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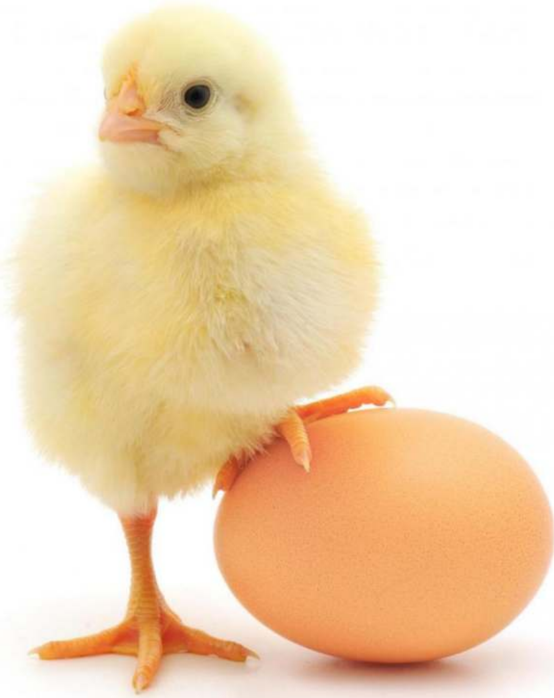

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Antibiotics Susceptibility Pattern of *Escherichia coli* Strains Isolated from Chickens with Colisepticemia in Tabriz Province, Iran

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Abstract: Antimicrobial agents are used extremely in order to reducing the enormous losses caused by *Escherichia coli* infections (colibacillosis) in Iran poultry industry. In this investigation fifty avian pathogenic *Escherichia coli* (APEC) strains isolated from broiler chickens with colisepticemia and examined for susceptibility to antimicrobials of veterinary and human significance. *In vitro* antibiotic activities of 32 antibiotic substances against the isolates were determined by disc diffusion test (Kirby Bauer method). Multiple resistances to antibiotics were observed in all the isolates. The highest rate of resistance was against Nalidixic acid (100%), Lincomycin (100%), Erythromycin (97%), Oxytetracycline (95%), Chlortetracycline (95%), Tetracycline (94%), Flumequine (94%), Tiamulin (91%), Doxycycline (88%), Difloxacin (83%), Neomycin (81%), Streptomycin (81%), Trimethoprim-Sulphamethoxazole (80%), Kanamycin (77%), Enrofloxacin (76%), Norfloxacin (68%), Ciprofloxacin (67%), Chloramphenicol (67%), Furazolidone (66%), Nitrofurantoin (56%), Amoxicillin (53%) and Ampicillin (47%). Resistance to Gentamicin wasn't observed and to Amikacin, Cefazolin, Colistin, Tobramycin, Ceftizoxime, Cefixime, Lincospectin, Ceftazidime and also Florfenicol were low. This study showed resistance rate against the antibiotics that are commonly used in poultry is very high but against them that are only used in human or less frequently used in poultry is significantly low. These findings confirm significant increase in the incidence of antimicrobial resistance in the *E. coli* strains is most probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry. This study also showed that the prevalence of Quinolone-Resistant *Escherichia coli* (QREC) is very high in broiler farms in Tabriz province. The high presence of QREC from broiler chickens probably is due to overuse of enrofloxacin in these farms for therapeutic purposes. The present study suggests introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed because other than animal health problems, transmission of resistant clones and resistance plasmids of *E. coli* from food animals (especially poultry) to humans can occur.

Key words: Antibiotics susceptibility, *Escherichia coli*, chicken, colisepticemia, Iran, Tabriz

Introduction

Escherichia coli is the most important agent causing secondary bacterial infection in poultry and may also be a primary pathogen (Gross, 1994). Colibacillosis is the most frequently reported disease in surveys of poultry diseases or condemnations at processing (Saif, 2003). Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia coli* (APEC), including colisepticemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), cellulites (inflammatory process), swollen-head syndrome, peritonitis, salpingitis, osteomyelitis/synovitis (turkey osteomyelitis complex), panophthalmitis, and omphalitis/yolk sac infection (Saif, 2003). Colisepticemia is the most common form of colibacillosis and is responsible for significant economic losses in aviculture in many parts of the world (Barnes and Gross, 1997; Ewers, *et al*., 2003). In the past few years, both the incidence and severity of colibacillosis have increased rapidly, and current trends indicate that it is likely to continue and become an even greater problem in the poultry industry (Altekruse *et al*., 2002; Blanco, 1997).

