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Studies on veterinary antibiotic residues in chicken eggs and their public health significance in Bangladesh perspective

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Abstract

Antibiotic resistance in pathogenic organisms has become as a serious global public health concern. Generally, same major groups of antibiotics are used in both animal and human medicine. Thus, antibiotic residues in animal originated food like eggs can result in severe public health hazards. Nevertheless, no comprehensive study on residual antibiotics in table eggs and their public health implications has been documented yet in Bangladesh. Therefore, the study was conducted to develop scientific evidence on residual antibiotics in table eggs and assist in securing better public health for the nation. Microbiological Four-Plate Test (FPT) was employed for detecting antibiotic residues in 300 egg samples. Media seeded with *Bacillus subtilis* at different pH values (6, 7.2 or 8) and a fourth medium seeded with *Staphylococcus aureus* at pH 8 were used for disc diffusion microbial inhibition test. Standard solutions of antibiotics were used as control. Out of the 300 samples, 81 (27%) samples contained antibiotic residues. Derivates of Macrolides and Betalactam were detected in 40.7% of positive cases followed by derivatives of Tetracyclines and Betalactam in 39.5% cases and antibiotics residues relevant to Aminoglycosides were detected in 19.8% of positive cases. However, no Sulfonamide residue was detected in eggs in this study. The presence of these residual antibiotics in eggs could cause mild to serious health hazards including development of resistant organisms to these antibacterial agents in consumers that are believed to have fatal public health impact in the long run. In conclusion, the antibiotic residues in commercial eggs have to be monitored as routine test. Proper maintaining of the withdrawal period and development of active surveillance system are highly recommended for public health security. It is anticipated that the findings of this study will contribute to the development of strategies for prudent use of antibiotics in Bangladeshi poultry farms.

Keywords: *Residual antibiotics; Table eggs; Bangladesh; Poultry farms.*

1. Introduction

Antibiotic resistance has now been recognized by the major world health organizations as one of the top global health challenges for this modern era (Nisha, 2008). Many of the antibiotics used to treat bacterial infections in humans also have veterinary applications like treatment, control and prevention of infection as well as to enhance animal growth and feed efficiency (Tollefson and Miller, 2000). Antibiotic usage has facilitated the efficient production and also enhanced the health and wellbeing by reducing the incidence of disease in poultry. Currently, approximately 80% of commercial poultry receive medication in their lives (Collignon, 2003). In Bangladesh, these antimicrobials are particularly used in poultry farming for therapeutic purposes and are added in feed and water in sub-therapeutic doses for prophylaxis and growth promotion (Islam et al. 2016a). The use of antibiotics in veterinary medicine for food producing animals

including poultry is thus considered as one of the causes resulting in antibiotic resistance (Donoghue, 2003). The use of antibiotics in commercial poultry in Bangladesh seems to be huge. The ignorance of the local farmers, lack of proper veterinary service at rural/remote areas and the desire for high profit provoke the farmers to use antibiotics indiscriminately in Bangladesh (Islam et al. 2016a). These antibiotics tend to accumulate in tissues and other products such as eggs and form residues at different concentrations. Presence of the antibiotics residues in eggs above the maximum level is considered as illegal which consumption could result in serious public health hazards including: development of resistant strains of microorganisms, hypersensitivity and distortion of intestinal microflora (FAO/IEAE, 1997). Residues of veterinary antibiotics in human food threaten human health by being acutely or cumulatively allergenic, organotoxic, mutagenic, teratogenic or carcinogenic (Doyle, 2006). Drug resistance appears to be the most important hazard of drug residues all over the world (Paige et. al., 1997). The resistant bacteria could then cause disease that is difficult to treat in humans and may also transfer the resistant gene to some other human pathogens (Tollefson and Miller, 2000; Doyle, 2006). Most importantly, poultry can also carry zoonotic bacteria which may develop resistance during treatment with antibiotics. Thus, the consequences to public health could increase disease severity, complications, and even mortality in humans. This seemingly reasonable scenario has resulted in a demand to understand the residual antibiotics in poultry origin food particularly in eggs which are an important source of animal protein in Bangladesh, very especially for children.

But surprisingly, no comprehensive study on the residual antibiotics in eggs in relation to their public health aspect has been documented yet in Bangladesh. Therefore, the study was conducted to develop a scientific evidence on the status of residual antibiotics in table eggs and provide a base for the researchers, academicians as well as policy makers to take necessary initiative to control the indiscriminate use of antibiotics and thus, to secure public health in the country.

