Isolation and identification of Microbial flora from Turkey and Antimicrobial sensitivity in Al-hamdanyah-Mosul

H.F.Al-Abidy

Department of microbiology, College of Veterinary Medicine / University of Mosul –Mousl-Iraq

عزل وتشخيص المايكروفلورا في الديك الرومي وحساسيتها للمضادات الحياتيه في منطقه الحمدانيه ,الموصل

حوراء فيصل العابدي

كليه الطب البيطري/جامعه الموصل

المستخلص

هدفت هذه الدراسه عزل وتوصيف المايكروفلورا من الرومي في منطقه الحمدانيه في الموصل . تم جمع (60) عينه (فم ,مخرج,اذن) اخذت المسحات من حقول تربيه الرومي للفتره مابين تشرين الاول 2013 ولغايه نيسان 2014 وتم العزل بزرع العينات على الاوساط الزرعيه وتم التشخيص اعتمادا على الخصائص الكيماوحيويه. بينت نتائج الدراسه عزل 85 عزله من جميع العينات المفحوصه وسجلت جراثيم *Bacillus* spp 13(21.6%), و 11 (18.3%) لكل من *E.coli ,Streptococcus pyogenes* تلتها *Staphylococcus aureus* 10 (16.6%) و *Corynebacterium pyogenes 10* (16.6%) و *6 Staphylococcus saprophyticus* (10%) و4 (6,6%) لكل من *Staphylococcus klossi* و *Proteus vulgris* وعزلت نسبه قليله من جرثومه *Staphylococcus caprae* 3(5%).كانت نتائج فحص الحساسيه مقاومه جميع العزلات للمضاد الحيوي Trimethoprim 100% في حين سجلت اغلب العزلات الجرثوميه المفحوصه حساسيه للمضاد الحيوي و Penicillin(84.7%) و Colistin (89.4%) .

Abstract

The research was worked conducted to isolate and identify the microbial flora from apparently healthy turkey in Al-hamdanyah –Mousl .Sixteen number of (oral, cloacal and ear) swabs were collected from fields breeding turkey during period October 2013 to April 2014 .The samples were inoculated onto different bacteriological media, and they were isolate and identified by their cultural and biochemical properties. Eigteey five isolates, ***Bacillus*** spp 13(21.6%) , ***E.coli , Streptococcus pyogenes*** 11(18.3%)for each, ***Corynebacterium pyogenes ,Staphylococcus aurues*** 10(16.6%) for each bacteria and ***Staphylococcus saprophyticus*** 6(10%), ***Klebsiella pneumonia*** 5(8.3%),***Staphylococcus klossi, Proteus vulgris*** 4(6.6%), and ***Staphylococcus caprae*** 3(5%).The results of Antibiotics sensitivity test resistant was revealed that all microbial isolates resistant to trimethoprim 100%.While, the most isolates were sensitive to penicillin (84.7%) and colistin (89.4%).

Key **word:** Microbial flora , turkey , sensitivity test.

Introduction

A turkey is a large bird in the family of phasiadnida in the taxonomic order of galliformes. Geneus meleagris was the only genus in the subfamily meleagridinae respecterly known as related to family meleagrididae, but now subsumed in family phasianidae ,which is native to the Americas .One species from them galloparo (commonly known as the wild turkey or domestic turkey) was coming to the forest of north America, mainly Mexico and United States .The other living species is meleagris ocellata, or the ocellata turkey native to the forest of the Yucatan peninsula (1,2). Growth performance and sustained flock health was a major economic important to commercial turkey producer. Microbial community gastro intestinal tract or microbiome is assumed to play a critical role in overall health of turkey and other poultry. Fewer studies have saught to understand the turkey microbiome, some works have focused on comparison of the fecal microbiomes of wild and domestic birds (3). A number of possible contributing factors had speculated in management practice .The presence of known bacterial or viral pathogens disruption of the gastro intestinal microbial communities' problems with nutrient absorption or dwarfed immune development in poults (4).This problem host not to our knowledge but identified in many states of USA. Many studies identified numerous bacterial and fungal species in the fecal sample obtained from turkey and duck. There are several billions of bacterial present in poultry faeces such as chickens, duck, turkey and geese including pathogenic and non-pathogenic species. Normal flora and the opportunistic ones(5).Bird had population of bacterial known as normal microbial flora ,which colonies the skin and mucous membrane of the respiratory and gastro intestinal tract ,gram positive bacteria are the predominant normal inhabitants of the crop ,cloaca ,skin and respiratory tract of clinically birds such *as lactobacillus, Bacillus* **,***Corynebacterium* ,*Staphylococcus* **,** *Streptococcus* **,** and *Enterococcus* ,gram negative bacteria may be present in very low numbers in clinically normal birds, when present in large number however. They are frequently associated with disease *Enterobacte*r ,*Escherichia coli , Proteus , klebsiella* and *Pseudomonas* are disease causing Gram's negative bacteria may be also be responsible for skin infection ,sinus infection, air sac infection ,crop infection and lower gastro intestinal tract problems (6).If bacterial infection was suspected, or if a bird presented for a healthy examination cultures of the crop and cloaca should be taken, it is important that .However, as the cloaca serves as both an excretory as well as copulatory organ microbes can be transmitted between mates during sexual contact (7).Gastrointestinal microflora plays role in health of animal ,the bacteria that individuals acquire inducing profound consequences for their future fitness .However, change in microbial community structure with host age remain poorly understand (8).The development of antibiotic resistance among bacteria is apiont concern in both human and animal medicine there are a number of factors that have been associated with increased development of antimicrobial resistance among bacteria as well as other emerging pathogens .Increased pathogenicity and antibiotic

