جامعة
بنغازي الحديثة

مجلة جامعة بنغازي الحديثة للعلوم والدراسات الإنسانية

مجلة علمية الكترونية محكمة

Journal of Benghazi Modern University of Sciences and Humanities

العـــدد العاشر

لسنة 2020

شروط كتابة البحث العلمي في مجلة جامعة بنغازي الحديثة للعلوم والدراسات الإنسانية

1- الملخص باللغة العربية وباللغة الانجليزية (150 كلمة).
2- المقدمة، وتشمل التالي:
- ❖ نبذة عن موضوع الدراسة (مدخل).
- ❖ مشكلة الدراسة.
- ❖ أهمية الدراسة.
- ❖ أهداف الدراسة.
- ❖ المنهج العلمي المتبع في الدراسة.

3- الخاتمة. (أهم نتائج البحث - التوصيات).
4- قائمة المصادر والمراجع.
5- عدد صفحات البحث لا تزيد عن (25) صفحة متضمنة الملاحق وقائمة المصادر والمراجع.

القواعد العامة لقبول النشر

1. تقبل المجلة نشر البحوث باللغتين العربية والانجليزية؛ والتي تتوافر فيها الشروط الآتية:

- أن يكون البحث أصيلاً، وتتوافر فيه شروط البحث العلمي المعتمد على الأصول العلمية والمنهجية المتعارف عليها من حيث الإحاطة والاستقصاء والإضافة المعرفية (النتائج) والمنهجية والتوثيق وسلامة اللغة ودقة التعبير.
- ألا يكون البحث قد سبق نشرة أو قُدم للنشر في أي جهة أخرى أو مستل من رسالة أو اطروحة علمية.
- أن يكون البحث مراعياً لقواعد الضبط ودقة الرسوم والأشكال - إن وجدت - ومطبوعاً على ملف وورد، حجم الخط (14) وبخط (Arial 'Body') للغة العربية. وحجم الخط (12) بخط (Times New Roman) للغة الإنجليزية.
- أن تكون الجداول والأشكال مدرجة في أماكنها الصحيحة، وأن تشمل العناوين والبيانات الإيضاحية.
- أن يكون البحث ملتزما بدقة التوثيق حسب دليل جمعية علم النفس الأمريكية (APA) وتثبيت هوامش البحث في نفس الصفحة والمصادر والمراجع في نهاية البحث على النحو الآتي:
- أن تُثبت المراجع بذكر اسم المؤلف، ثم يوضع تاريخ نشرة بين حاصرتين، ويلي ذلك عنوان المصدر، متبوعاً باسم المحقق أو المترجم، ودار النشر، ومكان النشر، ورقم الجزء، ورقم الصفحة.
- عند استخدام الدوريات (المجلات، المؤتمرات العلمية، الندوات) بوصفها مراجع للبحث: يُذكر اسم صاحب المقالة كاملاً، ثم تاريخ النشر بين حاصرتين، ثم عنوان المقالة، ثم ذكر اسم المجلة، ثم رقم المجلد، ثم رقم العدد، ودار النشر، ومكان النشر، ورقم الصفحة.

2. يقدم الباحث ملخص باللغتين العربية والانجليزية في حدود (150 كلمة) بحيث يتضمن مشكلة الدراسة، والهدف الرئيسي للدراسة، ومنهجية الدراسة، ونتائج الدراسة. ووضع الكلمات الرئيسية في نهاية الملخص (خمس كلمات).

3. تحتفظ مجلة جامعة بنغازي الحديثة بحقها في أسلوب إخراج البحث النهائي عند النشر.

إجراءات النشر

ترسل جميع المواد عبر البريد الالكتروني الخاص بالمجلة جامعة بنغازي الحديثة وهو كالتالي:

- ✓ يرسل البحث الكترونياً (Word + Pdf) إلى عنوان المجلة info.jmbush@bmu.edu.ly او نسخة على CD بحيث يظهر في البحث اسم الباحث ولقبة العلمي، ومكان عملة، ومجاله.
- ✓ يرفق مع البحث نموذج تقديم ورقة بحثية للنشر (موجود على موقع المجلة) وكذلك ارفاق موجز للسيرة الذاتية للباحث إلكترونياً.
- ✓ لا يقبل استلام الورقة العلمية الا بشروط وفورمات مجلة جامعة بنغازي الحديثة.
- ✓ في حالة قبول البحث مبدئياً يتم عرضة على مُحكِّمين من ذوي الاختصاص في مجال البحث، ويتم اختيارهم بسرية تامة، ولا يُعرض عليهم اسم الباحث أو بياناته، وذلك لإبداء آرائهم حول مدى أصالة البحث، وقيمته العلمية، ومدى التزام الباحث بالمنهجية المتعارف عليها، ويطلب من المحكم تحديد مدى صلاحية البحث للنشر في المجلة من عدمها.
- ✓ يُخطر الباحث بقرار صلاحية بحثه للنشر من عدمها خلال شهرين من تاريخ الاستلام للبحث، وبموعد النشر، ورقم العدد الذي سينشر فيه البحث.
- ✓ في حالة ورود ملاحظات من المحكِّمين، تُرسل تلك الملاحظات إلى الباحث لإجراء التعديلات اللازمة بموجبها، على أن تعاد للمجلة خلال مدة أقصاها عشرة أيام.
- ✓ الأبحاث التي لم تتم الموافقة على نشرها لا تعاد إلى الباحثين.
- ✓ الأفكار الواردة فيما ينشر من دراسات وبحوث وعروض تعبر عن أراء أصحابها.
- ✓ لا يجوز نشر إي من المواد المنشورة في المجلة مرة أخرى.
- ✓ يدفع الراغب في نشر بحثه مبلغ قدره (400 د.ل) دينار ليبي إذا كان الباحث من داخل ليبيا، و (200 $) دولار أمريكي إذا كان الباحث من خارج ليبيا. علماً بأن حسابنا القابل للتحويل هو: **(بنغازي – ليبيا - مصرف التجارة والتنمية، الفرع الرئيسي - بنغازي، رقم 001-225540-0011. الاسم (صلاح الأمين عبدالله محمد)**.
- ✓ جميع المواد المنشورة في المجلة تخضع لقانون حقوق الملكية الفكرية للمجلة.

info.jmbush@bmu.edu.ly

00218913262838

د. صلاح الأمين عبدالله
رئيس تحرير مجلة جامعة بنغازي الحديثة
Dr.salahshalufi@bmu.edu.ly

Study on antibiotic resistance strains of *Ecoli* recovered from ill chicken in ELmarj region

*** D. Ahmad .m.m. altalhy, ** Mosa khalifa Ibrahim lamin**

Agricultural and Animal Research Center in Libya (ACR, Libya). College of Human Medicine Elmarj Benghazi University

ABSTRACT.

