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Animal as Potential Reservoir of Diarrheagenic Escherichia coli



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Abstract

HIS study was designed to determine the type of bacteria in animal that my cause diarrhoea. This study includes 100 samples form sheep and cows, which were collected from private veterinary clinics. These samples were transferred to this laboratory, and all the bacterial isolates were diagnosed based on microscopic and phenotypic characteristics and biochemical tests. The E. coli bacteria was recorded the highest percentage of 59 [47%] of the bacterial isolates followed by Proteus spp. which recorded 36 [26%], while both bacterial isolates of Pseudomonas spp. and Salmonella enterica were 18 [13%] and 8 [5%], respectively. Out of 100 fecal samples from cultured calves and sheep, 126 bacterial isolates were examined and obtained. The E. coli bacteria recorded the highest percentage of 59 [47%] of the bacterial isolates. E. coli. Was recorded the highest percentage in the samples from children and animals [calves and sheep], they were 56% and 47%. The diarrhea samples contained different types of bacteria where the 65 [65%] of the samples were contained E. coli and 15 [15%] of them were contained Salmonella enterica and E. coli. [47%] of the stool samples were detect strains of E. coli bacteria. All isolates [47%] contained the uidA gene which confirmed the identification of E. It was found that the most important type of bacteria that causes diarrhea in children and might be transmitted from animals was E. coli. The most important diarrhea-causing strains of E. coli isolates in sheep calves were ETEC and EHEC.

Keywords: Calves, Sheep, Bacteria, Diarrhea, E. coli.

Introduction

Diarrhea is defined as a pathological condition resulting from a functional defect in the digestive system [1]. The normal rate in a watery or loose form leads to the loss of salts such as sodium and potassium from the body, which leads to an increase in the acidity of the blood and muscle contraction [2]. The Enterobacter family is one of the most widespread germs in nature, They inhabit the intestines of humans and animals naturally and cause simple or severe infections such as Salmonella, Shigella, and cause gastroenteritis such as *E.coli* [3].

One of the most important pathogenic intestinal species, Enterobacter, Proteus, Klebsiella, Salmonella, Yersinia, and Escherichia [4]. The *E.coli* germs live naturally in the intestines of humans and animals. At the same time, they are opportunistic germs that cause many diseases such as diarrhea and blood poisoning [5]. They are among the most common types of germs that cause intestinal poisoning [6].

The pathogenicity of these germs is due to the possession of many virulence factors, and among these factors is the possession of Shiga toxin, siderophores and cytotoxic necritizing factor, and the possession of surface structures such as flagella and capsules, lipopolysaccharides [LPs] which gives the bacteria antigenic characteristics, by producing flagellate antigen (H), somatic antigen (O), and capsular antigen (K), and also possesses pilli, fimbriae that help them adhere to the host tissues, thus giving them the ability to form a biofilm [7]. During the last decades, E.coli has been associated with many foodstuffs, such as meat, milk and dairy products, eggs, and mayonnaise [8]. Germs of E.coli are transmitted from ruminants to humans directly and indirectly through the contaminated foods such as uncooked meat and unpasteurized milk, or through direct contact with the animal during slaughter [9]. In his view, individual cases or herds, therefore, many studies indicated that the relationship between the E.coli and the host is symbiotic [10]. Isolating and diagnosing E.coli that causes diarrhea in children. Isolation and diagnosis of *E.coli* that causes diarrhea in animal

Material and Methods

Chemical Materials

The laboratory equipment and supplies that were used in the present study are grose from Biolab (USA). Green Master mix from Intron Bio /Korea,Primers from Oligomer /Turky DNA extraction Kit from Chelex Bio Rad (USA), Alphanaphthol from BDH(England). Methyl red from BDH(England) Ethanol from BDH(England), KOH from BDH(England), Red safe from BDH(England), Crystal violet from Biomerieux (France), Tetra methyle-para-phrn-dimine dihdrochloride from BDH (England), Urea from Fluka (Switzerland)

Culture media

- Culture Media from Manufacture (Origin).
- Nutrient agar from Mast Diagnostic (UK).
- MacConkey agar from Mast Diagnostic (UK).
- Eosin Methylene Blue agar from Mast Diagnostic (UK).
- Brain heart infusion agar fom Oxoid(UK).
- Brain heart infusion broth from Oxiod (UK).

Specimens Collection

In this study, 73 fecal animal's samples were collected, including calves and sheep that were infected, with ages ranging from one month to 8 months and the same period, and they were collected through private veterinary clinics within Tikrit and its districts. The samples were collected in swabs containing a preservative for the growth of bacteria for a period of 24 hours, sterilized and transferred under refrigerated conditions to the laboratory for laboratory tests and bacterial culture.

