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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.

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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%) .

عزل و تشخيص جراثيم الكومبايلوباكتر,اللستيريا ,الاشريشيا القولونية والسالمونيلا من لحوم الدجاج والابقار النيئة في مدينة بغداد بواسطة تفاعل سلسلة البلمرة المتعدد

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الخلاصة

تم تصميم الدراسة لتحديد مدى انتشار بعض الجراثيم الغذائية المهمة ومن ضمنها الكومبايلوباكتر والليستيريا مونوسايتوجينز والاشريشيا القولونية والسالمونيلا من عينات لحوم الدجاج و الابقار النيئة بطريقة تفاعل سلسلة البلمرة المتعدد .

جمعت 100 عينة من لحوم الدجاج والأبقار من الاسواق محلات القصابة ضمن منطقة الكرخ من بغداد لتحديد وجود البكتيريا , كما تم اجراء فحص حساسيتها لبعض المضادات الحيوية وأظهرت النتيجة تواجد بكتيرياالكومبايلوباكتر بنسبة 44% والليستيريا مونوسايتوجينز 8% و والاشريشيا القولونية 42% والسالمونيلا 7% من مجموع العينات الكلية .

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نتائج فحص الحساسية سجلت حساسية الكومبايلوباكتر جاجيناي للسايبروفلوكساسين والجنتمايسين والستربتومايسين والكلورمفينيكول بنسبة (87,87,80,80)%, الكومبايلوباكتر كولاي فكانت حساسة للسايبروفلوكساسين (84%) و والجنتمايسين والستربتومايسين والكلورمفينيكول والنيومايسين بنسبة (75%) واما الليستيريا مونوسايتوجينز كانت حساسة لمعظم المضادات الحيوية والتي تضمنت (الاميكاسين,الارثرومايسين,الاوكسيتتراسايكلين,حامض النالیدکسیك,السیفالوثین,الجنتمایسین,الامبسلین والستربتومایسین) 100%, الاشريشيا القولونية كانت حساسة لحامض النالیدکسیك والجنتمایسین بنسبة (98,%96% اما السالمونيلا فكانت حساسة لكل من, الجنتمايسين و السيفالوثين بنسبة(86%, 71%) .

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* l 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 in iced 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 (Mitsubish Gas Chemical Co., Inc,Japan) ,Bolton broth (Oxoid) for *Campylobacter spp,* buffered listeria enrichment broth Base (Oxoid) for *L. monocytogenes,* trypticase 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 37ºC, while *Salmonella* broth was incubated at 42ºC. At the end of the incubation period loopfull from each of the selective enrichment broths was streaked on prepared blood-free selective agar (Oxoid). After 48 h of incubation at 42°C, the plates were examined for typical *Campylobacter* colonies, which were small, gray, droplike and shiny then the suspected colonies were selected for Gram staining , oxidase and catalase tests., Columbia Blood Agar Base as the typical colonies of *L. monocytogenes* were small round mignonette colonies surrounded by brownish-black hydrolysis halo [6,7].

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% $NaCl_2$). after the centrifugation the supernatants were replaced with 50 µl of sterile distilled water and incubated for 10 minutes at 100ºC 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:H71 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 .

Bacterial name	Sequence used (5′→ 3′)	Target Gene	(bp)	Reference
C. jejuni	Forward GACTTCGTGCAGATATGGATGCTT 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:CTGGCACAAAATTACTTACAACGA 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 (30µg), Erythromycin (15 μg), Vancomycin (30 μg), Oxacillin (1 μg), Oxytetracycline (30 μg), Nalidixic acid (30 μg), Cephalothin (30 μg), Gentamycin (10 μg), Ampicillin (10μg) , Streptomycin (10 μg) ,Chloramphenicol (30 µg) and Neomycin (30µg). supplied by HiMedia Laboratories Pvt. Ltd., India, were placed onto Mueller-Hinton agar plates. The plates were incubated at 37°c 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

Types of Meat	Samples No.	*C.jejuni*	*C. coli*	*L. monocytogenes*	*E. coli* O157:H7	*Salmonella* spp
Chicken	50	17(34%)	5(10%)	3(6%)	22(44%)	3(6%)
Beef	50	14(28%)	7(14%)	5(10%)	20(40%)	4(8%)
Total	100	31(31%)	12(12%)	8(8%)	42(42%)	7(7%)

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 Gentamicinhe , 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, Ceftazidim, 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, Nitrofuranthoin, 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 .

