Aerobic bacteria isolated from eggs and day-old chicks and their antibacterial resistance in Shiraz, Iran

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Summary

To study the putative transfer of antibiotic resistance from broiler breeders to human, hen's eggs and their day-old chicks were examined for the presence of bacteria. The most frequently isolated organisms in decreasing order were: *Streptococcus spp.*, *Bacillus spp.*, *Staphylococcus spp.*, *Klebsiella spp.*, *Enterobacter spp.* and *Escherichia coli* followed by *Citrobacter spp.*, *Proteus spp.* and *Pseudomonas spp.* from the eggs and *E. coli*, *Enterobacter spp.* and *Citrobacter spp.* followed by *Klebsiella spp.* and *Bacillus spp.* from the chicks. Different detection methods were evaluated which use various enrichment and plating media for bacteria in eggs and day-old chicks. Sensitivity tests showed the presence of antibacterial resistant strains of bacteria. In comparison, resistance to all antibiotics in chicks' isolated bacteria were more frequent than eggs' isolates, but statistically no significant differences between patterns of antibacterial resistance were seen ($P \leq 0.05$). Twenty-three, 54, 55, 60, 24 and 10% of chicks' isolates were resistant to chloramphenicol, enrofloxacin, erythromycin, furazolidone, trimethoprim and tylosin, respectively. Whereas these data about eggs' isolates were as follows: 1, 12, 18, 18, 10 and 6%, respectively. This study revealed that eggs are often contaminated with different bacteria and could be potential vehicles for transmitting of these bacteria through their broilers. Our findings stress the need for increased implementation of hazard analysis of critical control points (HACCP) and consumer food safety education efforts.

Key words: Eggs, Day-old chicks, Antibacterial resistance, Public health

Introduction

Microbial food safety is an increasing public health concern worldwide. Data recorded in different countries have shown that the incidence of some of food-borne diseases have increased dramatically over the past few years, but because of under-responding, the data are of limited value and can not be compared between countries (Sackey *et al*., 2001; Zhao *et al*., 2001).

The relationship of the hen's egg to human and animal health depends to some extent on its microbial content, more especially on the microbiology of the freshly laid egg. Microbial contamination of eggs is a well-established phenomenon and has important economic implications to the poultry industry. Contamination of hatching eggs may reduce hatchability, be responsible for transmission of poultry pathogens and impair the quality of chicks produced. In the case of table eggs spoilage may occur and if the organism is of public health significance, the affected eggs may be the cause of spread of disease. The majority of the salmonellosis outbreaks is associated with consumption of eggs and egg dishes. There are two ways in which eggs can become contaminated, namely, by the transovarian and trans-shell routes. In transovarian contamination the egg becomes contaminated prior to oviposition, with the source of contamination originating in the egg-laying apparatus of the bird. In the case of trans-shell contamination, the organisms gain access to the egg after oviposition by penetration into the shell. These organisms could be derived from either the intestinal tract or the environment. Contact with contaminating organisms when the egg shell is wet may also facilitate the penetration of the pathogens. When eggs are

broken, bacteria present on egg shells may contaminate the contents. These con-taminants may grow rapidly in broken out egg if storage is at ambient temperature (Board and Fuller, 1994; Grijspeerdt, 2001; Hara-Kudo *et al*., 2001; Radkowski, 2001).

Eggs are produced by laying hens (layers); however, common approaches are applied in the supply of layers. As nearly all birds are derived from a very small number of elite/great-grandparent supply points, it is clear that any pathogens entering the population in these early stages will be able to spread throughout the layer populations very quickly indeed (Bell and Kyriakides, 2002). Among the available methods for the control of these pathogens, the one most widely practiced is the use of various antibiotics, fungicides and coccidiostats in the birds' diet. Nevertheless, it is well known that the extended and continuing use of a range of antimicrobial agents in animals' food has been an important factor in promoting the emergence of resistant strains of Gram-positive and Gram-negative bacteria (Papadopoulou *et al*., 1997; Aarestrup *et al*., 2000). Resistant organisms can spread from chicken to chicken and from chicken to man (Levy *et al*., 1976). In 1992, an article that was published in Science focused on antimicrobial resistance and listed the "top ten drug-resistant microbes": one-half were Gram-negative bacteria, including Enterobacteriaceae, *Haemophilus influenzae, Neisseria gono-rrhoeae, Pseudomonas aeruginosa* and *Shigella dysenteriae*. Although resistant Gram-positive bacteria, particularly enter-ococci, pneumococci and staphylococci are clearly a problem, resistant Gram-negative bacteria remain an important cause of morbidity and mortality. The cost of treatment is also a problem for infections caused by Gram-negative organisms (Gibbons, 1992).

