The understanding of the dynamics of plasmid conjugation in animal production systems, such as the poultry industry, could contribute to the mitigation of antibiotic resistance through the food chain. Although the exchange of genetic material among bacteria has been well documented in different environments, including soil, marine sediment, seawater, sewage wastewater and activated sludge (Davison 1999), information on putative factors affecting plasmid conjugation at the animal–environment interface is still scarce.

The aim of this study was to investigate the *in vitro* effects of low concentrations of AGPs in poultry litter on the frequency of plasmid conjugation among *E. coli*. Considering the increasing use of non-antibiotic feed additives in broilers in replacement of AGPs, we also investigated the frequency of plasmid conjugation in littler containing residual concentrations of sanguinarine, a quaternary benzophenanthridine alkaloid.

Results and discussion

Due to the importance of antimicrobial resistance, studies on the horizontal transfer of resistance genes have been increasingly frequent. Here, we tried to elucidate the influence of both AGPs and quaternary benzophenanthridine alkaloid on the frequency of IncFII-FIB plasmid conjugation. Although other plasmids were not investigated here, we strongly believe that the conjugation phenomenon is valid for other types of plasmids that carry ARGs. Similar methodologies to this study were applied with different plasmids (Licht *et al.* 1999; Johnsen and Kroer 2007; Alderliesten *et al.* 2020), which leads us to suggest that such methodology can be applied to different types of plasmids with the intent to evaluate their conjugative frequency.

According to the enumeration of both total recipient and transconjugant bacteria on MacConkey agar dishes, there was no difference in the total recipient *E. coli* counts between treatments, except a lower count ($P < 0{\cdot}0001$) on both sugarcane bagasse and wood shavings samples containing virginiamycin (Table 1).

The fact that the presence of antimicrobials did not inhibit the growth of recipient *E. coli* populations in the samples suggests that our experimental model was valid in providing appropriate conditions for bacteria maintenance and plasmid conjugation events. This was expected considering that antimicrobials were added at subinhibitory concentrations in the litter (10% of the the recommended use on animal feed), as previously reported (Zhao and Drlica 2001; Andersson and Hughes 2014).

The digestive tract is considered the optimal site in terms of nutrient availability and necessary conditions enabling cell-to-cell interactions in enterobacteria (Dumonceaux *et al.* 2006; Pan and Yu 2014). Interestingly, the conjugation frequencies observed in our study were higher than those reported in *in vitro* studies using gut models, with values ranging from 10^{-9} to 10^{-18}

Table 1 Enumeration (Log CFU g^{-1}) of recipient and transconjugant *Escherichia coli* in an *in vitro* conjugation assay using two plain poultry litter materials (sugarcane bagasse and wood shavings) added with sanguinarine or antimicrobial growth promoters at low concentrations (10% of the recommended use on animal feed)

Recipient *E. coli* counts (Log CFU g^{-1})†

	Litter material (l)		
Treatment	Sugarcane bagasse	Wood shavings	Treatment means
CON	8·58405 ± 0·078^{aB}	8·54435 ± 0·035^{aB}	8·564
SANG	8·59325 ± 0·062^{aB}	8·53166 ± 0·060^{aB}	8·562
MON	8·52642 ± 0·048^{aB}	8·50298 ± 0·059^{aB}	8·515
LCM	8·49866 ± 0·065^{aB}	8·56780 ± 0·168^{aB}	8·533
VIR	7·87456 ± 0·096^{bA}	7·7480 ± 0·0398^{aA}	7·811
Litter means	8·415	8·378	
P-value‡	T < 0·0001	L = 0·0060	T * L = 0·0002

Transconjugant *E. coli* counts (Log CFU g^{-1})†

	Litter material (l)		
Treatment	Sugarcane bagasse	Wood shavings	Treatment means
CON	4·44234 ± 0·054^{aA}	4·58508 ± 0·121^{aA}	4·514
SANG	4·31842 ± 0·080^{aA}	4·57993 ± 0·196^{bA}	4·449
MON	5·11039 ± 0·049^{aB}	5·17033 ± 0·059^{aB}	4·140
LCM	5·55787 ± 0·160^{aC}	5·75945 ± 0·168^{bC}	5·659
VIR	5·51298 ± 0·121^{aC}	5·72395 ± 0·040^{aC}	5·618
Litter means	4·988	5·164	
P-value‡	T < 0·0001	L < 0·0001	T * L = 0·0193

CON: control; SANG: sanguinarine (30 mg g^{-1}); MON: monensin (10 mg g^{-1}); LCM: lincomycin (1 mg g^{-1}); VIR: virginiamycin (1 mg g^{-1}); CFU: colony forming units.

