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DETECTION OF ANTIBIOTIC RESIDUES IN CHICKEN MEAT OF JANAKPUR

Microbiology

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ABSTRACT

Introduction: Use of antibiotics by the poultry industry nowadays has become common practice to enhance the health and productivity of flocks. Antibiotics used in poultry feed result in deposition of residues in meat , milk and eggs used for human consumption. Antimicrobial residues in food of animal origin produces potential threat to direct toxicity in human and may result in alteration of microflora and develop resistant strains which cause failure of antibiotic therapy in clinical situations. This study reveals the detection of antimicrobial residues in liver and breast muscles of chicken meat by three plate method as a screening method and high pressure liquid chromatography (HPLC) technique as a confirmatory method. **Materials And Methods:** Among 300 samples, 150 liver samples and 150 breast muscles samples were collected from different area of Janakpur. Screening test of samples were done by three plate method and confirmatory test of the samples were done by high pressure liquid chromatography technique. **Results:** 22.66 % liver and 17.33 % breast muscle sample showed presence of antimicrobial residues. Residues of tetracylines/β-lactams, sulphonamides and, macrolides were found in 21.33 %, 14.66 % and 8 % in liver respectively and 10.66 % tetracylines/β-lactams, 9.33 % sulphonamides and 6.66 % macrolides were detected in breast muscle.Out of all the positive samples in screening test, 13 liver samples and 1 breast muscle sample showed oxytetracycline residues by confirmatory test. **Conclusion:** Presence of antibiotic residues detected by three plate method and high pressure liquid chromatography method shows the use of antibiotics in poultry as a common practice. Chicken meat is one of the highly consumed food for human beings nowadays and contamination with antibiotic residues of chicken meat may become one of the indirect consumption of antibiotics to human food. The intake of antibiotic residues as a food may cause in alteration of microflora and develop antibiotic resistant pattern in commensals which affect antibiotic therapy.

KEYWORDS

Liver, Breast muscle, Tetracylines/β-lactams, Sulphonamides, Macrolides, High Pressure Liquid Chromatography

INTRODUCTION

Antibiotics are substances produced by living organisms that are able to kill or inhibit the growth of microorganisms. The term "antibiotic" originally referred to natural compounds produced by a fungus or other microorganisms that kills disease causing bacteria. Some of the natural antibiotics are benzyl penicillin, streptomycin, chloramphenicol, tetracyclines and macrolides (Praveen, 2010).

Antimicrobial drugs are classified according to their typical core structure. It can also be classified as broad or narrow spectrum, depending on the range of bacterial species against which they are active, or as bacteriostatic or bactericidal on the basis of their mechanism of action. The most commonly used antimicrobial drugs in food producing animals are β-lactams, tetracyclines, aminoglycosides, quinolones, macrolides and sulfonamides.[11] The use of antimicrobials for the treatment or prevention of disease in animals closely followed their uses in humans [6], and they were first employed in veterinary medicine for the treatment of mastitis in dairy cows.[5,3,13] The first compound with chemotherapeutic activity that was used therapeutically was prontosil rubrum, an azo dye structurally related to sulfanilamide.[4] Today antimicrobial drugs are used to control, prevent and treat infection, and to enhance animal growth and feed efficiency.[15] Currently, approximately 80% of all food-producing animals receive medication for part or most their lives .[8]

The initial intention for adequate consumer protection led to the desire to achieve complete elimination of all traces of drug residues in food commodities.[11] Use of antimicrobial drugs and its residues in foods of animal origin may cause the problems like allergic or anaphylactic reactions in sensitized individuals (sulfonamides and β-lactams), toxicity such as aplasia of the bone marrow (chloramphenicol), gastrointestinal disturbances (tetracyclines), genotoxic carcinogenicity (nitrofurans), effects on the human gut microbiota and immune systems, the emergence of resistant bacteria and the transfer of the antibiotic resistance genes to human pathogens.[9] Even some studies showed the evidences of threats like cancer, birth defects, and heart attacks due to antimicrobial residues .[16] These problems are considered as the major public health importance.

MATERIALS AND METHODS

Study Area

This study was conducted in Janakpur.

Sampling

Samples of breast meat and liver of broiler chicken were taken. A total of 300 samples were examined for antimicrobial drug residues, which included 150 breast muscle and 150 liver samples.

Agar Diffusion test (Screening test) (Method- Wolf, 1980)

Reference organisms

Bacillus subtilis BGA was the reference organisms used in this study. The pure culture of *B. subtilis* BGA was provided by Prof. Dr. Reinhard Fies via Dr. Kamal Acharya, Institute of Meat Hygiene and Technology, Free University, Berlin.

