SHORT COMMUNICATION

Study on quality and efficacy of commercial tylosin and doxycycline products against local isolates of mycoplasma in broilers

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Abstract: The present study was conducted to investigate the quality and efficacy of commercially available preparations of tylosin and doxycycline available in the local market at Peshawar for poultry. *In vitro* and *in vivo,* tests were conducted to check the quality of these antimicrobial drugs. *In vitro* quality control test was performed by High performance liquid chromatographic (HPLC) and micro dilution method. *In vivo,* efficacy of the test drugs was checked in broilers infected with *Mycoplasma gallisepticum*. Results of HPLC indicated that test drug-2 contains doxycycline hydrochloride within specified limits but contain high quantity of active ingredient (Tylosin tartrate 120%). Recovery percentage of test drugs (3, 4, 5) were below the pharmacopoeial limit, which contained low quantity of tylosin tartrate (85%, 87.5%, 85%) respectively however, percent recovery of doxycycline were in the appropriate limits. All the tested drugs were effective against *Mycoplasma gallisepticum* and showed minimum inhibitory concentration (MIC) at 1.9µg/ml. The *in vivo* result indicated that all tested drugs decreased morbidity and mortality in infected chicks. The birds treated with test drugs (3 and 5) showed mortality of 9.5%, which was slightly higher than the other test groups. The current study suggested that there are incidences of substandard drugs in Pakistan and the drug regularity authorities should take strict actions against the manufacturing companies.

Keywords: Tylosin, Doxycycline, *in vitro, in vivo,* morbidity, mortality, efficacy, *Mycoplasma.*

INTRODUCTION

Poultry is the fastest growing industry in Pakistan. It's per annum growth is 8 to 10% which is very fast in comparison to the other agriculture industries. Poultry meat and its products are an important source of protein. In poultry production Pakistan is on 11th position in Asia. It provides employment to 1.5 million people in Pakistan (Anonymous, 2011). People involved in poultry business get benefit from poultry but losses occur due to attack of different bacterial and viral diseases. These diseases are very important because they affect the poultry birds and severe economic losses occur to the farmer due to high mortality and high treatment expenses. *Mycoplasma gallisepticum* is a common respiratory pathogen in chickens, which causes heavy losses to poultry industry. *Mycoplasma gallisepticum* caused respiratory infection in avian species mainly commercial chickens (Ley, 2003) and birds showed clinical signs and symptoms like nasal and ocular discharge, sneezing, coughing, tracheal rales (Kleven, 1998a). Antibiotic therapy is very common to control *Mycoplasma gallisepticum* infection (Stipkovits *et al.,* 1993). *Mycoplasma gallisepticum* is sensitive to different antimicrobials *in vitro* and *in vivo* such as macrolides (Jordan and Horrocks, 1996), tetracyclines and quinolones (Bebear *et al.,* 1999; Wu *et al*., 2000).

Tylosin is used in veterinary medicine for the treatment and control of bacterial infections in different species and has a high margin of safety. Tylosin is macrolide antibiotic, which belongs to 16-member ring macrolide group. It blocks the translocation step to inhibit bacterial protein synthesis (Brisson-Noel *et al.,* 1998). It is produced by *Streptomyces fradiae*. It has a high degree of lipid solubility and very low degree of ionization. It is thus extensively disseminated throughout tissues and body fluids (Gingerich *et al.,* 1977). It is recommended for the treatment of arthritis, pneumonia, chronic respiratory disease and other infections caused by susceptible organisms (Taha *et al.*, 1999). It is used in infections caused by gram-positive bacteria *Staphylococci, Streptococci, Corynebacteria and Erysipelothrix*. It is also used against anaerobic bacteria, Gram-positive bacteria and *Mycoplasma* (Prescott and Baggot 1998; Prats *et al.,* 2001).

Similarly doxycycline has been commonly used in veterinary and poultry medicine for therapeutic as well as

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prophylactic purposes. It is a tetracycline derivative, which is broad spectrum in nature. Due to its wide spectrum it is active against various gram positive and gram-negative organisms (Cinquina *et al.,* 2003). It is used in the treatment of different infectious diseases caused by *Mycoplasma, Actinomyces species Spirochetes, Rickettsiae and Chlamydiae* (McGraw-Hill, 2001). In poultry it is used in combination with tylosin to treat chronic respiratory disease.

Despite of availability of so many antimicrobial drugs in the local market, there are complaints about ineffectiveness of some of these drugs. There might be different reasons for ineffectiveness, of which the prevalence of substandard and counterfeit medicines is a big issue, which is affecting both developing and developed countries. To get maximum benefits from any medicine or medicinal product its safety efficacy and quality is of paramount importance. Drug quality has always been of huge public and veterinary health concern, however during the last few decades the issue of counterfeit and low quality medicines became apparent for human and veterinary health in both developed and developing countries. According to recently published papers, there is incidence of substandard medicines from developing countries including Pakistan (Arie, 2012). Keeping in view the importance of the above two drugs used in poultry medicine and the substandard and counterfeit complaints, this study was undertaken to check the quality and efficacy of some of the commercially available poultry antimicrobials in district Peshawar.

