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## Detection of Bacterial Contamination of Imported Chicken Meat in Iraq

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### Abstract

With the constant increase in poultry meat consumption worldwide and the large variety of poultry meat products and consumer demand, guaranteeing the microbial safety of poultry carcasses and cuts is crucial.

During 2018; one hundred-ten chicken meat samples were collected randomly from local markets in Baghdad. Selective and differential media were used to isolate and identify the contaminant bacteria from the collected samples, the predominant species was *Klebsiella pneumoniae*, 47 isolates (42%), followed by *Escherichia coli* 35 isolates (31%), 13 (11%) *Citrobacter freundii*, 9 (8%) *Salmonella*, and 6 (5%) *Shigella*. Vitek -2 system used to confirm the identification of *Citrobacter spp.* and *Klebsiella spp.* while 16s rRNA gene amplification using PCR technique was applied to confirm the identification of *C. freundii*.

**Keywords:** *Citrobacter*, Isolation, Chicken, Bacterial contamination, *Klebsiella*.

### التحري عن التلوث البكتيري لحوم الدجاج المستوردة في العراق

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قسم علوم الحياه، كلية العلوم، جامعة بغداد، بغداد، العراق

### الخلاصة

مع الزيادة المستمرة في استهلاك لحوم الدواجن في جميع أنحاء العالم ، والتنوع الكبير في منتجات لحوم الدواجن و زيادة الطلب من قبل المستهلك ، فإن ضمان سلامة منتجات الدواجن أمر بالغ الأهمية. خلال 2018 ؛ تم جمع 110 عينة من لحوم الدجاج بشكل عشوائي من أسواق بغداد. تم استخدام الأوساط الزرعية الانتقائية والتفريقية لعزل وتحديد البكتيريا الملوثة من العينات التي تم جمعها ، وكانت الأنواع السائدة هي *Klebsiella pneumoniae* ، 47 عزلة (42 %) ، تليها *Escherichia coli* 35 (31 %) ، 13 (11 %) *Citrobacter freundii* و 9 (8 %) *Salmonella* و 6 (5 %) *Shigella*. تم استخدام نظام - Vitek 2 لتأكيد تشخيص *Citrobacter spp.* و *Klebsiella spp.* . في حين تم تطبيق سلسلة البلمرة لجين 16S rRNA لتأكيد تشخيص *C. freundii*

### Introduction

Poultry meat consumption is steadily increasing worldwide and the large variety of poultry meat products and consumer demand, ensuring the microbial safety of poultry carcasses and cuts is essential [1], in fact, during and after slaughtering, the bacteria from animal microbiota, the slaughterhouse environment, and the equipment used contaminate carcasses, their subsequent cuts, and processed meat products. Some of these bacterial contaminants can grow or survive during food processing and storage [2]. Bacterial contamination by equipment surfaces can take place early in the process. For example, the rubber fingers used for feather removal or conveyor belts can be sources of bacterial contamination [3, 4, 5], Even new rubber fingers can host bacteria and be a source of contamination

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for carcasses, cross contamination between carcasses or cuts may occur by direct contact or through contact with contaminated surfaces [5].

During the subsequent processing steps (deboning, cutting, mincing, and mixing) for meat-based foodstuff production, manipulators, air and equipment surfaces are the main sources of contamination, in fact, transformation operations increase the surface area of meat in contact with working surfaces and air, consequently, the level of bacteria is higher in transformed products than on primary cuts [6]

## Methods

### I-Samples Collection

One hundred-ten chicken meat samples were collected randomly from local markets in Baghdad city according to the instruction of the Iraqi Standard Criterion No.2/2270 in sampling, (2006) [7]; from September 2018 to December 2018.

### II-Bacterial Isolation

One gram of each chicken meat sample was suspended in 9 ml D.W., left for 30 minutes, then 1ml from each broth/sample was placed in the center of sterile Petri dish using a sterile pipette, Molten cooled agar (approx. 15ml) is then poured into the Petri dish containing the inoculum and mixed well [8].

After the solidification of the agar, the plate was incubated at 37°C for 24-48 hrs. Later the grown colonies were further investigated.

### III-Identification

Bacterial isolates were identified to the genus level using both microscopic and macroscopic characteristic on selective and differential media, according to [9], While the identification of *C.freundii* and *Klebsiella* isolates to species level was accomplished by vitek-2 system and PCR technique.

