College: Veterinary Medicine **Student's Name:** Ibrahim Hasan Madhloom

Dept.: Veterinary Microbiology & Parasitology **Supervisor's Name:** Assist. Prof. Dr. Rasha M. Othman

Certificate: Master **Specialization:** Microbiology

Title:

Phylogenetic Tree Constructed Amongst Mastitis Causing *E.coli* in Basra Province

Abstract:

This is the first study of its kind in Iraq which investigates the distribution of *E.coli* isolated from various origins into phylogenetic groups and sub phylogroups, and compare the prevalence of main phylogenetic groups and sub phylogroups. Also this study was aimed to investigate the phylogenetic relationship and construction of phylogenetic tree of *E. coli* in relation to clinical and subclinical mastitis in cows, sheep and goats.

During a period of four months (October 2016 to January 2017), a total of 180 samples were collected from cows, sheep and goats in different regions of Basra province. These samples were collected from (60) cows [subclinical mastitis (30) and clinical mastitis (30)], (60) from sheep [subclinical mastitis (30) and clinical mastitis (30)] and (60) from goats [subclinical mastitis (30) and clinical mastitis (30)].

All samples were screened for the presence of *E. coli* by cultured on differential media (MacConkey sorbitol agar) and selective media (EMB and Endo agars). A total of 30 (16.66 %) of suspected *E. coli* isolates were obtained; 2 (0.00%) from clinical mastitis and 9 (5.0%) from subclinical mastitis samples in cows; 7 (3.88%) from clinical mastitis and 5 (2.77%) from subclinical mastitis samples in sheep and 3 (1.66) from subclinical mastitis in goats.

Many techniques were used in this study to evaluate the presence of *E.coli*, these techniques included the traditional bacteriological assays, commercial identification kit (API 20 E System) and molecular techniques (multiplex and conventional PCR). Results of these techniques indicated the absence of *E.*coli in clinical mastitis samples from goats and showed higher occurrence of *E. coli* in mastitic samples from cows than mastitic samples in sheep. All suspected *E. coli* isolates were tested by API 20 E system and 9 (30 %) were confirmed as *E. coli*.

All the isolates of *E.coli* were tested in at least 10 antibiotics to which they were subjected according the method of Kirby-Bauer (disk diffusion assay). All *E. coli* isolates were resistant (100%) to cefuroxime, cloxacillin and lincomycin, while they showed (90.0%) resistance to ampicillin and novobiocin. Some of the isolates showed (83.3%) resistance to tetracycline, (46.7%) to polymixin and (33.3%) to neomycin. Some *E. coli* isolates showed sensitivity (90.0%) to streptomycin, (86.7%) to cephalothin, (66.7%) to neomycin and (16.7%) to tetracycline. The *E. coli* isolates

showed MDR at (6.66%).

E. coli were further examined by multiplex PCR technique using a set of three primers to amplify *chuA*, *yjaA* and TspE4.C2 fragment. The results revealed that 6.66% of the isolates were positive for *chuA* gene, 50% of isolates yielded amplification products with *yjaA* gene and 50% of isolates were positive for TspE4.C2 fragment.

E.coli isolates were assigned into four main groups and subgroups. The results showed that the most strains of group A (14 isolates, 46.7%) belonged to subgroup A_0 about (2 isolates, 20.0%), and (8 isolates, 26.7%) to A_1 subgroup. On the other hand, the results revealed that group A an equal B1, while group B1 (14 isolates, 46.7%) distributed into subgroup $B1_1$ included (8 isolates, 26.7%) and $B1_2$ about (2 isolates, 20.0%). In addition our results showed (1 isolate, 3.3%), assigned to B2 belonged to subgroup $B2_3$ and (1 isolate, 3.3%), fitted in D belonged to subgroup D_1. No isolates were found to belong to subgroups $B2_2$ and D_2.

BLAST analysis included sequenced of 25 PCR amplification products of genetic markers *chuA*, *yjaA* and TspE4.C2 fragment of *E. coli*. After analyzing and comparing the obtained sequences with ECOR sequences, the results showed identity (92.0% to 98.3%) and eleven strains showed changes in nucleotides sequence. These strains were composed of MC17 belong *chuA* gene, strain MG15 belong *yjaA* gene and strains MC3, MC5, MC6, MC7, MS8, MC23, MC27, MC28 and MS30 belong TspE4.C2 fragment.

So, the phylogenetic tree was constructed using sequences of 25 strains that belong to genetic markers and 6 strain that belong to ECOR. The results observed 60% from strains clustered into phylogroup A, 36% from strains belong to phylogroup B and 4% from strains assigned to phylogroup B2.