Antimicrobial susceptibility of one thousand bacterial isolates to five antibacterial agents commonly used in the Iranian poultry industry

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Abstract:

BACKGROUNDS: Different susceptibility rates of pathogenic bacteria to antimicrobial agents are considered major factors in the choice of drugs and the success of treatments. Concerns have been raised regarding the emergence of antimicrobial resistance among pathogenic bacteria that may result in unpredictable antimicrobial susceptibilities and therapy failure. **OBJECTIVES:** The purpose of this investigation was to determine the antimicrobial susceptibility of 1,000 bacterial isolates to five antibacterial agents commonly used in the Iranian poultry industry. **METHODS:** From July 2008 to June 2009, the antimicrobial susceptibility of 1,000 bacterial isolates to five antibacterial agents was tested. These agents that are commonly used in the Iranian poultry industry include colistin, doxycycline, enrofloxacin, florfenicol, and sulfamethoxazole + trimethoprime. The data were provided by 19 laboratories in eight Iranian provinces. **RESULTS:** The bacterial species belonged mainly to *Escherichia coli* and *Salmonella* spp. Of all tested samples, 55.5% were resistant to colistin, 61.5% to doxycycline, 41.5% to enrofloxacin, 34.5% to florfenicol, and 65.5% to sulfamethoxazole + trimethoprime. **CONCLUSIONS:** The findings of this survey represent the high frequency of resistance to antimicrobial agents commonly used in the Iranian poultry industry. They also highlight the need for the implementation of a national monitoring program for antimicrobial resistance and for a rational use of antimicrobial drugs.

Introduction

Most bacteria are able to develop resistance to antimicrobial agents commonly used against infections that they cause in humans and animals (Bennett, 2008). Antimicrobial resistance is an increasingly global problem, and this emerging resistance has become a public health issue worldwide. Both human and veterinary uses of some classes of drugs have probably contributed significantly to the emergence of resistant bacteria (Hawkey, 2008). The effects of antimicrobial drugs on the development of resistance among animal bacterial flora have gained much attention during the last decade (Smith et al., 2007). Resistance factors reduce the efficacy of antimicrobial therapy thus resulting in enhanced morbidity and mortality associated with disease outbreaks. These resistance elements may be later transferred to other animals or humans (Da Ccosta et al., 2009). It has been noted that an antimicrobial therapy with a particular agent has been either accompanied or followed shortly by the occurrence of resistant bacteria (Schwarz and Chaslus-Dancla, 2001). This observation underlines the remarkable capability of bacteria to quickly and efficiently respond to the selective pressure imposed by the use of that antimicrobial agent. Antimicrobial therapy is one of the primary control measures for reducing

morbidity and mortality to avian bacterial pathogens in poultry production. In chickens, apart from the therapeutic use of antimicrobial agents, sub-therapeutic application of these agents for prophylaxis and growth promotion contributes to the formation of resistances. Several procedures have been developed to determine the in vitro antimicrobial susceptibility of bacteria from clinical samples. However, these procedures are based on just two basic methods, agar disc diffusion and agar broth dilution (Forbes et al., 2007). The agar disc diffusion procedure (Kirby-Bauer method) is still the most commonly used technique in veterinary laboratories around the world including Iran. This is due to its flexibility in the types and numbers of drugs that can be tested on a daily basis and its relatively low cost. Pathogenic bacteria, such as *Escherichia coli* and *Salmonella* spp. cause a variety of infections in poultry, thereby resulting in considerable economic losses in the related industry (Barnes et al., 2008; Gast, 2008). *Salmonella* infection in poultry is also considered an important source of food-borne infections in humans (Gast, 2008).

In this survey, we analyzed the antimicrobial susceptibility of samples obtained from 19 veterinary diagnostic laboratories in eight Iranian provinces.

Materials and Methods

Between July 2008 and June 2009, susceptibility reports of different bacterial species (*E. coli*, *Salmonella* spp., *Klebsiella* spp., *Staphylococcus* spp, *Pseudomonas* spp.) to five common commercial antimicrobial agents were collected from 19 laboratories in eight Iranian provinces (Tehran, Mazandaran, Isfahan, Semnan, Golestan, Kurdistan, Kermanshah and Ghazvin). The susceptibility of the isolated bacteria was evaluated against enrofloxacin, florfenicol, doxycycline, sulfamethoxazole+trimethoprime, and colistin, using the standard disc diffusion method (Forbes et al., 2007). Statistical analysis was carried out using SPSS version 16.0 (SPSS, Chicago, IL, USA) where applicable.

