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Original article

Phenotypic and genotypic characterization of *Escherichia albertii* in chicken and human

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ABSTRACT

Background: *Escherichia albertii* (*E. albertii*) is a newly identified enteropathogen that affects humans and birds. It is a Gram-negative bacterium frequently mistaken for *E. coli*. **Objective**: To isolate *E. albertii* from chicken feces, products, and patients with diarrhea to assess its role in gastroenteritis and inflammatory bowel disease (IBD), also to assess antimicrobial susceptibility of this pathogen, and to identify it genetically by PCR**. Methodology:** 225 random samples from Assiut Governorate were tested, representing (100) chicken feces, (50) chicken products and (75) human feces from patients with gastroenteritis and IBD. The fecal samples were cultured on Hektoen enteric agar and xylose lysine deoxycholate plates. Biochemical identification of *E-albertii* was done by sulfur-indole motility (SIM), Simmons' citrate, urease test, triple-sugar iron (TSI), lysine iron and indole test. Genotypic detection of *E. albertii* was done by PCR for eae and mdh genes. The isolates were tested for antimicrobial susceptibility. **Results:** The prevalence of *E. albertii* was 21.7% by culture, 18.6 % by biochemical tests and 12.8 % by PCR. *Escherichia. albertii* was identified by PCR in 20% of chicken feces and 9% of human feces. No *E. albertii* was identified in chicken products. Out of 29 isolates, 65.5 %, 51.7% were resistant to tetracycline, nalidixic acid, respectively, while lower resistance rates were observed to other antibiotics**. Conclusion:** *Escherichia albertii* could be isolated from chicken and human feces, but not from chicken products. High resistance rate was observed for tetracycline, and nalidixic acid. *Escherichia. albertii* culture should be interpreted carefully and confirmed by PCR.

Introduction

Escherichia albertii (E. albertii) is a newly identified enteropathogenic bacteria that affects both humans and birds. It is a Gram-negative pathogen frequently mistaken for *Escherichia coli (E. coli*) due to its resemblance to other *Escherichia* genus members [1]. *Escherichia albertii* may cause symptoms like fever, abdominal pain, nausea, vomiting and diarrhea, when consumed in foods like ground beef, turkey and lettuce [2]. It is found in the corpses of farm-raised birds after slaughter, as well as chicken meat sold in grocery stores [3,4].

Escherichia albertii has previously been linked to human diarrhea but not as a zoonotic

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illness. But, in 2004, investigatory techniques revealed that *E. albertii* was most likely the reason of redpoll birds' deaths in Alaska. Isolates from human beings, previously identified as *E. coli* O86:K61 from dead birds were also found to be *E. albertii* [5]. The *eae* gene, coding for the integral membrane protein intimin, was present in *E. albertii* isolates, considering it a member of *Enterobacteriaceae* [1].This gene aids bacterial adhesion to tissues' epithelial cells, which eventually changes the cells and causes diarrhea [6]. *Escherichia albertii* also had cytolethal distending toxin (*cdtB*) but doesn't have Shiga toxin (*stx*) genes. *E. albertii* isolates from birds are variable but similar to those from humans, according to *eae* and *cdtB* sequencing [5].

Escherichia albertii's genome is remarkably similar to that of the rest of the *Escherichia* genus, making it difficult to distinguish them separately [1]. Through a series of experiments, the relationship between *E. albertii* infection and gastroenteritis has been hypothesized and studied [7]. Inflammatory bowel disease or IBD, is a group of persistent inflammatory gastrointestinal illnesses. IBD has typically been split into two categories, ulcerative colitis (UC) and Crohn's disease (CD). It has been hypothesized that a number of bacteria are involved in the etiology of IBD [8]. *Escherichia coli* has been associated with IBD and is thought to be responsible for relapses of the condition [9]. Numerous O antigens, including those of the O1, O2, O6, O18, and O75 serotypes, cause enhanced positive antibody reactivity in the majority of IBD patients [10]. The faecal microbiota is the source of these serotypes [11]. The exact prevalence of *E. albertii* is unknown; so, we needed to characterize the pathogenic nature and prevalence of *E. albertii* in order to prevent the occurrence of diarrhea in humans. This study also aimed to assess antimicrobial susceptibility to different antimicrobial agents and to identify *E. albertii* genetically by PCR.

