ISSN: 0067-2904

Isolation and identification of *Citrobacter freundii* from chicken meat samples using cultural and molecular techniques

Mohammed Hayder Hashim*[1], Marwa Hameed AlKhafaji[2]
*[1]Department of Biology, Al-Farabi University College, Baghdad, Iraq
[2]Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

Abstract

Because of *Citrobacter freundii* medical and economical importance and that there are only little local studies about it, this study aimed to isolate and identify this important bacterial species from others that have a similar biochemical and morphological characteristics. Twenty five chicken meat samples were collected randomly from local markets in Baghdad city during 2017; *Citrobacter* was isolated from the collected samples using selective and differential media and identified using biochemical tests, the identification was confirmed using Vitek 2 compact and polymerase chain reaction for 16S rRNA and the isolated bacteria identified as *C. freundii*.

Keywords: *Citrobacter*, Chicken, 16S rRNA, Sequencing.

عزل وتشخيص بكتريا *Citrobacter freundii* من عينات لحوم الدجاج باستخدام الطرق الزرعية والجزيئية

محمد حيدر هاشم*[1]، مروه حميد[2]
[1]قسم علوم الحياة، كلية الفارابي الجامعة، بغداد، العراق
[2]قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

الخلاصة

نظرا لاهمية بكتريا *Citrobacter freundii* من الناحية الصحية والاقتصادية وقلة الدراسات المحلية عنها هدفت هذه الدراسة الى عزل وتشخيص هذا النوع البكتيري المهم وتفريقه عن الانواع البكتيرية الاخرى التي تتشابه معه في بعض الصفات البايوكيميائية والمظهرية، اذ جمعت خمس وعشرون عينة من لحوم الدجاج بشكل عشوائي من الأسواق المحلية في مدينة بغداد خلال العام 2017 ، وتم عزل بكتريا *Citrobacter* من العينات التي تم جمعها باستخدام الاوساط الزرعية الانتقائية والتفريقية . شخصت البكتريا بالاعتماد على الاختبارت البايوكيمائية وتم تأكيد التشخيص بأستخدام Vitek-2وتفاعل سلسلة البلمرة لجين 16S rRNA.

1. Introduction

Citrobacter, a genus of the Enterobacteriaceae family, Gram-negative, facultative anaerobic bacteria that look as coccobacilli or rods [1]. *Citrobacter* spp. are motile using their peritrichous flagella, can ferment mannitol with making of H_2S, and can use citrate as their single source of carbon [2-4]. *Citrobacter* spp. are uncommon opportunistic nosocomial bacteria can cause urinary tract, hematologic, or neonatal infections (e.g. meningitis, sepsis, general bacteremia); intra-abdominal

*Email: mohammed.hydar@gmail.com

sepsis; brain abscesses; or pneumonia [5, 6]. *Citrobacter* spp. infections can be mortal with 33-48% overall death rates being reported including 30% for children [7, 8]. Children and immune deficiency, elderly, or weakened patients are at risk of infection [2, 9]. *Citrobacter* spp. is prevailing worldwide, as it is a part of the normal intestinal flora of humans [10, 11]. Less well known species that have also been implicated in foodborne disease like some strains of *Citrobacter* spp. (notably *C. freundii*), *Klebsiella* spp., *Providencia* spp. *Enterobacter* spp. and *Proteus* spp., may occasionally cause what is often described as opportunistic gastroenteritis [12], this study aimed to isolation and identification of *C. freundii* from chicken meat samples using cultural and molecular techniques.

2. Method and materials

2.1 Samples collection

Twenty five chicken meat samples were collected from local markets in Baghdad city using sterilized containers from July 2017 to October 2017.

2.2 Isolation

One gram of each chicken meat sample was suspended in 9 ml D.W., left for 1 minute, then 0.1ml of each sample suspension was inoculated on the Salmonella shigella (SS) agar medium, the plates were left to solidify at room temperature, and then were incubated at 37 °C for 24-48 hours. Later the grown colonies were further investigated

2.3 Identification

The *Citrobacter* isolates were identified to the level of species using the traditional morphological and biochemical tests [13]. The identification of isolates was confirmed by vitek2 compact system and PCR.

2.3.1 Cultural characteristics on selective and differential media.

2.3.1.1 SS, MacConkey and Xylose lysine deoxycholate (XLD) agar

The organisms were cultured on S.S agar media and incubated overnight at 37°C. The colonies of *C. freundii* appear with black center after 24hrs incubation period, The suspected colonies of *C. freundii* cultured on MacConky media, the positive result appears pink (Lactose fermenters) after 24hrs incubation period, pale colonies further incubated for 24hrs to identify the (late lactose fermenters). The selected colonies were cultured on Xylose lysine deoxycholate agar, after 24hrs, the positive result appeared as yellow colonies [13].