### III-Identification of Bacteria by PCR

#### DNA Extraction

Genomic DNA was isolated from Bacteria according to the protocol of Genomic DNA mini kit, Gene aid. A PCR reaction with a specific primer provided by Advanced Scientific Co alharthia , alkindi ST, Baghdad. (Table-1)

**Table 1-Primers and their sequences**

Primer Name	Sequences 5 → 3	Size (bp)
27F	AGAGTTTGATCCTGGCTCAG	1500 bp
1492R	TACGGTTACCTTGTTACGACTT	

(25µl) of PCR amplification mixture contained (12.5µl) Master mix, (1µl) forward primer, (1µl) reverse primer, (8.5µl) nuclease free water, and (2µl) 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 30 sec., extension 72 °C for 1 min. and final extension 72 °C for 7min, 32 cycles.

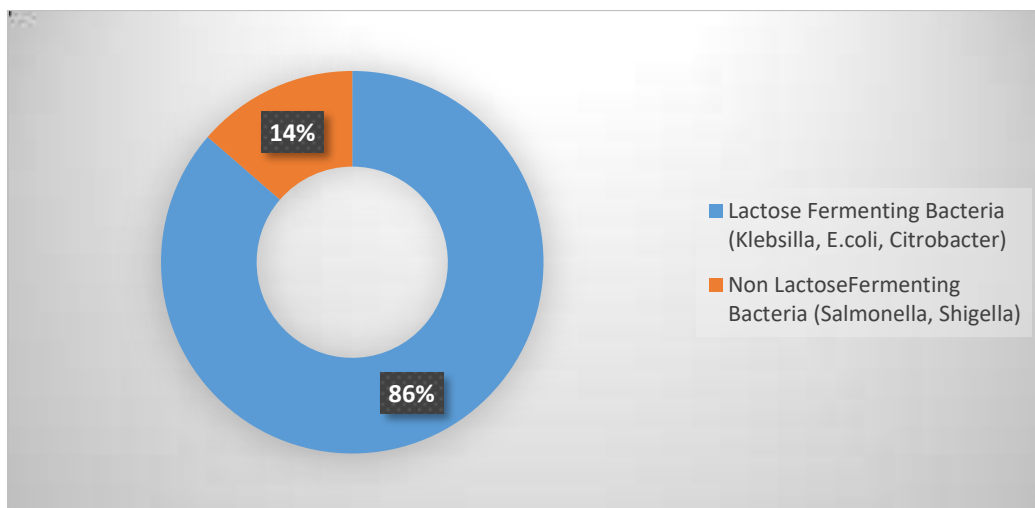
## Results and Discussion

### Isolation and Identification

The collected chicken meat samples were cultured on four selective and differential media; all isolates were purified by ABC streaking method on MacConkey agar.

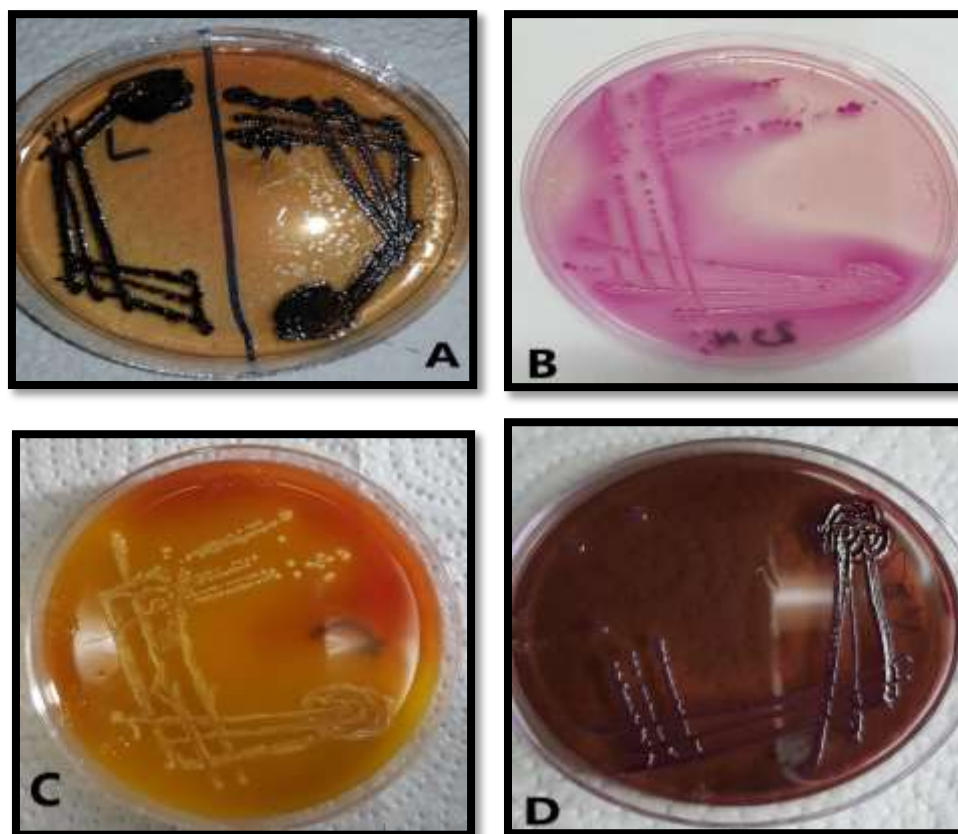
MacConkey agar medium is a selective and differential culture medium for bacteria designed to selectively isolate gram negative and enteric bacilli and differentiate them based on lactose fermentation. After 18 hrs. incubation at 37°C two types of colonies appeared, lactose fermenter pink colonies and non-lactose fermenter pale colonies.