Over all 200 samples from visceral organs were collected from fifty Chickens in different farms from Elmarj region. These chickens were ranged from 35 -50 days age and showed signs of loss of appetite, sneezing, coughing, air sacculitis, per hepatitis before collecting chicken organs the organ separately into sterile bags under refrigeration in coolers and analyzed within6h of sampling at most. According to results 73 samples (36.5%) Were confirmed to be E. coli positive, Twenty six O serogroups were identified by sero-diagnosis, the most prevalent serogroups were O78 then O2 and O1. Other infrequently encountered serogroups. Our study results of APEC strain showed that the resistance of avian *E.coli* strains to two or more antibiotics the penicillin, and doxycycline was 100% and 69% respectively while resistance rate of chloramphenicol was 30%. We also found that quinolone resistance among *E.coli* strain from chicken is increasing (36% to 49.3%)

*** د. احمد محمد موسى الطلحي، ** أ. موسى خليفة إبراهيم**

الملخص:

تم جمع أكثر من 200 عينة من الأعضاء الحشوية من خمسين دجاجة من مزارع مختلفة بمنطقة المرج. تراوحت أعمار هذه الدجاجات بين 35-50 يومًا وظهرت عليها علامات فقدان الشهية والعطس والسعال والتهاب الحويصلات الهوائية والتهاب الكبد قبل جمع أعضاء الدجاج بشكل منفصل في أكياس معقمة تحت التبريد في المبردات وتحليلها في غضون 6 ساعات من أخذ العينات على الأكثر وفقا للنتائج، تم التأكد من أن 73 عينة (36.5٪) إيجابية للإشريكية القولونية، وتم التعرف على ستة وعشرين مجموعة مصلية من O عن طريق التشخيص المصلي، وكانت المجموعات المصلية الأكثر انتشارا هي O78 ثم O2 و O1. المجموعات المصلية الأخرى التي نادرًا ما تمت مواجهتها. أظهرت نتائج دراستنا لسلالة APEC أن مقاومة سلالات الإشريكية القولونية للطيور لمضادات حيوية أو أكثر، البنسلين، والدوكسيسيكلين كانت 100٪ و 69٪ على التوالي بينما كان معدل مقاومة الكلورامفينيكول 30٪. وجدنا أيضًا أن مقاومة الكينولون بين سلالة الإشريكية القولونية من الدجاج تزداد (36٪ إلى 49.3٪)

1 .INTRODUCTION

E.coli is a Gram - negative, faculatively anaerobic bacterium that commonly found in the blooded organisms in the lower intestine (scheutez and Strockbine, 2005). Most strains of *E.coli* are harmless, but some serotypes can lead to food poisoning in their hosts. The harmless strains can benefit their hosts by producing vitamin K2 as they are normally found in flora of the gut, (Hudault el at., 2001) and preventing colonization of pathogenic bacteria in the intestine (Nataro and kaper, 1998).

E.coli and other facultative anaerobes represent about 0.1% of flora in the gut (Feng *et al*., 2002) and the major route of pathogenic strain transmission is through fecal- oral route which lead to serious diseases. *E.coli* consider potential indicator organisms for fecal

Differences between *E. coli* strains are the combination of different antigens they possess. There are three types of antigens: the somatic lipopoly saccharide antigen (O), the flagellar antigens (H), and the capsular antigens (K). There are about 174 O antigens, 56 H antigens, and 103 K antigens that have been identified. There are several E, coli strains including: the enteric E. coliare dividedon the basis of virulence properties into (ETEC), (EPEC), (EIEC), (VTEC) or (EHEC) and (EaggEC). ETEC can be found in human, sheep, pigs, cattle, goat, horses, and dogs; EPEC is found in human, dogs, cats, rabbits, and horses; EIEC and EAggEC are only found in humans; VTEC is found in dogs, and cats, pigs and cattle; while EHEC is found in humans, goats and cattle (Nataro and Kaper, 1998)

Avian pathogenic *E. coli* (APEC) is asub group of extrainestinal pathogenic *E.coli*, enters through different routes including genital and respiratory tracts and causes various extra intestinal diseases known as colibacillosis in chickens, which are responsible for high economic losses in the chicken industry (Matthijs *et al*., 2009). The pathogenicity of avian pathogenic *E. coli* that allows certain intestinal commensal *E. coli* to become APEC (Dziva and Stevens, 2008)

APEC strains cause complex of diseases mainly colibacillosis. APEC belong mostly to the serotypes O1, O2, O78. colibacillosis begins with an infection of the upper respiratory tract , end by septicemia . APEC can cause apotential zoonotic risk for human.

Antibiotics have been critical in the fight against infectious diseases (WHO) . Approaches to control APEC infections in the poultry industry include improved hygienic protocols, vaccination, and the introduction of immunopotentiators, however, each of these practices had low success (La Ragione *et al*., 2002). This makes it necessary to use antimicrobial therapy to control coli bacillosis out breaks; however, recent reports have described increased resistance to antimicrobial agents commonly used for treatment (Altekruse *et al.*, 2002; yang *et al.*, 2004)

The goal of this study is to detect antibiotic resistance strains of *E.coli*.

Which isolated from ill shicken in Elmarj region? Therefore, the aim of this study was:

1. Collection of samples from ill chicken in Elmarj city, Libya.
2. Identification of *E.coli* strains which isolated from ill chicken on monkey agar then sub cultured on EMB media.
3. Detection of antibiotic resistance to *E. coli* recovered from ill chicken.

2. REVIEW OF LITERATURE

2. 1 Morphological characters of *E. coli:*

Nakazato *et al.* (2009) confirmed that E.coli related to the Enterobacteriaceae family. It is facultative, anaerobic rod – shaped bacterium; it ferments lactose by producing acid and gas when incubated at 44C.

Moemen *et al.* (2014) examined 276 chicken samples and isolate 96 with a percentage of 34.8% strains which are gram negative bacilli, none sporulated, rod shapped bacterium.

2. 2 Culture characters of E .coli:

Mohammed (2012) confirmed the *E .coli* isolation as following analysis with API 20 E and found that API Rapid 20E profile of 29 (90 **.**6%) tested strains gave very good identification with identity 99.5 %, while 3 (9.4%) strain revealed atypical phenotype and showed the same results except for a single biochemical test

Momtaz et al. (2012) demonstrated on EMB media agreen metallic sheen isolate were considered to be gram- negative bactreia (E. *coli)*

Kunerfilho et al. (2015) confirmed that E.coli colonies usually appear a dark center and greenish metallic sheen due to the Rapid lactose fermentation, whereas other bacteria such as salmonella, which do not ferment saccharose or lactose, produce colonies that are colorless or have a transparent amber color.

2. 3 Biochemical characters of *E. coli*:

Nolan et al. (2013) confirmed that E .coli positive responses are observed in lysine, indole production and motility. Negative results are expected in tests like oxidase, gelatin liquefaction, urea hydrolysis, citrate utilization, and H2S production.

2. 4 Serotyping:

Zhao et al. (2005) reported that O78 (12%), O15 (5%), and O 53 (4 %) were the most common serogroups identified among the APEC isolates.

El-seedy et al (2011) isolated (O1, O2, O78, O26, and O126) FROM colibacillosis, with an incidence of (27.2 %, 9.09 % and 27.2 %) for O1, O2 and O78 respectively.

