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A comparison of antibiotic resistance in microorganisms isolated from chicken and ostrich faeces in Bulawayo, Zimbabwe

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ABSTRACT

Samples of fresh faeces were obtained from a free-range chicken source, three commercial chicken farms and a commercial ostrich farm, all located around Bulawayo City, Zimbabwe, in order to determine the antibiotic resistance profile of selected bacterial isolates of interest in food-related human infections. Samples were prepared at various dilutions and plated on selective media for Coryneforms, *Escherichia coli, Enterococcus faecalis* and *Pseudomonas*. The targeted bacteria were isolated as pure cultures and tested for antibiotic resistance to ampicillin, chloramphenicol, oxytetracycline, sulphonamide, streptomycin and tetracycline. Isolates from the faeces of chickens and ostriches in the commercial farms were found to be generally more resistant to streptomycin, tetracycline and oxytetracycline as compared to those from the free-range chickens. This study emphasizes the need to monitor antibiotic resistance genes in the environment and to curb/curtail antibiotic use for growth promotion in farm animals, particularly in developing countries, as continued use will only add to the growing problem of microbial antibiotic resistance.

Keywords: *Escherichia coli*, *Pseudomonas*, Coryneforms, *Enterococcus faecalis*, chicken, ostrich, faeces.

INTRODUCTION

There have been concerted efforts to draw attentions to the critical problem of microbial resistance to antibiotics in recent times (Amy et al., 2006). This is so because the development of antibiotic resistance among pathogenic bacteria is a major public health concern (Smith et al., 2002, Ohlsen et al., 2003). These antimicrobial agents that have human analogues increase the likelihood that bacterial pathogens in foods of animal origins will develop cross-resistance to antimicrobial agents used in human medicine (Anderson et al., 2003; Hayes et al., 2004).

The widespread use of antibiotics in medicine and intensive animal husbandry exerts selection pressure on resistant bacteria (Schwartz et al., 2003), thus, the rise of antibiotic resistance is considered to be closely linked with the widespread use of antibiotics in humans and animals (Amy et al., 2006) and is now recognized as a global problem, spreading so rapidly that there is a real threat of drifting back to the pre-antibiotic era (Chander at al., 2007). Antibiotics are used in agriculture for therapy and as growth promoters in low concentrations (Quinsey et al., 1990). It has been estimated that more than one half of the antibiotics used in the United States of America are administered to livestock for the purpose of growth promotion or infection treatment (Gaskins et al., 2002; Amy et al., 2006). Unfortunately, such uses have resulted in the emergence of antibiotic resistance in pathogenic bacteria (Lukasova and Sustackova, 2002), a phenomenon that has increased to the point of complicating therapy of diseases of farm animals (Quinsey et al., 1990).

Almost all animal feeds on the market contain antibiotics such as chlortetracycline, procaine penicillin and oxytetracycline alone or bacitracin and streptomycin in combination with other compounds (Quinsey et al., 1990) and it is thought to result directly in antibiotic resistant Coryneforms.

The concern over microbial antibiotic resistance has led to the banning of antibiotic use in feeds for poultry and livestock in various countries of Europe (Hayes et al., 2004) as well as in the U.S.A. Not much has been done in Africa, although countries such as Zimbabwe, that have their livestock and poultry markets in the European Union, have implemented the ban to some extent in order to have their products accepted (Poultry Producers Association of Zimbabwe, personal communication).

The current study therefore aimed to check the levels of antibiotic resistance and determine the differences in the proportions of antibiotic resistant bacteria isolated from commercial ostriches, free-range chickens and commercial poultry farms around Bulawayo, Zimbabwe.

MATERIALS AND METHODS.

Sample collection

Fresh droppings were aseptically picked from the pens of commercial chickens and ostriches while those of free-range chickens were collected from their feeding grounds and the chicken run.

Isolation and identification of bacteria

The work was carried out in the Department of Applied Biology and Biochemistry, National University of Science and Technology, Bulawayo, Zimbabwe.

