Bacteriological evaluation of freshly slaughtered chicken carcasses.

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A B S T R A C T

A grand total of 100 random samples of chicken carcasses were collected from local commercial retail shops in BeniSuef city. All samples were bacteriologically examined for determination of aerobic plate count (APC), psychrotrophic count, Enterobacteriaceae count, Coliforms count, Staphylococcus count and *Staphylococcus aureus* count. The mean values were 6.18 ± 0.67, 3.31 ± 1.33, 3.91 ± 0.96, 2.07 ± 1.87, 3.50 ± 1.68 and 2.71 ± 1.67 log cfu/g, respectively. Moreover, this study aimed to isolate and identify *Salmonella spp*., *Staphylococcus aureus*, *E. coli* and *Listeria monocytogens*, their prevalence percentages were 12%, 73%, 4% and 6 %, respectively, while *clostridium perfringens* and *E.coli* O157:H7 failed to be detected in the examined samples. Salmonella could be serologically identified as *S. typhimurium*, *S.virchow* and *S.enteric* with percentages of 41.70 %, 41.70 % and 16.60 %, respectively. Moreover, the isolated serotypes of E. coli were *E. coli* O55 and *E. coli* O86 A with percentages of 50% and 50% for each one, respectively.

Keywords: Chicken carcasses - Bacteriological quality - APC.

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1. INTRODUCTION

Poultry is a food that has been highly appreciated by man since time immemorial. It is an important, low-cost source of animal protein, rich in nutrients, phosphorus, other minerals, and B-complex vitamins (FAO 2010). Chicken carcasses have higher pathogenic and spoilage bacterial counts than most other foods, where carcass can be contaminated at several points throughout the processing operation during scalding, de-feathering and evisceration as well as cross contamination from other birds and processing equipment . Several indicators can be useful to evaluate hygiene level of meat such APC and total psychrotrophic counts. Total Enterobacteriaceae count and total coliforms count are more frequently used to assess enteric contamination and commonly used in slaughterhouses as indicators of faecal as well as environmental contamination (Gonzalez and Domingues, 2006). Moreover, total staphylococci count and *Staphylococcus aureus* counts, which are present on hand, mucous membrane and skin of man, birds and animals, are good indicators of poor personal hygiene, poor handling and temperature control (Rindhe et al., 2008). Contamination of poultry meat with foodborne pathogens remains an important public health issue, where many food poisoning bacteria contaminate chicken meat (Mbata, 2005). Therefore, the present study aimed to evaluate the bacteriological quality of some freshly slaughtered chicken carcasses through: Determination of APC, *Psychrotrophic* count, *Enterobacteriaceae* count *Coliform* counts, total *Staphylococci* count, and isolation and identification of *Salmonella, S. aureus, E. coli* O157:H7, *Listeria monocytogenes* and *Clostridium perfringenes*.

2. MATERIAL AND METHODS

2.1. Collection of samples

A grand total of one hundred random samples of chicken carcasses (slaughtered, plucked and eviscerated) were collected from local commercial retail shops in BeniSuef city. The collected samples were kept in separate plastic bags, transferred directly to the laboratory in an insulated ice box under complete aseptic conditions without any delay to evaluate their bacteriological quality.

2.2. Preparation of samples (USDA, 2011)

Twenty five grams of the examined samples were removed by sterile scissors and forceps after surface sterilization by hot spatula, transferred to a sterile polyethylene bag, and 225 ml of 0.1 % sterile buffered peptone water were aseptically added to the content of the bag. Each sample was then homogenized in a blender at 2000 rpm for 1-2 minutes to provide a homogenate of 1/10 dilution. One ml from the original dilution was transferred with sterile pipette to another sterile test tube containing 9 ml of sterile buffered peptone water 0.1 % and mixed well to make the next dilution, from which further decimal serial dilutions were prepared. The prepared dilutions were subjected to the following examinations.

2.3. Determination of APC (USDA, 2011)

It was done using standard plate count agar media.

2.4. Determination of total psychrotrophic count (USDA, 2011)

It was done using standard plate count agar media.

2.5. Determination of Enterobacteriaceae count (ISO, 2001)

It was done using violet red bile glucose agar media (VRBG).

2.6. Determination of total coliform count (FDA, 2002)

It was done using violet red bile agar media (VRB).

2.7. Determination of total Staphylococci and Staphylococcus aureus count (USDA, 2011)

It was done using Baird Parker agar media.