Lamyaa (2013) isolated (O1, O2, and O78) from colibacillosis, with an incidence of (21%, and 26.3 % and 26.3%) respectively

Moemen et al. (2014) detected those four serogroups (O78, O86, O128, and O111). The most common isolated serogroup of E.coli from the diseased cases were O78 and O86 which represented 60% of the obtained serogroups followed by O128 (20%).

2. 5 APEC:

Johnson et al. (2008) mentioned that the APEC isolates are becoming resistant to antibiotics, making control of colibacillosis very difficult.

Kemmett et al. (2014) confirmed that APEC, the causative agent of bird's extra-intestinal infections, is apathotype that belongs to the group of ExPEC.

Extraintestinal infections caused by APEC are known as colibacillosis and characterized by fibrinous lesions around visceral organs

Markland et al. (2015) documented that infection of APEC lead to decrease quality, yield, and hatching of eggs. The zoonotic potential of transmission must be considered, since poultry consider the main host for APEC and the undercooked poultry consumption may infect human, which can serve as reservoir of this pathotype.

2. 6 Pathogenicity in chicken:

Eid and Erfan (2013) mentioned that in poultry production, extra –intestinal infection with APEC causes colibacillosis which is an economically devastating disease for the poultry industry in Egypt and many parts of the world.

2. 7 Antibiotic resistance of *E. coli:*

Yang et al. (2004) reported that 70% of *E. coli* isolates were resistant to ciprofloxacin, naldixic acid (100%), tetracycline (98%), sulfa - methoxazole (84%), ampicillin (79%), streptomycin (77%) and levofloxacin (64%). fluoroquinolones have become in effective in veterinary medicine in china.

Zhao et al. (2005) demonstrated resistance of *E.coli* isolates to sulfamethoxazole (93%), tetracycline (87%), streptomycin (86%), gentamicin (69%), and naldixic acid (59%)

Kim et al. (2007) isolated 101 APEC isolates were resistant to tetracycline (84.2%), streptomycin (84%), ampicillin (67%)

Zang et al. (2010) isolated 164 E. coli isolates that were resistance to tetracycline and doxycycline with percentage (84.7%, 70.12%) respectively

Rndall et al. (2010) detected E.coli resistance to ampicillin (71.9%), sulphamides (67.3%) and streptomycin (65.3%)

Obeng et al. (2012) detected the resistance of *E.coli* to ampicillin, streptomycin, neomycin and tetracycline

Momtaz et al. (2012) found that *E. coli* isolates were resistance to tetracycline (91.2%), followed by resistance to sulfamethoxazol (45.6%)

Chloramphenicol and trimethoprim (29.8%). all *E. coli* isolates were susceptible to cephalothin, streptomycin, gentamicin and ampicillin.

Ahmed and Shimamoto (2013) revealed that antibiotic resistance of *E. coli* isolates was ampicillin (90%), streptomycin (87%) and tetracyclin (80%).

Moemen et al. (2014) tested 25 *E. coli* strains for their resistance to 30 antimicrobial drugs. They found that resistance percentage (100%) to gentamicin and amoxicillin (96%) to norfloxacin, (92%) to neomycin (88%) to streptomycin and doxycycline, (76%) to cefotaxime.

Ammar et al. (2015) mentioned that E. coli isolates displayed high rates to resistance to amoxicillin \ clavulanic acid, sulfamethoxazole \ trimethoprim and erythromycin, followed by streptomycin and tetracycline.

3. Materials and methods.

3.1. Materials:

3.1.1. Samples:

In this study, a total of 200 chicken samples from visceral organs Liver, lung, heart, spleen and intestinal swap were collected from fifty Chickens in different farms from Elmarj region giving a total of 200 samples (table 1). These chickens were ranged from 35 -50 days age and showed signs of loss of appetite, sneezing, coughing, air sacculitis, per hepatitis before collecting chicken organs; the external surfaces were disinfected with 70% alcohol to minimize surface contamination. using sterile scissors and tissue forceps, organ were collected separately into sterile bags and transferred to our laboratory in the department of bacteriology at faculty of veterinary medicine, Mansoura University under refrigeration in coolers and analyzed within6h of sampling at most.

Table 1. Number of examined samples

Samples type	Number of samples
Lung	50
Liver	50
Spleen	50
Heart	50
Total	200

3.1.2 Media

3.1.2.1 Media used for isolation of E.coli

. Nutrient agar

Nutrient agar is a general purpose medium supporting growth of a wide range of non-fastidious organisms. It typically contains (mass/volume):[1]

- 0.5% Peptone - this provides organic nitrogen
- 0.3% beef extract/yeast extract - the water-soluble content of these contribute vitamins, carbohydrates, nitrogen, and salts
- 1.5% agar - this gives the mixture solidity
- 0.5% Sodium Chloride - this gives the mixture proportions similar to those found in the cytoplasm of most organisms
- distilled water - water serves as a transport medium for the agar's various substances
- pH adjusted to neutral (6.8) at 25 °C (77 °F).

These ingredients are combined and boiled for approximately one minute to ensure they are mixed and then sterilized by autoclaving, typically at 121 °C (250 °F) for 15 minutes. Then they are cooled to around 50 °C (122 °F) and poured into Petri dishes which are covered immediately. Once the dishes hold solidified agar, they are

stored upside down and are often refrigerated until used. Inoculation takes place on warm dishes rather than cool ones: if refrigerated for storage, the dishes must be rewarmed to room temperature prior to inoculation.

Eosin methylene blue agar medium (EMB)

Eosin methylene blue (**EMB**, also known as "**Levine's formulation**") is a selective stain for Gram-negative bacteria. EMB contains dyes that are toxic to Gram-positive bacteria. EMB is the selective and differential medium for coliforms. It is a blend of two stains, eosin and methylene blue in the ratio of 6:1. EMB is a differential microbiological medium, which slightly inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., *E.coli*) and those that do not (e.g., *Salmonella*, *Shigella*). Organisms that ferment lactose display "nucleated colonies"—colonies with dark centers.

This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time.

- Rapid lactose fermentation produces acids, which lower the pH. This encourages dye absorption by the colonies, which are now colored purple-black.
- Lactose non-fermenters may increase the pH by deamination of proteins. This ensures that the dye is not absorbed. The colonies will be colorless.

On EMB if *E. coli* is grown it will give a distinctive metallic green sheen (due to the metachromatic properties of the dyes, *E. coli* movement using flagella, and strong acid end-products of fermentation). Some species of *Citrobacter* and *Enterobacter* will also react this way to EMB. This medium has been specifically designed to discourage the growth of Gram-positive bacteria.

EMB contains the following ingredients: peptone, lactose, dipotassium phosphate, eosin Y (dye), methylene blue (dye), and agar.

There are also EMB agars that do not contain lactose.

. MacConkey

It is a selective and differential culture medium for bacteria. It is designed to selectively isolate Gram-negative and enteric (normally found in the intestinal tract) bacilli and differentiate them based on lactose fermentation. Lactose fermenters turn red or pink on McConkey agar, and nonfermenters do not change color. The media inhibits growth of Gram-positive organisms with crystal violet and bile salts, allowing for the selection and isolation of gram-negative bacteria. The media detects lactose fermentation by enteric bacteria with the pH indicator neutral red.