2.3.1.2 Eosin Methylene Blue (EMB) agar

In order to differentiate *Citrobacter* from *E.coli*, the lactose fermenter isolates were subcultured on EMB for 24hr. at 37C^{o}. Brown colonies were the positive result [14].

2.3.2 Identification of bacteria by Vitek 2 compact system.

Vitek 2 compact was used to identify the bacterial isolates. It is a compact system of two parts, Instrument and computer. The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinisation, enzyme hydrolysis, and growth in the presence of inhibitory substances.

2.3.3 Identification of Bacteria by PCR

2.3.3.1 DNA Extraction

Genomic DNA was isolated from Bacteria according to the protocol of Wizard Genomic DNA Purification Kit, Promega. A PCR reaction with a specific primer (Table-1),

Table 1-Primers sequences

Primer Name	**Sequences** 5´ → 3´	**Tm˚C**	**Size (bp)**
27F	AGAGTTTGATCMTGGCTCAG	60	20mer
1492R	TACGGYTACCTTGTTACGACTT		22mer

For 16S rRNA was performed to identify *C. freundii* (Table-2).

Table 2-Reaction mixture

Master mix components	Stock	Unit	Final	Unit	1 sample
Master mix	**2**	X	**1**	X	**12.5**
Forward primer	**100**	µM	**10**	µM	**1**
Reverse primer	**100**	µM	**10**	µM	**1**
Template	**43**	ng/µl	**86**	ng/µl	**2**
Nuclease Free Water			**8.5**		

(25µl) of PCR amplification mixture contained (12.5 µl) Master mix, (1 µl) forward primer, (1 µl) reverse primer, (8.5 µl) nuclease free water, and (2 µl) DNA template. The protocol for PCR condition was initial denaturation 95°C for 5 min. denaturation 95°C for 30 sec., annealing 60 °C for 40 sec., extension 72 °C for 1 min. and final extension 72 °C for 7min.

3. Results and discussion

3.1 Bacterial Isolation and Identification

Twenty five chicken meat samples were collected from local markets in Baghdad city. *Citrobacter* was detected in 3 samples, were all samples cultured on S.S. agar for initial isolation, after incubation at 37°C for 24 hr ; different types of bacterial isolates appeared on S.S. agar, of them: small pale flattened colonies with black center due to their ability to produces H_2S on S.S agar, then these colonies sub-cultured on MacConkey, XLD and EMB to differentiate *Citrobacter* from *Salmonella* because both of them are H_2S, *Citrobacter* is lactose fermenter on MacConkey agar appeared as pink colonies while *Salmonella* is pale colonies (Non lactose fermenter) on XLD *Citerobacter* appeared as yellow colonies while *Salmonella* appeared as red colonies with black center . After incubation period; lactose fermenter (pink) on MacConkey and yellow colonies on XLD while on EMB they were brown in colour, these were depended as *Citrobacter*. To confirm the primary identification Gram stain was performed to examine the microscopic properties which were Gram negative bacilli. The ability of *Citrobacter* to produce urease enzyme was detected using urease test in order to differentiate it from the genus *Proteus* which was urease producer while *Citrobacter* isolates were non urease producers. Thus depending on colonial morphology; bacterial isolates were identified as *Citrobacter* Figure-1 (A, B, C, D) and Table -3 showed these biochemical tests used to identify *Citrobacter* as described by [15, 16].