Samples were weighed and suspended in ¼ strength Ringer's solution. Serial dilutions were prepared up to 10^{-5}. A volume of 0.1 ml of each of the 10^{-3}, 10^{-4}, and 10^{-5} dilutions were plated out on Cysteine Lactose Electrolyte Deficient (CLED) medium and incubated at 37 °C for 72 hours. Plates with the best isolates were selected and the isolates were streaked out to obtain pure cultures. These were kept on nutrient agar slants with 0.4% glucose. The colours of the selected organisms were noted. Typical colonies for the organisms under investigation were confirmed by the Gram stain. Gram -positive rods and club shaped organisms were subjected to the catalase test. Gram -negative rods were subjected to growth at 4 and 42 °C, urease activity, and gelatin liquefaction. Gram-negative rods that could not liquefy gelatin and showed no urease activity were subjected to the methyl red and Voges-Proskauer tests as well as indole, catalase and growth at 44 °C.

Antibiotic resistance evaluation

Confirmed isolates of *E. coli*, *E. faecalis*, *Pseudomonas* and Coryneforms were tested against 250 µg/ml tetracycline, oxy-tetracycline, sulphonamide, streptomycin, chloramphenicol and ampicillin using the disc diffusion method (Quinsey et al., 1990), and zones of inhibition noted.

Statistical analysis

Data was computed as mean ± SD and Student's t test was used to test for significance. A p value of <0.05 was considered statistically significant.

RESULTS

Based on their cultural and biochemical characteristics, four groups of bacterial isolates, which are common organisms in the poultry industry, were selected for resistance studies. These are *Escherichia coli, Enterococcus faecalis, Pseudomonas sp* and Coryneforms (Table 1).

E. coli isolated from commercial chicken and commercial ostrich faeces were more resistant to ampicilin when compared to that isolated from free-range chicken faeces ($p < 0.05$). No statistically significant differences in *E. coli* resistance to chloramphenicol, streptomycin, oxytetracyclin and sulphonamide were seen in free-range chicken, commercial chicken and commercial ostrich faeces (Fig 1).

Fig 2 shows a comparison of antibiotic activity of *E. faecalis.* Non-significantly ($p > 0.05$) higher resistance of *E. faecalis* to ampicilin was observed in isolates from commercial chicken and commercial ostrich faeces when compared to isolates from free-range chicken faeces. Significantly ($p < 0.05$)

Table 1: Appearance of microorganisms grown on CLED for 72 hours at 37 °C.

Colony Description	Species	AFRC	ACC	AO
Dark yellow centered colonies	*E. coli*	+ + + +	+ + + +	+ + + +
Green colonies with rough periphery	*Pseudomonas*	+ + +	+ +	+ +
Yellow colonies with diameter about 0.5 mm	*E. faecalis*	+ +	+ + +	+
Small grey colonies	Coryneforms	+	+	+

AFRC = Abundance in free range chickens; ACC = Abundance in commercial chickens; AO = Abundance in ostriches

