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Molecular detection of *uidA* gene in *Escherichia coli* isolated from the dairy farms in Nineveh governorate, Iraq

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Abstract

Escherichia coli is a gram-negative environmental micro-organism- which causes intramammary infections in mammals, in addition, to cause food poisoning in human. Four hundred samples were obtained from various areas of the dairy farm. Fifty samples from each of bedding, soils, feces, feed, milk, water, worker's hands, and flies were randomly collected from different regions located around Nineveh Governorate between September 2020 and January 2021. *E. coli* isolates were identified using the classical methods based on the morphological and biochemical methods for the detection the *E. coli* isolates and PCR had been used to detect the *uidA* gene in it. The results of our study revealed that out of 400 samples collected from different dairy herds, 140 (35%) of *E. coli* were positive. The percentage of *E. coli* isolated from flies, bedding, feces, milk, soils, feed, water, and worker's hands was 76% (38/50), 46% (23/50), 38% (19/50), 34% (17/50), 28% (14/50), 28% (14/50), 18% (9/50), and 12% (6/50), respectively. All the positive *E. coli* isolates have been possessing the *uidA* gene which is the species-specific gene. The results of classical methods had concurred with the results of the PCR test. The current study showed that milk, worker's hands, and flies played an important role to spread *E. coli* in the dairy farms. The PCR technique is a rapid method that helps to give the suitable drugs in the treatment of the cattle suffered from mastitis and prevent *E. coli* from distribution among the dairy herds.

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Introduction

Escherichia (*E.*) *coli* is considered an important pathogenic micro-organism that lives in the intestinal tract of the different hosts such as humans, warm-blooded mammals, and birds (1). The *E. coli* is characterized that it is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacteria located in the genus *Escherichia* and family *Enterobacteriaceae* that is generally located in the digestive system of animals (2). Dairy cattle are constantly exposed to the main infectious bacteria which causes mastitis, including *E. coli*, *Staphylococcus aureus*, and *Streptococcus agalactiae* (3). *E. coli* is the major causative agent responsible for all the types of mastitis in cattle (4). In addition, *E. coli* causes huge economic losses due to reject milk and its products because reduce the goodness and volume of milk, cost of therapeutics, and death or killing the infected cows (5). Also, *E. coli* has able to transmit from animals to human by direct contact such as large animals slaughterhouse workers, vocational exposure of farm, and consumption the contaminated food, also *E. coli* transmits to humans by the indirect method through the environment (6). Animals produce are several types of food such as meat, milk, cheese, yogurt, and other dairy products that reported as a possible source of *E. coli* (7). Humans may be exposed to the various foodborne illness such as hemorrhagic colitis, hemolytic uremic syndrome, bloody diarrhea, and thrombotic thrombocytopenic purpura that due to eating milk and its products contaminated by *E. coli* (8). In addition, *E. coli* had found in the farm environment such as pets, water, soils, and workers which regarded as a reservoir of *E. coli* and they had contributed to the spread of it in the farms (9).

In the developing countries, all the dairy farms use the open-house system to breed the cows, which allow birds to fly free that played an important role to spread *E. coli* on the farm (10). There are many methods used to identify *E. coli*, Classical methods based on the biochemical methods for the detection the *E. coli* isolates. In the last decade, there are various types of the molecular assays had been used to identify the *E. coli* isolates by detecting the species-specific gene (11).

The aims of this study was to isolate and detect *E. coli* from dairy farm in various regions in the Nineveh governorate. Additionally, the *E. coli* isolates were identified using the traditional methods as morphological and biochemical tests and confirmed the result by employed the PCR method depending on the *uidA* gene.