Within each bacterial count, different capital letters in the column are different, and different lowercase letters in the row are different by the two-way ANOVA with a Bonferroni multiple comparison test at 1% probability.

†Each value represents mean ± standard deviation of 15 observations

‡T = Treatment; L = Litter material; T * L = Interaction between main factors (treatment and litter material).

(Card *et al.* 2017; Saliu *et al.* 2020). According to our study, the type of materials used as litter had no direct effect on the plasmid conjugation frequencies when no coumpounds were added to the litter (CON) or in the presence of sanguinarine (SANG). However, the factorial analysis revealed a significant interaction ($P < 0·01$) between the type of material and the treatments. Differences in conjugation frequencies between sugarcane bagasse and wood shavings were only observed in the treatments containg antimicrobial residues. Higher plasmid conjugation frequencies were observed among *E. coli* in wood shavings compared with sugarcane bagasse regardless of the type of antimicrobial. A previous study showed higher plasmid conjugation frequencies in *E. coli* in wood shavings compared with sugarcane bagasse, even in the absence of antibiotics (Saraiva *et al.* 2020). It might be possible that the higher moisture retention of sugarcane bagasse (Teixeira *et al.* 2015) might affect the availability of antibiotic residues in the litter (Benabdeljelil and Ayachi 1996). Moreover, other factors could also affect the dynamics of plasmid conjugation. For instance, pH value is an important parameter affecting the degradation of antimicrobials under different treatment methods (Bilal *et al.* 2019; Wang and Zhuan 2019; Reis *et al.* 2020). Therefore, potential differences in pH values (Teixeira *et al.* 2015) among different litter materials could affect antimicrobial availability. A limitation of our study relies on the fact that we have not raised information on the physicochemical parameters of both types of litter. However, pH values that could potencially affect plasmid conjugation are far from those commonly observed in poultry litter under field conditions. Therefore, pH probably does not play a significant role in our study.

The presence of monensin (MON), lincomicyn (LCM) and virginiamycin (VIR) in the litter increased the presence of transconjugant bacteria (Table 1), corroborating previous reports showing that antimicrobial residues can indeed favour plasmid conjugation (Barr *et al.* 1986; Zatyka and Thomas 1998, Beaber *et al.* 2004). Although the mechanisms behind the modulation of conjugation

efficiency are still unclear (Zatyka and Thomas 1998; Lopatkin *et al.* 2016a, 2016b), our findings support the hypothesis that an acquired genetic framework for antimicrobial resistance is important for the survival of bacteria under antimicrobial pressure (Egorov *et al.* 2018).

Plasmid conjugation frequencies in *E. coli* are presented in Table 2. A significant interaction was observed between the type of litter and the presence of antimicrobial residues on the frequency of plasmid conjugation in *E. coli*. In the presence of AGPs (MON, LCM and VIR), higher conjugation frequencies were observed in wood shavings compared with sugarcane bagasse. This was not observed in non-treated litter (CON) or in the litter samples containing sanguinarine (SANG).

Among all the treatments containing AGPs, the highest plasmid conjugation frequencies were observed for virginiamycin in wood shavings. Considering the sugarcane bagasse in particular, the highest conjugation frequencies in *E. coli* were seen in litter containing monensin (MON) ($P < 0{\cdot}01$). Both virginiamycin and monensin compounds were frequently used as AGPs although their use is prohibited in some countries (Kelly *et al.* 2004; Chapman *et al.* 2010; Danzeisen *et al.* 2011).

Virginiamycin is still one of the mainly used AGPs, even in countries experiencing a reduction in the use of antibiotic feed additives in animal production. (Thibodeau *et al.* 2008). Although the use of this AGP has been associated with increased antimicrobial resistance in avian bacteria (Singer and Hofacre 2006; Furtula *et al.* 2010), there is still a lack of knowledge on its effects on plasmid conjugation or other mechanisms of horizontal gene transfer. Interestingly, Mathers *et al.* (2004) reported inhibition of the transfer of a multiresistance-conferring plasmid in bacteria exposed to different AGPs at concentrations normally added to animal feed. The authors also reported a positive correlation between drug concentration and its inhibitory effects on plasmid conjugation.

