

Detection of *Escherichia albertii* in urinary and gastrointestinal infections in Kermanshah, Iran

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Research article

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Abstract

Background

Escherichia albertii (*E. albertii*) is a Gram-negative and facultative anaerobe bacterium. In recent years, the bacterium has been isolated from the feces of people with gastroenteritis as a pathogen that causes diarrhea. Due to insufficient information on the phenotypic and biochemical characteristics of *E. albertii*, it is difficult to distinguish it from other species of the Enterobacteriaceae family. This is especially prevalent in the pathotypes of *Escherichia coli* (*E. coli*). Moreover, in clinical laboratories, it is mistakenly identified as *E. coli* or even *Hafnia alvei* (*H. alvei*). This study was performed for the first time in Iran to identify *E. albertii* by PCR method from a sample of urinary and gastrointestinal infections obtained from clinical laboratories in Kermanshah, which were distinguished as *E. coli*.

Methods

In this study, 60 urinary samples and 40 fecal samples that were identified as *E. coli* by phenotypic and biochemical methods in clinical laboratories. The samples were re-evaluated in the first step in terms of specific phenotypic and biochemical characteristics of *E. coli*. Subsequently, DNA was extracted from the isolates by the phenol method. Then, two *lysP* and *mdh* genes were detected for *E. albertii* and the *uidA* gene for *E. coli* by PCR using specific primers pairs.

Results

The results obtained from phenotypic and biochemical tests indicated that all samples were consistent with *E. coli* characteristics. However, findings from PCR showed that out of a total of 100 samples, specific genes of *E. coli* were identified in 6 samples (6%) and *uidA* gene in 94 remaining samples (94%). Of these 6 samples, 5 samples were urinary tract infections, and only one was a gastrointestinal infection.

Conclusion

The findings of this study show that *E. albertii* can be considered as one of the causes of urinary and gastrointestinal infections that are mistakenly identified in clinical laboratories as *E. coli*. Therefore, the use of molecular methods for accurate and definitive diagnosis of bacteria can be useful.

Background

Infectious diseases are always a threat to health and life, and they impose huge costs on families and the health-care system of the country every year. Infections related to gastrointestinal and urinary tract

infections are of particular importance. A high proportion of these infections are attributed to the genus *Escherichia* from the Enterobacteriaceae family (1–4).

Escherichia albertii is a relatively new member of this bacterial family, and there is strong evidence that the bacterium plays a role in many global conflicts. Its misdiagnosis as Enteropathogenic *Escherichia Coli* (EPEC), Enterohemorrhagic *Escherichia coli* (EHEC), or other pathogenic bacteria has continued from the past to the present. Recently, its pathogenic potential has been strengthened by identifying strains with multiple antibiotic resistance (5–7).

Epidemiology, transmissibility, prevalence, pathogenicity, virulence factors, and antibiotic resistance in this emerging bacterium remain a challenge, and information about it is very poor. Its misdiagnosis as *E. coli* and the lack of a reliable diagnostic method to differentiate it are important points that have led to further investigation of *E. albertii* to identify its unique behaviors and characteristics. Comprehensive knowledge of the features of *E. albertii*, as a type of intestinal pathogen in humans and awareness of its prevalence, requires more analysis from different sources, different hosts, and the use of more accurate identification methods.

The *uidA* gene is one of the specific housekeeping genes in *E. coli* that encodes beta-di-glucuronidase and is considered one of the virulence markers in *E. coli*. Numerous studies have used this gene to identify *E. coli*, and PCR methods based on the diagnosis of this gene have been repeatedly used to diagnose *E. coli* (8–11). On the other hand, to identify *E. albertii*, tracking of two genes of *mdh* (Encoding Malate dehydrogenase) and *lysP* (Encoding Lysine Permease) as specific and protected genes in *E. albertii* is performed in the form of a multiplex PCR method (8–10, 12, 13). Also *rpoB* gene sequence-based identification, Multilocus sequence typing (MLST), or Whole Genome Sequencing (WGS) methods have been proposed, one of the limitations of which is being time-consuming to complete the results (10, 13–15).

