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Isolation and Identification of *Escherichia coli* Producing Cytosine Deaminase from Iraqi patients

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Abstract

This study was aimed to isolate a locally higher cytosine deaminase producer *Escherichia coli.* Ten *E. coli* bacterial isolates were obtained from twenty-five urine samples collected from Al-Imamein AlKadhumain medical city hospital in Baghdad. These isolates were identified by cultural, microscopical and biochemical tests, identifications also done by vitek 2 system.
Cytosine deaminase activity was assayed for these isolates and it was found that all isolates were enzyme producer with variable activities and *E. coli* E9 was the most efficient with specific activity of (0.350 U/mg).

Keywords: *Escherichia coli*, cytosine deaminase, identification, vitek 2 system

Introduction

Enterobacteriaceae are a large and diverse family. Members of this family are Gram- negative facultative anaerobic bacilli approximately 0.3-1.0 x 1.0-6.0 µm in size, they are generally motile by peritrichous flagella. Most bacteria in this family grow well and are most metabolically active at 25-35°C, they are chemoorganotrophic, having both a respiratory and fermentative metabolism (1).It is usually characterized by two key properties; it is oxidase negative and glucose fermenters. Isolates initially identified as members of this family are classified according to their biochemical properties, to determine the genus and species (2). Members have been isolated from a wide variety of sources, including soil, fresh and salt water, fruits and vegetables, grains, meats and eggs, a wide variety of plants, insects, animals and humans (3). *Esherichea, Shigella, Salmonella, Citrobacter, Erwinia, Serratia, Proteus, Yersinia, Providencia, Morganella, Enterobacter* and *Klebsiella* are some of the most important genera that belong to family *Enterobacteriaceae (*2). Also, members of family *Enterobacteriaceae* are usually found in the human gastrointestinal tract (GIT) with some of them are considered part of the gut normal flora, they also inhabit the GIT of other animals, fish, and insects, as well as plants, soil, and water, also most species of this family are considered pathogenic to immunocompromised people (2). On the other hand, they possess an endotoxin (Lipopolysaccharide), which is the primary contributor to their ability to cause infections (4). The genus *Escherichia* is a Gram-negative, non-spore forming, facultatively anaerobic, rod-shaped bacteria from the family Enterobacteriaceae (5). In those species which are inhabitants of the gastrointestinal tracts of warm-blooded animals, *Escherichia* species provide a portion of the microbially derived vitamin K for their host. A number of the species of *Escherichia* are pathogenic (6). *E. coli* is responsible for the vast majority of *Escherichia*-related pathogenesis and other members of the genus have also been implicated in human disease (7). *Escherichia* are associated with the

imbalance of microbiota of the lower reproductive tract of women, these species are associated with inflammation (8).

One of the most notable features of *E. coli* is broad diversity of disease-causing genotypes. The diseases can encompass different symptoms and gastrointestinal tract pathologies, but there are also diseases at extraintestinal sites. These different genotypes and their disease-causing abilities lead to categories of *E. coli* often referred to as pathotypes. There are six intestinal and two extraintestinal pathotypes currently recognized (9). Specify an enterotoxigenic *E. coli*. (ETEC) as a frequent cause of diarrhea in both humans and animals, (ETEC) are estimated to cause 600 million cases of human diarrhea and 800,000 deaths worldwide principally in children under the age of 5(10). Since one of the principal virulence factors for this pathogen is the heat-labile enterotoxin (LT), which interestingly shares structural and functional similarity to the *Vibrio cholera* cholera toxin (11).

Materials and Methods

Specimens collection

A total of 25 specimens were collected from the urine of the patients, from Al-Imamein AlKadhumain medical city hospital in Baghdad. Swab specimens were aseptically transferred under cooling conditions to the laboratory for analysis.

Isolation of *Escherichia coli*

According to Macfadden, (12), swabs were taken carefully from the site of infection and placed in tubes containing transferred medium to maintain the swab wet during transferring to laboratory. Each specimen was inoculated on *Escherichia* isolation MacConkey agar. All plates were incubated aerobically in incubator at 37 ^{0}C for 24 hrs.

Identification of *Escherichia coli*

Cultural examination

Morphological characteristics of colonies were studied on MacConkey agar. Color; size and edge of colonies were recorded after 24 hrs of incubation at 37˚C.

Microscopic examination

A single colony of each isolate was fixed on a clean slide to study gram stain, under light microscope Atlas *et al.*, (13).

Biochemical tests

The suspected isolates were subjected to the biochemical tests as mentioned by Macfadden (12), and as follow:

A- Catalase test

A single colony of each bacterial isolate was taken and smeared on clean glass slide, then a drop of hydrogen peroxide (3drops was flooded with 1.0 ml of 3% hydrogen peroxide. Presence of gaseous bubbles indicate a positive result.

B- Oxidase test

This test was done using filter paper moistened with few drops of a freshly prepared solution of tetramethyl- ρ- phenylenediaminedihydrochloride. Aseptically, a clump of cells was picked up from the slant growth with a sterile wooden stick and smeared on the moistened paper. Development of a violet or purple color within 10 seconds indicates a positive result.

C- Indole test

Colony was inoculated into peptone water broth and incubated at 37°C for 24 hrs in a shaker incubator. After incubation, few drops of Kovac's reagent were added. Presence of pink colored ring indicates a positive result.

D- Citrate utilization test (Simmon's Citrate slant)

A loopful of colony was streaked onto a simmon citrate agar slant, and then incubated for 24 to 48 hrs at 37°C in incubator. Change in medium color to blue color indicates a positive result.

