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Clinical and microbial efficacy of antimicrobial treatments of experimental avian colibacillosis

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ABSTRACT

The clinical and microbial efficacy of antimicrobial treatments of avian colibacillosis was studied, using an experimental model on chickens previously inoculated with multi-resistant commensal *Escherichia coli* strains. One *E. coli* with pMG252 plasmid containing bla_{FOX5} and *qnrA1* genes and another *E. coli* with pMG298 plasmid containing $bla_{CTX\text{-}M15}$ and *qnrB1* genes were first orally inoculated to chickens Both isolates were also resistant to chloramphenicol, sulphamethoxazole, trimethoprim, streptomycin, gentamicin, kanamycin, and tetracycline. The birds were then experimentally infected with an avian pathogenic *E. coli* (APEC), via the air sac. Treatments (oxytetracycline (OTC), trimethoprim–sulfadimethoxin (SXT), amoxicillin (AMX) or enrofloxacin (ENR) were then offered at the therapeutic doses. Symptoms, lesions in dead or sacrificed birds, and isolation and characterization of APEC from internal organs were studied.

Results showed that OTC, SXT or ENR treatments could control the pathology. AMX worsened the disease, possibly due to endotoxin shock. All APEC re-isolated from internal organs showed the same antimicrobial susceptibility as the APEC inoculated strain, except for one APEC isolate from an infected OTC-treated bird, which acquired tetracycline resistance only, and one APEC isolate recovered from the air sacs of a chicken in the infected SXT-treated group, which acquired the pMG252 plasmid and became multi-resistant.

Thus three antimicrobials could control the disease but the experimental model enabled, to our knowledge, the first observation of plasmid transfer from a bacterium of the intestinal tract to a pathogenic isolate from the respiratory tract.

1. Introduction

Avian colibacillosis has been reported in all avian species, at all ages and in all types of poultry production (broilers, breeders, layers, etc.). Numerous forms of avian colibacillosis exist, such as colisepticemia, air sac diseases, coliform cellulitis, peritonitis, panophtalmitis and omphalitis/egg yolk infection. In birds, contrary to mammals, colibacillosis is typically a localized or systemic disease, and not an enteric disease. Economic losses result from mortality, retarded growth, condemnation at slaughterhouses (Barnes et al., 2003) and antimicrobial treatments. *Escherichia coli* forms part of the normal intestinal flora of birds at concentrations up to 10^6/g and can be found in the environment of all farms. In normal chickens, 10–15% of intestinal *E. coli* are avian pathogenic *E. coli* (APEC) serotypes such as O1, O2 or O78 (Barnes et al., 2003). These APEC isolates have virulence factors (fimbria, hemolysins, etc.). Stress due to virus infections, toxins, or nutritional deficiencies may compromise the bird's immune defenses in which case avian colibacillosis may develop, which will motivate antimicrobial administration.

Control of avian colibacillosis includes hygiene measures and flock management, but once the disease is

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present, an antimicrobial treatment may be required to improve animal welfare and reduce the economic consequences of the disease. The most commonly administered treatments include amoxicillin, trimethoprim–sulfadimethoxine, oxytetracycline or enrofloxacin (Chauvin et al., 2005). Treatments are usually given via the drinking water. The purpose of our trial was to compare the clinical and microbial efficacies of these antimicrobials in the treatment of experimental *E. coli* colibacillosis and the possible selection of resistant pathogenic bacteria. Moreover, as oral administration of antimicrobials to poultry may lead to the selection of resistant bacterial strains in the intestinal flora, the study protocol was designed to allow the impact of oral antibiotics on the intestinal flora to be monitored (to be reported in a future paper). Thus, the chickens were given strains of *Campylobacter*, *Enterococcus faecium* and two intestinal non pathogenic *E. coli* strains bearing plasmidic *qnr*, ESBL or AmpC genes (Bouder et al., 2009), by oral route.

2. Materials and methods

2.1. Bacterial strains

The pathogenic *E. coli* "Goren" strain was kindly provided by Dr Froyman (Bayer Animal Health Leverkusen, Germany). This O78K80 avian pathogenic strain was susceptible to the different antimicrobials that were used as treatments in this study (Table 1). It was cultured in Mueller Hinton broth (Biorad, Marnes la coquette, France).