It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria), and neutral red dye (which turns pink if the microbes are fermenting lactose).

Composition:

- Peptone – 17 g
- Proteose peptone – 3 g
- Lactose – 10 g

- Bile salts – 1.5 g
- Sodium chloride – 5 g
- Neutral red – 0.03 g
- Crystal violet – 0.001 g
- Agar – 13.5 g
- Water – add to make 1 litre; adjust pH to 7.1 +/− 0.2

3.1.2 .2 Media for biochemical identification

. Triple sugar iron agar medium (TSI)

Differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates.

As with the phenol red fermentation broths, if an organism can ferment any of the three sugars present in the medium, the medium will turn yellow. If an organism can only ferment dextrose, the small amount of dextrose in the medium is used by the organism within the first ten hours of incubation. After that time, the reaction that produced acid reverts in the aerobic areas of the slant, and the medium in those areas turns red, indicating alkaline conditions. The anaerobic areas of the slant, such as the butt, will not revert to an alkaline state, and they will remain yellow. This happens with *Salmonella* and *Shigella*.

Vigorous fermenters such as *Escherichia coli* and E*ntrobacter cloacae* will ferment all the available sugars and then begin using the amino acids. This will produce amine groups and cause the medium to turn alkaline.

. ***Simmon citrate agar medium ,(*** *Dehydrated Culture Media)*

Code: CM0155

An agar for the differentiation medium used for Enterobacteriaceae based on the utilisation of citrate as the sole source of carbon.

Typical Formula*	**gm/litre**
Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0
pH 7.0 ± 0.2 at 25°C	

Adjusted as required to meet performance standards

Technique
the medium may be used either as slopes in test tubes or as a plate medium in petri

dishes. In both cases the surface of the medium is lightly inoculated by streaking and, where slopes are used, the butt of medium is inoculated by stabbing. Incubation for 48 hours at 35°C is recommended.

Positive growth (i.e. citrate utilisation) produces an alkaline reaction and changes the colour of the medium from green to bright blue, whilst in a negative test (i.e. no citrate utilisation) the colour of the medium remains unchanged.

Escherichia coli including serotypes from epidemic infantile enteritis, as well as *Shigella, Yersinia* and *Edwardsiella* species do not grow on the medium. *Serratia* and the majority of the *Enterobacter, Citrobacter, Klebsiella, Proteus* and *Providencia* species, except *Morganella morganii* and *Klebsiella rhinoscleromatis* utilise citrate and produce the characteristic blue coloration.

Simmons Citrate Agar may be used to differentiate citrate-positive *Salmonella enteritidis* and members of *Salmonella* subgenus II, III and IV from the citrate-negative *Salmonella typhi, Salmonella paratyphi A, Salmonella pullorum* and *Salmonella gallinarum.*

Appearance
Dehydrated medium: Yellow coloured, free-flowing powder
Prepared medium: Dark blue-green coloured gel

Quality control

Positive control:	**Expected results**
Klebsiella pneumoniae ATCC® 13883*	Growth; colour change of medium to blue
Negative control:	
Escherichia coli ATCC® 25922*	Inhibited or no growth; no colour change of medium

Christensen urea agar base medium

Product Code: LAB130 **Thermo Fisher**

This is a modification of Christensen's urea base for the detection of rapid urease production by Proteus spp.

Other enterobacteria will split the urea, but this will be delayed. This delay is achieved by the incorporation of glucose and the introduction of a buffering system into the medium. The indicator for ammonia production is phenol red.

. Peptone water medium (Thermo Fisher)

Code: CM0009

A basal medium to which carbohydrates and indicator may be added for fermentation studies.

Typical Formula*	**gm/litre**
Peptone	10.0
Sodium chloride	5.0
pH 7.2 ± 0.2	

Directions
Dissolve 15g in 1 litre of distilled water. Mix well and distribute into final containers. Sterilized by autoclaving at 121°C for 15 minutes. When sterile solutions are to be

added after autoclaving, reduce the volume of water for reconstitution by an equal amount.

Description

Peptone Water may be used as a growth medium or as the basis of carbohydrate fermentation media, whilst a pure culture in Peptone Water is a convenient inoculum for a series of fermentation tubes or other diagnostic media.

Peptone Water, adjusted to pH 8.4, is suitable for the cultivation and enrichment of *Vibrio cholerae* from infected material[1]. The medium was formerly used for the performance of the indole test, but now better results can be obtained by the use of Tryptone Water CM0087.

Peptone Water may be modified for use in carbohydrate fermentation tests by the addition of Andrade's indicator. The indicator which is pink at pH 5.0 and yellow at pH 8.0 is prepared by adding sodium hydroxide to acid fuchsin until it becomes yellow. When added to Peptone Water it is colourless to slightly pink. Filter-sterilised `sugar' solutions are added to the base medium after sterilisation. These solutions are usually at 10% w/v concentrations and it is important to allow for dilution of the Peptone Water when making up the initial volume of medium. A final concentration of 1% w/v sugar in Peptone Water is normally used but more expensive sugar can be used at 0.5%.

Andrade's indicator may be made by adding 1N sodium hydroxide to a 0.5% solution of fuchsin until the colour just becomes yellow. Appropriate safety precautions must be taken to avoid inhalation of, and skin contact with, acid fuchsin.

Both Peptone Water and Andrade Peptone Water are prepared and sterilised in the same manner except that an inverted fermentation tube (Durham tube) to detect gas production is included in Andrade Peptone Water containing glucose. Some organisms will utilise carbohydrate to produce acid only without gas formation. It is unnecessary to add Durham tubes to Peptone Water sugars other than glucose.

Precautions for Andrade Peptone Water sugars

Each individual bottle of Peptone Water sugar was coded for the contained sugar.

Andrade Peptone Water is reddish-pink when hot; it returns to a colourless or a slightly pink colour when cooled to room temperature. Some sugar solutions may affect the pH of the Peptone Water.

Sub-cultures may be necessary to ensure purity of the inoculant. Mixed or contaminated cultures will give false reactions.

Appearance

Dehydrated medium: Straw coloured, free-flowing powder Prepared medium: Light straw coloured solution

Quality Control

Maintain stock cultures of organisms which have known positive and negative reactions in each sugar. Using fresh sub- cultures test each batch of sugar medium with the appropriate organisms.

Positive control:	**Expected results**
Escherichia coli ATCC® 25922 *	Turbid growth
Negative control:	
Uninoculated medium.	No change

3. 1. 2. 3 Media used for antibiotic sensitivity test:

. Muller –Hinton agar (oxoid),

Code: CM0337 **Thermo Fisher**

An antimicrobial susceptibility testing medium which may be used in internationally recognised standard procedures.

Typical Formula*	**gm/litre**
Beef, dehydrated infusion from	300.0
Casein hydrolysate	17.5
Starch	1.5
Agar	17.0
pH 7.3 ± 0.1 @ 25°C	

To 1 litre of distilled water we Add 38g. After that bring to the boil to dissolve the medium completely. Than sterilize by autoclaving at 121°C for 15 minutes.

