The Effect of the Administration of Different Antimicrobial Formulations on the Fungal Infestation of the Gastrointestinal Tract in Turkeys

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ABSTRACT

The gastrointestinal tract of poultry is inhabited by a diverse community of bacteria, fungi, protozoa and viruses. The aim of the study was to determine the influence of early antimicrobial treatment on the fungal microflora of the gastrointestinal tract of turkeys and analyze candidiasis occurrence in later age of the birds from treated flocks. The samples were collected from the oral cavity, crop and cloaca of turkey poults treated at first week of life with one of the following treatments: amoxicillin; amoxicillin and clavulanic acid; enrofloxacin; florfenicol; lincomycin and spectinomycin; amoxicillin and colistin; amoxicillin, clavulanic acid, and colistin; enrofloxacin and colistin. Sampling was again carried out during the 5th week of the turkey’s life. The most common isolated fungal were Candida and Trichosporon. Treatment with amoxicillin or amoxicillin and clavulanic acid provided the highest number of positive samples, while treatment with enrofloxacin provided the lowest number of positive samples. The inclusion of colistin in the treatment regimen resulted in a reduction of the number of positive fungal samples. Clinical candidiasis was not observed in any of the examined birds. The experiments demonstrated that early antimicrobial treatment in poultry can predispose to fungal colonization and that beta-lactams have the highest influence on gastrointestinal colonization by fungi.

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INTRODUCTION

The gastrointestinal tract of poultry is inhabited by a diverse community of bacteria, fungi, protozoa, and viruses. The development of the gut microbiota starts at the moment of hatching, when poults are exposed to microbes originating from the egg shell and the surrounding environment (Kwiatek et al., 2008; Okiki and Obigimi, 2010/2011). The microflora is stabilized by the second week of life in the crop and jejunum, and by the fourth week in the cecum (Amit-Romach et al., 2004). Hence, the microbial inoculum obtained at the early stage of the post hatch period is critical for the establishment of the gut microflora (Apajalathi et al., 2004). The gastrointestinal tract primarily consists of bacteria, which are natural occupants that have been well documented. In addition, the digestive tract is inhabited by resident mycobionta, which is still not well known (Yegani and Korver, 2008). From the gastrointestinal tract of poultry, both filamentous fungi and yeasts have been isolated, including a predominance of the Candida species, followed by Trichosporon spp., Geotrichum spp., Rhodotorula spp., and Saccharomyces cerevisiae (Ziółkowska and Tokarzewski, 2007). Although some fungi such as Saccharomyces cerevisiae and Saccharomyces boulardii are used as probiotics in animal production, others may be pathogenic under some conditions (Mikulski et al., 2008). Candida albicans is an opportunistic fungal pathogen that is involved in infections ranging from superficial mucosal infections to systemic candidiasis. Other species such as C. parapsilosis, C. rugosa, C. famata, C. tropicalis, and C. krusei also have been isolated from poultry with candidiasis (Singh et al., 2015).
The clinical signs of candidiasis in birds are non-specific, and include listlessness, inappetence, retarded growth and ruffled feathers. The signs may be masked by the clinical signs of primary viral and bacterial diseases. The affected mucosa is often diffusely or focally thickened with white to grey pseudomembranous or diphtheric patches and ulcers (Sokół et al., 2015). *Trichosporon* is a medically important genus whose members are able to colonize and proliferate in the gastrointestinal system, respiratory tract, and skin. This yeast-like pathogen may cause deep-seated, mucosa-associated, or superficial infections (Colombo et al., 2011). In recent years, the pathogenic role of strains of the genera *Rhodotorula* and *Geotrichum* have been confirmed (Fleming et al., 2002; Girmenia et al., 2005). Fungal infections in poultry can be associated with many features such as inadequate management, pre-existing diseases, and prolonged treatment with antimicrobials (Sokół et al., 2015). Early antimicrobial treatment may result in the microflora establishing disorders and fungal colonization, which may provide to candidiasis. Therefore, the aim of the present study was to determine the influence of various antimicrobial treatments on the fungal mycobiota of the gastrointestinal tract of turkey poults and analyze the occurrence of candidiasis in the birds from treated flocks.

