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RESEARCH ARTICLE

Molecular Study of *Escherichia albertii* in Pediatric Urinary Tract Infections

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Abstract:

Background:

There are insufficient data about the presence of *E. albertii* as a causative organism in urinary tract infection in pediatric patients. Objective: The present study aimed to detect *E. albertii* by polymerase chain reaction (PCR) for detection of *uidA*, *mdh*, and *lysP* genes among isolated *E. coli* from children with urinary tract infection.

Methods:

The present study was a cross-sectional retrograde study which was carried out on 100 isolates of phenotypically confirmed *E. coli* detected in urine samples of children suffering from urinary tract infection. The isolates were subjected to molecular identification by PCR for *uidA*, *mdh*, and *lysP* genes.

Results:

E. albertii was identified by PCR in 7% of the isolates and *E. coli* was identified in 93% of the isolates. Two *mdh* and *lysP* genes were detected for *E. albertii* and the *uidA* gene for *E. coli*. *E. albertii* isolates had marked resistance to gentamicin (71.4%), followed by resistance to ciprofloxacin (57.1%), meropenem and imipenem (42.9% each) and ESBL activity by double discs method was reported in 57.1% of the isolates. However, none of the isolates had shown resistance to nalidixic acid and only one isolate had resistance to norfloxacin. There was a statistically insignificant difference between resistance to the used antibiotics such as aztreonam (P=0.083), ampicillin/clavulanate (P=0.5), ciprofloxacin (P=0.69), gentamicin (P=0.3) and ceftazidime (P=1.00).

Conclusion:

The present study highlights the emergence of *E. albertii* as a pathogen associated with urinary tract infections in children. There is marked antibiotic resistance of this pathogen, especially toward extended spectrum beta-lactams antibiotics. The identification method depends mainly on genetic studies. Further longitudinal studies with large number of patients are required to verify the accurate prevalence of this bacterium.

Keywords: *E. albertii*, Children, PCR, *uidA*, *mdh*, *lysP* genes.

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1. INTRODUCTION

Urinary tract infections (UTI) represent a major infection in children with high incidence and tendency to relapse [1, 2]. This infection is associated with marked morbidity due to difficulty in diagnosis based upon clinical symptoms only and requires careful laboratory diagnosis based upon accurate urine collection and culture [3, 4]. The accurate diagnosis and appro-

priate antibiotics treatment reduce morbidity [5]. The most common bacteria associated with this infection is *Escherichia coli* (*E. coli*) causing 90% of UTI in children [6 - 10].

Escherichia coli is a Gram negative genus that belongs to the family of *Enterobacteriaceae*, which is formed of three species namely *Escherichia coli*, *Escherichia albertii* and *Escherichia fergusonii* and five cryptic clades *Escherichia* C-I to C-V [11]. *Escherichia coli* has been fully studied and identified biochemically and genetically besides its association with intestinal and extraintestinal infections in human and

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animals. The *Escherichia fergusonii* is classified as opportunistic infections in animals, birds and human with no reported intestinal infections [12]. The *Escherichia albertii* (*E. albertii*) has been recognized to be associated with enteropathogenic effects and fewer studies have described its association with extraintestinal infections [13, 14]. The main enteropathogenic strains of *E. coli* have been identified to be *E. albertii* [15]. Pathogenesis of *E. albertii* depends mainly on its ability to adhere to epithelial cells with formation of attaching-effacing lesions causing formation of dense microfilaments beneath the adherent strains which helps in the invasion process, the intracellular presence of *E. albertii* protect them from intestinal clearance and immune system causing prolongation of diarrheal disease, those isolates and their products also have the ability to translocation from the intestinal lumen to the mesenteric lymph nodes [16]. *E. albertii* strains are capable of production of Shiga toxins Stx2a and Stx2f) and causes outbreaks of gastroenteritis [17, 18]. The complete genomic structure and the virulence factors of *E. albertii* are not fully investigated [19]. *E. albertii* is associated with different intestinal and extraintestinal infections and even there are strains with multiple antibiotics resistance [3 - 5].

The diagnosis of infections associated with this species remains a challenge due to poor discrimination between *E. albertii* and *E. coli* based upon biochemical identification. The accurate method for identification of *E. albertii* depends mainly on identification of specific genes namely of *mdh* that produces malate dehydrogenase and *lysP* that produces Lysine permease. These genes are preserved genes in *E. albertii* and can be detected by a multiplex PCR method [20, 21]. The other molecular identification method depends on identification of *rpoB* gene by the use of multilocus sequence typing and the method of whole genome sequencing. However, these methods are tedious and time consuming and therefore cannot be used for routine diagnosis of infections [20, 21].