In our study, lower conjugation frequencies ($P > 0{\cdot}01$) were observed among *E. coli* in sugarcane bagasse litter containing sanguinarine (SANG) compared with all other treatments (Table 2). Quaternary benzophenanthridine alkaloids, which includes sanguinarine, have been used as an alternative to antibiotics in animal production to prevent pathogenic bacteria (Vieira *et al.* 2008a, 2008b; El-Sheikh *et al.* 2018). These compounds can modulate the microbiota of broiler chickens (Lemos *et al.* 2020) and present broad antimicrobial, anti-inflammatory, antifungal and anti-biofilm activities that have been studied since the 1980s (Lenfeld *et al.* 1981; Godowski 1989; Obiang-Obounou *et al.* 2011; Qian *et al.* 2020). Although sanguinarine has been previously reported to reduce plasmid conjugation frequency in bacteria (Hausner and Wuertz 1999; Watnick and Kolter 2000; Ghigo 2001), the molecular mechanisms are unknown.

It is plausible to consider that resistant bacteria from the animal gut follow the same dissemination routes than the antibiotics shed in the excreta in a given environment (Baquero *et al.* 2008). Therefore, the presence of bacteria, ARGs, and residual concentrations of antibiotics, as drivers for selective pressure, can lead to the emergence of antimicrobial drug resistance in a bacterial population through horizontal gene transfer mechanisms (Berglund 2015). In the case of poultry litter, this process could be facilitated by the presence of appropriate conditions for bacteria survival and multiplication, such as temperature, humidity and organic matter. While the concept of

Table 2 Logarithmic plasmid conjugation frequency between *Escherichia coli* H2332 (donor) and *E. coli* J62 (recipient) in two plain poultry litter materials (sugarcane bagasse and wood shavings) added with sanguinarine or antimicrobial growth promoters at low concentrations (10% of the recommended use on animal feed)

	Litter material (l)		
Treatment	Sugarcane bagasse	Wood shavings	Treatment means
CON	$-4{\cdot}40725 \pm 0{\cdot}075^{aB}$†	$-4{\cdot}53309 \pm 0{\cdot}066^{aA}$	−4·470
SANG	$-4{\cdot}59788 \pm 0{\cdot}123^{aA}$	$-4{\cdot}70714 \pm 0{\cdot}220^{aA}$	−4·653
MON	$-3{\cdot}45755 \pm 0{\cdot}133^{aD}$	$-3{\cdot}24223 \pm 0{\cdot}181^{bC}$	−3·350
LCM	$-3{\cdot}85377 \pm 0{\cdot}088^{aC}$	$-3{\cdot}54237 \pm 0{\cdot}192^{bB}$	−3·698
VIR	$-3{\cdot}49785 \pm 0{\cdot}167^{aD}$	$-3{\cdot}19562 \pm 0{\cdot}143^{bC}$	−3·347
Litter means	−3·963	−3·844	
P-value‡	T < 0·0001	L < 0·0001	T * L < 0·0001

CON: control; SANG: sanguinarine (30 mg g^{-1}); MON: monensin (10 mg g^{-1}); LCM: lincomycin (1 mg g^{-1}); VIR: virginiamycin (1 mg g^{-1}); CFU: coony forming units; ANOVA, analysis of variance.

The conjugation frequency was determined by the ratio between the logarithmic counts (Log CFU g^{-1}) of transconjugants and recipients.

Means followed by different capital letters in the column are different, and different lowercase letters in the row are different by the two-way ANOVA with a Bonferroni multiple comparison test at 1% probability.

†Each value represents mean ± standard deviation of 15 observations.

‡T = Treatment; L = Litter material; T * L = Interaction between main factors (treatment and litter material).

reducing horizontal gene transfer among bacteria in a given environment in order to effectively mitigate the dissemination of antimicrobial resistance seems to be plausible, as supported by this in vitro study, this still needs to be confirmed *in vivo*. Therefore, our findings should be interpreted carefully before conclusions could be made at the animal production level. For instance, a previous investigation on lake water reported no increase in the concentrations of genes conferring antimicrobial resistence even after 1000-fold increase in the amount of antibiotics commonly detected in wastewater (Berglund *et al.* 2014).

In summary, this study revealed that subinhibitory concentrations of commonly used AGPs in poultry production can favour the conjugation of plasmids containing ARGs among *E. coli* and that the litter may impact on conjugation as well. These findings raise questions whether a similar phenomenon could be observed for antimicrobials other than AGPs, such as drugs used for therapeutic and methaphylactic purposes in poultry production. Therefore, further knowledge on how these residues could putatively impact the dissemination of antimicrobial resistance in the animal raising environment would be important for improving antimicrobial stewardship programmes in veterinary medicine.