Laboratory Diagnosis (Isolation and identification of E. coli)

One colony was taken from each positive culture on MacConkey agar, then it is identified depending on the morphology properties that include colony shape, color, nature of pigments, edge, elevation and texture. The specific shape, type of reaction, aggregation and staining bacteria with Gram stain for microscopic examination [11]. The Gram negative bacilli were transferred to MacConkey, eosin methylene blue agar (EMB) and chrom agar to clearly differentiate between the colonies of *E.coli* and other. All plates were incubated aerobically at 37°C for 24 hrs..

Biochemical test

- The following tests were conducted.
- *Oxidase test
- *Catalase test
- *Methyle red test
- *Indole test

- *Voges -Proskauer test
- *Citrate utilization test
- *Urease test
- *H₂S test

All bacterial isolates were diagnosed based on microscopic and phenotypic characteristics and biochemical tests after growing them in aerobic conditions in high altitude McConkey medium, EMP, and other media mentioned in a tables (3-3) according to Mahon (2007) [11,12], in addition to diagnosis by biochemical tests.

Preservation and maintenance of bacterial isolates

After diagnosis, the bacterial isolates were preserved on slanted culture media from nutrient agar at a temperature of 4 °C, and the maintenance process continued on a monthly basis by renewing their culture on new media to ensure that they remain active throughout the study period. This preservation is short-term.

As for the long-term preservation of the isolates, a brain-heart infusion broth medium was used at which glycerol was added at a rate of 15 without the possibility of losing some of its genetic characteristics, as a test tube containing 5 ml of the medium was inoculated with one colony and the culture was incubated for 24 hours, then 0.85 ml of the culture was transferred to bottles with a tight-fitting cap containing 0.15 ml of sterile glycerol. The contents were mixed by turning the tube up and down several times and store the crops at -20 C until use.

Molecular Biology Experiments

Extraction of DNA

Bacterial DNA was extracted using Celex100 and transferred to tubes containing 200 μ L of Celex100 and 100 μ L of TE. The tubes were eluted, centrifuged, and the upper aqueous layer was removed, stored in tubes at -4°C.

Agarose gel electrophoresis: involves dissolving agarose powder in 1X TBE buffer, with varying concentrations for different purposes. The gel is then poured into a gel tray, comb placed, and dried. Samples are loaded into separate wells, and electrodes are connected. The run time is 45 minutes for genomic DNA and 1 hour and 30 minutes for PCR product.

Specific primers used in the Multiplex PCR reaction

The study used polymerase chain reaction (PCR) to amplify identified genes and identify virulence factors related to E. coli strains. The coding genes, such as vt1 and vt2, indicate pathological patterns from E. coli isolates. The PCR products were separated, and E. coli isolates were electrophoresed on a 1.5% agarose gel containing Ethidium Bromide (EtBr). Results were then taken under ultraviolet light to identify the types of E. coli strains.

Screening for virulence factor genes using multiplex polymerase chain reaction technology

PCR reactions were conducted under sterile conditions for approximately 80 isolates of Ecoli bacteria using specific primers (LT,ST,bfpA,EA,daaE,eae,vt1,vt2,SHIG,uidA) For genes encoding virulence factors for the purpose of diagnosing pathological types of DEC

Results

Identification of bacteria isolated from diarrhea samples

Out of 100 cultured animals (calve and sheep) stool samples, 140 bacterial isolates were examined and obtained. According to the results of routine biochemical and microscopic tests used in the process of identifying bacteria, the types of bacteria were identified through their phenotypic characteristics of cultural colonies. The *E. coli* recorded the highest percentage 78 (56%) of bacterial isolates, followed by *Proteus spp.* which recorded 36 (26%), while both bacterial isolates of *Pseudomonus spp.* and *Salmonella enterica* 18 (13%) and 8 (5%), respectively, as shown in Figures (1) and (2).

Analysis of the Table 1 reveals that samples were examined for four different bacterial species. Among these samples, *E. coli* was the most prevalent at 56%, with 78 isolates. Proteus spp. followed as the second most prevalent at 26%, with 36 isolates. Pseudomonas spp. ranked third at 13%, with 18 isolates. Lastly, Salmonella Enterica was the least prevalent at 5%, with only 8 isolates.

The analysis of the results reveals that out of the total 126 bacterial isolates obtained from fecal samples of calves and sheep, 59 isolates (47%) were identified as *E. coli*, while the remaining 67 isolates were categorized as "Other" and discarded.