For the purpose of studying antibacterial resistance, potentially transmitted from poultry to humans, hens' eggs and their day-old chicks were examined for the presence of bacteria. The eggs and chicks used came from a broiler breeder farm in Shiraz area.

Materials and Methods

Sample collection

Sampling visits were made in every other month for 8 months. A total number of 114 eggs and 120 day-old chicks were examined, from a broiler breeder company in Shiraz area. Each egg sample was aseptically removed and placed in a plastic container and transferred to the lab. The chicks (Aryan hybrid) were kept in special boxes at the laboratory, have been brought there immediately after hatching. Fertile eggs were hatched in the company's own hatchery.

Breaking of eggs for culturing

The egg-shell was wiped with a sterile cotton wool swab moistened with sterilized normal saline (0.85%), then wiped with a cotton ball soaked in 70% ethanol and finally it was sterilized by a quick passing over a flame. This procedure was followed to avoid contamination of the egg contents from the germs colonizing the egg-shell (Papadopoulou *et al*., 1997; Himathon-gkham *et al*., 1999).

Preparing the chicks for bacterio-logical examination

Killing the day-old chicks was performed following disarticulating cervical vertebrate method recommended by Strafuss (1988). Necropsy procedure was preformed following the method recommended by Strafuss (1988) and immediately after death the abdomen was quickly opened and the whole intestine removed and unraveled with sterile precautions (Smith, 1965).

Culturing method

After disinfection, each egg was cracked with a sterile surgical knife and its content (white and yolk) was dropped into glass container containing 150 ml of trypton soya broth (TSB, Merck) (Papadopoulou *et al*., 1997; Himathongkham *et al*., 1999). The intestinal samples were squeezed into a tube containing 9 ml of TSB (Barnes *et al*., 1972). After homogenization, the TSB cultures were incubated at 37ºC for 18-24 hrs and then subcultured to suitable selective media. These media were McConkey agar (Merck) for the cultivation of Enterobacteriaceae, double concentration selenite enrichment broth (Merck) as enrichment broth and brilliant green phenol red lactose agar (Merck) for *Salmonella* isolation. Mueller-Hinton agar (Merck) and blood agar

(Oxoid) were used for the cultivation of other Gram-positive bacteria (Papadopoulou *et al*., 1997; Himathon-gkham *et al*., 1999).

Selective enrichment for *Salmonella* was carried out using 225 ml of double concentration selenite enrichment broth for the content of each egg, or 25 ml liquid egg and 10 ml for intestinal contents and incubated at 37°C for 24 hrs under aerobic conditions. Enrichment was carried out for all samples (Hara-Kudo *et al*., 2001; Sackey *et al*., 2001). Selenite enrichment broth was then streaked onto McConkey agar and brilliant green phenol red lactose agar as indirect plating. *Escherichia coli* and other Enterobacteriaceae were isolated using McConkey agar and eosin methylene blue agar (BioMérieux). Incubation was done at 37°C for 24-48 hrs (Sackey *et al*., 2001).

After incubation an isolated colony was picked from a suitable plate and subcultured into another plate to obtain a pure culture. The identification of the isolated bacteria was based on standard bacteriological and biochemical procedures. Strains characte-rized by Gram stain and hemolysis on sheep blood agar. The Gram-positive cocci were first classified upon their reaction to the catalase test and then further identified using the oxidase test and other biochemical reactions. The Gram-negative bacteria were first classified upon their reaction to the oxidase test and then further identified by using the biochemical tests (Clarke and Bauchop, 1977; Quinn *et al*., 1994; Mahon and Manuselis, 1995; Papadopoulou *et al*., 1997; Himathongkham *et al*., 1999).

Antimicrobial susceptibility testing

Antimicrobials

A total of 6 antibacterials were used as the following: trimethoprim, tylosin, erythromycin and enrofloxacin (approved drugs in Iran) and chloramphenicol and furazolidone (not approved for use in animal in Iran).

Antimicrobial powders were obtained from different companies in Iran. Firstly, the purity of them was measured with diffusion method (Brooks *et al*., 1998) and then, antimicrobial stock solutions were prepared and stored in 95% ethanol solution (chloramphenicol and erythromycin), distilled water (trimethoprim, tylosin and enrofloxacin) and dimethyl formaldehyde-DMF (furazolidone) (Andrews, 2001).