Materials and methods

Study design

The experiment was performed according to a completely randomized design using a 2 × 5 factorial arrangement including two types of litter material (wood shavings and sugarcane bagasse) and five treatments with 15 repetitions each, including non-treated litter (CON) and litter treated with herbal alkaloid sanguinarine (30 mg g^{-1}, SANG), monensin (10 mg g^{-1}, MON), lincomycin (1 mg g^{-1}, LCM) or virginiamycin (1 mg g^{-1}, VIR).

Considering that 10–60% of the ingested AGPs is shed in the excreta, the concentrations of both the AGPs and the sanguinarine in the litter were calculated to represent 10% of their respective recommended concentrations, i.e., the minimum excreted amount under commercial field conditions.

Bacterial strains and growth conditions

Escherichia coli H2332 harbouring the plasmid pH2332-166 was used as the donor strain. This IncFII-FIB-plasmid harbours genes conferring resistance against amphenicols (*catA1*), aminoglycosides (*aadA1b*; *strAB*), β-lactams (bla_{TEM-1}), macrolides (*mph*-B), tetracyclines (*tetR*; *tetA*), trimethoprim (*dfrA1*) and sulphonamides (*sul1*; *sul2*) (Wang *et al.* 2014). The rifampicin/nalidixic acid-resistant non-lactose fermenting *E. coli* J62 strain was used as recipient (Niero *et al.* 2018). Both bacteria strains were cultured in 5 ml of Luria-Bertani broth under orbital incubation at 37°C for 24 h. They were individually transferred to buffered peptone water (10 ml) and bacteria concentration was adjusted to 1×10^7 and 3×10^7 colony froming units (CFU) ml^{-1} for the donor and the recipient strains, respectively.

Plasmid conjugation experiment

In each treatment, 10 g of litter was placed into a sterile plastic bag, and 10 ml of peptone water containing the recipient strain (3×10^7 CFU ml^{-1}) was added. After 1 h at room temperature, liquid excess was withdrawn and 10 ml of peptone water with the donor strain (1×10^7 CFU ml^{-1}) was added. After 1 h at room temperature, liquid excess was withdrawn and samples were cultured aerobically at 25°C for 24 h.

Conjugation frequencies

After incubation, bag contents were homogenized in 0·9% sterile saline (90 ml) and then serially diluted 1:9 (v:v) until 10^{-9}. Aliquots (20 μl) from each dilution were plated in triplicates onto two types of MacConkey agar dishes: one supplemented with 70 μg of nalidixic acid (NAL) + 40 μg of ceftriaxone (CRO) (for transconjugant bacteria recovery); and one supplemented with 70 μg of NAL (for recovering total bacteria: recipient+transconjugant). The dishes were incubated at 37°C for 24 h. The typical colonies of non-lactose fermenting *E.coli* were enumerated and logarithmically transformed (CFU g^{-1}). The conjugation frequency was obtained by dividing the number of transconjugants (Log CFU g^{-1}) by the number of total recipients (Log CFU g^{-1}).

Data analyses

Logarithmically transformed counts were evaluated for normal distribution by means of Shapiro–Wilks normality test. Means were compared within each type of litter material (wood shavings and sugarcane bagasse) and between the two types of littler materials within each treatment according to a two-way analysis of variance with a Bonferroni multiple comparison test ($P < 0{\cdot}05$). Statistical analyses were performed in GraphPad Prism 8 software.

Acknowledgements

The authors thank Prof. Paul Barrow (University of Nottingham) for kindly providing both H2332-166 and J62 strains. They are also thankful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES-finacial

code 001), to Conselho National de Pesquisa e Desenvolvimento (CNPq, proc. 313678/2020-0) and to the São Paulo Research Foundation (FAPESP 2018/03189-0; 2018/21301-2) for fellowships and financial support.

Author contributions

Conception and design of the study (O.C. Freitas Neto, A. Berchieri, P.E.N. Givisiez, W.A. Gebreyes, C.J.B. Oliveira), data aquisition and analysis (M.M.S. Saraiva; N.M.V. Silva; V.A. Ferreira; A.L.B. Moreira Filho), drafting the mansucript (M.M.S. Saraiva; N.M.V. Silva; V.A. Ferreira; A.L.B. Moreira Filho; C.J.B. Oliveira), critically revising the manuscript (P.E.N. Givisiez, O.C. Freitas Neto; A. Berchieri Júnior; W.A. Gebreyes), approval of the final submitted version (M.M.S. Saraiva; N.M.V. Silva; V.A. Ferreira; A.L.B. Moreira Filho; P.E.N. Givisiez, O.C. Freitas Neto; A. Berchieri Júnior; W.A. Gebreyes; C.J.B. Oliveira).