The majority of the bacterial isolates were lactose fermenters with a percentage of 86 while the remaining were unable to ferment lactose (Figure-1)



**Figure 1-**Percentages of lactose fermenting bacteria and lactose non-lactose fermenting bacteria isolated from chicken meat samples

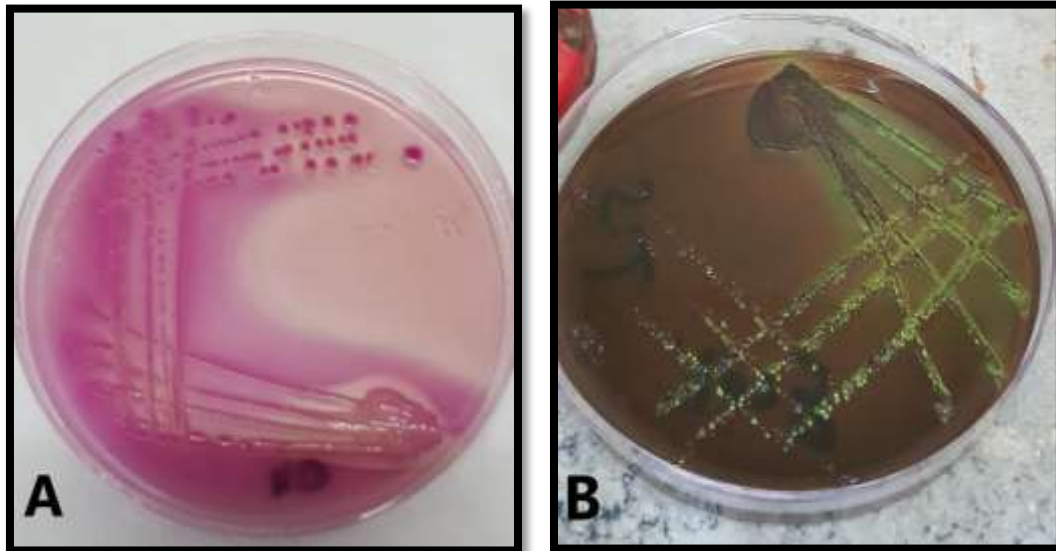
The pink colonies were cultured on EMB, XLD and S.S agar for further investigation, *Citrobacter freundii* appeared as brown colonies on EMB, and small pale flattened colonies with black center on S.S agar due to their ability to produce H<sub>2</sub>S, as described by [10], this result agrees with [11], who managed to isolate *C.frendii* from chicken meat using same selective and differential media. Figure-2 A, B, C, D



**Figure 2-**Different selective and differential media cultured with *Citrobacter spp.* after incubation at 37°C for 18 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

*Escherichia coli* identified as pink colonies on MacConkey agar, and with a distinctive green-metallic color on EMB. Figures-(3A, B).



