**Bacteriological studies on some bacterial strains isolated from imported and local frozen chicken meat**

**By**

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**ABSTRACT**

The present study was performed on 150 random samples of local frozen and imported frozen chicken meat (75 for each), collected from different shops at Kaliobia Governorate to throw light over the bacterial status of them beside the phenotypic characterization of the isolated bacterialstrains and detection of some virulence genes in some strains. The bacteriological examination of samples cleared that, a total of 93(62.0%) isolates of foodborne pathogens were recovered from 150 samples (37 from local frozen chicken meat and 56 from imported frozen chicken meat) where *S. aureus* were the most isolated (31=20.7%, 17 from imported frozen samples and 14 from local frozen ones ) followed by *Ps.* *aeruginosa* (18=12.0%, 10 from imported and 8 from local ones); *E.coli* (11=7.3%,7 from imported and 4 from local ones); *Enterobacter diversus* (8=5.3%, 5 from imported and 3 from local ones); *A. hydrophila* and *Kl*. *pneumoniae* (7=4.7% , 5 from imported and 2 from local ones for each); Micrococcus spp. (6=4.0%, 4 from imported and 2 from local ones) and *Proteus vulgaris* (5=3.3%,3 from imported frozen samples and 2 from local frozen ones) .Meanwhile, Salmonellae failed to be isolated from all samples. In addition, the results of antibiotic sensitivity tests for the isolated strains appeared that: For *E.coli*, they were highly resistant for amoxicillin; ampicillin and oxytetracycline followed by methicillin but they were highly sensitive to enrofloxacin and gentamycin followed by norfloxacin, cefotaxime and ciprofloxacin. For *S.aureus*, they were highly resistant for oxacillin followed by methicillin; nalidixic acid; ampicillin; amoxicillin and oxytetracycline but they were highly sensitive to gentamycin followed by enrofloxacin and norfloxacin. For *Ps. aeruginosa*, they were highly resistant for oxacillin followed by amoxicillin, ampicillin and methicillin but they were highly sensitive to enrofloxacin and norfloxacin followed by gentamycin and ciprofloxacin. In addition, for *A. hydrophila*, they were highly resistant for methicillin and oxacillin followed by amoxicillin, ampicillin, erythromycin, oxytetracycline and streptomycin. Meanwhile, they were highly sensitive to ciprofloxacin and enrofloxacin followed by norfloxacin and gentamycin. PCR results cleared that *eaeA* and *blaSHV* virulence genes were detected in the two studied *E.coli* strains; *clfA* and *mecA* virulence genes were detected in the two studied *S.aureus* strains and regarding to *A. hydrophila* strains; the haemolysin  virulence gene was detected in one strain, while *bla TEM* virulence gene was detected in the two studied strains.

**Key words:** Frozen chicken meat, bacterial status, *A. hydrophila*

1. **INTRODUCTION**

Poultry meat was a common vehicle of pathogenic microorganisms such as Enterobacter; Salmonella; Campylobacter; *S. aureus; E. coli* ;Listeria, Aeromonas and Pseudomonas species that considered as the most important causes of foodborne outbreaks in people (Noori and Alwan, 2016 ). Avian strains of *E.coli* show many similarities with human extra intestinal pathogenic *E. coli* (ExPEC) strains, in that most of the virulence genes they possess are similar to those identified in uropathogenic *E. coli* and new-born meningitis causing *E. coli* (NMEC) and some studies have also demonstrated that ( ExPEC) strains could belong to the same clones as human EPEC strains and it can be transferred to humans through consumption of contaminated food or food products causing a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Ewers *et al.*, 2007 and Gi *et al.,* 2009 ). *S.aureus* is one of the most common agents in bacterial food poisoning outbreaks. They produce disease when the bacteria contaminate food, they produce some enzymes which are implicated with Staphylococcus invasiveness and many extracellular substances some of which are heat stable enterotoxins that renders the food dangerous even though it appears normal and extensive cooking can be killed the bacteria but the toxins may not be destroyed because most of them are gene based i.e. they can be carried on the plasmid (Prescott *et al.,* 2005). The emergence of antimicrobial resistance among *E. coli*, Salmonella, Staphylococci, Pseudomonas and *A. hydrophila* strains of poultry origin has important public health implications. Several studies showed that drug-resistant *E. coli*, Salmonella, Staphylococci, Pseudomonas and *A. hydrophila* infections in humans were caused by strains from animals and poultry and that those infectious agents harbored the same mobile resistance genes as were found in diverse bacterial species from a variety of animal sources (Khan *et al.*, 2009; and Husain, 2010). Beside the antimicrobial resistance among food borne pathogens, the pathogenicity of them could be attributed to the virulence factors the bacteria produce. For *E.coli*, these factors including those encoding for adhesions (F1, P, and *stg* fimbriae, curli, and EA/I), anti-host defense factors (*omp*A, *iss*, lipopolysaccharide, and K1), iron acquisition systems (aerobactin, Iro proteins, yersiniabactin, and the Sit iron acquisition locus), auto transporters (*tsh*, *vat*, and *aat*A), the phosphate transport system, sugar metabolism, and the *ibe*A protein (Le Bouguenec and Schouler,2011 and Van.der and Bragg, 2012) . Meanwhile, for *S. aureus* could be attributed to intracellular adhesion (*icaA*) ; clumping factors A(*clfA*); toxins (enterotoxins, toxic shock syndrome toxin-1, Panton-Valentine Leukocidin); haemolysin ; coagulase, thus clot blood; protease ; hyaluronidase, and staphylokinase (Bokarewa *et al.,*2006 and Abdalrahman *et al.,*2015 ).Moreover, for *A. hydrophila* strains,the pathogenicity is associated with the liberation of virulence factors and cell associated endotoxin. Virulence factors include the production of exotoxins (cytotoxin or enterotoxin); haemolysins; protease; Aerolysin is a significant virulent toxin protein secreted by *A. hydrophila*; it produces deep wound infections and hemorrhagic septicemia (Singh, 2010), ability to bind and to invade epithelial cells (Aravena *et al.,* 2014). The aim of this work is to determine the prevalence of bacterial isolates in imported and local broiler frozen meat, Phenotypic characterization of the isolated bacterial strains, In-vitro antimicrobial sensitivity tests for the isolated bacterial strains,Genotypic characterization and detection of some virulence factors of some isolated *E.coli, S. aureus*  and *A. hydrophila* strains by PCR technique.

