

ISOLATION AND IDENTIFICATION OF BACTERIA FROM KIDNEY LESIONS IN BROILERS IN SULAIMANIA PROVINCE

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ABSTRACT

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A total of ninety seven broiler chicks in Sulaimania/ Kurdistan region/ Republic of Iraq, were collected from different flocks at different dates suffering from nephritis. Samples of affected kidneys were cultured on blood, Mannitol salt and MacConkey agar plates, the isolates were confirmed by API 20E system. The isolated microorganism included *E.coli* (89.69%), *Proteus mirabilis* (9.27%) and *Klebsiella pneumonia* (1.03%). Antimicrobial susceptibility test for *E.coli* showed high sensitivity to Ciprofloxacin and Co-trimoxazole. Histopathological changes of the kidneys were characterized by enlargement of epithelial cells of renal tubules and pale cytoplasm, engorgement of blood capillaries in both renal glomeruli and interstitial tissues with atrophy of renal glomeruli.

Key words: Broiler, Kidney lesions, Bacteria

INTRODUCTION

Bacteria are pathogenic living organisms but, not all bacteria are detrimental to poultry health. In fact, many bacteria are beneficial and necessary for such processes as food digestion and manufacturing of some dairy products. Classification of bacteria into species is done so disease producing organisms may be separated from those that are harmless or beneficial, (Berry, and Whitenack, 2011).

Bacterial nephritis usually occurs when bacteria enter the kidney secondary to systemic disease through the renal arteries or the renal portal system (Speer, 1997 and Phalen *et al.*, 1990). A wide range of bacteria has been reported to cause bacterial nephritis including Enterobacteriaceae, Pasteurella spp, Pseudomonas spp, Streptococcus spp, and Staphylococcus spp (Speer, 1997, Schmidt *et al.*, 2003, Phalen *et al.*, 1990 and Lierz, 2003). *Listeria monocytogenes* has been reported (Schmidt *et al.*, 2003), whereas *Erysipelothrix rhusiopathiae* has been reported in quail and chicken (Schmidt *et al.*, 2003 and Mutalib *et al.*, 1995). *Mycobacterium avium* can, rarely, cause renal lesions (Schmidt *et al.*, 2003 and Sato *et al.*, 1996).

Among bacterial infections, colibacillosis is a worldwide major cause of morbidity and mortality in

poultry and responsible for significant economic losses to the poultry industry, (Stordeur, and Mainil, 2002) they also reported that the causative bacteria, avian pathogenic *Escherichia coli* (APEC), induce various syndromes including respiratory tract infection (airsacculitis), acute colisepticemia, salpingitis, and cellulitis. The most common form of colibacillosis occurs in 3- to 10-week old chickens. It is characterized by an initial respiratory infection usually induced by mycoplasmal and/or viral infections and enhanced by a high level of ammonia in poultry houses. The disease evolves as a systemic infection (perihepatitis, pericarditis, and septicemia) due to the invasive abilities of the *Escherichia coli* strains (Dho-Moulin, and Fairbrother, 1999), hence, can infect both kidneys and cause damage to the kidneys and impair its function (Al-Hiyali *et al.*, 2005).

Successful control of bacterial diseases entails isolating and identifying disease-producing species, if present, and preventing multiplication and spread of the organism within the chick's body or to other chicks, also antibacterial resistance has profound clinical implications. The objectives of the present study are to isolate and identify bacterial species infect broiler chicks kidney, furthermore to investigate antibacterial susceptibility and describe

the changes in the kidneys by means of histopathology.

MATERIALS and METHODS

A total of ninety seven broiler chicks suffering from nephritis were collected from different flocks in Sulaimania region at different dates. Samples of Affected kidneys were cultured on 5% blood agar base, Mannitol salt agar, MacConkey agar plates (Quiun *et al.*, 2002) the inoculated plates were incubated at 37c°for 24-48 hours. Primary cultures were evaluated by visual examination of the morphology of the bacterial colonies and were sub cultured for further examination. The identification of the isolated colonies was performed using standard bacteriological and biochemical procedures as described by carter and Cole (1990) and barrow and felthem (1993) and conformed by API 20E system (biomerieux, Marcy-1 Etoile, france) as per manufactures directions.