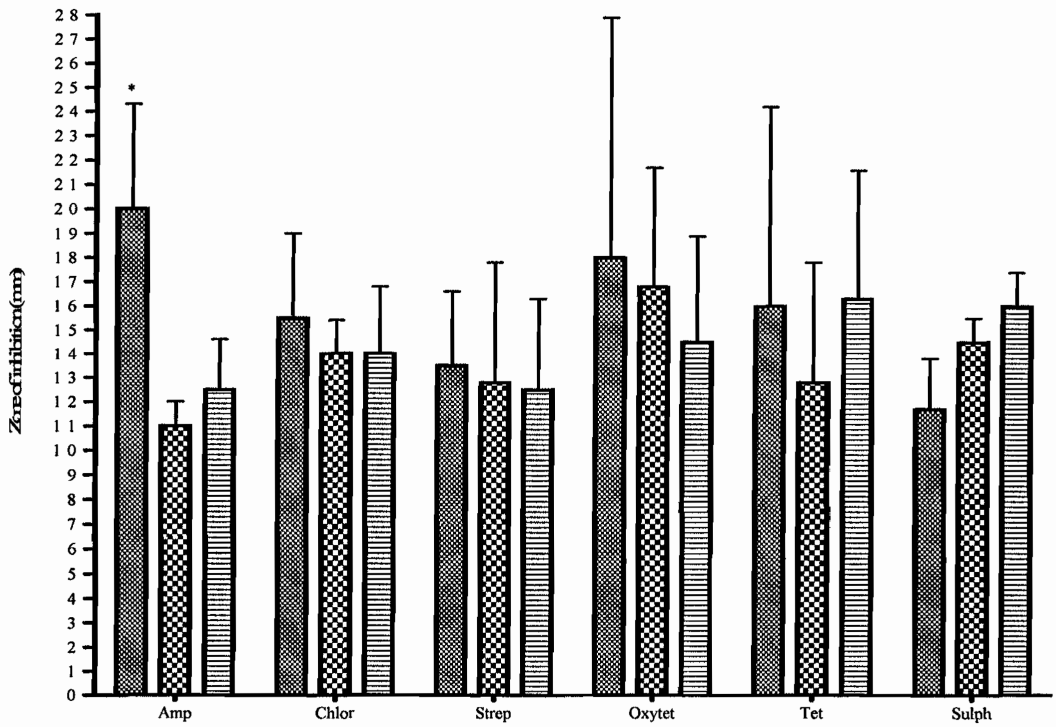
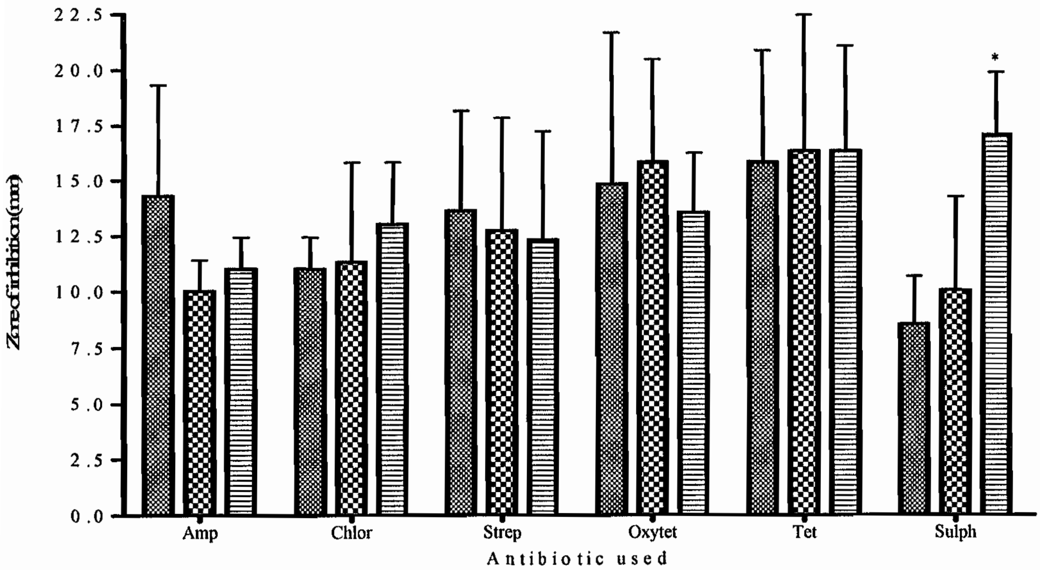


Fig 1. A comparison of antibiotic activity on *E. coli* isolated from free range chicken , commercial chicken and commercial ostrich faeces. Values are means ± SD. * $p < 0.05$ significantly different from commercial chicken and ostrich, Student's t test.

higher resistance to sulphonamide was, however, observed in isolates from commercial ostrich faeces when compared to those from free-range and commercial chickens (Fig 2). No differences in resistance to the other antibiotics were observed.

Pseudomonas sp isolated from faeces of commercial chicken and commercial ostrich showed significant ($p < 0.05$) resistance to ampicilin when compared to that isolated from faeces of free-range chicken (Fig 3). However, higher resistance to chloramphenicol was exhibited by isolates from free-range chickens when compared to the isolates from commercial chickens. No difference in resistance was observed for the other antibiotics.

Resistance to ampicilin in Coryneforms isolated from free-range chicken faeces was significantly ($p < 0.05$) higher when compared to isolates from faeces of commercial chicken and commercial ostriches (Fig 4). No differences in resistance to the other antibiotics were observed.



Amp = ampicilin Chlor = chloramphenicol Strep = Streptomycin
Oxytet = oxytetracyclin Tet = tetracyclin Sulph = sulphonamide

Fig 2. A comparison of antibiotic activity on *E. faecalis* isolated from free range chicken, commercial chicken and commercial ostrich faeces. Values are means ± SD. *$p < 0.05$ significantly different from commercial and free range chicken, Student's t test.

