BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, August 2014. Vol. **11**(2), 999-1003

Effect of Different Factors of Time on the Antimicrobial Agent Resistance

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doi: http://dx.doi.org/10.13005/bbra/1374

(Received: 15 June 2014; accepted: 10 July 2014)

In this study, 318 commercial broiler flocks from 18 Parent Stocks with different breeder age were investigated to assess the changes of antimicrobial agent resistance. All samples were collected from April 2009 to March 2012 and Parent Stocks age was between 30 to 63 week years old. The majority of isolates were resistant to Tylosin and Erythromycin, the minimum resistant to Gentamicin and Ciprofloxacin were found and other antimicrobial susceptible test showed different resistance percentage from 20.75 to 43.40. These data suggested that the time passing as an independent factor whether in the study as seasonal changes or as passing of different years and without effect of other factors cannot be effective on increase of the bacterial resistance and, also, antimicrobial agent resistances found in chickens' showed a significant increase when the age of breeders is increased.

Key words: Antimicrobial Agents, Resistance, Broiler, Parent Stocks, Age.

Debate over resistance observed amongst Gram-negative bacteria including *E. coli* and *Salmonella* has started the most robust disagreement to antimicrobials (Giovanardi *et al*. 2005). Antimicrobials have customarily been utilized in the poultry manufacturing to make better the function and health of birds by changing or decreasing the bacterial populations in the GI tract (Zanella *et al*. 2000). The administrations of the antimicrobials change the intestinal microflora during the grow-out period and induce a selective pressing supporting the resistant bacteria (Diarra *et al*. 2007).

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Earlier studies have implicated that a vertical transfer of *E. coli* isolates as of broiler breeding chickens to their progeny (Giovanardi *et al*. 2005; Petersen *et al*. 2006; Bortolaia *et al*. 2010). Furthermore, horizontal transmission may be took place at the abattoir. Resistant strains from the gut may contaminate poultry carcasses and, consequently, poultry meats are often dealt with multi resistant *E. coli.* In addition, eggs are contaminated within laying (Miles *et al*. 2006) showing that infectivity of broilers and broiler meat with resistance isolates can cause human colonization and, consequently, human infection with resistance pathogens. These isolates are able to share their genes with other microbes in the gastro-intestinal tract by plasmid-transmitting performing as a origin of infections for other parts of the body (Dierikx *et al*. 2013).

The broiler production has a pyramidal organization in which lineage chickens and Great Grandparent Stock (GGPS) on the begin via breeding chickens (Grandparent Stock (GPS) and Parent Stock (PS)) generate the broiler chickens on the end stages of the pyramid.

Vertical transmission of *E. coli* in pyramidal structure leads resistance bacteria from poultry meat to human.(Mesa *et al*. 2006; Smet *et al*. 2008; Cohen Stuart *et al*. 2012; Dierikx *et al*. 2013).

The study aimed to determine whether age of poultry breeder affected the antimicrobial agent resistance of *E. coli* isolates from 7-14 days old chicken flocks.

MATERIALS AND METHODS

Sampling procedure and Bacterial isolates

In this study, 318 commercial broiler flocks from five industrial broiler chicken farms located in Shahrekord Industrial Poultry Company were examined from April 2009 to March 2012. The chickens in each trial received the same general management, including the same feed and water, and were exposed to the same environment on each industrial farm. Suspected samples with clinical signs of septicemia during 7 to 14 days old were cultured on MacConkey and EMB agar and the colonies suspected to *E. coli* were recognized by standard methods(Gonzalez and Blanco 1989). All of broiler chickens were provided from different Parent Stock (PS) in Iran and demographic breeder data of chicks were obtained from Cell CART and classified.

Antimicrobial susceptibility determination

Detection of antimicrobial sensitivity was done by the standard disc diffusion method based on the Clinical and Laboratory Standards Institute (CLSI) (Wayne 2006). The *E. coli* strains were tested against the antimicrobial agents of veterinary significance. The following antimicrobial agent discs on Mueller Hinton agar were applied: Chloramphenicol (C/30 µg), Chlortetracycline (CTe/30µg), Ciprofloxacin (CP/5 µg), Danofloxacin(D/30 µg), Difloxacin (DIF/25 µg), Doxycycline (D/30 µg), Enrofloxacin (NFX/5 µg), Erythromycin (E/15 µg), Florfenicol (FFc/30 µg), Gentamicin (GM/10µg), Oxytetracycline(T/30/µg), Trimethoprim-Sulphamethoxazole (SXT/25 µg) and Tylosin (TYC/30 µg).