MATERIAL AND METHODS

The present study involved *in vitro* and *in vivo* evaluation of tylosin and doxycycline containing commercial poultry antimicrobial drugs. Most part of the proposed research work was conducted at the Department of Poultry Science, Faculty of Animal Husbandry and Veterinary Sciences, The University of Agriculture Peshawar while National Veterinary Laboratory Islamabad and University Diagnostic Center, University of Veterinary and Animal Sciences Lahore extended help in the analysis of commercial drugs samples and procurement of seed culture of *Mycoplasma gallisepticum*.

Collection of samples

Products of different manufacturers containing Tylosin tartarte and Doxycycline hydrochloride in combination were purchased from the local market at Peshawar and were used in further experiments

Chromatographic system

The LC equipment (L-7000 Series, Hitachi Japan) consisted of L-7100 pump, L-7200 Autosampler, column oven L-7300, L-7400 UV detector, Degasser L-7610 and D-7000 interface. EZ Chrome Elite 4.0 (Hitachi) software was used for data acquisition. A Kromasil 100C18 (25×0.46, 5µm) (Teknokroma) column was used as the stationary phase.

Preparation of mobile phase

A buffer of 0.04M potassium dihydrogen phosphate was prepared. The pH was adjusted to 2.60±0.2 through pH meter (Jenway), using diluted solution of phosphoric acid. Acetonitrile was mix with buffer solution with a ratio of 30:70. The solution was stirred through magnetic stirer (Velp Scientifica) for 10 minutes, filtered through 0.45µm size filter paper (Polyamide Sartorius Germany) and was sonicated for 10 minute in sonicator (Ultrasonic LC 30H Elma). Mobile phase for doxycycline was composed of 0.01 M oxalic acid, Acetonitrile and methanol with a ratio of (5:2:3 v/v/v). The solution was stirred through magnetic stirer (Velp Scientifica) for 10 minutes, filtered through a 0.45µm size filter paper (Polyamide Sartorius Germany) and was sonicated for 10 minute in sonicator (Ultrasonic LC 30H Elma).

Preparation of standard stock / Working solutions

Stock solution for tylosin was prepared by dissolving 25mg tylosin tartrate (weight was corrected according to assay) in 25ml acetonitrile and stored in a special refrigerator at -10°C. Working solution was prepared from standard solution with different concentration of tylosin 100ppm, 10ppm, 1ppm, 0.1ppm and 0.01ppm in acetonitrile. Similar solution was also prepared for doxycycline.

Preparation of samples

Samples were prepared by dissolving 25mg sample in 25ml de-ionized water and stored in refrigerator at 4°C. Working solution was prepared from stock solution in de-ionized water with different concentrations of test drug 100µg/ml, 10µg/ml, 1µg/ml, 0.1µg/ml and 0.01µg/ml**.**

HPLC protocol for tylosin determination

Tylosin determination in samples was done according to the method described by Kowalski *et al*. (2006). The HPLC instrument was run under the following experimental conditions. The mobile phase was composed of acetonitrile and 0.04M potassium dihydrogen phosphate with pH adjusted to 2.60±0.02 (30:70 v/v). The flow rate was 1ml/min at ambient temperature. The UV detector was set at 280nm and injection volume was 10µl.

HPLC protocol for determination of doxycycline

Determination of doxycycline was done according to the method developed by (Mitic *et al.,* 2008). The HPLC instrument was run under the following experimental conditions: mobile phase was composed of 0.01M oxalic acid: methanol and acetonitrile (5:3:2 v/v/v). The flow rate was 1.25ml per minute at 25°C. The detector wavelength was set at 350 nm and injection volume was 30µl.

Table 3: Label claim, actual and recovery percentage of tylosin tartrate and Doxycyline in test samples

Sample ID	Label claim (g)	Actual (g)	Tylosin tartrate (%)	Label claim (g)	Actual (g)	Doxycycline (%)
Test drug-1	20	22	120	40	39	97.5
Test drug-2	10	12	110	20	19.5	97.5
Test drug-3	10	8.5	85	20	19	95
Test drug-4	20	17.5	87.5	40	37	92.5
Test drug-5	10	8.5	85	20	18	90
Test drug-6	20	19	95	40	39	90.5
Test drug-7	14	13.5	96.5	16	14.5	90.5
Test drug-8	10	9	90	20	19	95

Table 5: Morbidity and mortality in broiler infected with *Mycoplasma gallisepticum*.

Group	Morbidity (%)	Mortality (%)
Control negative	0	0
Control positive	95	14.5
Test group-1	90.5	4.5
Test group-2	90.5	4.5
Test group-3	95	9.5
Test group-4	81	4.5
Test group-5	81	9.5
Test group-6	71	4.5
Test group-7	90.5	4.5
Test group-8	71.5	4.5

In vitro determination of drugs efficacy

In vitro efficacy of the pharmaceutical products was determined against *Mycoplasma gallisepticum*. Frey's medium was prepared according to (Kleven *et al*., 1998b). Both part A and part B was prepared separately. Component of part A medium was accurately weighted through electric balance and dissolved in 200ml distal water in a sterilized bottle (Simax Czech Republic). The pH was adjusted to 7.8 and autoclaved (Sanyo, Japan) at 121°C and 15 PSI pressure for 15minutes. Components of part B medium were accurately weighted through an electric balance and dissolved in 20ml distal water in a sterilized test tube. The pH was adjusted to 0.47 and was passed through a 0.2um size membrane filter (nitrocellulose) with the help of a vacuum pump (Cast manufacture USA) in a sterilized flask. Horse serum 30ml was passed through membrane filter in the same sterilized flask. Both part A and part B media were mixed with each other. The whole work was done in Bio Safety cabinet class A2 (Technico Pakistan). A small amount of media was incubated at 37°C in carbon dioxide incubator (New Brunswick scientific) for 24 hour in a sterilized test tube to check the sterility.