**2. MATERIAL AND METHODS**

2.1. Samples collection:

 A total of 150 random samples of local frozen and imported frozen chicken meat (75 for each), were collected from different shops at Kaliobia Governorate and transferred with minimum delay to the laboratory for studying its bacteriological status. Each examined sample was taken alone in sterile plastic bags, kept in icebox and transferred with minimum delay to the laboratory for bacteriological examination.

2.2. Bacteriological examination

Preparation of samples(APHA, 2001)for isolation and identification of some food-borne pathogens:

2.2.1. Isolation of *E.coli* strains:

Isolation and identification morphologically by Gram stain, biochemically, serologically by slide agglutination test using *E.coli* antisera of DENKA SEIKEN CO., LTD.TOKYO, Japan and their In-Vitro anti-microbial sensitivity using the disc and agar diffusion method according to Edward and Ewing (1972); Koneman *et al.*( 1997) and Quinn et al. (2002).

2.2.2. Isolation and identification of *S. aureus* strains:

Isolation and identification morphologically by Gram stain, biochemically, and their In-Vitro anti-microbial sensitivity using the disc and agar diffusion method according to Koneman *et al.*( 1997) and Quinn *et al.* (2002) .

2.2.3. Isolation and identification of Pseudomonas species:

Isolation and identification morphologically by Gram stain, biochemically, and their In-Vitro anti-microbial sensitivity using the disc and agar diffusion method according to Koneman *et al.*( 1997) ; Quinn et al. (2002) and Markey *et al*.( 2013).

2.2.4. Isolation and identification of Aeromoneus species:

Isolation and identification morphologically by Gram stain, biochemically, and their In-Vitro anti-microbial sensitivity using the disc and agar diffusion method according to Koneman *et al.*( 1997) ; Quinn *et al.* (2002) and Nicky( 2004).

2.2.5. Isolation and identification of Salmonella strains:

Isolation and identification morphologically by Gram stain and biochemically following Quinn *et al.* (2002).

2.2.6. Virulence genes of *E. coli*; *S.aureus* and *A. hydrophila* detection by PCR:

PCR was applied by using 6 sets of primers for detection of 6 virulence genes that may play a role in virulence of *E. coli*; *S.aureus* and *A. hydrophila* strains, 2 random strains from each type. These genes were intimin or *E.coli* attaching and effacing gene (*eaeA*) and *blaSHV* gene for *E. coli*; clumping factor (*clfA*) and methicillin resistant gene (*mecA*) for *S.aureus* and haemolysin (*hly*) and *bla TEM* for *A. hydrophila* strains following QIAamp® DNA Mini Kit instructions (Catalogue no. 51304), Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A and 1.5% agarose gel electrophoreses (Sambrook *et al.,* 1989).

**3-RESULTS**

The results of bacteriological examination of studied samples revealed that, foodborne pathogens were isolated from 70 positive samples (46.7%); represented as 26 positive samples (34.7%) from local frozen chicken meat samples, where 7 (26.9%) were single pure cultures and 19 (73.1%) were mixed cultures; meanwhile, 44 (58.7%) from imported frozen chicken meat samples where 12 (**27.3**%) were single pure cultures and 32 (72.7%) were mixed cultures (Table, 1). Moreover, 93(62.0%) isolates of foodborne pathogens were recovered from 150 samples (37 from local frozen chicken meat and 56 from imported frozen chicken meat) where *S. aureus* were the most isolated (31=20.7%) followed by *Ps. aeruginosa* (18=12.0%), *E.coli* (11=7.3%), *Enterobacter diversus* (8=5.3%), *Kl. pneumoniae* and *A. hydrophila* (7=4.7% for each), Micrococcus spp. (6=4.0%) , *Proteus vulgaris* (5=3.3%) and Salmonellae failed to be isolated as shown in Table (2) .

The results of *E. coli* isolation (Table, 2) showed that, 11 strains were isolated, 4 (5.3%) from local frozen chicken meat and 7 (9.3%) from imported frozen chicken meat. The results ofserological examination Table (3) showed that, out of 11 *E.coli* strains, 4 (36.3%) strains were typed as 4 O55:H7, two from each samples; 3 O111:H4 (27.3%), one from local frozen samples and two from imported ones; 2 O125:H18 (18.2%), one from each samples; one O128:H2 and one O26:H11 (9.1% for each) from imported samples only. Moreover, the results of in- vitro sensitivity tests for the isolated *E.coli* were present in Table (4). In addition, PCR results for studied *E.coli* strains showed that, *eaeA* and *blaSHV* virulence genes were detected in two studied strains (100.0%) as shown in Fig. (1 and 2).

The results of *S. aureus* isolation (Table, 2) revealed that, 31 strains were isolated, 14(18.7%) from local frozen chicken meat and 17(22.7%) from imported frozen chicken meat. The results of in- vitro sensitivity tests for the isolated *S.aureus* were present in Table (5). Also, PCR results for studied *S. aureus* strains showed that, *clfA* and *mecA* virulence genes were detected in two studied strains (100.0%) as shown in Fig. (3 and 4).

