

Detection of antibiotic residues in poultry meat

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Abstract: The antibiotic residues in poultry meat can pose certain hazards to human health among them are sensitivity to antibiotics, allergic reactions, mutation in cells, imbalance of intestinal micro biota and bacterial resistance to antibiotics. The purpose of the present paper was to detect antibiotic residue in poultry meat. During the present study a total of 80 poultry kidney and liver samples were collected and tested for detection of different antibiotic residues at different pH levels *Escherichia coli* at pH 6, 7 and *Staphylococcus aureus* at pH 8 & 9. Out of 80 samples only 4 samples were positive for antibiotic residues. The highest concentrations of antibiotic residue found in these tissues were tetracycline (8%) followed by ampicillin (4%), streptomycine (2%) and aminoglycosides (1%) as compared to other antibiotics like sulfonamides, neomycine and gentamycine. It was concluded that these microorganism at these pH levels could be effectively used for detection of antibiotic residues in poultry meat.

Keywords: Antibiotic residues, poultry meat, well diffusion method.

INTRODUCTION

Meat is one of the most important constituents of the human diet as, it provide protein, energy, vitamins and minerals. However, meat could also become source of health hazards if it contains excess fats or harmful materials such as toxins or residues of chemical agent (Mahgoub *et al.*, 2006).

Red meat is not very friendly to human consumption pattern, in the sense it also raises the levels of cholesterol, fat, uric acid, hormonal secretion (Alison *et al.*, 2010). It also allows the body to become an open ground for a variety of cancer like cancer of colon, stomach, intestine, pancreas even breast, especially due to high levels of uric acid that increase with consumption of red meat (United States Department of Agriculture, 2007).

In contrast the white meat, generally found within the breast muscles of the birds, are used for quick bursts of power, which require little of the meat darkening myoglobin. White meat is far safer in this respect. It has been found by medical practitioners that white meat especially the lean variety does not have too high levels of fats and so does not contribute to cause cardio vascular diseases (Frank *et al.*, 2002).

A wide range of antibiotics are used in poultry not only to treat diseases but also to maintain health, promote growth and enhance feed efficiency (Noppe *et al.*, 2007). Existence of the antibiotic residues in foodstuff can pose hazards to human health. Among them are sensitivity to antibiotics, allergic reactions and imbalance of intestinal microbiota (Javadi *et al.*, 2011).

Veterinary drug residues tend to be higher in liver and kidney than in muscles. All the antibiotics have the potential to cause allergic reactions, penicillin are most commonly implicated affecting upto 10% of people receiving these drugs therapeutically. Sulfonamide may cause allergic reactions upto 3%. Allergic reactions may involve skin rashes or asthma and in the worst cases, anaphylactic shock. Some drugs have been shown to have adverse effects at high doses, much higher than residue levels detected in meat. These include hear loss and kidney toxicity for neomycin, gentamycin and streptomycin possible carcinogenicity for arsenicals, and effects on thyroid and pituitary functions by sulfonamides (Doyle, 2006).

Tetracycline is a broad spectrum antibiotics used to treat a variety of infections and is also used as a growth promoter in animals. About 60% of an ingested dose of oxytetracyclin is absorbed from the gastrointestinal tract and widely distributed in the body, particularly to liver, kidney, bones and teeth (Doyle 2006).

Staphylococcus aureus are gram-positive bacteria, spherical in shape, arranged in irregular grape like cluster. Some strains are capsulated, non-motile and non-spore forming. They ferment sugar and hemolyzes red blood cell. They are catalase positive and facultative anaerobes. It causes diseases by both producing toxin and also by multiplying in the tissue and causing inflammation (Mansoor 2010).

Escherichia coli are gram-negative bacilli belong to *Enterobacteriaceae*. They are rod- shaped and arranged in chains or pairs. They are flagellated, motile, non- spore forming, capsulated, use acetate as a main source of energy and facultative anaerobes. They are glucose and

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lactose fermenters, lysine decarboxylase and indole positive, urease and citrate negative (Mansoor 2010).

