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Plasmids Prevalence and Patterns in Diarrheagenic Escherichia Coli Reflect

the Bacterial Transmission Possibility

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Abstract

Diarrheagenic Escherichia coli are the most common strains of E. coli that animals and humans are exposed to. It can be classified into six pathogenic groups, regarding their antibiotic resistance ability and the presence of virulence genes. Most of them are located on mobile genetic elements such as plasmids. Thus, this study aimed to identify the plasmid DNA patterns from E. coli isolated from dogs and humans each of which has diarrhoea. In order to search for similarities between the plasmids distributed among them, plasmid patterns as bands on agarose gel and plasmid incompatibility replicons detection were applied. A multiplex PCR-based method was used to detect the presence of the most common plasmid replicons (B/O, FIA, FIB, II and Frep). The results showed that almost all E. coli isolates contain plasmids. The IncF (FIA and FIB) replicons were the most prevalent Inc (60%) among human isolates. In contrast, the dominant Inc replicon in dog isolate was Frep followed by FIB and FIA in rates reached 35% and 25% respectively. Whereas, the II replicon was the least common in both sources. The dual plasmids Inc Frep+FIB and Frep+FIA were found to be the shared types. In contrast, one of the triple Incs patterns (FIB+Ferp+B/O) was common among dog and human isolates. This pattern's similarity indicates the bacterial transmitted ability and triggered a preliminary idea about the possibility of plasmid transmission between E. coli from different hosts.

Keywords: Escherichia coli, Plasmid, Bacterial Transmission.

Introduction

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium, which is an important part of the healthy intestinal tract of humans and animals (1). Generally, E. coli is considered a non-pathogenic bacterium, but some serotypes can cause diseases inside and outside the intestinal tract (2). The most common diseases caused by E. coli that affect both animals and humans are the various cases of diarrhoea (3). Diarrheagenic E. coli (DEC) are the major strains that animals and humans are exposed to since transmitted they can be through contaminated food, water and direct contact with animals or persons (4). Diarrheagenic E. coli is classified into six pathogenic groups; Enteropathogenic Escherichia coli (EPEC), Enterotoxigenic Escherichia coli Enterohemorrhagic (ETEC), coli (EHEC/STEC), Enteroaggregative Escherichia coli (EAEC), adherent-invasive Escherichia coli (AIEC) and entero-invasive Escherichia coli (EIEC) (5). All of these types infect humans however, only the first three are common between humans and animals (6). Diarrheagenic pathotypes differ regarding their preferential colonisation virulence host sites. mechanisms, and the ensuing clinical symptoms and consequences (4).

The ability of these bacteria to cause infection is related to several factors. The most intuitive of them is the existence of virulence factors, which are associated with a wide range of activities (7). Some of those related to bacteria colonization and others correlated to virulence factors include; adhesins, toxins, iron acquisition factors, lipopolysaccharides, polysaccharide capsules, and invasions, which are usually encoded by plasmids and other mobile genetic elements (8).

Plasmids are extrachromosomal DNA molecules that replicate autonomously. They

are inherited stably and can exist in several copies per cell (9). Plasmids usually include at least one gene, which is always useful to the host cells. They can allow bacteria to survive in an extreme environment, encode antibiotic resistance, heavy metal resistance and produce virulence factors that help a bacterium colonize a host and overcome its defenses (10). Some of them can carry antibiotic encoding agents, which would help the cells fight other organisms and survive in the challenging environment (11). Plasmids can be classified into several class types. One of the most plasmid recognition methods is the incompatibility (Inc) groups. Based the plasmid replication on mechanism, they can be categorized into several Inc groups such as B/O, FIA, FIB, II and Frep Inc (12). A microbe can have a variety of plasmids, but only compatible plasmids can coexist in a single bacterial cell. If two plasmids are incompatible, one of them will be quickly eliminated from the cell. Distinct plasmids may be classified into distinct incompatibility groups based on their ability to coexist. Incompatible plasmids (those belonging to the same incompatibility group) typically use the same replication or partition mechanisms and so cannot coexist in a single cell (13).