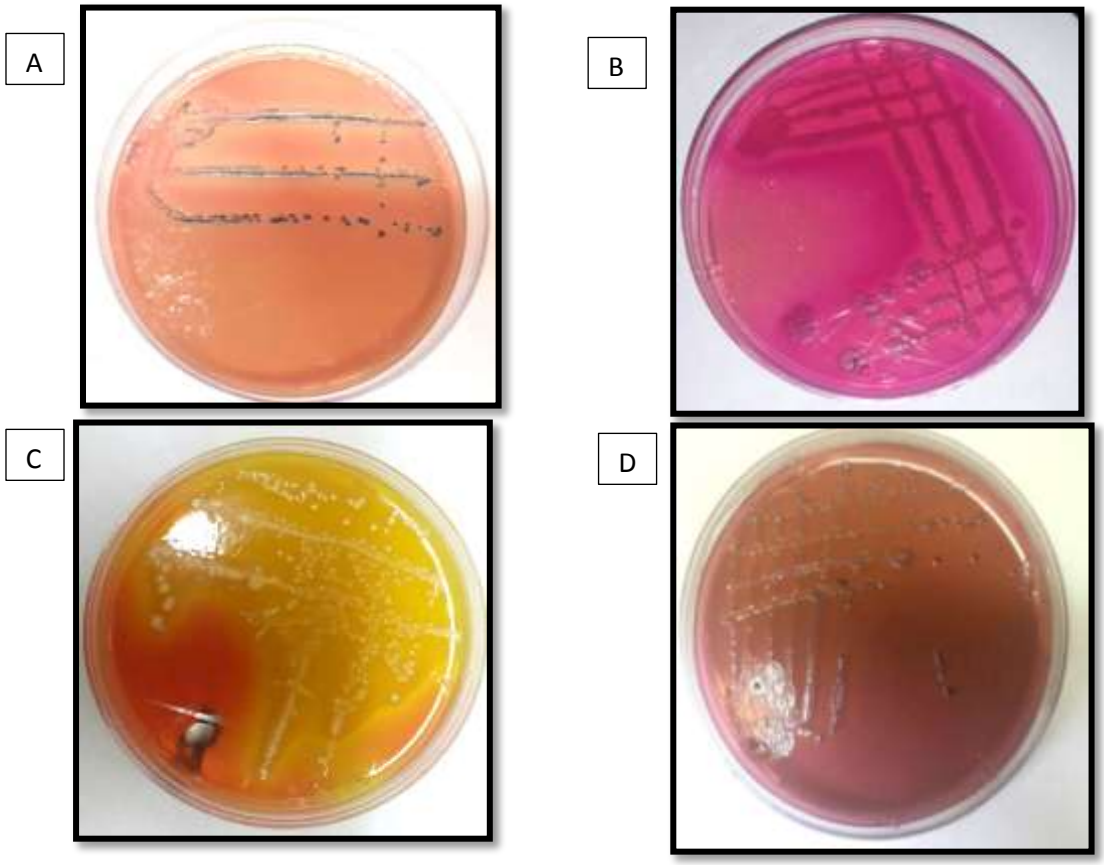

Figure1-Different selective and differential media cultured with *Citrobacter* spp. after incubation at 37°C for 24 hr.

A. Pale colonies with black center on S.S. agar

B. Small pink (Lactose fermenter) colonies on MacConkey agar

C. Yellow colonies on XLD agar

D. Brown colonies on EMB

Table 3-Result of biochemical tests

Test	Result
Growing on MacConkey agar	Dry Pink colonies
Growing on EMB	Not forms green metallic sheen
Gram stain reaction	Gram negative bacteria
Urease	Non urease producer
S.S agar	Pale colonies with black center
XLD agar	Yellow colonies

To confirm the identification of *Citerobacter* spp. Vitek 2 compact system was depended and the result showed that the isolated bacteria in this study was *Citerobacter* and the species *freundii* as shown in Table-4.

Table 4-Identification of *Citrobacter* spp. by Vitek 2 compact system

McFarland: (0.50 - 0.63)

Identification Information	Card:	GN	Lot Number:	2410131403	Expires: Apr 6, 2018 13:00 CDT
	Completed:	Nov 15, 2017 00:28 CST	Status:	Final	Analysis Time: 5.80 hours
Organism Origin	VITEK 2				
Selected Organism	97% Probability		Citrobacter freundii		
	Bionumber:	4407610455520210		Confidence:	Excellent identification
SRF Organism					
Analysis Organisms and Tests to Separate:					
Analysis Messages:					
Contraindicating Typical Biopattern(s)					
Citrobacter freundii	H2S(76),PHOS(86),				

Biochemical Details

2	APPA	-	3	ADO	-	4	PyrA	+	5	IARL	-	7	dCEL	-	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	-	39	5KG	+
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	IMLTa	-	62	ELLM	(-)	64	ILATa	-			

In order to confirm the identification of *Citrobacter* to species level 16SrRNA gene amplification was performed using monoplex PCR technique, 1.5 % agarose gel electrophoresis was used to detect the positive result as shown in Figure-2.