2. Methodology

Study area and period: The study was conducted on samples collected from different parts of Dhaka, Bangladesh for a period of 6 months from July 2016 to June 2017.

Collection and preservation of sample: A total of 300 table egg samples were randomly collected from local markets in Dhaka city. Collected samples were carried to the laboratory of Department of Medicine and Public Health, Sher-e-Bangla Agricultural University, Dhaka maintaining standard procedure. Especial emphasis was given to the screening of as many samples as possible. For all sources, eggs were processed within 24 hours of collection.

Preparation of bacterial standards: Pure cultures of *Bacillus subtilis* and *Staphylococcus aureus* was isolated and identified using standard protocol. Their antibiotic sensitivity against commonly used antibiotics in the layer production was checked using antibiotic sensitivity test. A standard antibiotic sensitivity pattern for those bacteria was established for referral use.

Preparation of inoculums and media: Primary cultures were sub cultured into a nutrient or brain heart infusion broth for about 8 to 12 hours before inoculating into a nutrient agar under an aseptic condition. Nutrient agar was prepared according to producers' guidelines. The pH was adjusted using a pH meter by adding drops of NaOH or HCL solutions. The adjusted media was then introduced into a universal bottle and sterilized using an autoclave at 121^{o}C for 15 min. The sterilized media was poured onto a Petri dish and allowed to solidify. It was then incubated for 18-24 hour at 37^{o}C before inoculating the test bacteria.

Preparation of egg samples: Surface of each egg was manually cleaned using a sterile hand towel soaked in 70% (v/v) alcohol. A small opening was made at the tip of the egg using sterile forceps. The albumen was then drained out carefully through the pore and the yolk was transferred into a sterile falcon tube. The homogenization step was performed by adding 10 ml of phosphate buffer (pH 7) to the egg yolk. Special sterile paper discs, 12 mm in diameter, were then dipped into the falcon containing egg yolk. Using sterile forceps, paper discs were brought out, allowed to soak and excess yolk was drained from the discs before placing them on the surface of seeded agar plates.

Screening of antibiotic residues in the samples: The European Four Plate Test (FPT) as described by Oboegbulem and Fedelis (1996) was employed to determine the level of residues in raw eggs. Briefly, prepared samples were placed on to four plates of agar media, three of which are pre-inoculated with *Bacillus subtilis* at pH 6 (Plate 1), pH 7 (Plate 2), pH 8 (Plate 3) and the fourth plate (Plate 4) at pH 8 with *Staphylococcus aureus* using sterile swabs. The plates were incubated at optimal temperature for growth of the test bacteria. After incubation at 37°C for 24 h, samples with inhibition zones were considered as positive and their diameters were measured. Four different antibiotic groups were considered: β-lactams, tetracyclines, aminoglycosides and macrolides. Tetracycline (30 µg), trimethoprim (25 µg), gentamicin (10 µg) and erythromycin (15 µg) discs were used as control positive standards.

Samples having residual antibiotics were identified by the presence of a complete inhibition of growth in an annular zone not less than 2 mm around the piece of sample. Less than 2 mm of inhibitory zone was considered as samples with insignificant amount of antibiotic residue. Test bacterium has known susceptibility to antibiotics. Presumptively, positive result at pH 6 indicates the presence of tetracycline antibiotics, streptomycines at pH 7 and penicillines at

pH 8. These antibiotics readily diffuse at the respective pH inhibiting bacterial growth around zone of paper discs. At significantly high concentration (MRL)* zone of inhibition is ≥ 2 mm.
*Maximum residue limit (MRL) or violative residue is the residue above tolerance level.

3. Result and discussion

A total of 300 egg samples were studied in the laboratory for the detection of residual antibiotics. Inhibition zone of about 2 mm around a disc was considered as a positive result. As many as 81 (27%) of the study samples were found positive for the residual antibiotics (Figure-1).