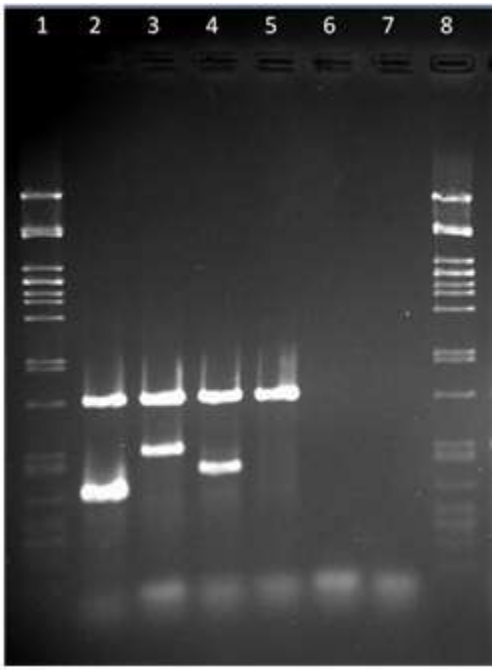

Figure 1:- Gel electrophoresis of Multiplex PCR results. Lane 1 and M: 100bp DNA ladder; lane 2 C. jejuni *hipo* gen; lane 3 C. coli *asp* gen; lane 4 l.monocytogens *iap* gen; lane 5 S typhymurium. *stx* gen; lane 6 E. coli *inv A* gen; lane 7 reagent blank

Table 3:- Antibiotic sensitivity and resistance of isolates.

Antibiotics	*C.jejuni* n=31			*C. coli* n=12		
	S	I	R	S	I	R
Ciprofloxacin	27(87%)	1(3%)	3(10%)	10(84%)	1(8%)	1(8%)
Erythromycin	24(77%)	2(7%)	5(16%)	8(66%)	2(17%)	2(17%)
Neomycin	24(77%)	2(7%)	5(16%)	9(75%)	1(8%)	2(17%)
Oxytetracycline	5(16%)	4(13%)	22(71%)	2(17%)	2(17%)	8(66%)
Nalidixic acid	2(7%)	1(3%)	28(90%)	2(17%)	3(25%)	7(58%)
chloramphenicol	25(80%)	2(7%)	4(13%)	9(75%)	1(8%)	2(17%)
Gentamycin	27(87%)	1(3%)	3(10%)	9(75%)	2(17%)	1(8%)
Ampicillin	17(55%)	2(7%)	12(38%)	9(75%)	-	3(25%)
Streptomycin	26(84%)	1(3%)	4(13%)	9(75%)	-	3(25%)

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

Antiboitics	*L. monocytogenes* n=8		
	S	I	R
Amikacin	8 (100%)	0	0
Erythromycin	8(100%)	0	0
Vancomycin	6	0	2
Oxacillin	4	3	1
Oxytetracycline	8(100%)	0	0

Nalidixic acid	8(100%)	0	0
Cephalothin	5	2	1
Gentamycin	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.

Antiboitics	*E. coli* O157:H7 n=42			*Salmonella* spp n=7		
	S	I	R	S	I	R
Amikacin	7(17%)	2(4%)	33(79%)	2(29%)	0	5(71%)
Vancomycin	8(19%)	4(10%)	30(71%)	1(14%(	0	6(86%)
Oxacillin	0	2(4%)	40(96%)	0	2(29%)	5(71%)
Oxytetracycline	3(7%)	1(2%)	38(91%)	0	0	7(100%)
Nalidixic acid	41(98%)	1(2%)	0	4(57%)	2(29%)	1(14%)
Cephalothin	36(86%)	2(4%)	4(10%)	5(71%)	0	2(29%)
Gentamycin	40(96%)	1(2%)	1(2%)	6(86%)	1(14%)	0
Ampicillin	6(15%)	4(10%)	31(75%)	4(57%)	1(14%)	2(29%)
Streptomycin	6(15%)	1 (2%)	35(83%)	1(14%)	0	6(86%)

