RESEARCH ARTICLE

A STUDY OF ISOLATION AND IDENTIFICATION OF CAMPYLOBACTER SPECIES, LISTERIA MONOCYTOGENES E. COLI O157:H7 AND SALMONELLA SPECIES FROM RAW CHICKEN CARCASSES AND BEEF MEAT BY MULTIPLEX POLYMER CHAIN REACTION IN BAGHDAD CITY.

Zena K. Khalil.

Medical Technical Institution /Al-Mansur.

Manuscript Info

Abstract

The study designed to determine the distribution of a major important food pathogens including Campylobacter spp, Listeria monocytogenes, E. coli O157:H7 and Salmonella spp from raw chicken carcasses and beef meat by using multiplex PCR. A total of 100 raw chicken carcasses and beef meat samples were collected from different markets and butcher's shops in Al-Karkh side of Baghdad city and analyzed for the presence of these types of bacteria and their susceptibilities to some antibiotics was investigated, the results showed that the prevalence of C.jejuni was (31%) , C. coli (12%), L. monocytogenes (8%), E. coli O157:H7 (42 %) and Salmonella spp (7%) from the total samples. The result of the susceptibility test showed that C.jejuni isolates were susceptible to Ciprofloxacin, Gentamycin, Streptomycin and chloramphenicol, (87,87,84,80)% respectively and both Erythromycin,Neomycin (77%) , C. coli isolates were susceptible to Ciprofloxacin (84%) and (75%) for each Gentamycin, Streptomycin ,chloramphenicol and Neomycin ,while L. monocytogenes isolates were susceptible to the most used antibiotics as following Amikacin, Erythromycin, Oxytetracycline, Nalidixic acid ,Cephalothin, Gentamycin, Ampicillin and Streptomycin (100%). E. coli O157:H7 isolates were susceptible to Nalidixic acid and Gentamycin (98%,96%) and Salmonella spp isolates were susceptible to Gentamycin ,Cephalothin and (86%,71%) .

Copy Right, IJAR, 2017. All rights reserved.

Corresponding Author:- Zena K.Khalil.
Address:- Medical Technical Institution /Al-Mansur.
Introduction:

Many risky pathogens are transmitted through contaminated food and water but protein in many developing countries remains as the main source of energy, this led to increased production and consumption of meats [1]. Although these pathogens usually cause mild to moderate self-limiting gastroenteritis, invasive diseases but complications can occur and resulting in many severe cases. Such as, *Campylobacter* which considered the predominant cause of Guillain-Barre` syndrome and reactive arthritis [2].

*Campylobacter, Salmonella,* and pathogenic *E. coli* colonized at the gastrointestinal tracts of a wide range of the domestic animals, especially farm animals which raised for human consumption [3].

WHO has announced that the majority of listeriosis cases are caused by the species *L.monocytogenes* in humans and animals and this pathogen has a severe role to threat the consumer’s safety [4].

Food contamination by these pathogens can occur at multiple steps beginning with the food chain, production processing, then distribution, retail marketing, ending with handling or preparation [5].

The goal of this study was to determine the occurrence of *Campylobacter spp*, *Salmonella spp*, *E. coli* O157:H7 and *Listeria monocytogenes* in raw chicken and beef meat from butcher’s shops and markets in Al-Karkh side of Baghdad city by using a multiplex PCR assay and to determine the susceptibility of the bacterial isolates to some selective antibiotics.

Material and Methods:-

A total of 100 random samples of raw chicken carcasses and beef meat were collected from different butcher’s shops and markets in Al-Karkh side of Baghdad city between February and September 2016. Samples were transported to the laboratory iniced boxes within 2 hours as a 25 g of each sample were homogenized by using a stomacher with 225 ml of enrichment broths, the isolation and culturing of *Campylobacter* spp was done under micro aerobic conditions by using AnaeroPak system (Mitsubishi Gas Chemical Co., Inc,Japan) Bolton broth (Oxoid) for *Campylobacter spp*, buffered listeria enrichment broth Base (Oxoid) for *L. monocytogenes*, trypsinase soy broth (TSB) containing 0.5 mg/ml novobiocin as an enrichment broth for *E. coli* O157:H7 and Rappaport-Vassiliadis enrichment broth for *Salmonella*. Enrichment broths were incubated for 24 hours at 37°C, while *Salmonella* broth was incubated at 42°C. At the end of the incubation period loopfull from each of the selective enrichment broth was streaked on prepared blood-free selective agar (Oxoid). After 48 h of incubation at 42°C, the plates were examined for typical colonies of *Salmonella* which were transparent colonies with black centers then biochemical tests were used for complete characterization.