As can be seen, the species of *E. albertii* is considered a human pathogen that may be ignored due to weakness in diagnosis. Moreover, the prevalence of this species in Iran is unknown. Therefore, the present study aimed to investigate the prevalence of *E. albertii* and its differentiation from *E. coli* in clinical samples of patients with diarrhea and urinary tract infections in Kermanshah using molecular methods.

Methods

This is a cross-sectional-analytical study. The study population was the cultures of urine and fecal clinical samples collected from laboratories in Kermanshah. 180 culture samples belonging to 180 clinical samples were collected during 6 months (From February 2018 to July 2018), which after initial tests, a total of 100 samples infected with *E. coli* were identified. Finally, these 100 samples were examined to track study objectives. Samples of urine and fecal culture included 60 and 40 cases, respectively.

Culturing of samples

In the research laboratory, subcultures were prepared from cultures suspected of containing *E. coli* obtained from fecal and urinary specimens (Positive lactose and negative lactose) and biochemical tests including TSI, SIM, MRVP, Simon citrate, Urease, lysine, phenylalanine, and culture on the MacConkey agar were performed to identify *E. coli* (16).

Preparation of microbial stock

To make the isolates usable in later stages, including molecular tests, the microbial stock was prepared from pure isolates that were detected *E. coli* using biochemical tests. A preservation medium (Including glycerol and liquid BHI medium) was used for this purpose. The pure bacterial isolates were then placed in tubes and after two hours of heating stored in a 20°C freezer (17).

Genomic DNA extraction

In the present study, DNA extraction was performed by the Phenol-Chloroform-Isoamyl method (16).

Determination of quality and quantity of extracted genomic DNA

To determine the quantity and quality of DNA, electrophoresis was used on 0.8% agarose gel, and the quality of the band was assessed using Ethidium bromide and placing the gel inside the Gel Document device.

Polymerase chain reaction (PCR)

Isolates that belonged to *E. coli* (Negative lactose and positive lactose) based on biochemical tests were nominated to track down *E. albertii*. For this purpose, three genes of *uidA*, *mdh*, and *lysP* were evaluated using the PCR method.

In the present study, the polymerase chain reaction was performed using a thermocycler device in a volume of 20 µl. The materials required for each reaction are shown in Table 1.

Primer selection

The primers of the three required genes (*uidA*, *mdh*, and *lysP*) were purchased from CinnaGen Company (Iran). The selected primer pairs were diluted (1:10) according to the defined standard by adding specific amounts of sterile distilled water and a working solution was prepared from them (Table 2).

Thermal cycling of PCR reaction

The thermal cycle was first performed with an initial denaturation at 95°C for 5 min, followed by 35 cycles. During these 35 cycles, denaturation at 95°C for 30 s, the primer annealing at different temperatures for each primer (Annealing temperatures in this study for *uidA*, *mdh*, and *lysP* primers were 67, 65, and 64°C, respectively) was performed for 30 s and the extension step was performed at 72°C for 60 s. Finally, after the cycles were completed, the final extension of the samples was performed at 72°C

for 5 min (Table 3). After the cycles were completed, the samples were removed from the device and stored at 4°C until electrophoresis was performed (10 and 11 with minor modifications).

Electrophoresis of PCR products on agarose gel

DNA electrophoresis was performed horizontally in 2% agarose gel. The gel was placed inside the gel documentation device and the proliferated DNA bands were compared by the indicator DNA to view the desired bands.

Results

As mentioned earlier, during the initial processing, 100 samples (Negative lactose and positive lactose samples suspected of *E. coli*), including 60 urine samples and 40 fecal samples, were sent to the research laboratory for phenotypic and complementary biochemical diagnosis and molecular testing to identify *E. albertii*.

Results from phenotypic and biochemical tests

Biochemical tests performed on 100 samples, including TSI, SIM, MRVP, Simon citrate, urease, lysine, phenylalanine, and culture on MacConkey agar indicated that they belonged to *E. coli*.

The PCR results of *uidA*, *mdh*, and *lysP* genes

The results of the polymerase chain reaction of 100 samples showed that 94% of them were *E. coli* (Positive *uidA* gene) (Figs. 1) and 6% of them were positive in terms of tracking two specific genes of *E. albertii* (*mdh* and *lysP*) (Fig. 2 and 3). On the other hand, these 6 isolates were negative for the *uidA* gene. Five isolates of these 6 cases were related to the urinary tract and one to gastrointestinal infections.

