DIAGNOSIS OF *E.COLI* ISOLATED FROM ARTHRITIS IN CHICKENS BY VITEK AND MOLECULAR METHODS

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ABSTRACT

This study was aimed to identify and confirm of *E.coli* isolated from arthritis in chickens. Samples for the isolation of the bacteria were taken from broiler chickens with arthritis symptoms (swelling hock joint), and then examined by culturing, VITEK test as well as molecular assay. Antimicrobial susceptibility of bacterial isolate was done. The results showed pink colour colonies on MacConkey agar. The VITEK system was used to identify those colonies. The results revealed that the isolate gave 97 % parallel to those features of *E. coli* equally recognized by the standard gram negative card. The isolate was found to be sensitive to ticarcillin/clavulanic acid and many others antibiotics and resistance to ticarcillin as well as for other types of antibiotics .The results of the 16Sr RNA gene revealed that *E.coli* primers of the16S rRNA gene had successfully targeted the respective gene and have shown the single bands of the16S RNA gene at 1500 bp. Sequencing of this gene was performed for the isolate. The results of nucleotide sequencing were submitted in Gene bank database and have accession number: ID: MT012194.1. The phylogenetic analysis Of the isolate was 100% similar to USA:CP048605, Pakistan:GU594300, VitNamHatey: AP022650 and China:MN208222, however, it was 99% similar to India:LCO58573.

Keyword: broiler, hock joint, PCR, phylogenetic

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تشخيص وعزل ايشريكيا القولون من التهاب المفاصل في الدجاج بواسطة VITEK والفحص الجزيئي

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المستخلص

تم إجراء هذه الدراسة لعزل وتوصيف ايشريكيا القولون من دجاج مصاب بالتهاب المفاصل وتم اخذ عينات للعزل البكتيري تم جمعها من حقل دجاج لحم مصاب بتورم مفصل العرقوب ، ثم تم فحصها عن طريق الزرع الجرثومي , اختبار VITEK و الفحص الجزيئي ثم فحصت حساسية العزلة البكتيرية تجاه المضادات الحياتية .أظهرت النتائج ظهورنمو مستعمرات وردية اللون في اكار MacConkey وعندما حددت هذه المستعمرة بواسطة VITEK تم الكشف عن أن العزلة كانت97 ٪ مشابهة مع السلالة المرجعية *E.coli* القياسية، اما نتائج اختبار فحص الحساسية فكانت العزلة حساسة ل ticarcillin/clavulanic acid, وعدد اخر من المضادات الحياتيه وكانت مقاومة ل ticarcillin وعدد اخر من المضادات الحياتيه المستخدمه اما نتائج التشخيص الجزيئي باستخدام بادئات خاصة لهذا الجين 16SrRNA قد استهدفت بنجاح الجين المعني وأظهرت النطاقات الفردية من هذا الجين عند 1500 زوج قاعدي اما نتائج التسلسل الجيني لهذه العزله بارسال تسلسل النوكليوتيدات الى قاعدة بيانات بنك الجينات NCBI وقد سجلت برقم القبول:MT012194.1 واظهرت نتائج التطور الشكلي تطابق هذه العزلة لعزلة الولايات المتحدة الأمريكيةCP048605: ,عزلة الباكستان:GU59430 ,عزلة فيتنام :AP022650وعزلة الصين:MN208222 في حين كان التطابق ٩٩٪ مع عزلة الهند : LCO58573

الكلمات المفتاحية: لاحم , مفصل العرقوب ,تفاعل البوليميراز المتسلسل ,التطور السلالي.