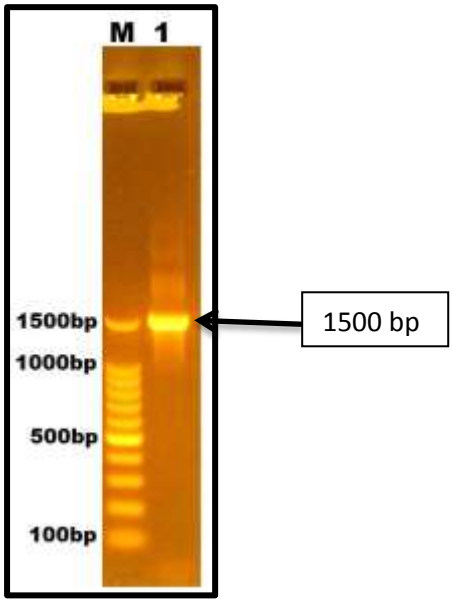

Figure 2- Amplified PCR products of 16SrRNA gene (1500 bp): Agarose gel electrophoresis, ethedium bromide stained, 1.5 % agarose, electrophoresed in 75 volt for 2 hrs and photographed under ultraviolet trans-illuminator. M: The DNA molecular weight marker (100 bp ladder) and 1: the amplified PCR product of 16SrRNA of C10 isolate of *Citrobacter freundii*

One of the most gorgeous likely uses of 16Sr RNA gene sequence informatics is to offer genus and species or tax identification for isolates [17]. Although 16SrRNA gene sequencing is highly valuable in regards to bacterial classification [18]. PCR products were exposed to direct sequencing, both strands of PCR products were sequenced with an automatic sequencer. Sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) (Table-5).

Table 5-16S rRNA gene of C10 isolate *C. freundii* BLAST with reference sequences

Score	Expect	Identities	Gaps	Strand
2534 bits(1372)	0.0	1380/1384(99%)	0/1384(0%)	Plus/Minus

```
Query  3        GCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCCGATGGAGG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4665323  GCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAATGTCTGGGAAACTGCCCGATGGAGG

Query  61       GGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4665263  GGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGA

Query  121      CCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4665203  CCTTCGGGCCTCTTGCCATCGGATGTGCCCAGATGGGATTAGCTAGTAGGTGGGGTAACG

Query  181      GCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4665143  GCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAACTGA
```

```
Query  241      GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4665083  GACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGC

Query  301      CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4665023  CTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGA

Query  361      GGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATTGACGTTACTCGCAGAAGAAGCACCG
                |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct  4664963  GGAGGAAGGTGTTGTGGTTAATAACTGCAGCAATTGACGTTACTCGCAGAAGAAGCACCG

Query  421      GCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664903  GCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACT

Query  481      GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664843  GGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAATCCCCGGGCTCAACCT

Query  541      GGGAACTGCATCCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664783  GGGAACTGCATCCGAAACTGGCAGGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTG

Query  601      TAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664723  TAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACA

Query  661      AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664663  AAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTC

Query  721      CACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664603  CACGCCGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAA

Query  781      CGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664543  CGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACG

Query  841      GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACC
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664483  GGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACC
```

```
Query  901      TACTCTTGACATCCAGAGAAGTTGGCAGAGATGCGAACGTGCCTTCGGGAGCTGTGAGAC
                ||||||||||||||||||||||| |||||||||||||||||||||||| || ||||||
Sbjct  4664423  TACTCTTGACATCCAGAGAAGTTTGCAGAGATGCGAACGTGCCTTCGGGAACTCTGAGAC

Query  961      AGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664363  AGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGA

Query  1021     GCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664303  GCGCAACCCTTATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGT

Query  1081     GATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664243  GATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTA

Query  1141     CACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCA
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664183  CACACGTGCTACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCA

Query  1201     TAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664123  TAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCT

Query  1261     AGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4664063  AGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCG

Query  1321     TCA  1323
                |||
Sbjct  4664003  TCA  4664001
```

Outbreaks caused by *Citrobacter freundii* in the United States have been related to the eating of semi-soft cheeses. In Germany an outbreak associated with *C. freundii* caused gastroenteritis between children, followed by haemolytic uraemic disease with acute renal disaster. Contaminated infant formula has also been occupied as the transporter of infection in an outbreak of *C. freundii* infection [12]. For food control processes fast, sensitive and specific detection technique for pathogens is necessary. For this resolution, PCR technique can be a used [19]. In this study, *C. freundii* was detected using primers pairs based 16S rRNA gene that belongs to *C. freundii* chromosomes and produces 1500 bp.

Conclusions

The usage of microbiological approaches allows the isolation and identification of *Citrobacter*. PCR for 16S rRNA can allow a fast and dependable means of measuring the bacteriological safety of food and waters and should provide another methodology to conventional viable culture method. PCR for 16S rRNA may also allow necessary sensitivity and specificity for the direct detection of *Citrobacter* in food samples.