Discussion

The share of Escherichia, especially *E. coli*, is significant in urinary and gastrointestinal infections (3, 4, and 18). In recent years, *E. albertii* has been reported to cause gastrointestinal infections in some cases of epidemics, and the results indicated that *E. albertii* resembles *E. coli* pathotypes (19, 20). This has challenged a range of studies to verify the diagnosis of *E. coli* pathotypes, especially EPEC, in recent years. Thus, it was proved that *E. albertii* was mistakenly identified as EPEC due to its unknown features and similar phenotypic and biochemical characteristics to *E. coli* (12, 21, and 22). The reliable classification of diarrhea-causing bacteria into distinct pathotypes requires molecular tools, and the unavailability of this equipment in clinical laboratories has led to misdiagnosis and neglect of some pathogens, including *E. albertii* (22). Recently, researchers, have examined this unknown species in terms of prevalence, biochemical characteristics, pathogenicity, and virulence factors. Although studies in this area are increasing day by day, there is still insufficient information about these indicators in *E. albertii*.

The study of this bacterial species is of particular importance since some findings have obscured the information from previous research. Therefore, given the importance of *E. albertii* as a lesser-known bacterial species and its importance in gastrointestinal and urinary tract infections and its neglect in diagnosis, for the first time in Iran (As far as the authors know) the present study identified *E. albertii* among urinary and fecal samples collected from clinical laboratories in Kermanshah province. These specimens were previously identified as *E. coli*.

Of the 100 clinical samples, 94 were positive for the *uidA* gene (Specific for *E. coli*) and using the specific genes of *E. albertii* (*lysP* and *mdh*), 6 cases (6%) of *E. albertii* were finally identified. The *uidA* gene was not found in these six samples. Of the 6 cases of *E. albertii*, 5 cases belonged to urinary tract infections. It is believed that the origin of *E. coli*, which causes urinary tract infections, is intestinal flora (23). As a result, the gastrointestinal tract can be the origin of *E. albertii* identified from urinary samples in the present study.

To identify *E. albertii*, similar studies have traced two genes of *lysP*, and *mdh*. Nimri reported that out of a total of 250 isolates obtained in about ten years from the feces of people with diarrhea, 48 cases of *E. albertii* were identified using the *lysP* and *mdh* genes. The *uidA* gene was not found in these 48 isolates. It should be noted that these specimens had previously been identified as *E. coli* (8).

Aoshima also reported 6 *E. albertii* isolates from 20 phenotypically recognized *E. coli* samples by identifying the *lysP* and *mdh* genes in a population with a gastrointestinal infection of food origin (12).

By tracking the *eae* gene and sequencing it, Ooka identified 21 out of 31 samples related to the gastrointestinal infection as *E. albertii*. They were initially diagnosed as *E. coli* (27). In another study, Ooka reported that out of a total of 278 samples from the human, animal, and environmental sources previously identified as *E. coli* using common diagnostic methods, 26 were identified as *E. albertii* using MLST analysis of the *eae* gene. 14 cases of them belonged to human samples (21).

Hinenoya re-examined 20 strains of *E. coli* isolated from diarrheal infections. He identified all 20 isolates as *E. albertii* by MLST analysis of housekeeping genes of *E. albertii* (24).

Ori et al., in a 6-year care program re-examined diarrhea-causing *E. coli* isolates. They identified 10 *E. albertii* cases out of a total of 693 isolates by tracking specific genes including *cdgR*, DNA-binding transcription activating gene in cysteine biosynthesis, and palmitoyl-acyl carrier protein-dependent acetyltransferase gene (22).

In a research conducted by Lindsey, out of a total of 1,644 samples of chicken carcasses over one year at the slaughterhouse, *lysP* and *mdh* genes were positive in 61 isolates, which were identified as possible *E. albertii* species. However, the sequencing of the *rpoB* gene reduced the number of *E. albertii* to 27 (14).