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INTRODUCTION

Escherichia coli (E. coli) is often responsible for flock outbreaks of arthritis and osteomyelitis in chickens which are sequelae of septicemia, and these outbreaks may be associated with enteric or respiratory disease(8). *E. coli* which have an importance role in broiler breeders and considered contagions via *it* represent main anxieties to the poultry production globally, the most common lesions seen were swelling and inflammation of joint and/or tendons which are accountable for significant economic losses in the production of poultry (3, 4, 13). Bacterial arthritis was also be associated with another bacterial agents such as *Erysipelothrix, Listeria, Mycoplasma and Staphylococcus* (2, 17, 18). *E.coli* could be colonized in the vesicular buds that attack the growing bone provoking demagogic condition, that resulting in osteomyelitis, this type of arthritis is almost always curable. *E. coli* localization in skeletons and synovial membrane is a communal outcome of coli septicemia affecting birds likely have insufficient resistance to completely clear bacteria (21). The yearly losses due to skeletal problems in the USA were 80$ to 120$ million dollar (22, 23). There is no information about molecular features of "*E. coli* strains isolated from lesions in the locomotor system of broilers" in Iraq, therefore, the aim of this study was planned to identify *E.coli* isolated from arthritis in broiler chickens by VITEK system and molecular assay.

MATERIALS AND METHODS

A cotton swab was taken from chickens with arthritis (hock joint) inoculated into nutrient broth then raised at 37oC for 18hr an incubator. A loopful of the culture suspension was streaked onto MacConkey agar and incubated at 37^{o}C for (24-48) hrs. following a day, those pinkish colonies most likely an *E. coli* were subcultured onto nutrient agar to acquire a pure colony, followed by subculture, putative *E. coli* colonies were further identification was done by VITEK (identification of bacteria and yeast as well as susceptibility for antibiotic) testing by means of commercially obtainable proof of identity cards for gram-negative bacteria "in accordance to the manufacturer's recommendations "also examined antibiotic susceptibility by VITEK card (9). Genomic DNA extraction was isolated from bacterial growth according to the of manufacturer protocol of the kit (ABIO, USA) (19). PCR assay was performed depending on 16SrRNAgene using 27F (5`AGAGTTTGATCCTGGCTCAG-3`) and 1492R(5`TACGGTTACCTTGTTACGACTT-3`) primers, these primers were supplied by Macrogen Company in a lyophilized form (26) , PCR products of 16S rRNA gene were sent for Sanger sequencing using Applied Biosystems ABI3730XL, automated DNA sequencer, by Macrogen Corporation. The results were analyzed using Geneious software (5). Sequence analysis of 16S rRNA gene were compared with those existing in GenBank, nucleotides sets were used to obtain the identity score of our isolate strain with the other world references strains by the MEGA 6+NCBI program as previously described (24) after that investigate the results for structure phylogenetic tree by the use of neighbor-joining method for contraction tree with the MEGA 6 software (25).

RESULTS AND DISCUSSION

Arthritis is one of the most economic problems facing poultry industry worldwide, many different types of infections can produce joint inflammation (12).The results showed pink colour colonies in MacConkey agar as shows in fig.1 and when identified this colony by the VITEK, the results revealed that the isolate gave 97 % likeness to those features of *E. coli*, similar to those identified by the standard gram negative card elsewhere, as shows in Table (1).