The results of *Ps. aeruginosa* isolation (Table, 2) showed that, 18 strains were isolated, 8 (10.7%) from local frozen chicken meat and 10 (13.3%) from imported frozen chicken meat. The results of in- vitro sensitivity tests for the isolated *Ps. aeruginosa* were present in Table (6). The results of *A. hydrophila* isolation (Table, 2) showed that, 7 strains were isolated, 2 (2.7%) from local frozen chicken meat and 5 (6.7%) from imported frozen chicken meat. The results of in- vitro sensitivity tests for the isolated *A. hydrophila* were present in Table (7). Also, PCR results for studied *A. hydrophila* strains showed that, *hly* virulence gene was detected in one strain(50.0%) , while *bla TEM* virulence gene was detected in 2 studied strains (100.0%) as shown in Fig. (5 and 6).

**4- DISCUSSION**

The results of Food borne pathogens isolation showed in (Table, 1 ) revealed that,. These results came in accordance with that obtained by Olukemi *et al*. (2015) and Noori, and Alwan (2016).

The results of bacteriological examination of examined samples showed in (Table ,2) revealed that, it is similar to the results were recorded by Olukemi *et al*. (2015); Noori and Alwan (2016) and Suleiman *et al*.(2016).

The results of *E.coli* isolation (Table, 2) showed that 11 strains were isolated, 4 (5.3%) from local frozen chicken meat and 7 (9.3%) from imported frozen chicken meat. Nearly similar results were obtained by Adeyanju and Ishola (2014)); Olukemi *et al*. (2015); Rasmussen *et al.*(2015); Noori and Alwan (2016); Suleiman *et al*.(2016) and Zogg *et al.*(2016) .(Table, 3) showed that, out of 11 *E.coli* strains, 4 (36.3%) strains were typed as O55:H7, two from each samples; 3 O111:H4 (27.3%), one from local frozen samples and two from imported ones; 2 O125:H18 (18.2%), one from each samples; one O128:H2 and one O26:H11 (9.1% for each) from imported samples only. These results came in harmony with those of Lee *et al.* (2009) and Abd El-Tawab *et al*.(2015). The results of antibiotic sensitivity tests for the isolated *E.coli* (Table, 4) showed that, they were highly resistant for amoxicillin; ampicillin and oxytetracycline (72.7% for each) followed by methicillin (63.6%) and erythromycin, nalidixic acid, oxacillin and streptomycin (54.5% for each). Meanwhile, they were highly sensitive to enrofloxacin and gentamycin (90.9%) followed by norfloxacin (81.8%); cefotaxime (72.7%) and ciprofloxacin (63.6%). Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol (54.5%).These results go in parallel with those obtained by Adeyanju and Ishola (2014) ; Abd El-Tawab *et al*. (2015); Olukemi *et al*. (2015) and Rasmussen *et al*. (2015).

The results of *S. aureus* isolation (Table, 2) cleared that, 31 strains were isolated, 14(18.7%) from local frozen chicken meat and 17(22.7%) from imported frozen chicken meat. These results were nearly similar to Zargar *et al*.(2014); Suleiman *et al*.(2016) and Zogg *et al*.(2016) .. Meanwhile, the results disagreed with previous studies that failed to isolate *S.aureus* from chicken meat (Kabour, 2011). The in- vitro sensitivity tests for the isolated *S.aureus* (Table, 5) showed that, the isolated *S.aureus* were highly resistant for oxacillin (83.9%) followed by methicillin (80.7%); nalidixic acid (74.2%); ampicillin (67.7%); amoxicillin (64.5%); oxytetracycline (54.8%) and cefotaxime (51.6%). Meanwhile, they were highly sensitive to gentamycin (87.1%) followed by enrofloxacin and norfloxacin (83.9% for each) and ciprofloxacin (67.7%). Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol (61.3%); erythromycin (54.8%) and streptomycin (51.6%). These results were agreed with (Momtaz *et* *al.,*2013; Abd El-Tawab *et al*., 2015; Olukemi *et al*., 2015 and Zogg *et al.,* 2016).

The results of *Ps. aeruginosa* isolation (Table, 2) showed that, 18 strains were isolated, 8 (10.7%) from local frozen chicken meat and 10 (13.3%) from imported frozen chicken meat. These results were nearly similar to Elnawawi *et al*. (2012). The in- vitro sensitivity tests for the isolated *Ps. aeruginosa* Table (6) revealed that, the isolated *Ps. aeruginosa* were highly resistant for oxacillin (88.9%) followed by amoxicillin, ampicillin and methicillin (83.3% for each); cefotaxime (77.7%) and erythromycin (72.2%). Meanwhile, they were highly sensitive to enrofloxacin and norfloxacin (94.4% for each) followed by gentamycin (88.9%) and ciprofloxacin (72.2%). Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol (66.7%); oxytetracycline and nalidixic acid (61.1% for each) and streptomycin (55.5%). These results were agreed with Efuntoye *et al*. (2012) and Musefiu and Olasunkanmi (2015).

The results of *A. hydrophila* isolation (Table, 2) appeared that, 7 strains were isolated, 2 (2.7%) from local frozen chicken meat and 5 (6.7%) from imported frozen chicken meat. These results were nearly similar to Dallal *et al*. (2012). The in- vitro sensitivity tests for the isolated *A. hydrophila* Table (7) revealed that, the isolated *A. hydrophila* were highly resistant for methicillin and oxacillin (85.7% for each) followed by amoxicillin, ampicillin, erythromycin, oxytetracycline and streptomycin (71.4% for each) and nalidixic acid (57.1%). Meanwhile, they were highly sensitive to ciprofloxacin and enrofloxacin (85.7for each) followed by norfloxacin (71.4%) and gentamycin (57.1%). Moreover, they were intermediate sensitive to cefotaxime and trimethoprim/ sulphamethoxazol (57.1% for each). These results were agreed with (Awan *et al*., 2009 and Jayavignesh *et al*., 2011).