In general, due to the lack of a specific diagnostic protocol for *E. albertii*, different studies have used different methods and specific genes to identify it, and this information is constantly changing. It can be argued that although most studies have identified the two genes of *lysP* and *mdh* as specific genes in the

diagnosis of *E. albertii*, in several studies these two genes have not been able to identify all *E. albertii*. Therefore, efforts have been made to design more specific areas of the genome (10, 13–15).

Also, there is a discrepancy in the findings of the phenotypic and biochemical characteristics of *E. albertii*. For example, *E. albertii* was previously considered as a type of negative lactose, but a recent study found that strains of *E. albertii* could ferment the lactose (26). One of the reasons for the limited recognition of the phenotypic and biochemical properties of *E. albertii* is the small number of known strains to date (14).

In general, there is no sufficient information about the features of *E. albertii* to isolate and diagnose it optimally. As a result, it is difficult to identify the true prevalence of infections associated with *E. albertii* (24).

In several studies, the role of *E. albertii* as a potential and related pathogen in cases of gastroenteritis and diarrhea in humans has been confirmed (14 and 25–30). In countries such as Japan and Norway, the number of infections caused by *E. albertii* is increasing, which is a warning sign that the bacterium is causing problems around the world (24).

E. albertii is important not only for its pathogenicity and its role in gastroenteritis in the world but also for its resistance to certain antibiotics (6, 7). Therefore, it is important to continuously examine patients with diarrhea and urinary tract infections to detect *E. albertii*. Deaths among birds are another reason for global attention to *E. albertii*. The bacterium has also been isolated from animals such as pigs, cats, and in some cases from environmental and food contamination (21, 31–33, 98–100). As a result, epidemiological studies should include not only the clinical level but also animal and environmental patterns such as water and food.

In the first step, the study of the frequency of *E. albertii* in the world can help further identify it. Epidemiological studies also help identify pathogenic strains, biochemical characteristics, and virulence genes. Finally, the integration of data and results leads to a comprehensive and accurate definition of the characteristics of *E. albertii*. In general, it can be said that in the future, diagnostic tests for *E. albertii* will be routinely performed in clinical laboratories to differentiate it from other members of the Enterobacteriaceae family.

Conclusions

As far as the authors know, the present study is the first report on the identification and separation of *E. albertii* from gastrointestinal and urinary tract infections in Iran. The results show that *E. albertii* is one of the possible causes of diarrhea, gastroenteritis, and urinary tract infections. Therefore, it is necessary to study and recognize this bacterial species as much as possible. Extensive and comprehensive epidemiological studies in Iran and other parts of the world are recommended using well-known molecular methods to achieve preventive and therapeutic goals before major conflicts caused by *E. albertii*.

Abbreviations

PCR: Polymerase Chain Reaction.

Declarations

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Authors' contributions

All authors contributed to the study conception and design. AF designed the study, performed the statistical analysis, conducted data analysis and wrote the manuscript. AN and BRZ participated in sample collection and carried out the bacteriological and molecular tests. All authors read and approved the final manuscript.

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Availability of data and materials

Data in this study is available upon request from corresponding author.

Ethics approval and consent to participate

Bacterial samples were collected from clinical laboratories which patients have previously been approved for use in medical research. Explanations were written to Editorial manager.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Optimized Reaction Components

(Volume (µl	Sample components
12.6	DD water
2	Buffer (10X) PCR
1.5	(MgCl ₂ (50 mM
0.4	(dNTPs (10 mM
(From each one) 1.2	Primers
0.3	(Taq DNA Polymerase (5 U/µl
2	Template DNA (10 ng/µl)
20	Sum

Table 2. Primer Sequences Used in This Study

Gene	Sequence ('3-'5)	Product size (bp)	Reference
<i>uidA</i>	5'-GCGTCTGTTGACTGGCAGGTGGTGG -3' 5'-GTTGCCCGCTTCGAAACCAATGCCT -3'	503	11
<i>mdh</i>	5'-CTG GAAGGC GCA GAT GTG GTA CTG ATT-3' 5'-CTT GCT GAA CCA GAT TCT TCA CAA TAC CG-3'	115	10
<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	10