References:-

1. Germini A., Masola A., Carnevali P. and Marchelli R. **2009.** Simultaneous detection of *Escherichia coli* O175: H7, *Salmonella spp*., and *Listeria monocytogenes* by multiplex PCR. Food Cont.,20(8):733–738.
2. Centers for Disease Control and Prevention. **2007**. Preliminary FoodNet data on the incidence of foodborne illnesses–selected sites, United States,2000. Morb. Mortal. Wkly. Rep. 50**:**241–246..
3. Madden, R. H., L. Moran, and P. Scates**. 2009**. Frequency of occurrence of *Campylobacter* spp. in red meats and poultry in Northern Ireland and their subsequent subtyping using polymerase chain reaction-restriction fragment length polymorphism and the random amplified polymorphic DNA method.*J. Appl. Microbiol*. 84**:**703–708..
4. Vitas A.I., Aguado V., Garcia-Jalon I. **2010.**: Occurrence of *Listeria monocytogenes* in fresh and processed foods in Navarra (Spain) *Int J Food Microbiol*, 90, 449-356
5. Todd, E. C. **2007**. Epidemiology of food borne diseases: a worldwide review.World Health Stat. Q 50**:**30–50
6. Nilsson P. and Ripa T.**2006.** *Staphylococcus aureus* throat colonization is more frequent than colonization in the anterior nares. *J. Clin. Microbiol*. 44:3334–3339
7. ROBERT W."Microbiology with diseases by body system **2015 .** forth Ed. Inc., Permissions Department, 1900 E. Lake Ave.,Glenview, IL 60025.
8. Richmond J. and McKinney R. **2007**. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Government Printing Office, Washington, D.C.
9. Karami A., Ahmadi Z., Safiri Z. and Pourali F. **2012** .Detection of *Salmonella* strain by rapid-cycle multiplex PCR. *Jundishapur J. Microbiol*.,4(2):91–8.
10. *Garrido A., Chapela M., Román B., Fajardo P., Lago J., Vieites J.,* **2013**. *A new multiplex real-time PCR developed method for Salmonella spp. and Listeria monocytogenes detection in food and environmental samples. Food Control 30(1): 76-85.*
11. Persson S.and Olsen K.. **2005**. Multiplex PCR for identification of Campylobacter coli and Campylobacter jejuni from pure cultures and directly on stool samples. *J Med Microbiol*.; 54(Pt 11):1043-7.
12. Nakari U., Puhakka A. and Siitonen A. **2008** .Correct identification and discrimination between Campylobacter jejuni and C. coli by a standardized hippurate test and species-specific polymerase chain reaction. *Eur J Clin Microbiol Infect Dis*.; 27(7):513-8.
13. Kim J., Lee G., Park J., Jung Y., Kwak H. and Kim S. **2007**.A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli O157:H7, Salmonella, Staphylococcus aureus, Listeria monocytogenes,* and *Vibrio parahaemolyticus. J. of Food Prot.,*70(7):1656–62.
14. Kahraman T. and Aydin A. **2009**. Prevalence of *Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes* in meat and meat products in Turkey. Arch. Lebensmittelhyg.,60:6–11.
15. Nyenje M. , M. E. Tanih M., Green E. and Ndip R**. 2012**.Current status of antibiograms of *Listeria ivanovii* and *Enterobacter cloacae* isolated fromready-to-eat foods in Alice, South Africa. *Int. J. of Enviro. Res. and Pub. Health*, 9(9), pp. 3101–3114.