Methods to test susceptibility

The susceptibility tests were performed following the dilution antimicrobial tests recommended by Quinn *et al*., (1994) and Hirsh and Zee (1999). This test was performed by preparing two-fold dilutions of an antibiotic in a series of tubes containing Mueller-Hinton broth. From each bacterial strain an inoculum previously adjusted to 0.5 Unit of the McFarland scale and then diluted 1 : 100 to obtain 10^4 and 10^5 bacteria/ml concentration. Each tube was inoculated with a suspension of the test bacterium. The inoculated tubes of broth were incubated at 35-37°C for 16-18 hrs. The highest dilution of the antibiotic to inhibit visible growth of bacterium (no turbidity in the tube) was used as the minimum inhibitory concentration (MIC). To determine the resistant isolated bacteria, following breakpoints of antibiotics were considered: chloramphenicol ≥ 3200 µg/ml (Aarestrup *et al*., 2000; White *et al*., 2003); enrofloxacin ≥ 400 µg/ml (White *et al*., 2000); erythromycin ≥ 800 µg/ml (Aarestrup *et al*., 2000; White *et al*., 2003); furazolidone ≥ 200 µg/ml (National Committee for Clinical Laboratory Standards, NCCLS, Guidelines, Chicago Department of Public Health, 1998); trimethoprim ≥ 1600 µg/ml (Aarestrup *et al*., 2000) and tylosin ≥ 3200 µg/ml (White *et al*., 2003).

Statistical analysis

Data were analysed using the SPSS statistic software version 11.5. Independent t-test was used to find the significant differences between the two groups ($P \leq 0.05$).

Results

Various Gram-positive and Gram-negative bacteria were isolated. Thirty-three (28.94%) out of 114 examined eggs were contaminated with 46 bacteria (9 species) that 27.3% of these eggs were contaminated with more than one bacterium. The rate of microbial contamination of eggs with *E. coli* in this study was 8.7% (n = 4). Our study indicated the following rates for contamination with other Entero-bacteriaceae: *Klebsiella spp.* 15.22% (n = 7), *Proteus spp*. 2.17% (n = 1),

Enterobacter spp. 10.87% (n = 5) and *Citrobacter spp*. 2.17% (n = 1). Also one *Pseudomonas spp*. (2.17%) was detected. Gram-positive bacteria had these rates of contamination: 21.74% *Streptococcus spp*. (n = 10), 17.4% *Staphylococcus spp.* (n = 8) and 19.56% *Bacillus spp*. (n = 9).

Ninety (75%) out of 120 tested day-old chicks were contaminated with 144 bacteria (5 species) that 55.56% of these chicks were contaminated with more than one bacterium. In this case, *E. coli* had the most incidence (68.05%) followed by *Enterobacter spp.* (16.67%), *Citrobacter spp.* (11.11%), *Klebsiella spp.* (3.47%) and *Bacillus spp.* (0.7%). At last, the resistance of all isolated bacteria against several antibiotics commonly used in chicken industry in Iran was examined. The antibiotic susceptibility of isolates to the different antibiotics is shown in Tables 1 and 2.

Ten percent of egg isolates (n = 1) were resistant to chloramphenicol. This resistance was pertaining to *Streptococcus spp*. No resistance was seen in other isolates. 55.56, 25, 14.28, 25 and 30% of *Bacillus spp.*, *E. coli*, *Klebsiella spp.*, *Staphylococcus spp.* and *Streptococcus spp*. isolates from eggs were resistant to enrofloxacin, respectively. Among resistance to erythromycin in bacteria isolated from eggs, these results were obtained: *Bacillus spp.* (55.56%), *E. coli* (75%), *Klebsiella spp.* (14.28%), *Staphylococcus spp.* (37.5%) and *Streptococcus spp*. (60%).

With exception of *Proteus spp.* and *Pseudomonas spp*., all isolated bacteria from eggs were resistant to furazolidone in the following order: *Citrobacter spp.* (100%), *Bacillus spp.* (66.67%), *Klebsiella spp.* (42.85%), *Streptococcus spp.* (40%), *E. coli* (25%), *Staphylococcus spp.* (25%) and *Enterobacter spp*. (20%). Resistance to trimethoprim was seen in 11.14% of *Bacillus spp.*, 20% of *Enterobacter spp.*, 25% of *E. coli*, 28.58% of *Klebsiella spp.*, 25% of *Staphylococcus spp.* and 30% of *Streptococcus spp.* isolates from eggs. 11.14% of *Bacillus spp.*, 25% of *E. coli*, 14.28% of *Klebsiella spp.*, 12.5% of *Staphylococcus spp.* and 20% of *Streptococcus spp.* isolates from eggs were resistant to tylosin.