Conflict of Interest

The authors declare that they have no conflict of interest.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

References

Aarestrup, F. (2012) Get pigs off antibiotics. *Nature* **486**, 465–466. https://doi.org/10.1038/486465a

Alderliesten, J.B., Duxbury, S.J.N., Zwart, M.P., de Visser, J.A.G.M., Stegeman, A. and Fischer, E.A.J. (2020) Effect of donor-recipient relatedness on the plasmid conjugation frequency: a meta-analysis. *BMC Microbiol* **20**, 1–10. https://doi.org/10.1186/s12866-020-01825-4

Andersson, D.I. and Hughes, D. (2014) Microbiological effects of sublethal levels of antibiotics. *Nat Rev Microbiol* **12**, 465–478. https://doi.org/10.1038/nrmicro3270

Baquero, F., Martínez, J.L. and Cantón, R. (2008) Antibiotics and antibiotic resistance in water environments. *Curr Opin Biotechnol* **19**, 260–265. https://doi.org/10.1016/j.copbio.2008.05.006

Barr, V., Barr, K., Millar, M.R. and Lacey, R.W. (1986) Beta-lactam antibiotics increase the frequency of plasmid transfer in *Staphylococcus aureus*. *J Antimicrob Chemother* **17**, 409–413. https://doi.org/10.1093/jac/17.4.409

Beaber, J.W., Hochhut, B. and Waldor, M.K. (2004) SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427**, 72–74. https://doi.org/10.1038/nature02241

Benabdeljelil, K. and Ayachi, A. (1996) Evaluation of alternative litter materials for poultry. *J Appl Poult Res* **5**, 203–209. https://doi.org/10.1093/japr/5.3.203

Berglund, B. (2015) Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infect Ecol Epidemiol* **5**, 28564. https://doi.org/10.1016/j.scitotenv.2019.07.224

Berglund, B., Khan, G.A., Lindberg, R., Fick, J. and Lindgren, P.E. (2014) Abundance and dynamics of antibiotic resistance genes and integrons in lake sediment microcosms. *PLoS One* **9**, e108151. https://doi.org/10.1371/journal.pone.0108151

Bilal, M., Ashraf, S.S., Barceló, D. and Iqbal, H.M. (2019) Biocatalytic degradation/redefining "removal" fate of pharmaceutically active compounds and antibiotics in the aquatic environment. *Sci Total Environ* **691**, 1190–1211. https://doi.org/10.1016/j.scitotenv.2019.07.224

Blaak, H., van Hoek, A.H.A.M., Hamidjaja, R.A., van der Plaats, R.Q.J., Kerkhof-de Heer, L., de Roda Husman, A.M. and Schets, F.M. (2015) Distribution, numbers, and diversity of ESBL producing *E. coli* in the poultry farm environment. *PLoS One* **10**, e0135402. https://doi.org/10.1371/journal.pone.0135402

Borges, C.A., Tarlton, N.J. and Riley, L.W. (2019) *Escherichia coli* from commercial broiler and backyard chickens share sequence types, antimicrobial resistance profiles, and resistance genes with human extraintestinal pathogenic *Escherichia coli*. *Foodborne Pathog Dis* **16**, 813–822. https://doi.org/10.1089/fpd.2019.2680

Card, R.M., Cawthraw, S.A., Nunez-Garcia, J., Ellis, R.J., Kay, G., Pallen, M.J., Woodward, M.J. and Anjum, M.F. (2017) An in vitro chicken gut model demonstrates transfer of a multidrug resistance plasmid from *Salmonella* to commensal *Escherichia coli*. *MBio* **8**, e00777-17. https://doi.org/10.1128/mBio.00777-17

Castanon, J.I.R. (2007) History of the use of antibiotic as growth promoters in European poultry feeds. *Poult Sci* **86**, 2466–2471. https://doi.org/10.3382/ps.2007-00249

Chapman, H.D., Jeffers, T.K. and Williams, R.B. (2010) Forty years of monensin for the control of coccidiosis in poultry. *Poult Sci* **89**, 1788–1801. https://doi.org/10.3382/ps.2010-00931

Dame-Korevaar, A., Fischer, E.A.J., Stegeman, A., Mevius, D., van Essen-Zandbergen, A., Velkers, F. and van der Goot, J. (2017) Dynamics of CMY-2 producing *E. coli* in a broiler parent flock. *Vet Microbiol* **203**, 211–214. https://doi.org/10.1016/j.vetmic.2017.03.024