**Figure 3-**Selective and differential media cultured with food origin *E. coli* isolate after incubation at 37°C for 18 hr.: (A) pink (Lactose fermenter) colonies on MacConkey agar, (B) green metallic sheen colonies on EMB

Other researchers [12], also isolated food origin *E. coli* using these selective and differential media in order to characterize it from other lactose fermenting bacteria.

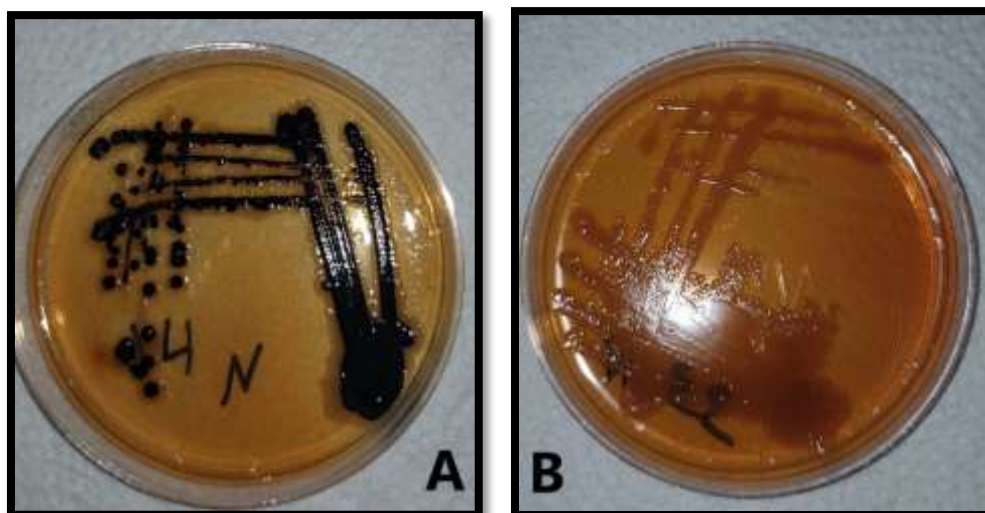
*Klebsiella pneumoniae* have two distinguishing characteristics are lactose fermentation on the medium and the viscosity of the colonies. Encapsulated strains of *Klebsiella* spp. are also mucoid in appearance, which is a characteristic of the strains of this genus other studies which used MacConkey as a selective media for *K. pneumoniae* identification [13] (Figure-4).



**Figure 4-**pink mucoid colonies of *K. pneumoniae* on MacConkey agar after incubation at 37°C for 18 hr.

SS Agar is a highly selective agar used for the isolation of *Salmonella* and *Shigella* species from contaminated samples, *Salmonella* appeared as colorless colonies, with a black center, *Shigella* appeared as colorless colonies on S.S agar. Figures-(5) A, B





**Figure 5**-selective and differential S.S media cultured with *Salmonella* and *Shigella*. after incubation at 37°C for 18 hr.

A- *Salmonella* colorless colonies, with black center

B- *Shigella* colorless colonies, no H<sub>2</sub>S

Other studies isolated *Salmonella* and *Shigella* using S.S agar [14, 15]

**Table 2**-Distribution of samples according to different bacteria isolated from meat chicken

Isolated	No.	Percentage (%)
<i>K.pneumoniae</i>	47	42.73
<i>E. Coli</i>	35	31.82
<i>C. freundii</i>	13	11.82
<i>Salmonella</i>	9	8.18
<i>Shigella</i>	6	5.45
Total	110	100%
Chi-Square ( $\chi^2$ )	---	9.027 **
** (P<0.01).		

*K. pneumoniae* is not only a major hospital-acquired pathogen but also an important food-borne pathogen that can cause septicaemia, liver abscesses, and diarrhea in humans. *K.pneumoniae* was the highest containment (42.73%) found in chicken meat samples, according to other researchers *K.pneumoniae* was found in high numbers in different food product including dairy product, meat and retail food These findings are in agreement with previous studies [16] which reported that A total of 78 samples of street foods in Malaysia were examined for the presence of *K. pneumonia* contamination was recorded in 32% of the samples examined.

Vitek -2 system was used to confirm the identification. Figures-(6 A, B).