The PCR results for *E.coli* showed that, intimin or *E.coli* attaching and effacing gene (*eaeA*) virulence gene in *E.coli* isolates was amplified in two (100.0%) *E.coli* strains giving a PCR product of 248 bp as shown in Fig. (1). These results came in accordance with those recorded by Hideki *et al.*(2009); and Dutta *et al*.(2011).Meanwhile these results disagreed with the findings of Wen-Jie *et al.* (2008) and Olsen *et al.* (2011) who found no *eaeA* gene detected in all APEC isolates. Moreover, *blaSHV* virulence gene was amplified in two (100.0%) *E.coli* strains giving a PCR product of 392 bp as shown in Fig. (2). These results came in accordance with those recorded by Schaumburg *et al.* (2014); Rasmussen *et al.* (2015) and Zogg *et al.* (2016).

The PCR results for *S.aureus* showed that, clumping factor A(*clf*A) gene was amplified in two (100.0%) *S.aureus* strains giving product of 638 bp as shown in Fig. (3). Similar findings were recorded by Yang *et al*. (2012) and Momtaz *et al.* (2013). Moreover, *mec*A gene was amplified in two (100.0%) *S.aureus* strains giving product of 310 bp as shown in Fig. (4). Similar detection of *mecA* gene in *S.aureus* strains (MRSA) isolated from chicken meat and its products were recorded by Momtaz *et al.* (2013); Kraushaar and Fetsch (2014) ; Krupa *et al.* (2014) and Zogg *et al.* (2016) .

Regarding to *A. hydrophila* studied strains; the PCR results showed that, *hly* virulence gene was amplified in one (50.0%) *A. hydrophila* strain giving product of 1500 bp as shown in Fig. (5). similar results were recorded by Yu *et al.* (2004) and Aravena *et al.* (2014). In addition, *bla TEM* virulence gene was amplified in two (100.0%) *A. hydrophila* strains giving a PCR product of 516 bp as shown in Fig. (6). These results came in accordance with those recorded by Janda and Abbott (2010) and Ndi and Barton (2011).

**5. CONCLUSION**

The results proved that multiple antibiotic resistances are widely spread among isolated strains of *A. hydrophila; E.coli; Ps. aeruginosa* and *S.aureus*. Moreover, all studied strains with PCR technique were virulent strains as for *E.coli*, *eaeA* and *blaSHV* virulence genes were detected in the two studied strains; for *S.aureus*, *clf*A and *mecA* virulence genes were detected in the two studied strains and for *A. hydrophila*  , *hly* virulence gene was detected in one strain , While *bla TEM* virulence gene was detected in two studied strains. Therefore, it was concluded that *A. hydrophila; E.coli*; coagulase positive *S. aureus* and *Ps. aeruginosa* are meat-borne pathogens of public health important.

**6. References.**

Abd El-Tawab, A.A. , Maarouf,A.A., El-Hofy,Fatma ,I. and El-Said,Aya,A., 2015 .Bacteriological studies on some food borne bacteria isolated from chicken meat and meat products in Kaliobia Governorate.B.V.M.J.,29(2) ,47-59.

Abdalrahman, L.S , Stanley, A., Wells,H. and Fakhr,M.K., 2015.Isolation, virulence, and antimicrobial resistance of methicillin-resistant Staphylococcus aureus (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA) Strains from Oklahoma retail poultry meats. Int. J. Environ. Res. Public Health12, 6148-6161.

Adeyanju, G.T. and Ishola ,O. , 2014. Salmonella and Escherichia coli contamination of poultry meat from a processing plant and retail markets in Ibadan, Oyo State, Nigeria. SpringerPlus 3,139-147.

APHA American Public Health Association’’ 2001 .Compendium of Methods for the Microbiological examination of Foods. 4th Ed. F.P. Downes and K. Ito(editors), APHA. Washington D.C., USA.

[Aravena,R. M](http://www.ncbi.nlm.nih.gov/pubmed?term=Aravena-Rom%C3%A1n%20M%5BAuthor%5D&cauthor=true&cauthor_uid=24866333)., [Inglis, T.J](http://www.ncbi.nlm.nih.gov/pubmed?term=Inglis%20TJ%5BAuthor%5D&cauthor=true&cauthor_uid=24866333)., [Riley, T.V](http://www.ncbi.nlm.nih.gov/pubmed?term=Riley%20TV%5BAuthor%5D&cauthor=true&cauthor_uid=24866333). and [Chang, B.J](http://www.ncbi.nlm.nih.gov/pubmed?term=Chang%20BJ%5BAuthor%5D&cauthor=true&cauthor_uid=24866333). , 2014 . [Distribution of 13 virulence genes among clinical and environmental Aeromonas spp. in Western Australia.](http://www.ncbi.nlm.nih.gov/pubmed/24859908) [Eur. J. Clin. Microbiol. Infect. Dis.](http://www.ncbi.nlm.nih.gov/pubmed/?term=Aravena-Rom%C3%A1n+M%2C+Inglis+TJ%2C+Riley+TV%2C+Chang+BJ+%28.2014+%29%3A), 33(11),1889-1895.

Awan, M. B. ; Maqbool, A. ; Abdul Bari and Krovacek, K. ( 2009) : Antibiotic susceptibility profile of Aeromonas spp. isolates from food in Abu Dhabi, United Arab Emirates. New Microbiologica, 32, 17-23.

Bokarewa, M.I. , Jin, T. and Tarkowski, A. , 2006 . *Staphylococcus aureus*. Staphylokinase. The International Journal of Biochemistry and Cell Biology 38(4), 504-509.

Dallal ,M. M. S. , Yazdi ,M. K. S. and Avadisians ,S. ,2012. Study of prevalence and antibiotic resistance in Aeromonas species isolated from minced meat and chicken samples in Iran. African J. of Microbiology Research Vol. 6(2), 460-464.