Micro broth dilution

Three (3) sterilized microtiter plates were taken and labeled properly. A 100µl medium was dispensed in each well of microtiter plate through multi channel pipette (Eppendorf). Final concentration of the test drugs in different wells was adjusted to 1, 0.5, 0.25, 0.12, 0.062, 0.031, 0.015, 0.0078, 0.0039, 0.0019 $mg.ml^{-1}$ respectively. A 20µl *Mycoplasma gallisepticum* culture was dispensed in all wells except negative control. Each antibiotic was tested in three replicates and the inoculums size for MIC was 10^5 CFU/ml. Plates were incubated at 37°C for 24 hour in carbon dioxide incubator having 5% carbon dioxide (New Brunswick scientific). After 24 hours, the plates were read through Elisa plate reader (Bio-Rad) with detection wavelength set at 600nm.

In vivo determination of efficacy of testing products

A total of 210day old chicks were used in the trial. All the chicks were allocated to ten major groups with 3. All birds were kept at optimal environmental conditions. After two days of rearing the chicks in all groups except negative control were inoculated with virulent strain of *Mycoplasma gallisepticum* intra-tracheally @ 0.1ml of the PPLO (Pleuro Pneumonia like Organism) broth containing 1.2 X 10^8 CFU/ml. The birds were examined for clinical signs on daily basis and were treated for four days after the appearance of the clinical signs with the experimental products. Morbidity and mortality in all experimental groups were recorded.

STATISTICAL ANALYSIS

Data was statistically analyzed with the help of Microsoft excel (version 2007).

RESULTS

The main purpose of this study was to check the quality and efficacy of different antimicrobial products of tylosin and doxycycline preparations used for treatment of

different bacterial diseases in poultry especially Mycoplasmosis in Peshawar. Quality was determined through *in vitro* and *in vivo* tests. *In vitro* quality control test was determined by liquid chromatographic and micro dilution methods while *in vivo* efficacy was checked in broilers infected with *Mycoplasma gallisepticum*.

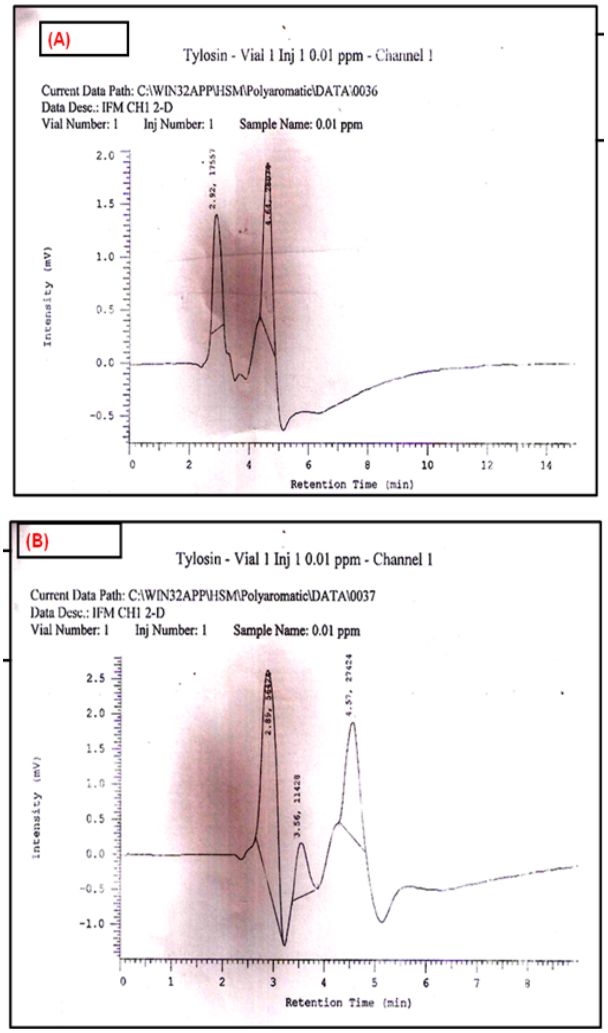

Fig. 1: Representative chromatograms of samples: (A) Tylosin tartrate standard; (B) Test drug-1

Determination of tylosin and doxycycline contents in pharmaceutical products

Tylosin and doxycycline contents of different pharmaceutical products of national and international origin were determined on high performance liquid chromatography (HPLC) adopted from (Kowalski *et al*., 2006) and (Mitic *et al*., 2008) with minor modification. A (HPLC) method was developed for the quantification of tylosin tartrate and doxycycline hydrochloride. HPLC analysis was performed on C18 column, the detection wave length was set at 280nm. Run time for samples and standards was 15 minutes, flow rate was 1ml/min and retention time was 4.64±0.20 minutes for tylosin while for doxycycline the detection wavelength was 350nm and flow rate was 1.25ml/min. The retention time was 3.50±0.20 for doxycycline. The active ingredients were identified by matching retention time of standard and samples. The active ingredients were quantified from a standard curve, which was based on measurement of the peak area of serial dilutions of tylosin tartrate and doxycycline hydrochloride. The representative chromatogram of standard and samples is given in figs. 1, 2, 3 and 4.

