Prevalence of Enterococcus species in chicken meat in Sharkia Governorate

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Abstract

A total of 175 samples of fresh chicken breast, chicken thigh, chicken liver, chicken gizzard, chicken heart, cloacal skin and neck skin (25for each) were randomly collected from different markets and poultry shops at variable sanitation levels in Zagazig city. All collected samples were immediately transferred in an icebox container, aseptically handled and moved promptly to postgraduate student laboratory, Food Control department, Faculty of Veterinary Medicine, Zagazig University, Egypt. The bacteriological examination was applied for enumeration and isolation Enterococcus species. The enterococci detected in chicken samples within different percentages ranged from 15/25(60%) in chicken heart to 25/25(100%) in cloacal skin. The descending prevalence arranged cloacal skin > neck skin > gizzard > liver > thigh > breast > heart. The counts of enterococci ranged from 2.12 to 5.15, 3.19 to 5.64, 3.54 to 5.95, 3.68 to 6.32, 3.11 to 6.26, 4.82 to 6.3 and 3.24 to 6.7 with mean values 3.04 ±0.37, 3.47 ±0.48, 4.05 ±0.38, 4.15 ±0.41, 3.72 ±0.54, 5.03 ±0.13 and 4.8 ±0.25 log $_{10}$ CFU/g in examined chicken breast, thigh, liver, gizzard, heart, cloacal skin and neck skin, respectively. The biochemical identification of enterococcus species declared that *E. faecalis, E.faecium, E. durans, E. avium and E. hirae* were detected in 69 (48.9%), 50 (35.5%), 16 (11.4%), 3 (2.1%) and 3 (2.1%), respectively.

Key words: Enterococci, Chicken meat, Chicken liver, *Enterococcus faecalis*, Chicken thigh

1.Introduction

Poultry meat products constitute an excellent source of high quality, easily prepared, cooked and digested animal protein, which contains all essential amino acids besides many vitamins and minerals which are required for human development, hence it represents an important food article in most countries and has a considerable share in Egyptian's diet for its competitive price with that of other meats **(Hussein *et al*.,2018)**. Chicken meat is characterized by a lower caloric value as it contains less fat, which is rich in unsaturated fatty acids, so it can be used for feeding young children and some patients. Also they are low in price with a comparison to beef and mutton.

The consumption of poultry meat is increasing every year and consumers want a safe and a good quality product without the presence of pathogenic microorganisms. Enterococci are Gram-positive, oval cocci, facultative anaerobic bacteria and belong to the group of lactic acid bacteria. Most species are resilient and versatile, being able to survive at 6.5% NaCl, at pH 9.6 and at a wide range of temperatures (10 to 45ºC), with the optimum growth at 35-37ºC **(Ludwig *et al.*, 2009)**. Initially, *Enterococcus* spp. were considered as harmless commensal inhabitants of the gastrointestinal tract of humans, widely used in the food industry as probiotic or starter cultures **(Moreno *et al.*, 2006)**. However, for the last two decades, enterococci became one of the most common pathogens to be associated with healthcare-associated infections. Enterococci is the dominant commensal in the chicken gut microbiome and an expected contaminant of postharvest or retail chicken **(Jackson *et al.*, 2015)**. *Enterococcus* spp. are found in a wide variety of environments, including dairy and food products, humans, and animals **(Van den Bogaard *et al.*, 2002)**. *Enterococcus* can be used as an indicator of both fecal contamination of foods and the dissemination of antimicrobial resistance related to the use of antimicrobials in poultry farming **(Van den Bogaard *et al.*, 2002)**. Enterococcus species are also recognized as human and animal opportunistic pathogens. Enterococci are responsible for several infections in immunocompromised patients, such as urinary tract infections, endocarditis, surgical wound infection, bacteremia and neonatal sepsis **(Billington *et al.*, 2014)**. Moreover, today they are one of the leading causes of major nosocomial infections **(Tsai *et al.*, 2005)**. The study conducted to evaluate the rate of chicken and giblets contamination with enterococcus species.

2. Materials and Methods

2.1- Samples Collection:

A total of 225 samples of fresh chicken breast, chicken thigh, chicken liver, chicken gizzard, chicken heart, cloacal skin and neck skin (25for each) were randomly collected from different markets and poultry shops at variable sanitation levels in Zagazig city. All collected samples were immediately transferred in an icebox container, aseptically handled and moved promptly to postgraduate student laboratory, Food Control department, Faculty of Veterinary Medicine, Zagazig University, Egypt. The bacteriological examination was applied for enumeration and isolation Enterococcus species.

2.2- Preparation of samples and serial dilution according to ISO 6887-2:(2003) :

Twenty five grams of each sample was homogenized aseptically for 1 min with 225 ml of 0.1 % peptone water in a stomacher **(Colworth, 400)** then serially diluted to 10-fold in the same diluent.