References

1. Abbott, S. L. **2011.** *Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas*, and other Enterobacteriaceae. In *Manual of Clinical Microbiology, 10th Edition* American Society of Microbiology. 639-657.
2. Doran, T. **1999.** "The Role of Citrobacter in Clinical Disease of Children: Review." *Clinical Infectious Diseases*, **28**(2): 384-394.
3. Knirel, Y., Kocharova N., Bystrova, O., Katzenellenbogen, E. and Gamian, A. **2002.** Structures and serology of the O-specific polysaccharides of bacteria of the genus *Citrobacter*. , *rchivum Immunologiae et Therapiae Experimentalis,* **50**(6): 379-392.
4. Thompson, R.,; Perry, J., Stanforth, S. and Dean, J. **2018.** Rapid detection of hydrogen sulfide produced by pathogenic bacteria in focused growth media using SHS-MCC-GC-IMS. *Microchemical Journal*, **140**: 232-240.
5. Ryan, K., Ray, C. and Sherris, J. **2004.** Sherris medical microbiology: an introduction to infectious diseases, McGraw-Hill. 237
6. Raphael, E. and Riley, L. W. **2017.** Infections caused by antimicrobial drug-resistant saprophytic Gram-negative bacteria in the environment. *Frontiers in medicine*, **4**: 183
7. Pepperell, C., Kus, J., Gardam, M **2002.** Low-virulence Citrobacter species encode resistance to multiple antimicrobials. *Antimicrob Agents Chemother*, **46**(11): 3555-3560
8. Owoseni, M. and Okoh, A. **2017.** Assessment of chlorine tolerance profile of Citrobacter species recovered from wastewater treatment plants in Eastern Cape, South Africa. *Environmental monitoring and assessment*, **189**(4): 201
9. Chen, Y., Wong, W, Fung, C., Yu, K. and Liu, C. **2002.** Clinical features and antimicrobial susceptibility trends in *Citrobacter freundii* bacteremia. *Journal of microbiology, immunology, and infection*, **35**(2): 109-114.
10. Holmes, B. and Aucken, H. **1998.** *Citrobacter, Enterobacter, Klebsiella, Serratia* and other members of the Enterobacteriaceae, in Collier L, Balows A, Sussman M (eds):Microbiology and Microbial Infections: Systematic Bacteriology (ed 9). *London, Arnold*, (999-1033).
11. Card, R., Mafura, M., Hunt, T., Kirchner, M., Weile, J., Rashid, M. amd Anjum, M. **2015.** Impact of ciprofloxacin and clindamycin administration on Gram-negative bacteria isolated from healthy volunteers and characterization of the resistance genes they harbor. *Antimicrobial agents and chemotherapy*, **59**(8): 4410-4416.
12. Lawley, R., Curtis, L. and Davis, J. **2012.** The food safety hazard guidebook. *Royal Society of Chemistry*.
13. MacFaddin, J. **2000.** *Biochemical tests for identification of medical bacteria*, 3rd ed. Lippincott Williams and Wilkins, Philadelphia, PA.
14. Bettelheim, K., Evangelidis, H., Pearce, J., Sowers, E. and Strockbine, N. A. **1993.** Isolation of a *Citrobacter freundii* strain which carries the *Escherichia coli* O157 antigen. *Journal of clinical microbiology*, **31**(3): 760-761.
15. Janda, J., Abbott, L., Cheung, W. and Hanson, D. **1994.** Biochemical identification of *Citrobacteria* in the clinical laboratory. *Journal of Clinical Microbiology*, **32**(8): 1850-1854
16. Gwida, M., Hotzel, H., Geue, L. and Tomaso, H. **2014.** Occurrence of Enterobacteriaceae in raw meat and in human samples from Egyptian retail sellers. *International scholarly research notices*. **2014**:1-6.
17. Janda, J. and Abbott, S. **2007.** 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *Journal of clinical microbiology*, **45**(9): 2761-2764.

18. Bosshard, P., Zbinden, R., Abels, S., Böddinghaus, B., Altwegg, M. and Böttger, E. **2006.** "16S rRNA gene sequencing versus the API 20 NE system and the VITEK 2 ID-GNB card for identification of nonfermenting Gram-negative bacteria in the clinical laboratory." *Journal of clinical microbiology*, **44**(4): 1359-1366.

19. Aabo, S., Rasmussen, O., Roseen, L., Sørensen, P. and Olsen, J. **1993.** *Salmonella* identification by the polymerase chain reaction. *Molecular and cellular probes*, **7**(3): 171-178.