Materials and methods

Samples collection

Four hundred samples were gathered randomly from various areas of the dairy farm (50 samples from each of bedding, soils, feces, feed, milk, water, worker's hands, and flies) were collected from different regions including (Namrod, Al Hawy, Al Kaser, Kaukagly, and the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Mosul University) located around Nineveh Governorate between September 2020 and January 2021. The milk samples were gained directly from the udder as following: all the teats were cleaned then dried by using a single towel, the teat end was dipped using 70% ethanol. One to three foremilk streams were discarded, then the subsequent milk was placed in antiseptic tubes. While others types of samples were collected by using the sterile sacs. All the samples stored in the cool box (12) and transported to the Department of Veterinary Public Health, College of Veterinary Medicine.

Identification of bacterial cultures

All the types of samples were inoculated in the nutrient broth (LAB, United Kingdom) and incubated for 24 h at 37°C. After that, 0.1 ml of the broth was streaked on the EMB agar and MacConkey agar (LAB, United Kingdom) and incubated for 24 h at 37°C. The *E. coli* colonies were distinguished depended on the morphology of colonies which able to form the metallic sheen on the EMB agar and dark pink color on the MacConkey agar. All suspected colonies were identified by using biochemical methods such as Gram,s stain, oxidase, catalase, triple sugar iron agar, Indole test, methyl red test, citrate utilization test, and Voges-Proskauer test (13).

DNA extraction and template preparation

The suspected *E. coli* were streaked on EMB agar. The Extracted DNA of *E. coli* isolates was extracted according the instructions of DNeasy Blood and tissue kit (Geneaid, Korea).

PCR reaction

For the identification of *E. coli*, the *uidA* gene of *E. coli* was amplified by using the polymerase chain reaction method. The molecular weight of the *uidA* gene that amplified to detect the *E. coli* isolates is 623 bp (14). The whole volume of the PCR reaction was 25 µL which including: (i) 1 µL primer F 5-CCAAAAGCCAGACAGAGT-3 10 pmol/µL (Eurofins Genomics, Germany), (ii) 1 µL of primer R 5-GCACAGCACATCCCCAAAGAG-3 10 pmol/µL (Eurofins Genomics, Germany), (iii) 12.5 µL of 2×Go Taq Green Mix Master including (1 unit GoldStar DNA polymerase, 400 µM dNTPs, 3 µM $MgCl_2$, 20 µM $(NH4)_2SO4$, 75 µM TrisHCl (pH 8.5), green dyes which function as loading dye (Promega Corporation, USA), (iv) 8 µL of nuclease-free water (Promega Corporation, USA), and (v) 2.5 µL DNA template of *E. coli*. The mixture was placed in PCR reaction tube (Biozym, Oldenhorf, Germany). The thermocycler program was based on three steps: (i) 5 minutes at 95°C for the denaturation, (ii) 35 cycles, where each cycle consisted of denaturation (for 60 sec at 94°C); annealing (for 60 sec at 53°C); and extension (for 60 sec at 72°C), and (iii) 5 minutes at 72°C for the final extension. Finally, the amplicons were determined by gel electrophoresis together with DNA marker 100 bp ladder in 2% agarose gel (Peqlab, Erlangen, Germany).

Results

The results of our study showed that out of 400 samples collected from different dairy herds, 140 (35%) of *E. coli* were positive. All the *E. coli* isolates appeared positive results according to the biochemical tests used to identify the *E. coli* isolates. The high proportion of the *E. coli* isolated was from the flies 76% (38/50), while the low percentage of *E. coli* was from worker's hands 12% (6/50). The percentage of *E. coli* isolated from bedding, feces, milk, soils, feed, and water was 46% (23/50), 38% (19/50), 34% (17/50), 28% (14/50), 28% (14/50), and 18% (9/50), respectively (Table 1).

Table 1: Number and proportion of *E. coli* isolates from dairy farms

Samples	No. of sample	No. of isolate (%)
Feces	50	19 (38%)
Milk	50	17 (34%)
Water	50	9 (18%)
Soils	50	14 (28%)
Bedding	50	23 (46%)
Fly	50	38 (76%)
Worker's hands	50	6 (12%)
Feed	50	14 (28%)
Total	400	140 (35%)

Additionally, the results of the present studies declared which all the positive *E. coli* have been possessing the *uidA* gene that is the species-specific gene for *E. coli* by using the PCR assay (Figure 1). The results of classical methods had concurred with the results of the PCR test.