2.8. Isolation and Identification of Salmonella (FDA, 2011a)

It was done using Rappaport Vassilidis broth and Xylose Lysine Desoxycholate (XLD) agar.

2.9. Isolation and Identification of E. coli O157:H7 (FDA, 2011b)

It was done using *E.coli* broth supplemented with novobiocin and sorbitol MacConkey agar media.

2.10. Isolation and Identification of Clostridium perfringens (FDA, 2001)

It was done using cooked meat medium and Tryptose-sulfite-cycloserine (TSC) agar containing egg yolk emulsion.

2.11. Isolation and Identification of Listeria monocytogenes (Hitchins, 2003)

It was done using buffered Listeria enrichment broth and Oxford agar media.

3. RESULTS

Table (1) and Fig.(1) reported that APC (log cfu/g) in the examined samples varied from 4.30 to 7.85 with a mean value of 6.18 ± 0.67. The total psychrotrophic count varied from <1 to 5.00 with an average value of 3.31 ± 1.33 , the total coliforms varied from <1 to 4.95 with an average value of 2.07 ± 1.87 , the total staphylococci count varied from <1 to 4.90 with a mean value of 3.50 ± 1.68 and *S. aureus* count varied from 0 to 4.60 with an average value of 2.71 ± 1.67 . Results given in table (2) showed that the incidences of isolated *Salmonellae, St.aureus, E. coli* and *listeria monocytogenes* were 12, 73, 4 and 6 %.

Clostridium perfringenes failed to be detected in the examined samples. Regarding the results in table (3) the incidence of pathogenic *E. coli* serotypes isolated from the examined chicken carcasses were *E. coli O55* (50%) and *E. coli O86A* (50%). Table (4) reported that *Salmonellae* could be identified serologically as *S.typhimurium* (41.70%), *S. virchow* (41.70%) and *S. enteric* (16.60%).

Table (1): Statistical analytical results of bacterial counts (log_{10}cfu/g) in the examined chicken carcasses (n=100)

	Min.	Max.	Mean ± S.D
APC (TCC)	4.30	7.85	6.18 ± 0.67
Psychrotrophic count	< 1	5.00	3.31 ± 1.33
Enterobacteriaceae count	< 1	5.95	3.91 ± 0.96
Coliform count	< 1	4.95	2.07 ± 1.87
Total Staphylococci count	< 1	4.90	3.50 ± 1.68
Staph. aureus count	< 1	4.60	2.71 ± 1.67

Table (2): Incidence of some food borne pathogens in the examined chicken carcasses (n= 100)

	Positive Samples	
	No.	%
Salmonella spp	12	12
Staphylococcus aureus	73	73
Escheichia coli	4	4
Listeria monocytogenes	6	6
Clostridia Perfringens	0	0

Table (3): Incidence of pathogenic *E.coli* serotypes isolated from the examined chicken carcasses (n= 100)

	Positive Samples	
	No.	%
E. coli O55	2	50
E. coli O86A	2	50
Total	4	100

N.B.: % was calculated according to the positive number of samples

Table (4): Incidence of *Salmonella* serotypes isolated from the examined chicken carcasses (n= 100)

	Positive Samples	
	No.	%
S. typhimurium	5	41.70
S. virchow	5	41.70
S. enteric	2	16.6
Total	12	12

N.B.: % was calculated according to the positive number of samples

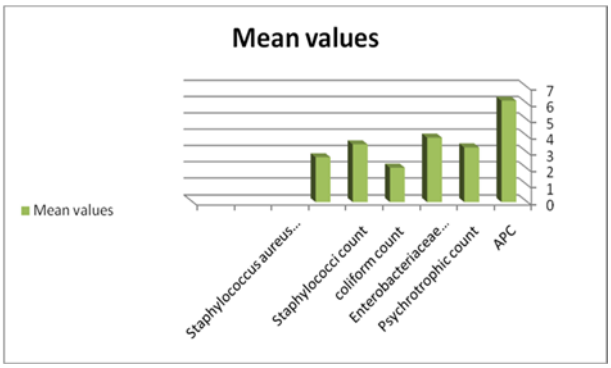

Fig. (1): Mean values of bacterial counts (log_{10}cfu/g) in the examined chicken carcasses