Plasmids can be transferred from one bacterial cell to another using one of the genetic transfer methods (14). Thus, the spread of these genes (carried by plasmids) between the bacterial cells from different hosts would cause real health and economic issues and raise one health concern much more than those spreading among the bacterial cells from the same host type.

For these reasons, this study included extracting plasmids of *E. coli* bacteria isolated from diarrheic dogs (Since dogs are one of the most common pets that accompany humans) and patients. This helps to compare the number of types and patterns

of plasmids distributed among them. In order to verify the presence of similarities which give preliminary confirmation idea about the transmission of bacteria or plasmids themselves between different hosts. This would help in how to control, resist and treat these bacteria.

Materials and methods Sample collection

Forty-two faecal samples were evenly collected from two different groups, twenty-one samples from dogs and twenty-one samples from humans from April 2024 to August 2024. The dog samples were collected from various veterinary clinics and hospitals in Basrah Governorate. The human samples were obtained from patients in Al-Sadr Teaching Hospital. Samples collection was done using swabs and collection cups, then transported directly to the central laboratory at the College of Veterinary Medicine, University of Basrah within a period not exceeding two hours to perform bacterial culture.

Isolation and identification of *E. coli*

Samples were directly swabbed on MacConkey agar and nutrient agar (as a control), and incubated at 37°C for 24 hrs. After the first incubation, well-defined single pink colonies were picked up and streaked on Eosin-methylene blue (EMB) agar using a sterile loop. Plates were then incubated at 37°C for 24 hrs.

DNA Extraction

Genomic DNA (gDNA) of all bacterial isolates was extracted using the boiling method (15). Bacteria was cultured in 5ml nutrient broth containing falcon tubes, incubated in a shaking incubator at 37°C, 180rpm for 24 hrs. The bacterial pellet was harvested by centrifugation at 5000 rpm for 5 min, then resuspended with 100μL molecular grade water and transported into a

clean 1.5ml Eppendorf tube. Subsequently, the Eppendorf tube was incubated in a water bath at 100° C for 10min. Then 450μ L of molecular grade water was added to the components and mixed well, followed by a 10 min centrifugation at 12,000rpm. The supernatant (containing the gDNA) was collected in a new Eppendorf tube and stored at -20° C until it was used.

Molecular Detection of E. coli

A species-specific pair of primers was used to confirm the isolated E. coli identity by partially amplifying the Maltose operon protein B (malB) gene (16). A 20µL PCR reaction mix was prepared by adding (10µL) GoTaq green master mix, (1µL of 10pmol/ uL) forward primer and reverse primer, (3µL) template DNA and (5µL) nucleasefree water. The mixture was subjected to a PCR amplification program started with an initial denaturation step at 95°C for 5min, followed by 35 cycles for each of the following steps: denaturation 94°C/ 30sec, annealing 62°C/ 30sec, extension 72°C/ 40sec, ending with a final extension step at 72°C/ 5min and held at 4°C. The produced PCR products were analysed and sizefractionated by running on 1.5% agarose gel. The amplicon's sizes were measured by loading a standard DNA ladder (100bp Promega) alongside the samples. The gel was run in a TBE buffer at 100V (constant voltage) for 45min. The DNA bands (replicons) were displayed on the UV transilluminator safety imaging system in the presence of DNA safe dye. The Band's size was estimated by comparison to the DNA ladder bands.

Plasmid DNA extraction

Plasmid DNA was extracted using a commercial kit (Solarbio, China) designed to lyse the cell by alkali pyrolysis, and then specifically adsorb DNA using a centrifugal adsorption column in a high-salt condition.