Results

Most of the isolated bacteria were from broiler farms, but some isolates were from other sources such as hatcheries, layer flocks, and pet birds. Out of 1,000 isolates, 943 *E.coli*, 49 *Salmonella spp*., 5 *Pseudomonas* spp., 2 *Klebsiella* spp., and one isolate of *Staphylococcus* spp. could be identified. Antimicrobial susceptibility patterns observed for isolates are shown in Tables 1 and 2. Among *E. coli* isolates, the highest and lowest resistance frequencies were observed to sulfamethoxazole + trimethoprime (67.4%) and to florfenicol (34.3%), respectively. For *Salmonella* isolates, the highest and lowest resistance rates were observed to doxycycline (51%) and to florfenicol (8.1%), respectively. Except for colistin, *E. coli* isolates demonstrated higher resistance rates to antimicrobials than *Salmonella*. Because of the low numbers of *Pseudomonas* spp., *Klebsiella* spp., and *Staphylococcus* spp. isolates found in this study, the susceptibility patterns observed for these species did not provide sufficient data for an analysis.

The sample size of bacterial species within the investigation period varied markedly. Of the samples analyzed in this survey, 77.7% were obtained in spring, 3.8% in summer, 12.1% in fall, and 6.4% in winter. Resistance to more than one drug, also known as multi-drug resistance (MDR) was detected in 75% of all bacterial isolates. MDR was found in 76% and 48% of *E. coli* and *Salmonella* isolates, respectively.

Discussion

Previous reports from different provinces of Iran indicated the high frequency of resistance to different antimicrobial agents among bacterial isolates. Zahraei and Farashi (2006) observed that 88%, 80%, 76%, 27%, and 6% of *E. coli* isolates were resistant to doxycycline, sulfamethoxazole + trimethoprime, enrofloxacin, florfenicol, and colistin, respectively. In another study, it was found that among 150 *E. coli* isolates, the frequencies of resistance to antibacterial agents were as follows: colistin, 96.7%; tetracycline, 94.0%; sulfamethoxazole + trimethoprime, 72.6%; enrofloxacin, 66.0%; and chloramphenicol, 46.7% (Khoshkhoo and Peighambari, 2005). A report from Northern Georgia indicated that the majority of 95 APEC isolates displayed resistance to sulfamethoxazole (93%), tetracycline (87%), and enrofloxacin (52%) (Zhao et al., 2005). In the period of 2006-2008, the development of resistance to quinolones was evaluated in 317 *E. coli* isolates provided from healthy broilers in different farms. Resistance

Table 1. Susceptibility patterns of 1000 bacterial isolates to five antimicrobial agents.

Antimicrobial agents	% of isolate		
	Susceptible	Intermediate susceptible	Resistant
Colistin	9.2	35.3	55.5
Doxycycline	11.3	27.2	61.5
Enrofloxacin	28.6	29.9	41.5
Florfenicol	47.0	18.5	34.5
Sulfamethoxazole + trimethoprime	19.9	14.6	65.5

Table 2. Susceptibility patterns*1* of each bacterial species to five antimicrobial agents. *1*S = susceptible, I = intermediate susceptible, R = resistant.

Antimicrobial agents	*Escherichia coli*			Salmonella			*Klebsiella*			*Pseudomonas*			*Staphylococcus*		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Colistin	35.2	55.7	9.0	38.7	48.9	12.2	100	-	-	60.0	40.0	-	100	-	-
Doxycycline	60.6	28.5	10.8	51.0	26.5	22.4	100	-	-	60.0	20.0	20.0	100	-	-
Enrofloxacin	42.7	31.2	25.9	24.4	24.4	51.0	100	-	-	20.0	20.0	60.0	-	-	100
Florfenicol	34.3	21.4	44.2	8.1	16.3	75.5	-	-	100	40.0	-	60.0	-	-	100
Sulfamethoxazole + trimethoprime	67.4	16.0	16.5	26.5	22.4	51.0	-	-	100	60.0	-	40.0	-	-	100

frequencies of 52%, 42%, and 22 % to enrofloxacin was observed among isolates sampled in 2006, 2007, and 2008, respectively (Kmet and Kmetova, 2010).

The susceptibility of *Salmonella* isolates to antimicrobial agents has been also determined in several studies. Zahraei et al., 2005 reported that all 30 *Salmonella* isolates from poultry in their study were susceptible to colistin and enrofloxacin. Intorre and colleagues (2005) found that all *Salmonella* isolates were susceptible to colistin and chloramphenicol, but the incidence of susceptibility to enrofloxacin, cotrimoxazole, and doxycycline were 93%, 76%, and 55%, respectively (Intorre et al., 2005). A recent study from Iran found that among 29 poultry *Salmonella* isolates, 6.9%, 3.4%, and 17.2% were resistant to enrofloxacin, florfenicol, and sulfamethoxazole + trimethoprime, respectively (Morshed and Peighambari, 2010).