**MATERIALS AND METHODS**

**Birds and sample collection:** The turkeys used in this study originated from British United Turkeys Big 6 flocks produced in western Poland. The flocks were managed by one company and were raised under uniform managemental condition on 26 poultry houses. All farms were provided with food, litter, medicine from the same sources. The birds were monitored during the rearing period for gastrointestinal disorders. Fifty-one flocks were monitored over two years.

Poults that died during transport from the hatchery to the farms were subjected to necropsy, and swabs from the liver, heart, lung, and yolk sac were taken for microbiological examination. The 24 flocks with confirmed bacterial growth were chosen for this experiment, and after microbiological examination, were treated according to the antibiogram.

The examined flocks, 3-5 thousand birds each, were treated from the third day of life for five days with the most popular chemotherapeutics used in turkey flocks: amoxicillin; amoxicillin and colistin; amoxicillin and clavulanic acid; amoxicillin, clavulanic acid, and colistin; enrofloxacin; enrofloxacin and colistin; florfenicol; lincomycin and spectinomycin (lincopectin), according to the manufacturer’s instructions (Table 1). As a control group, three non-treated flocks of the same birds number and age (nine-day-old turkeys) was chosen. The day after the treatment was finished, swabs from the oral cavity, crop, and cloaca of ten randomly chosen birds from each flock were taken. The same procedure was repeated in the 5th week of the turkey’s life, to check for fungal infestation in the gastrointestinal tract.

All dead birds were subjected to necropsy and from birds which showed lesions of gastrointestinal tract suggesting candidiasis, the mycological examination was performed.

**Mycological examination:** The swabs were cultured on Sabouraud glucose agar with chloramphenicol (Emapol, Poland) and incubated at 37°C for 48 h. A sample was considered positive if any fungal growth was observed. The total number of fungal colonies was counted, and the fungal growth was determined as low intensity (<10 colonies; +), medium intensity (10–50 colonies; ++), or high intensity (>50 colonies; +++). All cultures were examined daily over the next two weeks to ensure that the negative samples remained negative. Identification of the genera of the fungi was based on the morphological characteristics of the isolates (colony morphology, microscopic examination) (Kurtzman and Fell, 2000) and their growth on chromagar media (ChronAgar Candida, Emapol).

**Statistical analysis:** Statistical analysis was carried out using Statistical 12 Software. Fisher’s chi-squared test was used to compare data between the treated groups and the control group. To compare the groups treated with antibiotics with and without colistin addition, the chi-squared test was used. Statistical significance was considered when P<0.001.

**RESULTS**

Fungal growth in turkeys after antimicrobial treatment was observed among all treated flocks, while no fungal growth was detected in the untreated control flock (Table 1). The most intensive fungal growth was observed in the groups treated with amoxicillin; amoxicillin and clavulanic acid; amoxicillin and colistin; or amoxicillin, clavulanic acid, and colistin. In those groups, fungi were isolated from 51-79% of samples. In contrast, the samples taken from turkeys treated with enrofloxacin; enrofloxacin and colistin; florfenicol; or lincomycin and spectinomycin, the fungal growth was less intense and the number of positive samples was 19-29% (Table 1).

The addition of colistin to the treatment resulted in a reduction of the number of positive fungal samples. The reduction occurred in all groups in which colistin was used simultaneously with antibiotics, compared with the use of antibiotics on their own. In particular, the highest difference in the number of positive samples among the colistin and the no-colistin groups was observed between the amoxicillin group and the amoxicillin and colistin group, which had 30.8% fewer positive samples than the amoxicillin only group (P<0.001). Lower differences in the number of positive samples were noted in the other two colistin groups; there were 15% fewer positive samples in the enrofloxacin and colistin group (P=0.58) and 5% fewer positive samples in the amoxicillin, colistin, and clavulanic acid group, compared to the amoxicillin and clavulanic acid only group (P=0.21).