In the present study it was focused to find out the antibiotic residues in poultry meat and aware the people about these residues to adopt preventive measures for their own health and welfare of the animals.

MATERIAL AND METHOD

A total of 80 liver and kidney samples were collected (2013) from different poultry markets and brought under refrigeration to Microbiology Laboratory of Abasyn University Peshawar (Pakistan). These samples were then tested for the presence of antibiotics residues.

Media used

The nutrient agar and brain-heart infusion agar were used.

Preparation of Inoculums

The microbes *Staphylococcus aureus* and *Escherichia coli* were obtained from the Microbiology Laboratory of Abasyn University Peshawar. The test microbes were isolated from poultry samples and farm environment (feeders and drinkers) and were previously characterized for antibiotic sensibility by standard techniques. The primary cultures were refreshed by sub-culturing into nutrient agar and brain-heart infusion agar for about 8-12 hours before inoculation. Then the media were autoclaved for about 121°C for 15min. The sterilized media were then poured into Petri dishes and the pH of the medium were adjusted 6,7,8 and 9 by adding drops of NaOH or HCL, respectively and allowed to solidify.

Well diffusion method

The microorganisms were inoculated on the media by using sterile swab. Then through cork borer wells (10mm) were made in the medium, discs of meat were applied on the media plates and incubated at 37°C for production of zone of inhibition for 18 to 24 hours and were observed at the completion of incubation period. Positive samples were indicated by a complete inhibition of growth in an annular zone not less than 2mm around the piece of meat. While less than 2mm of inhibitory zones were indicated as negative result (Karrauan *et al.*, 2009 & Official Method of Analysis, Association of Official Analytical Chemists, International, Gaithersburg, Maryland, USA, 2000).

RESULTS

Antibiotic detection in liver samples by using *E. coli*

A total of 21 liver samples were tested for antibiotic detection out of which 2 were positive for tetracycline at pH 6 & 9 with a zone of 12mm. A single sample gave a very slight zone of inhibition (1mm) at pH 6&8 which was out of the recommended range of zone of inhibition for positive samples (table 1).

Antibiotic detection in liver samples by using *S. aureus*

A total of 19 liver samples were tested for antibiotic detection out of which 1 were positive for tetracycline with a zone of 12mm and 1 were positive for Ampicillin with a zone of 13mm. (table 2).

Antibiotic detection in Kidney samples by using *E. coli*

A total of 21 Kidney samples were tested for antibiotic residues, out of which 1 were positive for tetracycline with a zone of 12mm. A single sample gave a very slight zone of inhibition (2mm), which was out of the recommended range of zone of inhibition for positive samples (table 3).

Antibiotic detection in Kidney samples by using *S. aureus*

A total of 18 Kidney samples were tested for antibiotic detection out of which 1 was positive for Ampicillin with a zone of 13mm and 2 samples gave a very slight zone of inhibition (2mm) which was out of the recommended range of zone of inhibition for positive samples (table 4).

Zones of inhibition measured

In total 80 samples only 10 samples shown zone of inhibition both in liver and kidney. In kidney samples, 2 shows 13mm zone of inhibition while 3 shows intermediate zone of inhibition. In liver out of 5 samples, 2 were positive with 12mm zone of inhibition while 3 again show an intermediate zone of inhibition.

DISCUSSION

Antibiotics residues were detected through Well Diffusion Method. For detection of antibiotics residues in poultry Liver and Kidney a total of 80 samples were run on Nutrient Agar at pH 6 and 7 for *Escherichia Coli* and Brain Heart Infusion Agar at pH 8 and 9 for *S. Aureus*. In total 80 samples only four samples were positive and the zones of inhibition detected were 12mm and 13mm for Tetracycline and Ampicillin, respectively. In this study about 8% of liver and 4% of kidney samples were positive in which 8% of Tetracycline and 4% of Ampicillin were detected.