Danzeisen, J.L., Kim, H.B., Isaacson, R.E., Tu, Z.J. and Johnson, T.J. (2011) Modulations of the chicken cecal microbiome and metagenome in response to anticoccidial and growth promoter treatment. *PLoS One* **6**, e27949. https://doi.org/10.1371/journal.pone.0027949

Davison, J. (1999) Genetic exchange between bacteria in the environment. *Plasmid* **42**, 73–91. https://doi.org/10.1006/plas.1999.1421

Dibner, J.J. and Richards, J.D. (2005) Antibiotic growth promoters in agriculture: history and mode of action. *Poult Sci* **84**, 634–643. https://doi.org/10.1093/ps/84.4.634

Dumonceaux, T.J., Hill, J.E., Hemmingsen, S.M. and Van Kessel, A.G. (2006) Characterization of intestinal microbiota and response to dietary virginiamycin supplementation in the broiler chicken. *Appl Environ Microbiol* **72**, 2815–2823. https://doi.org/10.1128/AEM.72.4.2815-2823.2006

Egorov, A.M., Ulyashova, M.M. and Rubtsova, M.Y. (2018) Bacterial enzymes and antibiotic resistance. *Acta Naturae* **10**, 33–48. https://doi.org/10.2172/1213182

El-Sheikh, S.M., Khairy, M.H., Eleiwa, N.Z.H., Abdalla, O.E. and El-Monsef, A.G.A. (2018) Effect of sanguinarine phytobiotic, sodium butyrate compared to ampicillin on controlling necrotic enteritis in broiler chickens. *Slov Vet Zb* **55**, 405–419.

Fischer, E.A., Dierikx, C.M., van Essen-Zandbergen, A., Van Roermund, H.J., Mevius, D.J., Stegeman, A. and Klinkenberg, D. (2014) The IncI1 plasmid carrying the blaCTX-M-1 gene persists in in vitro culture of a *Escherichia coli* strain from broilers. *BMC Microbiol* **14**, 77. https://doi.org/10.1186/1471-2180-14-77

Furtula, V., Farrell, E.G., Diarrassouba, F., Rempel, H., Pritchard, J. and Diarra, M.S. (2010) Veterinary pharmaceuticals and antibiotic resistance of *Escherichia coli* isolates in poultry litter from commercial farms and controlled feeding trials. *Poult Sci* **89**, 180–188. https://doi.org/10.3382/ps.2009-00198

Ghigo, J.M. (2001) Natural conjugative plasmids induce bacterial biofilm development. *Nature* **412**, 442–445. https://doi.org/10.1038/35086581

Godowski, K.C. (1989) Antimicrobial action of sanguinarine. *J Clin Dent* **1**, 96–101.

Hausner, M. and Wuertz, S. (1999) High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. *Appl Environ Microbiol* **65**, 3710–3713. https://doi.org/10.1128/AEM.65.8.3710-3713.1999

Heuer, H., Schmitt, H. and Smalla, K. (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr Opin Microbiol* **14**, 236–243. https://doi.org/10.1016/j.mib.2011.04.009

Johnsen, A.R. and Kroer, N. (2007) Effects of stress and other environmental factors on horizontal plasmid transfer assessed by direct quantification of discrete transfer events. *FEMS Microbiol Ecol* **59**, 718–728. https://doi.org/10.1111/j.1574-6941.2006.00230.x

Kelly, L., Smith, D.L., Snary, E.L., Johnson, J.A., Harris, A.D., Wooldridge, M. and Morris, J.G. (2004) Animal growth promoters: to ban or not to ban? A risk assessment approach. *Int J Antimicrob Agents* **24**, 205–212. https://doi.org/10.1016/j.ijantimicag.2004.04.007

Lemos, M.P.L., Saraiva, M.M.S., Leite, E.L., Silva, N.M.V., Vasconcelos, P.C., Giachetto, P.F., Freitas Neto, O.C., Givisiez, P.E.N. *et al.* (2020) The posthatch prophylactic use of ceftiofur affects the cecal microbiota similarly to the dietary sanguinarine supplementation in broilers. *Poult Sci* **99**, 6013–6021. https://doi.org/10.1016/j.psj.2020.06.078

Lenfeld, J., Kroutil, M., Marsálek, E., Slavík, J., Preininger, V. and Simánek, V. (1981) Antiinflammatory activity of quaternary benzophenanthridine alkaloids from *Chelidonium majus*. *Planta Med* **43**, 161–165.