Table 3. PCR Temperature cycles

Cycle (s)	Time duration	Temperature	Step
1	5 m	95°C	Heating
35	30 s	95°C	Denaturing
	30 s	67°C	<i>uidA</i>
		65°C	<i>mdh</i>
		64°C	<i>lysp</i>
60 s	c°72	Extension	
1	5 m	c°72	Final extension

Figures

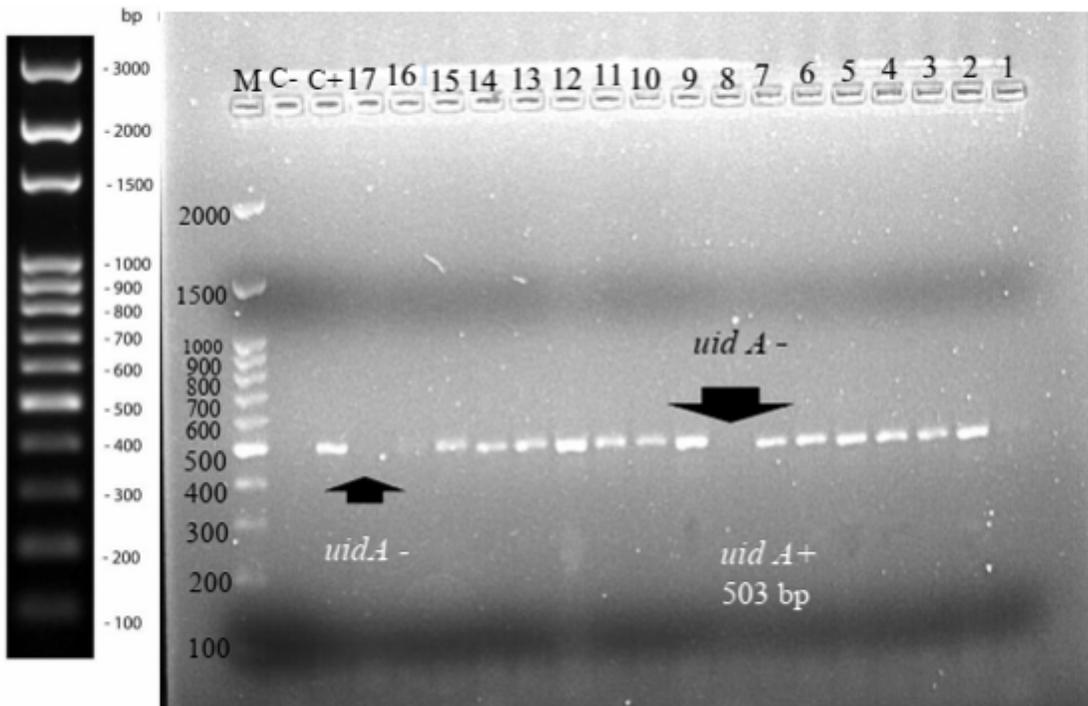


Figure 1

Electrophoresis of PCR products related to *uidA* gene (503 bp) on agarose gel 2%, M: Ladder 100 bp, C+: Positive control, C-: Negative control. Samples 8 and 17 are negative for *uidA*.

