Original Article

The use of antibiotics as food additives in poultry and their effect on antibiotic resistance

Musa HA[1], Shikieri AB[2], Alfaki AM[3]

Abstract:
The widespread use and misuse of antimicrobials beyond human medicine, assisted in the alarming emergence of resistance amongst the bacterial strains.

Aim: to determine the effect of the use of antibiotics as food additives on the intestinal flora of poultry.

Methods: one hundred and eighty chickens at the age of two weeks were divided equally into six groups. Five of these groups were used for the test and the sixth served as a control group. Each of the test groups was fed with a different type of antibiotic which served as food additives. The control group was fed an antibiotic-free diet. The antibiotics used were amoxycillin, ciprofloxacin, tetracycline, gentamicin and co-trimoxazole. Rectal swabs were collected from the chickens at the age of two and six weeks respectively. The specimens were processed for the isolation and antibiotic sensitivity of *E. coli* from each group. After slaughtering the chickens, the presence of the antibiotic residue in the tissues was tested. Pieces of tissues were applied on a sensitivity agar using standard *E coli* as a test organism.

Results: significant increase in antibiotic resistance was noticed in the test groups ($P < 0.05$). The change in resistance was insignificant in the control group ($P > 0.05$). The pieces of tissues from the test groups inhibited the growth of *E coli* indicating the presence of antibiotic residue. No inhibition of growth was detected in the control group.

Conclusion: the use of antibiotics as food additives in animals and poultry can lead to the emergence of resistant bacterial strains in their intestinal flora and can leave antibiotic residue in their tissue.

Key words: Antibiotics, Food additives, Poultry.

One of the recent concerning public debates is the use of antibiotics in veterinary practice and agriculture. The reason behind this concern is the fact that the same antibiotics are also used for human therapy. The mechanism of how antibiotics improve the growth of farmed animals or poultry is not fully understood. It is unclear whether these antibiotics inhibit the subclinical infections, suppress the intestinal anaerobic bacteria or enhance the immune system by releasing cytokines [1,2,3].

1. Department of Microbiology, The National Ribat University
2. Department of Community Medicine, The National Ribat University
3. Private Technologist, Sudan
Correspondence. E-mail: hasanaziz15@yahoo.com

In several countries, antibiotics such as penicillin, erythromycin and tetracycline are approved for the growth promotion as well as therapeutic use in animals [3,4]. It is estimated that about half of the total amount of antimicrobials produced globally is used in animals' food. It has also been estimated that approximately 70% of all the antimicrobials used in the United States are administered to animals for non-therapeutic purposes. Moreover, the rise in meat production in many developing countries is mainly due to the intensified farming which is often coupled with the increased use of antimicrobials [5]. Many of the antibiotics including the previously mentioned ones that are given to animals are similar to or closely related to medically important human drugs [6]. Thus it is possible that the irresponsible use of antibiotics for non-human use e.g. as food additive, can lead to the development of resistance which could then be passed to human pathogens [4].

Studies have revealed that 75% of the antimicrobials fed to animals might be excreted unmetabolized into their waste products [7]. Moreover, studies indicated that the antibiotic-resistant bacteria from animals' food may colonize the human gut and transfer resistance genes to ordinary pathogens or other bacteria present in the human gut [8]. The transfer of these genes is thought to cause tremendous harm to human life [8].

Fey *et al* discovered that a strain of salmonella resistant to 13 antibiotics infecting a 12-year old boy was identical to an isolated one from cattle in the father's farm [9]. In the Denmark, an outbreak of a multi-drug resistant salmonella food poisoning was traced back to swine herd [10]. Similarly in Canada, multi-drug resistant bacterial water-born outbreak of enteritis was related to bacterial contamination of the drinking water source from nearby livestock farms [11].

The aim of the current study is to investigate the effects of the use of antibiotics as food additives in the bacterial flora of poultry and to determine the presence of any antibiotic residual in their meat.

Materials and Methods:

One hundred and eighty chickens aged 2 weeks were selected for the study. They were divided into six equal groups and kept in separate cages. Five of these groups were studied for the effect of one type of antibiotic as a food additive and the sixth group served as the control. Rectal swabs were collected from all the chickens and transported to the laboratory for processing. The formers were then inoculated on Mac Conkey's agar and eosin-methylene blue agar for the isolation of *E. coli*.

After an overnight incubation, lactose-fermenting colonies were identified as *E. coli* by their colonial morphology, staining and biochemical tests. Antibiotic sensitivity was carried out according to the NCCL method [12] using tetracycline (30 µg), co-trimoxazole (25 µg), ciprofloxacin (5 µg), gentamicin (10 µg) and amoxycillin (30 µg). The isolated *E. coli* from each group was tested for the sensitivity of only one type of the previously mentioned antibiotics. Each group was then fed with the same antibiotic as a food additive for four weeks.