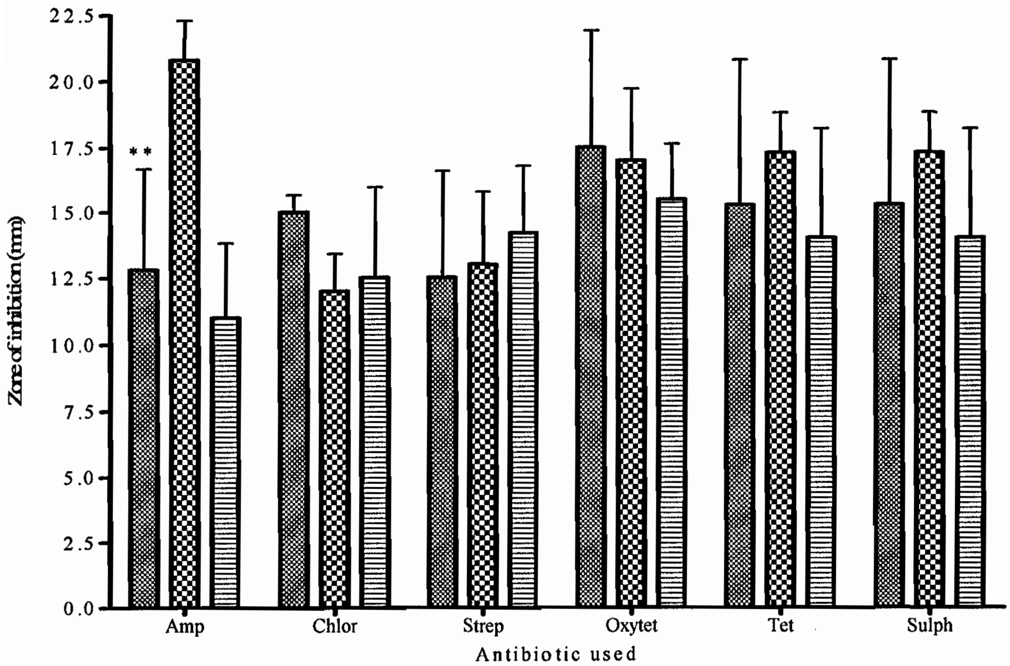
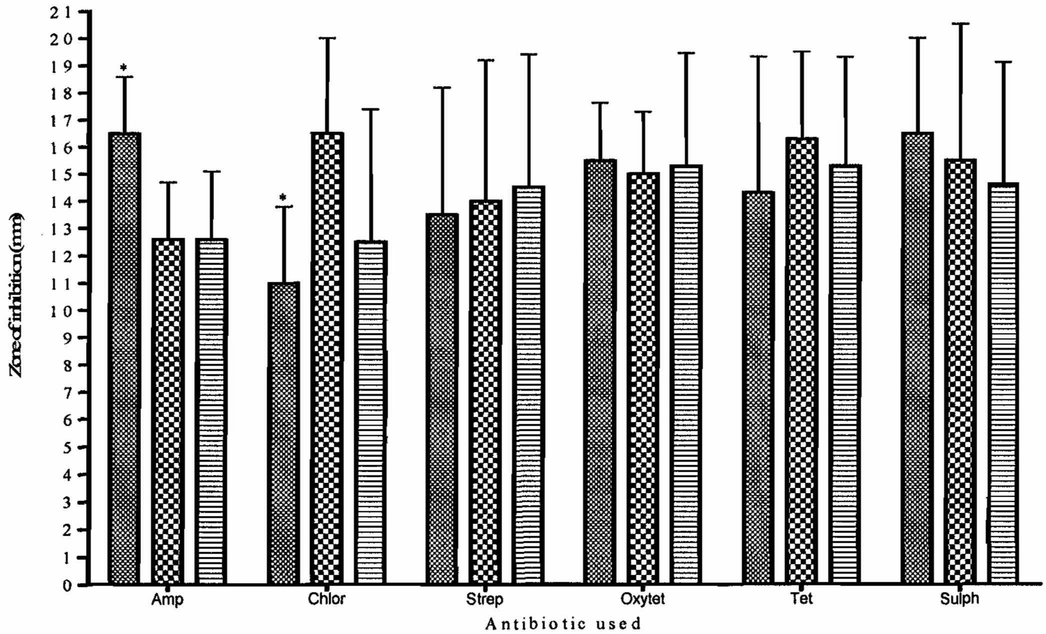


Fig 3. A comparison of *antibiotic* activity on Coryneforms isolated from free range chicken, commercial chicken and commercial ostrich faeces. Values are means, ± SD. **$p < 0.05$ significantly different from commercial chicken, Student's t test.