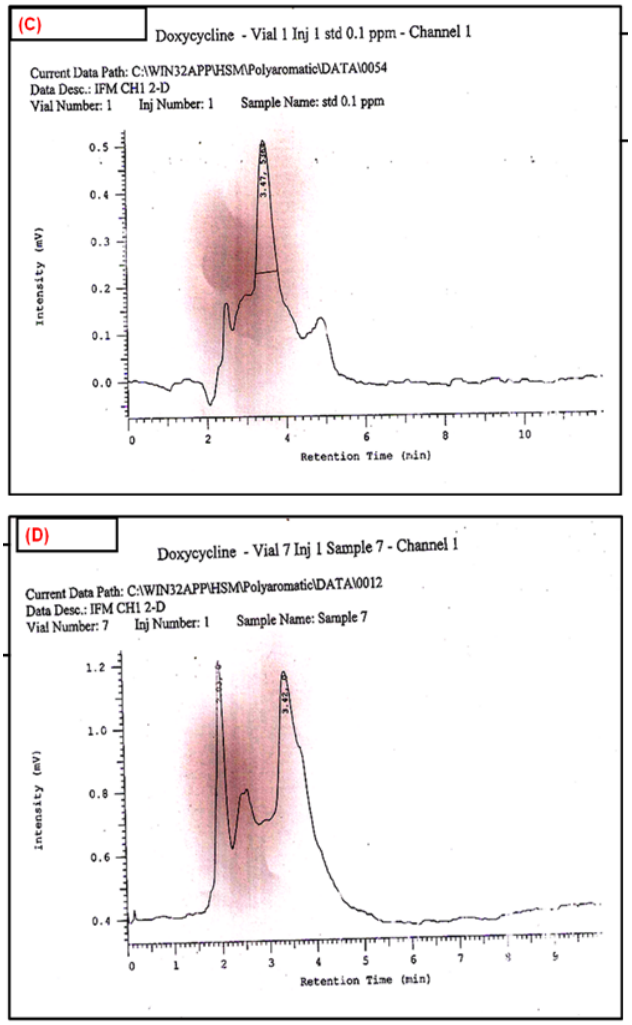

Fig. 2: Representative Chromatograms of samples: (C) Doxycycline Hydrochloride standard (D) Test drug-7

Pharmacopoeial specification for drug quality is 100±10% (British pharmacopeia). The statistical data results obtained in the analysis of commercially available drugs samples as shown in table 3 and 4, represents that the drugs samples which fill-full the specification of the British Pharmacopeia and the drugs which failed to meet the standard specifications. Test drug-1 was found to have good quality which contained tylosin tartrate and doxycycline hydrochloride within the specified limits. Test drug-2 contains doxycycline hydrochloride within specified limits but contain high quantity of active

ingredient (tylosin tartrate 120%) which is above the specified limits. Recovery percentage of test drugs (3, 4) was less then pharmacopoeial limit and contained low quantity of active ingredient tylosin tartrate (85%, 87.5%), respectively, however, percent recovery of doxycycline hydrochloride was in the appropriate limits. Test drug-5 contained doxycycline hydrochloride in appropriate quantity but tylosin tartrate contents was below the specified limits. The other test drugs (6, 7 and 8) contained appropriate quantity of tylosin tartrate and doxycycline hydrochloride and full-fill the standard specification of British pharmacopoeia and of quality standard.

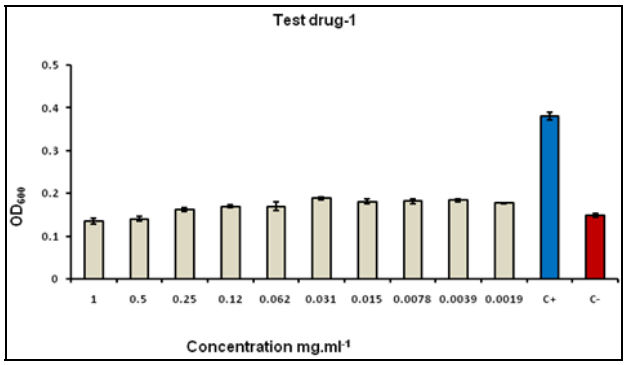

Fig. 3: *In vitro* sensitivity of different concentration of test drug-1 against mycoplasma gallosepticum at OD 600

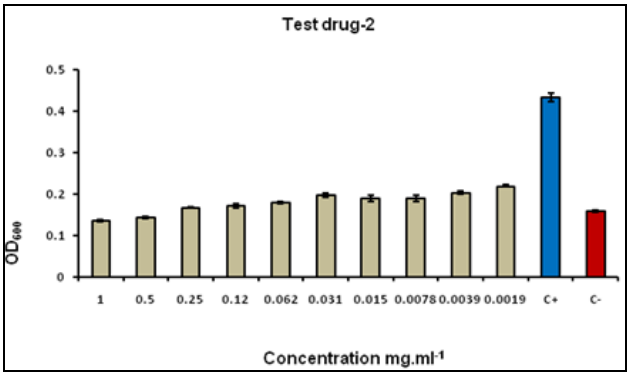

Fig. 4: *In vitro* sensitivity of different concentry of test drug-2 against mycoplasma gallisepticum at OD 600