resistance patterns may result from genetic changes among bacterial strains ,change in host ,populations ,populations health and ecology also effect interaction with potential pathogens(9).

Antibiotic resistance genes are transferred between bacteria by horizontal transfer involving the mechanism of conjugation, transduction, transformation and transposition. Also occur from commensals bacteria with inherent resistance, containing antimicrobial resistance genes occur via direct contact between, and within human and animal population or via zoonotic bacteria along the food chain (10).Antibiotic over use in humans ,animal and agriculture has fuelled the emergence of resistance phenotypes in bacteria such as staphylococci from both human and animal sources due to significant increase in Kansas's wild turkey population ,and the greater likelihood that these birds will be indirectl exposed to antibiotics(11). During an investigation of the microbiological aspects connected with the use of streptomycin and other antibiotic supplements in turkey nutrition, it was observed cases of considerable drug fatness in the intestinal microflora, since the development of antibiotic resistance strains of microbes has obearing on the successful use of antibiotics nutritional supplements (12). .

Aim: The study aimed to isolate the microbial flora associated with turkey population .And Antibiotic sensivity of its

Material and method

1- Collection of samples

Samples were collected from 60 turkey clinically healthy, by cotton swabs were taken from oral ,cloacal and ear during the period from October 2013 to the April 2014 ,the swabs sample were placed on sterile nutrient broth test tube and labeled appropriately with the source ,date of collection number of sample and transported to the Microbiology Laboratory ,Veterinary University of Mousl for bacteriological examination upon arrival incubated at 37 Cº for 24 h.

2- Primary culture of organism

Bacteriological examination was carried by used standard method for bacteria (13)) from the nutrient broth (oral, cloacal and ear) swabs they were placed on nutrient agar plate ,and incubated at 37Cº for 24 h typical bacteria colonies were randomly selected examined microscopically for their morphology and recultivated to obtain pure culture(14).

3- Subculture

All swabs sample were subculture in nutrient agar, sheep blood agar,eosin methylene blue ,brain heart agar , mannitol salt agar , Edward agar and tellurite potassium agar. A small amount of inoculum for the nutrient agar was spread into culture media agar was spread into culture media and incubated at 37 cº for 24 h. The microorganism were identified by colony, morphology staining characterized (microscopic examination)

and biochemical characteristic (15).Isolated bacteria from each sample was biochemically identified by sugar fermentation (trehalose, mannitol ,maltose ,sucrose, xyloses ,arabinose) indole test , MR-VP test ,catalase and coagulase test ,oxidase test and nitrate broth test as per method described by (16).

Antibiotic sensitivity test

Isolated were tested for susceptibility test using disk diffusion method was based on the orginal work of (19).Antibiotic disk (oxoid) used were erythromycin (15 mg), trimethoprim(5mg),ciprofloxacin(10mg),ampicillin(10),florfenicol(30),penicillin(10) and colistin(10).