In the isolated bacteria from day-old chicks, resistance to chloramphenicol was limited to *Enterobacter spp.* (12.5%) and *E. coli* (20.4%). 18.75% of *Citrobacter spp.*, 48.97% of *E. coli* and 12.5% of *Enterobacter spp*. isolated from chicks were resistant to enrofloxacin. In the isolated bacteria from chicks, resistance to erythromycin was seen in 55.1 and 6.25% of *E. coli* and *Citrobacter spp*., respectively. All bacteria isolated from chicks showed resistance to furazolidone as the following percentages: *Bacillus spp.* (100%), *Citrobacter spp.* (18.75%), *Enterobacter spp.* (29.16%), *E. coli* (48.97%) and *Klebsiella spp.* (20%). 21.42, 8.34 and 6.25% of *E. coli*, *Enterobacter spp.* and *Citrobacter spp*. isolated from chicks were resistant to trimethoprim, respectively. Resistance to tylosin had the least level in chicks' isolated bacteria: 9.2% of *E. coli* and 4.16% of *Enterobacter spp*.

A very frequently occurrence of resistance to tested antibiotics was observed among both groups, but statistically no significant differences between the pattern of antibacterial resistance were seen ($P \leq 0.05$).

Discussion

The present study was conducted to determine the species distribution and susceptibility to antimicrobial agents among bacteria isolated from eggs and day-old chicks in Shiraz, Iran. The use and misuse of antibiotics contribute to the development of resistance and it is generally in agreement that this is a function of the span of time and use; therefore, it is of basic importance to implement monitoring systems. A common limitation of monitoring systems is that they usually consider the resistance only to antimicrobial drugs of clinical isolates. In view of the expected correlation between animal food and human clinical disease, we decided to direct our investigation towards the strains isolated from food instead of clinical isolates. In this way, we could establish an Iranian database to be periodically updated to foresee the trend of bacterial resistance to antibiotics. Thus, selected strains of bacteria were recovered from eggs and day-old chicks. Also an assessment of the incidence and type of bacterial contamination occurring in eggs produced and hatched in commercial hatcheries is essential for understanding the role that microorganisms play in influencing

hatchability.

The results of the present study indicate the existence of a variety of bacteria in the egg which can transfer to human via different foods or their chicks. It was also observed that of all eggs examined (114), 28.94% were found to be contaminated with more than one organism with the combination of Enterobacteriaceae and *Streptococcus spp.* occurring more frequently than would be expected by chance. This can be explained by assuming that the organism originates from a common source, namely faeces. It should also be noted that the various pathogens were isolated from egg-yolk after sterilization of the shell egg to minimize contamination from germs colonizing the egg shells.

In this study, the bacterial flora recorded from the eggs showed that the predominant species were *Streptococcus spp., Bacillus spp., Staphylococcus spp.* and *Klebsiella spp.* comprised 21.73, 19.56, 17.39 and 15.21% of the total bacteria isolates, respectively and other groups present lower levels. All these bacteria (*Bacillus spp., Citrobacter spp., Enterobacter spp., E. coli, Klebsiella spp., Proteus spp., Pseudomonas spp., Staphylococcus spp*. and *Streptococcus spp*.) have been isolated from eggs in other studies and our results are in general agreement with the results obtained by Taku *et al*., (1986), Board and Fuller (1994), Papadopoulou *et al*., (1997) and Zhao *et al*., (2001).

Moreover, the bacteria were isolated from eggs coming from large industry-scale broiler breeder plant, where antibiotics are not widely used. Taking into consideration that furazolidone had used for the control of infections before laying period for a week and comparing the resistance of the isolated bacteria to this specific antibiotic, it is quite possible that resistant bacteria could be passed to human through the food chain (Papadopoulou *et al*., 1997). As showed in Table 1, except of *Proteus spp.* and *Pseudomonas spp*., all of other isolated bacteria from eggs were resistant to this antibiotic in high percentage. This high resistance to furazolidone also was seen in all isolated bacteria from chicks (Table 2).