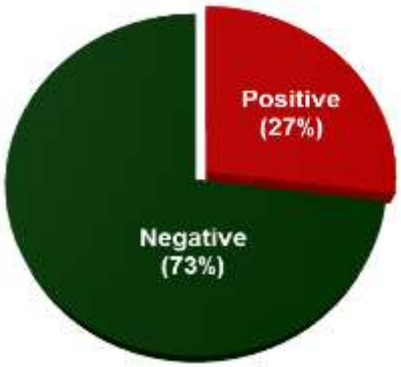

Figure (1): Existence of antibiotic residues in table eggs

Laboratory analysis of the egg samples revealed the existence of residual drugs belonging to four families of antibiotics namely; Macrolides, Tetracyclines, Aminoglycosides and Beta lactam.

Among the 81 samples found positive for antibiotic residues, 32 samples (39.5%) were positive at pH 6 of *Bacillus subtilis* plates, showing penicillin and tetracycline residues and 16 samples (19.8%) were positive at pH 8 of *Bacillus subtilis* plates, showing aminoglycoside residues (Figure-2).

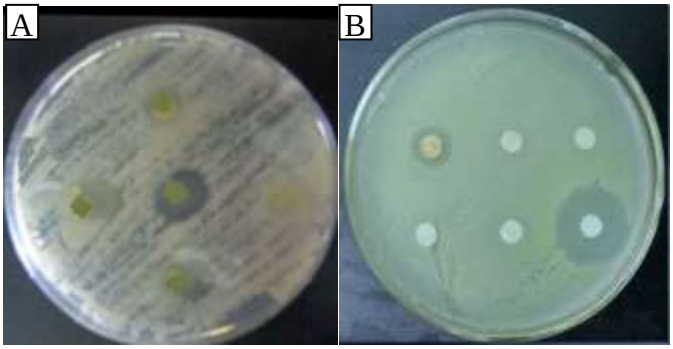

Figure (2): *Bacillus subtilis* plates at pH 6 showing the existence of penicillin and tetracycline residues (A) and pH 8 (B) showing the existence of aminoglycoside residues in table eggs

Besides, 33 samples (40.7%) were positive at pH 8 of *Staphylococcus aureus* plates indicating the presence of residual Macrolides and Betalactam antibiotics (Figure-3).

Figure (3): *Staphylococcus aureus* plate at Ph 8 showing the existence of antibiotic residue in table eggs

However, no Sulfonamide residue was detected in eggs in this study as samples at pH 7.2 of *Bacillus subtilis* plates produced no or less than 2mm zone of inhibition (Figure-4).

Figure (4): *Bacillus subtilis* plates at pH 7.2 showing no or less than 2mm zone of inhibition indicating no or insignificant Sulfonamide residues in table eggs

Table-1 below summaries the findings of detected antibiotics residues at different plates

Table (1): Detection of antibiotics residues by FPT method

pH of culture medium	Tested bacteria	Inferred antibiotics	No. of positive Samples	% of total positive cases
6	*Bacillus subtilis*	Penicillin and Tetracyclines groups	32	39.5%
7.2	*Bacillus subtilis*	Sulfonamide group	0	0
8	*Bacillus subtilis*	Aminoglycoside group	16	19.8
8	*Staphylococcus aureus*	Penicillin and Macrolide groups	33	40.7

The study was designed to unveil the residual antibiotics in table eggs in Bangladesh. The impact of antibiotic residues on the emergence of antibiotic resistant bacteria has become the global public health concern. But surprisingly, no comprehensive study on the residual antibiotics in table eggs and their public health aspect has been documented yet in Bangladesh. Therefore, the objectives set in the proposed study seem to be highly related and time demanded which would unveil the common residual antibiotics in eggs and their potential threats to public health.

Different antibiotics are used frequently in food producing animals including poultry for various

purposes like treatment, control and prevention of diseases, growth promotion, weight gain, higher efficiency, etc. (Vazquez-Moreno et al. 1990). Food with residual antibiotics is harmful for human health that causes allergy and antibiotic resistance (Ansari and Khatoon, 1994). Therefore, screening of residual antibiotics in food is the first step to prove drug residues. The methods should be inexpensive, applicable for multiple samples and should be able to show the lowest false negative and false positive results (Mariël et al. 2008). Qualitative microbiological methods to screen out antibiotic residues in food, especially food from animal origin are well known and recognized. FPT is a reference method for food screening and detecting the main category of antibiotic residues in the world (Kilinc and Cakli, 2008).