After incubation, *E. coli* O157:H7 colonies have black or gray coloration on Rainbow Agar(RBA) and *Salmonella-Shigella* (SS) agar and incubated at 37°C for 24 h. The plates were examined for the presence of typical colonies of *Salmonella* which were transparent colonies with black centers then biochemical tests were used for complete characterization.

Extraction of DNA and Multiplex Polymerase Chain Reaction:-

The extraction of DNA was done at the laboratories of the Iraqi Biotechnology Co. in Baghdad using boiling method. [8] After incubation a 1 ml of the enrichment broths were centrifuged for 3 minutes. Then they formed bacterial pellets were suspended in 1 ml of sterile saline solution (0.85% NaCl), after the centrifugation the supernatants were replaced with 50 μl of sterile distilled water and incubated for 10 minutes at 100°C for DNA
extraction, then the clear supernatants centrifuged for 5 minutes at 14000 rpm, they were stored at -20ºC till using. The extracted DNA were mixed and used for the multiplex PCR reactions. The following oligonucleotide primers are used in this study showed in Table 1, they were synthesized by Sigma Company (Singapore). Two primers pairs were used: stx, which is specific primers to various stx1 and stx2 gene for E. coli O157:H7 and the multiplex PCR procedure is done as reported by Karami et al 2012 [9]. In the multiplex PCR with mixed DNA samples, the thermal cycler of the reaction steps are initial denaturation at 94ºC for 3 minutes and denaturation at 94ºC for 45 seconds then primer annealing at 54ºC for 45 seconds and extension at 72ºC for 60 seconds. The final cycle included a 5-minute additional extension at 72ºC. 2% agarose gels using to observe the PCR products [10].

Table 1: types of Oligonucleotide primers that used in the study .

<table>
<thead>
<tr>
<th>Bacterial name</th>
<th>Sequence used (5’ → 3’)</th>
<th>Target Gene</th>
<th>(bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| C. jejuni     | Forward GACTTCGTGCAGATGGATGCTT  
Reverse GCTATAACTATCCGAAGAAGCCATCA | hipO | 397 | [11] |
| C. coli       | Forward GGT ATG ATT TCT ACA AAG CGA  
Reverse ATA AAA GAC TAT CGT CGC GTG | asp | 325 | [12] |
| L monocytogenes | Forward:CTGGCACAAAAATTACTTACAACGA  
Reverse:AACTACTGGAGCTGCTTGTTTTTC | iap | 454 | [13] |
| E. coli O157:H7 | Forward:GATAGACTTTTCGACCCAACAAAG  
Reverse:TTGCTCAATAATCAGACGAAGATG | stx | 208 | [14] |
| Salmonella spp. | Forward:GAATCCTCAGTTTTTCAACGTTTC  
Reverse:TAGCCGTAACAACCAATACAAATG | invA | 678 | [15] |

Antibiotic susceptibility:–
Antibiotic susceptibility was monitored with the disk diffusion assay (Kirby–Bauer) recommended by the National Committee for Clinical Laboratory Standards on Muller Hinton agar (Oxoid, Milan, Italy), the zone of inhibition was interpreted according to NCCLS guidelines [16].

These antibiotic discs were used ciprofloxacin (5 μg), Amikacin (30 μg), Erythromycin (15 μg), Vancomycin (30 μg), Oxacillin (1 μg), Oxytetracycline (30 μg), Nalidixic acid (30 μg), Cephalothin (30 μg), Gentamycin (10 μg), Ampicillin (10 μg), Streptomycin (10 μg), Chloramphenicol (30 μg) and Neomycin (30 μg), supplied by HiMedia Laboratories Pvt. Ltd., India, were placed onto Mueller–Hinton agar plates. The plates were incubated at 37ºc for 24 h. The zone diameter was measured and results were interpreted based on CLSI [17].

The reference strains C.jejuni ATCC 33560, C. coli ATCC 33559 and E. coli ATCC 25922 were used as a control.