Preparation of Standard antimicrobial Discs

Timethoprim solution (٥٠µg/ml)
Oxytetracycline HCl (Reference standards)
Penicillin G Sodium (1IU/ml) (Elysinn Pharmaceuticals Limited, India)
Sulphadimidine (50µg/ml) (Piramal Healthcare Limited, India)
Streptomycin ((50µg/ml) (Safe Parenterals Limited)

Preparation of standard antimicrobial discs:

Penicillin G Sodium, Streptomycin Sulphate and Sulphadimidine Sodium were purchased from Medicine shop in Kathmandu. Proper dilutions of Penicillin G Sodium (1IU/ml), Sulphadimidine (50µg/ml), Streptomycin ((50µg/ml) were prepared. Paper discs of 6 mm diameter were cut and 10µl of each diluted antimicrobial solutions were inoculated on the paper discs accurately and then air dried for 10 minutes. The antibiotic containing paper discs of Penicillin G Sodium, Sulphadimidine and Streptomycin were laid on Petri dishes of pH 6.0, 7.2 and 8.0 respectively (Wolf, 1980). For the test result to be valid the inhibition zone by these discs should be between 5 and 10 mm.

Three Plate Test method (Method- Wolf, 1980)

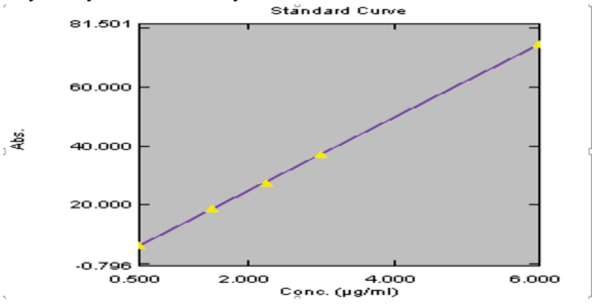
The Three Plate Test method is simple to perform and inexpensive as compared to other method. This method is used for the screening of antibiotic residues present in the meat, milk, eggs, etc. In this thesis work, this method was applied for the screening of antibiotics in chicken meat. Although this method resembles the EU four plate technique, this method includes test agar media of pH 6.0, pH 7.2 and pH 8.0. The reference organism used in this method is *B. subtilis* BGA. In this method, each samples, liver and breast meat, cut as above (8 mm in diameter and 2 mm in thickness) and placed properly on each plate of pH 6.0, 7.2 and 8.0 in duplicate.

High Pressure Liquid Chromatography (Confirmatory test)
Sample Extraction (Method- Sarica *et al*., 2000)

Two grams of sample (liver and breast meat) was homogenized in mortar and pestle for 5 minutes and then 0.1 gm citric acid was added. To this mixture, 1 ml nitric acid (30%), 4 ml methanol and 1 ml distilled water were added respectively. The suspension with solid particles was put in vortex for good mixing, kept in ultrasonic bath for 15 minutes and then centrifuged for 10 minutes at 5000 rpm. After filtering through a 0.45 µm nylon filter, 50 µl of solution was injected into HPLC for analysis.

Analytical Method Validation (Method- Sarica *et al*., 2000 & USP'31)

Analytical method validation of the HPLC method has been done by obtaining calibration curve . Calibration standards were prepared using concentrations of 0.25, 0.5, 1.5, 2.25, 3.0 and 6.0 mg/L of Oxytetracycline in 0.01N Hydrochloric acid.



Chromatographic Conditions (Method-USP'31)

Isocratic separation was achieved using Hypersil BDS C_{18} (5µm, 250 x 4.6 mm) Column. The mobile phase consisting of 200 ml HPLC grade water, 50 gms of tertiary butyl alcohol in a 1000 ml volumetric flask. 60 ml of pH 7.5 Phosphate buffer , 50 ml of Tetrabutylammonium hydrogen sulfate solution and 10 ml of Edetate disodium solution were added and diluted with HPLC water to make up the volume.

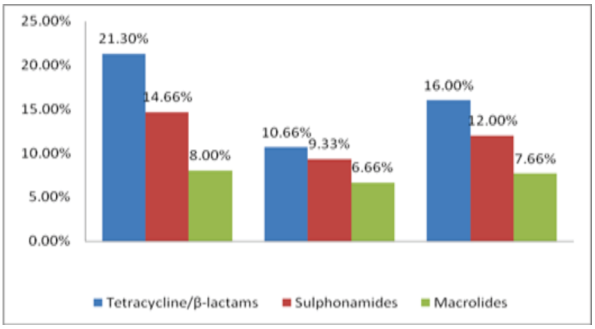
RESULTS

Detection of antimicrobial drug residues by three plate method

The antimicrobial residues detected in the liver and breast muscle samples were Tetracyclines/β-lactams, Sulphonamides and Macrolides. Tetracyclines/β-lactams residues were found higher in liver samples followed by sulphonamides and macrolides. Similarly, Tetracylines/ β-lactams were found higher in breast muscle followed by sulphonamides and macrolides.