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed *et al*., 1993; Goren, 1990; Watts *et al*., 1993). *E. coli* may be sensitive to many antibiotics. However, isolates of E. coli from poultry are frequently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over a long period (e.g., tetracyclines) (Allan *et al*., 1993; Blanco *et al*., 1997; Watts *et al*., 1993). Antibiotics once effective at controlling *E. coli* infections are now ineffective due to the bacterium's acquired resistance to these compounds. Resistance to two or more classes of antibiotics is now commonplace in both veterinary (Gonzalez and Blanco, 1989; Harnett and Gyles, 1984; Irwin *et al*., 1989) and human (Dennesen *et al*., 1998) medicine.

Resistance generally occurs following response to prior contact with the antimicrobial but can occur naturally in the absence of previous exposure. Resistance to florfenicol, an antibiotic related to chloramphenicol that has never been used in poultry in the United States was found in *E. coli* isolates from chickens (Keyes *et al.,* 2000).

Antibiotic usage is possibly the most important factor that promotes the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Neu, 1992; Witte, 1998). Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals (animals and humans) or populations (Piddock, 1996; Hinton *et al*., 1982).

Concern has been expressed about possible harmful effects on humans through the use of drugs in agriculture, as follows: 1) increased microbial drug resistance, 2) drug residues in food, 3) allergic reactions and sensitisation to antimicrobials, and 4) drug toxicity (Bazile-Pham-Khac *et al*., 1996). Concern about antibiotic resistance and its transmission to human pathogens is important because these resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. Colonization of the intestinal tract with resistant *E. coli* from chicken has been shown in human volunteers (Linton *et al*., 1977). The result of a study was done by van den Bogaard *et al*. (2001) strongly indicates that transmission of resistant clones and resistance plasmids of *E. coli* from poultry to humans commonly occurs (van den Bogaard, 2001). Furthermore, in some previous studies, spread of an antibiotic resistance plasmid, pSL222-6, in *E. coli* from chickens to human handlers (Levy, 1976), finding the same O serotype in chickens from a commercial rearing centre, in oven-ready birds and in humans (Linton *et al*., 1977; Linton, 1977), direct transmission of *E. coli* resistant to streptomycin, sulphonamides and tetracycline from poultry to poultry attendants in Nigeria (Ojeniyi, 1985, Ojeniyi, 1989), evidence that animals are a reservoir for *E. coli* found in humans(Cooke, 1971), chickens as a source of antibiotic resistance in humans in Saudi Arabia, Morocco and northern India (Al Ghamdi *et al.,* 1999; Amara *et al*., 1995; Singh *et al*., 1992) was described. Of course, in contrast, others have concluded that human and poultry isolates belong to two distinct pools of resistant *E. coli* (Bass *et al*., 1999; Caya *et al.,* 1999; Kapoor *et al*., 1978; Kariuki *et al*., 1997; Nijsten *et al*., 1995; Parsonnet and Kass, 1987; Shooter *et al*., 1974; Smith, 1969).

On the other hand, fluoroquinolones are highly efficacious antimicrobial agents, which because of their low toxicity and relatively broad-spectrum coverage, are extremely valuable for treating human infections (Angulo, *et al.,* 2000; Livermore *et al.,* 2002). In the past years they are used in treatment of certain bacterial diseases in animals, including acute bovine respiratory disease and avian colibacillosis (Hooper, 2001; White *et al*., 2000; Yang *et al*., 2004). However, a growing number of studies report an association between the emergence of fluoroquinolone-resistant zoonotic pathogens, such as *Salmonella*, *E. coli*, and *Campylobacter*, and the subsequent approval and use of these agents in veterinary medicine (Blanco *et al.,* 1997; Guerra *et al.,* 2003; Saenz *et al.,* 2003; Yang *et al.,* 2004). Garau *et al.* (1999) suggested that the high prevalence of fluoroquinolone-resistant avian *E. coli* in the stools of healthy humans in their area (Barcelona, Spain) could be linked to the high prevalence of resistant isolates in poultry and pork (Garau *et al*., 1999).