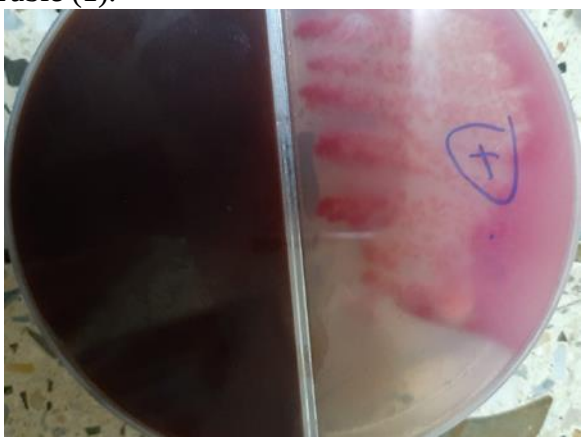

Figure 1. shows pink colour colonies in MacConkey agar

The most commonly isolated bacteria were *E. coli*, that described by Mamza *et al*.,(15) and Rasheed (20) whom isolated *E.coli* from hock joint and digital pad samples of chicken. At current study, VITEK and molecular tests after culturing method are intended for the confirmation of *E.coli*. Garcia *et al*., (10) confirmed that the VITEK system is a stress-free to handle system that offers a fast and practically accurate means for the identification of microbial agents. Bacteria isolate testing was found to be sensitive to ticarcillin/clavulanic acid, piperacillin/tazobactam, imipenem, meropenem ,amikacin, gentamicin and obramycin and the isolate was resistance to Ticarcillin, Piperacillin, Ceftazidime, Cefepime, Aztreonam, Ciprofloxacin and Trimethoprim/Sulfamethoxazole (table 2). Bacteria isolate was found to be sensitive to ticarcillin/clavulanic acid while resistance to Ticarcillin alone by reason of the synergistic effect between ticarcillin and clavulanate, therefore, it could be suggested for the treatment of severe contagious of *Enterobacteriaceae* and *Pseudomonas* (14) although cefepime was broad spectrum beta-lactams antibiotic and beta-lactamase inhibitor combinations, such as ticarcillin , but *E.coli* isolate was resistance to them because the extensive usage of antibiotics in farming and medication is putative as a major selective force in the high occurrence of antibiotic resistance among gram-negative bacteria (16).The results of the 16Sr RNA gene revealed that *E.coli* primers of the16S rRNA gene had successfully targeted the respective gene and shown the single band of the16S RNA gene at 1500 bp as shows in fig. (2).

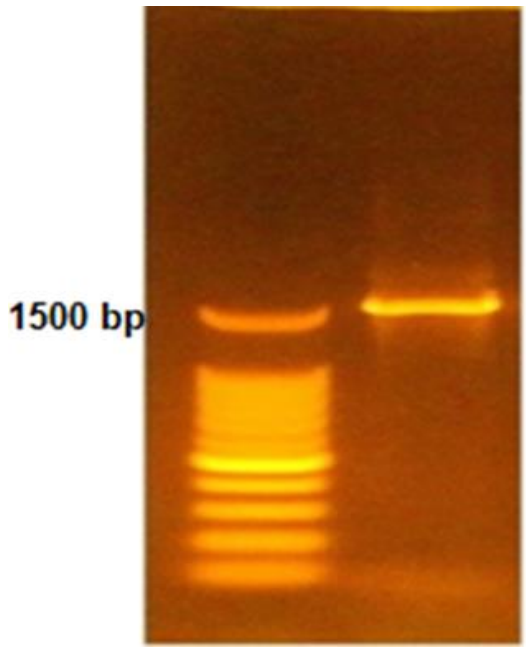

Figure 2. electrophoresis of amplicon PCR products of field isolate of *E.coli* with a single band at size1500bp represent a 16sRNA gene (M: marker 1500bp)

Sequencing of 16SrRNA gene of *E.coli* was performed to the isolate, the nucleotide sequence of chicken *E.coli* 16SrRNA gene was submitted in GenBank database and have accession number: ID: MT012194.1, the phylogenetic analysis post sequencing of the Iraqi *E.coli* isolate that was placed in the NCBI and based on the nucleotide phylogenetic tree of 16Sribosomal RNA gene found that this isolate ,MT012194.1, was100% similar to USA:CP048605, Pakistan: GU594300, VitNamHatey : AP022650 and China:MN208222 while 99% similar to India:LCO58573 fig. (3). "PCR is well optimized with respect to the sensitivity, specificity, and repeatability of the amplification of a target gene, as well as detect pathogens more quickly than bacterial culture"(1). Clinical microbiology laboratory is increasingly relying on partial 16S rRNA gene sequencing for bacterial identification (13) and (11). Many scientists have demonstrated that the accuracy has been improved with 16S rRNA gene sequencing using GenBank databases (4). The phylogenetic tree depicts close proximity between the *E. coli* obtained from Iraq and other countries based on their evolutionary dynamics (6) In conclusion, *E coli* infections were widely distributed among poultry and by means of conventional and molecular

techniques, *E. coli* was confirmed in the present study and this could be correlated with the poor disinfected conditions, deserted technological requirements and immunosuppressive diseases.