Distribution of Antimicrobial drug residues in different test samples

All the samples of liver and breast muscle were tested for four antimicrobial families i.e. tetracyclines, β-lactams, sulphonamides and macrolides. The positive number and its percentage are shown in Figure 1. This study showed that 21.3%, 14.66%, 8.0% of tetracyclines/ β-lactams, sulphonamides and macrolides are present in liver sample respectively. Similarly, 10.66%, 9.33% and 6.66% of tetracyclines/ β-lactams, sulphonamides and macrolides are present in breast muscle respectively. Among the total test samples tested for antimicrobial drug residues, 16% samples were found positive for tetracyclines/ β-lactams, followed by sulphonamides (12%) and then macrolides (7.66%). The highest drug residues found in both test sample tissues were tetracyclines/ β-lactams followed by sulphonamides and macrolides.



Fig; Distribution of Antimicrobial drug residues in different test samples

Antimicrobial drug residues in meat samples

Out of all the total samples, tetracyclines/β-lactams, sulphonamides and macrolides all the three antimicrobial residues were detected in 12 liver samples and 4 breast muscle samples. Liver samples showed large number of antimicrobial residues than that of breast muscle. Statistical analysis of association between antimicrobial drug residues and meat samples showed significant difference (P value < 0.05).

Results of Confirmatory test (HPLC method)

Confirmatory test was performed in the positive samples for tetracycline/ß-lactaobtained from three plate technique (TPT). High Pressure Liquid Chromatography was applied for antibiotics of tetracycline group, Oxytetracycline. All the 32 positive liver samples obtained from three plate method for tetracylines/ß-lactams were subjected for HPLC analysis. Among them, 13 samples were identified and confirmed for the presence of Oxytetracyclines. All the 16 positive breast muscle samples obtained from TPT method were subjected for HPLC analysis and 1 sample identified and confirmed for the presence of oxytetracycline.

Result of HPLC test for Oxytetracycline in Test samples

Tissue Sample	Screening test (Positive)	Confirmatory test(Positive)
Liver	32	13
Breast Muscle	16	1

DISCUSSION

Recent decades have seen significant progress in the development of quantitative confirmatory methods for the detection of antimicrobial residues. According to Kuhne and Ebrecht (1994) an orientation to immunological and chromatographic methods would lead to a drastic reduction of the numbers of analyses. Although the method is widely known as the "Pour-plate method", many variations are made and most laboratories apply a specific approach with a different number and types of bacterial strains and therefore a different number of test plates.[7]

In agar diffusion method, the presence of antimicrobial substances in the sample inhibits the growth of the test organisms. The presence of inhibition halo zone around the sample and its comparison with standard disc indicates the presence of the corresponding antibacterial family.[1] In this three plate method, the growth of *Bacillus subtilis* was found better in pH 6.0, 7.2 and 8.0 in Mueller Hinton agar (MHA) which is similar with the findings of Okerman *et al.*, 2001. This is because *B. subtilis* are versatile chemoheterotrophs capable of respiration using a variety of simple organic compounds and grows in minimal defined medium with no added growth factors at various ranges of temperature.[14] The standard disc of Penicillin, Streptomycin and Sulphadimidine were found highly sensitive to the test organism. In this study, tetracycline/β-lactams, sulphonamides and macrolides were detected in liver and breast muscle samples.The detection and identification of antimicrobial residues in test samples were based on the size of zone of inhibition of antimicrobial residues and the pH of the culture media.. The samples showing inhibition zone of antimicrobial residues ≥ 2mm in culture media of pH 6.0, 7.2 and 8.0 indicates the positive residues. This might be due to the presence of antimicrobial residues in chicken meat higher than the acceptable limits of maximum residues levels (MRLs) which shows the zone of inhibition ≥ 2mm as reported for meat tissues (EC, 1996). The inhibition zone of antibiotics < 2 mm has been observed (Table 1) which might be due to the presence of trace residues or other inhibitors.[2,10]

Out of total 300 samples, 59 (19.66%) meat sample were found positive. Among 59 (19.66%) positive samples, the residues were found higher in liver (22.66 %) than breast muscle (17.33%).The antimicrobial residues present in test samples were found significantly different (P<0.05) in liver and breast muscle. The study reveals the presence of two or more antimicrobial residues in the same sample. Samples positive for tetracyclines/β-lactams were also positive for macrolides and some samples found positive for all the three antibiotic families. This may be due to the recent trend in the treatment and prevention of poultry diseases by using the combination of different antimicrobial drugs.[15]