Plasmid profiling using the replicon typing method

Plasmids were originally classed based on their incompatibility with other plasmids, which is related to replication site sequences. Incompatibility (Inc) typing is based on the fact that two plasmids sharing replication common and partitioning components cannot proliferate stably in the same cell line (17). The extracted plasmid DNA of each E. coli isolate was subjected to a multiplex PCR of five pairs of primers to examine the presence of the five most frequent different replicons (B/O, FIA, FIB, II and Frep) located on Diarrheagenic E. coli. This could indicate how many types of plasmids exist, despite their copy number, which would help generate a plasmid profiling, subsequently, comparing similarities of the obtained patterns between the two sample groups. A 30µL PCR reaction mix was prepared by adding (15µL) green master mix, (1µL of 10pmol/μL) forward primers and reverse primers, (2µL) template DNA and (3µL) nuclease free water. The mixture was subjected to a PCR amplification program started with an initial denaturation step at 95°C for 1min, followed by 30 cycles for each of the following steps: denaturation 94°C/ 30sec, annealing 60°C/ 30sec, extension 72°C/ 45sec, ending with a final extension step at 72°C/ 10 min and held at 4°C. The produced PCR products were analysed and size-fractionated by running on 1.5% agarose gel. The amplicon's sizes were

measured by loading a standard DNA ladder (100bp Promega) alongside the samples. The gel was run in a TBE buffer at 100V (constant voltage) for 50min. The DNA bands (replicons) were displayed on the UV transilluminator safety imaging system.

Detection of some virulence genes on plasmids

Five genes (iutA, stx1, stx2, eaeA and cnf1) among the genes that are supposed to exist and which some recent studies have confirmed their role in virulence enabling E. coli to cause infection were investigated (Table, 1). A multiplex PCR was done to detect the presence of these genes (18). A 30µL PCR reaction mix was prepared by adding (15µL) GoTaq green master mix, (1μL of 10pmol/ μL) forward primers and reverse primers(table,2), (2µL) template DNA and (3µL) nuclease-free water. The subjected mixture was to a **PCR** amplification program started with an initial denaturation step at 95°C for 4min, followed by 30 cycles for each of the following steps: denaturation 94°C/ 30sec, annealing 63°C/ 30sec, extension 72°C/30sec, ending with a final extension step at 72°C/5 min and held at 4°C. The produced PCR products were analyzed and size-fractionated by running on 1.5% agarose gel. The amplicon's sizes were measured by loading a standard DNA ladder (100bp Promega) alongside the samples. The gel was run in a TBE buffer at 100V (constant voltage) for 50min. The DNA bands (replicons) were displayed on the UV transilluminator safety imaging system.

Table 1: Role and function of targeted virulence genes

Targeted genes	Function	Reference	
iutA	iron acquisition	(19)	_
Stx1, Stx2, Cnf1	Toxicity	(20)	
eaeA	attachment of EPEC to epithelial cells.	(21)	

Table 2: PCR primers used in this study

Primers name	primer sequence $5' \longrightarrow 3'$	Amplicon size	Reference
malB	F: GACCTCGGTTTAGTTCACAGA R: CACACGTGACGCTGACCA	585 bp (16)	
B/O	F: GCGGTCCGGAAAGCCAGAAAAC R: TCTGCGTTCCGCCAAGTTCGA	159 bp	
FIA	F: CCATGCTGGTTCTAGAGAAGGTG R: GTATATCCTTACTGGCTTCCGCAG	462 bp	
FIB	F: GGAGTTCTGACACACGATTTTCTG R: CTCCCGTCGCTTCAGGGCATT	702 bp	(22)
II	F: CGAAAGCCGGACGCAGAA R: TCGTCGTTCCGCCAAGTTCGT	139 bp	
Frep	F: TGATCGTTTAAGGAATTTTG R: GAAGATCAGTCACACCATCC	270 bp	
iutA	F: GGCTGGACATGGGAACTGG R: CGTCGGGAACGGGTAGAATCG	300 bp	
stx1	F: ACGTTACAGCGTGTTGCRGGGATC R: TTGCCACAGACTGCGTCAGTRAGG	121 bp	
Stx2	F: TGTGGCTGGGTTCGTTAATACGGC R: TCCGTTGTCATGGAAACCGTTGTC	102 bp	(18)
eaeA	F: TGAGCGGCTGGCATGAGTCATAC R: TCGATCCCCATCGTCACCAGAGG	241 bp	
cnf1	F: GGCGACAAATGCAGTATTGCTTGG R: GACGTTGGTTGCGGTAATTTTGGG	552 bp	

Results

The current study showed that forty-one (97.61%) *E. coli* suspected isolates out of 42 samples appeared with a metallic-green sheen appearance (figure,1). All of these forty-one isolates were confirmed as *E. coli*

bacteria using a molecular technique (a PCR-based method), as each sample appeared as a single band at the right size (585bp) of partially amplified *malB* gene on agarose gel electrophoresis (figure, 2).