4. DISCUSSION

Microbial contamination of poultry carcasses is a natural result of different procedures necessary to produce retailed products from living birds. Most of bacterial contaminants are non pathogenic; however, poultry are known to harbour a large number of bacteria that are pathogenic to human being (Zhang et al., 2001). Several indicators can be useful to evaluate hygiene levels during meat slaughtering process. Aerobic plate count (APC) is commonly used to evaluate the hygiene of the entire meat production process. Nearly similar results were reported by Santosh Kumar et al. (2012) (6.23 log cfu/g), Sengupta et al.(2012) (6.39 log cfu/g) and Omorodion and Odu (2014) (5.96 log cfu/g) .On the other hand, higher counts were reported by Barbuddhe et al. (2003) (7.34 log cfu/g), Huong et al. (2009) (11.1 logcfu/g) and Bhandari et al. (2013) (7.24 log cfu/g). Lower counts were reported by Rindhe et al. (2008) (3.67 log cfu/g), Chaudhry et al. (2011) (5.07 log cfu/g),

Javadi and Safarmashaei (2011) (5.5 log cfu/g) and Al-jasser (2012) (4.03 log cfu/g).The level of APC in chicken meat indicates improper hygiene during processing and incorrect storage conditions, which can lead to proliferation of pathogens. Relatively higher psychrotrophic counts were recorded by Chaiba et al. (2007) (5.63 log cfu/g) and Santosh K. et al. (2012) (4.07 log cfu/g) comparatively lower results were recorded by Barbuddhe et al. (2003) (2.87 log cfu/g). Psychrotrophic bacteria present on the carcass immediately after processing were present also in the feather, feet of life birds in the water used in the processing plant (especially the chill tank) and on equipments. Therefore, psychrotrophic bacteria may be used as a general index of plant sanitation. However, poultry products were subjected to variations in holding temperature during processing, storage, distribution, and while being displayed for retail sale Barnes (1976). Nearly similar Enterobacteriaceae counts were reported by Capita et al. (2000) (3.04 log cfu/g) while lower counts were reported by Cegielskaradziejewska, et al. (2008) (2.7 log10 cfu/g). On the other hand, higher counts were reported by Rindhe et.al. (2008)(6.27 log cfu/g) and Bhandari et.al. (2013) (8.5 log cfu/g). Evisceration is a step that, if carried out badly, can cause a significant increase in the microbial levels on carcasses. A certain level of contamination is unavoidable because of the natural variation in birdsize that is responsible for some degree of breakage of intestines and also because of the spillage of intestinal content that can occur during evisceration (Mead, 2004). Total coliform count agrees with the results reported by Capita et.al. (2002) (2.7 log cfu/g) and Northcutt et al., (2003) (2.6 log cfu/g). Lower counts were reported by Selvan et al. (2007), (1.13 log cfu/g) and Joshi and Joshi (2010), (1.03 log cfu/g). On contrary, higher counts were reported by Santosh Kumar et al. (2012) (4.97 log cfu/g), Sengupta et al. (2012) (32.2 log cfu/g) and Bhandari et.al. (2013) (6.5 log cfu/g). Presence coliforms in greater number may be responsible for inferior quality of chicken meat resulting in economic losses and possibility of presence of other enteric pathogens, which constitute at time public health hazard (Chaem et. al., 2002). Total staphylococci count nearly resembles the mean count reported by Sengupta et al. (2012) (3.7 log10 cfu/g). On the other hand higher counts were reported by Selvan et al. (2007) (4.88 log cfu/g), Joshi and Joshi (2010) (4.46 log cfu/g), Ruban and Fairoze (2011) (4.4 log cfu/g), Al-jasser (2012) (4.9 log cfu/g) and Bhandari e.al. (2013) (6.5 log cfu/g. The presence of staphylococci could be due to the insanitary condition of the butcher and absence of the health services in butcheries. Contamination takes place during the handling and preparation of the meat and also from air dust and personal contact, external sources during bleeding, handling and cutting. Additional contamination took place in the retails markets and containers (Harrigan and McCance, 1976). Nearly similar results of *S.aureus* count were reported by Huong et al. (2009) (2.38 log10 cfu/g). Moreover, lower counts were reported by Chaiba et al. (2007) (1.87 log10 cfu/g) and Cegielska-radziejewska et al. (2008) (1.01 log cfu/g). On contrary, higher counts were reported by Rindhe et.al. (2008) (6.11 log cfu/g) and Al-jasser (2012) (4.5 log cfu/g). The prevalence of *St.aureus* in this study nearly similar to results reported by Kitai (2005) (65.8%) and Javadi and Safarmashaei (2011) (65%). While higher percentage was reported by Joshi and Joshi (2010) (100 %). On the other hand, many studies reported lower percentages asGundogan et al. (2005) (57%), Kozačinski et. al. (2006) (30.3%), Koluman et.al. (2011) (52%), Kozačins et.al. (2012) (17.9%) and Momtaz (2013) (22.77%). The presence of *St. aureus* could be as a result of it being a common organism on the skin and hands hence their presence in Chicken products may be as a result of contamination due to handling, processing, transportation and storage. Its presence in