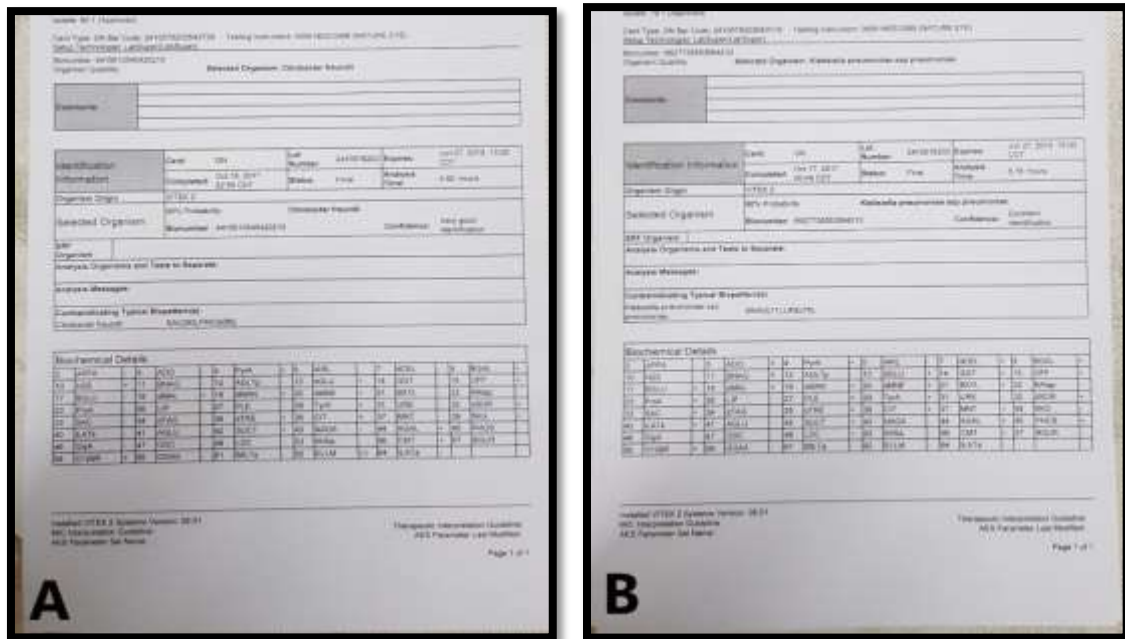


Figure 6-Identification of *C.freundii* (A) and *K. pneumoniae* (B) by Vitek 2 compact system

Another study [17] on Retail Foods in China had also reported the presences of *K.pneumoniae*. Followed by *E.coli* (31.82%) this result in agreement with previous studies [18] on poultry meat in Nigeria *E.coli* contamination was at 43.4%, and *C.freundii* (11.82%). Another study was able to isolate *C.freundii* from chicken meat samples in Iraq [11], *Salmonella* (8.18%), other studies [18] managed to isolate *Salmonella* from poultry meat and it was found in high numbers up to (33%). *Shigella* were found in low numbers (5.45%) These findings are in agreement with previous studies [15], which reported low numbers of *shigella* in food samples in Tunisia only six *Shigella* spp. strains were isolated from 280 food samples.

In order to confirm the identification of *Citrobacter*to species level 16S rRNA gene amplification was performed using monoplex PCR technique, 1.5 % agarose gel electrophoresis was used to detect the positive result as shown in Figure-7.

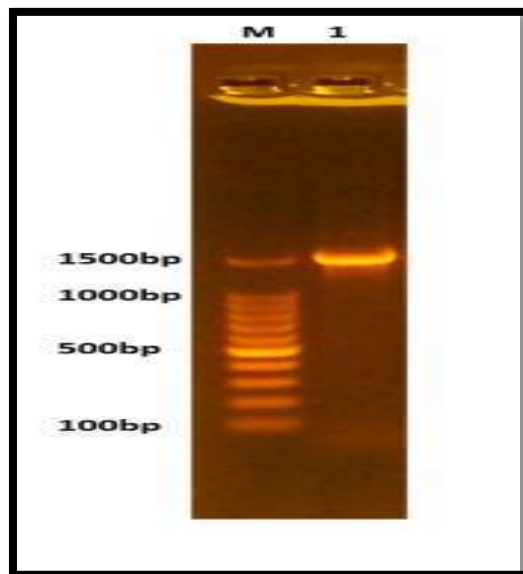


Figure 7-Amplified PCR products of 16SrRNA gene (1500 bp): Agarose gel electrophoresis, ethidium bromide stained, 1.5 % agarose, electrophoresed in 75 volts for 2 hrs. and photographed under ultraviolet trans-illuminator. M: The DNA molecular weight marker (100 bp ladder) and 1: the amplified PCR product of 16SrRNA of C4 isolate of *Citrobacter freundii*.