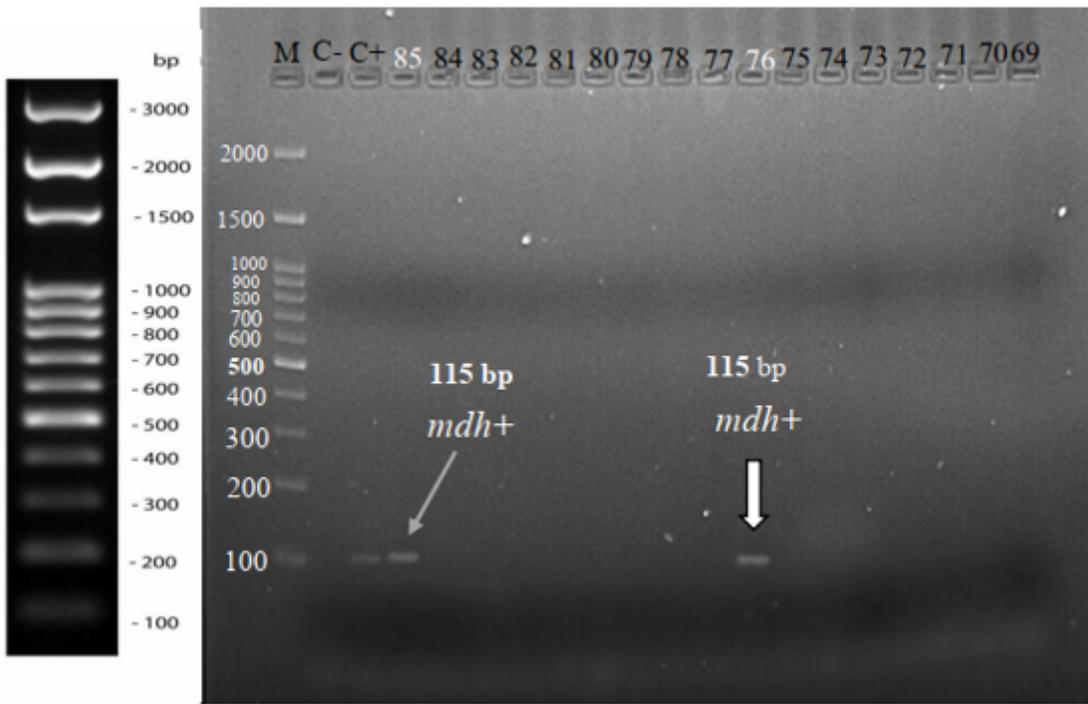


Figure 2

Electrophoresis of PCR products related to *mdh* gene (115 bp) on agarose gel 2%, M: Ladder 100 bp, C+: Positive control, C-: Negative control. Samples 8 and 17 are positive for *mdh*.

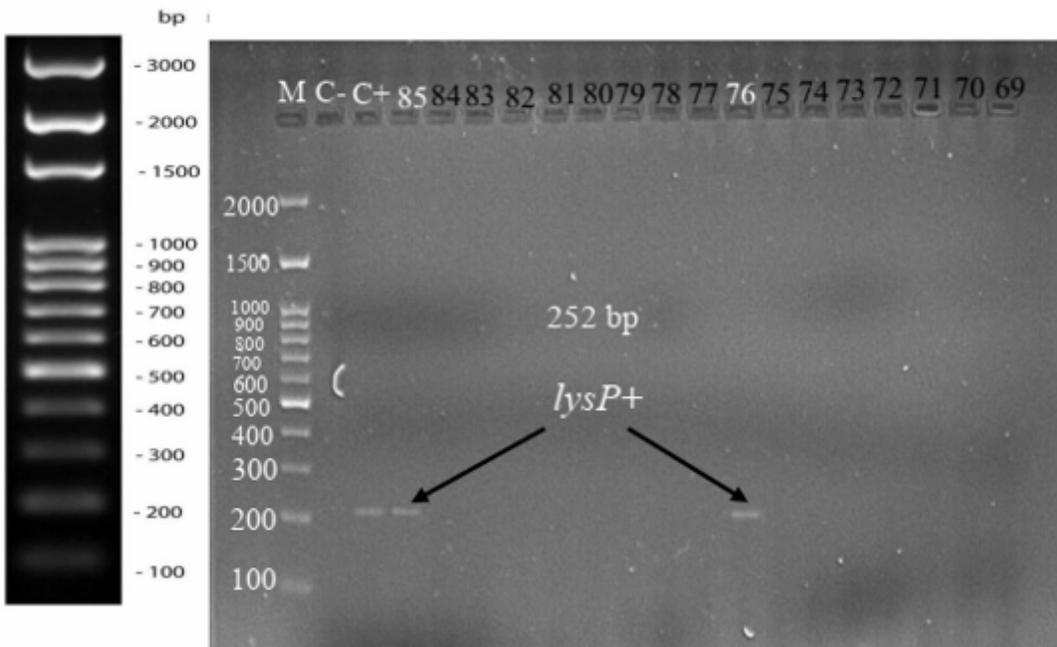


Figure 3

Electrophoresis of PCR products related to lysP gene (252 bp) on agarose gel 2%, M: Ladder 100 bp, C+: Positive control, C-: Negative control. Samples 8 and 17 are positive for lysP.

Supplementary Files

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