Dutta, T. K. , Roychoudhury, R., Bandyopadhyay, S., Wani, S. A. and Hussian, I., 2011 Detection and characterization of shiga toxin producing *Escherichia coli* (STEC), enteropathogenic *Escherichia coli*(EPEC) in poultry birds with diarrhoea. Indi .J. Res., 133(5),541-545.

Edward,P.R.and Ewing, W. H. ,1972.Edwards and Ewing's identification of Enterobacteriacae. 3rd Ed. Burgess, Minneapolis.

Efuntoye,M.O, Olurin,K.B and Jegede,G.C ., 2012 . Bacterial Flora from Healthy Clarias Gariepinus and their Antimicrobial Resistance Pattern. Advance Journal of Food Science and Technology 4(3), 121-125.

Elnawawi, F. A. , Attala , O. A. and Saleh,S . , 2012 .Enteropathogens of Public Health Importance in Imported Frozen Meat and Chicken. International Journal of Microbiological Research 3 (1), 59-63.

Ewers, C., Li, G., Wilking-Kiessling, S., Alt, K. and Antao, E. M. ,2007. Avian pathogenic, Uropathogenic, and newborn meningitis-causing *Escherichia coli.* how closely related are they?. Int. J. Med. Microbiol., 297,163-176.

 Gi, Y. L., Hye, I. J., In, G. H. and Min, S. R., 2009. Prevalence and classification of pathogenic *E.coli* isolated from fresh beef, poultry and pork in Korea. International J. Food Microbiology134(3).196-200.

Hideki, K., Mika, K., Eiji, H. and Masanori, K., 2009. Prevalence and characteristics of eae- and stx- positive strains of *Escherichia coli* from wild birds in the immediate environment of Tokyo bay. Applied and environmental Microbiology 75(1), 292-295.

### Husain, A. ,2010. Prevalence and antimicrobial resistance of *Salmonella*  serovars isolated from poultry and meat in Hyderabad, Pakistan. Turk. Vet. Anim. Sci. 34(5), 455-460.

Janda, J. M. and Abbott, S. L., 2010. The genus Aeromonas. taxonomy, pathogenicity, and infection. Clin. Microbiol. Rev., 23,35–73.

 Jayavignesh, V., Sendesh K. K. and Abhijith D. ,2011. Biochemical characterization and cytotoxicity of the *A.* *hydrophila* isolated from Catfish Arch. Appl. Sci. Res. 3 (3) , 85-93.

 Kabour, G.A. (2011). Evaluation of Microbial Contamination of Chicken Carcasses during Processing in Khartoum State. M.V.Sc. Thesis Sudan University of Science and Technology, the Sudan.

###  Khan, E., Jabeen, K., Ejaz, M., Siddiqu, J., Shezad, M. F. and Zafar, A., 2009. Trends in antimicrobial resistance in Shigella species in Karachi, Pakistan. J. Infection in Developing Countries, 3,798-802.

Konemann, E., Allen, S., Janda, W., Schreckenberger, C. and Winn, W., 1997 .Color Atlas and text book of Diagnostic Microbiology. Fifth Edition.Lippincott, Philadelphia, New York.55-73.

Kraushaar, B. and Fetsch, A. ,2014. First Description of PVL-Positive Methicillin-Resistant Staphylococcus aureus (MRSA) in Wild Boar Meat. International Journal of Food Microbiology, 186,68-73.

Krupa, P., Bystro . J., Bania, J., Podkowik , M. , Empel , J. and Mroczkowska , A. ,2014. Genotypes and oxacillin resistance of Staphylococcus aureus from chicken and chicken meat in Poland . Poultry Science 93 ,3179–3186.

Le Bouguenec, C. and Schouler, C. , 2011.Sugar metabolism, an additional virulence factor in enterobacteria. International Journal of Medical Microbiology, 301 (1), 1-6.

Lee, G. Y., Jang, H. I., Hwang, I. G. and Rhee, M. S., 2009. Prevalence and classification of pathogenic *Escherichia coli* isolated from fresh beef, poultry, and pork in Korea. Inter. J. Food Microbiol., 134 , 196–200

Markey,B.K.; Leonard,F.C. ; Archambault,M. ;Cullinane,A. and Maguire,D. ( 2013): Clinical Veterinary Microbiology . Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto

Momtaz ,H., Dehkordi,F.S., Rahimi,E. Asgarifar ,A. and Momeni ,M. , 2013. Virulence genes and antimicrobial resistance profiles of Staphylococcus aureus isolated from chicken meat in Isfahan province, Iran. J. Appl. Poult. Res., 22 ,913–921.

Musefiu,T.A and Olasunkanmi,S.M ., 2015 . Antibiotics Resistance in Bacteria Strains Isolated From Fish Potential Health Risk. Journal of Biology, Agriculture and Healthcare. 5(10) , 2224-3208 .

Ndi ,O. L. and Barton ,M .D. ,2011 . Incidence of class 1 integron and other antibiotic resistance determinants in Aeromonas spp. from rainbow trout farms in Australia .Journal of Fish Diseases 34 , 589–599

Nicky, B. B. , 2004. Bacteria from fish and other aquatic animals (a practical identification manual).CABI publishing is a division of CAB international. QR106.B85 , (83 – 116).

 Noori, T.E. and Alwan, M. J., 2016 .Isolation and Identification of Zoonotic Bacteria from Poultry Meat. Int. J. Adv. Res. Biol. Sci., 3(8),57-66.

 Olsen, M.S. C. and Christensen,J.P., 2011 .Clonality and virulence traits of *Escherichia coli* associated with haemorrhagicsepticaemia. Avian Pathology, 40 (6), 587-595.

Olukemi, A. Y. , Osas , I. M. , Olaitan, J. O.& Jeremiah, O ,I. ,2015 .Bacterial Contamination Associated with Retail Chicken Carcasses in Osogbo, Nigeria, Nitte University Journal of Health Science(NUJHS) 5(4 ), 45-50.

