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Monitoring of Antibiotic Residue in chicken eggs in Tabriz city by FPT

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Abstract

Despite of beneficial effects of antibiotics in treatments of infectious diseases, antibiotic residues in meat, milk, eggs and another products can cause serious side effects for human health. It is clear that, antibiotic residues in food stuff, cause bacterial resistance, allergic reactions, toxicity, carcinogenic effects and change of natural micro flora of intestine in consumers. So, the aim of present study is detection of antibiotic residues contamination rate in chicken eggs. Four-plate test (FPT) is one of the microbiologic methods for detecting antibiotic residues in food stuff, which is based on inhibition zone formation around the sample in four culture media with different pH and test bacteria. For this purpose, 60 samples of chicken eggs obtained randomly from market of Tabriz city. As a result, after different phases of four-plate test, from total 60 samples, 18 (30%) cases are diagnosed to be contaminated to antibiotic residues that 11 (61.11%) cases relevant to Macrolides, 4 (22.22%) cases relevant to Aminoglycosides and in 3 (16.66%) cases contaminated to Tetracycline. But, There isn't Fluoroquinolones residue in eggs, And the most contamination to antibiotic residues were related to Macrolides groups ($P<0.05$). Concluding, the antibiotic residue in chicken eggs has to be monitored as routine test due to their side effects on human health.

Key words: Antibiotic residue, Chicken eggs, FPT, Tabriz

Introduction

Antibiotics are essential drugs considered as the final strategy to treat human infection. Their effectiveness is, however, threatened by wide and unfit use, not only in medicine but also in agriculture (Smith et al., 2009). Currently, more than forty thousand types of Antibiotics have been identified and 80 of them are consumed in the agricultural and aquaculture industries (Martos et al., 2010). In the veterinary practice, antibiotics are used for treatment and prevention of disease as well as to promote growth in the live stock and fish farms (Lopes et al., 2012). Antibiotics are used by the poultry industry and poultry veterinarians to enhance growth and feed efficiency and reduce disease. Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality

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meat and eggs. Antibiotic usage has also enhanced the health and well-being of poultry by reducing the incidence of disease. Although these uses advantage all involved, unfortunately, consumer perceptions are that edible poultry tissues are contaminated with harmful concentrations of drug residues. In a recent consumer survey, Resurreccion and Galvez (1999) reported that 77% of consumers responding considered animal drug residues in meats to be an extreme health concern. Although antibiotics are approved for use in poultry, there is extensive regulatory oversight to ensure the safety of our foods.

Over the last few years, many diagnostic methods for the determination of drug residues in food and consumable organs of animals is known such as: Microbiological Methods, Immunochemistry and Quantitative measurements of drug residues with High performance liquid chromatography apparatus, Gas chromatography, Thin layer chromatography and Mass spectrometry. Each of these methods has its own advantages and disadvantages (Fabianssn S., Rutergrad A., 1979). Among these methods, Microbiological methods are the most common and practical method to determine antibiotic residues in food, because, these methods are compared with other methods in terms of cost and time savings are more Advantageous (Mariël et al., 2008). FPT method that, this article is written based on it, that used in some researcher's studies and Its sensitivity to antibiotic residues were confirmed and Recently approved as a standard method for antibiotic residues is used in Europe Union (Khannazer H., H.Kahba, 1999). In this study, we determine of antibiotic residues contamination rate in eggs supplied by Tabriz market. Four-plate test (FPT) is one of the microbiologic methods for detecting antibiotic residues in food stuff, which is based on inhibition zone formation around the sample in four culture media with different pH and test bacteria.

Materials and Methods

A. Sample preparation:

Sampling of 60 eggs with an average weight of 60-65 (g) was randomly assigned in summer 1392 in Tabriz city's market. Samples moved to the Food Industry laboratory of Islamic Azad University of Tabriz and sampling of the egg yolk was performed in the sterile conditions and under biological hood.

B. Preparation of culture medium

The bacteria used in the four plate test such as: *Bacillus Subtilis* with code: PTTC 1365, *Micrococcus luteus* with code: PTTC 1169 and *E.coli* with code: PTTC 1165, that have been prepared from Fungal and bacterial infections collectors and industrial center of Iran Scientific and Industrial Research Organization. The bacteria were cultured in nutrient agar culture medium, after multiplying bacteria suspension with concentration of 0/5 McFarland (108 * 1.5) has been prepared (Berna et al., 2008). The culture medium used in FPT method was Moler Hinton agar made from QUELAB Laboratories inc. (England). Culture Medium was prepared by using a digital pH meter by acetic acid and sodium hydroxide is equivalent to 6, 7.2 and 8. Then the culture medium at a temperature of 121 ° C for 15 minute was autoclaved.