In vitro determination of drug efficacy

In vitro efficacy of testing products containing tylosin and doxycycline was determined against *Mycoplasma gallisepticum* through micro broth technique in 96-well microtiter plates. Each well of the microtiter plates contained stepwise twofold dilutions of the test drugs. The inoculum size for MIC was 10^5 CFU/ml. The lowest dilution in the study was 1.9µg/ml and sensitivity at lowest possible dilution was taken as minimum inhibitory concentration (MIC). The microtiter plates were incubated at 37°C for 24 hours and analyzed through ELISA plate reader with detection wave length set at 600 nm. The results showed that test drug-1 was effective against *Mycoplasma gallisepticum.* Sensitivity at different dilutions was compared with positive control (Students "t" test), which showed significant difference (P>0.05). The test drug-1 showed MIC at 1.9µg/ml. The drug was effective at every dilution as shown in fig. 5. Test drug-2 was significantly active against *Mycoplasma gallisepticum* and showed MIC at 1.9µg/ml. *Mycoplasma gallisepticum* was inhibited in every well of micro titer plates as shown in fig. 6. Test drug-3 was also effective at various dilutions and showed MIC at 1.9µg/ml, the values at different dilutions were compared with positive control (Students "t" test) which presented a statistical significant difference (P>0.05) and control growth of *Mycoplasma gallisepticum* in the medium as presented in fig. 7. Similarly test drug-4 was effective at every dilution and showed MIC at lowest dilution as shown in fig. 8. Sensitivity at different dilutions was compared with positive control (Students "t" test) for test drug-5 and showed similar sensitivity as the other drugs shown (fig. 9). Other tested drugs 6, 7 and 8 were also active at every dilution and showed MIC at 1.9µg/ml as shown in the fig. 10, 11, 12.

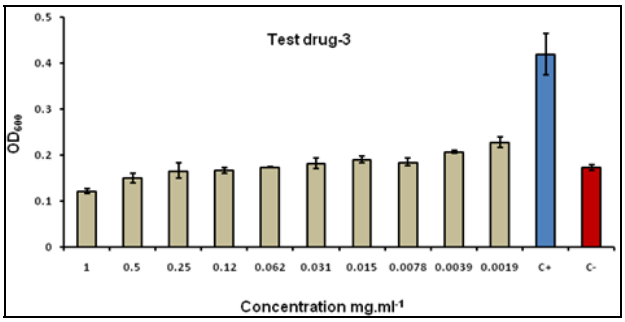

Fig. 5: *In vitro* sensitivity of different concentry of test drug-3 against mycoplasma gallisepticum at OD 600

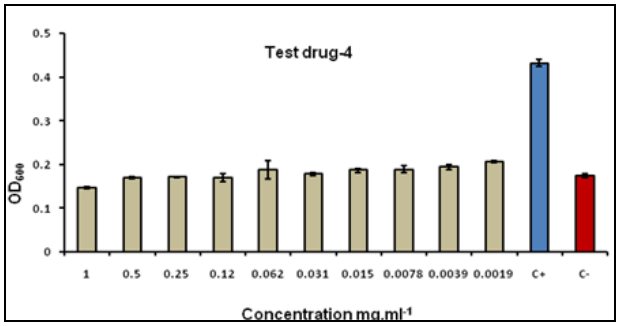

Fig. 6: *In vitro* sensitivity of different concentry of test drug-4 against mycoplasma gallisepticum at OD 600

In vivo determination of drug efficacy

In vivo, efficacy of the test drugs was determined against *Mycoplasma gallisepticum* in broiler chicks infected with virulent strain of *Mycoplasma gallisepticum* in a controlled environment.

The results as summarized in table 5, showed that negative control group remained healthy throughout the study and showed no sign of disease. The positive control

chickens exhibited the clinical sign and symptoms (sneezing, rales, coughing, & lacrimation) throughout the study and showed a higher morbidity and mortality of 95 and 14.5%, respectively. The test drug-1 treated birds (test group-1) showed a morbidity of 90.5%, where as mortality was 4.5%, Similarly in the test drug-2 treated broiler chicken (test group-2), a morbidity of 85.5% and mortality of 4.5% was noted. The birds treated with test drug-3 (test group -3) showed a morbidity and mortality of 95 and 9.5% respectively which is slightly higher than the other test groups, Test group-4 broiler chicken which was treated with test drug-4 exhibited morbidity of 81 % and mortality of 4.5%. Likewise there was 81% morbidity in birds treated with test drug-5 (test group -5) while a high mortality rate of 9.5% was noted. The other groups (6, 7 and 8) which were treated with test drug (6, 7 and 8), showed a morbidity of 71, 90.5 and 71.5%, respectively where as mortality values were 4.5% each.