The isolated *E. coli* from the control chickens were tested for their sensitivity to all antibiotics. During the next four weeks, the control chickens were fed with an antibiotic-free diet. At the end of the four weeks, rectal swabs were collected from all the chickens and processed as described above. Pieces of muscle tissue were taken from the chickens after being slaughtered and applied directly on sensitivity agar plates using standard *E. coli* as a control organism. The plates were then incubated overnight at 37°C and examined for the presence of inhibition zones.

Results:

There was a statistically significant ($P < 0.05$) change in the antibiotic sensitivity of the isolated E. coli from the test groups of chickens at the age of 2 weeks and 6 weeks (Table 1). However, there was no significant change in antibiotic sensitivity of the isolated E. coli from the control group of chickens at 2 and 6 weeks (Table 1).

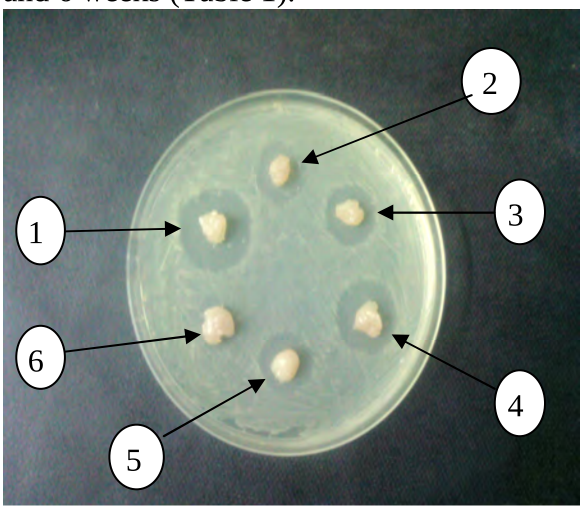

Fig 1: The effect of antibiotic residues, present in chicken's muscle tissues, on standard *E coli* as a control organism.

1-5: pieces of muscles from the chickens fed on antibiotics showing the inhibition zones

6: piece of chicken muscle from the control group showing no inhibition zone

The pieces of muscle tissue from the test groups showed zones of inhibition indicating the presence of antibiotic residue in the chickens' muscle. The pieces of muscles from the control group showed no inhibition zones (Fig 1).

Table 1: Antibiotic sensitivity of the isolated *E. coli* from the control and the test groups of chickens at the age of 2 and 6 weeks*

Antibiotics	Test group		Control group	
	% of resistance before antibiotic (age = 2 wks)	% of resistance after food additive (age = 6 wks)	% of resistance at 2 wks	% of resistance at 6 wks
Amoxycillin	53.3	96.6	36.6	46.6
Ciprofloxacin	3.3	46.6	6.6	13.3
Tetracycline	63.3	96.6	50.0	56.6
Gentamicin	23.3	46.6	40.0	43.3
Co-trimoxazole	56.6	93.3	43.3	50.0

*$P < 0.05$ for the test groups, $P > 0.05$ for the control group

Discussion:

It was perceived that by the discovery of antibiotics, infectious diseases have been conquered and are no longer a major threat to humans' and animals' health. It was soon observed that bacteria could develop resistance to antimicrobial agents [3]. It is well known now that antibiotics are used as food additives for animals to promote growth and enhance meat production [6]. There are more than 24 antimicrobials available for veterinary use and as much as 16 of them are used in human medicine. The allowed concentration of antibiotics as growth promoters is relatively low (2.5-12.5mg/kg) depending on the type of the antibiotic and the animal species.

The World Health Organisation fact sheet reported that some of the newly emerging resistant bacteria in animals are transmitted to human beings mainly via food of animal origin or through direct contact with farm animals [14]. In the current study, the detection of the high rate of resistance amongst the isolates of *E. coli* from chicken after the use of antibiotics as food supplement is not surprising. Hence, it could be predicted that the increase in antibiotic resistance might occur when antibiotics are used for meat production in poultry. The close contact of humans who are caring for chicken may expose the formers to highly resistant faecal bacteria.

Bogaard in the Netherlands found that the enterococci isolated from faecal samples from poultry, poultry farmers and poultry slaughterers were highly resistant to antibiotics [14]. Moreover, in Ethiopia, Molla and Kleer related the high resistance of salmonella isolated from human samples to the resistance in salmonella isolated from chicken [15]. From the current study, it was clear that the use of antibiotics as food additives in poultry leaves antibiotic residue in their tissues. Therefore, the consumption of such chicken will expose the human beings to antibiotics that may affect the resistance of their intestinal normal flora.

Conclusion:

The use of antimicrobials as food additives in poultry production and prophylaxis can contribute to the development of antibiotic resistance in the microflora of poultry as well as human beings. Hence, all the opportunities to reduce and limit the use of antibiotics in poultry production and the overuse and misuse of antibiotics in general, should be encouraged so as to prevent the appearance of multidrug-resistant bacteria.

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