high numbers is a good indication of poor hygiene and poor temperature control. Higher percentage of *Salmonella* were reported by Abdellah et al. (2009) (57%), Huong et. al. (2009) (62.79%), Bhandari et al. (2013) (46.2%) and Lertworapreecha et. al. (2013) (67.5%). Moreover, Joshi and Joshi (2010) reported that *Salmonella* was isolated from all examined chicken carcasses (100%). On contrary, *Salmonella* was isolated in low percentage from chicken carcasses as reported byCohen et al. (2007) (1.6%), Abdellah et al.(2008) (2.08%) , Colmegna et al. (2009) (1.1%) and Kozacins et al. (2012) (7.46%) . On the other hand, *Salmonella* could not be isolated from chicken carcasses as reported byVaidya et al. (2005) , Lindblad et al.(2006) , Selvan et al. (2007), Shaltout,F.A.(2009) and Javadi and Safarmashaei (2011). Presence of *salmonellae* in chicken meat may be attributed to the healthy state of the living bird which carries salmonellae, bad hygienic conditions during slaughtering, cross contamination either from other birds, instruments, machines, workers, scalding tanks, defeathering machines, crop removal, manual evisceration , during slaughter, intestinal contents can spill and contaminte the muscle and organs of the chicken, which is the important source of presence of *Salmonella* in meat and chilling tanks (Paiao et al., 2013). *E.coli* O_{157}:H_7 failed to be detected in the examined samples. Such results agrees with results reported by Baran and Gulmez (2000) , Joa et al.(2004),Hajian et al. (2012) and Kalin et al.(2012). On the other hand, *E.coli O157:H7* was isolated in low percents byAkkaa et al. (2006) (1.05%) and Kiranmayi and Krishnaiah (2010) (2%). On contrary, higher results were obtained by Chang et al. (2013) (40%). Although *E.coli*0_{157}:H_7 is mostly found in ruminant animal and it is occasionally associated with other livestock and various foods of animal origin , experience suggests that it is rare in poultry ,whether in the live birds or on processed products (Mbata 2005) .

Similar prevalence of *L. monocytogenes* was reported Kozačinski et al. (2006) (3%), Colmegna et al. (2009) (3%) and Kozacins et al. (2012) (4.5 %). Lower percentages were reported by Molla et al. (2004) (1.9%) and Cohen et al. (2007) (0.5%). *L. monocytogenes* is an important foodborne pathogen, which has been isolated from many natural environments, such as water, soil, sewage, mud, gut of poultry and feces Yeh (2004), Donnelly et al. (1992) and Nickelson and Finne, 1992). It is considered an environmental contaminant, therefore, cross contamination easily occurs in traditional shops during preparation of chicken carcasses ready to be sold.

Clostridium perfringenes (C.perfringenes) failed to be isolated from examined samples. Such results agrees with Shaltout (2009). On Contrary, *C. perfringens* was isolated in higher percentages by Hall and Angelotti (1965) (58%), Miwa et al. (1998) (84%), Singh et al. (2005) (70.4%) and Nowell et. al. (2010) (66%). On the other hand, other studies could detect *C.perfringens* in lower percentages as Craven et al. (2003) (4%), Cohen et al. (2007) (7.2%) and Thangamani and Subramanian (2012) (3.81%).The organism is an obligate anaerobe that is relatively tolerant to oxygen and can be found in low numbers in the alimentary tract of poultry. When present in meat, growth is favoured by conditions in which oxygen has been dispelled. However, growth of the organisms cannot occur if the meat is held below 15^{O}C, the problem is easily avoided by refrigerated storage. Regarding all the discussed results, chicken carcasses could be contaminated in all stages of production starting from the live bird carrying microorganisms during transportation to the slaughter house , during stages of slaughtering , bleeding , scalding , defeathering , evisceration , washing and storage . Therefore, good hygienic practices should be followed in every step of processing.

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