Statistical Analysis:–
The Chi-square test used for statistical analysis. A P value <0.05 was used for statistical significance to compare rate of isolation of the various pathogens in chicken and beef raw meat. [18].

Results and discussion:–
The results showed that the prevalence of C.jejuni was (31%) , C. coli (12%), L. monocytogenes (8%), E. coli O157:H7 (42%) and Salmonella spp (7%) from the total samples there were no significant differences between two groups at P < 0.05 as shown in Table 2. And figure 1.

Table 2: The incidence of C.jejuni, C. coli, L. monocytogenes, E. coli O157:H7 and Salmonella spp. in chicken and beef raw meat.

<table>
<thead>
<tr>
<th>Types of Meat</th>
<th>Samples No.</th>
<th>C.jejuni</th>
<th>C. coli</th>
<th>L. monocytogenes</th>
<th>E. coli O157:H7</th>
<th>Salmonella spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken</td>
<td>50</td>
<td>17(34%)</td>
<td>5(10%)</td>
<td>3(6%)</td>
<td>22(44%)</td>
<td>3(6%)</td>
</tr>
<tr>
<td>Beef</td>
<td>50</td>
<td>14(28%)</td>
<td>7(14%)</td>
<td>5(10%)</td>
<td>20(40%)</td>
<td>4(8%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>31(31%)</td>
<td>12(12%)</td>
<td>8(8%)</td>
<td>42(42%)</td>
<td>7(7%)</td>
</tr>
</tbody>
</table>
The presence of Campylobacter was common in many studies especially raw meats with contamination rates as high as 100% [19]. In Hanoi, they found that from 100 breast part of chicken carcass the most frequently isolated were C. jejuni (45.2%) followed by C. coli (25.8%) and this was highest than our results may be because the increase contamination levels of retail chicken products [20]. Another study in Campobasso, from 104 chicken samples, the prevalence of C. jejuni was (25.2%) and C. coli was (15.8%) by using the polymer chain reaction which conceded close to our findings [21]. Many studies agreed with the low prevalence of Campylobacter in beef meats as they isolated from only 2 to 10% of the beef samples analyzed and this may be related to some environmental stresses including transporting, processing and storage of the products [22].

The study showed that L. monocytogenes was (8%) in chicken raw meat and this is agreed with other studies in Jordan and Germany as the ratio were (9.4%) and (6%) chicken meat samples and this may be related to presence of other kinds of bacteria [23,24]. In Buenos Aires L. monocytogenes was (10.7%) from raw beef samples and In Thailand the prevalence of L. monocytogenes in raw meats marketed was 15.4% from the total samples and 3% from total beef samples by using PCR technique [25,26]. Meat in different butcher’s shops have brought from different sources beside the absence of good hygienic meat processing and handling, the pH and water activity play significant role in the growth of L. monocytogenes and this might be one of the reasons behind the different prevalence of L. monocytogenes found in the studies [27].

The prevalence of E. coli O157:H7 (44%) in chicken and (40%) in beef raw meat samples and this disagree with other study that found the highest prevalence rate was found in beef (13.32 %), followed chicken (3.28 %), another study in Riyadh showed that (27.27%) E. coli O157:H7 isolates from raw beef while (45.45%) E. coli O157:H7 isolates from raw chicken by PCR [28,29].

The incidences of Salmonella spp in raw meats varies in different countries, in the previous study it was (6,8) % in chicken and beef meat but in United States Salmonella spp isolated from 43% of raw chicken meat samples and in raw beef meat seems to be lower, ranging from 0.8 to 10.4% by PCR [30]. Other study found that the rates of Salmonella spp contamination 1.9% for beef samples and 4.2% for chicken samples. The difference might be due different portions of samples treated [31].

The results of antibiotic susceptibility showed that C. jejuni isolates were susceptible to Ciprofloxacin, Gentamycin, Streptomycin and chloramphenicol, (87, 87, 84, 80) % respectively and both Erythromycin, Neomycin (77%) while C. coli isolates were susceptible to Ciprofloxacin (84%) and (75%) for each Gentamycin, Streptomycin, chloramphenicol and Neomycin and these results were similar to other studies in Iran and Ethiopia [32,33].