Resulting in 65 samples (65%) testing positive for *E. coli*. Among these samples, 15 (15%) showed a co-infection of *E. coli* and Salmonella Enterica. Additionally, 20 samples (20%) did not contain any of the common bacteria associated with children's diarrhea.

Genetic content of E. coli isolates in human and animal diarrhea samples

The 78 *E. coli* isolates from children's stool samples and 59 from calves and sheep fecal samples were examined and identified by molecular analysis after genomic (DNA) was extracted as the previously described

Detection of diarrheagenic E. coli strains in animals using Multiplex PCR technique

Regarding animal samples (calves and sheep), 59 (47%) of their stool samples were analyzed to detect strains of *E. coli* bacteria. All isolates (47%) contained the *uidA* gene which confirmed the

identification of E. coli. The results were positive for diarrhea-causing E. coli strains tested using Multiplex PCR for the *uidA* gene. According to the type of diarrhea-causing strains isolated in the current study, it is clear from Figures (2 and 3) that the genes that indicate the most important diarrhea-causing strains of *E. coli* isolates in sheep calves were ETEC and EHEC.

Discussion

Diarrhea is considered one of the public health problems that children still suffer from in all countries of the world, the contamination of the home environment by the presence of animals such as calves, sheep, and rabbits, or contact with them, and the presence of the feces of these animals, leads to direct or indirect contamination [12, 13, 14].

The findings indicate that *E. coli* was the most prevalent bacterium among the studied samples, highlighting the importance of studying this bacterium and analyzing its potential impact on animal health and public safety.

Such studies provide information about the nature of the common disease relationship between humans and animals [14, 15, 16].

The results showed a high prevalence of *E. coli* in the feces of calves and sheep, followed by Salmonella bacteria. The reason for this may be attributed to the fact that *E. coli* is widely spread among field animals and is transmitted through contamination to humans [17 and 18].

Investigating the presence of various germs in the digestive system of animals is important for controlling diseases that affect their digestive system [19].

Our current results indicated that *E. coli* was ranked first in the feces of calves and sheep, out of 100 stool samples from which we obtained 126 isolates, and the percentage of *E. coli* was 47%.

The results showed that children's stool samples also recorded high rates of *E. coli* infection, close to what was recorded in animals, and there may be common mechanisms for the paths taken by these bacteria, as well as commonalities between them [20 and 21].

The infection caused by this bacteria in animals may be different from that in humans and possess specificity, although the possibility of infection from animals remains[22].

E. coli is a bacteria widely spread in the environment. Organic pollution with animal waste and animal and organic fertilizers may increase the contamination of fruits and vegetables, which eating them without washing and sterilizing leads to the transfer of the bacteria to the human digestive system. Also, direct contact between humans and

animals and living together may lead to the spread of infection [23].

The results emphasize the importance of paying attention to personal hygiene, isolating animals from humans, and paying attention to sanitary isolation of contaminated water in hospitals and sewage, as well as paying attention to the environment of calves and sheep and the water provided to them, and identifying sources of infection to reduce them.

According to these results, the E. coli strains detected in the feces of calves and sheep were ETEC and EHEC, where 42 (71%) of the isolates contained ETEC strains, which were the most common, while only 17 (29%) of the isolates contained EHEC strains. Also, this result is consistent with the results of previous studies, including a study conducted in Ethiopia and a study conducted in Iran. They mentioned in their results that the most common strain of E. coli isolates detected in young calves was ETEC, followed by the EHEC strain [24, 25]. While this result did not agree with the results of a study conducted in Ethiopia, which reported the absence of the ETEC strain in E. coli isolates in calves, this study is consistent with the results of our study as it reported the detection of the EHEC strain in E. coli isolates in calves, and was (30%) [26]. which is close to the result the study obtained.

The results of the current study confirmed that there are strains of *E. coli* isolates, ETEC and EHEC, present in samples from children and animals. In addition, bacterial diseases and infections were transmitted to humans in several ways, including through direct contact with these animals, contamination of food and water with their feces, and the spread of infection in the places where these animals live, especially when they suffer from diarrhea. In other words, from the results obtained

for both human and animal samples, animals can be a reservoir for human infection with Escherichia coli strains that cause diarrhea.

Conclusion

There is a type of transmission connection between *E. coli* bacteria between animal strains in calves and sheep, as well as humans, and the research requires further research and investigation.

Acknowledgment

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Funding statement

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical approval

Ethical approval was obtained according to No. 7/23/67/55 on 20/11/2023, and scientific methods were followed in ethical dealing with animals, according to the instructions of the Ministry of Higher Education and Scientific Research in Iraq.

Author's contributions

The first researcher carried out the practical aspect and prepare the manuscript for submitting. The second participated in designing the research, completed the task of statistical analysis, making tables, and writing.

