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Article

Antibiotic sensitivity and resistant pattern of bacteria isolated from table eggs of commercial layers considering food safety issue

Mahfuzul Islam[1*], Mirza Synthia Sabrin[1], Md. Hazzaz Bin Kabir[1] and Md. Aftabuzzaman[2]

[1]Department of Microbiology and Parasitology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh
[2]Department of Poultry Science, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh

*Corresponding author: Mahfuzul Islam, Department of Microbiology and Parasitology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh. E-mail: mislam.mipa@sau.edu.bd, mislam.micro.sau@gmail.com

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Abstract: The present study was carried out during the period of June 2016 to June 2017 to evaluate the antibiotic sensitivity and resistant pattern of bacteria isolated from table eggs of commercial layers considering food safety issue. A total of 200 egg samples (100 for egg shell surface and 100 for egg content) were collected from different retail markets of Dhaka city in sterile polythene bags in a view to prevent extraneous contamination and transported to the laboratory immediate after collection using icebox. The samples were inoculated onto nutrient broth and nutrient agar plates aerobically at 37°Cfor isolation. The isolated organisms were identified based on staining, motility, colony morphology and biochemical tests. The isolated bacteria were also subjected to characterize their antibiotic sensitivity. About 74% egg samples (148 out of 200 samples) were positive for microbial contamination. Among them 100 (100 %) samples had their shells contaminated with microbes of different genera; however, only 48 (48%) growths were observed from the egg contents. The major contaminants are *Escherichia coli* (34.64%), Coagulase positive *Staphylococcus* (24.29%), *Salmonella* spp. (20.71%) followed by Coagulase negative *Staphylococcus* (10%), *Pseudomonas* spp. (6.43%) and *Bacillus* spp. (3.93%). The isolated bacteria *E. coli*, Coagulase positive *Staphylococcus*, *Salmonella* spp. and *Pseudomonas* spp. showed their greatest sensitivity against ciprofloxacin, ceftriaxone and azithromycin whereas resistant against tetracycline, amoxicillin and ampicillin. There is potential for these antibiotic-resistant bacteria to be transferred to humans through contaminated eggs and are of public health concern from food safety point of view.

Keywords: antibiotic; sensitivity; resistance; table eggs; commercial layers; food safety

1. Introduction

Microbial contamination of egg has important outcome to the poultry industry and illness from contaminated egg is a serious public health problem around the world. Treatment of these infections is very difficult due to development of multi drugs resistant bacteria of public health significance. Many investigations around the world reported the outbreak, contamination of egg by the *Salmonellas* spp., *Campylobacter jejuni*, *Listeria monocytogens* and *E. coli* (Cox Stern *et al*., 2002; Gorman and Adley, 2002 and Cortés *et al*., 2004). The significance of these diseases in humans can vary from mild symptoms to life threatening situation (Kaneko *et al*., 1999). The egg shell can already be infected when passing through the vent, but many researchers suggest that the main bacterial contamination occurs within a short period after lay due to contact with dirty surfaces (Gentry and Quarles, 1972). Messens *et al*. (2005) and De Reu*et al*. (2006a) reported that increasing numbers of micro-organisms on the eggshell consequently increase the risk of microbial eggshell penetration and egg content contamination. Beside this horizontal route of bacterial infection of eggs, egg contamination also occurs through the vertical or transovarian route. In the transovarian route (vertical transmission), the yolk (very infrequently the yolk itself), the albumen and/or the membranes are directly contaminated as a result of bacterial infection of the reproductive organs. Stepień-Pyśniak *et al*. (2009) studied the occurrence of bacteria of the genus *Staphylococcus* in table eggs descended from different sources. Mohammad *et al*. (2011) reported *Escherichia coli* O157:H7 facilitates the penetration of *Staphylococcus aureus* into table eggs.

Chousalkar *et al*. (2010) conducted a research on recovery of *Salmonella* and *Escherichia coli* (*E. coli*) from commercial egg shells and effect of translucency on bacterial penetration in eggs. This experiment was conducted to study the prevalence of *Salmonella* and *E. coli*. from the surface of egg shells, egg shell membranes or pores, and internal contents from unwashed eggs collected from commercial caged layer farms in Australia. De Reu *et al*. (2006b) studied eggshell factors influencing eggshell penetration and whole egg contamination by different bacteria, including *Salmonella enteritidis*. Moats (1980) isolated a total of 432 bacteria from washed and unwashed eggs, egg-washer surfaces, and wash waters from five egg-grading plants in Maryland and southeastern Pennsylvania were classified. De Reu *et al*. (2008) conducted an experiment on bacterial contamination of table eggs and the influence of housing systems. Musgrove *et al.* (2008) conducted a research on enterobacteriaceae and related organisms isolated from shell eggs collected during commercial processing. Kone *et al*. (2013) analyzed egg surfaces for the presence of *Bacillus cereus* group bacteria. Abdullah (2010) also studied isolation and identification of some bacterial isolates from table egg.