L. monocytogenes isolates were susceptible to the following antibiotics Amikacin, Erythromycin, Oxytetracycline, Nalidixic acid, Cephalothin, Gentamycin, Ampicillin and Streptomycin (100%) as shown in Table 4. And this agree with other study that found all L. monocytogenes strains were susceptible to 90% from the tested antibiotics [34]. While disagree with another studies that found L. monocytogenes strains have a natural resistance phenotypes to the cephalosporins and nalidixic acid, the resistance for this antibiotic could be due to the illegal using in animal’s farms [35,36].

E. coli O157:H7 isolates were susceptible to Nalidixic acid and Gentamycin (98%,96%), and these finding were disagree with other result of E. coli serotype O157:H7 they were 70% resistant to Nalidixic acid and all the isolates were sensitive to Gentamicin and high level of resistance to these antimicrobials was probably an indication of their extensive usage in the veterinary sector for therapeutic and prophylactic purpose both for E. coli and other infections. [37]. Our findings on some of the effective antibiotics agree with the other reports [38,39].

The susceptibility might have contributed to the effectiveness of these antimicrobials mostly against gram negative bacteria like those of the family of Enterobacteriaceae to which E. coli O157:H7 belongs.

On the other hand, Salmonella spp isolates were susceptible to Gentamycin and Cephalothin, (86, 71) % and resistant to Oxytetracycline (100%). As shown in Table 5. And this agree with other study in morocco as they found that 71% (75/105) of Salmonella spp isolates were susceptible to Ciprofloxacin, Cefazidim, Cefotaxime, Cefamandol, Gentamycin and Mecillinam while the most common resistance observed was to tetracycline and Ampicillin. Another study in Iran, all isolates, from chicken meat samples were resistance to Ampicillin, Amoxicillin, Nitrofurantoin, Tetracycline, and were susceptible to Gentamycin and Ceftriaxone [40,41].
Conclusion:
The results indicate that the beef and chicken raw meat is considered as a reservoirs of many food pathogens at both markets and the butcher's shops and this maybe because the absence of sanitary hygiene and due to the potential hazard of these pathogenic bacteria, it is necessary to put more emphasis on meat hygiene, so, the surveillance of potential contaminant bacteria in different kinds of meat is crucial to safeguard the public health, and the isolated bacteria were highly susceptible to a number of antibiotics which could use as a treatment of infections caused by these pathogens.

![Gel electrophoresis of Multiplex PCR results. Lane 1 and M: 100bp DNA ladder; lane 2 C. jejuni *hipo* gen; lane 3 C. coli *asp* gen; lane 4 L. monocytogenes *iap* gen; lane 5 S typhymurium. *stx* gen; lane 6 E. coli *inv A* gen; lane 7 reagent blank.](image)

Table 3: Antibiotic sensitivity and resistance of isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>C. jejuni n=31</th>
<th>C. coli n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Ciprofloxacine</td>
<td>27(87%)</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>24(77%)</td>
<td>2(7%)</td>
</tr>
<tr>
<td>Neomycin</td>
<td>24(77%)</td>
<td>2(7%)</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>5(16%)</td>
<td>4(13%)</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>2(7%)</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25(80%)</td>
<td>2(7%)</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>27(87%)</td>
<td>1(3%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>17(55%)</td>
<td>2(7%)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>26(84%)</td>
<td>1(3%)</td>
</tr>
</tbody>
</table>

Table 4: Antibiotic sensitivity and resistance of *L. monocytogenes* isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>L. monocytogenes n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>8(100%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>6</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>4</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>8(100%)</td>
</tr>
</tbody>
</table>
Nalidixic acid  8(100%)  0  0
Cephalothin  5  2  1
Gentamicin  8100%)  0  0
Ampicillin  8(100%)  0  0
Streptomycin  8(100%)  0  0

Table 5- Antibiotic sensitivity and resistance of E. coli O157:H7 and Salmonella spp isolates.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. coli O157:H7 n=42</th>
<th>Salmonella spp n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>I</td>
</tr>
<tr>
<td>Amikacin</td>
<td>7(17%)</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>8(19%)</td>
<td>4(10%)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>3(7%)</td>
<td>1(2%)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>41(98%)</td>
<td>1(2%)</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>36(86%)</td>
<td>2(4%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>40(96%)</td>
<td>1(2%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>6(15%)</td>
<td>4(10%)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>6(15%)</td>
<td>1(2%)</td>
</tr>
</tbody>
</table>

References:
