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Identification of Ostriches (*Struthio camelus*) Gastrointestinal Bacterial Flora and Characterization of the Antibiotic Resistance Profile of *Salmonella* Serovars Isolated from North-West of Iran

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ABSTRACT

This study was aimed to update knowledge on the prevalence and antimicrobial resistance characteristics of *Salmonella* spp. isolated from ostriches in the North-west of Iran. All 140 samples were collected from feces, feeds and different segments of gastrointestinal tract (GIT) of 5 healthy adult ostriches. Diagnostic methods used during this study allowed isolation of sixteen *Salmonella* strains, belonging to different serotypes. The most frequent serotypes were *S. typhimurium* (37.5%) followed by *S. enteritidis* (31.25%). Among the 16 *Salmonella* isolates tested for resistance to 12 different antimicrobials, 8 (50%) isolates belonging to four different serotypes were multidrug resistant. The first critical component to comprehensive farm-to-fork strategies in reducing the burden of foodborne illness in the identification of the pathogenic bacteria in foodstuff with animal source. The different serotypes and antibiotic resistance profiles that were observed highlights the substantial diversity of *Salmonella* spp. in Iran, the contribution of poultry isolates to human salmonellosis and the capacity of *Salmonella* spp. to colonize all types of environment worldwide.

KEYWORDS: *Salmonella*; Antibiotic resistance; Ostriches; Foodborne.

INTRODUCTION

In recent years, increasing attention has been developed for ostrich breeding in Iran. This interest has focused on application of ostriches as meat producers. Ostriches farming in Iran plays a major role in agriculture, economy and meat production system. Ostriches are susceptible to numerous diseases of bacterial, fungal or parasitic origin.[1]

Enteric diseases are important concern in the poultry industry because of decreasing productivity, increased mortality and the associated hazard of poultry products for human food safety. Prebiotics and probiotics are two of several approaches with the potential to reduce enteric diseases and subsequent contamination of poultry products.[1,2]

The first critical component in comprehensive farm-to-fork strategies is to reduce the burden of foodborne illnesses by identification of the pathogenic bacteria in foodstuff with animal source and reduction of human pathogen contamination in the food production.[3,4]

The gastrointestinal tract (GIT) is the main digestive and absorbing organ which

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plays an important role in animal growth and health. The lower GIT of most poultry species including ostrich is normally populated by large numbers of microorganisms. On the other hand identification of multi drug resistance (MDR) in various species and in food has led to concerns about the role of animals, especially livestock, in the epidemiology of drug resistance and bacterial colonization in humans. Some groups of individuals who work closely with animals, including veterinarians, farmers, and slaughterhouse workers might have high MDR species colonization rates.[5-8]

However, there is a lack of published research characterizing the bacterial flora of digestive tract of food producer animals especially domesticated ostriches. The aims of this study were to identify the resident gram-negative bacteria in the GIT of ostriches and determine the antimicrobial susceptibility of *Salmonella* spp. isolates from north-west of Iran.

MATERIAL AND METHODS

Ostriches and Sampling Procedure

Samples were collected from feces (40), feeds (20) and different parts of GIT of 5 healthy adult ostriches (80). All birds in each sampled farms were healthy with no clinical sings of GIT disease; likely, they had no time to suffer immunodepression due to the mulnutritional-associated stress and, in consequence, no signs for spreading any subclinical infection in the studied farm. In the slaughterhouses, the carcasses were immediately opened and sections from the preventriculus to the anus were taken, the samples were transported in an insulated ice bag to the laboratory without delay.