In this study, *E. coli* strains isolated from broiler chickens having died from colisepticemia were analyzed to determine their susceptibilities to antimicrobial agents used in veterinary and human.

Materials and Methods

Bacterial isolates: Isolation and identification of *E. coli* were performed by standard bacteriological methods. Specimens were cultured on McConkey and EMB agar and the colonies suspected to be *E. coli* were identified by standard methods (27, 80 and 81). 50 strains of *E. coli* were isolated from 23 Commercial broiler flocks, from July 2005 till October 2005 in Tabriz province of Iran. The number of isolates ranged from 1 to 5 per flock. All strains were isolated from broiler chickens that had died from colisepticemia in which bacteria were obtained in profuse culture both from heart and liver. The chickens were between 14 to 55-days of age.

Antimicrobial susceptibility determination: Antimicrobial susceptibility determination was routinely tested by the single-disc diffusion method (10). The *E. coli* strains were tested against the antibiotics of human and veterinary significance. The following antibiotic discs on Mueller Hinton agar were applied: Amikacin (AN/30 µg), Ampicillin (AM/10 µg), Amoxicillin (AMX/30 µg), Cefazolin (CZ/30 µg), Cefixime (CFM/5 µg), Ceftazidime (CAZ/30 µg), Ceftizoxime (CT/30 µg), Chloramphenicol (C/30 µg), Chlortetracycline (CTe/30µg), Ciprofloxacin (CP/5 µg), Colistin (Cl/10 µg), Difloxacin (DIF/25 µg), Doxycycline (D/30 µg), Enrofloxacin (NFX/5 µg), Erythromycin (E/15 µg), Florfenicol (FFc/30 µg), Flumequine (Flu/30 µg), Furazolidone (FR/100 µg), Gentamicin (GM/10 µg), Kanamycin (K/30 µg), Lincomycin (L/2 µg), Lincospectin (LIN/SE), Nalidixic acid (NA/30 µg), Neomycin (N/30 µg), Nitrofurantoin (FM/300 µg), Norfloxacin (NOR/10 µg), Oxytetracycline (T/30 µg), Streptomycin (S/10 µg), Tetracycline (TE/30 µg), Tiamulin (TM/ 30µg), Tobramycin (TOB/10 µg) and Trimethoprim-Sulphamethoxazole (SXT/25 µg).

The diameters of the zones of inhibition were interpreted by referring to the table which represents the NCCLS subcommittee's recommendation (NCCLS, 2001).

Results

The highest rate of resistance was against Nalidixic acid (100%), Lincomycin (100%), Erythromycin (97%), Oxytetracycline (95%), Chlortetracycline (95%), Tetracycline (94%), Flumequine (94%), Tiamulin (91%), Doxycycline (88%), Difloxacin (83%), Neomycin (81%), Streptomycin (81%), Trimethoprim-Sulphamethoxazole (80%), Kanamycin (77%), Enrofloxacin (76%), Norfloxacin (68%), Ciprofloxacin (67%), Chloramphenicol (67%), Furazolidone (66%), Nitrofurantoin (56%), Amoxicillin (53%), and Ampicillin (47%). Low levels of resistance were against Florfenicol (27%), Ceftazidime (18%), Lincospectin (15%), Cefixime (14%), Ceftizoxime (7%), Tobramycin (7%), Colistin (6%), Cefazolin (4%), Amikacin (3%), and Gentamicin (0%). Susceptible(S), intermediate (I) and resistant (R) percentages of the isolates to the antimicrobial agents were showed in Table 1. Multiple resistances were observed in all of the isolates.