Methodology

Ethical considerations

The study was authorized by the Assiut University, Faculty of Medicine's Ethical Committee in accordance with the Declaration of Helsinki, the code of ethics of the World Medical Association; IRB number: 17101418. An informed written consent was obtained from the included patients.

Study design

This cross-sectional study was conducted to isolate *E. albertii* from chicken feces, chicken products, and patients' feces. The tested chicken were brought from different localities in Assiut Governorate.

The recruited patients presented with acute gastroenteritis and inflammatory bowel disease, either ulcerative colitis or Crohn's disease, at the outpatient clinics of Al-Rajhi Liver University Hospital, in Assiut University.

1.Clinical assessment of the included patients

A thorough history was taken from the recruited patients, including demographics and clinical data like age, sex, nausea, vomiting, diarrhea, abdominal pain, distension, fever, and the type of the received treatment. Patients who received antibiotics were excluded.

2. Identification of *E. albertii*

The fecal swabs were inoculated in Luria-Betani (LB) broth and incubated at 37º C for 24 hrs then inoculated on xylose lysine deoxycholate agar (XLD) and Hektoen enteric agar (HEA) and incubated over-night at 37°C. *Escherichia albertii* colonies are pink with a slightly yellow or cream-colored center on XLD and green on HEA [3].

3. Biochemical reactions of *E. albertii*

The isolates were tested on indole test (Hi media, India) and five biochemical agar slants; sulfur-indole motility (SIM) (Hi media, India), Simmons' citrate agar (Hi media, India), urease test (Hi media, India), triple sugar iron agar (TSI) (Hi media, India) and lysine iron test (Hi media, India) [3].

4. Genotypic detection of *E. albertii*

Preparation of *E. albertii* isolates from chicken and human faeces for PCR

DNA extraction was achieved by boiling method as follows: *E. albertii* isolates were cultured at 37 °C for 24 hrs. Bacterial isolates were suspended in 200 ml of sterile deionized water and treated in a thermal cycler (Biometra, UNO II, Göttingen, Germany) at 95 °C for 20 min. After centrifuging for 10 min, the supernatant was used as template DNA and stored at -20 °C till use [3].

Preparation of chicken products for PCR

Chicken liver, stomach and meat of thigh, and chest samples were minced in a sterile mortar, and 25 g of the minced flesh was suspended in 225 ml of *E. coli* broth (EC). One ml of the enrichment sample was centrifuged at 1500 g for one min to pellet large meat fragments following a 24 hrs incubation period

at 20 º C on a shaking plate form (220 rpm). After centrifuging the supernatant at 1300 g for 2 min, the pellet was suspended in 100 µl of lysis buffer and boiled for 10 minutes before being centrifuged once more. The resulting supernatant was used for PCR [12].

Conventional PCR for *eae* and *mdh* genes

Components of PCR:10µl of master mix (Promega Co., USA),0.5 µl of each forward and reverse primer (Invitrogen Co., UK),3 µl of boiled colony lysate ,6 µl of sterile deionized water with a final volume equal to 20 µl.

Primer selection: The primers for *eae* and *mdh* are stated in **table (1).** The primers were diluted in sterile distilled water in proportion equal to 1:10. **The PCR condition for *eae* gene:** Denaturation at 95°C for 15 min, followed by 30 cycles of 94°C for 30 sec, 55°C for 45 sec, and 70°C for 25 sec with a final extension at 72°C for 10 min [13].

