Detection of Bacterial Contamination of Imported Chicken Meat in Iraq

Mustafa Basil Abdul Qader*, Marwa Hameed AlKhafaji
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq

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.

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

*Email: mustafabasil1993@yahoo.com
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 alharthi , alkindi ST, Baghdad. (Table-1)

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequences 5 (\rightarrow) 3</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27F</td>
<td>AGAGTTTGATCCTGGCTCAG</td>
<td>1500 bp</td>
</tr>
<tr>
<td>1492R</td>
<td>TACGGTTACCTTGTTACGACTT</td>
<td></td>
</tr>
</tbody>
</table>

(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)
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 H2S, as described by [10], this result agree with [11], who managed to isolate *C.freundii* from chicken meat using same selective and differential media. Figure-2 A, B, C, D

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

**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 H₂S

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

<table>
<thead>
<tr>
<th>Isolated</th>
<th>No.</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>47</td>
<td>42.73</td>
</tr>
<tr>
<td>E. Coli</td>
<td>35</td>
<td>31.82</td>
</tr>
<tr>
<td>C. freundii</td>
<td>13</td>
<td>11.82</td>
</tr>
<tr>
<td>Salmonella</td>
<td>9</td>
<td>8.18</td>
</tr>
<tr>
<td>Shigella</td>
<td>6</td>
<td>5.45</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>100%</td>
</tr>
<tr>
<td>Chi-Square (χ²)</td>
<td>---</td>
<td>9.027 **</td>
</tr>
</tbody>
</table>

** (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).
Another study [17] on Retail Foods in China had also reported the presence of K. pneumonia. Followed by E. coli (31.82%) this result is 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 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.
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

<table>
<thead>
<tr>
<th>Score</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>2590 bits(1402)</td>
<td>0.0</td>
<td>1402/1402(100%)</td>
<td>0/1402(0%)</td>
<td>Plus/Plus</td>
</tr>
</tbody>
</table>

Query 1
GTCGAACGGTAGCACAGAGGAGCTTGCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAA

Sbjct 5
GTCGAACGGTAGCACAGAGGAGCTTGCTCCTTGGGTGACGAGTGGCGGACGGGTGAGTAA

Query 61
TGTCCTGGGAAAACCTGCCCGATGGAGGGGGATAACTACTGGAACGTTAGCTAATACCAGAT

Sbjct 124
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Query 181
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Sbjct 304
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Query 301
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Sbjct 364
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Query 361
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Sbjct 424
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Query 421
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Sbjct 484
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Query 481
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

Sbjct 540
GGATTAGCTAGTGGTGGAACGTCCAGCAAGGAGGGACCTTCTGGGCTCTTGGCGATGTGCCAGATG

<table>
<thead>
<tr>
<th>Score</th>
<th>Expect</th>
<th>Identities</th>
<th>Gaps</th>
<th>Strand</th>
</tr>
</thead>
</table>
Sbjct 1145
ATCATGGCCCTTACGAGTGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCG 1204
Query 1201
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAAC 1260

Sbjct 1205
ACCTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATTGGAGTCTGCAAC 1320
Query 1261
TCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAATGCCACGGTGAATACGT 1324

Sbjct 1265
TCGACTCCATGAAGTCG
Query 1321
TCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAAGAAGTAGGT 1380
Sbjct 1385
AGCTTAACCTTCGGGAGGGCGC 1406
Query 1381
AGCTTAACCTTCGGGAGGGCGC 1402

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 16S rRNA sequencing.

References


