MICROBIAL SCREENING OF EGGS FOR DETECTION OF ANTIMICROBIAL DRUG RESIDUES - A SIMPLE AND COST-EFFECTIVE METHOD

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ABSTRACT
This study was carried out to determine the antimicrobial drug residues in commercial chicken eggs obtained from various outlets of layer farms in Tamil Nadu. Microbial tube test method was performed using the bacterial strain Micrococcus luteus. Sixty commercial eggs were collected randomly and examined for antimicrobial drug residues. The results of this study revealed 20 (33.3%) egg samples were positive for antimicrobial residues related to pleuromutilin group (Tiamulin). However, residues detected in 33.3% of eggs were all below the MRL set by European Union (EU). The presence of these residues in eggs could cause health hazards includes toxicological, carcinogenic and genotoxic impacts, effect on normal gut flora, hypersensitivity reactions and bacterial resistance. Thus, the antibiotic residues in commercial eggs must be monitored as routine test in order to guarantee food safety due to their side effects on human health.

KEYWORDS: antimicrobial drug, chicken eggs, Tiamulin, EU.

INTRODUCTION
Poultry industry is growing rapidly in India and the consumption of eggs has also been increasing resulting in more demand. The increasing demand for poultry eggs has necessitated the large scale production of poultry and subsequent use of veterinary drugs, especially antimicrobials. However, the use of these antimicrobial agents has recently become a serious public health issue (Jafari, 2007). These antimicrobials are used for therapeutic purposes and are added in feed and water in sub-therapeutic doses for prophylaxis and growth promotion and its residues can be detected in poultry eggs, if proper withdrawal protocols are not followed (Ezenduka et al., 2014; Diaz-Sanchez et al., 2015). The consumption of eggs containing residues could result in public health hazards including: development of antimicrobial resistance, alteration of intestinal microflora (Salehzadeh et al., 2006) and hypersensitive reactions (Samanidou et al., 2008). To ensure consumer safety, worldwide regulatory authorities have set the Maximum Residual Limits (MRL) for several veterinary drugs including antimicrobials (European Union, 2010; Codex Alimentarius Commission, 2012) and it is necessary to abide by these set limits to make sure that these residues are not present at levels in animal products intended for human consumption.

For antibiotic residue detection, numerous methods are available but the easy, fast and cheap method is the microbiological assay which is the first step in screening samples to prove the existence or absence of antibiotic residues (Hakimzadegan et al., 2014). 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 (Pikkemaat, 2009). As a screening method, the microbiological method minimizes the number of test samples that would eventually be tested with a more sensitive test for confirmation. Although not always specific, these methods are recommended for screening as they can detect many classes of drug residues in animal products in a single test (Fagbamila et al., 2012). This study therefore was designed to provide information on antimicrobial drug residues in commercial eggs collected from Tamil Nadu.

MATERIALS & METHODS
Sample collection
A total number of 60 egg samples were collected from various commercial layer farm outlets in Tamil Nadu, India. Then the egg samples were kept in the refrigerator until further use.

Sample preparation
The surface of each egg sample was cleaned using 70% alcohol. A small opening was made at the tip of the egg using sterile forceps and the eggs were separated into 3 parts—Egg yolks, Egg albumen and whole egg was drained out carefully, extracted and analysed for antibiotic residues.

Extraction of Egg Samples
The chemicals and solvents used for the extraction of antibiotic residues from egg samples were of analytical and purchased from Merck and Sigma-Aldrich. Extraction of antibiotic residues from eggs samples were done by using the method described by Godfred Darko et al., 2015 with some modifications.

Preparation of culture medium
The following culture media were used: Nutrient agar and Nutrient broth obtained from HIMEDIA. Nutrient broth (1.3 g) was weighed into a conical flask and 100 ml of distilled water added, as per the manufacturer’s
instructions. It was then sterilized in an autoclave at a pressure of 15 mm Hg and a temperature of 121°C for 15 minutes, after which it was cooled to about 50°C. Then Bromo Cresol Purple indicator (0.002 g/l) was added in the autoclaved nutrient broth medium. (Shelef et al., 1997, Jambalang, A.R., 2012)

**Preparation of bacterial strain**
The bacterial strain used was *Micrococcus luteus* (MTCC 7590), obtained from Microbiological Type Culture Collection (MTCC). The freeze dried bacterial culture was activated according to the instructions given by MTCC. Single colony obtained from petriplate was inoculated into 5ml nutrient broth and incubated at 37°C for 18 -24hrs. Broth suspension of test organism was adjusted with a sterile physiological saline to a concentration approximately equal to 0.5 McFarland standard equivalents to 1.5×10⁶CFU/ml. All the procedures were done aseptically under biosafety cabinet (Kilinc et al., 2008).

**Experimental Design**

<table>
<thead>
<tr>
<th>Egg samples collected</th>
<th>Egg samples separated into</th>
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<tbody>
<tr>
<td>Commercial chicken eggs</td>
<td>Whole Egg Egg</td>
</tr>
<tr>
<td>60</td>
<td>60 60 60</td>
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**Analysis of Egg Samples by Microbial Screening**
Screening of antimicrobial drug residues were conducted by using microbiological tube test method with a suitable indicator organism for the antibiotic studied (Ref: Kirbis, 2006)

<table>
<thead>
<tr>
<th>Antibiotic tested</th>
<th>Indicator bacteria</th>
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<tbody>
<tr>
<td>Tiamulin</td>
<td>Micrococcus luteus</td>
</tr>
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</table>

**Microbiological tube test method**

**Procedure**
1800µl of nutrient broth with pH indicator bromocresol purple was taken in a test tube and then 100µl of egg sample and 100µl of test bacterium was pipetted into the test tube and mixed thoroughly. The test tubes with positive growth controls containing culture organism in the broth culture and a negative control containing broth also kept. All the test tubes were incubated at 37°C for 18-24hrs depending on the culture organism and their growth. The test tubes that remained purple after the incubation were recorded as positive for antimicrobial residues, and those that turned yellow were recorded as negative for antimicrobial residues (Jambalang, A.R., 2012)

**RESULTS**
Out of 60 egg samples analyzed, 20(33.3%)were found to be positive for antibiotic drug residues against the test organism *Micrococcus luteus*. A positive result was indicated by color change i.e., test tubes that remained purple after the incubation were recorded as positive and those that turned yellow were recorded as negative for antimicrobial residues. In this study, antibiotic residues were analyzed for albumen and yolk separately because some antimicrobials would occur in the yolk and some in the albumen and testing of only the egg yolk or egg albumen could lead to false negative results (Jambalang, A.R., 2012). Tiamulin residues were detected in both egg yolk and egg white of all the positive samples. Physicochemical properties of the drugs, the physiology of the hen and egg formation will determine how much drug will be deposited (Woodward KN., 1991). The LOD for the antibiotic tiamulin was found to be 100 µg/kg which is below the accepted maximum residue level (MRL) for the tested antibiotic (1000µg/kg) set by European Union.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Bacterial strain</th>
<th>LOD st.s. (µg/ kg)</th>
<th>LOD egg (µg/kg)</th>
<th>MRL egg (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tiamulin</td>
<td>Micrococcus luteus</td>
<td>100</td>
<td>100</td>
<td>100000</td>
</tr>
</tbody>
</table>

**FIGURE 1: Micrococcus luteus**
DISCUSSION
Veterinary drugs are essential in poultry production to prevent and control infectious and non-infectious diseases, assist in reducing stress due to environmental changes, vaccination, debeaking and other management practices (Kabir et al., 2004). The increased demand for eggs as an alternative and cheapest source of animal protein places a lot of pressure on the growing poultry industry and compel the indiscriminate use of veterinary drugs particularly the antimicrobials to ensure disease free growth promotion in broilers and egg production in layers, with farmers failing to observe the recommended withdrawal periods (Fagbamila et al., 2010). Nevertheless, the use of antibiotics in food producing animals can leave their traces in food stuffs such as meat, milk and eggs. The results obtained in this study indicated the presence of antimicrobial residue tiamulin in egg samples tested. Tiamulin is mainly used for swine and poultry and it is indicated for the prophylaxis and treatment of dysentery, pneumonia, and mycoplasmal infections (Wang et al., 2011). Tiamulin is commonly used to treat Mycoplasma infection in laying hens (Smith et al., 2006) even though the residue level of the positive samples were not above the MRL level but still the development of antibiotic resistance in humans is unavoidable. Tiamulin resistance is rare but emerging and cross-resistance to tylosin and erythromycin occurs (Krasucka et al., 2010). Since the mechanism of development of resistance to antibiotic is due to the usage of antibiotic at sub-therapeutic doses, so there may be possibility of development of resistance among the consumers (human) due to continuous ingestion of milk, meat and eggs with antibiotic residues even below the MRL (Singh et al., 2014).

CONCLUSION
The detection of antimicrobial residues in animal products (milk, meat and eggs) is of immense importance because of their direct impact on public health. Microbial screening methods are essential to know about the existence or absence of drug residues in foods of animal origin and have been recommended as official and conventional methods because of their simplicity. Also, it is not feasible to employ sophisticated instruments when large number of samples needs to be analysed, as they are very expensive and consume lot of time. Currently, there are very few reports available describing the screening methods for the detection of antimicrobial residues in eggs and extension of this method to identify various classes of antimicrobial drugs is need of the hour.

Conflicts of interest
The authors declared no conflict of interest in this research.

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REFERENCES