The PCR condition for *mdh* gene: Denaturation at 95°C for 5 min, followed by 35 cycles of 50°C for 60 sec, 72°C for 20 sec, and 72°C for 20 sec with a final extension at 72°C for 10 min [14].

Electrophoresis of PCR products was done in 2% agarose gel.

Antibiotic susceptibility test of *E. albertii* isolates

The modified Kirby-Bauer disc method was used to test the susceptibility of *E. alberii* isolates. Ten antibiotics (Oxoid; Basingstoke, UK) were used; ampicillin 10 µg (AMP), chloramphenicol 30 µg (C), ciprofloxacin 5 µg (CIP), kanamycin 30 µg (K), streptomycin 10 µg (S), sulfisoxazole 25 µg (ST), gentamycin 10 µg (CN), and cefixime 5 µg (FEP), tetracycline 30 µg (TE) and nalidixic acid 30 µg (NA) [15].

Statistical analysis

The Statistical Package for Social Sciences, version 16, was used to do the statistical analysis (SPSS Inc., Chicago, USA). Comparisons of both categorical and continuous variables was made using the Chi-square test and the student's T-test. Statistical significance was defined as a *p*-value less than 0.05.

Table 1. Primers used for detection of *E. albertii.*

Target gene	Sequence of primers	Amplicon size	References
***eae* F** ***eae* R**	5'- ATA TCC GTT TTA ATG GCT ATC T -3' 5'- AAT CTT CTG CGT ACT GTG TTC A -3'	425 bp	[13]
***mdh* F** ***mdh* R**	5'- CTG GAA GGC GCA GAT GTG GTA CTG ATT -3' 5'- CTT GCT GAA CCA GAT TCT TCA CAA TAC CG -3'	115 bp	[14]

Result

This study was conducted on 150 samples from chicken, which included 100 fecal swabs and 50 samples from chicken products (liver, stomach, thigh and chest).

In this study, a total of 75 patients with diarrhea were recruited. They were categorized into two groups; 50 patients with acute gastroenteritis and 25 patients with inflammatory bowel disease (IBD).

Phenotypic characterization of *E. albertii*

Escherichia albertii colonies appeared on XLD as pink colonies with a slightly cream-colored center as in **figure (1)** and on HEA as green colonies as in **figure (2).**

Biochemical identification of *E-albertii* was done by sulfur-indole motility (SIM), Simmons' citrate, urease test, triple-sugar iron (TSI), lysine iron and indole test **(Table 2).**

Genotypic characterization of *E.albertii*

Conventional PCR was done for detection of 2 specific genes of *E. albertii; eae* **(Figure 3)** and *mdh* **(Figure 4)** genes.

While 49/225 of isolates (21.7%) were positive for *E. albertii* by conventional methods, 29/49 isolates (12.8%) were positive for both specific genes.

Antimicrobial susceptibility test of *E. albertii* isolates

The susceptibility of *E. albertii* isolates was performed using Kirby Bauer method. Nineteen out of 29 isolates (65.5 %) were resistant to tetracycline and (15/29) 51.7 % of isolates were resistant to nalidixic acid, while lower resistance rates were observed to ampicillin (10/29) 34.4%, chloramphenicol (11/29) 37.9%, ciprofloxacin (7/29) 24.13%, kanamycin (8/29) 27.5%, streptomycin (14/29) 48.2%, sulfisoxazole rate (6/29) 20.6%, gentamycin rate (5/29) 17.2% and cefixime (13/29) 44.8 as in **figure (5).**

Demographic and clinical data of patients with acute gastroenteritis positive for *E. albertii*

Patients with gastroenteritis positive for *E. albertii* by conventional culture technique (n=15) suffered from diarrhea, abdominal pain and fever.Four patients were positive for *E. albertii* by PCR; one male and three females. Their age ranged between (20-60) years old. The diarrhea in three of them was non bloody, while the fourth patient had bloody diarrhea. Fever and abdominal pain were observed in all four cases but without vomiting as in **table (3).**

Demographic and clinical data of patients with inflammatory bowel diseases positive for *E. albertii*

Patients with IBD who were positive for *E. albertii* by conventional culture technique (n=6) suffered from ulcerative colitis and Crohn's disease. Five patients were positive for *E. albertii* by PCR; one female and four males. Their age ranged between (20 -85) years old. Four patients suffered from ulcerative colitis and one suffered from Crohn's disease.