The growth method can be selected 3-5 well isolated colonies of the same morphological type from an agar plate culture. Touch the top of each colony with sterile loop and transfer the growth into a tube containing 4-5 ml of a sterile nutrient broth medium incubated the broth culture at 37 c^o for 6 h to reach log phase of growth, after turbidity of inoculum suspension dip a sterile cotton swab into the adjusted suspension rotate the swab several times inoculate the dried surface of an Muller Hinton agar plate by streaking the swab over the entire sterile agar surface. Then placed the antimicrobial disk on the surface of the inoculated agar plate with sterile forceps and inoculated 37 c^o for 24h. Measured zone of no growth around disk was measured ,using ruler, the plate was held over aback surface, and examined used reflected light from a desk light were measured .Individual antibiotic was recorded as highly sensitive moderately sensitive resistant depended on the area of antibiotic of bacterial growth (17,10).

Results

A total of 60 samples were examined in this study and identify of the microflora from apparently healthy turkey. Eighty five microflora isolated and identified were comprised of 11 species namely ***E*.coli,** Bacillus spp, *Klebsiella pneumonia, Corynebacterium pyogenes, Staphylococcus aureus, Staphylococcus klossi, Corynebacterium renal, Staphylococcus saprophyticus Proteus vulgris, Streptococcus pyogenes, Staphylococcus caprae* (tablet 1) . The results for isolation of microflora from oral ,cloacal and ear turkey sampled examined are represented in (table 2) The major type of bacteria found in the cloacal swab of turkey were identified as *Corynebacterium pyogenes, Bacillus spp, Corynebacterium renal, Staphylococcus saprophyticus, E.coli, Proteus vulgris, Streptococcus pyogenes*. The more oral and ear isolated *klebsiella pneumonia, Staphylococcus aureus, Staphylococcus caprae*. Isolated were tested for susceptibility test to different antibiotic using the disk diffusion method according to the national committee for clinical laboratory standards antibiotic disk (table3)

Table (1): Microflora isolated from Turkey

Microflora isolated	No	percentage%
Bacillus spp	13	21.6
E.coli	11	18.3
Streptococcus pyogenes	11	18.3
Corynebacterium pyogenes	10	16.6
Staphylococcus aureus	10	16.6
Corynebacterium renal	8	13.3
Staphylococcus saprophyticus	6	10
Klebsiella pneumonia	5	*8.3*
Proteus vulgris	4	6.6
Staphylococcus klossi	4	6.6
Staphylococcus caprae	3	5

Table (2): Microflora isolated from ear ,cloacal and era of turkey

Microflora isolated	Number isolated microflora from oral	Number isolated microflora from cloacal	Number isolated microflora from ear	Total	%
Bacillus spp	3	7	3	13	21.6
E.coli	1	8	2	11	18.3
Streptococcus pyogenes	5	4	2	11	18.3
Corynebacterium pyogenes	5	4	1	10	16.6
Staphylococcus aureus	6	4	-	10	16.6
Corynebacterium renal	2	3	3	8	13.3
Staphylococcus saprophyticus	1	2	3	6	10
Klebsiella pneumonia	2	3	-	5	8.3
Proteus vulgris	-	4	-	4	6.6
Staphylococcus klossi	-	2	2	4	6.6
Staphylococcus caprae	1	-	2	3	5

Table (3): Antibiotic susceptibility of isolated microflora

Antibiotic drugs / Isolated microflora		Corynebacterium pyogenes	Bacillus spp	Klebsiella pneumonia	Staphylococcus aureus	Corynebacterium renal	Staphylococcus saprophyticus	E.coli	Proteus vulgris	Streptococcus pyogenes	Staphylococcus caprae	Staphylococcus klossi	total	Percentage %
No		10	13	5	10	8	6	11	4	11	3	4	85	
E	S	—	—	3	6	—	3	2	—	4	3	4	25	29.4
(15)	I	—	—	—	2	—	—	1	—	—	—	—	3	3.5
	R	10	13	2	2	8	3	8	4	7	—	—	57	67
Tri	S	—	—	—	—	—	—	—	—	—	—	—		—
(5)	I	—	—	—	—	—	—	—	—	—	—	—		—
	R	10	13	5	10	8	6	11	4	11	3	4	85	100
Cip	S	6	5	1	—	3	3	8	—	4	—	—	30	35.2
(10)	I	1	3	3	—	—	—	—	—	—	—	—	7	8.2
	R	3	5	1	10	5	3	3	4	7	3	4	4	56.4
Am	S	9	—	5	—	3	—	2	4	—	—	4	27	31.7
(10)	I	1	—	—	—	1	—	4	—	—	—	—	6	7
	R	—	13	—	10	4	6	5	—	11	3	—	52	61.7
FFC	S	—		—	—	—	2	—	—	4	—	—	6	7
(30)	I	—	—	—	—	—	—	—	—	—	—	—		
	R	10	13	5	10	8	4	11	4	7	3	4	79	92.9
P	S	10	13	5	10	8	6	5	4	4	3	4	72	84.7
(10)	I	—	—	—	—	—	—	6	—	—	—	—	6	7
	R	—	—	—	—	—	—	—	—	7	—	—	7	8.2
Col	S	10	13	5	10	8	6	2	4	11	3	4	76	89.4
(10)	I	—	—	—	—	—	—	2	—	—	—	—	2	2.3
	R	—	—	—	—	—	—	7	—	—	—	—	7	8.2