RESULTS

The obtained results of antimicrobial resistance of 318 *E. coli* strains isolated from diseased broiler chickens are shown in Figure 1 by yearly configuration and arranged one on season configuration in Fig 2.

Table 1. Statistical relationship between the age increase in mother hens and the antimicrobial agent resistance

		Mather age	Resistancy
Breeder age	Pearson Correlation	1	0.462*
	Sig. (2-tailed)		0.001
Resistance	Pearson Correlation	0.462*	1
	Sig. (2-tailed)	0.001	

*. Correlation is significant at the 0.01 level (2-tailed)

Table 2. Mean±SE of percentage of antimicrobial resistance and Early chicken mortality on three range of breeder age

	Breeder Age		
	Young	Middle-aged	Old
Percentage of anti microbial resistance	28.02±3.19[a]	31.50±3.22[a]	45.15±3.35[b]

[a,b] Numbers with different superscripts in the same column differ significantly (P<0.05).

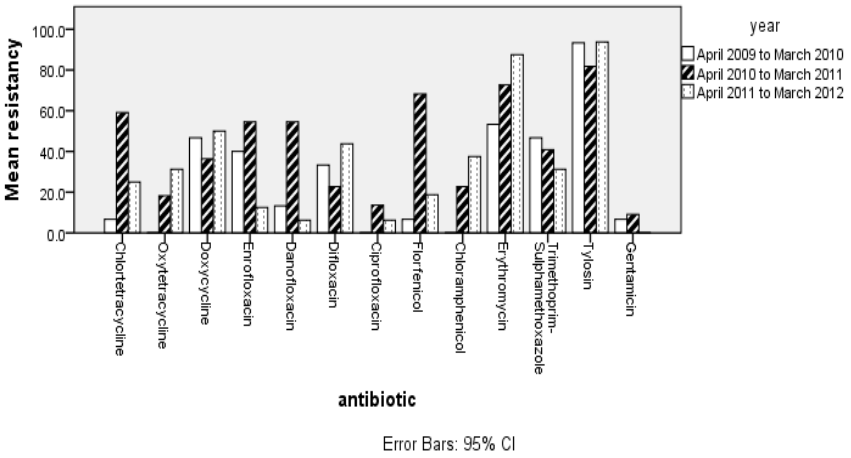

Fig. 1. Antimicrobial resistance of 318 *E. coli* strains isolated from diseased broiler chickens