The molecular identification of *E. coli* depends on the detection of the *uidA* gene, housekeeping and virulence gene. Molecular identification of *E. coli* used this gene in laboratory diagnosis [20, 21].

There are insufficient data about the presence of *E. albertii* as a causative organism in urinary tract infection in pediatric patients. Hence, the present study aimed to detect *E. albertii* by polymerase chain reaction (PCR) for detection of *uidA*, *mdh*, and *lysP* genes among isolated *E. coli* from children with urinary tract infection.

2. MATERIALS AND METHODS

The present study was a cross-sectional retrograde study which was carried out on 100 isolates of phenotypically confirmed *E. coli* detected in urine samples of children suffering from urinary tract infection recruited from Mansoura University Children Hospital, Egypt. The isolates are obtained from March 2019 till May 2020. The study was approved by Mansoura Faculty of Medicine Ethical committee (R.20.12.1110) and approval was taken from parents of the patients.

Urine specimens obtained from children by clean catch method and from children trained to use toilet clean catch mid-stream samples were obtained. The urine is collected in a sterile container and transported within 30 minutes to the laboratory for microscopic examination, urine culture and colony counts [22]. Isolated *E. coli* was identified by Gram stain and manual biochemical reactions that include the use of triple sugar iron agar, Simmon citrate, urease, lysine, phenylalanine, Methyl red voges-proskauer test and subculture on MacConkey agar [23]. For molecular studies, pure suspension of the isolated *E. coli* was prepared in brain heart infusion broth and glycerol kept frozen at -20°C.

2.1. Antibiotics Sensitivity Testing by Disk Diffusion Method

Testing for antibiotic susceptibility was conducted by Kirby-Bauer diffusion technique. The suspension of *E. coli* isolates was prepared by the use of Mueller-Hinton broth to obtain 0.5 McFarland concentrations and spread on Mueller Hinton plates. The used discs of antibiotics were amoxicillin-clavulanate (20/10µg), imipenem (10 µg), meropenem (10 µg), piperacillin (30 µg), ampicillin/sulbactam (20 µg), cefepime (30 µg), cephalothin (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), gentamicin (10 µg), aztreonam (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), nalidixic acid (30 µg), levofloxacin (5 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), trimethoprim/sulfamethoxazole (25 µg) (Oxoid, United Kingdom). Then, incubation of the plates was done aerobically for 24 hours at 37°C. Interpretation of inhibition zone diameters was done according to the Clinical Laboratory Standards Institute (CLSI) guidelines [24].

2.2. Determination of the Isolates Producing Extended Spectrum Beta Lactamase (ESBL)

The isolated *E. coli* which was resistant to ceftazidime and/or cefotaxime were evaluated for production of ESBL by the double discs method (CLSI). The prepared dilution of 0.5 McFarland was spread over Mueller-Hinton plates and cefotaxime + cefotaxime/clavulanic acid and ceftazidime + ceftazidime/clavulanic acid discs were added and the plates were incubated at 37°C for 24 hours. Positive ESBL production was considered if there was an increase of inhibition zone diameter around antibiotics ≥ 5 mm after combination of antibiotic with clavulanic acid [24]. *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as the ESBL-negative and positive control strains, respectively.

2.3. Extraction of Genomic DNA

DNA was extracted by the use of QIA amp DNA Mini Kit (Qiagen- Wade Road Basingstoke Hampshire RG24 8PW United Kingdom).

2.4. Polymerase Chain Reaction (PCR)

The identified *E. coli*, according to biochemical reaction, was subjected to PCR intended for detection of *uidA*, *mdh*, and *lysP* genes to differentiate *E. albertii* from *E. coli*.

Table 1. The sequences of the studied genes primers and the amplified base pair (Bp).

Gene	Sequences of the Primers	bp	References
<i>uidA</i>	5'-GCGTCTGTTGACTGGCAGGTGGTGG -3' 5'-GTTGCCCGCTTCGAAACCAATGCCT -3'	503	[25]
<i>mdh</i>	5'-CTG GAAGGC GCA GAT GTG GTA CTG ATT-3' 5'-CTT GCT GAA CCA GAT TCT TCA CAA TACCG-3'	115	[26]
<i>lysP</i>	5'-GGG CGC TGC TTT CAT ATA TTC TT-3' 5'-TCC AGA TCC AAC CGG GAG TAT CAG GA-3'	252	[26]

Table 2. Demographic and clinical data of the studied children.