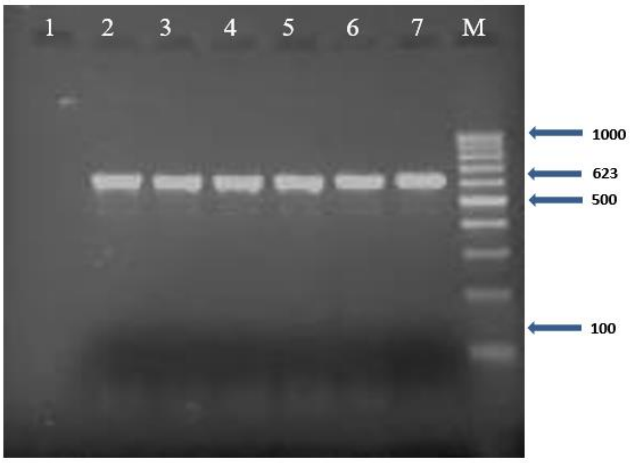

Figure 1: Agarose gel electrophoresis (2%) showing the typical amplicon of the *uidA* gene product of *E. coli* isolates (623 bp).

Discussion

The importance of *E. coli* is concentrated on human public health and animals because they have many virulence factors that cause more morbidity and mortality and they had resistant to many antibiotics that cause reduced treatment chances. *E. coli* may be transferred from animals to humans by consumer's milk and its products, direct contacts with animals, and indirect through they spread in the environment so that, there is a relationship between the *E. coli* isolated from humans and animals. The current study was purposed to investigate and detect the *uidA* gene in the *E. coli* isolates. Four hundred samples were gained from the various dairy farm areas (50 samples from each of bedding, soils, feces, feed, milk, water, worker's hands, and flies) randomly collected from different regions around Nineveh Governorate between September 2020 and January 2021.

The result of our study revealed the percentage of *E. coli* isolated from the dairy plant was 35%. The result of our study is more than other studies which were appeared the percentage of *E. coli* isolated from the dairy farms was 4.7% and 5% (15,16). The difference of the results between the studies due to applying the farms' management practices in the dairy farm which lead to prevent the pathogenic bacteria to spread among the dairy herds (17). The clean the floor of the farm, all the utensils used , and provide the safety water for the dairy farms are important to prevent the *E. coli* to growth and proliferation among cows that helps to reduce risk of bacterial contaminations and infection (18). In addition, our study appeared that *E. coli* has been isolated highly percentage from fly 76%, bedding 46%, and feces 38%. Many of studies referred that the flies, bedding, and feces are played an importance role for spreading *E. coli* in the dairy farms and caused the infections in the dairy herds. Most of dairy cattle breeder in the open-house system which allows the flies and birds to fly freely to find food and water that they may cause contaminated, so that used the disinfectant and sterilizers to control of all the flies in the farms (19).

Additionally, our result appeared that *E. coli* found in the milk was 34%, soils and feed was 28%. The rate of *E. coli* isolated from milk in this study is high than in other studies which had recorded rate of *E. coli* isolated from milk in Ethiopia was 26.6% (20), and In India was 18.5% (21). The various rate of *E. coli* isolated from milk in many studies is due to apply the sanitary conditions during milking, cleaning the milking machines, and clothes of workers which play the role for transfering of *E. coli* to milk (22). The percentage of *E. coli* from the feed in this study was nearby with Lynn *et al.* (23) that recorded *E. coli* isolated from feed was 30.1%, while the result of our study is lower than the other studies which recorded 48% and 50% (24,25). The feed will contaminate with *E. coli* due to use the dirty vehicles for transferring feed and storage in the bad conditions as well as feed may contaminate by exposed to feces of a birds and flies contaminated with *E. coli* (26).