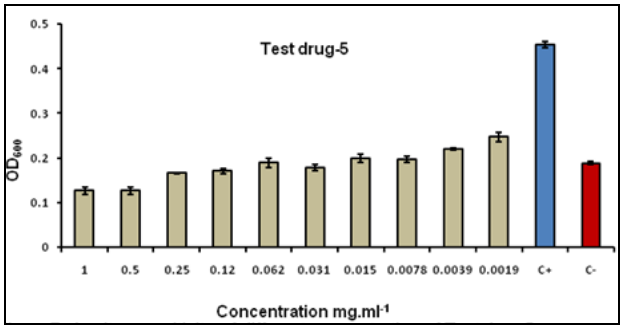

Fig. 7: *In vitro* sensitivity of different concentry of test drug-5 against mycoplasma gallisepticum at OD 600

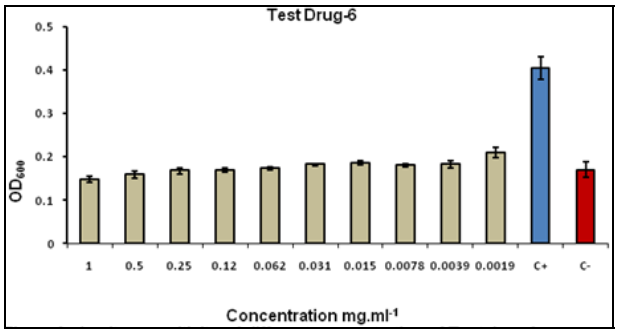

Fig. 8: *In vitro* sensitivity of different concentry of test drug-6 against mycoplasma gallisepticum at OD 600

DISCUSSION

Tylosin and doxycycline content in pharmaceutical products

The quality of drugs of human and veterinary health has been a major issue for over years but it became more prominent during the last few years. The production of counterfeit is a global and under-recognized problem that contributes to morbidity, mortality and drug microbial resistance and leads to spurious reporting of resistance and toxicity and loss of assurance in health-care systems (Newton *et al.,* 2006). The most important criterion in establishing the quality of a given drug preparation is its content of active ingredients. We used the limits specified in the British Pharmacopeia because the manufactures follow the specification of *BP* for preparation. Quality control test was determined by liquid chromatography because the method was more precise, specific and accurate. The data showed that most of the preparations tested in the present study contained almost the correct quantity of active drug but some of the drugs are not of standard quality and outside the pharmacopoeial limits. Failure to meet the specifications was as likely to be a result of too high concentration of active ingredient and/or of too low concentration. Our findings confirmed that the test drug 2 contained tylosin tartrate in high quantity, which leads to toxicity and increased drug residues level. Results agreed with previous findings of (Silverman *et al.,* 1990); however, if the amount of active ingredient is not too far outside the official limits, then it does not suggest that the preparation is counterfeit. Some of the preparations contained active ingredients below the pharmacopoeial specification; however they were close to pharmacopoeial limits. In current study, the test drug (3, 4 and 5) contained active ingredient tylosin tartrate which was less than the labeled amount. Substandard preparations have been attributed to poor manufacturing practices by other workers (Arya, 1995; Petralanda, 1995), and this was consistent with our findings. Decomposition of the drug is a probable cause of a reduced amount of drug in a preparation and some investigators (Hogerzeil *et al.,* 1992) have sought to determine the extent of such degradation in adverse climatic conditions. A Word Health Organization report described criteria for counterfeit and substandard drugs. The report described that a counterfeit product include drugs with the wrong ingredients, without active ingredients, with insufficient active ingredient or with fake packaging. Analysis of the content of a drug preparation to identify the actual drug and its quantification is necessary to distinguish counterfeit drugs (WHO, 1992). In our study all the products were in suitable packs with clear labels and were within their expiry dates. Poor quality and ineffective drugs produce health complications. If the amount of active ingredient is below the standard in a preparation, use of such drugs lead to therapeutic failure and drug microbial resistance. Finding of the study are accordance with Masland & Marshall, 1990 and Ten, 1992, who described that substandard preparations may lead to adverse clinical results both in terms of low efficacy and encouraging drug resistance.

In vitro efficacy of tested products

In current study efficacy of pharmaceutical products containing tylosin and doxycycline were tested in 96 wells microtitre plates in the laboratory against the local isolates of *Mycoplasma gallisepticum*. Micro broth dilution method was used because it is simple, easy to perform and need very small quantity of media (Taylor-

Robinson, 1967), moreover it was easy to test different drugs at various dilutions in a single plate. In our experiments it was confirmed that all the preparations were significantly active against *Mycoplasma gallisepticum.* Results were in accordance with the work of early researcher of Burch and Valks, 2002 and Burch and Stipkovits, 1993, who stated that tylosin and doxycycline are significantly active against all the isolates of *Mycoplasma gallisepticum*. Similar results were also reported by Jordan *et al*., 1998 and Ziv., 1980. In the present study it was noted that using tylosin and doxycycline in combination; significantly inhibit *Mycoplasma gallisepticum* growth and showed minimum inhibitory concentration at 1.9µg/ml. Findings of the study are in accordance to the reports of Lin, 1987 and Kleven *et al.,* 1971.