Table 1. Identification of *E. coli* by VITEK test

Biochemical tests	result	Biochemical tests	result
beta-galactosidase	+	Adonitol	-
l-pyrrolydonyl-arylamidase	-	d-trehalose	-
l-malate assimilation	-	d-glucose	+
H2S		alpha-galactosidase	+
O/129 resistance	+	d-glucose	+
glycine arylamidase	-	lysine decarboxylase	+
gamma-glutamyl-transferase	-	fermentation/glucose (OFF),	+
Succinate alkalinization	+	beta-glucoronidase (BGUR	+
Ellman	-	phosphatase	-
l-lactate assimilation	-	d-mannose	+
courmate	+	l-proline arylamidase	+
d-mannitol	+	ornithine decarboxylase	+
Urease	-	Citrate (Sodium)	-
tyrosine arylamidase	+	d-maltose	+
Ala-phe-proaramidase	-	β-N-Acetyl	-
Palatinose	-	Glutamyl Arylamidase	-
d-cellobiose	-	5-Keto-DGluconate	-
D-Sorbito	+	Lipase	-
β-Xylosidase	-	Malonate	-
β-N-Acetyl-Galactose aminidase	-	α-Glucosidase	-
L-Histidine assimilation	-	β-Alanine Arylamidase	
L-Arabinose	-		

Table 2. Antibacterial sensitivity test by VITEK

Antibacterial	results	Antibacterial	results
Ticarcillin	R	Amikacin	S
Ticarcillin/Clavulanic Acid	S	Gentamicin	S
Piperacillin	R	Tobramycin	S
Piperacillin/Tazobactam	S	Ciprofloxacin	R
Ceftazidime	*R	Pefloxacin	
Cefepime	*R	Minocycline	I
Aztreonam	*R	Colistin	
Imipenem	S	Rifampicin	
Meropenem	S	Trimethoprim/Sulfamethoxazole	R

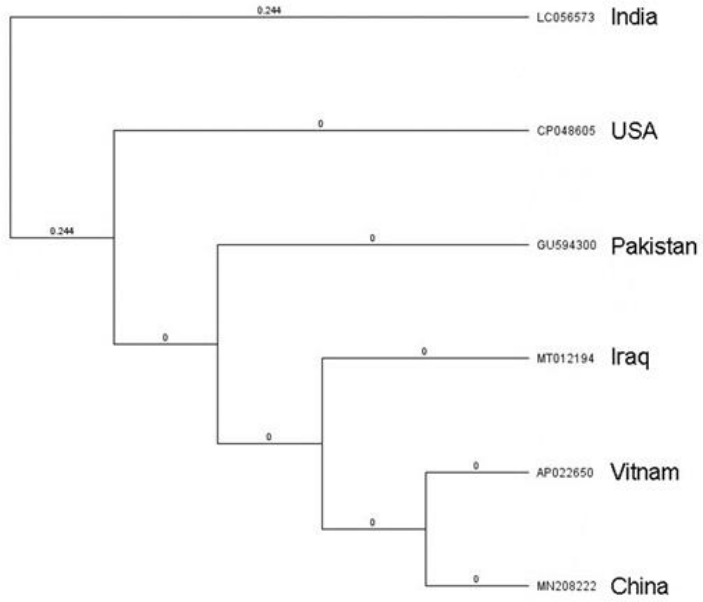

Figure 3. phylogenic tree of *E.coli* isolate using Mega6+NCBI

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