Abdul-Salam Ibrahim *et al.* 2010 also reported the same antibiotic residues whose result were as 16(32%) of Liver and 6(12%) of Kidney which were positive in which Tetracycline is 8% and Ampicillin 4% detected. For samples with the multiple antibiotics residues, 8% contain Tetracycline, Streptomycine and Penicillin, 4% Tetracycline and Streptomycine, 1% Tetracycline and Penicillin and 4% Penicillin and Streptomycine. Olatoyo *et al.* 2010 also detected the antibiotic residues, which have 54.44% of Oxytetracycline and 22.32% of Penicillin. Shareef *et al.* 2009 also detected the antibiotic residues which have 7% of Liver and Breast muscles were positive for Tetracycline and 4% samples were positive for

Table 1: Liver samples tested for detection of antibiotic residues through *E. coli* by using Nutrient agar with 6, 7 and BHI agar with 8 and 9 pH.

S. No	pH		Zone of Inhibition (mm)	Antibiotic
	pH	pH		
1	6	9	12	Tetracycline
2	6	9	12	Tetracycline
3	6	8	1	-

Key: The Streptomycin gave zone of inhibition of 20-30mm on nutrient agar. Ampicillin gave < 13mm, Aminoglycosides gave < 14mm, Neomycin gave < 15mm, Tetracycline gave < 12mm and Sulfonamides gave < 10mm (Salehi et al., 2006).

Table 2: Liver samples tested for detection of antibiotic residues through *S. aureus* by using Nutrient agar with 6, 7 and BHI agar with 8 and 9 pH.

S. No	pH		Zone of Inhibition (mm)	Antibiotic
	pH	pH		
1	6	9	12	Tetracycline
2	7	8	13	Ampicilin

A total of 19 liver samples were tested for antibiotic detection out of which 1 were positive for tetracycline with a zone of 12mm and 1 were positive for Ampicilin with a zone of 13mm. (table 4).

Table 3: Kidney samples tested for detection of antibiotic residues through *E. coli* by using Nutrient agar with 6, 7 and BHI agar with 8 and 9 pH.

S. No	pH		Zone of Inhibition (mm)	Antibiotic
	pH	pH		
1	6	9	12	Tetracycline
2	6	8	1	-

Table 4: Kidney samples tested for detection of antibiotic residues through *S. aureus* by using Nutrient agar with 6, 7 and BHI agar with 8 and 9 pH.

S. No	pH		Zone of Inhibition (mm)	Antibiotic
	pH	pH		
1	6	9	13	Ampicilin
2	7	9	2	Negative
3	6	8	2	Negative

Table 5: Positive Liver & kidney samples with antibiotic residues detected through *E. coli* and *S. aureus* by using Nutrient agar with 6, 7 and BHI agar with 8 and 9 pH.

S. No	pH				Zones of inhibition (mm)	Antibiotic	Microorganisms used
	N.A	pH	BHI	pH			
1	-	6	-	8	12	Tetracycline	<i>E. coli</i>
2	-	6	-	9	1	-	<i>S. aureus</i>
3	-	6	-	8	12	Tetracycline	<i>E. coli</i>
4	-	6	-	9	13	Ampicilin	<i>S. aureus</i>
5	-	6	-	8	2	-	<i>E. coli</i>
6	-	7	-	9	1	-	<i>E. coli</i>
7	-	7	-	8	13	Ampiciline	<i>S. aureus</i>
8	-	7	-	8	12	Tetracycline	<i>E. coli</i>
9	-	7	-	9	2	-	<i>S. aureus</i>
10	-	6	-	8	2	-	<i>S. aureus</i>

Sulfadiazine. Mahgoub et al. 2006 detected antibiotics residues in which Tetracycline were about 49.8%, Choramphenicol 0.02% and Streptomycin were 11%.

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