Prescott, M., Harley, P. and Klein, D. A. ,2005 .Microbiology. 6th Ed. McGraw Hill. New York U.S.A. , 910.

 Quinn, p., Markey, B., Carter, M., Donelly, W. and Leonard, F., 2002. Veterinary microbiology and microbial disease. Black Well Science. chapters 26-36.

Rasmussen, M. M. , Opintan, J. A. , Møller, N. F. and Styrishave , B. ,2015. Beta-Lactamase Producing Escherichia coli Isolates in Imported and Locally Produced Chicken Meat from Ghana. PLOS ONE 10(10),1-15.

 Sambrook, J., Fritsch, E.F. and Montias, T. ,1989. Molecular Biology. In. Molecular cloning. Laboratory manual, Second Edition.Cold Spring Harbor Laboratory press, USA., 268.

Schaumburg ,F. , Alabi, S. A. , Frielinghaus, L. , Grobusch, M. P. , Köck ,R. , Becker, K. , Issifou, S. , Kremsner, P. G , Peters , G. and Mellmann , A. , 2014 . The risk to import ESBL-producing Enterobacteriaceae and Staphylococcus aureus through chicken meat trade in Gabon . BMC Microbiology 14,286-292.

 Shalini, M. and Rameshwar, S. , 2005 . Antibiotic resistance in food lactic acid bacteria - a review. International Journal of Food Microbiology, 105, 281-295.

 [Singh, V](http://www.ncbi.nlm.nih.gov/pubmed?term=Singh%20V%5BAuthor%5D&cauthor=true&cauthor_uid=19763901)., [Somvanshi, P](http://www.ncbi.nlm.nih.gov/pubmed?term=Somvanshi%20P%5BAuthor%5D&cauthor=true&cauthor_uid=19763901), [Rathore ,G](http://www.ncbi.nlm.nih.gov/pubmed?term=Rathore%20G%5BAuthor%5D&cauthor=true&cauthor_uid=19763901) ,[Kapoor, D](http://www.ncbi.nlm.nih.gov/pubmed?term=Kapoor%20D%5BAuthor%5D&cauthor=true&cauthor_uid=19763901). and [Mishra, B.N](http://www.ncbi.nlm.nih.gov/pubmed?term=Mishra%20BN%5BAuthor%5D&cauthor=true&cauthor_uid=19763901)., 2010. Gene cloning, expression, and characterization of recombinant aerolysin from *A. hydrophila.* Apr. 160(7),1985-1991.

Suleiman ,M.A. , Wasa ,A.A. , Na’ilatu, M.I. , Titus, I. and Adamu, S . ,2016. BACTERIOLOGICAL Analysis of Chicken Parts of Fesh and Frozen Poultry Chicken Sold in GOMBE METROPOLIS, NIGERIA., *International Journal of Adulteration* 1(3), 58-73.

 Van. Der, W. and Bragg,R.R. ,2012.Multiplex polymerasechain reaction for screening avian pathogenic *Escherichia coli* for virulence genes. Avian Pathology 41 (1), 33-40.

 Wen-Jie, J., Zhi-Ming, Z., Yong-Zhi, Z., Ai-Jian, Q., Hong-Xia, S., Yue-Long, L., Jiao, W. and Qian-Qian, W., 2008. Distribution of virulence-associated genes of avian pathogenic *Escherichia coli* isolates in China. Agricultural Sciences in China 7 (12),1511-1515.

 Yang, F.L., Shan, X. L., Liang, X.W., Zhang, X.F., Qiu, G.S. and Yang, B.Z. 2012 Detection of virulence associated genes in *S. aureus* isolated from bovine clinical mastitis milk sample in Gaungxi. Trop. Anim. health prod. (10), 1243-1250.

Yu, H.B., Rao, P.S.S., Lee, H.C., et al., 2004. A type III secretion system is required for *Aeromonas hydrophila*AH-1 pathogenesis. Infection and Immunity 72 (3),1248–1256.

Zargar, M , H. ,S . , Doust, R. H. and Mobarez, A. M. ,2014. *Staphylococcus aureus* Enterotoxin A Gene Isolated From Raw Red Meat and Poultry in Tehran, Iran. Int J Enteric Pathog. 2(3) e16085.

Zogg , L. , Zurfluh, K., Inderbinen ,M. N. , and Stephan, R. , 2016.Characteristics of ESBL-producing Enterobacteriaceae and Methicillinresistant *Staphylococcus aureus* (MRSA) isolated from Swiss and imported raw poultry meat collected at retail level. J.SAT ASMV 158(6),451-456.

Table (1): Total number and Percentage of positive samples for pathogens isolation from studied samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Samples | Number of sample | Negative samples | Positive samples | Prevalence of single and mixed cultures |
| Single | Mixed |
| No. | %\* | No. | %\* | No. | %\*\* | No. | %\*\* |
| Local frozen chicken meat | 75 | 49 | 65.3 | 26 | 34.7 | 7 | 26.9 | 19 | 73.1 |
| Imported frozen chicken meat | 75 | 31 | 41.3 | 44 | 58.7 | 12 | 27.3 | 32 | 72.7 |
| TOTAL | 150 | 80 | 53.3 | 70 | 46.7 | 19 | 27.1 | 51 | 72.9 |

%\* Percentage in relation to total number of each sample in each row (75 for each sample &150 for total).