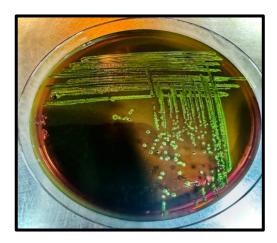


Figure 1: Escherichia coli colonies on EMB agar. Small rounded distinctive metallic green sheen colonies can be clearly notified after 24 h incubation period.

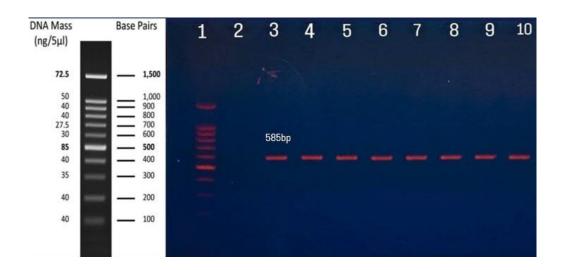


Figure 2: An agarose gel image displays the partially amplified *malB* promoter gene of the tested *E. coli* using a species-specific pair of primers. One clear band was reported at approximately 585 bp for each bacterial sample. Lane 1: DNA ladder 100bp (Promega, USA), lane 2: negative control, lanes 3–10: single amplicon approximately 585bp.

The extracted plasmid was migrated directly onto an agarose gel to detect their pattern regarding the shape, size and number of the resulting bands. Plasmids appear in many patterns. The majority of them 36(87.8%) have appeared in one band pattern form. Meanwhile, 2(4.87%) was the rate of two-

band and three-band plasmid patterns. Followed by 1(2.43%) of plasmids that appeared as the five bands pattern. Almost all of these plasmids exceeded the size of the ladder used, which confirms that they are larger than 1500bp.

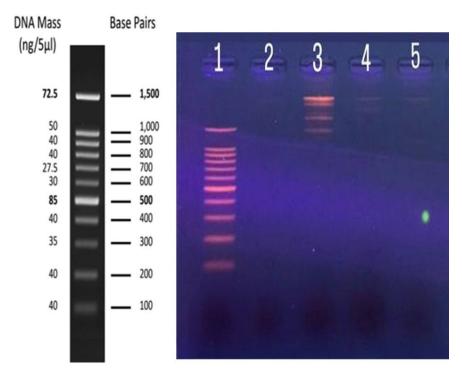


Figure 3: agarose gel electrophoresis image of extracted plasmid DNA. Lane1: DNA ladder 100bp (Promega, USA), lane2: negative control, Lane 3-5: different bands (with different Molecular weights) that showed different plasmid patterns for each isolate.

The results of plasmid replicons (Inc groups) displayed three types of plasmids present in one isolate as a maximum number of incompatibility plasmids. The *FIA* and *FIB* replicons were the most prevalent Inc (60%) among human isolates followed by *B/O*

(50%) and *Frep* (40%). In contrast, the dominant Inc replicon in dog samples was *Frep* followed by *FIB* and *FIA* in rates reached 35% and 25% respectively. Whereas, the *II* replicon was the least common in both (table, 3) (Figure, 4).

Table 3: Prevalence rates of replicons types in the investigated *E. coli* isolates from humans and dogs

Replicons	Dogs	Human	Statistical
B/O	3 (14.2%)	10 (47.6%)	p < 0.05*
FIA	5 (23.8%)	12 (57.1%)	p > 0.05**
FIB	7 (33.33%)	13 (61.9%)	p > 0.05**
I1	3 (14.2%)	1 (4.76%)	p > 0.05**
Frep	11 (52.38%)	8 (38%)	p > 0.05**