E-Methyl-red test

Colony was inoculated in MR-VP broth and incubated at 37°C for 24 hrs. After incubation, 3-4 drops of methyl red reagent were added. Converting media color to red is a positive result .

F-Vogas-Proskauer test

Colony was inoculated in MRVP broth and incubated at 37°C for 24 hrs. Then two drops of VP1 and four of VP2 were added. Appearance of red color after 15 min indicates a positive result.

Identification of suspected bacteria by VITEK 2 system

The VITEK 2 is an automated microbiology system utilizing growth-based technology. Used for bacterial identification (14).

Determination of specific cytosine deaminase activity for *Escherichia coli*

Cytosine deaminase activity was determined according to katsuragi *et al.,* (15) by adding 0.4 ml of enzyme solution to 1 ml of cytosine solution prepared in and 0.6 ml of potassium phosphate solution. Then incubated for 30 minutes at 37 ^{o}C in water bath. The reaction was stopped by adding 6 ml of the 0.1M HCL. The solution was centrifuged at 9000 rpm for 15 minutes. Then the absorbance was measured for the supernatant at 280 nm using UV-spectrophotometer.

The blank was prepared using the same steps except the addition of stop solution into cytosine before the addition of enzyme solution.

Protein concentration was determined according to the method described by Bradford, (16)

Results and Discussion

Isolation and identification of bacterial isolates

3.1.1 Cultural characteristic

Clinical specimens of urine were cultured onto MacConkey agar. Ten isolates were found to be able to ferment lactose as determined by the presence of pink colonies. These characteristics come in accordance with the corresponding cultural characteristic of *E. coli* that mentioned by Mohammed, (17).

Also, Van Belkum*et al.*, (18) mentioned that rapid identification of *E. coli* and processing of Enterobacteriaceae, colonies were first examined for lactose fermentation (direct examination on MacConkey agar).

Microscopical examination and biochemical characteristics

Pure colonies were identified according to their Gram staining and other microscopically characteristic. The bacterial isolates were gram negative rods in shape, non-spore forming, with peritrichous flagella.

Bacterial isolates suspected to be *E. coli* according to microscopical characteristics were subjected to the related biochemical tests. Results illustrated in Table (1) showed that all the ten isolates were had given positive test for catalase, indole, methyl red and for lactose fermentation, but negative test for oxidase, citrate and Vogas-Proskauer. Kithar R. *et al*., (19) and Forbes *et al*., (20) mentioned that such characteristics usually are coming in accordance with those belonging to *E. coli.*

Table (1): Biochemical tests for bacterial isolates.

Biochemical tests	Isolated									
	E6	E7	E8	E9	E11	E15	E16	E17	E18	E21
Catalase test	+	+	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-	-	-	-
Indole test	+	+	+	+	+	+	+	+	+	+
Citrate utilization test	-	-	-	-	-	-	-	-	-	-
Methyl-red test	+	+	+	+	+	+	+	+	+	+
Vogas-Proskauer	-	-	-	-	-	-	-	-	-	-
Lactose fermentation	+	+	+	+	+	+	+	+	+	+

***(+) Positive results (-) negative results**

Identification of *E. coli* by VITEK system

The ten isolate were subjected to identification by VITEK 2 system. Table (2) shows that these isolates gave 96 % similarity to those characteristics of *E. coli* as identified by the standard GN (gram negative) card.

Garcia-Garrote *et al*. (21) pointed that the VITEK 2 system is an easy to handle system that provides a rapid (during 4 to 15 h) and reasonably accurate means for the identification of bacterial species.

Joyanes *et al*., (22) tested 146 routinely isolated strains of non-fermenting gram-negative rods using VITEK 2 system and ID-GNB cards with 91.6% similarity in identification. Also, Ines *et al*., (23) founded that the correct identification rates of *E. coli* were 90.1%using the same VITEK 2 identification card.

Table (2): Biochemical tests for identification of *E. coli* by VITEK 2 system

Test	Result	Test	Result	Test	Result	Test	result	test	Result
APPA	-	ADO	-	PyrA	-	IARL	-	dCEL	-
BGAL	+	H_2S	-	BNAG	-	AGLTp	-	dGLU	+
GGT	-	OFF	+	BGLU	+	dMAL	+	dMAN	+
dMNE	+	BXYL	-	BAIap	-	ProA	-	LIP	-
PLE	-	TyrA	+	URE	-	dSOR	+	SAC	+
dTAG	-	dTRE	+	CIT	-	MNT	-	5KG	+
ILATK	+	AGLU	-	SUCT	+	NAGA	-	AGAL	+
PHOS	-	GLyA	-	ODC	+	LDC	+	IHISa	-
CMT	+	BGUR	-	O129R	+	GGAA	-	IMLTa	-
ELLM	+	ILATa	-						

***(+) Positive results (-) negative results**

Screening ability of *Escherichia coli* for cytosine deamiase production

The ability of local *Escherichia coli* isolates for cytosine deaminase production was screened by determining the Enzyme Activity Katsuragi *et al.*, (15).

Ten of twenty-five isolates are cytosine deaminase producing with different specific activities (Table 3). Depending on these results, the isolate named E9 was found to be the most efficient in the production of cytosine deaminase with specific activity about 0.350 U/mg protein, therefore it was chosen for further study.

Table (3) Specific activity of cytosine deaminase produced by 10 local isolates of *Escherichia coli.*

Isolate number	Specific activity (U/mg)
E6	0.150
E7	0.173
E8	0.333
E9	0.350
E11	0.339
E15	0.157
E16	0.122
E17	0.113
E18	0.338
E21	0.163

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