( ) concentration of antibiotic of mg , R= resistance , I= intermediate , S=sensitive

ERY=Erythromycin ,Tri=Trimethoprim , Cip=Ciprofloxacin, Aim=Ampicillin , Fc=Florfenicol ,P=Penicillin ,C=Colistin

Discussion

In the present study, microflora were identified in the cloacal, oral and ear samples obtained from turkey *includes Bacillus spp, Staphylococcus aureus, Staphylococcus klossi, Staphylococcus caprae, Proteus vulgris, E.coli, Streptococcus pyogenes, Corynebaterium pyogenes ,Corynebacterium renal, Staphylococcus saprophyticus, klebsiella pneumonia* (table 1). These identified microflora species were in accordance with the Adegunloye and Adejumo (5)study also isolated a large group of bacteria includes *Escherichia coli* 15.55%, *Staphylococcus* spp 13.74%, *Streptococcus* spp 3.23% from faeces (droppings) turkey (meleagris ocellata) in Akure metropolis. In the study of (11) which reported a large number *Staphylococcus lentus* (57 isolated) were isolated from faeces of 26 wild turkey were a common normal flora of both humans and other animals. This results difference in this research were isolated *Staphylococcus spp* (table 1) , this difference depends on, a number of samples ,type of procedure and isolation .The microbial population of the samples varies from one location of sampling to another differences in environmental condition such as water activity ,ph , oxidation reduction and potential nutrient content may be responsible for the difference in the microbial population (18). Other reports Barnes and Impey (19) higher isolates more than 80% of the total flora from caeca of turkey this might be due to several anaerobic technique and number of different media. (Table 3) All *Staphylococcal* isolates were resistance to trimethoprim ,florfenicol which higher than those reported by Dobeer et al (11) the *Staphylococcus* isolates susceptibility to trimethoprim ,and in this study all isolates susceptibility 100% to penicillin is comparable to those reported by Dobeer et al(11) in contract coagulase negative staphylococci (CoNs) are normally considered benign organisms, that are part of the normal flora .However, in recent year the number CoNs implicated in human and animal disease has risen dramatically coupled with the observation that bacteria of all genera are increased in developing resistance tall classes of antibiotic bacteria. Previously, it is considered to be harmless such as CoNs ,could pose a significant health threat and need to be examined (20). Exposure from animals treated with antibiotics causes increased risk of resistance colonization or infection in humans consumption of food contaminated with antibiotic –resistance, bacteria causes on outbreak of resistant diarrheal disease. Consumption of antibiotic containing meat products induces resistance in normal flora of the human gastrointestinal tract (21). The studies (22) detected clinical isolates of avian *Escherichia coli* molecular typing demonstrated, that is the florfenicol resistance gene. There are signs that other pathogenic strains Escherichia coli could have a zoonotic potential between birds and humans (23). Found similar traits in a cluster of *E-coli* causing colibacillosis among birds and urinary tract infection and neonatal meningitis in humans .In the study (24) tracing transfer of *E.coli* between poultry and humans has also been done by studying antibiotic–resistance E.coli isolates from poultry farmers and slaughterers .Although, the pulsed field gel electrophoresis (PEGE) patterns from the isolates from the different populations

were quite heterogeneous *E.coli* with identical PFGE patterns were isolated at two farms from a turkey and the farmer, indicating a direct transfer of certain *E.coli* strains between humans and poultry.

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Conclusion

This study showed difference type of microflora which opportunities different growth circumstances. could not in our studies compared all result which recommended because did not found studies included all this group of microflora and rare research about microflora infected turkey and did not found similar studies in Mousl governorate conclusive important of safety of turkey flock, because healthy turkey flock is a major economic importance to commercial turkey producer fields .In addition ,further studies are needed to understand the microflora gravity that infected turkey population regions .

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