From the gastrointestinal tracts of the treated turkey poults, *Candida* spp., *Trichosporon* spp., *Rhodotorula* spp., *Geotrichum* spp., and *Saccharomyces* spp. were isolated (Table 2). The most frequently isolated genera of fungi were *Candida* spp. and *Trichosporon* spp. The greatest diversity of fungi was found in the samples from poults in the amoxicillin group, in which *Candida* spp., *Trichosporon* spp., *Geotrichum* spp., and *Saccharomyces* spp. were cultured. Moreover, mostly *Candida* spp. were isolated in groups with colistin treatment (Table 2).
The significant influence of the early treatment on the fungal infestation of the gastrointestinal tract was noted in 3 groups treated with amoxicillin; amoxicillin with clavulanic acid; amoxicillin with clavulanic acid and colistin (P<0.001). In the control group, positive samples were noted, but in lower number that in the experimental groups. Lesions suggesting candidiasis of the beak cavity, crop or intestines were not noted in any of the autopsied birds.

### DISCUSSION

Many researches confirmed that antibiotic treatment promotes the development of fungal infections in humans and animals (Dollive et al., 2013; Sokól et al., 2015).
Multiple studies have examined the effects of antibiotic treatment on the gut microbiome in vertebrates and have shown that the gut communities in adult individuals may return to their initial states once the treatment is completed; however, some scientists have found that detectable microbiome differences remain long time after treatment has ended (Jernberg et al., 2010; Panda et al., 2014). The microfloral imbalance is especially dangerous in immature individuals, in whom the gastrointestinal microflora is not fully established.

Our research demonstrated that antimicrobial treatment in the first week of life of turkey poults significantly affects the intestinal tract colonization of fungi. According to our predictions, it was observed that the groups treated with amoxicillin or amoxicillin and clavulanic acid, which are broad-spectrum antimicrobials, showed the highest levels of fungal colonization. These observations are in agreement with other studies in which high fungal colonization was observed, mostly by yeast in humans after amoxicillin treatment (Lin et al., 2005; Noni et al., 2015). Previous studies on the impact of amoxicillin on human gut microflora have shown that the treatment significantly decreases the number of Bifidobacterium spp. and Lactobacillus spp. These groups of bacteria, especially the Lactobacillus genus, have beneficial effects. For example, they help to sustain resistance to colonization by producing acetic and lactic acids, which lower intestinal pH, thus preventing overgrowth of many potentially pathogenic microorganisms (Fuller, 1973). Lactobacillus spp. also produce hydrogen peroxide, which prevents the development of yeasts (Lidbeck and Nord, 1993). In birds, it probably works the same way.

Unexpectedly, low fungal colonization was observed after enrofloxacin treatment. Enrofloxacin, one of the most popular antibiotics used to treat poultry, has antibacterial activity against a broad spectrum of Gram-negative and Gram-positive bacteria and also reduces the number of bacteria in the gastrointestinal tract which should predispose to fungal colonization. Similar results have been observed with amoxicillin treatment. Panda et al. (2014) have observed that fluoroquinolones and β-lactams significantly decrease microbial diversity and reduce the core phylogenetic microbiota of the gastrointestinal tract. Similarly, Lin et al. (2005) have found that almost one-third of examined humans with blood candidemia were previously treated with fluoroquinolones. We expected a similar high correlation between enrofloxacin treatment and fungal colonization of the gastrointestinal tract. However, in our study, the connection between enrofloxacin treatment and fungal growth was the lowest of all groups tested.