The Parameters	
Sex	46 46%
Male (No.-%)	54 54%
Female (No.-%)	
Age (Years)	
Median	4.1
Minimum	0.7
Maximum	14.00
Percentile	
25%	2.1
50%	4.1
75%	7.3
Dysuria (No.-%)	39 39%
Fever (No.-%)	46 46%
Abdominal pain (No.-%)	36 36%
Lethargy (No.-%)	27 27%
Irritability (No.-%)	25 25%
Poor feeding (No.-%)	27 27%
Vomiting (No.-%)	35 35%

The used sequences of the used genes primers of *lysP*, *uidA* and *mdh* are listed in Table 1 [25, 26]. The used amplification mixture ready to use kit was supplied from Qiagen (Wade Road Basingstoke Hampshire RG24 8PW United Kingdom). The amplification procedures were carried out for each gene separately with the following amplification conditions: an initial denaturation for 5 minutes at 95°C, then 35 cycles with denaturation for 30 seconds at 95°C followed by primer annealing for 30 seconds using different temperatures for each primer, the annealing temperatures were 67°C, 65°C and 64°C for *uidA*, *mdh*, and *lysP*, respectively and the extension step was done for 60 seconds at 72°C. Final extension was performed for 5 minutes at 72°C. Then electrophoresis was performed for 30 minutes

Electrophoresis in 2% agarose gel stained with ethidium bromide was performed to visualize DNA.

2.5. Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 22 software (SPSS Inc., Chicago, IL). Chi-square was run to verify the P-value. Statistically significant P-value was considered if it was less than 0.05.

3. RESULTS

The study included 100 isolates that were identified by biochemical methods as *E. coli*. The isolates were separated from urine samples of children complaining of urinary tract infection. The children were 46 males and 54 females with an age range from 0.7 up to 14 years old. The most common symptoms were fever (46%), dysuria (39%), abdominal pain (36%) and vomiting (35%) (Table 2).

E. albertii was identified by PCR in 7% of the isolates and *E. coli* was identified in 93% of the isolates.

E. albertii isolates had marked resistance to gentamicin (71.4%), followed by resistance to ciprofloxacin (57.1%), meropenem and imipenem (42.9% each). ESBL activity by double discs method was reported in 57.1% of the isolates. However, none of the isolates had shown resistance to nalidixic acid and only one isolate had resistance to norfloxacin. There was statistically insignificant difference between resistance to the used antibiotics such as azteronam (P=0.083), ampicillin /clavulanate (P=0.5), ciprofloxacin (P=0.69), gentamicin (P=0.3) and ceftazidime (P=1.00) (Table 3).

There was a statistically insignificant difference in clinical symptoms between children infected with *E. albertii* and those infected with *E. coli* (Table 4).

Table 3. Comparison between *E. albertii* and *E. coli* as regards antibiotics resistance.

	<i>E. albertii</i> (n=7) No. %	<i>E. coli</i> (n=93) No. %	Odds Ratio	95%CI	P
Ampicillin/ Clavulanate	3 42.9%	46 49.5%	0.766	0.16-3.615	0.5
Aztreonam	4 57.1%	23 24.7%	0.25	0.05-1.18	0.083
Cefepime	1 14.3%	32 34.4%	1.97	0.22-17.2	0.5
Cefotaxime	3 42.9%	37 39.8%	0.88	0.19-4.2	0.58
Ceftazidime	3 42.9%	38 40.9%	0.9	0.19-4.34	1.00
Ceftriaxone	3 42.9%	46 49.5%	1.3	0.27-6.15	1.00
Cefuroxime	2 28.6%	31 33.3%	1.3	0.24-7.14	0.9
Cephalothin	2 28.6%	31 33.3%	1.25	0.22-6.81	1.00
Ciprofloxacin	4 57.1%	41 44.1%	1.7	0.35-7.98	0.69
Gentamicin	5 71.4%	44 47.3%	2.7	0.5-15.1	0.3
Imipenem	3 42.9%	41 44.1%	0.95	0.2-4.49	1.00
Levofloxacin	2 28.6%	39 41.9%	1.8	0.33-9.7	0.7
Meropenem	3 42.9%	37 39.8%	1.13	0.24-5.35	1.00
Nalidixic acid	0 0%	17 18.3%	1.09	1.02-1.16	0.59
Norfloxacin	1 14.3%	17 18.3%	0.74	0.036-6.6	1.00
ESBL	4 57.1%	39 41.9%	1.8	0.39-8.7	0.45

Table 4. Comparison between *E. albertii* and *E. coli* as regards demographic and clinical data.