Our study revealed the *E. coli* isolates found in the water 18% that was lower than another study which isolated *E. coli* in the water was 23% (27). The water used to drink is contaminated with feces of infected cattle by *E. coli*, also the fresh water may be contaminated by flies carried by different types of an organism such as *E. coli* (28). In addition, *E. coli* found in worker's hands 12%. the result of our study is lower from other study which showed that *E. coli* found in had workers was 26% (29). The workers may be infected with *E. coli* by direct contact with infected dairy herds and indirect transmission through the environment also, most workers do not wash their hands or not use the disinfectants and the antiseptics after contact with animals that prevent or reduce transport *E. coli* from animals to the workers (22). Many studies showed that there are similarities between *E. coli* strains isolated from humans and animals (30,31).

Conclusion

The result of the present study indicates that the high rate of *E. coli* was isolated from flies, bedding, and feces so that they may be a reservoirs of *E. coli*. The PCR assay is a modern technique used to confirm that all isolates detected by classical methods were *E. coli*. All the owners must use the new practical programs to manage the dairy farms and used the disinfectants and antiseptics to kill all the flies and rodents which play a role to spread the *E. coli* in the dairy farms.

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Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this manuscript.

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التشخيص الجزيئي لجين *uidA* في سلالات الايشريكيا القولونية المعزولة من مزارع الأبقار في محافظة نينوى، العراق

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الخلاصة

تعد الايشريكيا القولونية كائن حي مجهري بيئي، سالبة الكرام مسببة لالتهاب الثدي في الأبقار والتسمم الغذائي للإنسان. جمعت ٤٠٠ عينة من مناطق مختلفة للفترة من شهر أيلول ٢٠٢٠ إلى شهر كانون الثاني ٢٠٢١ من مزارع الأبقار إذ شملت ٥٠ عينة عشوائية لكل من الفراش، الرمل، والبراز، الأعلاف، الحليب، المياه، أيدي العمال، والذباب من مناطق مختلفة حول محافظة نينوى. شخصت السلالات اعتمادا على الطرق التقليدية باستخدام الفحوصات الشكلية والكيمياء الحياتية، كما استخدمت تقنيه تفاعل السلسلة المتبلمرة لتحديد الجين *uidA* فيها. أظهرت نتائج هذه الدراسة أنه من بين ٤٠٠ عينة تم جمعها من قطعان الأبقار، كانت ٣٥% (١٤٠) من الإشريكية القولونية إيجابية. بينما كانت النسبة المئوية للإشريكية القولونية المعزولة من الذباب، والفراش، والبراز، والحليب، والرمل، والأعلاف، والمياه، وأيدي العمال:٧٦٪ (٥٠/٣٨)، ٤٦٪ (٥٠/٢٣)، ٣٨٪ (٥٠/١٩)، ٣٤٪ (٥٠/١٧)، ٢٨% (٥٠/١٤)، ٢٨% (٥٠/١٤)، ١٨٪ (٥٠/٩)، ١٢٪ (٥٠/٦) على التوالي. جميع سلالات الإشريكية القولونية الإيجابية تمتلك الجين *uidA* وهو الجين الخاص بنوع الإشريكية القولونية والذي تم تحديده باستخدام اختبار تفاعل السلسة المتبلمرة. وقد توافقت نتائج الطرق الكلاسيكية مع نتائج اختبار تفاعل البلمرة المتسلسل. واكدت الدراسة ان الحليب وأيدي العمال والذباب لعبت دورًا مهمًا في انتشار الإشريكية القولونية في مزارع الأبقار. تعتبر تقنية تفاعل البلمرة المتسلسل هي طريقة سريعة الذي يساعد على إعطاء الأدوية المناسبة في علاج الماشية المصابة بالتهاب الضرع ومنع انتشار الإشريكية القولونية بين قطعان الأبقار.