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Extended spectrum β-lactamase producing *Escherichia coli* in broiler breeding roosters: Presence in the reproductive tract and effect on sperm motility

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ABSTRACT

Extended spectrum β-lactamases (ESBL)-producing *Escherichia coli* have emerged worldwide in animal husbandry and they were reported from different ecosystems. The purpose of this study was firstly, to investigate the presence of ESBL-producing *E. coli* in the gastrointestinal (GIT) and reproductive (RT) tracts of broiler breeding roosters, and secondly to study the impact of an ESBL-producing *E. coli* on artificially infected semen. A total of seventeen ESBL-producing *E. coli* strains were isolated from the gastrointestinal and reproductive tracts of nine broiler breeding roosters. All isolates were identified to the species level by API 20E system and MALDI-TOF, serotyped, and genetically characterized for ESBL production. Semen was artificially infected with *E. coli* ATCC25922 or with an ESBL-producing *E. coli* strain recovered from the reproductive tract. A computer aided semen analyzer (CASA) was used to compare different spermatozoa motility parameters in each sample. All ESBL-producing *E. coli* isolates could not be typed with the currently used sera and they were harboring a $bla_{CTX\text{-}M}$ gene alone or in combination with a bla_{TEM} gene. The semen quality was notably less affected in samples infected with ESBL-producing *E. coli* strain compared to the control and sample infected with *E. coli* ATCC25922. The present study revealed that ESBL-producing *E. coli* can be isolated from both reproductive and digestive tracts of broiler breeding roosters. Contamination of the reproductive tract with ESBL-producing *E. coli* could lead to contamination of semen and could be an important factor in the dissemination of ESBL-producing *E. coli* in poultry.

1. Introduction

The threat of antimicrobial resistant bacteria in both human and veterinary medicine has become a worldwide issue (Veldman et al., 2011; Tansarli et al., 2014).

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Many researchers have reported resistant bacteria recovered from different bird species (Schwaiger et al., 2008; Randall et al., 2011; Ma et al., 2012; Álvarez-Fernández et al., 2013) with an increasing emphasis on the presence of extended spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* in the avian gut (Smet et al., 2008).

Even though antimicrobial resistance in intestinal *E. coli* isolates is well studied in broilers (Olsen et al., 2014), little is published at broiler breeding level, especially concerning ESBL-producing *E. coli*. In birds, the intestines and the reproductive tract are in connection by means of the cloaca, therefore, there is a possibility that intestinal bacteria, for example *E. coli*, can colonize the reproductive tract of both male and female birds and, thus, may decrease fertility of the infected animals (Boone and Hughes, 1970; Reiber et al., 1995).

Because poultry is probably the only industrial species where reproduction occurs naturally with no quality control of the semen, and considering the large number of females that can be fertilized by one male, the presence of resistant bacteria in the intestines or in the reproductive tract of broiler breeding roosters could be a determining factor in the dissemination of antimicrobial resistance in broilers.

However, to the best of our knowledge, no data on the presence of antimicrobial resistant *E. coli* in the gut or the reproductive tract of broiler breeding roosters are available. Even though results obtained in birds are not conclusive (Lombardo and Thorpe, 2000), it has been extensively shown in human medicine that the presence of *E. coli* not only affects sperm motility, but also sperm structure which leads to a decrease in male fertility (Fraczek et al., 2012). *E. coli* reduces sperm motility through sperm adhesion and agglutination (Wolff et al., 1993; Monga and Roberts, 1994) and causes morphological changes of the midpiece, plasma membrane, and acrosome (Köhn et al., 1998; Diemer et al., 2000).

In birds, *E. coli* could be transmitted to the progeny through copulation. Microorganisms present in the seminal fluid or attached to spermatozoa are transmitted to females (Barnes and Gross, 1997; Jacobs et al., 1978; Poiani, 2010) causing reproductive tract infections (Askienazy-Elbhar, 2005) and embryonic mortality (Reid et al., 1961).

Despite the numerous publications concerning sperm infection, to date, there are no data on the potential impact that could be related to resistant *E. coli* contamination. Indeed, it has been reported that the bacterial virulence could be modulated by the acquisition of antimicrobial resistance. In this regard, contradictory results have been reported; both positive (Buckley et al., 2006; Bialek et al., 2010; Pitout, 2012) and negative correlations (Branger et al., 2005; Horcajada et al., 2005) have been suggested between resistance and virulence. Thus, in such situation, this could be expressed in terms of variability when comparing the impact of resistant and sensitive strains on spermatozoa motility.

Therefore, the present study aimed to explore the presence of ESBL-producing *E. coli* in the digestive and reproductive tracts of broiler breeder roosters, and investigate the potential effects of an ESBL-producing *E. coli* on semen motility parameters of broiler breeding roosters.

2. Material and methods

2.1. ESBL-producing E. coli characterization

2.1.1. Sampling and isolation

All animal experimentation was conducted according to the national and international guidelines for animal welfare. Nine broiler breeding roosters of 56 weeks-old, weighing 4–6 kg, collected at the end of the production cycle from a breeding farm in Bejaia (Algeria) were used in this experiment to investigate the presence of ESBL-producing *E. coli* in the digestive and reproductive tracts. The animals were humanly killed, after which a 70% solution of ethanol was applied to decontaminate the stripped carcasses. The gastrointestinal and reproductive tracts were subsequently removed aseptically. The gastrointestinal tract was sampled at different locations (crop, proventriculus, gizzard, duodenum, jejunum, ileum, caecum, and cloaca). The contents were transferred into 15 mL Falcon tubes and incubated aerobically without agitation overnight at 37 °C in 10 mL nutrient broth (HiMedia®, India). Testes surfaces were also disinfected by 70% solution of ethanol before cutting with a sterile knife and then swabbed with a dry sterile cotton swab to collect semen. Segments of the reproductive tract (ductus deferens and testes) were decontaminated, processed and incubated as described above. After overnight incubation, 10 μL of the nutrient broth was streaked onto two MacConkey agar plates (HiMedia®, India), one supplemented with 1 μg/mL cefotaxime and one supplemented with 1 μg/mL ceftazidime for selective isolation of ESBL positive *Enterobacteriaceae*. After overnight incubation at 37 °C, one lactose positive colony from each plate was purified and identified by the API®20E system (bioMérieux, Marcy-l'Etoile, France).

2.1.2. MALDI-TOF identification and serotyping

Identification by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was conducted for all ESBL-producing *E. coli* isolates; a Bruker Daltonics Biotyper instrument on ethanol-washed cell pellets extracted with formic acid and acetontirile solutions was used as recommended by the manufacturer (Bruker Daltonics Inc., Billerica, MA).

Isolates confirmed as *E. coli* were then serotyped by agglutination in micro-titer plates with monospecific antisera toward 27 different somatic O antigens: O1, O2, O5, O6, O8, O9, O11, O12, O14, O15, O17, O18, O20, O35, O36, O45, O53, O78, O81, O83, O88, O102, O103, O115, O116, O132, and O109, purchased from the *E. coli* Reference Laboratory of the University of Santiago de Compostela (Lugo, SPAIN), according to the procedure of Blanco et al. (1996).

2.1.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing against 14 antimicrobials was conducted in all *E. coli* isolates by the disk diffusion method on Mueller Hinton agar (HiMedia®, India) according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2014). The following antibiotics were tested: amoxicillin/clavulanic acid (AMC: 20/10 μg), cefotaxime (CTX: 30 μg), ceftazidime (CAZ: