

# NOVEL RAPID SCREENING TECHNIQUE FOR ANTIBIOTIC RESIDUES IN DESI-CHICKEN EGGS

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**ABSTRACT-** Extensive use of antibiotics in chickens causes a serious public health issue because of reasonable alarm regarding harmful concentrations of residues to be present in the edible animal products (meat, milk and eggs). Currently, the trend is increasing towards consumption of desi chicken eggs because of awareness about the presence of antibiotic residues in commercial eggs. However, there is a possibility that some small-scale poultry farmers who are rearing desi chicken for the commercial purpose may also use antibiotics. Therefore, this study was carried out to determine the antimicrobial drug residues in desi chicken eggs obtained from Tamil Nadu. Microbial tube test method was performed using the bacterial strain *Bacillus subtilis* and Sixty desi eggs were collected randomly and examined for antimicrobial drug residues. The results of this study revealed that no egg samples were positive for antimicrobial residues related to any class of antibiotics.

**KEYWORDS** Antibiotic residues, Desi chicken eggs, Microbial screening, Public health.

## INTRODUCTION

Antimicrobial drugs are used to control, prevent and treat infection and they are also added in feeds as growth promoters to enhance animal growth and feed conversion efficiency (Haihong *et al.*, 2014). The increased demand for eggs by the growing poultry industry and the consumption of eggs as a source of animal protein places a lot of pressure on the poultry farms that use veterinary drugs indiscriminately to boost egg production resulting in deposition of antibiotic residues in eggs (Fagbamila *et al.*, 2010). Since eggs are consumed by almost every individual, therefore, the deposition of drug residues in various egg components is of considerable concern (Mund *et al.*, 2017). The possible adverse effects of antibiotics residues include allergic/anaphylactic reactions, chronic toxic effects occurring with prolonged exposure to low levels of antibiotics, development of antibiotic resistance bacteria in treated animals and disruption of normal human flora in the intestine (Hamann *et al.*, 1979, Darwish *et al.*, 2013).

There are various analytical methods which are widely used for screening, confirmation and quantitative analysis of antibiotic drug residues in milk, meat and eggs. 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 (Pikkemaat, 2009). In order to address and manage food safety, it is imperative to have knowledge on the current situation and trends with regard to the occurrence and spread of residues in the food chain. Therefore, this study was undertaken to analyse antimicrobial drug residues in desi chicken eggs by microbial screening.

## 2. MATERIALS AND METHODS

### 2.1 Sample collection

A total number of 60 egg samples were collected from backyard desi chickens in Tamil Nadu, India. Then the egg samples were kept in the refrigerator until further use.

### 2.2 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 yolk, Egg albumen and whole egg was drained out carefully, extracted and analysed for antibiotic residues.

### 2.3 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 Kodimalar *et al.*, 2018 with some minor modifications.

### 2.4 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)

## 2.5 Preparation of bacterial strain

The bacterial strain used was *Bacillus subtilis* (MTCC 121<sup>T</sup>) 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 equivalent to  $1.5 \times 10^8$ CFU/ml. All the procedures were done aseptically under biosafety cabinet (Kilinc *et al.*, 2008).

## 2.6 Experimental Design

Egg samples collected	Egg samples separated into		
	Whole egg	Egg yolk	Egg albumen
Desi chicken eggs 60	60	60	60

## 2.7 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 *Bacillus subtilis* for the antibiotic class Tetracyclines and Aminoglycosides (Kirbis, 2006)

## 2.8 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 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 18-24 hrs 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 analysed, none of the samples were found to be positive for antibiotic drug residues against the test organism *Bacillus subtilis*. All test tubes were changed into yellow color which indicated the absence of antimicrobial residues. In this study, antibiotic residues were analyzed for both 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). Results obtained from this study showed that desi eggs obtained from the areas of study are generally safe for human consumption because of absence of any antimicrobial residues. Till now there are no reports about screening of antimicrobial residues in desi eggs in our state so may be this study will help the consumers to consume desi eggs instead of commercial chicken eggs.

Figure 1 *Bacillus subtilis*

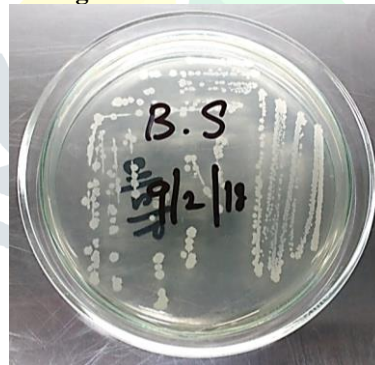


Figure 2 Egg samples showing Negative result



1 ←  
2 ←

1 – Positive control  
2 – Negative control

**DISCUSSION:**

Poultry eggs provide an important source of human nutrition and they are considered to be very significant in combating the problem of low protein intake of the populace (Dipeolu, 2004, Sasanya *et al.*, 2005). Antibiotic residues in foods of animal origin (milk, meat and eggs) that are meant for human consumption are the cause of numerous health concerns in humans (Shareef *et al.*, 2009). This is because man is the ultimate consumer of these toxic antibiotic residues in these animal products (Donoghue, 2003; Dipeolu, 2004; Sasanya *et al.*, 2005; Doyle, 2006; Olatoye and Ehinmowo, 2009; Shareef *et al.*, 2009). It is therefore of utmost importance that such food of animal origin is safe and wholesome for human consumption (Dipeolu, 2004; Olatoye and Ehinmowo, 2009).

Health hazards resulting from consumption of antibiotic residue in foods ranges from direct toxicity on consumers exhibiting allergic reactions, immunopathological diseases, carcinogenic effect, mutagenicity, nephropathy, hepatotoxicity, reproductive disorders, bone marrow toxicity, allergy and the destruction of useful microflora present in the gastro-intestinal tract especially of children leading to indigestion (Nisha, 2008; Nonga *et al.*, 2010); to indirect hazard through the generation of resistant strains of pathogenic bacteria which can be transferred to humans and the residual contamination of manures used in crop production (Kaitlin, 2013). Moreover, the consumption of drug residues via chicken products may result in the production and proliferation of drug-resistant bacteria in human beings, which could lead to therapeutic failures among such infected individuals (Karmi, 2014 and Mund *et al.*, 2017). Taking into consideration of the above facts, desi egg samples were analyzed to check the presence of any antimicrobial residues. The present study revealed that no egg samples analyzed for antimicrobial residues were found to be positive. It can be inferred from this study desi eggs are safe to consume as desi chickens were raised in environmentally friendly manner in free range extensive system and the chance of having any diseases are rare so the farmers are not having any need to use antibiotics.

**CONCLUSION**

The results from the study indicated that, out of 60 egg samples analyzed for the class of antibiotics Tetracyclines and Aminoglycosides, none of the samples were found to be positive for these residues. The usage of desi eggs in the name of organic eggs has now become popular among the public and need for them has also increased, thus makes the commercial desi egg producers to use antibiotics in feed as well as to combat diseases. So the possibility of occurrence of antimicrobial drug residues in desi eggs could not be avoided. As on now, there is no regulations for monitoring the antimicrobial residues in desi eggs. Hence, regulations has to be made by regular examination of desi eggs for veterinary drug residues to ensure safety consumption and to remove international trade barriers.

**Conflicts of interest**

The authors declared no conflict of interest in this research.

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