Leungtongkam, U., Thummeepak, R., Tasanapak, K. and Sitthisak, S. (2018) Acquisition and transfer of antibiotic resistance genes in association with conjugative plasmid or class 1 integrons of *Acinetobacter baumannii*. *PLoS One* **13**, e0208468. https://doi.org/10.1371/journal.pone.0208468

Licht, T.R., Christensen, B.B., Krogfelt, K.A. and Molin, S. (1999) Plasmid transfer in the animal intestine and other dynamic bacterial populations: the role of community structure and environment. *Microbiology (Reading)* **145**, 2615–2622. https://doi.org/10.1099/00221287-145-9-2615

Lopatkin, A.J., Huang, S., Smith, R.P., Srimani, J.K., Sysoeva, T.A., Bewick, S., Karig, D.K. and You, L. (2016a) Antibiotics as a selective driver for conjugation dynamics. *Nat Microbiol* **1**, 16044. https://doi.org/10.1038/nmicrobiol.2016.44

Lopatkin, A.J., Meredith, H.R., Srimani, J.K., Pfeiffer, C., Durrett, R. and You, L. (2017) Persistence and reversal of plasmid-mediated antibiotic resistance. *Nat Commun* **8**, 1689. https://doi.org/10.1038/s41467-017-01532-1

Lopatkin, A.J., Sysoeva, T.A. and You, L. (2016b) Dissecting the effects of antibiotics on horizontal gene transfer: analysis suggests a critical role of selection dynamics. *BioEssays* **38**, 1283–1292. https://doi.org/10.1002/bies.201600133

Mathers, J.J., Clark, S.R., Hausmann, D., Tillman, P., Benning, V.R. and Gordon, S.K. (2004) Inhibition of resistance plasmid transfer in *Escherichia coli* by ionophores, chlortetracycline, bacitracin, and ionophore/antimicrobial combinations. *Avian Dis* **48**, 317–323. https://doi.org/10.1637/7105

Millet, S. and Maertens, L. (2011) The European ban on antibiotic growth promoters in animal feed: from challenges to opportunities. *Vet J* **187**(2), 143–144. https://doi.org/10.1016/j.tvjl.2010.05.001

Niero, G., Bortolaia, V., Vanni, M., Intorre, L., Guardabassi, L. and Piccirillo, A. (2018) High diversity of genes and plasmids encoding resistance to third-generation cephalosporins and quinolones in clinical *Escherichia coli* from commercial poultry flocks in Italy. *Vet Microbiol* **216**, 93–98. https://doi.org/10.1016/j.vetmic.2018.02.012

Obiang-Obounou, B.W., Kang, O.-H., Choi, J.-G., Keum, J.-H., Kim, S.-B., Mun, S.-H., Shin, D.-W., Kim, K.W. *et al.* (2011) The mechanism of action of sanguinarine against methicillin-resistant *Staphylococcus aureus*. *J Toxicol Sci* **36**, 277–283. https://doi.org/10.2131/jts.36.277

Pan, D. and Yu, Z. (2014) Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes* **5**, 108–119. https://doi.org/10.4161/gmic.26945

Pulss, S., Stolle, I., Stamm, I., Leidner, U., Heydel, C., Semmler, T., Prenger-Berninghoff, E. and Ewers, C. (2018) Multispecies and clonal dissemination of OXA-48 carbapenemase in Enterobacteriaceae from companion animals in Germany, 2009–2016. *Front Microbiol* **9**, 1265. https://doi.org/10.3389/fmicb.2018.01265

Qian, W., Yang, M., Li, X., Sun, Z., Li, Y., Wang, X. and Wang, T. (2020) Anti-microbial and anti-biofilm activities of combined chelerythrine-sanguinarine and mode of action against *Candida albicans* and *Cryptococcus neoformans* in vitro. *Colloids Surf B Biointerfaces* **191**, 111003. https://doi.org/10.1016/j.colsurfb.2020.111003

Reis, A.C., Kolvenbach, B.A., Nunes, O.C. and Corvini, P.F. (2020) Biodegradation of antibiotics: the new resistance determinants–part II. *New Biotechnol* **54**, 13–27. https://doi.org/10.1016/j.nbt.2019.08.003

Saliu, E., Zentek, J. and Vahjen, W. (2020) In vitro conjugation kinetics of AmpC, broad spectrum and extended-spectrum beta-lactamase-producing *Escherichia coli* donors and various *Enterobacteriaceae* recipients. *BMC Microbiol* **20**, 133. https://doi.org/10.1186/s12866-020-01787-7

Saraiva, M.M.S., Moreira Filho, A.L.B., Vasconcelos, P.C., Nascimento, P.V., Azevedo, P.S., Freitas Neto, O.C., Givisiez, P.E.N., Gebreyes, W.A. *et al.* (2020) Chemical treatment of poultry litter affects the conjugation of plasmid-mediated extended-spectrum beta-lactamase resistance genes in *E. coli*. *J Appl Poult Res* **29**, 197–203. https://doi.org/10.1016/j.japr.2019.10.006