All of them had diarrhea, one of them had bloody diarrhea, and one had diarrhea with mucus. The frequency of diarrhea in four cases was between 2 - 3 times and one had 8-10 times motions. Three patients had abdominal pain, one patient had weight loss and another one had vomiting. Fever was not observed in any case. Three patients received treatment in the form of 5-ASA (5-aminosalicylic acid), azathioprine, steroids and biological treatment. No patient received antibiotic therapy as in **table (4).**

Comparison between both groups of patients positive for *E. albertii* by PCR

Patients who had gastroenteritis and IBD showed no statistically significant difference regarding age and sex. However, they had statistically significant difference regarding presence of fever which was higher among patients with gastroenteritis (p= 0.008). Otherwise they showed no statistically significant difference as regard to the clinical presentations or the prevalence of *E. albertii* isolated by culture, biochemical tests or PCR as in **table (5).**

The prevalence of *E. albertii* isolated from chicken feces was 28 % by culture, 26% by biochemical tests and 20 % by PCR with no statistically significant difference between them (p=0.393). The prevalence of *E. albertii* isolated from human stool who suffered from gastroenteritis was 8% by PCR with statistically significant difference between them (p = 0.021), while the prevalence of *E. albertii* isolated from human stool that suffered from IBD was 20% by PCR with no statistically significant difference between them (p=0.924). Totally, the prevalence of isolated *E. albertii* was 21.7 % by culture, 18.6 % by biochemical tests and 12.8% by PCR with statistically significant difference between them (p = 0.044).

Table 2. Biochemical tests for identification of *E. albertii*.

Test name	**Result**
Sulfur-indole motility (SIM)	Non motile
Simmons' citrate	Green color (-ve)
Urease test	Yellow color (-ve)
Triple-sugar iron (TSI)	Yellow butt and red slant with no H2S production
Lysine iron agar	Purple butt and purple slant with no H_2S production
Indole test	No red ring (-ve)

Table 3. Demographic and clinical data of patients with acute gastroenteritis positive for *E. albertii* by PCR.

Demographic Data		**Number of cases**	
		(n=4)	**(%)**
Ages	< 20 y	1	25
	20 -40 y	1	25
	41 -60 y	1	25
	> 60 y	1	25
Sex	(Males)	1	25
	(females)	3	75
Clinical Data		**Number of *E. albertii* positive cases by PCR**	
		(n=4)	**(%)**
Diarrhea		4	100
Non –bloody diarrhea		3	75
Bloody diarrhea		1	25
Abdominal pain		4	100
Fever		4	100
Vomiting		0	0

Table 4. Demographic and clinical data of IBD patients positive for *E. albertii* by PCR.

Demographic Data		**Number of cases**	
		(n=5)	**(%)**
Ages	< 20 y	3	60
	20 -40 y	1	20
	41 -60 y	0	0
	> 60 y	1	20
Sex	(Males)	4	80
	(females)	1	75
Clinical Data		**Number of cases**	
		(n=5)	(%)
Ulcerative colitis		4	80
Crohn's disease		1	20
Diarrhea		**5**	**100**
Frequency of diarrhea		**4 (2-3 times)**	**80**
		1(8-10 times)	**20**
Diarrhea	**With blood**	**1**	**20**
	With mucus	**1**	**20**
Abdominal pain		**3**	**60**
Fever		**0**	**0**
Vomiting		**1**	**20**
Weight loss		**1**	**20**
Treatment		**3**	**60**

*

Table 5. Comparison between both groups of patients positive for *E. albertii* by PCR.