Description

Mueller-Hinton Agar was designed to be a reproducible culture medium for the isolation of pathogenic *Neisseria* species. The inclusion of starch ensures that toxic factors found during growth will be absorbed and its presence is often essential to establish growth from very small inocula.

Mueller-Hinton Agar and Broth are used as the basis of solid and liquid media containing cefoperazone, trimethoprim, piperacillin and cycloheximide for selective isolation of *Arcobacter* spp. from meats.

Quality control

Positive controls:	**Expected results**
Escherichia coli ATCC® 25922 *	Good growth; pale straw coloured colonies
Pseudomonas aeruginosa ATCC® 27853 *	Good growth; straw coloured colonies
Staphylococcus aureus ATCC® 25923 *	Good growth; cream coloured colonies
Negative control:	
Uninoculated medium	No change

3.1.3. Differential stain:

. Gram stain

Created by Monica Z. Bruckner

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups by coloring these cells red or violet. Gram positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls, which retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.

Gram staining involves three processes: staining with a water-soluble dye called crystal violet, decolorization, and counterstaining, usually with safanin. Due to differences in the thickness of a peptidoglycan layer in the cell membrane between Gram positive and Gram negative bacteria, Gram positive bacteria (with a thicker peptidoglycan layer) retain crystal violet stain during the decolorization process, while Gram negative bacteria lose the crystal violet stain and are instead stained by the safranin in the final staining process

Reagents:

- Crystal violet (primary stain)
- Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall)
- Decolorizer (e.g. ethanol)
- Safranin (secondary stain)
- Water (preferably in a squirt bottle)

3 . 1 .4 Reagent for biochemical examination

. pepton water , H_2O_2 , Oxidase reagent , Kovac reagent

. Tryptophan, Methyl red pH indicator

. Voges proskauer test reagents , Urea solution 40%

3. 1. 5 Diagnostic antisera used for serotyping

Rapid diagnostic E.coli antisera set (Denka Seiken Co ., japan)

For diagnosis of the enteropathogenic types .

Company Name: DENKA SEIKEN Co. Ltd

Address: 1359-1, Kagamida, Kigoshi, Gosen-shi, Niigata, 959-1834, Japan Division: Quality Assurance Division Telephone: +81-0250-43-4136 Facsimile :+81-0250-43-3789

General use: In vitro diagnostic use only MSDS No.: 200000-02

Ingredient CAS Registry No Concentration Each serum Rabbit/Swine serum Disodium hydrogen phosphate 12H2O Potassium dihydrogen Phosphate Sodium chloride Sodium azide Glycerin --- 10039-32-4 7778-77-0 7647-14-5 26628-22-8 56-81-5 250mL/L 2.3g/L 0.2g/L 5.4g/L 0.8g/L 0.15g/L

Features of this test is none of the components in these products requires special labeling in accordance with The Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and we need only Personal protection: Normal laboratory clothing should be worn and standard safety procedures should be followed according to local rules. Accidental release spillage measures: After spillage mop up with absorbent material e.g. cotton, wool or paper towel.

Bacterial Antisera Item Products Name Size

Item	Name	Size	Item	Name	Size	Item	Name	Size	Item	Name	Size
215741	poly 9	2mL	215802	O 161	2mL	295392	H-16	5mL	295521	H-45	5mL
215758	O 74	2mL	292490	poly II	2mL	295446	H-21	5mL	295538	H-5	5mL
215765	O 91	2mL	292506	poly III	2mL	295453	H-27	5mL	295545	H-51	5mL
215789	O 121	2mL	292513	poly Iv	2mL	295460	H-28	5mL	295552	H-6	5mL
215796	O 145	2mL	2925	H-10	5mL	295477	H-34	5mL	295569	H-7	5mL
215819	O 165	2mL	295378	H-11	5mL	295484	H-4	5mL	295576	H-9	5mL
215826	O 104	2mL	295385	H-12	5mL	295491	H-40	5mL	295583	O 1	2mL
295408	H-18	5mL	295422	H-2	5mL	295507	H-41	5mL	295590	O 111	2mL
295415	H-19	5mL	295439	H-20	5mL	295514	H-42	5mL	295606	O 1 12	2mL
295736	O 146	2mL		O 136	2mL	295651	O 125	2mL	295613	O 114	2mL
295743	O 148	2mL	295705	O 142	2mL	295668	O 126	2mL	295620	O 115	2mL
295750	O 15	2mL	295712	O 143	2mL	295675	O 127	2mL	295644	O 119	2mL
295767	O 151	2mL	295729	O 144	2mL	295682	O 128	2mL	295651	O 124	2mL
295774	O 152	2mL	295781	O 153	2mL	295804	O 158	2mL	295798	O 157	2mL
295811	O 159	2mL	295958	O 55	2mL	295835	O 166	2mL	295996	O 8	2mL
295828	O 164	2mL	295965	O 6	2mL	295842	O 167	2mL	296009	O 86a	2ml
295866	O 169	2mL	295972	O 63	2mL	295899	O 168	2mL	295880	O 20	5mL
295903	O 26	2mL	295910	O 27	2mL	295873	O 18	2mL	295897	O 25	5mL
295927	O 28ac	2mL	295934	O 29	2mL	295941	O 44	2mL			

3. 1. 6. Antibiotic disc:

Sulfamethoxzole (100mg /disk), levofloxacin (5 mg /disc), chloromphinicol (30mg /disc), norfloxacin (10mg /disc)

Tetra cycline (30mg), streptomycin (10mg), neomycin (30mg/disc)

Cefoxitin (30mg), amoxicillin (10mg), naldixic acid (30mg /disc)

Cefotaxime (30mg), penicillin (10mg /disc), doxycycline (30mg /disc)

3.2. Methods

3.2.1. Collection of samples:

In this study , a total of 200 chicken sample from visceral organs (liver , lung , heart , spleen , and intestinal swap) were collected . the common lesions detected in postmortem examination were pericarditis , air sacculitis, per hepatitis , ascites , splnitis and peritonitis . Sample were taken from diseased freshly dead chickens after clinical and post mortem examination. Before collecting chicken organs , the external surface were disinfected with 70% alcohol to minimize surface examination . using sterile scissors and tissue fore ceps , organs were collected separately into sterile bags and transported in a cooled for further processing .

3.2.2 Bacteriological examination:

3.2.2.1. Enrichment:

A total volume of 2 gm of each samples were homogenized with 225 ml of sterile Macon key broth (oxoid), the resultant suspension was then incubated at 37C for 18 – 20 h

3.2.2.2. Isolation of E.coli strains:

Loopfuls from positive Macon key broth tubes were separately streaked onto sterile plates of Macon key agar for isolation of E.coli strains and incubated at 37 C then were examined for any E.coli growth at 24 hrs.