16. Kahraman T. and Aydin A. **2009**. Prevalence of *Salmonella spp., Escherichia coli O157:H7 and Listeria monocytogenes* in meat and meat products in Turkey. Arch. Lebensmittelhyg.,60:6–11.
17. Nyenje M. , M. E. Tanih M., Green E. and Ndip R**. 2012**.Current status of antibiograms of *Listeria ivanovii* and *Enterobacter cloacae* isolated fromready-to-eat foods in Alice, South Africa. *Int. J. of Enviro. Res. and Pub. Health*, 9(9), pp. 3101–3114.
18. Bagdonavicius, V., Nikulin, S. **2011**. "Chi-squared goodness-of-fit test for right censored data". *Int .J. of App. Math. and Static.*, p. 30-50.
19. Atanassova, V., and Ring C. **2009**. Prevalence of *Campylobacter* spp. in
20. poultry and poultry meat in Germany. Int. J. Food Microbiol. 51**:**187–190.
21. Luu Q., Tran T., Phung D.and Nguyen T. **2006**. Study on the prevalence of Campylobacter spp. from chicken meat in Hanoi, Vietnam. *Ann N Y Acad Sci*. 1081:273-5.
22. Sammarco ML, Ripabelli G, Fanelli I, Grasso G. and Tamburro M**. 2010.**Prevalence and biomolecular characterization of *Campylobacter* spp. isolated from retail meat. *J Food Prot.*;73(4):720-8
23. KLER, J., WIELAND, M. WITTWER, R. STEPHEN, V. **2007**.(Distribution and genetic variability among *Campylobacter spp* isolated from different animal species and humans in Switzerland. Zoonoses Public Hlth. 54, 2-7.
24. Osaili, T., Alaboudi, A. and Nesiar, E. **2011**. Prevalence of *Listeria* spp. and antibiotic susceptibility of *Listeria monocytogenes* isolated from raw chicken and ready to eat chicken product in Jordan. *Food Control,* 22(3-4), 586-590.
25. Meyer, C., Ahomaa, M. F., Kleta, S., Ellerbroek, L., Thiel, S., and Mörtlbauer, E. **2012**. Occurence of *Listeria monocytogenes* in ready to eat poultry products available on the German Market. *Food Research International*, 48(2), 944-947.
26. Wang J, Ray A., Hammons S.and Oliver H. **2015** .Persistent and transient Listeria monocytogenes strains from retail deli environments vary in their ability to adhere and form biofilms and rarely have *inlA* premature stop codons. Food Path Dis;12: 151–158.
27. Jalali M. and Abedi D. **2008**. Prevalence of *Listeria* species in food products in Isfahan,. *Int. J. Food Microbiol.*,122(3):336–40.
28. Rouquette C. and Berche P. **2006**. The pathogenesis of infection by *Listeria monocytogenes*. *J.Microb.*,12(2):245–58.
29. Zhang S. , Zhu X. , Wu Q. , Zhang J. , Xu X .and Li H. **2015**. Prevalence and characterization of Escherichia coli O157 and O157:H7 in retail fresh raw meat in South China *Annals of Microbiology* 65(4):1993-1999
30. Ashgan M., , Abdullah A. , Adel M. , Jakeen K. , Onizan G. , Hassan A. ,[f] and Ihab M. **2015**.Molecular characterization of *Escherichia coli* O157:H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia *Saudi J Biol Sci*.; 22(6): 725–729.
31. Chiu L., Chiu C., Horn Y., Chiou C., Lee C. and, Yeh C.. **2010** .Characterization of 13 multi-drug resistant Salmonella serovars from different broiler chickens associated with those of human isolates. *BMC Microbiol*.;10:86.
32. Doyle, M. P., and J. L. Schoeni. **2007**. Isolation of *Escherichia coli* O157:H7from retail fresh meats and poultry. *Appl. Environ. Microbiol*. 53:2394–2396.
33. Hossein D.,; Shadi A.; Hossein G.; Maryam N.;Manouchehr A. Seyed M. **2014** .Prevalence and Antibiotic Susceptibility of *Campylobacter* species Isolated From Chicken and Beef Meat *Int J Enteric Pathog* 2(2): e17087.
34. Dadi L and Asrat D. **2008** .Prevalence and antimicrobial susceptibility profiles profiles of thermotolerant Campylobacter strains in retail raw meat products in Ethiopia. *Ethiop J Health Dev*.;22(2):195–200.
35. Sangvik M., Olsen R., Olsen K., Simonsen G., Furberg A. and Sollid J. **2011**. Age- and gender-associated *Staphylococcus aureus* found among nasal carriers in a general population: the Tromso Staph and Skin Study. *J. Clin. Microbiol*., 49:4213–4218
36. Olsen K., Sangvik M., Simonsen G., Sollid J., Sundsfjord A., Thune I. and, Furberg A**. 2013**. Prevalence and population structure of *Staphylococcus aureus* nasal carriage in healthcare workers in a general population. The Tromsø Staph and Skin Study. Epidemiol.Infect., 141:143–152
37. Karakolev R. **2009.**.Incidence of *Listeria monocytogenes* in beef, pork, raw-dried and raw-smoked sausages in Bulgaria. Food Cont.,20:953-5.
38. George F., Anders J., Joachim F. andVincent P. **2009** .Prevalence,genetic diversity and antimicrobial susceptibility of *Listeria monocytogenes* isolated from open-air food markets in Greece. Food Cont.,20:314-7.
39. Handa S., Kimura B., Takahashi H., Koda T., Hisa K. and Fuji T. **2005**. Incidence of *Listeria monocytogenes* in raw seafood products in Japanese retail stores. *J. Food Prot.*,68:411-5.

40. Igwe J., Onaolapo J., Ehimidu O., Bolaji R., Tytler B., Ojiego O., Kachallah M., Okafo C., Musa A., Sidi T. and Salihu S. **2016**. Antibiotic Susceptibility Profile of *E. coli* Serotype 0157:H7 in ABUTH, Zaria, *Int. J. of Trop. Dis.*, 11(1): 1-8.
41. Bello M., Lawan M., Kwaga J. and Raji M . **2011**.Assessment of carcass contamination with
42. *E. coli* O157 before and after washing with water at abattoirs in Nigeria. *Int.J.of F. Microbiol*., 150.
43. Umolu P., Omigie O., Tatfeng Y., Omorogbe F., Aisabokhale F. and Ugbodagah O. **2006**.Antimicrobial susceptibility and plasmid profiles of *Escherichia coli* isolates obtained. *J.of Ameri. Scie.*,2(4). 184–186.