Sex		**Gastroenteritis (n=4) n (%)**	**IBD (n=5) n (%)**	***p*-value**
Males		1 (25%)	4 (80%)	0.206
Females		3 (75%)	1 (20%)	
Age				
(q< 20) y		1 (25%)	3 (60%)	0.591
(30 -40) y		1 (25%)	1 (20%)	
(40 -50) y		1 (25%)	0 (0%)	
(> 60) y		1 (25%)	1 (20%)	
Clinical data				
Diarrhea with	Blood	1 (25%)	1 (20%)	1.000
	Mucus	0 (0%)	1 (20%)	1.000
Abdominal pain		4(100%)	3 (60%)	0.444
Fever		4(100%)	0 (0%)	0.008*
Vomiting		0 (0%)	1 (20%)	1.000
Weight loss		0 (0%)	1 (20%)	1.000
Lab diagnosis				
No. of isolated *E.albertii* by culture		15 (30%)	6 (24%)	0.585
No. of positive isolated *E.albertii* by biochemical tests		11 (22%)	5 (20%)	0.842
No. of isolated *E. albertii* by PCR		4 (8%)	5 (20%)	0.150

* Significant *p*-value

Table 6. Comparison between culture, biochemical tests and PCR identification of isolated *E. albertii.*

Type of samples		**Total No. of samples**	**No. of isolated *E.albertii* by culture**		**No. of positive isolated *E. albertii* by biochemical tests**		**No. of positive isolated *E. albertii* by PCR test**		***p*-value**
			No.	**%**	**No.**	**%**	**No.**	**%**	
Chicken feces		100	28	28.0	26	26.0	20	20.0	0.393
Chicken products		50	0	0.0	0	0.0	0	0.0	--
Human stool	**Gastro-enteritis**	50	15	30.0	11	22	4	8.0	0.021*
	IBD	25	6	24.0	5	20	5	20.0	0.924
Total samples in the study		225	49	21.7	42	18.6	29	12.8	0.044*

* Significant *p*-value

Figure 1. *Escherichia albertii* colonies on XLD.

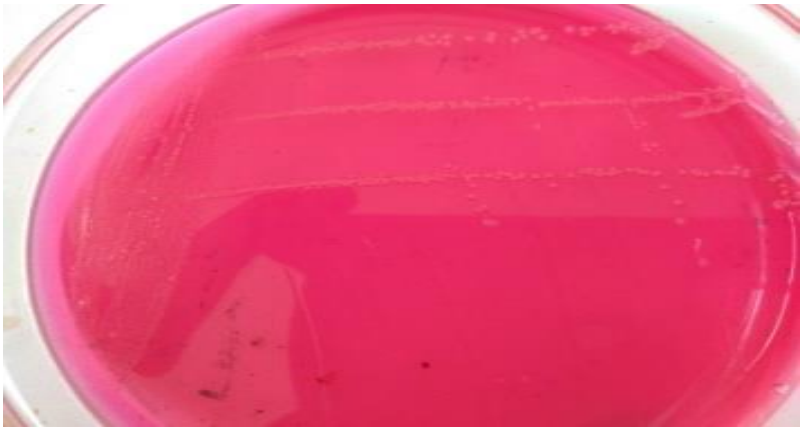
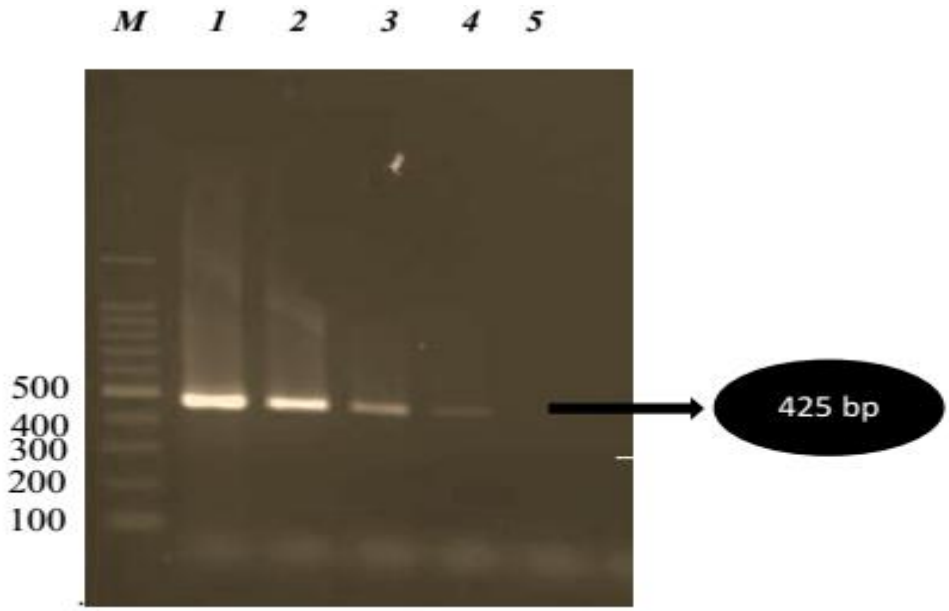


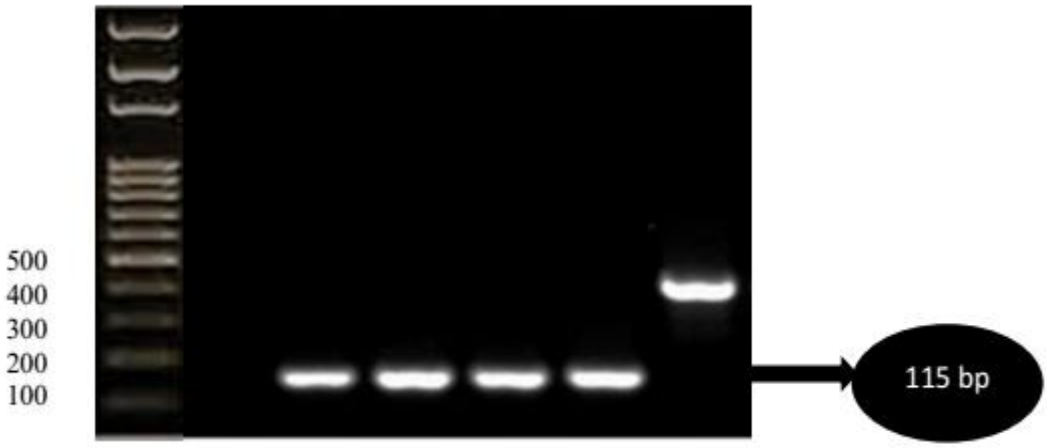
Figure 2. *Escherichia albertii* colonies on HEA.

Figure 3. Amplification results of *eae* gene in *E. albertii* isolates.



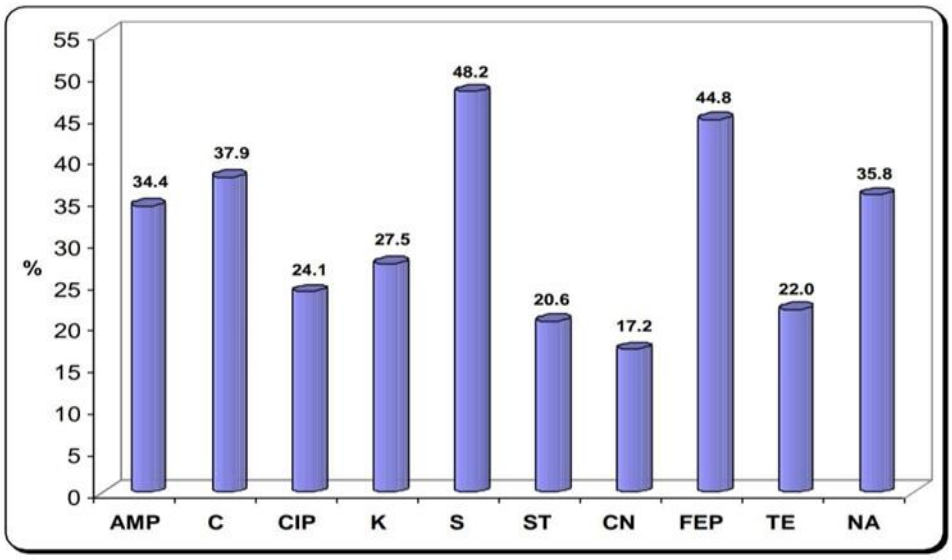
Lane M = 100 bp DNA ladder; Lanes 1,2,3 and 4 = positive results and lane 5 = negative result.

Figure 4. Amplification results of *mdh* gene in *E. albertii* isolates.



Lane M = 100 bp DNA ladder; Lanes 1,2,3 and 4 = positive results and lane 5 = negative result.

Figure 5. Antibiotic resistance pattern of all *E. albertii* isolates.



Discussion

Escherichia albertii is a Gram-negative enteropathogen linked to human gastroenteritis. Although the biochemical tests were used to identify *E. albertii*, it was misidentified as *E. coli*, *Shigella boydii*, *Yersinia ruckeri* or *Hafnia alvei* [7]. The clinical importance, prevalence, epidemiology, reservoirs and modes of transmission of *E. albertii* are all poorly understood.

In this study, 225 random samples representing 100 chicken feces ,50 chicken products (liver, stomach, thigh and chest) and 75 human stool samples were included. Chicken feces and human stool samples were cultured on XLD and HEA. *Escherichia albertii* colonies were pink with a cream-colored center on XLD and green on HEA. Then, the positive culture isolates were confirmed by PCR for *eae* and *mdh* genes.

The XRM-MacConkey agar; modified type of MacConkey agar supplemented with xylose (X), rhamnose (R), and melibiose (M) in place of lactose; was created and tested as a selective medium for *E. albertii* [16,17]. However, it wasn't available to use in this study.

In this study, *E. albertii* isolates were negative for SIM Simmon´s citrate, urease test, alkaline butt /acid slant with no H_2S production for TSI and alkaline butt/alkaline slant with no H_2S production for lysine iron agar, this is in concordance with a previous study by **Lindsey et al.**[3] who reported that all *E. albertii* isolates were non motile at 37°C and negative for citrate, urease and H_2S production.

Negative results regarding indole production in the current study are different from results by **Huys et al.** [18] who reported that 35.4% of *E. albertii* isolates were positive indole producers and also **Oaks et al.** [5] and **Asoshima et al.** [19] who reported that indole production was observed in *E. albertii* isolates.

As regard to motility, **Asoshima et al.** [19] reported that the tested *E. albertii* strains were non motile. This is in harmony with our study.

Escherichia albertii has been related to the pathogenesis of disease in a variety of migratory and domestic bird species, occasionally producing epidemics globally [20].