The occurrence of tetracyclines/ß-lactams residues in liver is higher (21.33%) than breast muscle (10.66%) and the presence of tetracyclines/ß-lactams found is significant different in liver and breast muscle (P < 0.05). This is probably due to the frequent use of this group of antibiotics compared with others and to the high tissue affinity and

slow elimination of some tetracyclines, especially doxycycline in poultry.[1] Presence of sulphonamides in test samples were found comparatively lesser than tetracyclines/ß lactams. Similarly, sulphonamides residues were found statistically insignificant (P>0.05) with test samples. Macrolides were detected in 13 liver (8.66%) and 10 breast muscle samples (6.66%). This might be due to the increased use of sulphonamides in modern practice and used in poultry sector for reducing mortalities due to fowl typhoid, fowl cholera and coccidiosis.[12]

CONCLUSION:

Presence of antibiotic residues detected by three plate method and high pressure liquid chromatography method shows the use of antibiotics in poultry as a common practice. Chicken meat is one of the highly consumed food for human beings nowadays and contamination with antibiotic residues of chicken meat may become one of the indirect consumption of antibiotics to human food. The intake of antibiotic residues as a food may cause in alteration of microflora and develop antibiotic resistant pattern in commensals which affect antibiotic therapy.

REFERENCES

1) Ferrini, A.M., V. Mannoni and P. Aureli. 2006. Combined Plate Microbial Assay (CPMA): a 6-plate-method for simultaneous first and second level screening of antibacterial residues in meat. Food Addit. Contam.23(1):16-24.
2) Ferrini, A.M. V. Mannoni and P. Aureli. 1997. The combined plates microbial assay (CPMA) for the detection of beta-lactam, sulfonamide, streptomycin and tetracycline residues in meat. Arch. lebensmittelhyg.48:133-135.
3) Foley, E. G., Lee, S. W.and Epstein, G. A. 1946. The effect of penicillin on staphylococci and streptococci commonly associated with bovine mastitis. J. Milk Food Technol. 8, 129-133.
4) Forth, W., D. Henschler, and W. Rummel. 1983. Allgemeine und spezielle, Phamakologie. Bibl. Institut, Mannheim. pp 566-570.
5) Gildow, E. M., Fourt, D. L. and Shaw, A. O. 1938. Sulfanilamide in the treatment of streptococcic mastitis. J. Dairy Sci. 21, 759-766.
6) Gustafson, R. 1993. Historical perspectives on regulatory issues of antimicrobial resistance. Vet Hum. Toxicol. 35, 2-5.
7) Kirbis, A. 2006. Microbiological 5-plate Screening Method for Detection of Tetracyclines, Aminoglycosides, Cephalosporins and Macrolides in Milk, Slov Vet Res; 43(4):161-68.
8) Lee, H. J., Lee, M. H. and Ruy, P. D. 2001a. Public health risks:chemical and antibiotic residues. Asian-Aust. J. Anim. Sci. 14, 402-413
9) Mitchell, J. M., Griffiths, M. W., McEven, S. A., McNab, W. B. and Yee, J. 1998. Antimicrobial drug residues in milk and meat: causes, concerns, prevalence, regulations, tests, and test performance. J. Food Prot. 61, 742-756.
10) Myllyniemi, A.L., Rintala, R., Backman, C., Niemi, A. 1999. Microbiological and chemical identification of antimicrobial drugs in kidney and muscle. Food Additives and Contaminants. 16:339-351.
11) Myllyniemi, A.L., 2004. Development of microbiological methods for the detection and identification of antimicrobial residues in meat,Faculty of Veterinary Medicine, University of Helsinki.
12) Saif YM. 2003. Disease of poultry.11th ed. Iowa State University Press. Black well publishing company.
13) Spencer, G. R. 1950. Recent progress in mastitis control. J. Milk Food Technol. 13, 44-49.
14) Todar, K. 2005. The Genius Bacillus, Todar's online Textbook of Bacteriology (online) Available:http://www.textbookofbacteriology.net/Bacillus.html.
15) Tollefson, L. and Miller, M. A. 2000. Antibiotic use in food animals: controlling the human health impact. J. AOAC. Int. 83, 245-256.
16) Young, R. 2001. Regulators hide true extent of dangerous drug levels in poultry products (online)Available:http://wwwlsoilassociation.org/web/sa/saweb.nsf/librarytitles/Antibiotics 30052001.