Natural contamination of egg contents with bacteria such as *Salmonella* and *E. coli* can occur in two ways. One is penetration from outside the egg into the content. Another mode of natural contamination occurs in the reproductive tract, probably the upper oviduct, with the most important sites for contamination being the outside of the vitelline membrane and the surrounding albumen (Humphrey and Whitehead, 1993; Humphrey, 1994). It was suspected that the contamination was a result of incomplete disinfection of the shell and/or membrane resulting in transfer of bacteria to the content during the egg breaking procedure.

The normal gut flora of chicks is highly complex and not yet fully understood. A number of naturally occurring and artificial factors are able to affect the composition of the flora. These factors include age, the immune response, diet and orally administered antibiotics (Board and Fuller, 1994). Although the alimentary tract of the healthy newly hatched chick is usually sterile it rapidly becomes colonized by facultative anaerobic bacteria, particularly coliforms and streptococci (Board and Fuller, 1994). Work by Fuller and Jayne-Williams (1968) demonstrated bacterial contamination of the peritoneal cavity, 38% and yolk sac infections, 23% in 121 conventional chicks examined during the first 5 days of life. Considerable variation, related to incubator hygiene was observed between different batches of chicks. The more frequently isolated organisms were streptococci followed by, in decreasing order, micrococci and coliform organisms (mainly *E. coli*). The micrococci were considered to be the characteristic of the

Table 1: Antimicrobial susceptibility of isolated bacteria from eggs in Shiraz, Iran

Antimicrobial agent	Bacterial species	Number of isolates with MIC (µg/ml)										Resistance (%)
		6.25≥	12.5	25	50	100	200	400	800	1600	≥3200	
Chloramphenicol *	*Bacillus spp.*	1	1	2	2	1	1	-	1	-	-	0
	Citrobacter	-	-	-	-	-	1	-	-	-	-	0
	Enterobacter	-	2	-	1	-	1	-	-	1	-	0
	Escherichia	1	-	1	1	-	-	-	1	-	-	0
	Klebsiella	-	2	-	1	1	2	1	-	-	-	0
	Proteus	-	-	-	1	-	-	-	-	-	-	0
	Pseudomonas	-	1	-	-	-	-	-	-	-	-	0
	Staphylococcus	-	1	-	1	2	3	-	1	-	-	0
	Streptococcus	-	1	1	1	2	1	2	1	-	1	10
Erythromycin *	*Bacillus spp.*	1	1	-	1	-	1	2	2	1	-	55.56
	Citrobacter	1	-	-	-	-	-	-	-	-	-	0
	Enterobacter	2	1	-	-	1	1	-	-	-	-	0
	Escherichia	1	-	1	-	-	1	-	1	-	-	25
	Klebsiella	2	-	-	1	1	2	1	-	-	-	14.28
	Proteus	-	-	1	-	-	-	-	-	-	-	0
	Pseudomonas	-	1	-	-	-	-	-	-	-	-	0
	Staphylococcus	1	2	1	-	1	1	1	-	1	-	25
	Streptococcus	2	1	1	2	1	-	2	-	1	-	30
	Bacillus spp.	-	-	1	-	2	-	1	3	1	1	55.56
	Citrobacter	-	-	-	1	-	-	-	-	-	-	0
	Enterobacter	1	-	-	2	-	1	1	-	-	-	0
	Escherichia	-	-	1	-	-	-	-	1	-	2	75
	Klebsiella	1	1	-	-	1	1	2	-	1	-	14.28
	Proteus	-	-	-	-	1	-	-	-	-	-	0
	Pseudomonas	-	-	-	-	-	1	-	-	-	-	0
	Staphylococcus	1	-	-	2	-	1	1	-	1	2	37.5
	Streptococcus	-	-	-	-	1	2	1	1	3	2	60

Table 1 (Cont): Antimicrobial susceptibility of isolated bacteria from eggs in Shiraz, Iran