Antibiotics have been used for more than half a century in poultry feed for improving performance, reducing some pathogenic microorganisms and increasing some useful microorganisms in intestinal tract of these birds (Gibson and Fuller, 2000). However, antibiotics used as growth promoters in animal feeds have been banned recently due to potential development of antibiotic resistant human pathogenic bacteria (Patterson and Burkholder, 2003). So, the present study was conducted on antibiotic sensitivity and resistant pattern of bacteria isolated from egg shells and egg contents of table eggs of commercial layers from different retail markets of Dhaka city in Bangladesh that may have public health significance.

2. Materials and Methods

The whole study will be conducted in the laboratory of the department of Microbiology and Parasitology, Sher-e-Bangla Agricultural University (SAU), Dhaka-1207 during the period from June, 2016 to June, 2017.

2.1. Sources and collection of samples

A total of 200 egg samples (100 for egg shell surface and 100 for egg content) were collected from different retail markets of Dhaka city in sterile polythene bags. In laboratory, egg shell surface samples were collected by using wet cotton swabs with gentle rubbing and egg content samples were collected by using sterile cotton swabs or inoculating loop after disinfecting the egg shell surface and subsequent broke the air shell.

2.2. Isolation and identification of Bacteria from egg shell surface and egg content

For isolation and identification of bacteria, the procedure suggested by Cheesbrough (2006) was followed throughout the experiment.

2.3. Isolation and preservation of bacteria

At first, the samples were inoculated onto nutrient broth and incubated at 37°C for 24 hours immediate after collection. After primary growth in nutrient broth the bacteria were then inoculated onto nutrient agar (NA) plates by steak plate method and incubated at 37°C for 24 hours. From primary culture individual single colony of different characters was inoculated onto NA plates to obtain pure culture (subculture). The subculture technique will be followed up to obtaining pure culture (Cheesbrough, 2006). Stock cultures were maintained in both agar slant and 20% sterile buffered glycerin (Merchant and Packer, 1967).

2.4. Identification of bacteria

The isolated organisms were identified based on staining, motility, colony morphology and different biochemical tests such as sugar fermentation test, Catalase test, Coagulase test, Methyle red test, Voges-Proskauer test and Indole test according to standard laboratory methods (Cheesbrough, 2006).

2.5. Antibiotic sensitivity tests

To perform this test, a total of 18 isolates were selected from each of the isolated *E. coli*, *Salmonella* spp. Coagulase positive *Staphylococcus* and *Pseudomonas* spp. Antibiotic sensitivity tests were done by using disc diffusion test following the method described by Kirby-Bauer (Bauer *et al*., 1966) with minor modification. Briefly, 0.5 McFarland standards concentration of freshly growing broth culture were poured on NA plate and spread uniformly. Antibiotic discs were placed apart onto the surface of the inoculated plates aseptically with the help of a sterile forceps and incubated at 37°C for 24 hours. After incubation, the plates were examined and the diameters of the zone of inhibition were measured. Then these diameters were interpreted with the standard diameters of NCCLS, (1999) and were recorded as sensitive (S), intermediate (I) and resistant (R). The following antibiotics will be used for disc diffusion test: ceftriaxone (30μg), azithromycin (15 μg), Ciprofloxacin (5 μg), Tetracyclin (30 μg), chloramphenicol (30 μg), amoxycillin (10 μg), ampicillln (10 μg), gentamycin (10 μg), nalidixic acid (30 μg) and kanamycin (30 μg). These antibiotics were randomly selected based on their commercial availability and widely used in poultry industry not only in Bangladesh but also worldwide.