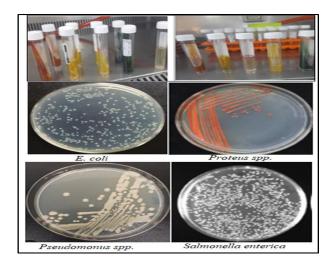
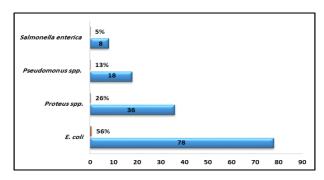


Fig. 1. The different colonies of bacteria isolated from diarrhea samples.



 $Fig.\ 2.\ The\ percentages\ of\ distribution\ of\ bacterial\ types\ that\ were\ isolated\ from\ diarrhea\ samples.$

TABLE 1. The distribution of bacterial isolates

Bacterial Species	Number of Isolates	Percentage of Total Isolates
E.coli	78	56%
Proteus spp.	36	26%
Pseudomonas spp.	18	13%
Salmonella enterica	8	5%

TABLE 2. Out of 100 fecal samples from cultured calves and sheep.

Bacterial Species	Number of Isolates	Percentage of Total Isolates
E. coli	9	47%
Other (discarded)	7	53%

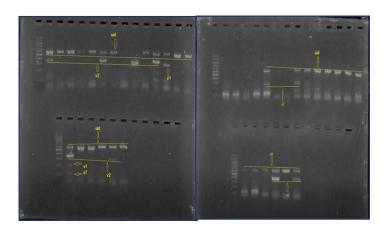


Fig. 3. Electrophoresis on agarose gel by 1.5% concentration for the mPCR reaction product for E. coli isolates from calves' samples.

TABLE 3. The prevalence of bacterial causes in samples from calves and sheep.

Bacterial Causes	Number of Samples	Percentage of Total Samples
E. coli	65	65%
E. coli and Salmonella enterica	15	15%
Other (no common bacteria with children's diarrhea)	20	20%

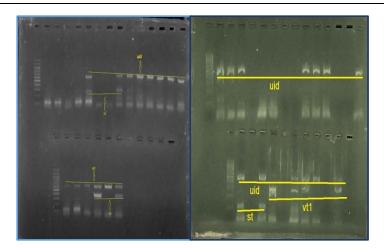


Fig. 4. Electrophoresis on agarose gel by 1.5% concentration for the mPCR reaction product for E. coli isolates from sheep's samples.

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الحيوان كمستودع محتمل للإشريكية القولونية المسببة للإسهال

رحمة صلاح طلحة و نهاد عبدالحسين جعفر

فرع الاحياء المجهرية - كلية الطب البيطري - جامعة تكريت - تكريت - العراق.

الملخص

صممت هذه الدراسة لتحديد نوع البكتيريا الموجودة في الحيوان والتي تسبب الإسهال. شملت هذه الدراسة 100 عينة من الأغنام والأبقار تم جمعها من العيادات البيطرية الخاصة. تم نقل هذه العينات إلى هذا المعمل، وتم تشخيص جميع العزلات البكتيرية بناء على الصفات المجهرية والمظهرية والاختبارات الكيموحيوية. سجلت 100 أعلى نسبة 100 والميل العزلات البكتيرية تليها Proteus على المعوية كانت 100 والتي سجلت 100 والميل المعوية كانت 100 المستزرعة. والتي سجلت 100 المستزرعة تليم المستزرعة من أصل 100 على التوالي. تم فحص 100 عزلة بكتيرية من أصل 100 عينة براز من العجول والأغنام المستزرعة. سجلت بكتيريا 100 أعلى نسبة 100 (100 ألم العزلات البكتيرية. بكتيريا قولونية. وقد سجلت أعلى نسبة في العينات من الأطفال والعجول والأغنام) حيث بلغت 100 والحيات الإسهال على أنواع مختلفة من البكتيريا حيث احتوت 100 (100) من العينات على الإشريكية القولونية و100 (100) منها احتوت على السالمونيلا المعوية والإشريكية القولونية. والمسببة الإسهال عند الأطفال والتي قد تنتقل من الحيوانات هي 100 الذي أكد تشخيص بكتيريا 100 المسببة للإسهال عند الأطفال والتي قد تنتقل من الحيوانات هي E. coll . وحد أن أهم نوع من البكتيريا المسببة للإسهال عند الأطفال والتي قد تنتقل من الحيوانات هي E. coll .

ا**لكلمات الدالة:** العجول، الأغنام، البكتيريا، الإسهال، الإشريكية القولونية.