Discussion

Colibacillosis is the primary cause of morbidity, mortality, and condemnation of carcasses in the poultry industry worldwide (Allan *et al.*, 1993; Cloud *et al.*, 1985; Goren, 1990) and colisepticemia is the most usual form of colibacillosis and is responsible for significant economic losses in poultry industry (Barnes and Gross, 1997; Ewers *et al.*, 2003). To control and prevent poultry diseases especially colibacillosis, subtherapeutic and therapeutic levels of antimicrobial agents are administered to chickens via food and water. This practice also improves feed efficiency and accelerates weight gain (Bower and Daeschel, 1999). The treatment of whole flocks with antimicrobials for disease prevention and growth promotion has become a controversial practice (van den Bogaard *et al.*, 1999; Witte, 1998). However, administration of antimicrobial agents provides a selective pressure which causes detection of resistant bacteria. Therefore, the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently their faecal flora contains a relatively high proportion of resistant bacteria and resistance to existing antimicrobials is widespread and of concern to poultry veterinarians (Cloud *et al.*, 1985; Goren, 1990; Hinton *et al.*, 1987; Peighambari *et al.*, 1995).

There is also concern that antimicrobial use in food animals can lead to the selection of antimicrobial resistant zoonotic enteric pathogens which may then be transferred to people by the consumption of contaminated food or by direct animal contact. At slaughter, resistant strains from the gut readily soil poultry carcasses and as a result poultry meats are often contaminated with multi-resistant *E. coli* (Caudry and Stanisich, 1979; Turtura *et al.*, 1990); likewise eggs become contaminated during laying (Lakhotia and Stephens, 1973). Hence, resistant faecal *E. coli* from poultry can infect humans both directly and via food. These resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. It was conclusively shown by Linton (1977) that antibiotic-resistant *E. coli* could be transferred from poultry to a food-handler's hands during food preparation and, finally, to the foodstuff (Linton, 1977). The transmission of enteric bacteria to consumers via this route has been established, and prevention of food poisoning is the basis for food hygiene and public health regulations in many countries (Piddock, 1996).

In this study, multiple antibiotic resistance was observed in all of the examined strains similar to the findings of previous studies had done in Iran and other countries (Bass *et al.*, 1999; Bazile-Pham-Khac *et al.*, 1996; Blanco *et al.*, 1997; Guerra *et al.*, 2003; Miles *et al.*, 2006; Saenz *et al.*, 2003; Zahraei Salehi, 2005). Almost all the *E. coli* isolates showed high percentage of resistance to the antibiotics. High levels of resistance were against Nalidixic acid (100%), Lincomycin (100%), Erythromycin (97%), Oxytetracycline (95%), Chlortetracycline (95%), Tetracycline (94%), Flumequine (94%), Tiamulin (91%), Doxycycline (88%), Difloxacin (83%), Neomycin (81%), Streptomycin (81%), Trimethoprim-Sulphamethoxazole (80%), Kanamycin (77%), Enrofloxacin (76%), Norfloxacin(68%), Ciprofloxacin(67%), Chloramphenicol (67%), Furazolidone (66%), Nitrofurantoin (56%), Amoxicillin (53%) and Ampicillin (47%). Low levels of resistance were against Florfenicol (27%), Ceftazidime (18%), Lincospectin (15%), Cefixime (14%), Ceftizoxime (7%), Tobramycin (7%), Colistin (6%), Cefazolin (4%), Amikacin (3%), and Gentamicin (0%). So far, Tetracyclines, Enrofloxacin, Streptomycin, Neomycin, Tiamulin, Flumequine, and Trimethoprim-Sulphamethoxazole were extremely used in Tabriz poultry industries. For this reason, these antibiotics are inactive against avian pathogenic *E. coli* strains at the present time. Despite the fact that administration of Chloramphenicol and Furazolidone is forbidden in veterinary, resistance to this antibiotics was high. This is probably because of persistence of previous resistances or illegal use of these agents. At the beginning of this study, resistance rate against Florfenicol (fluorinated analogue of chloramphenicol) that has been used in Tabriz poultry industries only one year ago (2004), was low but at the end (only four months later); isolation of resistant *E. coli* stains were significantly high. This event was due to extremely use of Florfenicol for treatment of the disease in poultry because of its very good effect against *E. coli*. Ceftazidime, Cefixime, Ceftizoxime, Cefazolin,