3.2.2.3. Identification of E.coli isolates:

Suspected E.coli colonies were transferred before being over grown by contaminants. Cultures on selective differential media were examined

After 24hrs. Suspected colonies were sub cultured onto Eosin methylene blue agar (EMB) the plates were incubated at 37C for 24 hrs. . Then colonies were sub cultured onto nutrient agar slope and identified using a combination of biochemical tests:

1- Morphological characters of the colonies :

Bright pink colonies on Macon key agar and characteristic metallic sheen colonies of Eosin methylene blue (EMB)

2- Microscopical examination:

. Gram stain:

1. To make the slide of cell sample to be stained. We fixed the sample of suspected *E.coli* colonies to the slide by the heat under passing the slide carefully with a drop or small piece of sample on it through a Bunsen burner three times.
2. The smears covered with primary stains (crystal violet). After that, the slides incubate for 0ne minute. Then we rinse slide with a gentle stream of water for a maximum of five seconds to remove unbound crystal violet.
3. The slide covered with Gram's iodine solution for one minute- this is a mordant, or an agent that fixes the crystal violet to the bacterial cell wall.
4. Rinse sample/slide with alcohol 95% for ~three seconds and rinse with a gentle stream of water. The alcohol will decolorize the sample if it is Gram negative, removing the crystal violet. However, Add the secondary stain, safranin, to the slide and incubate for 1 minute. Wash with a gentle stream of water for a maximum of 5 seconds and dried between blotting paper. If the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (safranin), causing it to look violet/purple under a microscope. If the bacterium is Gram negative, it will lose the primary stain and take the secondary stain, causing it to appear red when viewed under a microscope.

E.coli organism appears gram negative bacilli. medium size, stained evenly coccobacilli were suspected to be *E.coli*

3 – Biochemical tests:

. Catalase test:

It was performed by taking 2 -3 drops of H2O2 on clean glass slide and single colony from EMB plate was mixed with the help of wire loop.

Immediate formation of gas bubbles was considered as positive test

. Oxidase test:

Loopful culture from single colony was just touched on the kovac reagent. No change in color was considered as positive test.

Indole test: tested colonies were inoculated into peptone water plus 1% tryptophan and were incubated for 37C for 24 hrs. Then kovac reagent was added down the inner wall of the tube. Formation of red color at the interface between reagent and broth was considered the positive result.

. Methhyl red test:

Glucose phosphate broth was inoculated by tested colonies and was incubated at 37 C for 48hrs. Then 5 drops of methyl red indicator were added. red color was considered positive result.

4- serological identification of *E.coli:*

The isolates were serologically identified by using rapid diagnostic E.coli antisera sets (Denka seiken Co., Japan) for diagnosis of the Enteropathogenic types.

Technique:

. Two separate drops of saline were put on a glass slide and apportion of the colony from the suspected culture was emulsified with the saline solution to give a smooth fairly dense suspension.

. To one suspension, control, one loopful of saline was added and mixed. To the other suspension one loopful of un diluted antiserum was added and titled back and forward for one minute.

. Agglutination was observed using indirect lighting over a dark background. When a colony gave a strongly positive agglutination with one of the pools of polyvalent serum, a further portion of it was inoculated on to a nutrient agar slant and incubated at 37C for 24 hrs. To grow as culture for testing with mono –Valente sera.

. A heavy suspension of bacteria from each slope culture was prepared in saline, and slide agglutination tests were performed with the diagnostic sera to identify the O antigen.

The diagnostic *E.coli* antisera sets used for identification include the following sets :

Set 1 : O antisera :

Polyvalent antisera 1 : O1 , O26 ,O86a , O111, O119 , O127 a , O128

Polyvalent antisera 2 :O44 , O55 , O125 , O126 , O146 , O166

Polyvalent antisera 3 : O18 , O114 , O142 , O151, O157 , O158

Polyvalent antisera 4 : O2 , O6 , O27 , O78 ,148 , O159 , O168

Polyvalent antisera 5 : O20 , O25 , O63 O153 , O167

Polyvalent antisera 6 : O8 , O15 , O115 , O169

Polyvalent antisera 7 : O28ac ,O112ac , O124 , O136 , O144

Polyvalent antisera 8 : O29 , O143 , O152 ,O164

Set 2 : H- antisera H2 , H4 , H6 , H7 , H11 , H18 , H21

3 – 2 -3 . Antibiotic sensitivity test :

3-2-3-1 .Technique

It was performed by disc diffusion method using Muller Hinton agar, minimal inhibition concentration (MIC) Values were applied to evaluate the sensitivity of bacterial isolates to antibiotics

3 -2-3-2 . Antibiotics tested:

The antimicrobial agents tested and their corresponding concentration were as follows :

Sulfamethoxzole (100mg /disk), levofloxacin (5 mg /disc), chloromphinicol (30mg /disc), norfloxacin (10mg /disc)

Tetra cycline (30mg), streptomycin (10mg), neomycin (30mg/disc)

Cefoxitin (30mg), amoxicillin (10mg), naldixic acid (30mg /disc)

After incubating the inoculated plates aerobically at 37C for 18 to 24 hrs

The susceptibility of the *E. coli* isolates to each antimicrobial agent was measured and the results were interpreted.

3-2-3-3. Bacterial strains:

Arepresentive field strains isolated will purified and identified biochemically and serologically then tested *in vitro* for their sensitivities to different types of antibiotics and commonly used in veterinary field using standard procedures as described by Quinn et al.(1994)

3-2-3-4 . Preparation of standard inoculums:

A smooth single colony was inoculated in 5 ml Muller Hinton broth after that the sample incubated at 37C for 18 hrs., then turbidity was adjusted to 0.5 M.C. Farland contain (1.5 x10) colony forming unit /ml, then a few drops of inoculated broth were flooded on to surface of Muller –Hinton agar plates

Excess of cultural fluid was removed aseptically and the plates were allowed to stand for 15 minutes at 37C for dryness.

3-2-3-5. Inoculation of the test plate:

Identified *E.coli* isolates inoculated into the surface of agar media and spread over the whole plate by a sterile bent glass rod in three directions over the entire surface of the agar with the objective of obtaining a uniform inoculation. The tested agar was Muller – Hinton. the inoculated plates will be allowed to stand for 3-5 minutes but no longer than 15 minutes for any excess moisture from the inoculate to absorbed .

3-2-3-6. Application of discs and inoculation of the plates:

The discs were placed onto the agar surface using sterile forceps. Each disc was gently pressed with the point of sterile forceps. The discs were placed no closer together than 23mm (center to center) and 15 mm from the edge of the plates to ovoid

overlapping of inhibition zones and give more wide area for the zone of inhibition. The plates were inverted and incubated aerobically at 37C for 24 hrs. .

3-2-3-7. Interpretation:

Inhibition zones were measured in millimeters using aruler . the diameter

Was read from the back of the plate when the test was done on the comparatively clear Muller Hinton agar medium. The diameter of the zones was read across the center of the disc. The result was interpreted in comparisons to the interpretative standards of CLSI (Clinical and Laboratory Standards Institute, (2002).