Antibacterial susceptibility tests performed to detect the susceptibility of the isolated bacteria to antibiotic

which included, Doxycycline 30mg, Ciprofloxacin 5mg, Neomycin 30mg, Ampicillin 10mg, Chloramphenicol 30mg, Co-trimoxazole 25mg (England) (Brown, 2005).

To study histopathological changes, 1 cubic centimeter size were obtained from the affected kidneys samples of all chicks and kept in 10% neutral buffered formalin for at least 24 hours. After that, these samples were exposed as described by (james, 1976) for dehydration, clearing, paraffin embedding, blocking, sectioning, mounting and staining with Hematoxylen and Eosin stains and were examined by light microscope.

RESULTS and DISCUSSION

bacteriological and biochemical tests results showed 89.69 % (87) of isolates were *E.coli*, 9.27 % (9) isolates were *Proteus milabilis* and 1.03 % (1) isolates was *Klebsiella pneumonia* as shown in table (1). The isolates were confirmed by The API 20E test kit (biomerieux, Marcy-1 Etoile, france).

Table 1: Shows bacterial isolates from kidneys samples.

Bacteria	Number of isolates	%
<i>E.coli</i>	87	89.69
<i>Proteus milabilis</i>	9	9.27
<i>Klebsiella pneumonia</i>	1	1.03

to conditions such as chronic cloacitis (Speer, 1997, Lierz 2003 and Phalen *et al.*, 1990).

Other bacteria including *Proteus mirabilis* and *Klebsiella pneumonia* were isolated out of several kidneys samples but in a lower proportion, mentioned in Table (1), these results were similar to results reported by (Al-Hiyali *et al.*, 2005) in chicken and also similar to results showed by (Quinn *et al.*, 1997) in cattle, buffalo and dog.

In vitro Antimicrobial susceptibility test of the most frequent bacterial Isolates (*E.coli* isolate) were susceptible to a (Ciprofloxacin and Co-trimoxazole) but showed resistance to (Doxycycline, Ampicillin and -Chloramphenicol) as shown in Table (2) and figure (1), The results of susceptibility testing are identical with those mentioned by Carter and Cole (1990), Carter *et al.* (1995).

The isolation results of the present study revealed high percentage of *E.coli* which agree with results mentioned by (Al-Hiyali *et al.*, 2005) and (Sokker *et al.*, 1998), the high percentage of the *E.coli* isolates (89.69%) (Table 1), confirmed the importance of this bacteria for inducing the lesions in kidneys of broiler chicks which is identical to the results of (Al-Hiyali *et al.*, 2005) and (Al- Sultan *et al.*, 1998) in other animals (calves) and also resemble the result revealed by (Al Rajab and Al qazzaz, 1984) in urinary tract infection of human; the explanation of this results is thought due to the absence of lymph nodes and the presence of renal and hepatic portal systems which increase the risk of systemic or gastrointestinal microbes affecting the kidney (Lierz, 2003 and Lumeij 2000), also bacterial nephritis usually occurs when bacteria enter the kidney secondary to systemic diseases through the renal arteries or the renal portal system, Rarely, bacteria ascend the ureters secondary

Table 2: Shows Antimicrobial susceptibility test of *E.coli* isolate in the present study.