Table 1: Percentages of antibiotic susceptibility of isolated *E. coli* from broiler with colisepticemia in Tabriz province of Iran. Susceptible (S), intermediate (I) and resistant (R), to antimicrobial agents by disc diffusion method

Class and Antibiotic (Abbreviation)	Disc content (µg)	Diffusion zone breakpoint (mm) (NCCLS breakpoint)	S (%)	I (%)	R (%)
ß-lactam					
Penicillins					
Ampicillin (AM)	10	≤ 13	31	22	47
Amoxicillin (AMX)	30	≤ 14	9	38	53
Cephalosporins					
Cefazolin (CZ)	30	≤ 14	82	14	4
Cefixime (CFM)	5	≤ 15	68	18	14
Ceftazidime (CAZ)	30	≤ 14	61	21	18
Ceftizoxime (CT)	30	≤ 14	89	4	7
Polymyxins					
Colistin (Cl)	10	8	55	39	6
Aminoglycosides					
Amikacin (AN)	30	≤ 14	84	13	3
Gentamicin (GM)	10	≤ 12	97	3	0
Kanamycin (K)	30	≤ 13	13	10	77
Neomycin (N)	30	≤ 12	11	8	81
Streptomycin (S)	10	≤ 11	19	0	81
Tobramycin (TOB)	10	≤ 12	91	3	6
Tetracyclines					
Doxycycline (D)	30	≤ 12	8	4	88
Chlortetracycline (Cte)	30	≤ 14*	0	5	95
Oxytetracycline (T)	30	≤ 14	3	2	95
Tetracycline (TE)	30	≤ 14	6	0	94
Nitrofurans					
Nitrofurantoin (FM)	300	≤ 14	35	9	56
Furazolidone (FR)	100	≤ 14	31	3	66
Fenicols					
Chloramphenicol (C)	30	≤ 12	31	2	67
Florfenicol (Ffc)	30	≤ 19*	68	5	27
Lincosamides					
Lincomycin (L)	2	≤ 14	0	0	100
Lincospectin (LIN/SE)	150	≤ 19*	85	**	15
Macrolides					
Erythromycin (E)	15	≤ 13	0	3	97
Pleuromutilins					
Tiamulin (TM)	30	≤ 16	0	9	91
Quinolones and Fluoroquinolones					
Quinolones					
Flumequine (Flu)	30	19*	6	**	94
Nalidixic acid (NA)	30	≤ 13	0	0	100
Fluoroquinolones					
Ciprofloxacin (CP)	5	≤ 15	17	16	67
Difloxacin (DIF)	10	≤ 17	14	3	83
Enrofloxacin NFX)	5	≤ 16	12	12	76
Norfloxacin (NOR)	10	≤ 12	24	8	68
Sulfonamides and potentiated sulfonamides					
Trimethoprim-Sulphamethoxazole (SXT)	25	≤ 10	15	5	80

* No NCCLS breakpoint. ** The differentiation between intermediate and resistant was not possible because of the limited range of the antibiotic tested.

Tobramycin and Amikacin commonly used in human but aren't used in Tabriz poultry industries, also only available drug composition for Lincospectin and Gentamicin is injectable solution and they aren't used as mass medication in poultry. This subject can explain the high susceptibility rate of the *E. coli* strains for these agents. Kanamycin is also used only in human but high level resistance against it is probably due to cross resistance. Kanamycin is susceptible to the largest number of enzymes but conversely, resistance to Gentamicin is mediated by modifications at few sites on the molecule. These findings confirm significant increase in the incidence of antimicrobial resistance in the *E. coli* strains is most probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry.