In this study, *E.albertii* was detected by culture in 28% chicken feces while by PCR detected only in 20% (positive for *eae* and *mdh* genes). Similarly, **Gordon and cowling** [21] isolated *E. albertii* from 22% of chicken feces in Australia. **Lindsey et al.** [3] reported that 89% of isolates were positive for *mdh* and *eae. Escherichia albertii* was found in 0.9% and 1.4% of birds in Australia and Korea respectively [16, 21]

In this study, there was no *E. albertii* isolates from chicken products by PCR. This is in agreement with a prior study reported in Fukuoka City, Japan. However, **Asoshima et al.** [19] found that the 3 PCR positive isolates from chicken liver sample were *eae* positive. Also, **Maeda et al.** [4] reported 2 chicken liver samples and 1chicken meat sample tested positive for *E. albertii*. **Asoshima et al.** [22] detected *E. albertii* in 0.88 % of chicken liver samples and 1.8 % raw chicken meat samples. **Oh et al.** [23] detected *E. albertii* in 1.6% of broiler chickens

In the current study, 8% of patients with gastroenteritis had *E. albertii*. This is similar to another Egyptian study conducted by **Ghandour et al.** [24] who found *E. albertii* in 11.8 % of children with gastroenteritis. On the other hand, **Sulaiman et al.** [25] found *E. albertii* at lower prevalence (1.3%) among patients with gastroenteritis in Kano State, Nigeria. Also, **Ori et al.** [26] found *E. albertii* in 0.2% of the total isolates (10/5047) in Brazil, while **Ooka et al.** [7] found *E .albertii* at higher prevalence (50%).

For *eae* gene, **Ooka et al.** [2] isolated *E. albertii* from 67.7% of patients with gastroenteritis. While for *mdh* gene, **Nimri et al.** [27] isolated *E. albertii* from19.2% of cases with diarrhea and **Aoshima et al.** [19] isolated *E. albertii* from 30% of samples which previously identified phenotypically as *E. coli*.

Luo et al. [28] reported that the prevalence of *E.albertii* was equal among males and females. This is in disagreement with our results that reported gastroenteritis more in females than males and more in males in patients with inflammatory bowel disease.

Similar to the current study**, Ooka et al.** [7] reported that the prevalence of *E.albertii* was equal among different age groups.

In this study, diarrhea and abdominal pain were the prominent manifestations of the included patients. Meanwhile, **Ooka et al.** [7] reported that both fever and abdominal pain were prominent among patients infected with *E. albertii* in Japan with the prevalence of 38% and 76%, respectively.

Susceptibility to antimicrobials was determined by Kirby–Bauer disk diffusion method on Mueller Hinton agar (MHA). In our study, the results of the susceptibility of *E. albertii* isolates to these antibiotics showed that the highest resistance was observed to tetracycline and nalidixic acid, while low resistance rates were observed to streptomycin 48.2%, ampicillin 34.4%, cefixime 44.8%, chloramphenicol 37.9%, ciprofloxacin 24.13%, kanamycin 27.5%, sulfisoxazole 20.6%, gentamycin 17.2% . This is concordance with **Perez et al.** [15] who reported that tetracycline resistance was observed for all tested strains but sensitive to ampicillin, amoxicillin and clavulanic acid, cephalosporins and gentamicin.

Similarly, **Li et al.** [29] reported that the highest resistance was to tetracycline (62.7%) followed by resistance to nalidixic acid (56.9 %) and streptomycin (51%). Lower resistance was observed for ampicillin/sulbactam, cefepime, cephalothin, ceftriaxone,aztreonam, kanamycin, gentamicin, norfloxacin, ciprofloxacin and trimethoprim/sulfamethoxazole, with a rate ranging from 17.6 to 39.2%.

The urgency to determine distinctive characteristics of *E. albertii* in order to consistently differentiate this microbe from other members of the Enterobacteriaceae has increased by the difficulties in differentiating *E. albertii* from *E. coli* strains **Egan et al.** [30].

Conclusion

Escherichia albertii was isolated from chicken and patients with gastroenteritis and inflammatory bowel diseases. High resistance rate was observed for tetracycline, and nalidixic acid. Despite the fact that *E. albertii* strains are still being mislabeled, mounting evidence points to this microbe as being a significant pathogen for both humans and animals. However, *E. albertii* culture should be interpreted carefully and confirmed by PCR testing.

Conflict of interest

The authors report no conflicts of interest in this work.

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