2.3- Enterococci count: according to ISO 7899-2 (2000)

Enumeration of Enterococci was carried out on a bile esculin ager (BEA) Himedia (M340). The agar was inoculated by spreading 0.1 ml of the decimal dilution onto the surface. The agar plates were incubated for 24 hrs at 37 ± 0.5°C aerobically. typical black colonies on the underlying black agar with colony diameters of 1 mm were enumerated as total Enterococci. The Identification of Enterococcus species carried according to **Cruickshank et al. (1975)**

3. Results and discussion:

Even though it is known that enterococci are ubiquitous organism in the gut, it is one of the emerging organism causing nosocomial infections in humans. Recent studies confirmed enterococci contamination in a wide range of foods including cheese, sausages, meat, milk, and cereals due to improper handling **(Koluman et al., 2009)**. Studies conducted by **Olsen et al. (2012)** have provided strong evidence that enterococci originating from foods of animal origin had a remarkable degree of similarity in virulence characteristics with human isolates implicating animal meat as an important source for virulent enterococci strains for human colonization.

Table (1) Prevalence of Enterococcus in examined chicken samples(n = 25 of each).

Samples	Number	Percentage
Chicken breast	16	64%
Chichen thigh	19	76%
Chicken liver	21	84%
Chicken gizzard	22	88%
Chicken heart	15	60%
Cloacal skin	25	100%
Neck skin	23	92%

Prevalence of Enterococci in chicken samples:

The data in table 1 and figure 1declared that enterococci detected in chicken samples within different percentages ranged from 15/25(60%) in chicken heart to 25/25(100%) in cloacal skin. The descending prevalence arranged cloacal skin > neck skin > gizzard > liver > thigh > breast > heart. The high prevalence in chicken samples attributed to presence of enterococci in the digestive tract of chicken supports a presumption of meat contamination during the slaughter process in poultry processing

plant (**Franz et al., 1999)**. The resistance of enterococci to pasteurization temperatures (they belong to the most thermotolerant microorganisms among non-sporulating bacteria) may tolerate the scalding water temperature **(Moreno et al., 2006)**.

Enterococci previously detected from raw food samples 97% in pork and 100 ground beef **(Hayes et al., 2003)**, frozen chicken 45.2% **(Tansuphasiri *et al.*, 2006)**, retail chicken 95.0%, ground turkey 94.4% , ground beef 92.7%, and pork 85.8% **(Tyson et al., 2018)**. Commensal bacteria, including *Enterococcus* spp. in commercial livestock and poultry, could contaminate the food chain during processing or find their way into the environment **(Diarra et al., 2007)**. Because of their relative abundance and their resistance to environmental adversity, enterococci have been proposed as indicator indicators for the hygienic quality of food and water **(Pierson et al. 2007)**.

Table (2) Statistical results of Enterococcus count log $_{10}$ CFU/g of examined chicken samples(n = 25 of each).

Samples	Minimum	Maximum	Mean ±SD
Chicken breast	2.12	5.15	3.04 ± 0.37^{c}
Chichen thigh	3.19	5.64	3.47 ± 0.48^{bc}
Chicken liver	3.54	5.95	4.05 ± 0.38^{b}
Chicken gizzard	3.68	6.32	4.15 ± 0.41^{b}
Chicken heart	3.11	6.26	3.72 ± 0.54^{bc}
Cloacal skin	4.82	6.3	5.03 ± 0.13^{a}
Neck skin	3.24	6.7	4.8 ± 0.25^{ab}

Means of the same columns carrying different superscript letters are significantly different (P< 0.05). SE: Standard Error.

The data in table 2 showed that the counts of enterococci ranged from 2.12 to 5.15, 3.19 to 5.64, 3.54 to 5.95, 3.68 to 6.32, 3.11 to 6.26, 4.82 to 6.3 and 3.24 to 6.7 with mean values 3.04 ±0.37, 3.47 ±0.48, 4.05 ±0.38, 4.15 ±0.41, 3.72 ±0.54, 5.03 ±0.13 and 4.8 ±0.25 log $_{10}$ CFU/g in examined chicken breast, thigh, liver, gizzard, heart, cloacal skin and neck skin, respectively. Enterococci were counted in different food of animal origin 0.5 x 10^1 and 7.1 x10^2 CFU/g of minced meat **(Klein et al., 1998)**, 1.2 × 10^3 – 6.2 × 10^4 CFU/g and from 0 –10^4 CFU/g in chilled and frozen meat **(Šustáčková et al., 2004)**, (3.18 log CFU/g) in organic chicken meat, (2.06 log CFU/g) conventional chicken meat and (1.23 log CFU/g) conventional turkey meat **(Miranda et al., 2007)**,

Cloacal skin significantly higher ($p< 0.05$) than other examined samples, which attributed to the contamination with the fecal and cecal content during evisceration these results, supported by the finding of **Diarra et al., (2010)**whom recorded that enterococcus counts were 6.82 ± 0.27 and 5.53± 0.31 log CFU/g of fecal and cecal samples, respectively. Moreover, Enterococci are found in the gastrointestinal tracts of animals, birds, and humans, as well as in soil and water **(Hancock and Gilmore 2006)**. In the human intestine, the density of enterococci ranges from 5 to 8 log CFU/g of intestinal content (**Tannock and Cook 2002)**.