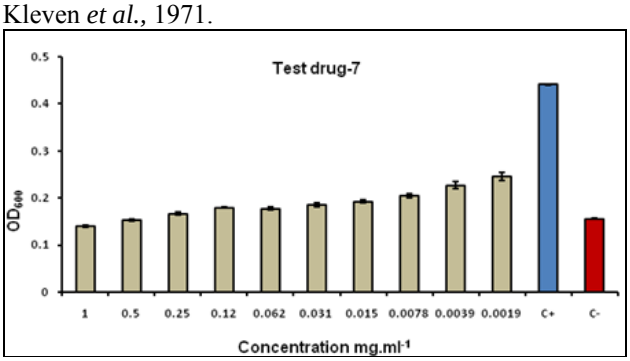

Fig. 9: *In vitro* sensitivity of different concentry of test drug-7 against mycoplasma gallisepticum at OD 600

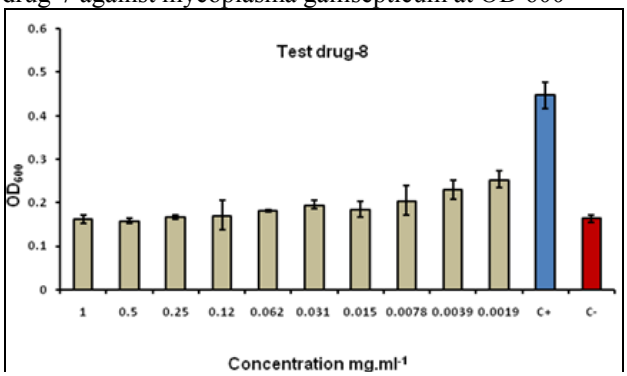

Fig. 10: *In vitro* sensitivity of different concentry of test drug-8 against mycoplasma gallisepticum at OD 600

In vivo efficacy of tested products

For *in vivo* efficacy the drugs were offered to the broiler chickens infected artificially with *Mycoplasma gallisepticum* in a controlled environment. In our study it was noticed that the disease produced in test drug-2(infected untreated) was very severe as shown by the mortality of 14.5% and morbidity of 95%. Results agreed with the previous findings of Jordan *et al.,* 1998, who evaluated *in vitro* and *in vivo* comparisons of different antibiotics (valnemulin, tiamulin, tylosin, enrofloxacin, and lincomycin/spectinomycin). Mortality, clinical signs, and gross lesions were significantly higher in infected un-medicated group than un-infected and infected medicated groups. The data showed that most of the pharmaceutical products we used in the present study were significantly active. The birds in all test groups (infected medicated) showed clinical sign of disease and the recovery rate was significantly high. Mortality was reduced by medications in all the test groups. The result was in accordance with previous findings of Khan *et al.,* 2006, who determined efficacy of tylosin, Oxytetracyline and tiamulin in broilers infected with the virulent strain of *Mycoplasma gallisepticum*. The cure rate was significantly higher ($P \leq 0.05$) and tiamulin and tylosin were proved to be the drug of choice to control *Mycoplasma gallisepticum* infection. Similar results were also reported by Ziv, 1980 and Arzey Arzey, 1992. The findings of our study are also is in line with Timms *et al.,* 1989, who determined the efficacy of chlortetracycline for the treatment and control of chronic respiratory disease caused by *E. coli* and *Mycoplasma gallisepticum.*

CONCLUSION AND RECOMMENDATIONS

Some of the preparations were either marginally exceeded or contained less amount of active ingredients specified in pharmacopeia. All the tested drugs were significantly active against *Mycoplasma gallisepticum.* All drugs used in this study, decreased the morbidity and mortality in broiler birds infected with *Mycoplasma gallisepticum*.

1. On the basis of results of the research study following recommendations are forwarded
2. Provincial government should appoint veterinary drug inspectors on district level for inspection of veterinary drugs in the market.
3. Before use of any antimicrobial in the field, it is recommended to first assess their sensitivity *in vitro* against susceptible pathogens.
4. High sensitive methods like liquid chromatography should be used to check the quality of drugs before marketing.

REFERENCES

Anonymous (2011). Pakistan Economic Survey.

Arie S (2012). Contaminated drugs are held responsible for 120 deaths in Pakistan. *British. Med. J.,* p.344.

Arya SC (1995). Inadvertent supply of substandard drugs. *World Health Forum.,* 16(3): 269.

Arzey GG and Arzey KE (1992). Successful treatment of Mycoplasmosis in layer chickens with single dose therapy. *Australian Vet. J.,* **69**: 126-128.

Bebear CM, Renaudin J, Charron A, Renaudin H, Barbeyrac B, Schaeverbeke T and Bebear C (1999). Mutations in the gyrA, parC and par E genes associated with fluoroquinolones resistance in clinical isolates of Mycoplasma hominis. *Antimicrob. Agents Chemoter*., **43**: 954-956.

Brisson-Noel A, Trieu-Cuot P and Courvalin P (1998). Mechanism of action of spiramycin and other machrolides. *J. Antimicrob. Chemotherapy*., **22**: 13-23.

British Pharmacopoeia (1993). HM Stationery Office, London.

Burch DGS and Stipkovits L (1993). Enhancement effect of tiamulin and chlortetracycline or tiamulinand doxycycline combinations against mycoplasmas. Proceedings of the 10th World Poultry Association Congress, Sydney. pp.167-188.

Burch DGS and Valks M (2002). Comparison of minimal inhibitory concentrations (MIC) against chicken Mycoplasma of tiamulin and other antimicrobials and their concentrations in the blood. Presentation at the World Veterinary Poultry Association, Cairo, Egypt, p.322.

Cinquina AL, Longo F, Anastasi G, Giannetti L and Cozzani R (2003). Validation of a high performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscles. *J. Chromatogr. A.*, **987**: 227-233.

Gingeric DA, Baggot JD and Kowalski JJ (1997). Tylosin antimicrobial and pharmacokinetics in cows. *Canadian. Vet. J*., 18: 96-100.