%\*\* Percentage in relation to total number of positive samples in each row

Table (2): Prevalence of foodborne pathogens in examined samples

|  |  |  |  |
| --- | --- | --- | --- |
|  Isolates | Local frozen chicken meat | Imported frozen chicken meat | TOTAL |
|  |  |  |  |
|  | No. | %\* | No. | %\* | No. | %\*\* |
| *A.hydrophila* | 2 | 2.7 | 5 | 6.7 | 7 | 4.7 |
| *E.coli* | 4 | 5.3 | 7 | 9.3 | 11 | 7.3 |
| *Enterobacter diversus* | 3 | 4.0 | 5 | 6.7 | 8 | 5.3 |
| *Kl. pneumoniae* | 2 | 2.7 | 5 | 6.7 | 7 | 4.7 |
| Micrococcus spp. | 2 | 2.7 | 4 | 5.3 | 6 | 4.0 |
| *Proteus vulgaris* | 2 | 2.7 | 3 | 4.0 | 5 | 3.3 |
| *Ps. aeruginosa* | 8 | 10.7 | 10 | 13.3 | 18 | 12.0 |
| Salmonellae | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| *S. aureus* | 14 | 18.7 | 17 | 22.7 | 31 | 20.7 |
| Total | 37 | 49.3 | 56 | 74.7 | 93 | 62.0 |

%\* Percentage in relation to total number of each sample (75)

%\*\*Percentage in relation to total number of samples (150)

Table (3): Serological typing of *E.coli* strains isolated from different examined samples.

|  |  |  |  |
| --- | --- | --- | --- |
| Isolates | Local frozen chicken meat | Imported frozen chicken meat | TOTAL |
|  |  |  |  |
| *E.coli* serotype | No. | %\* | No. | %\* | No. | %\* |
| O26:H11 | 0 | 0.0 | 1 | 9.1 | 1 | 9.1 |
| O55:H7 | 2 | 18.2 | 2 | 18.2 | 4 | 36.3 |
| O111:H4 | 1 | 9.1 | 2 | 18.2 | 3 | 27.3 |
| O125:H18 | 1 | 9.1 | 1 | 9.1 | 2 | 18.2 |
| O128:H2 | 0 | 0.0 | 1 | 9.1 | 1 | 9.1 |
| Total | 4 | 36.4 | 7 | 63.6 | 11 | 100.0 |

% Percentage in relation to total number of examined *E.coli* (11).

Table (4): In-Vitro anti-microbial Sensitivity test for isolated *E.coli*.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antimicrobial agents | Diskconcentrations | Sensitive | Intermediate | Resistant | AA |
| No. | % | No. | % | No. | % |
| Amoxicillin | 25µg | 0 | 0.0 | 3 | 27.3 | 8 | 72.7 | R |
| Ampicillin | 20 µg | 1 | 9.1 | 2 | 18.2 | 8 | 72.7 | R |
| Cefotaxime | 30 µg | 8 | 72.7 | 3 | 27.3 | 0 | 0.0 | S |
| Ciprofloxacin | 5 µg | 7 | 63.6 | 2 | 18.2 | 2 | 18.2 | S |
| Enrofloxacin | 5 µg | 10 | 90.9 | 1 | 9.1 | 0 | 0.0 | S |
| Erythromycin | 15 µg | 3 | 27.3 | 2 | 18.2 | 6 | 54.5 | R |
| Gentamicin | 10 µg | 10 | 90.9 | 1 | 9.1 | 0 | 0.0 | S |
| Methicillin | 5 µg | 1 | 9.1 | 3 | 27.3 | 7 | 63.6 | R |
| Nalidixic acid | 30 µg | 1 | 9.1 | 4 | 36.4 | 6 | 54.5 | R |
| Norfloxacin | 10 µg | 9 | 81.8 | 1 | 9.1 | 1 | 9.1 | S |
| Oxacillin | 1 µg | 1 | 9.1 | 4 | 36.4 | 6 | 54.5 | R |
| Oxytetracycline | 30 µg | 1 | 9.1 | 2 | 18.2 | 8 | 72.7 | R |
| Streptomycin | S/10 | 1 | 9.1 | 4 | 36.4 | 6 | 54.5 | R |
| Trimethoprim/ SulphamethoxazolTrimethoprim/ Sulphamethoxazol  | 1.25/23.75 mcg | 3 | 27.3 | 6 | 54.5 | 2 | 18.2 | IS |

 No.: Number of isolates AA: Antibiogram activity

% Percentage in relation to total number of isolated *E.coli* (11)

Table (5): In-Vitro anti-microbial Sensitivity test for isolated *S.aureus* strains

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antimicrobial agents | Diskconcentrations | Sensitive | Intermediate | Resistant | AA |
| No. | % | No. | % | No. | % |
| Amoxicillin | 25µg | 3 | 9.7 | 8 | 25.8 | 20 | 64.5 | R |
| Ampicillin | 20 µg | 4 | 12.9 | 6 | 19.4 | 21 | 67.7 | R |
| Cefotaxime | 30 µg | 6 | 19.4 | 9 | 29.0 | 16 | 51.6 | R |
| Ciprofloxacin | 5 µg | 21 | 67.7 | 3 | 9.7 | 7 | 22.6 | S |
| Enrofloxacin | 5 µg | 26 | 83.9 | 3 | 9.7 | 2 | 6.4 | S |
| Erythromycin | 15 µg | 6 | 19.4 | 17 | 54.8 | 8 | 25.8 | IS |
| Gentamicin | 10 µg | 27 | 87.1 | 3 | 9.7 | 1 | 3.2 | S |
| Methicillin | 5 µg | 1 | 3.2 | 5 | 16.1 | 25 | 80.7 | R |
| Nalidixic acid | 30 µg | 3 | 9.7 | 5 | 16.1 | 23 | 74.2 | R |
| Norfloxacin | 10 µg | 26 | 83.9 | 4 | 12.9 | 1 | 3.2 | S |
| Oxacillin | 1 µg | 2 | 6.4 | 3 | 9.7 | 26 | 83.9 | R |
| Oxytetracycline | 30 µg | 4 | 12.9 | 10 | 32.3 | 17 | 54.8 | R |
| Streptomycin | S/10 | 5 | 16.1 | 16 | 51.6 | 10 | 32.3 | IS |
| Trimethoprim/ Sulphamethoxa-zolTrimethoprim/ Sulphamethoxazol  | 1.25/23.75 mcg | 8 | 25.8 | 19 | 61.3 | 4 | 12.9 | IS |

No. : Number of isolates AA: Antibiogram activity

%: Percentage in relation to total number of isolates (31).