^{*} There are significant differences

^{**} There are no significant differences

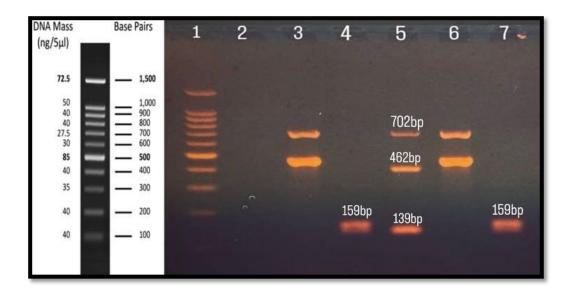


Figure 4: agarose gel electrophoresis image of multiplex PCR for *E. coli* plasmid profiling. Lane1: DNA ladder 100bp (Promega, USA), lane2: negative control, Lanes 3 and 6: two bands approximately 702bp and 462bp of FIA and FIB replicons which indicated the presence of two types of plasmids. Lanes 4 and 7: one band of approximately 159bp of B/O replicon indicates that one type of plasmid is present. Lane 5: three bands approximately 702bp, 462bp and 139bp of FIA, FIB and I1 replicons, respectively, which indicated the presence of three types of plasmids.

The results of virulence genes detection showed the presence of three of the investigated genes(figure,5) spread in the extracted plasmids. Over all the most common genes that found to be distributed among isolates were *iutA* (71.42%, 28.57%)

and *Cnf1* (19%, 23.8%%) in human and dog samples, respectively. Which are responsible for iron acquisition and toxicity. Whereas *eaeA* and *Stx2* genes have not been detected in any sample (table 4).

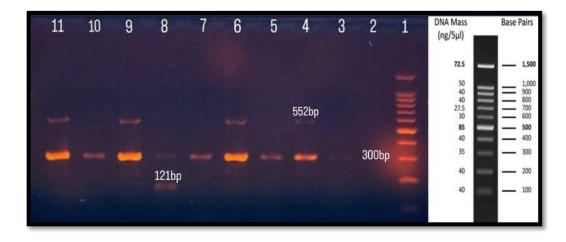


Figure 5: agarose gel electrophoresis image of multiplex PCR for virulence genes (*iutA*, *stx1*, *stx2*, *eaeA* and *cnf1*) detection. Lane1: DNA ladder 100bp (Promega, USA), lane2: negative control, Lane 3,5,7 and 10: a single band of approximately 300 which is the positive result for *iutA* gene presence. Lane 4, 6 and 9: two bands approximately 552 bp and 300 bp which represent the positive result of *Cnf1* and *iutA* genes presence, respectively. Lane 8: two bands approximately 300 bp and 121 bp which represent the positive result of *iutA* and *STX1* genes presence, respectively

Table 4: the percentages of Prevalence rates of virulence genes in Escherichia coli plasmids

Virulence genes	Dogs (%)	Human (%)
iutA	6 (28.57%)	15 (71.42%)
Stx1	1 (4.76%)	0 (0%)
Stx2	0 (0%)	0 (0%)
Cnf1	5 (23.8%)	4 (19 %)
eaeA	0 (0%)	0 (0%)

Discussion

The majority of *E. coli* strains are commensals inhabiting the intestinal tract of humans and animals (1). However, some strains are found to be pathogenic and can cause a group of diseases, the most important of which is diarrhea (4).

The current study reveals that almost all the collected faecal samples (from humans and dogs) are suspected to contain *E. coli* bacteria. The growth characteristics of the forty-two samples showed that 42 (100%) isolates had pink colonies when grown on MacConkey agar, however, only 41 (97.61

%) isolates appeared with metallic-green sheen colonies when grown on Eosin-methylene blue (figure,1). These forty-one isolates were confirmed as *E. coli* using a molecular genetic detection technique that included a partial amplification of the *malB* gene utilizing species-specific primers in polymerase chain reaction technology (figure, 2). The results of this study indicate that *E. coli* isolates play an important role in causing diarrhoea in humans and dogs. Even though, the prevalence rate of this bacteria seems to be higher than what has been

reported by (23) .this finding could be related to the time of sample collection during the diarrheal illness (24) and contamination of the consumable material such as food products, particularly frozen beef and chicken contamination (25) in addition to the quality control of the drinking water (26).