According to the results of this study, the highest contamination rate of antibiotic residues was related to penicillin and macrolide groups because of their physicochemical and pharmacokinetic properties and physiological condition of poultry (Donoghue et al. 1996; Furusawa, 1999). Present study findings are in good agreement with other researchers who found macrolides group as the major residual antibiotics in the egg yolk by FPT method. Hakimzadegan et al. (2014) investigated the presence of antibiotic residues in egg yolk using the FPT method and reported that 16.66% of eggs were contaminated with Tetracycline but major contaminants in their study were Macrolides (61.11%). Ehsani and Hashemi (2015) reported that 12.5% of the eggs were positive for antibacterial substances which were related to Macrolides group. In some other studies on egg yolk using the FPT method, Macrolides were also the major detected antibiotics and aminoglycosides and tetracycline were detected as minor contaminants (Smith et al. 2007). Different results in former studies can be due to the various antibiotics used to cure poultry diseases in different geographical regions.

Similarly, in Bangladesh, Islam et al.2016b carried out a study on residual antibiotics in poultry meat and eggs in Chittagong city by Microbial inhibition test (MIT) and Thin Layer Chromatography (TLC). They reported that the overall prevalence of antibiotic residues in eggs were 60% and 36% by MIT and TLC, respectively. According to the study, Ciprofloxacin, Enrofloxacin and Amoxicillin residues were present in 30%, 26% and 24% of eggs, respectively. Although a little variation is existing, present study findings are in good agreement with the findings of Islam et al. 2016b. The variation may be due to the use of different antibiotics at different regions.

Tiamulin, tylosin and erythromycin of macrolides group are commonly used against poultry diseases in Bangladesh (Islam et al. 2016a). Erythromycin is used for treatment of arthritis caused by *staphylococcus aureus* and tylosin and tiamulin are frequently used to treat *Mycoplasma* infections in poultry, especially in laying hens (Smith et al. 2007). Likewise, Amoxycillin and Tetracyclines are regularly being used in poultry farming of Bangladesh due to the broad spectrum of activities (Islam et al. 2016a).

However, Macrolides, Aminoglycosides, Tetracyclines and Penicillins are approved for treating poultry. The results of the present investigation indicate that there is a widespread misuse of these antibiotics in egg layer chicken farms in Bangladesh due to their broad spectrum against gram positive and gram-negative bacteria.

The possible adverse effects of widespread use of antimicrobials in livestock production have been reported (Van den Bogaard and Stobberingh, 2000; Molla et al. 2003; Oyekunle et al. 2003; Alo and Ojo, 2007). As observed in this study, the widespread use of antibiotics can lead to the transfer of such drugs into the human food chain in form of drug residues in animal source foods. This can occur when withdrawal periods are not observed appropriately before selling animal products for human consumption. This can lead to lower efficacy of such drugs when used in for treatment of human illness. Besides, antimicrobial usage can also select for antibiotic resistant bacterial strains with the possibility of transfer of resistant genes to other pathogenic and non-pathogenic bacteria. Human can thus acquire antimicrobial resistant bacteria following consumption of poultry products (Anon.1999).

As high as 27% of the study samples were found positive for the residual antibiotics which might not happen if proper withdrawal period was followed. The results obtained in this survey indicated a high incidence of veterinary drug residues in the eggs in Dhaka city, Bangladesh. Drug residues would be stored by hens' days to weeks after dosing period even without any additional drug transfer. This is of concern, because eggs are continuously supplied to markets for human consumption while no official program monitors drug residues and consumers response to the drug residue hazards is sustainable and passive (Alm El Dein and Elhearon, 2010; Donoghue et al.1996).

4. Conclusion

Many public health hazards such as teratogenic and carcinogenic effects, allergic reactions and antibiotic resistance can threaten human health due to presence of residues in food originated from animals. Although, producing animal origin food free from chemicals or drug residues is almost impossible, deep consideration is needed towards proper and controlled drug use, and safer animal food products. First step is the monitoring of withdrawal time of drugs followed by systematic education of farmers and regular veterinary supervision. In order to diminish the frequent usage of antibacterial drugs in poultry, occurrence of residues in food and the bacterial resistance to drugs, alternative management options like vaccinations, etc. should be developed. Obviously, there is a need for a national residue avoidance and control program in Bangladesh in accordance with international

regulations. It is anticipated that the findings of this study will contribute to the development of strategies for prudent use of antibiotics in poultry farms.

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Conflict of interest: The authors declare no conflict of interest.

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