Hogerzeil HV, Battersby A, Srdanovic V and Stjernstrom NE (1992). Stability of essential drugs during shipment to the tropics. *British Med J.*, **304**: 210-212.

Jordan FT, Forrestor CA, Ripley PH and Burch DG (1998). *In vitro* and *in vivo* comparisons of valnemulin, tiamulin, tylosin, enrofloxacin and lincomycin/ spectinomycin against *Mycoplasma gallisepticum*. *Avian Dis*., **42**: 738-745.

Jordan FTW and Horrocks BK (1996). The minimum inhibitory concentration of tilmicosin and tylosin for *Mycoplasma gallisepticum* and *Mycoplasma synoviae* and a comparison of their efficacy in the control of Mycoplasma gallisepticum in broiler chicks. *Avian. Dis*., **40**: 326-334.

Khan MA, Khan MS, Younus M, Abbas T, Khan I and Khan NA (2006). Short communication comparative therapeutic efficacy of tiamulin, tylosin and oxytetracyline in broilers experimentally infected with mycoplasma gallisepticum. *Int. J. Agriculture & Bio*., Vol: 1560-8530.

Klevan SH and Anderson DP (1997). *In vitro* activities of various antibiotics against Mycoplasma synoviea. *Avian Dis*., **15**: 551-557.

Kleven SH, Swayne DE, Glisson JR, Jackwood MW, Pearson JE and Reed WM (1998b). Mycoplasmosis. A laboratory manual for the isolation of avian pathogens. American Association of Avian Pathologists: Kennett Square, PA., pp.74-80.

Kleven SH (1998a). Mycoplasmas in the etiology of multifactorial respiratory disease. *J. Poult. Sci.,* **77**: 1146-1149.

Kowalsk C and Pomorska M (2006). Evaluation of bioequivalence of two tylosin formulations after oral administration in broiler chickens. *Polonia*, p.61.

Ley DH, Saif YM, Barnes HJ, Glisson JR, Fadly M, McDougald LR and Swayne DE (2003). Mycoplasma gallisepticum Infection. Diseases of Poultry. Eleventh edition. Ames, Iowa, Iowa State Press, A Blackwell Publishing Company, USA. Pp.722-744.

Lin MY (1987). *In vitro* comparison of the activity of various antibiotic and drugs against new Taiwan isolates and standard strain of Avian Mycoplasma. *Avian Dis*., 31: 705-712.

Masland T and Marshall R (1990). The pill pirates. *Newsroeek*., pp.18-23.

McGraw-Hill, Goodman & Gilman's (Ed.10th) (2001). The Pharmacological Basis of Therapeutics, New York.

Mitic SS, Miletic GZ, Kostic DA, Dokic DCN, Arsic BB and Rasic ID (2008). A rapid and reliable determination of doxycycline hyclate by HPLC with UV detection in pharmaceutical samples. *J. Serb. Chem. Soc.,* **73**: 665-671.

Newton NP, Green MD, Fernandez FM, Day NPJ and White NJ (2006). Counterfeit anti-infective drugs. *Lancet Infect Dis.,* 6: 602-613.

Petralanda I (1995). Quality of antimalarial drugs and resistance to Plasmodium vivax in Amazonian region. *Lancet Infect Dis*., **345**: 1433.

Prats C, Francesh R, Arboix M and Perez B (2001). Determination of tylosin residues in different animal tissues by high performance liquid chromatography. *J. Chromatogr. B.,* **766**: 57-65.

Prescott JF and Baggot DJ (1998). Antimicrobial therapy in veterinary medicine. Blackwell Scientific Publication, Boston.

Silverman MM, Lydeckerand M and Lee PR (1990). The drug swindlers. *Int. J. Health Services,* **20**: 561-572.

Stipkovits L, Laber G and Burch DGS (1993). Comparative studies on efficacy of MG bacterin and tiamulin treatment of breeder layers. Proceedings 10th World Veterinary Poultry Association Congress, pp.155-160.

Taha AA, Elsheikh HA and Khalafalla AE (1999). Deposition kinetics of tylosin administration intravenously and intramuscularly in Desert sheep and Nubian goats. *Vet. T*., **158**: 210-215.

Taylor-Robinson D (1967). Mycoplasmas of various hosts and their antibiotic sensitivities. *Postgrad. Med. J*., **43**: 100-104.

Ten HM (1992). Counterfeit drugs: Implications for health. Adverse drug reactions and toxicology. *Reviezos*., **11**: 59-65.

Timms LM, Marshall RN and Breslin MF (1989). Evaluation of the efficact of chortetracyline for the control of chronic respiratory disease caused by E. Coli and Mycoplasma gallisepticum. *Res. Vet. Sci*., **47**: 377-382.

World Health Organization (1992). Counterfeit Drugs. Report of a joint WHOIIFPMA workshop, WHO/ DMP/CFD/92, World Health Organization, Geneva, Switzerland

Wu CC, Shryock TR, Lin T and Faderan M (2000). Antimicrobial susceptibility of *Mycoplasma hyorhinis*. *Vet. Microbiol*., 76: 25-30.

Ziv G (1980). Preliminary clinical pharmacological investigations of tylosin and tiamulin in chickens. *Tijdschr Diergeneeskd*., **105**: 206-210.