Isolation and Identification

The samples were isolated and identified by conventional techniques, GIT (small intestine and large intestine) were separated under sterile conditions, it was opened up and repeatedly washed with sterile distilled water to collect the entire contents and intestinal content was homogenized in a storage medium using a vortex mixer. One ml of the gut homogenate suspension, feces and feed samples was pipetted and spread with 9 ml sterile double strength PBS onto Nutrient, Rappaport-Vassiliadis, MacConkey and peptone water (BPW) in a ratio of 1:10 (w/v) (Merck Co., Darmstadt, Germany). In brief, isolation of bacteria was carried out using XLD Medium, SS Agar, EMB Agar, Brilliant Green Agar, Violet red bile agar, KF streptococcus agar, Baird Parker agar and Mannitol salt agar (Merck Co., Darmstadt, Germany). All the plates were incubated at 37 °C for 24h-48h and the number of grown colonies was determined. Then suspected colonies were sub-cultured and further identified by biochemical tests. In these tests the following properties or activities were recorded: gram stain, motility, oxidase activity, catalase activity, oxidation/fermentation, glucose acid, glucose gas, pigment production and citrate utilization. Only the bacterial isolates that were confirmed to be *Salmonella* spp. based on the results of the biochemical tests were selected for antimicrobial agent sensitivity testing. Serological testing was performed for obtained *Salmonella* spp. according to generally accepted rules by a slide agglutination test according to the Kauffmann-White scheme.

Antimicrobial Susceptibility Testing

The antibiotic resistance was determined by Kirby-Bauer disc diffusion technique using Mueller-Hinton agar (Difco), according to the recommendations of National Committee for Clinical Laboratory Standards (CLSI 2011). After overnight incubation at 37 °C, the diameter in millimetres of the zones of inhibition around each of the antimicrobial discs was recorded and categorized as resistant or sensitive in accordance with company recommendations. *Salmonella* isolates were tested for sensitivities to (12 of routine and practical antibiotics) ampicillin (10 mμ/g), amoxicillin-clavulanate (30 mμ/g), cefixime (5 mμ/g), polymyxin E (10 mμ/g), ceftriaxone (30 mμ/g), ciprofloxacin (5 mμ/g), chloramphenicol (30 mμ/g), gentamicin (10 mμ/g), kanamycin (30 mμ/g), and tetracycline (30 mμ/g). The disks were purchased from national company. The results were interpreted by special manufacturer's tables. Stringent criteria were adopted for defining multi-antibiotic resistance (MAR), including resistance to at least four classes of antimicrobial agents.

Statistical Analysis

Data were analyzed using SPSS version 21. The chi-square (X^2) test was used to assess statistical differences between the groups. A *p*-value less than 0.05 were statistically considered significant.

RESULTS

A total of 200 bacteria isolates were obtained from the 120 (140) samples and the mean numbers of bacterial species were summarized in Table 1. Bacterial isolates belonged to 12 genera and predominant isolates were: *Escherichia coli* (15%), *Proteus* spp. (12.5%), *Pseudomonas* spp. (10%), *Corynebacterium* spp. (8.5%), *Salmonella* spp. (8 %) and *Enterobacter* spp. (7.5%). The total obtained bacteria in the cecum were higher than other parts. Among *Salmonella* isolates, *S. typhimurium* was the most predominant isolate following by *S. enteritidis*. From the 16 *Salmonella* isolates tested for resistance to 12 different antimicrobials, 8 (50%) isolates belonging to different serotypes were multi-antibiotic resistant. This multi-resistance concerned 3 isolates of *S. enteritidis* that exhibited decreased susceptibility to AMP, STR, CEFTRO, CIP. Five isolates of *S. typhimurium* were resistant to five antimicrobials (AMP, CEF, KA, TET, KA, CHL). The most commonly encountered resistant panel was AMP, AMO, COL, TET and STR (Tables 1 and 2).

The results of antibiotic testing are summarized in Table 2. As many as 15 (93.75%) isolates were resistant to at least one antimicrobial agent tested. Fifty percent (n=8) belonging to five