-	Children with <i>E. albertii</i> Infection (n=7) No. %	Children with <i>E. coli</i> Infection (n=93) No. %	Odds Ratio	95%CI	P
Male	1 14.3%	45 48.4%	1.09	.02-1.5	0.12
Female	6 85.7%	48 51.6%			
Dysuria	1 14.3%	38 40.9%	0.24	0.028-3.08	0.2
Fever	2 28.6%	44 47.3%	0.44	0.082-2.14	0.4
Abdominal pain	3 42.9%	33 35.5%	1.4	0.3-6.46	0.7
Lethargy	2 28.6%	25 26.9%	1.09	0.19-5.97	1.000
Irritation	3 42.9%	22 23.7%	2.4	0.5-11.6	0.4
Poor feeding	2 28.6%	25 26.9%	1.09	0.19-5.9	1.00
Vomiting	5 71.4%	30 32.3%	5.2	0.96-28.63	0.049

4. DISCUSSION

E. albertii is a species derived from *E. coli* with difficulty to differentiate with biochemical or phenotypic characterization. Some reports identify *E. albertii* on the base of negative lactose fermentation, however, some strains can ferment lactose [27]. From genetic studies conducted on *E. albertii*, two genes of *lysP* and *mdh* have been identified as diagnostic markers for the identification of *E. albertii*. *E. albertii* has been reported to be associated with mortality in animals such as cats, pigs and birds. Moreover, environmental and food contamination may be associated with this bacterium [28].

In the present study, by using *mdh* and *lysP* genes, seven isolates had been identified as *E. albertii* in 100 isolates from urinary tract infections. A previous study had reported identification of 5 isolates as *E. albertii* among 60 isolates from urinary tract infections [6]. Higher prevalence of *E. albertii* was reported in previous studies from patients with diarrhea by the use of these two genes among isolates recognized as *E. coli* by biochemical reactions and phenotypic methods. Nimri reported 48 isolates as *E. albertii* from total of 250 isolates

with the absence of *uidA* gene [29], and Aoshima identified six isolates of *E. albertii* detected from 20 *E. coli* recognized phenotypically in specimens from a gastrointestinal tract infection of food origin [30]. These studies highlight the emergence of *E. albertii* as a causative organism in human infections with the requirement of application of specific genetic studies for identification of *E. albertii* [19].

Specific identification of *E. albertii* is an important issue for determination of its resistance to antibiotics [31]. Until now, little data is known about antibiotic resistance of *E. albertii*. In the present research, *E. albertii* isolates had marked resistance to gentamicin, followed by resistance to ciprofloxacin, meropenem and imipenem. Another study from China reported different resistance pattern of 51 isolates of *E. albertii*, as those isolates were found to be either sensitive or exhibited intermediate susceptibility to amoxicillin-clavulanic acid, levofloxacin, imipenem and meropenem. The difference in the resistance pattern can be attributed to the difference in the number of isolates and the difference in the protocol of antibiotics prescription between different countries.

ESBL activity detected in *E. albertii* by double discs

method was reported in 57.1% of the isolates. This is higher than that reported in a previous study from China, as only 15 isolates from 51 *E. albertii* isolates had ESBL activity [31].

There was statistically insignificant difference in resistance to the used antibiotics between *E. coli* and *E. albertii* isolates. There was insufficient data about the resistance difference between *E. coli* and *E. albertii*. Therefore, there is a need for more epidemiological studies on the patterns of drug resistance of *E. albertii* and to determine the mechanisms of molecular resistance for the management of infections caused by those isolates [19].

Urinary tract infection signs in children are highly heterogeneous, and the symptoms can be relatively unclear. The gold standard diagnostic test for urinary tract infection is still urine culture [32]. The management of UTI in children has been changed from the use of aggressive radiological exploration and prophylactic antimicrobial therapy to a more controlled approach [33]. In the present study, the common symptoms were fever, dysuria, abdominal pain and vomiting. However, these symptoms are widely shared by many clinical conditions in children.

CONCLUSION

The present study highlights the emergence of *E. albertii* as a pathogen associated with urinary tract infections in children. There is marked antibiotic resistance of this pathogen, especially toward extended spectrum beta-lactams antibiotics. The identification method depends mainly on genetic studies. Further longitudinal studies with a large number of patients are required to verify the accurate prevalence of this bacterium.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee of Mansoura Faculty of Medicine, Egypt (R.20.12.1110).

HUMAN AND ANIMAL RIGHTS

No animals were used for studies that are base of this research. All the humans used were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013 (<http://ethics.iit.edu/ecodes/node/3931>).

CONSENT FOR PUBLICATION

The approval was taken from the parents of the patients.

STANDARDS OF REPORTING

STROBE guidelines and methodologies were followed for this study.

AVAILABILITY OF DATA AND MATERIALS

The data supporting findings of this study is available at: <https://data.mendeley.com/v1/datasets/publish-confirmation/cstdcfkwm2/1?folder=14dcb646-ab2b-498f-888f-83abda129b2e>.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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