E. coli could become harmful bacteria because of the virulence genes that they can acquire horizontally and pass to the next forming different types of generation. (enterohemorrhagic E. pathogens enteropathogenic E. coli, enteroaggregative E. coli, entero-invasive E. coli and enterotoxigenic E. coli), they are collectively called diarrheagenic E. coli (27), (28) and (4). As most of these virulence factors are plasmid-borne genes, particularly molecular weight plasmids (4), plasmids DNA were extracted from the isolated E. coli strains. Interestingly, the majority of these strains were found to be harbouring plasmid(s), the majority of them were large plasmids (figure, 3). To create a plasmid profile the extracted pDNA was run on 1.5% agarose gel electrophoresis. For further investigation, the plasmids type number (regardless of their copy number) present in each isolate was verified by subjecting to the multiplex PCR-based method. which included five specific primers to detect the most prevalent five types (B/O, FIA, FIB, II and Frep) of replicons genes based on their incompatibility (22).

The current results showed that 39 (97.5%) of the isolated *E. coli* contained at least one of these replicons (one plasmid), whereas 2 (4.87%) isolates did not contain any of them. The possible reason could be the number of tested Inc during this study (the common

five types) for the fact that other (less common) replicons can be detected in the replicon typing method. In general, the prevalence of the Inc group was greater in number and type in human isolates than in dog isolates. These findings reflect those of (29) and (30) who also reported that human *E. coli* isolates have more plasmids than other source isolates. Generally, *E. coli* is able to harbour a variable number of plasmids (31). Which could play a role in bacterial antibiotic resistance and the severity of their pathogenic trait.

Plasmid pattern on agarose gel results showed that the maximum number of plasmids per isolate was 5. In contrast, the minimum was 1 regarding the number of bands, in addition to non-plasmid isolates. Most of them were higher than 1500bp (figure, 3). Interestingly, some E. coli isolates share the same plasmid pattern even though they were isolated from different sources (human and dog). A possible explanation of this phenomenon is the bacterial transmission between the two different sources and being zoonotic (32). Another explanation would be the possibility of plasmid transmission between E. coli isolates, either they came from the same or different sources. Which would suggest the term zoonotic plasmid.

During this study, plasmid replicons were detected as the maximum number of Inc present in one isolate either in human or dog isolates. The IncF FIA (57.14) and FIB (61.9%) replicons were the most prevalent Inc among human isolates. These findings corroborate the results of a great deal of the previous studies in Egypt (33) and (34) which reported that the IncF was the dominant type of *E. coli* plasmids Inc among

the tested human isolates. In contrast, the dominant Inc replicon in dog isolate was *Frep* followed by *FIB* and *FIA* in rates reached 35% and 25% respectively. Whereas, the *II* replicon was the least common in both human and dog samples (table, 3).

The presence of one or more Inc in a single investigated was further comparing the resulting pattern between human and dog isolates. For example, the common single pattern between human and animal isolates containing one type of plasmids (regarding their numbers) included either FIB or FIA replicon. Whereas the dual Inc plasmid Frep+FIB or Frep+FIA was found to be the shared type. In contrast, one of the triple Inc pattern was found to be common among dog and human isolates which was FIB+ Ferp+ B/O. This similarity in Inc patterns between dog and human isolates would give a preliminary idea about the similarity between the isolates. This suggests that the diarrheagenic E. coli may have been precisely the same species transmitted from animals to humans (and vice versa) or the plasmid DNA conjugation ability diffused some specific genes among E. coli isolates of different sources, which suggested the zoonotic plasmid term.

The current study showed that the presence and prevalence of some important virulence genes (*iutA*, *Stx1*, *Stx2*, *Cnf1*, *and eaeA*) in *Escherichia coli* isolates from human and canine faecal samples are variable. The results revealed that the *iutA* gene has significant differences in distribution between the two hosts. Precisely, this gene was detected in 6 (28.57%) canine isolates and 15 (71.42%) human isolates. This

finding is consistent with a previous study reporting its higher prevalence in ExPEC strains from humans (35). This gene, characteristically plasmid-borne, is known as a bacterial fitness enhancer through effective iron acquisition, leading to underscore its importance in bacterial pathogenicity and the host adaptation ability (36).