Bacteria	Small intestine No. (%)	Large intestine No. (%)	Cecum No. (%)	Rectum No. (%)	Feces	Feed	Total No. (%)
Bacillus spp.	0	2(1)	6(3)	3(1.5)	0	0	11(5.5)
Citrobacter koseri	2(1)	3(1.5)	5(2.5)	3(1.5)	0	0	13 (6.5)
Corynebacterium Spp.	4(2)	4(2)	4(1.5)	3(1.5)	2(1)	0	17(8.5)
Escherichia coli	5(2.5)	7(3.5)	6(3)	5(2.5)	4(2)	3(1.5)	30(15)
Enterobacter spp.	2(1)	3(1.5)	5(21.5)	2(1)	3(1.5)	0	15(7.5)
Proteus spp.	4(2)	5(2.5)	6(3)	3(1.5)	4(2)	3(1.5)	25(12.5)
Pseudomonas spp.	3(1.5)	2(1)	5(2.5)	4(2)	2(1)	4(2)	20(10)
Shigella spp.	2(1)	3(1.5)	5(2.5)	2(1)	3(1.5)	0	15 (7.5)
Yersinia spp.	2(1)	3(1.5)	4(2)	1(.5)	1(.5)	1(.5)	12(6)
Salmonella spp.	2(1)	3(1.5)	4(2)	4 (2)	2(1)	1(.5)	16(8)
Staphylococcus gallinarum	2(1)	2(1)	5(2.5)	3(1.5)	0	0	12(6)
Klebsiella spp.	0	4(2)	5(2.5)	3(1.5)	2(1)	0	14(7)
Total	28(14)	41(20.5)	60(30)	36(18)	23(11.5)	12(6)	200(100)

Table 1: Frequency of bacteria species isolated from different parts of gut tract of ostriches.

Salmonella isolates	Small intestine No. (%)	Large intestine No. (%)	Cecum No. (%)	Rectum No. (%)	Feces	Feed	Total No. (%)	Antimicrobial resistance phenotypes
S. typhimurium	1(6.2)	1(6.2)	2(12.5)	2(125)	1(6.25)	0	7(43.75)	AMP,CEF,KA,TET,KA,CHL, CIP,AMO
S. enteritidis	1(6.2)	2(12.)	2(12.5)	2(12.5)	1(6.25)	1(6.25)	9(56.25)	AMP, STR,CEFTRO, CIP, GEN, TET
Total	2(12.)	3(18.75)	4(25)	4(25)	2(12.5)	1(6.25)	16(100)	-

AMP: ampicillin; AMO: amoxiclov; CEF: Cefixime; COL: colistin; CEFTRO: ceftriaxone; CIP: ciprofloxacin CHL: chloramphenicol; GEN: gentamicin; KAN: kanamycin; STR: streptomycin; ENR: enrofloxacin; TET: Tetracycline.
Pan-susceptible means susceptible to all antibiotics (12) tested.

Table 2: Antibiotic susceptibility of 16 *Salmonella* strains isolated From under study ostriches.

different serotypes were resistant to at least four antimicrobial agents. Ampicillin and tetracycline were the most common resistance property encountered (68.75%; n=11), followed by colistin resistance (50%; n=8), and amoxicillin-clavulanate resistance (43.75%; n=7). The highest susceptible rates were noted for ciprofloxacin (93.75%; n=15).

DISCUSSION

Before our study, there was no recent information available on Iranian ostriches GIT bacterial flora. The ostrich is an important animal in the commercial farming sector. Like other livestock, productivity of this bird is at threat from diseases.[9] Ostrich chicks are particularly susceptible to bacterial diseases especially salmonellosis. Meat-producing farm animals, including poultry, pigs and ostriches, can be carriers of *Salmonella* and can shed them fecally without any signs of disease, which leads to their further spread along the meat chain.[2,10]

The lower GIT of most animal species including poultry and ostriches is normally populated by large numbers of microorganisms.[11] Historically, the microbial composition of the GIT of ostriches has not been extensively defined compared to what is known about microorganisms in poultry. On the other hand the presence of a microbiota has several impacts on the digestive system of the host. However, review in literature showed there are limited data on the bacterial flora of ostriches GIT also these organisms antibiotic resistance.[12-14]

In this study, a range of bacterial flora was isolated from the GIT samples, indicating the presence of these organisms in the healthy ostriches GIT that living in arid regions of northwestern Iran. A total of two-hundred bacteria obtained from ostriches samples. The majority of the bacterial species isolated in this work are ubiquitous and most of the genera match with those reported in human GIT and in the GIT of other avian and mammalian species.[4,5,15] Over 200 different bacteria have been isolated and these bacteria are known to be influenced by various factors including diet, health, and age. These findings are in accordance with some report about birds and ruminant that reported as potential pathogens for humans and animals.[4,8]

In our study, the comparison between the obtained bacteria from different parts of GIT showed the cecum of

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ostriches had a major number of bacteria than the ileum. However, the literature contains discrepancies with respect to the genera and species that dominate the different areas of the animals GIT. On the other hand, our study results showed ostriches are potential reservoirs for *Klebsiella* spp., *Shigella* spp. and *Yersinia* spp. that, these bacteria are important pathogens for human and other animals.