Two other *E. coli* strains (*E. coli* 177pMG252 and *E. coli* 43pMG298) were used. They were obtained by conjugation between two intestinal avian *E. coli* strains previously isolated from healthy broilers in slaughterhouses, made resistant to rifampicin, and *E. coli* strains harboring plasmids, J53pMG252 and J53pMG298, kindly given by Prof. G. Jacoby, Lahey Clinic, Burlington, MA, USA (Jacoby et al., 2003, 2006). The pMG252 plasmid contains *qnrA1* and bla_{FOX-5} genes, which respectively confer reduced susceptibility to fluoroquinolones and cephamycins, as well as resistance genes against streptomycin, sulphonamide, trimethoprim, chloramphenicol, gentamicin and kanamycin but not tetracycline. The tetracycline resistance gene in *E. coli* 177pMG252 is a *tet*(A) gene borne by the chromosome or a other non conjugative plasmid (Le Devendec et al., in press). The pMG298 plasmid contains *qnr*B1 and $bla_{CTX-M15}$ genes which respectively confer reduced susceptibility to fluoroquinolones and resistance to cephalosporins, as well as resistance genes against sulphonamide, chloramphenicol, streptomycin, kanamycin, gentamicin, tetracycline and trimethoprim (Table 1).

Two avian *Campylobacter* (one *C. jejuni* and one *C. coli* strains) and two avian *E. faecium* strains from our collection were also used for oral inoculation (Bouder et al., 2009). All these strains had been isolated from healthy broilers in slaughterhouses.

2.2. Experimental model

Male and female specific pathogen free (SPF) Leghorn one-day-old chicks, 360 in all, were obtained from AFSSA, LERAPP, Ploufragan. On days 14 (D14) to D16, the birds received cultures of *E. coli* 177pMG252 and 43pMG298, and cultures of the two *Campylobacter* strains and of the two *E. faecium* strains, daily by oral route. On D17, they were vaccinated with infectious bronchitis vaccine (Bioral® H120, Merial, Lyon, France). One dose of vaccine was given through the sinus and one dose was given intratracheally to exacerbate the effects of the APEC administration three days later. Just before APEC inoculation, on D20, the birds were randomly divided into twelve replicates of 30 birds. The birds were allocated to the replicates by using a weight histogram to assemble groups with equal weight and variation. Six pressure-controlled animal rooms, with filtered air and controlled temperature, were used. Each room contained a single experimental group, kept in two cages, each cage containing one replicate of 30 chickens. Birds from five groups (ten replicates) were inoculated in the left air sac with 0.1 mL of a culture of APEC 078K80, and one group was left uninfected. The titer of the APEC inoculum was determined by dilution. The medication was initiated one day later, as the inoculated birds began to show depression and respiratory signs. Birds from four groups were treated with therapeutic doses of 20 mg/kg oxytetracycline (Terramycine, Pfizer, Paris, France), 28–6 mg/kg sulfadimethoxin–trimethoprim (Trisulmix liquid, Coophavet, Ancenis, France), 10 mg/kg amoxicillin (Suramox 10, Virbac, Carros, France) or 10 mg/kg enrofloxacin (Baytril® 10%, Bayer, Puteaux, France). Thus, the different groups of birds were: NINT (non-infected, non-treated birds), INT (infected, non-treated birds), I-AMX (infected, amoxicillin-treated birds), I-SXT (infected, trimethoprim–sulfadimethoxin-treated birds), I-OTC (infected, oxytetracycline-treated birds) and I-ENR (infected, enrofloxacin-treated birds) (Table 2). The antibiotics were given in the

Table 1
Characteristics of strains: serotype and MICs (in mg/L).

Strain	Serotype	AMP	AMC	TIO	FOX	NAL	CIP	TET	SXT	CHL	STR	KAN	GEN	RIF
J53pMG252	Neg	>32	>32/16	>8	>32	16	1	≤4	>4/76	>32	>64	32	8	≤16
177 pMG252	Neg	>32	>32/16	>8	>32	32	1	>32	>4/76	>32	>64	64	16	>128
43 pMG298	Neg	>32	32/16	>8	32	8	2	>32	>4/76	>32	>64	>64	>16	>128
O78K80	O78K80	4	4/8	0.5	8	4	0.03	≤4	≤0.12/2.38	8	≤32	16	1	≤16
472SA	O78K80	>32	>32/16	>8	>32	16	0.5	≤4	>4/764	>32	>64	64	>16	≤16
297F	O78K80	4	4/8	0.5	8	4	0.03	>32	≤0.12/2.38	8	≤32	≤8	1	≤16

Neg: negative results with O78K80, O2K1 and O1K1 antisera; AMP: ampicillin; AMC: amoxicillin, clavulanic acid; TIO: ceftiofur; FOX: cefoxitin; NAL: nalidixic acid; CIP: ciprofloxacin; TET: tetracycline; SXT: trimethoprim–sulfamethoxasol; CHL: chloramphenicol; STR: streptomycin; KAN: kanamycin; GEN: gentamicin; RIF: rifampicin.