Table (6): In-Vitro anti-microbial Sensitivity test for isolated *Ps.* *aeruginosa* strains

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antimicrobial agents | Diskconcentrations | Sensitive | Intermediate | Resistant | AA |
| No. | % | No. | % | No. | % |
| Amoxicillin | 25µg | 1 | 5.6 | 2 | 11.1 | 15 | 83.3 | R |
| Ampicillin | 10 µg | 2 | 11.1 | 1 | 5.6 | 15 | 83.3 | R |
| Cefotaxime | 30 µg | 3 | 16.7 | 1 | 5.6 | 14 | 77.7 | R |
| Ciprofloxacin | 5 µg | 13 | 72.2 | 2 | 11.1 | 3 | 16.7 | S |
| Enrofloxacin | 5 µg | 17 | 94.4 | 0 | 0.0 | 1 | 5.6 | S |
| Erythromycin | 15 µg | 2 | 11.1 | 3 | 16.7 | 13 | 72.2 | R |
| Gentamicin | 10 µg | 16 | 88.9 | 2 | 11.1 | 0 | 0.0 | S |
| Methicillin | 5 µg | 0 | 0.0 | 3 | 16.7 | 15 | 83.3 | R |
| Nalidixic acid | 30 µg | 1 | 5.6 | 11 | 61.1 | 6 | 38.9 | IS |
| Norfloxacin | 10 µg | 17 | 94.4 | 1 | 5.6 | 0 | 0.0 | S |
| Oxacillin | 1 µg | 0 | 0.0 | 2 | 11.1 | 16 | 88.9 | R |
| Oxytetracycline | 30 µg | 1 | 5.6 | 11 | 61.1 | 6 | 33.3 | IS |
| StreptomycinTrimethoprim/ Sulphamethoxazol  | 10 µg | 1 | 5.6 | 10 | 55.5 | 7 | 38.9 | IS |
|  | 1.25**/**23.75 mcg | 4 | 22.2 | 12 | 66.7 | 2 | 11.1 | IS |

No.: Number of isolates AA: Antibiogram activity

%: Percentage in relation to total number of isolates (18).

Table (7): In-Vitro anti-microbial Sensitivity test for isolated *A. hydrophila* strains

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Antimicrobial agents | Diskconcentrations | Sensitive | Intermediate | Resistant | AA |
| No. | % | No. | % | No. | % |
| Amoxicillin | 25µg | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 | R |
| Ampicillin | 10 µg | 0 | 0.0 | 2 | 28.6 | 5 | 71.4 | R |
| Cefotaxime | 30 µg | 1 | 14.3 | 4 | 57.1 | 2 | 28.6 | IS |
| Ciprofloxacin | 5 µg | 6 | 85.7 | 0 | 0.0 | 1 | 14.3 | S |
| Enrofloxacin | 5 µg | 6 | 85.7 | 1 | 14.3 | 0 | 0.0 | S |
| Erythromycin | 15 µg | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 | R |
| Gentamicin | 10 µg | 4 | 57.1 | 2 | 28.6 | 1 | 14.3 | S |
| Methicillin | 5 µg | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 | R |
| Nalidixic acid | 30 µg | 1 | 14.3 | 2 | 28.6 | 4 | 57.1 | R |
| Norfloxacin | 10 µg | 5 | 71.4 | 2 | 28.6 | 0 | 0.0 | S |
| Oxacillin | 1 µg | 0 | 0.0 | 1 | 14.3 | 6 | 85.7 | R |
| Oxytetracycline | 30 µg | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 | R |
| Streptomycin | 10 µg | 1 | 14.3 | 1 | 14.3 | 5 | 71.4 | R |
| Trimethoprim/ Sulphamethoxazol | 1.25/23.75 mcg | 1 | 14.3 | 4 | 57.1 | 2 | 28.6 | IS |

No.: Number of isolates AA: Antibiogram activity

%: Percentage in relation to total number of isolates (7)

****

Fig (2): *blaSHV* gene.

Lane L: 100-600 bpDNA Ladder.

Neg.: Negative control.

Pos.: Positive control (at 392 bp)..

Lane 1 and 2: *E.coli* (Positive).



Fig. (1) Intiman or E.coli attaching and effacing (eaeA) gene.

 Lane L: 100-600 bpDNA Ladder.

Neg.: Negative control.

 Pos.: Positive control (at 248 bp).

 Lane 1 and 2: *E.coli* (Positive).

****

Fig. (3): Clumping factor (*clfA*) gene.

Lane L: 100-1000 bpDNA Ladder.

Neg.: Negative control.

Pos.: Positive control (at 638 bp).

Lane 1 and 2:*S.aureus* (Positive).



Fig. (4): Methicillin resistant gene (mecA).

 Lane L: 100-600 bpDNA Ladder.

Neg.: Negative control.

Pos.: Positive control (at 310 bp).

Lane 1 and 2:*S.aureus* (Positive).

****

Fig. (5): Haemolysin (hly) gene.

 Lane L: 100-1500 bpDNA Ladder.

Neg.: Negative control.

 Pos.: Positive control (at 1500 bp).

Lane1: *A. hydrophila* (Positive).

 Lane 2: *A. hydrophila* (Negative).

****

Fig. (6): *bla TEM*gene

 Lane L: 100-600 bpDNA Ladder.

Neg.: Negative control.

 Pos.: Positive control (at 516 bp).

Lane1 and 2: *A. hydrophila* (Positive).