4. Results.

4 -1 Table (5) Incidence of E.coli in the examined chicken samples

No . examined samples	No . of positivsamples	Percentage of positive
200	73	%36

4. 2. Table (9) serogroups of avian pathogenic E.coli

Serial no	No.of strains	Type of sample	Sero-diagnosis	Strain characterizations
1	7	Lungs(2),spleen, liver Intestinal content	O2:H6	E P E C
2	12	Lungs(2) spleen(2),liver Intestinal content	O78	E P E C
3	2	Intestinal swab , liver	O166	E P E C
4	1	Lungs	O153:H2	E P E C
5	2	Spleen , lungs	O114:H4	E P E C
6	4	Heart (2) , lung , liver	O91:H21	E P E C
7	3	Lungs(2) , heart	O126:H21	E P E C
8	2	Lungs , heart	O117:H7	E P E C
9	1	Liver	O121:H7	E I E C
10	3	Spleen , liver ,lungs	O145	E P E C
11	4	Spleen , liver ,lungs	O8:H21	E P E C
12	1	Lungs	O6:H4	E P E C
13	7	Lung(2),heart(3), spleen	O1:H7	E P E C
14	2	Heart , spleen	O26:H11	E P E C
15	3	Spleen ,liver, heart	O44:H18	E P E C
16	1	Lungs	O159:H21	E P E C
17	1	Liver	O119:H6	E P E C
18	1	Spleen	O63	E P E C
19	2	Intestinal swab , lung	O55:H7	E I E C
20	2	Liver	O111:H2	E I E C
21	2	Lungs , heart	O128:H2	E I E C
22	1	Spleen	O15:H2	E P E C
23	1	Heart	O124	E P E C
24	1	Spleen	O158	E I E C
25	1	Liver	O114:H4	E I E C
26	1	Spleen	O171:H2	E I E C

27	1	Heart	O158	E P E C
28	1	Lungs	O26:H11	E I E C
29	1	Spleen	O146:H21	E P E C
30	1	Spleen	O87	E I E C
31	1	Spleen	O124	E I E C

4.3. Table (7) Frequency of antimicrobial resistance profiles of *E. coli* from chickens:

Antimicrobial class	Antimicrobial agent	E.coli (N =73)	Percentage %
Cephalosporins	Cefipime	70	95 .8 %
	Cefoxitin	66	90.8 %
	Cefotaxime	56	76.7 %
Aminoglycosides	Neomycin	56	89 %
	Streptomycin	65	73.9 %
Tetracycline	Tetracycline	39	53.4 %
	Doxycycline	51	69.8 %
Chloramphinicol	Chloramphinicol	22	30 %
Quinolones	Levofloxacine	31	42 %
	Norfloxacine	27	49 %
	Naldixic acid	36	49 %
Penicillins	Amoxillin	69	95 %
	Ampicillin / salbactam	34	47%
	Penicillin	73	100%
Sulfanoamides	Sulphamethoxazole	66	90 %

5- DISCUSSION.

5. 1. Incidence of E.coli in the examined chicken samples:

In this study, among 200 tested chicken samples, 73(36.5%) samples.

Were confirmed to be E. coli positive, our results were nearly similar to a study done by Momtaz and Jamshidi (2013) that showed recovery

Rate (34.59). On the other hand, Kilic et al. (2007) showed higher recovery rate (48%) of 100 chicken samples were *E .coli positive*. furthermore, another study revealed that only 49 E.coli isolates (20%) were recovered from 242 samples (Ammar et al., 2015)

The prevalence of the *E.coli* isolates recovered from different samples differ among different studies due to diversity of sampling and methods used for screening E.coli isolates (lee et al ., 2009) or other factors may affect contamination rate (Xia et al ., 2010)

5. 2. Serotyping of E.coli isolates:

Twenty six O serogroups were identified among the 73 avian pathogenic E.coli isolates which isolated from 200 chicken samples. Among the isolates that could be

typed, the most prevalent serogroups were O78 then O2 and O1. Other infrequently encountered serogroups included shown in Table 6.

According to results of sero-diagnosis, the *E.coli* isolates from chicken were serotyped into 26 serogroups (O1, O2 , O78 , O26 , O153 , O114, O91 , O 121 , O44 , O 63 , O 158 , O171 , O 146 , O 124 , O 15 , O8 , O145 , O 117 , O166, O 128 , O 111 , O 55 , O119 , O159 , O6 , O126)

As shown in table (6). also, the obtained results detected serotypes (O78 , O1, O2) were the most common with an incidence of (50% ,26%, 26.9 %) respectively . these results were confirmed by El-Seedy et al .,(2011) who isolated (O1 , O2 , O 78 , O26 , O126) from colibacillosis, O1, O2, O78 with incidence of (27.2 %, 9.09%, 27%) respectively. also the results agree with Lamyaa (2013) who isolated (O78, O1, O2) from Avian colibacillosis, with an incidence of (26.3% , 21% , 26.3%) respectively.

5. 3. Antimicrobial resistance phenotypes of APEC isolates:

Out of 200 specimens collected, 73 (36.5%) *E.coli* isolates were identified based on morphological and biochemical characteristic. Using Gram stain method, the 73 positive isolates showed short –rod, gram negative bacteria. The 73 isolates showed pink colonies on Macon key agar and green metallic sheen colonies on EMB agar, which are typical for E.coli. The results showed that the resistance pattern of 73 E.coli isolates to 15 antimicrobial agents tested in this study. All *E.coli* isolates were resistant to one or more antimicrobial agent. resistance to penicillin was the most common finding (100%), followed by resistance to cefipime (95%), and the lowest finding norfloxacine (37%) and chloramphenicol (30%)**resistance to the antimicrobial drugs has been described in Table 7**

All isolates of the E.coli showed resistance to two or more antibiotics the

First group includes the antibiotics to which there were resistance with high levels of of (73% to 100%) these are penicillin (p): (100%), Cefipime (FEP): 95.8%, Amoxicillin (AX) : 94.5%, Sulfamethoxazole (SMZ): 90.4%, Cefoxitin (FOX): 90.4% , Neomycin (N) : 89% group includes the antibiotics to which were moderate levels of resistance (42% to 69%), these are doxycycline (DO): 69.8% , Tetracycline (TE) : 53.4%

Naldixic acid (NA): 49.3%, Ampicillin / salbactam (SAM): 46.5% and Levofloxacine (LEV) : 42.4%. The third group includes the antibiotics to which gives low levels of resistance (0% to 36%), these are Norfloxacin

(NOR): 36.9% and chloramphenicol (C) : 30%

Antibiotics are commonly used in the prevention and control of disease and can also use as growth promoting agents of animals. Under the selective pressure of antibiotic lead to multidrug resistant bacteria to appear. In this study we showed an increased rate of resistance to the most of the examined antibiotics. 100% of the tested isolates of *E.coli*

Showed resistance against penicillin, 95.8% against cefipime and 94.5% against amoxicillin followed by considerable resistance to the rest of the Antibiotics examined. Most of these antimicrobial agents are usually used as growth promoters or as prophylactic agents in Libya in the poultry industry.