The Stx1 gene was identified in only one canine plasmid isolate but was absent in human isolates. Whereas, the Stx2 gene was not detected in either group (humans and dogs). These findings are in line with previous studies that reported that Shiga genes (Stx1 and Stx2) predominantly phage-encoded genes and suggest limited involvement of these toxins in the sampled *E. coli* populations (37), (38). Correspondingly, the cnfl gene distinguished in dogs and humans isolates at 23.8% and 19%, respectively. This prevalence highlights its possibility as a common virulence gene across host species. The Cnfl gene is frequently harboured on plasmids or pathogenic islands and has been associated with host tissue attacks and infection severity (39). It has been recorded that the eaeA gene is absent in all the tested plasmid isolates. This finding suggests that the eaeA gene is chromosomally encoded, as demonstrated by (40). Who reported that the eaeA gene associated with the locus of enterocyte effacement (LEE), is a hallmark of EPEC and contributes to intestinal adhesion and effacement.

Conclusion

The current study findings highlight the differences in the presence and prevalence of the plasmid Inc type group among *E. coli*

isolated from different sources (human and canine), in addition to the virulence genes existing and distribution between dogs and human E. coli plasmid isolates. Furthermore, they emphasize the possible role of plasmids in gene distribution. The observed frequency of plasmid Inc type and plasmid-borne virulence genes (especially iutA and cnf1) highlighted the importance of plasmids as a horizontal gene transfer factor. Such results would provide a valuable understanding of the antimicrobial resistance profiles and zoonotic potential of E. coli strains, leading to the development of control and treatment of such pathogens and mitigating public health risks.

Conflicts of interest

The authors declare that there is no conflict of interest.

Ethical clearance

This work is approved by the Research Ethical Committee.

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انتشار وأنماط البلازميدات في الإشريكية القولونية المسببة للإسهال يعكس إمكانية انتقال البكتيريا

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الخلاصة

تعد الإشريكية القولونية الإسهالية أكثر سلالات الإشريكية القولونية شيوعًا التي يعترض لها الانسان والحيوان. ويمكن تصنيفها إلى ست مجاميع مسببة للأمراض، فيما يتعلق بقدرتها على مقاومة المضادات الحيوية ووجود جينات الفوعة. والتي تقع معظمها على العناصر الوراثية المتنقلة مثل البلازميدات. ولذلك هدفت هذه الدراسة إلى التعرف على أنماط الحمض النووي البلازميد للأشريكية القولونية المعزولة من الكلاب والمرضى المصابين بالإسهال. من أجل البحث عن أوجه التشابه بين البلازميدات الموزعة فيما بينها، تم تطبيق أنماط البلازميد كحزم على هلام الاغاروز والكشف عن مجاميع نسخ عدم التوافق تم استخدام طريقة تعتمد على PCR المتعدد للكشف عن وجود النسخ المتماثلة البلازميدية الأكثر شيوعًا (BO) و BO) و FIB و FIB و FIB و التشابئة المسيطر المتحدد المتماثلة المسيطر المتحدد المتماثلة المسيطر المتحدد المتماثلة المسيطر المتحدد المتماثلة المتعاشلة المتحدلات تصل إلى 35% و 25% على التوالي. حيث أن النسخة المتماثلة المتعاشلة المتعاشلة المتماثلة المتعاشلة المتعاشلة المتعاشلة المتعاشلة المتعاشلة المتعاشلة المتعاشلة المتعاشلة المتعاشلة المتعدد الأقل شيوعًا في كلا المصدرين. تم العثور على البلازميدات المزدوجة (FIB+FIA) المتحددين. تم العثور على البلازميدات المزدوجة (FIB+FIA) المتعددين، تشابه هذا المصدرين. على الانتقال وأثار فكرة أولية حول إمكانية انتقال البلازميد بين الإشريكية القولونية بين مضيفين المتطالي قدرة البكتيريا على الانتقال وأثار فكرة أولية حول إمكانية انتقال البلازميد بين الإشريكية القولونية بين مضيفين

الكلمات المفتاحية: الإشريكية القولونية البلازميد انتقال البكتريا.