The most common isolated *Salmonella* stereotype in this study was *S. enteritis* (9/56.25%) followed by *S. typhimurium* (7/43.75%). These results were in agreement with other reports in poultry and animals. It is estimated that *Salmonella* serotypes cause 93.8 million human infections and 155,000 deaths annually through the world.[16,17]

Salmonellosis is the main zoonotic disease associated with ostriches, as well as several other meat producer animals. Occurrences of *Salmonella* spp. have been previously reported in poultry and birds by other authors.[18-20]

Animal-to-human transmission occurs when bacteria, such as *Salmonella,* are introduced into the food preparation process or through direct contact with infected animals and fecally contaminated environments.[21,22]

In the last years, predictive microbiology has focused on foodborne pathogens, whereas predictive modeling of bacterial flora in ostriches GIT had not received the same level of attention.[22,23]

Recently, increasing concern on the antibiotic resistance in bacteria has led to greater interest in the use of probiotics in poultry production to control bacterial infection and reduce the use of antibiotics. Full understanding of bacterial flora of the bird GIT is required for the development of probiotics.[24]

There are several factors that influence the microbe population such as different feed ingredients and subtherapeutic levels of antibiotics in their diets. Also, we know during dressing of slaughtered animals, bacteria can be transferred from the most heavily contaminated parts (hides, gut content) onto carcass meat *via*: a) direct fecal contamination of meat due to spillage from guts or contact with hides; b) indirectly, due to hand/equipment contaminated from hides/guts and consecutively used on meat; and c) through airborne transfer of contaminated dust (e.g. from hides) or droplets (e.g. from washing).[23,25]

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In the present study, among different obtained isolates, *Salmonella* spp. was one of the most frequent species isolated from different parts of ostriches GIT, in particular in intestine. The isolation of *Salmonella* spp. has been recorded earlier; also its involvement in foodborne pathogens and GIT infection in human is well documented.[25,26]

The bacteria isolated in this study, and their relative frequency, both demonstrate the similarity between healthy digestive tract of these birds and those of other animal species.

Our results showed a high prevalence of *E. coli* species (15%) in GIT, feces and feed samples of examined ostriches. The predominance of the enterobacteriacea was expected, since their role as members of GIT flora has been reported as a natural condition of humans and animals such as other poultry and birds. There are evidences that animal meat production can be the source of pathogenic bacterial infections in humans. Based on the reports, it would appear that *Salmonella* spp. are substantially represented in the total microbial ecology of spoiled poultry carcasses.[27-29]

Regarding to the above mentioned points, intestinal microbiota are referred to as commensal as they coexist without initiating inflammatory or infectious responses. It is becoming clear that these bacteria provide at least three key functions in the poultry intestine including epithelial cell health, nutrient metabolism and breakdown, and indirect mucosal defense against pathogenic bacterial strains.[5,8]

There are several studies of microbiota carried out with samples from domestic animals, like ruminant and poultry that in all these studies, the most frequently isolated bacterial genera were classified as gram negative (mainly *E.coli, Proteus* and *Salmonella)* which is competent by our studies.[23,28,29]

Household, workers, veterinarian and persons with specific medical conditions such as a chronic illness, immunodeficiency and pregnancy may be at higher risk of developing disease or complications from a zoonotic bacterial disease by contact with poultry and ostriches at the household and the industrial level.[25]

CONCLUSION

The results collected during this study provide the first baseline data on the prevalence of contamination by *Salmonella* spp. in ostriches in Iran. In conclusion, our study provides the information on the GIT bacterial flora of domestic ostriches in Iran, demonstrating a large number of bacteria and antibiotic resistance *Salmonella* spp. in different parts of GIT. In order to obtain the exact GIT bacterial flora in ostriches, this study should be continued by high population in different farms with defined variable into the future.

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CONFLICTS OF INTEREST

The authors have no conflict of interest.

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