Antimicrobial agents	Code	Disk potency	Inhibition
Doxycycline	DX	30mg	R
Ciprofloxacin	CX	5mg	S
Neomycin	NE	30mg	I
Ampicillin	AMP	10mg	R
Chloramphenicol	C	30mg	R
Co-trimoxazole	SXT	25mg	S

(S: sensitive, R: resistant, I: intermediate)

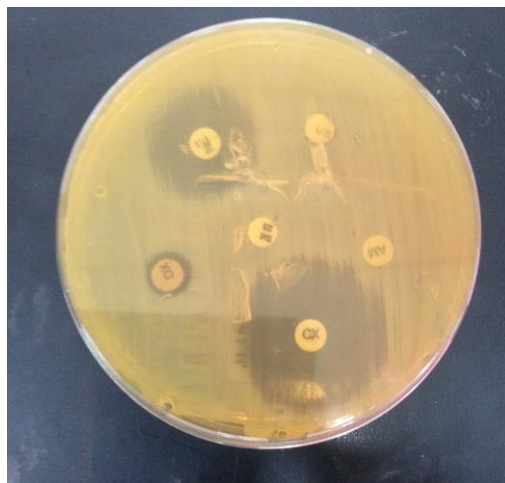


Figure 1: Antimicrobial susceptibility test of *E.coli* isolate shows susceptible to a (*Ciprofloxacin and Co-trimoxazole*) but resistant to (*Doxycycline, Ampicillin and -Chloramphenicol*)

The histopathological examination of affected kidney included the enlargement of epithelial cells of renal tubules with pale cytoplasm, blood capillaries were engorged with large numbers of Red blood cells in both renal glomeruli and interstitial tissues, and congestion of blood vessels in addition to reduction in the size (atrophy) of renal corpuscles as shown in figure (2), the results of histopathological examination of the current study are identical with those mentioned by (Sokker *et al.*, 1998).

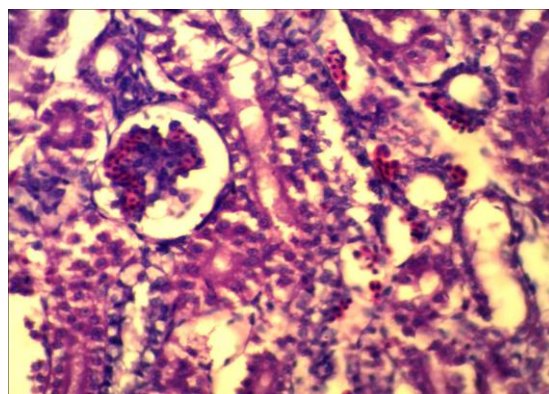


Figure 2: Congestion of blood vessels and atrophy glomeruli (400X)

In conclusion, this work revealed that the *E.coli* infections are the most real important causes of kidneys lesions leading to economic losses in the broiler chickens thus prevention and control of *E.coli* infections in broiler farms are very important.

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العزل والتعرف على البكتيريا من إصابات الكلية في الفروج بمحافظة السلبيانية

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تم جمع ٩٧ عينة من كلي فروج اللحم المصابة بالتهاب الكلية من أماكن مختلفة لتربية الدواجن وفي فترات مختلفة في مدينة السلبيانية/ إقليم كردستان/ العراق. زرعت العينات المأخوذة زرعاً أولياً على أوساط زرع مختلفة والتي شملت وسط الدم، وسط المانيتول الملحي ووسط الماكونكي ليتم تشخيصها باستخدام الفحوصات البيوكيميائية التقليدية. ثم تأكيد التشخيص باستخدام API 20E system. تم عزل ٣ أنواع من البكتيريا وهي *E.coli*, *Proteus mirabilis* و *Klebsiella pneumonia* وبنسبة ٦٩,٨٩% ، ٩,٢٧% و ١,٠٥% على التوالي. استخدمت ٦ أنواع من المضادات البكتيرية في اختبار الحساسية للمضادات البكتيرية والتي تضمنت Doxycycline, Ciprofloxacin, Neomycin, Ampicillin, Chloramphenicol and Co-trimoxazole واعطت بكتيريا ال *E.coli* حساسية عالية لكل من Ciprofloxacin و Co-trimoxazole. شملت التغيرات النسيجية للكلية تضخم الخلايا الطلانية للنيبيات الكلوية مع شحوب السيتوبلازم ، امتلاء وتضخم الأوعية الدموية الشعيرية في كل من الكبيبات الكلوية والأنسجة الخلالية مع ضمور في الكبيبات الكلوية.