The obtained data of antibiotic resistance in this study, in comparision with several published reports indicated that most recovered *E.coli* from poultry are resistant to antimicrobial agents with equal or lower percentages as recorded in Egypt (Moemen et al., 2014), Korea (Kim et al., 2007), China (Yang et al., 2004), united kingdom (Randall et al., 2010), united states (Johnson et al., 2005)

Our study results of APEC strain showed that the resistance of avian *E.coli* strains to penicillin, and doxycycline was 100% and 69% respectively while resistance rate of chloramphenicol was 30%. In the present study, we also found that quinolone resistance among *E.coli* strain from chicken is increasing (36% to 49.3%) which agreed with previous study (zhoa et al., 2005). But our results disagree with yang et al., (2004) who isolated 71 strains of E.coli from the livers of broilers from 10 different barns in China and found that Fluoroquinolones, Naldixic acid, Tetracycline, and other eight antibiotics were resistant to more than 80% of E.coli strains.

References

1. **Ahmed, A.M., and T. Shimam0to. (2013)**: Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septic emic broilers .Int.j.Med.microbial. 303:475 – 483.
2. **Altekruse ,S. F., Evinger, F., Lee, K.Y., Tollefson, L.K., Pierson,E.W., Eifert , j ., Sriranganathan , N ., (2002)**: Antimicrobial susceptibilities of *Escherichia coli* strains from a turkey operation . j . Am . Vet . Med . Assoc . 221: 411 – 416 .
3. **Ammar, A. M ., El – Hamid , M.I., Eid , S.E . A ., & El Oksh , A. S. (2015)** : Insight into antimicrobial resistance and virulence of emergent multidrug resistance avian pathogenic *Escherichia coli* in Egypt : how closely related are they .REVUE DE MEDICINE VETERNAIRE , 166 (9-10), 304 – 314 .
4. **Dziva, F., and M. P. S Tevens. (2008)**: Colibacillosis in poultry: Unravelling the molecular basis of virulence of avian pathogenic *Escherichia coli* in their natural host . Avian Pathol : 355 – 366 .
5. **Eid S. A., Erfan A.M. (2013)**: Characterization of *Escherichia coli* associated with high mortility of poultry flocks. Assiut Vet. Med . J.,59: 51-61.
6. **El-seedy F. R .,Hassan W.H.,Salama S.S. and Fatma El-Zahraa Gamal (2011)**: Optimization of Polymerase chain reaction for direct detection of colibacillosis in infected chickens . Vet . Med . J ., Giza , 59 (2) : 307-318.
7. **Feng P , Weagant S , Grant, M . (2002)** : Enumeration of *Escherichia coli* and the coliform bacteria . Bacteriological Analytical Manual (8thed) . FDA / Center for food Safety &Applied Nutrition.
8. **Johnson , T.J., Wannemuehler , Y., Doetkott, C., Johnson , S. J., Rosenberger, S . C.,&Nolan , L . K . (2008)**: Identification of minimal predictors of avian pathogenic E.*coli* virulence for use as arapid diagnostic tool. *Journal of clinical microbiology*, 46(12) , 3987- 3996.
9. **Kemmett , K., Williams, N . J ., Chaloner , G ., Humphrey (2014)** : The contribution of systemic *Escherichia coli* infection to the early mortilities of commericial broiler chickens . *Avian pathology* ,43 (1) m 37 – 42.
10. **Kim , T. E.,Jeong , Y. W .,CHO .(2007) :** Chronological study of antibiotic resistance and their relevant genes in Korean avian pathogenic
11. *Escherichia coli* isolates. *Journal of clinical microbiology*, 45(10), 3309-3315.

12. **Kunert Filho HC , Carvalho D , Grassotti TT.(2015):** Avian pathogenic *E . coli* – method improved diagnosis . world poultry science journal . ,71: 249 – 258.
13. **La ragione , R.M.,Woodward . J .,(2002)** Virulence factors of *Escherichia coli* serotypes associated with avian colisepticaemia . Res Vet. Sci73 : 27 – 35
14. **Lamyaa, M . R . (2013) :** Studies on antibiotic resistance of *Escherichia* coli isolated from poultry and children .SCVMJ , XVIII (2)
15. **Markland , S.M.,LeStrange, K. J.,Sharma .(2015) :** Old friends in new places : Eploring the role extra intestinal *E.coli* in intestinal disease and food borne illness. Zoonosis and public health, 62(7) , 491-496 .
16. **Matthijs , M., Ariaans, M. P .,Dwar R (2009)** : Course of infection and immune response in the respiratory tract of IBV infected broilers after super infection with *E. coli* "Veterinary Immunology and Immunopathology ., 127, 1-2 , 77-84.
17. **Moemen A. Mohamed, Mostafa A . Shehata.(2014)**: Virulence genes content and Antimicrobial Resistance in *E.coli* from broiler chickens . Hindawi publishing Corporation Veterinary Medicine International 195189,6.
18. **Mohammed , M . A .M . (2012)**: Molecular characterization of diarrhea genic *E. coli* isolated from meat products sold at Mansoura city Egypt . J. Food Control , 25 : 159- 164
19. **Momtaz , H.,Rahimi, E., & Moshkelani, S. (2012)** : Molecular detection of antimicrobial resistance genes in *E.* coli isolated from slaughtered commercial chickens in Iran. Veterinary Medicina , 57(4), 193-197
20. **Nakazato, G., Campos, T. A. D., Stehling.(2009)**: Virulence factors of avian pathogenic E.coli (APEC) . Pesquisa veterinarians Brasília , 29 (7) 479-486 .
21. **Nataro, J. P ., and Kaper , J.B . (1998)** :Diarrhea genic E.coli . J . Clin. Microbial Rev , 11 : 132 – 201
22. **Nolan LK, Barnes HJ, Vaillancourt JP, Abdul-Aziz T (2013)**: Colibacillosis in: Swayne DE,editor. Diseases of poultry. Ames: Wiley Blackwell, 13th ed , p 751 – 806.
23. **Obengg, H. Rickard, O. Ndi , M. (2012)** : Antibiotic resistance , polygenetic grouping and virulence potential of *E.coli* isolated from faeces of intensively farmed and free range poultry . Vet . Microbial . 154: 305 – 315 .
24. **Randall, L, P., Clouting , C., Horton, R . A., Coldham (2010)** : prevalence of *E.coli* carryng extended-spectrum B- lactmase from broiler chicken and turkeys in great Britain between 2006 and 2009 . Journal of Antimicrobial chemotherapy , dkq 396 .
25. **Scheutz F and Strockbine NA. (2005)** : Genus 1. Escherichia . In : Brenner , D .J., et al (Eds) The protebacteria part B, the Gammproteobacteria . Springer,(Bergy Manual of System Bacteriology Vol 2 part B). pp. 607 – 623.
26. **Yang , H., Chen , S., White , D. G., Zhao , S., (2004)** : Characterization of multiple- antimicrobial- resistance Escherichia coli isolates from diseased chickens and swine in china. Journal of clinical Microbiology, 42 (8), 3483-3489.
27. **Zhang WQ, pan WJ , Chen X, Geng SZ , Huang JL , (2010)** : Detection of plasmid – mediated Quinolone Resistance among Escherichia coli from avian in china . prog . Vet . Med . 31: 74-78 .
28. **Zhao, S., Maurer, J. J., Hubert, S., De Villena. (2005)** Antimicrobial susceptibility and molecular characterization of avian pathogenic Escherichia coli isolates, Veterinary microbiology, 107 (3), 215 – 224.