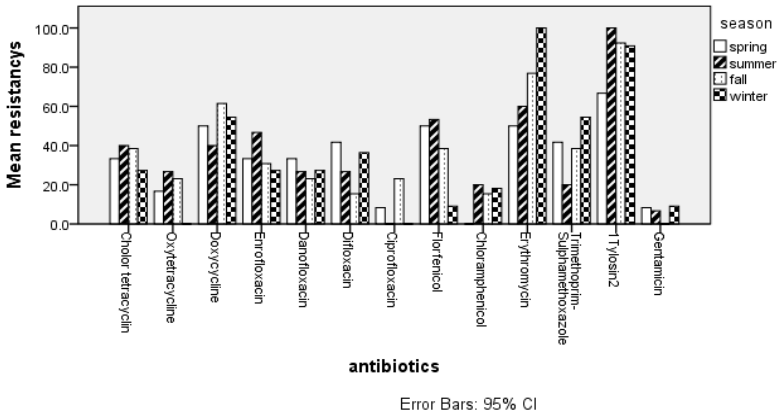

Fig. 2. Seasonal relationship between antimicrobial agents resistances

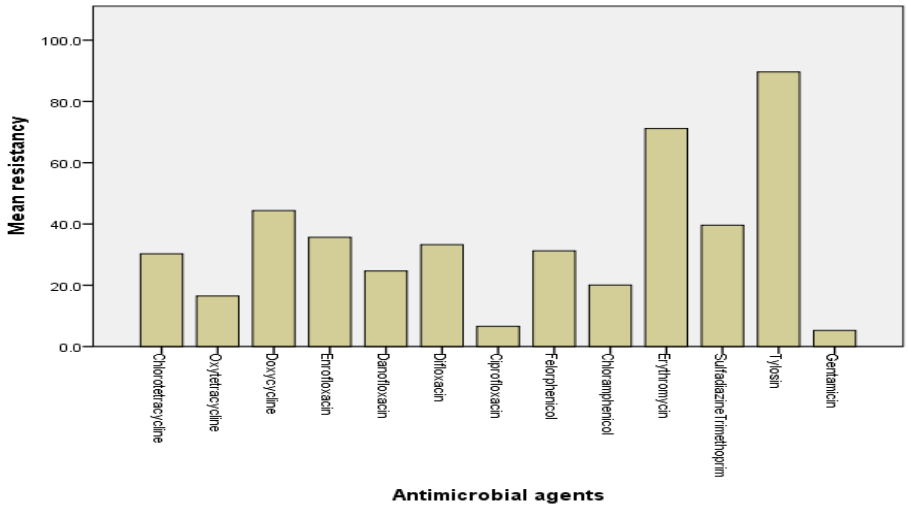

Fig. 3. Antimicrobial resistance and susceptibility of *E.coli* isolated from 7-14 days old chicken

Study the seasonal relationship between antimicrobial agents resistances, as was shown in the Figure 2, did not show the significant relationship for all antimicrobial agents except enrofloxacin.

Study the statistical relationship of antimicrobial agents resistances regarding the studied years only showed significant increase about tetracycline and while for doxycyclin and chloramphenicol antimicrobial agents the increase trend was found without the statistical relationship but this trend was decreasing for sulfamethoxazole/trimethoprim (sulfatrim) without the statistical relationship. The available trend for other antimicrobial agents showed a variable trend.

Figure 3 summarizes the resistance pattern of *E. coli* isolates to thirteen antimicrobial agents tested in this study. Of the 318 *E. coli* isolates tested, all were resistant to one or more antimicrobial agent. The majority of isolates were resistant to Tylosin and Erythromycin, minimum resistant to Gentamicin and Ciprofloxacin were found and other antimicrobial susceptible test shown different resistance percentage from 20.75 to 43.40.

The studied statistical relationship by the correlation method as not classified showed the direct statistical relationship between the age increase in mother hens and the antimicrobial agent resistance increase as significant (Table 1). Also in result of classifying age of the mother hens in 3 class, 30-35 weeks (young), 36-45 weeks (middle-aged) and 45 weeks to up (old), increase in antimicrobial agent resistance in the old group showed a significant relationship than other groups (ANOVA) Table 2.

DISCUSSION

Four major factors that influence poultry performance include breeder hen age, genetic strain, hatchery management, and brooding management. But there was still little understanding of how the factors interact in poultry production (Fairchild. A.S 2000). In this study we demonstrated the relationship between breeder age and anti microbial resistance that can impress the efficiency of chicken production process.

Many articles suggested *E coli* is major pathogenic organism can effect poultry health and performance also it can transmit the resistance genes with horizontal and vertical transduction.

Increase of resistance is the important world problem that many countries enactment limiting use of antimicrobial agents and they have several programs to resistance monitoring of micro organisms, nevertheless the broiler production system seems very uncomplicated having only a few elementary breeding company at the apex of the pyramid producing broilers around the world. However, according to transference and commerce of the same eggs and chickens to various countries all over the world, it can be resulted in a vulnerable system, too. If a disease or, even, antimicrobial agent resistant bacteria comes in to the production chain, it may be possible to be transferred all around the world.

Although at the top of the pyramid, the outbreak is lower than that in the longitudinal study at broiler manufacture farms at the bottom of the pyramid, it may worry us that antimicrobial resistance findings are already can be a sign of a wide-spreading outbreak (Dierikx *et al*. 2013). Extensive studies always have been performed in relation to antimicrobial agent resistance changes. Many researchers have reported available resistances in different times increasing (Heller and Smith 1973; Lambie *et al*. 2000). Also some researchers have reported differences in the bacterial resistances in relation to the season(Fairchild.A.S 2000). But the present research showed that the time passing as an independent factor whether in the study as seasonal changes or as passing of different years and without effect of other factors cannot be effective on increase of the bacterial resistance. In other words the obtained statistical relationships indicate that the time passage in most of breeder age will be effective in this regard and by increasing breeder age and with regard to possibility of being involved in different diseases that cause need to antibacterial treatments it will causes organisms that by the selective pressure resulted from consumption of antimicrobial agents in the bacterial population of the GI tract flora in the mother birds and firstly this resistance is extended among other bacteria and also resistant bacteria will be increased between the flowers population by using the horizontal transfer possibility. With regard to the vertical transfer possibility of these microorganisms by infecting the egg shell or the

uterus infection, when the egg is formed, the bacterial transfer resistant to the genetic will be increased. So, antimicrobial agent resistances found in the chickens shows a significant increase when the age of breeders is increased. So, it should be considered as an effective factor in efficiency of the raised chickens.

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