Sarmah, A.K., Meyer, M.T. and Boxall, A.B.A. (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. *Chemosphere* **65**, 725–759. https://doi.org/10.1016/j.chemosphere.2006.03.026

Singer, R.S. and Hofacre, C.L. (2006) Potential impacts of antibiotic use in poultry production. *Avian Dis* **50**, 161–172. https://doi.org/10.1637/7569-033106R.1

de Souza, M.M.S., Coelho, S.M.O., Coelho, I.S., Soares, B.S., Motta, C.C., Melo, D.A., Dubenczuk, F.C., Santiago, G.S. *et al.* (2016) Antimicrobial resistance in animal production: an overview. *Rev Bras Med Vet* **38**, 68–74. https://rbmv.org/BJVM/article/view/332

Teixeira, A.S., Oliveira, M.C., Menezes, J.F., Gouvea, B.M., Teixeira, S.R. and Gomes, A.R. (2015) Poultry litter of wood shavings and/or sugarcane bagasse: animal performance and bed quality. *Rev Colom Cienc Pecua* **28**, 238–246. https://doi.org/10.17533/udea.rccp.v28n3a4

Thibodeau, A., Quessy, S., Guévremont, E., Houde, A., Topp, E., Diarra, M.S. and Letellier, A. (2008) Antibiotic resistance in *Escherichia coli* and *Enterococcus* spp. isolates from commercial broiler chickens receiving growth-promoting doses of bacitracin or virginiamycin. *Can J Vet Res* **72**, 129–136. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2276897/pdf/cjvr72_pg129.pdf

Vieira, S.L., Berres, J., Reis, R.N., Oyarzabal, O.A., Coneglian, J.L.B., Freitas, D.M., Peña, J.E.M. and Torres, C.A. (2008a) Studies with sanguinarine like alkaloids as feed additive in broiler diets. *Rev Bras Cienc Avic* **10**, 67–71. https://doi.org/10.1590/S1516-635X2008000100010

Vieira, S.L., Oyarzabal, O.A., Freitas, D.M., Berres, J., Pena, J.E.M., Torres, C.A. and Coneglian, J.L.B. (2008b) Performance of broilers fed diets supplemented with sanguinarine-like alkaloids and organic acids. *J Appl Poult Res* **17**, 128–133. https://doi.org/10.3382/japr.2007-00054

Wang, J., Stephan, R., Power, K., Yan, Q., Hächler, H. and Fanning, S. (2014) Nucleotide sequences of 16 transmissible plasmids identified in nine multidrug-resistant *Escherichia coli* isolates expressing an ESBL phenotype isolated from food-producing animals and healthy humans. *J Antimicrob Chemother* **69**, 2658–2668. https://doi.org/10.1093/jac/dku206

Wang, J. and Zhuan, R. (2020) Degradation of antibiotics by advanced oxidation processes: an overview. *Sci Total Environ* **701**, 135023. https://doi.org/10.1016/j.scitotenv.2019.135023

Watnick, P. and Kolter, R. (2000) Biofilm, city of microbes. *J Bacteriol* **182**, 2675–2679. https://doi.org/10.1128/JB.182.10.2675-2679.2000

WHO - World Health Organization & WHO Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR). (2017) Critically important antimicrobials for human medicine: ranking of antimicrobial agents for risk management of antimicrobial resistance due to non-human use, 5th rev. World Health Organization. https://apps.who.int/iris/handle/10665/255027

Woolhouse, M., Ward, M., van Bunnik, B. and Farrar, J. (2015) Antimicrobial resistance in humans, livestock and the wider environment. *Philos Trans R Soc Lond B Biol Sci* **370**, 20140083. https://doi.org/10.1098/rstb.2014.0083

Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., Zhang, R., Walsh, T.R. *et al.* (2017) Novel plasmid-mediated colistin resistance gene mcr-3 in *Escherichia coli*. *MBio* **8**, e00543-17. https://doi.org/10.1128/mBio.00543-17

Zatyka, M. and Thomas, C.M. (1998) Control of genes for conjugative transfer of plasmids and other mobile elements. *FEMS Microbiol Rev* **21**, 291–319. https://doi.org/10.1111/j.1574-6976.1998.tb00355.x

Zhao, Z. and Drlica, K. (2001) Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. *Clin Infect Dis* **33**, 147–156. https://doi.org/10.1086/321841