Table (3) Prevalence of Enterococcus species according to biochemical identification.

Samples	Number	*E. faecalis*	*E.faecium*	*E. durans*	*E. avium*	*E. hirae*
Chicken breast	16	4	9	3	-	-
Chichen thigh	19	9	9		1	
Chicken liver	21	9	6	4		2
Chicken gizzard	22	12	6	3	1	
Chicken heart	15	8	5	2	-	-
Cloacal skin	25	14	6	3	1	1
Neck skin	23	13	9	1	-	-
Total	141	69 (48.9%)	50 (35.5%)	16 (11.4%)	3 (2.1%)	3 (2.1%)

The biochemical identification of enterococcus species declared that *E. faecalis, E.faecium, E. durans, E. avium and E. hirae* were detected in 69 (48.9%), 50 (35.5%), 16 (11.4%), 3 (2.1%) and 3 (2.1%), respectively as shown in table 3.

Nearly similar isolation rate was obtained by **Tyson *et al*. (2018)** who found that *E. faecalis* and *E. faecium* constituted the majority of the *Enterococcus* strains isolated from retail meat (64.0% and 28.6%, respectively), although additional enterococcal species were also isolated, including *Enterococcus hirae* (5.4%), *Enterococcus durans* (1.4%), and *Enterococcus gallinarum* (0.3%). Moreover, the common identified enterococcus species worldwide from food samples was *E. faecalis* (45.2%**)** in chicken **(Tansuphasiri *et al.*, 2006)**, (62.6%) in poultry samples (**Fracalanzza *et al*., 2007)**, (36.67%), organic chicken **(Miranda et al., 2007)**. On contrary *Enterococcus faecium* was the predominant species recovered (61%), followed by *E. faecalis* (29%), and *E. hirae* (5.7%). *E. faecium* was the predominant species recovered from ground turkey

(60%), ground beef (65%), and chicken breast (79%), while E. faecalis was the predominant species recovered from pork chops (54%) **(Hayes et al., 2003)** , 48 *E. faecium* isolates, 7 *E. faecalis* isolates, seven E. gallinarum isolates, six E. durans isolates, and one *E. avium* isolate **(Diarra et al. 2010)**. The variation in isolation rate may be attributed to the level of contamination with the cecal or intestinal content during preparation of poultry.

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الملخص العربى

مدى تواجد أجناس المكورات السبحية فى لحوم الدجاج بمحافظة الشرقية

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تم جمع ما مجموعه ١٧٥ عينة من صدور الدجاج الطازج ، فخذ الدجاج ، كبد الدجاج ، قانصة الدجاج ، قلب الدجاج ، وجلد المزرق وجلد العنق (٢٥ لكل منهما) بشكل عشوائي من الأسواق المختلفة ومحلات الدواجن في مستويات صحية مختلفة في مدينة الزقازيق. تم نقل جميع العينات التي تم جمعها ونقلها مبردة ، وتم التعامل معها بطريقة معقمة ونقلها على الفور إلى مختبر طلاب الدراسات العليا ، قسم مراقبة الأغذية ، كلية الطب البيطري ، جامعة الزقازيق ، مصر. تراوحت المكورات المعوية التى تم تحديدها في عينات الدجاج ضمن بنسب مختلفة من ٢٥/١٥ (٦٠٪) في قلب الدجاج إلى ٢٥/٢٥ (١٠٠٪) في جلد المزرق. وكان الترتيب التنازلي جلد المزرق> جلد العنق> القانصة> الكبد> الفخذ> الصدور> القلب. تراوحت أعداد المكورات المعوية من ٢.١٢ إلى ٥.١٥ ومن ٣.١٩ إلى ٥.٦٤ ومن ٣.٥٤ إلى ٥.٩٥ ومن ٣.٦٨ إلى ٦.٣٢ ومن ٣.١١ إلى ٦.٢٦ ومن ٤.٨٢ إلى ٦.٣ ومن ٣.٢٤ إلى ٦.٧ مع متوسط القيم ٣.٠٤ ± ٠.٣٧ و ٣.٤٧ ± ٠.٤٨ و ٤.٠٥ ± ٠.٣٨ و ٤.١٥ ± ٠.٤١ ، ٣.٧٢ ± ٠.٥٤ ، ٥.٠٣ ± ٠.١٣ و ٤.٨ ± ٠.٢٥ وحدة لوغاريتمية/جرام في صدر الدجاج الذي تم فحصه ، الفخذ ، الكبد ، القانصة ، القلب ، جلد المزرق وجلد العنق ، على التوالي. وتم تصنيفها عن طريق الأختبارات الكيميائية لأنواع المكورات المعوية أنه تم اكتشاف E. faecalis و E.faecium و E. durans و E. avium و E. hirae في ٦٩ (٤٨.٩ ٪) و ٥٠ (٣٥.٥ ٪) و ١٦ (١١.٤ ٪) و ٣ (٢.١ ٪) و ٣ (٢.١ ٪) ، على التوالي.