Amp = ampicilin Chlor = chloramphenicol Strep = Streptomycin
Oxytet = oxytetracyclin Tet = tetracyclin Sulph = sulphonamide

Fig 4. A comparison of antibiotic activity on *Psedomonas sp* isolated free range chicken , commercial chicken and commercial ostrich faecess. Values are means ± SD. *$p < 0.05$ significantly different from commercial chicken and ostrich, Student's t test.

DISCUSSION

Antibiotic resistance genes are currently considered as emerging environmental contaminants (Amy et al., 2006). Indeed, Amy et al. (2006) observed that as with other dangerous pollutants that spread in the environment and threaten human health, there is a need for concerted effort to help address the critical problem of microbial resistance to antibiotics.

All the targeted bacterial isolates were abundantly present in the faeces of the birds studied (Table 1) with the possibility of widespread contaminations of the environment. As may be noted from the figures, all the isolates showed varying degrees of resistances to the antibiotics tested.

Resistance to ampicillin was higher in the *E. coli, Pseudomonas sp* and *E. faecalis* isolates from the commercial chickens, and commercial ostriches. This result would be expected as animals reared under commercial conditions are exposed to the antibiotic for treatment of some ailments. It follows that the bacteria would have developed resistance as a result of this exposure.

Pseudomonas sp. isolated from free-range chicken faeces showed higher chloramphenicol resistance when compared to commercial chicken. Similarly, *E. faecalis* and Coryneform isolates from free-range chicken faeces showed higher resistance to sulphonamide and ampicilin when compared to isolates from commercial chicken faeces. This is not unexpected because such birds have unhindered access to the environment, particularly agriculture-influenced and urban-influenced treated and untreated water. Such water samples have all been reported to harbour either bacteria, which carry (multiple) antibiotic resistance genes, or plasmids carrying these genes (Schwartz et al, 2003; Pei et al., 2006; Amy et al., 2006). Improper use and disposal of the antibiotics lead to residues of these being released to the environment. Ampicilin, chloramphenicol and sulphonamide are common antibiotics used by humans for various ailments.

Previous studies have established strong links between the rise of antibiotic resistance and the widespread use of antibiotics in humans and animals (Teuber, 2001; Gaskins et al, 2002; Smith et al., 2002; Schwartz et al., 2003), thus justifying a need for restriction of widespread, non-medical uses of antibiotics. The restrictions of antibiotic use are intended to preserve the effectiveness of antibiotics for human use. Low level feeding of antibiotics to animals creates the ideal conditions for the development of antibiotic resistant bacteria.

According to Smith et al. (2002), agricultural antibiotic use may cause antibiotic-resistant bacterial infection in humans by two different processes. First, agricultural antibiotic use increases the frequency of antibiotic resistance in zoonotic pathogens which are typically acquired through exposure to contaminated animal food products, and secondly, antibiotic resistant bacteria from foods of animal origin may facilitate the development of antibiotic resistance in human commensal bacteria which ordinarily colonize humans without causing infections. Commensal bacteria typically have long persistence times; frequent human-to-human transmission and high bacterial loads that are associated with good health, but not disease. If such antibiotic resistant bacteria eventually infect humans, for example immuno-compromised hosts as in HIV/AIDS patients, they can result in severe or fatal illness.

In the present study, no other differences in resistance of organisms isolated from faeces of free-range chicken, commercial chicken and commercial ostrich were observed. This could be as a result of the fact that the antibiotics are more available (hence the increase in the desire to use them i.e. antibiotic-use pressure) in the environment either due to their well known inclusion in animal feeds (Quinsey et al., 1990) or as a result of direct environmental contamination (Amy et al., 2006). The almost uniform behaviour of the bacterial isolates from commercial farm birds to the antibiotics known to be widely used in commercial agriculture (streptomycin, oxytetracycline and tetracycline as growth promoters) may be attributed to the fact that they must have all been exposed to either antibiotic-enriched feed lots which are readily available in sale outlets in Bulawayo or through treatment for various ailments.

There is therefore a need to restrict the inappropriate use of antibiotics in both the agricultural and medical sector as this can save lives and costs. Perhaps it is time alternative treatment regimes were sought for poultry such as the traditional African treatment of poultry using crushed aloe in water to treat various poultry ailments. This seems to be or have been quite effective (Masika and Afolayan, 2003).

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