4. Results and Discussion

4.1. Overall prevalence of bacteria isolated from egg shells and egg contents

The results from this study revealed that 74% samples (148 out of 200 samples) were positive for microbial contamination isolated from the 20 randomly selected retail markets in Dhaka city, Bangladesh. Among them 100 (100 %) samples had their shells contaminated with microbes of different genera; however, only 48 (48%) growths were observed from the egg contents. A total of 280 isolates were isolated from eggs of which 188 isolates were obtained from shell surface (67.14%) and 92 isolates were from the egg content samples (32.86%) (Table 1). The identified isolates were tabulated and presented in Table 1. The major contaminants are *E. coli* (34.64%) followed by Coagulase positive *Staphylococcus* (24.29%), *Salmonella* spp. (20.71%) Coagulase negative *Staphylococcus* (10%), *Pseudomonas* spp. (6.43%) and *Bacillus* spp. (3.93%). This is in agreement with Chaemsanit *et al*. (2015) who reported microbial contamination of chicken eggs and its contents with isolates from 15 different genera, included *Staphylococcus* spp., *Micrococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Corynebacterium* spp., *Acinetobacter* spp., *Neisseria* spp., *Salmonella* spp., *Proteus* spp., *Citrobacter* spp., *Escherichiacoli*, *Klebsiella* spp., *Enterobacter* spp. and *Serratia* spp. Similarly, Salihu *et al*. (2015) reported 100% egg samples were positive for bacterial (nine different genera) contamination included *Escherichia coli*, *Salmonella* spp, *Shigella* spp, *Corynebacteria*, *Proteus* spp., *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp, and *Klebsiella* spp. The present study is supported by Hang' Ombe *et al*. (1999), who reported microbial contamination of chicken eggs with predominantly members of the family enterobactericiae. This study is also agreeing with USDA (2011), that micro-organisms can be found on the outside and inside of the egg shell. This may be due to the fact that the egg emerges from the hen's body through the same passageway the faeces is excreted, micro-organisms inside an un-cracked egg or intact egg may be due to the presence of pathogen within the hen's ovary or through oviduct, before the shell forms around the yolk and albumin. Faecal contaminants could also occur through the pores on the shell after they are laid.

In present study, it was also revealed that the percentages of isolated bacteria were higher in case of egg shell surface in comparison to egg contents. Similar findings were also found by Chaemsanit *et al*. (2015) and Hang' Ombe *et al*. (1999). Ansah *et al*. (2009), reported that, as eggs stayed longer, their resistance reduced which enabling these organisms to penetrate into the egg content. Several factors have been implicated in egg contamination. Among these are faeces of the birds, litter material, improper handling of the eggs by retailers, unhygienic conditions of the markets where these eggs are being sold, contaminated egg crates, packing and poor storage method (Bruce and Drysdale, 1994). Others are cloths and hands of poultry workers, the environment, dust transporting marketing, poor storage in retail shop, weather condition, etc.

4.2. Antibiogram

The results of in-vitro antibiotic sensitivity and resistant pattern of different organisms are presented in Table 2. In the present study, *E. coli* showed highest resistance against tetracycline followed by amoxicillin and ampicillin which were 100%, 88.89% and 77.78% respectively on the other hand highest sensitivity against ciprofloxacin (100%) followed by ceftriaxone (88.89%)and azithromycin (66.67%). In this study, *Salmonella* spp. showed the highest resistance patterns against tetracycline (94.44%), amoxicillin (94.44%) and ampicillin (77.78%). While the highest sensitivity rate was recorded against ciprofloxacin, ceftriaxone, gentamycin and azithromycin as 88.89%, 83.33%, 66.67% and 61.11% respectively. In case of Coagulase positive *Staphylococcus*, the greatest number of isolates showed resistance to tetracycline (94.44%), amoxicillin (88.89%) and ampicillln (83.33%), on the contrary sensitive to ceftriaxone (88.89%), azithromycin (83.33%), ciprofloxacin (72.22%). In case of *Pseudomonas* spp., the highest resistance rate was detected against tetracycline (100%) followed by ampicillln (94.44%), amoxicillin (88.89%) and chloramphenicol (66.67%)

whereas highest sensitivity rate (94.44%) were recorded against ceftriaxone followed by ciprofloxacin (88.89%) and azithromycin (66.67%).

Table 1. Name and percentage of bacteria isolated from egg shells and egg contents.

Name of isolated bacteria	Types of Sample				Total	
	Egg Shells		Egg Contents			
	No. of isolated bacteria	% of isolated bacteria	No. of isolated bacteria	% of isolated bacteria	No. of isolated bacteria	% of isolated bacteria
Escherichia coli	60	21.43	37	13.21	97	34.64
Salmonella spp.	38	13.57	20	7.14	58	20.71
Coagulase positive Staphylococcus	48	17.14	20	7.14	68	24.29
Coagulase negative Staphylococcus	19	6.79	9	3.21	28	10.00
Pseudomonas spp.	13	4.64	5	1.79	18	6.43
Bacillus spp.	10	3.57	1	0.36	11	3.93
Total	**188**	**67.14**	**92**	**32.86**	**280**	**100.00**

Legends: No. = Number and % = Percentage

Table 2. Antibiotic sensitivity and resistant pattern of selected bacteria isolated from egg shells and egg contents.