Perhaps the most striking finding from this study was the widespread resistance to quinolones and fluoroquinolones. This study showed that the prevalence of Quinolone-Resistant *Escherichia coli* (QREC) is very high in our area farms. Resistance to Nalidixic acid, Flumequine, Difloxacin, Enrofloxacin, Norfloxacin and Ciprofloxacin were 100%, 94%, 83%, 76%, 68% and 67%, respectively. Although identification of fluoroquinolone-resistant avian *E. coli* isolates has been reported in other places, for example in Saudi Arabia, Spain, the United States and China (Allan *et al.*, 1993; Blanco *et al.*, 1997; Saenz *et al.*, 2003, White *et al.*, 2000; Yang *et al.*, 2004). To our knowledge, this is the highest prevalence of resistance to quinolones in *E. coli* that has ever been reported. This high presence of QREC from the broiler chickens probably is due to overuse of enrofloxacin in this animal population for therapeutic purposes in our area. Consequently, fluoroquinolones have become ineffective in treatment of colibacillosis in Tabriz poultry flocks. Resistance to fluoroquinolonse in these isolates, coupled with the observation of widespread multiple-antimicrobial resistance in all of the isolates.

There is mounting evidence that antimicrobial use in veterinary medicine may select for antimicrobial-resistant zoonotic bacterial pathogens (e.g., *Salmonella* and *Campylobacter*). This has led to increased pressure to limit fluoroquinolones in animals to preserve the value of these drugs in the treatment of human infections but unfortunately, there is no any program to restriction antibiotic administration in veterinary in Iran and other under developing countries (Endtz *et al.*, 1991; Glynn *et al.*, 1999; Smith *et al.*, 1999). In addition to the human health concerns, antimicrobial-resistant pathogens also pose a severe and costly animal health problem in that they may prolong illness and decrease productivity through higher morbidity and mortality (Xu, 2001). Therefore the introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria strongly is needed.

The major factor selecting for antimicrobial resistance in bacteria is antibiotic use, and additionally, crowding and poor sanitation. These three factors are typical of intensive poultry farming and explain the high prevalence and degree of resistance in faecal *E. coli* of poultry in this and other studies (van den Bogaard and Stobberingh, 1999).

Other than antimicrobials, other approaches to prevent and control APEC infections in the poultry industry include improved hygienic methods, vaccination, use of competitive exclusion products, and the introduction of novel immunopotentiators. However, each of these practices have had limited success (Gomis *et al.,* 2003; Kwaga *et al.,* 1994; La Ragione *et al.,* 2001; La Ragione *et al.,* 2004; McGruder and Moore, 1999) and it has necessitated the use of antimicrobial chemotherapy to control outbreaks of colibacillosis. In vitro antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy. Also research to identify new ways to minimize antimicrobial use in poultry farms is essential.

In conclusion, these findings showed that multiple-antimicrobial-resistant *E. coli* isolates, including fluoroquinolone-resistant variants, are commonly present among diseased broiler chickens in Tabriz-Iran. Resistance to existing antimicrobials is widespread and of concern to poultry veterinarians. The significant increase in the incidence of resistance against antibiotics in the *E. coli* strains isolated from broiler chickens is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry. Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in Iran and other under developing countries because in addition to animal health problems, transmission of resistant clones and resistance plasmids of *E. coli* from food animals (especially poultry) to humans can occur. Since the use of cotrimoxazole and fluoroquinolones in poultry may cause cross-resistance with human enteric pathogens (especially with *Salmonella* and *Campylobacter* spp.), prudent use of these antimicrobial agents in avian species is highly recommended. To deal with multi-drug resistant organisms, it is usually recommended that potentially synergistic antimicrobial combinations be used, preventive strategies, such as appropriate husbandry and hygiene, routine health monitoring, and immunization, should be emphasized, poultry producers should approach the treatment of diseases with antibiotics very cautiously. The producer and veterinarian should work closely when antibiotic therapy is needed in a flock and both must continue to work toward ensuring a safe food supply for consumers.

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