According to the FPT method, after preparing the plate, *Bacillus subtilis* in three pH, such as: 6, 7.2 and 8, *E.coli* in pH= 6 and *Micrococcus luteus* in pH= 8 prepared from Bacterial suspensions with concentrations of 0/5 McFarland using cotton swab and were cultured under sterile conditions at the plate surface. To ensure the sensitivity of the bacteria used in testing, nine antibiogram discs from different antibiotics as a positive control samples in each of the plates are placed in different pH.

C.Preparation of test samples

Samples (egg yolk) were collected aseptically and under the biological hood of sterile swabs taken. According to Five pH that referred them, samples tested. Thereafter, Tags were placed on each plate that on which Name of the bacteria tested, pH of culture medium, Place of sampling and Number of samples was written. So, Plates incubated for 24 hour at 37 ° C were placed. *Micrococcus luteus* plates containing bacteria due to the slow growth have been in oven for 48 hours. Finally, the results were read using a digital caliper. Inhibition zone was created around the raw samples in culture medium for the presence of antibiotic residues positive detected was measured by using a digital caliper, the size of the zone of inhibition was recorded and by helping of FPT test, five group of antibiotics including: β-lactam, tetracycline, sulfonamides, amino glycosides and Macrolides were identified (Okerman et al., 2007 and Koenen-Dierick et al.,1995).The ability to detect based on the type of culture medium and bacteria used in that summarized in Table 1.

Table 1: The ability to detect based on the type of culture medium and bacteria used in.

pH of culture medium	Bacteria tested	Antibiotics identified
6.0	*Bacillus Subtilis*	Penicillin group Tetracycline group
7.2	*Bacillus Subtilis*	Sulfonamide group
8	*Bacillus Subtilis*	Amino glycoside group
8	*Micrococcus Luteus*	Penicillin group Macrolide group
6	*E.Coli*	Enrofloxacin

D. Statistic test:

Results obtained from experiments performed using SPSS software (Version 17) for statistical analysis. Inhibition zone due to the presence of antibiotic residues in egg yolk is shown in Figure 1, Figure 2, Figure 3 and Figure 4.

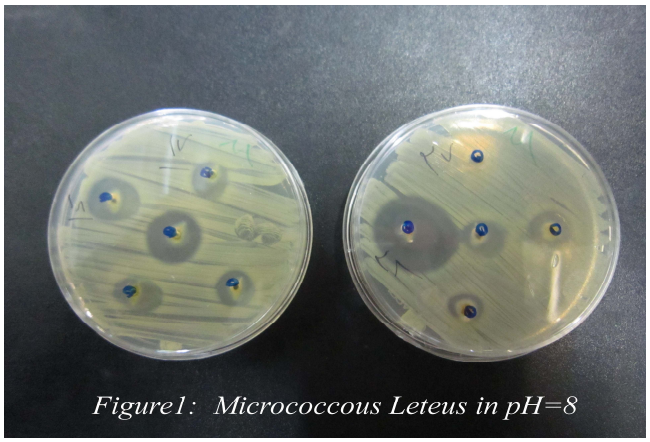


Figure1: Micrococcous Leteus in pH=8

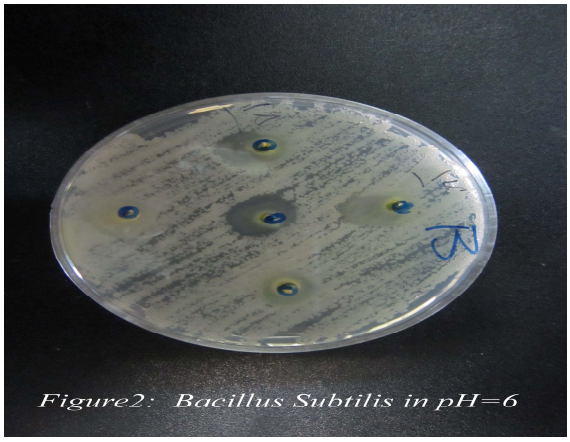
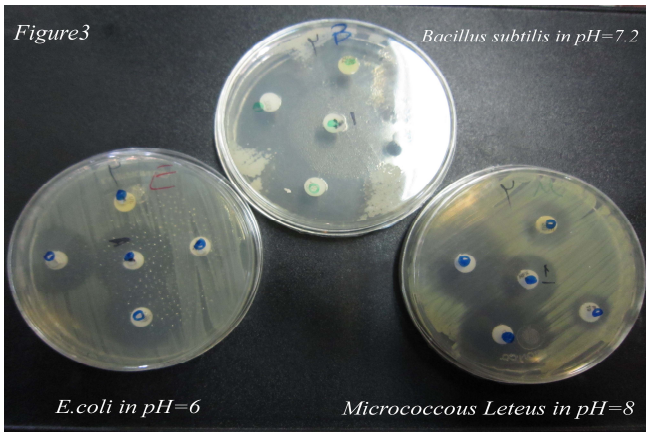


Figure2: Bacillus Subtilis in pH=6



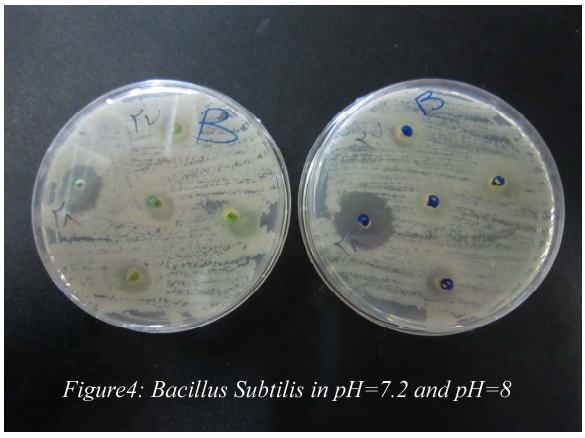


Figure4: Bacillus Subtilis in pH=7.2 and pH=8

Results:

According to studies, Inhibition zone in FPT Method only seen the remains of antibiotics is exceeded. Because the sensitivity of the test is able to detect antibiotic residues below or at the limit value is not allowed. Thus, creating a halo around the sample, at each pH as a result of positive or Antibiotic residue contamination of the sample was considered. The amount of inhibition zone created by the positive control samples are summarized in Table 2.

It is noteworthy that, this test is only used to check the sensitivity of the bacteria used and their results have not been involved in the data analysis.

Tabel 2: The results of positive control samples in millimeters

N	Antibotics	*E.Coli*			*Bacillus Subtilis*			*Micrococcus Luteus*		
		pH=6	**pH=7.2**	**pH=8**	**pH=6**	**pH=7.2**	**pH=8**	**pH=6**	**pH=7.2**	**pH=8**
1	**Enrofloxacin**	30-S	31-S	21-S	40-S	40-S	40-S	Blight	25-S	**20-S**
2	**Tetracycline**	0-R	0-R	0-R	37-S	25-S	20-S	Blight	22-S	**15-I**
3	**Trimethoprim**	21-S	25-S	25-S	36-S	36-S	40-S	Blight	20-S	**26-S**
4	**Gentamicin**	20-S	21-S	22-S	26-S	35-S	40-S	Blight	30-S	**30-S**
5	**Erythromycin**	0-R	0-R	0-R	21-S	21-S	25-S	Blight	21-S	**12-R**

After different phases of four-plate test, from total 60 samples, 18 (30%) cases are diagnosed to be contaminated to antibiotic residues that 11 (61.11%) cases relevant to Macrolides, 4 (22.22%) cases relevant to Aminoglycosides and 3 (16.66%) cases contaminated to Tetracycline. But, There isn't Fluoroquinolones residue in eggs, And the most contamination to antibiotic residues were related to Macrolides groups ($P<0.05$). The number and percentage of positive cases of egg yolks are summarized in Table 3.

Table 3: The result of samples to Separation, bacteria were used and pH of culture medium as the number and percentage.

	E.Coli	*Micrococcus Luteus*		*Bacillus Subtilis*		**Bacteria tested**	
	6	8	8	7.2	6	**pH of culture media**	
Total	**Number and percentage of positive samples**					**Number**	**Sample**
18	**0**	4	6	4	4	60	Egg yolk
30%	**0%**	6.66%	10%	6.66%	6.66%		

As can be seen, 18 samples (30%) of 60 samples diagnosed to be contaminated to antibiotic residues and on the other hand, considering FPT method, 14 cases (23.32%) using *Bacillus Subtilis* and 4 cases (6.66%) using *Micrococcus Luteus* were determined, and none of the samples were determined by using *E. coli*. Statistical analysis of the results showed that the highest rate of

contaminated to antibiotic residues were in pH=8 that According to Table 1, is due to the deposition of macrolides group (P<0.005).

In general, considering the 18 positive samples, the frequency of contamination with antibiotic groups studied in terms of pH, was plotted in Diagram 1.

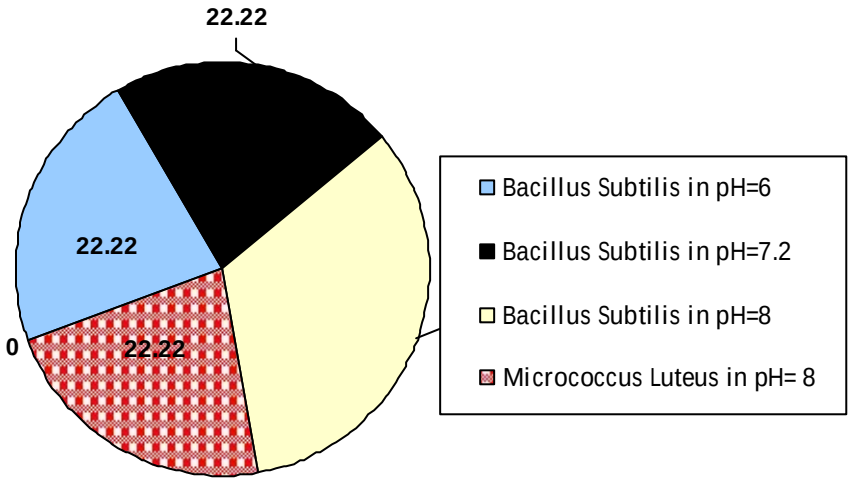


Diagram1: The overall prevalence of contamination with antibiotic groups under study in terms of pH

Discussion and Conclusion

The presence of antibiotic remains in meat, egg and edible viscera of food-producing animal has attracted extensive worldwide attention from national and international public health agencies (Salehzadeh et al., 2006; Asperger et al., 2009). Many reports have shown that bacterial resistance to antibiotics may be due to animal exposure to these drugs form and the resistance gene may be transmitted to human pathogenic bacteria. Furthermore, consumption of a large amount of various animal products containing antibiotic residues by human may cause undesirable changes in bowel micro flora and create immunological reaction in susceptible persons (Mottier et al.,2003; Salehzadeh et al., 2006; Nafisi et al., 2008). The screening procedure is the first step in studying samples to prove the existence or absence of drug residues. This method must be inexpensive, Ability to perform a large number of samples and have minimal false positive and false negative results. Also, all samples contained Antibiotic residues that more than maximum residue level (MRL) should showed positive (Mariel, 2008). This value is based on the type and size of residual drug in such a manner intended that isn't any risk of toxicological perspective to human health. Today microbiological testing mostly used in screened food samples at the macro level. Therefore, these tests are easy to perform and towards the cost of consumption, have an ability to detect multiple types of antibiotic residue. This method Cause to reduce the number of Samples sent for tests. But, it should be noted that these tests should be tuned to have minimize false negatives.

According to the results obtained from the implementation of present study, determined that using *Bacillus Subtilis* at pH = 8 has led to the greatest results. So, pH changes in the medium and type of

bacteria has the greatest impact on the detection of inhibitory effects of antibiotics (Table 3). Also, the most contamination to antibiotic residues was related to Macrolides groups. Since, Erythromycin is most frequently used in poultry to treat staphyloccus areus arthritis. Tylosin and Tiamulin are considered to be highly effective in the treatment of Mycoplasma infection in laying hens to restore egg production and reduce transovarian transmission (Smith et al., 2007). The second most contamination to antibiotic residues was related to Aminoglycosides groups that three Aminoglycosides are used in poultry such as: Gentamicine, Neomycin and Streptomycin. Neomycin is commonly used to treat entric infection and is administrated either in feed or water. Gentamicine is the most widely used aminoglycoside and it is used sub cutaneusly in day-old chicken or turkey chicks. Stereptomycine is partially absorbed from the intestine and therefore can be used to treat systematic E.coli infection (Smith et al., 2007). The less contamination to antibiotic residues was related to Tetracyclines groups that the Tetracyclines are the most widely used antimicrobials in poultry. This is largely due to their affordability, a wide margin of safety and broad-spectrum (Mycoplasma Gram-Positive and Gram-Negative bacteria) and intracellular activity. They are easily administered en mass in either feed or water. The tree tetracycline most commonly used in poultry is: chlortetracycline, Oxytetracycline, Doxycycline (Smith et al., 2007). In present study, there isn't Fluoroquinolones residue in eggs. Research conducted by scholars within and outside the country in recent years indicates that there is the potential risk in animal products. In the study on cows' meat, the levels of antibiotic residues 2.5% have been declared (Masztis et al., 1984). A study in southwestern Nigeria on meats of goats, cows, pigs, respectively, the rate of contamination with Streptomycin to 17.22%, 16.11%, 6.67% have been reported (Dipeolu et al., 2002). In another study, were determined on muscle chicks in north of Iran with using chromatography method that more than 50% of chicken samples contained considerable amounts of antibiotics (Tajik et al., 2006). In a study of antibiotic residues in rainbow trout market in Tabriz showed that 7/22% of skin samples and 10% of meat samples are contaminated with residues of antibiotics (Javadi et al., 2010). As a result, Plates containing antibiotic residues of *Bacillus subtilis* used for the definitive diagnosis of Beta-lactams and Macrolides and show high levels of MRL in this groups. Therefore, the major contaminants in the egg yolk with antibiotics under study based on the four-plate test are Macrolides group, that now Tiamulin,Tylosin and Erythromycin of Macrolides group are commonly used in this area for poultry diseases.

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