Name of antibiotics	No. of tested isolates	Sensitivity and Resistant patterns	Name of isolates							
			Escherichia coli		***Salmonella* spp.**		**Coagulase positive *Staphylococcus***		***Pseudomonas* spp.**	
			No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Ceftriaxone		S	16	88.89	15	83.33	16	88.89	17	94.44
		I	1	5.56	2	11.11	1	5.56	1	5.56
		R	1	5.56	1	5.56	1	5.56	0	0.00
Azithromycin		S	12	66.67	11	61.11	15	83.33	12	66.67
		I	2	11.11	4	22.22	1	5.56	4	22.22
		R	4	22.22	3	16.67	2	11.11	2	11.11
Ciprofloxacin		S	18	100.00	16	88.89	13	72.22	16	88.89
		I	0	0.00	1	5.56	2	11.11	2	11.11
		R	0	0.00	1	5.56	3	16.67	0	0.00
Tetracyclin		S	0	0.00	0	0.00	0	0.00	0	0.00
		I	0	0.00	1	5.56	1	5.56	0	0.00
		R	18	100.00	17	94.44	17	94.44	18	100.00
Chloramphenicol		S	8	44.44	9	50.00	2	11.11	0	0.00
		I	3	16.67	2	11.11	4	22.22	6	33.33
	18	R	7	38.89	7	38.89	10	55.56	12	66.67
Amoxycillin		S	2	11.11	1	5.56	1	5.56	0	0.00
		I	0	0.00	0	0.00	1	5.56	2	11.11
		R	16	88.89	17	94.44	16	88.89	16	88.89
Ampicillln		S	2	11.11	3	16.67	1	5.56	0	0.00
		I	2	11.11	1	5.56	2	11.11	1	5.56
		R	14	77.78	14	77.78	15	83.33	17	94.44
Gentamycin		S	10	55.56	12	66.67	10	55.56	2	11.11
		I	4	22.22	2	11.11	2	11.11	6	33.33
		R	4	22.22	4	22.22	6	33.33	10	55.56
Nalidix acid		S	8	44.44	7	38.89	8	44.44	3	16.67
		I	6	33.33	6	33.33	4	22.22	5	27.78
		R	4	22.22	5	27.78	6	33.33	10	55.56
Kanamycin		S	9	50.00	10	55.56	9	50.00	5	27.78
		I	3	16.67	2	11.11	5	27.78	5	27.78
		R	6	33.33	6	33.33	4	22.22	8	44.44

Legends: S = Sensitive; I = Intermediate sensitive; R= Resistant; No. = Number and % = Percentage

The present study is in close agreement with the findings of Papadopoulou (1997) who reported the presence of resistant strains of *Staphylococcus aureus* (to penicillin-G, tetracycline, erythromycin, clindamycin, cefalosporins, oxacillin, gentamycin, chloramphenicol and tobramycin), *Enterococcus faecalis* (to ampicillin, ciprofloxacin, clindamycin, gentamycin and tetracyclin), *Escherichia coli* (to tetracycline, erythromycin, ampicillin and cefalosporins), *Enterobacter cloacae* (to ampicillin, amoxycillin plus clavunalic acid, erythromycin and tetracycline), *Pseudomonas stutzeri* (to erythromycin and chlorampenicol) and *Citrobacter freundii* (to ampicillin, amoxycillin plus clavunalic acid, cefalosporins and co-trimoxazole). Jain and Yadav (2017) reported that isolated bacteria from eggs revealed the highest resistance rate against Cefixime (86.66%) whereas highest sensitivity rate (100%) were recorded against Gentamicin, Levofloxacin and Ciprofloxacin. Also, most of the isolates (73.3%) were found to be multi drug resistant as these showed resistance against three or more antibiotics tested. Eid (2015) reported 94% of *E. coli* isolates were resistant to five and more antimicrobial drugs. Adesiyun (2007) also reported 46.6% of *E. coli* isolates were resistant to three or more antimicrobial agents. Kilonzo-Nthenge *et al*. (2008) showed *Salmonella* isolates were resistant to ampicillin, streptomycin, and tetracycline, whereas *E. coli* isolates were resistant to ampicillin and nalidixic acid. *Salmonella* spp. isolated from chickens have also been reported to be resistant to ampicillin, tetracycline, and gentamycin (Wilson, 2004). Recent reports (Schroeder *et al.,* 2004) have shown that *E. coli* isolated from meat and poultry demonstrated resistance to at least one antimicrobial drug. Pyzik and Marek (2013) showed *S. aureus* strains were found to be resistant to at least one of the antibiotics tested, while some (55.55%) showed resistance to five or more of the 17 therapeutic agents. The greatest number of strains showed resistance to erythromycin (66.66%), tetracycline (66.66%), oxytetracycline (61.11%), penicillin G (50%), and amoxicillin (44.44%). The development of these greatest resistant bacteria may be due to their indiscriminate, haphazard and repeated use in poultry farms in different part of the World including Bangladesh.

5. Conclusions

In conclusion, the table eggs of commercial layers are contaminated with antibiotic resistant *E. coli, Staphylococcus* and *Salmonella* spp., *Pseudomonas* spp. and *Bacillus* spp. There is a potential for these antibiotic-resistant bacteria to be transferred to humans through contaminated eggs. This developing antibiotic resistance in bacteria from chicken eggs should be considered as public health concern.

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Conflict of interest

None to declare.

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