One of the most attractive potential uses of 16S rRNA gene sequence informatics is to provide genus and species or taxa identification for isolates [19]. Although 16S rRNA gene sequencing is highly useful in regards to bacterial classification [20]. PCR products were subjected 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 the National Center for Biotechnology Information (NCBI) website (<http://www.ncbi.nlm.nih.gov>) (Table-3).

**Table 3-**16S rRNA gene of *C. freundii* isolate BLAST with reference sequences

Score	Expect	Identities	Gaps	Strand
2590 bits(1402)	0.0	1402/1402(100%)	0/1402(0%)	Plus/Plus
Query 1				
GTCGAACGGTAGCACAGAGGAGCTTGCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAA				60
Sbjct 5				
GTCGAACGGTAGCACAGAGGAGCTTGCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAA				64
Query 61				
TGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCAT				120
Sbjct 65				
TGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCAT				124
Query 121				
AACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCAGATG				180
Sbjct 125				
AACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGTGCCAGATG				184
Query 181				
GGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA				240
Sbjct 185				
GGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGA				244
Query 241				
GGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG				300
Sbjct 245				
GGATGACCAGCCACACTGGAAGTGGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTGG				304
Query 301				
GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCT				360
Sbjct 305				
GGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCT				364
Query 361				
TCGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATT				420
Sbjct 365				
TCGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGTGTTGTGGTTAATAACCGCAGCAATT				424
Query 421				
GACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAG				480
Sbjct 425				
GACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAG				484
Query 481				
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG				540

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Sbjct 485
GGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCG 544
Query 541
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCGAAACTGGCAGGCTAGAGTCTT 600
|||||
Sbjct 545
GATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCGAAACTGGCAGGCTAGAGTCTT 604
Query 601
GTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC 660
|||||
Sbjct 605
GTAGAGGGGGGTAGAATTCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACC 664
Query 661
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCA 720
|||||
Sbjct 665
GGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCA 724
Query 721
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTTCGACTTGGAGGTTGTGCC 780
|||||
Sbjct 725
AACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTTCGACTTGGAGGTTGTGCC 784
Query 781
CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCA 840
|||||
Sbjct 785
CTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCA 844
Query 841
AGGTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAAAT 900
|||||
Sbjct 845
AGGTTAAAACCTCAAATGAATTGACGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAAAT 904
Query 901
TCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCT 960
|||||
Sbjct 905
TCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCT 964
Query 961
TTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTGA 1020
|||||
Sbjct 965
TTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTGTGA 1024
Query 1021
AATGTTGGGTAAAGTCCC GCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGC 1080
|||||
Sbjct 1025
AATGTTGGGTAAAGTCCC GCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGGTTAGGC 1084
Query 1081
CGGGAAC TCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTC 1140
|||||
Sbjct 1085
CGGGAAC TCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTC 1144
Query 1141
ATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCG 1200
|||||

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Sbjct 1145
ATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCG 1204
Query 1201
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAAC 1260
|||||
Sbjct 1205
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAAC 1264
Query 1261
TCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGT 1320
|||||
Sbjct 1265
TCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGT 1324
Query 1321
TCCC GGCCTTGTACACACCCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGT 1380
|||||
Sbjct 1325
TCCC GGCCTTGTACACACCCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGT 1384
Query 1381 AGCTTAACCTTCGGGAGGGCGC 1402
          |||||
Sbjct 1385 AGCTTAACCTTCGGGAGGGCGC 1406

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### Conclusions:

Although *Citrobacter freundii* is a food borne bacterium but it is so difficult to be differentiated from other closely related bacterial species, and its isolation from imported chicken meat samples was accompanied with so many difficulties one of which; competition with other bacteria e.g. *Klebsiella*, *E.coli*, *Salmonella* and *Shigella*, so the complete identification using vitek-2 system is very necessary to confirm its identification to the species level since it was further confirmed using 16SrRNA sequencing.

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