Chen et al. (2011) have investigated the effects of enrofloxacin treatment on the human intestinal microbiota in vitro. They have shown that the gut microflora demonstrates natural resistance against fungal colonization and that the effect of enrofloxacin on microflora colonization resistance to C. albicans is dosage-dependent. They suggest that a low enrofloxacin concentration slightly affects the microflora, which still possesses the capability to inhibit the growth of pathogens. Using average concentrations, the fungal growth was significant; but at high concentrations, the growth was low. The low number of fungi could be caused by the high residual level of enrofloxacin, which prevented Candida from growing due to the reconstructed ecology.

Anadón et al. (1995) have shown that enrofloxacin given orally is slowly and incompletely absorbed from the gastrointestinal tract of poultry. The high concentration of enrofloxacin in water (50 mg/L of water) may cause favorable conditions for fungal growth in the gastrointestinal tract.

Our research revealed that colistin may reduce the fungal colonization of the gastrointestinal tract of poultry. The antifungal effect was especially prominent between the group treated with amoxicillin and that treated with amoxicillin and colistin. Although the exact mode of action of colistin on fungi remains unclear, it has been proposed that it acts at the fungal membrane. Indeed, colistin has been shown to trigger the rapid efflux of ATP from germlings of Mucorales as well as induce alterations of the membrane structure (Zeidler et al., 2013). The antibiotic colistin also has shown synergism with other substances such as aminocandin, echinocandin, and caspofungin against Candida spp. (Schemuth et al., 2013; Zeidler et al., 2013). The confirmed antifungal activity of colistin against Candida spp., Cryptococcus spp., and Mucorales spp. has been shown previously (Schemuth et al., 2013). In our research, its impact on Trichosporon spp. colonization was observed.

Candida spp. and Trichosporon spp. were the most common fungi isolated from the examined parts of the gastrointestinal tract of the treated poults. These two genera are opportunistic fungal pathogens in vertebrates, and early colonization of the gastrointestinal tract may have an effect on poultry health.

Candidiasis of the gastrointestinal tract is a frequently noted type of mycosis in birds. Birds are quite susceptible to candidiasis of the buccal region, crop, and upper alimentary tract, and it resembles human thrush clinically and histologically (Moretti et al., 2000). Normally, the disease in poultry is not present in its severe form, and the outbreaks reported in the literature are connected to alterations in the host’s immune defenses, such as the integrity of the mucocutaneous barrier, the balance among the different components of the normal flora present, or the perfect response of T-cell-mediated immunity (Rinaldi et al., 1994). The effects of fungi on intestinal inflammation as well as gut bacterial constitution are visible, but they still remain unclear (Qiu et al., 2015).

In humans, a significant increase of C. albicans has been demonstrated in the guts of patients with Crohn’s disease, causing delayed mucosal healing (Underhill and Iliev, 2014). In addition, Iliev et al. (2012) have confirmed that the invasion of Candida spp. and Trichosporon spp. accompanies severe colitis in a mouse model. It also has been noted that increases in fungal invasion cause bacterial enteritis in poultry (Skraban et al., 2013). Moreover, factors causing alterations in host immunity, i.e., stress, resident microbiota, or antimicrobial treatment, can lead to fungal overgrowth, causing a wide range of infections (Moretti et al., 2000).

Conclusions: The experiments demonstrated that early antimicrobial treatment in poultry can highly predispose the gastrointestinal tract to fungal colonization and that β-lactams have the highest influence on gastrointestinal
colonization by fungi. Intestinal fungi are increasingly believed to greatly influence gut health. More research on the role of different fungal species will enrich our understanding of the role of the fungal microbiota in the gastrointestinal tract of poultry.

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**Authors contribution:** IS, AG, ST and KB conceived and designed the experiments; IS and ST performed the experiments; IS, ST, KB, AG contributed in sampling, writing, and analysis tools; The manuscript was written by KB, IS, AG. All the authors read the manuscript and approved the contents.

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