Due to a lack of standardization in sampling and methodology of tests, it is difficult to compare the results of antimicrobial susceptibility during different years, and to achieve a resistance profile among bacterial species. However, the high rates of resistance observed in recent years require the planning of new strategies to control them. There is mounting evidence that both human and veterinary uses of some classes of antimicrobial drugs have significantly contributed to the emergence of resistant strains of bacterial pathogens. Horizontal transfer and clonal spread of resistance genes among humans and food producing animals may occur (Hawkey, 2008). In addition to the human health concerns, drug resistant bacterial pathogens also impose a severe and costly animal health problem in that they may increase the illness duration and decrease the productivity through higher morbidity and mortality (Xu, 2001). To reduce the problem of antimicrobial resistance in veterinary medicine, it is necessary to implement a monitoring plan for the development of antimicrobial resistance in both healthy and diseased animals. It is furthermore required to carry out preventive measures to avoid disease and health problems in animal populations, thereby reducing the need for antimicrobial substances (Grugel and Wallmann, 2004). If the use of antimicrobial agents is to be continued to combat diseases, a rationalized plan based on the knowledge of each agent, its indications and the correct dosage should be followed to achieve a successful outcome (Mateu and Martin, 2001). A variety of factors influencing the development and persistence of resistance among bacterial population in vivo may be out of our control, but a

rational antimicrobial administration is mandatory to reduce the spread of resistant clones among bacterial species. To optimize the efficacy and to minimize opportunities for resistance, some researchers have proposed the integration of population kinetic (PK) data obtained in clinical subjects with both pharmacodynamic (PD) parameters such as minimum inhibitory concentration (MIC) and the clinical outcome in order to provide a basis for setting dose schedules of antimicrobial drugs in veterinary medicine (Lees and Shojaee, 2002).

Estimates of the proportions of antimicrobial resistance reported in this survey may not be an accurate representation of the proportions among bacterial isolates in all of Iran. Many provinces were not included in this survey and although the standard disc diffusion method was followed in all laboratories, the preferences of different laboratories in running and reporting the results of antimicrobial susceptibility tests may have influenced the estimation of antimicrobial resistance rates. To overcome these problems, further comprehensive surveillance programs that include large numbers of samples provided from all Iranian provinces should be implemented, and the selected laboratories should be strictly controlled for a uniform application of antimicrobial susceptibility techniques.

The findings reported in this survey emphasize on the high frequency of resistance to common antimicrobial agents used in the Iranian poultry industry, especially in broilers. This study also highlights the need for the implementation of a national monitoring program for antimicrobial resistance and for the rational use of antimicrobial drugs.

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حساسیت ضدمیکروبی ۱۰۰۰ جدایه باکتریایی نسبت به پنج آنتی بیوتیک رایج در صنعت طیور ایران

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چکیده

زمینه مطالعه: تفاوت در الگوی حساسیت باکتری های پاتوژن نسبت به آنتی بیوتیک ها، عامل مهمی در انتخاب دارو و موفقیت درمان محسوب می شود. نگرانی های زیادی راجع به ظهور مقاومت باکتری های پاتوژن نسبت به عوامل ضدمیکروبی ایجاد شده که این موضوع منجر به الگوهای غیرقابل پیش بینی حساسیت و شکست در درمان گردیده است. **هدف:** هدف از این تحقیق تعیین حساسیت ضدمیکروبی ۱۰۰۰ جدایه باکتریایی نسبت به پنج آنتی بیوتیک رایج در صنعت طیور ایران بوده است. **روش کار:** ما نتایج آنتی بیوگرام ۱۰۰۰ جدایه باکتریایی را نسبت به ۵ آنتی بیوتیک رایج در صنعت طیور ایران شامل: کلیستین، داکسی سیکلین، انروفلوکساسین، فلورفنیکل و سولفامتوکسازول+تری متوپریم در فاصله زمانی تیرماه ۱۳۸۷ تا خرداد ماه ۱۳۸۸ جمع آوری نمودیم. داده ها از طریق ۱۹ آزمایشگاه از ۸ استان ایران فراهم شده و مورد آنالیز قرار گرفتند. **نتایج:** باکتری های جداشده عمدتاً متعلق به گونه های اشریشیاکلی (۹۴۳ جدایه) و سالمونلا (۴۹ جدایه) بودند. الگوی مقاومت در بین تمامی جدایه ها بدین قرار بود: کلیستین ۵۵/۵٪، داکسی سیکلین ۶۱/۵٪، انروفلوکساسین ۴۱/۵٪، فلورفنیکل ۳۴/۵ ٪ و سولفامتوکسازول+تری متوپریم ۶۵/۵٪. **نتیجه گیری نهائی:** نتایج این بررسی حاکی از آن است که مقاومت بالایی نسبت به آنتی بیوتیک های رایج در صنعت طیور ایران وجود داشته که نیازمند اجرای طرح مانیتورینگ ملی برای مقاومت ضدمیکروبی و مصرف عقلانی داروها می باشد.

واژه های کلیدی: اشریشیاکلی، سالمونلا، حساسیت ضدمیکروبی، مقاومت ضدمیکروبی، ایران.

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