Antimicrobial agent	Bacterial species	Number of isolates with MIC (µg/ml)										Resistance (%)
		6.25≥	12.5	25	50	100	200	400	800	1600	≥3200	
	Bacillus spp.	-	2	1	-	-	1	2	1	1	1	66.67
	Citrobacter	-	-	-	-	-	-	1	-	-	-	100
	Enterobacter	1	-	-	2	1	-	1	-	-	-	20
	Escherichia	1	-	-	1	1	-	-	-	1	-	25
Furazolidone ***	*Klebsiella*	-	1	-	1	2	-	1	1	-	1	42.85
	Proteus	-	-	-	-	1	-	-	-	-	-	0
	Pseudomonas	-	-	-	-	1	-	-	-	-	-	0
	Staphylococcus	1	1	-	1	3	-	-	-	1	1	25
	Streptococcus	1	1	-	3	1	1	-	2	-	1	40
	Bacillus spp.	-	2	1	1	2	1	-	1	1	-	11.14
	Citrobacter	-	-	-	1	-	-	-	-	-	-	0
	Enterobacter	-	1	-	-	1	1	-	1	-	1	20
	Escherichia	-	-	1	-	-	1	-	1	-	1	25
	Klebsiella	-	-	-	1	2	1	1	-	2	-	28.58
	Proteus	-	-	-	-	-	-	-	1	-	-	0
	Pseudomonas	-	-	-	-	1	-	-	-	-	-	0
	Staphylococcus	1	-	1	-	1	1	2	-	-	2	25
	Streptococcus	-	1	1	1	-	2	1	1	2	1	30
	Bacillus spp.	1	1	-	1	2	1	-	2	-	1	11.14
	Citrobacter	-	-	-	-	1	-	-	-	-	-	0
	Enterobacter	-	1	1	-	1	1	-	1	-	-	0
	Escherichia	-	-	1	-	-	1	1	-	-	1	25
Tylosin *****	*Klebsiella*	2	-	2	1	-	-	-	1	-	1	14.28
	Proteus	-	-	-	-	1	-	-	-	-	-	0
	Pseudomonas	-	-	-	-	-	-	1	-	-	-	0
	Staphylococcus	1	-	2	-	2	1	1	-	-	1	12.5
	Streptococcus	1	-	2	-	1	1	2	-	1	2	20

Bold lines indicate breakpoints for resistance according to: *Aarestrup *et al*., (2000) and White *et al*., (2003); **White *et al*., (2000); ***National Committee for Clinical Laboratory Standards (NCCLS) Guidelines (Chicago Department of Public Health, 1998); ****Aarestrup *et al*., (2000); *****White *et al*., (2003)

Table 2: Antimicrobial susceptibility of isolated bacteria from day-old chicks in Shiraz, Iran

Antimicrobial agent	Bacterial species	Number of isolates with MIC (µg/ml)										Resistance (%)
		6.25≥	12.5	25	50	100	200	400	800	1600	≥3200	
	Bacillus spp.	-	-	1	-	-	-	-	-	-	-	0
	Citrobacter	2	2	1	1	1	6	1	2	-	-	0
Chloramphenicol *	*Enterobacter*	2	7	2	1	2	3	2	1	1	3	12.5
	Escherichia	12	6	9	14	10	5	3	8	11	20	20.4
	Klebsiella	-	2	1	1	-	1	-	-	-	-	0
	Bacillus spp.	-	-	-	-	-	1	-	-	-	-	0
	Citrobacter	6	2	1	3	1	-	1	2	-	-	18.75
Enrofloxacin **	*Enterobacter*	8	1	3	2	5	2	1	2	-	-	12.5
	Escherichia	7	5	14	8	3	13	7	14	9	18	48.97
	Klebsiella	2	1	-	1	-	1	-	-	-	-	0
	Bacillus spp.	-	-	1	-	-	-	-	-	-	-	0
	Citrobacter	1	3	1	1	5	2	2	1	-	-	6.25
	Enterobacter	2	4	3	7	2	4	2	-	-	-	0
	Escherichia	4	12	5	7	9	3	4	17	11	26	55.1
	Klebsiella	-	2	-	1	-	1	1	-	-	-	0
	Bacillus spp.	-	-	-	-	-	1	-	-	-	-	100
	Citrobacter	2	1	3	4	3	1	2	-	-	-	18.75
Furazolidone ***	*Enterobacter*	4	5	-	3	5	3	2	1	1	-	29.16
	Escherichia	17	14	5	10	4	14	11	2	9	12	48.97
	Klebsiella	1	1	-	-	2	-	1	-	-	-	20
	Bacillus spp.	-	1	-	-	-	-	-	-	-	-	0
	Citrobacter	2	2	1	3	2	1	2	2	1	-	6.25
Trimethoprim****	*Enterobacter*	1	4	1	2	5	3	2	4	1	1	8.34
	Escherichia	8	10	19	5	4	11	6	14	6	15	21.42
	Klebsiella	1	-	-	2	1	-	1	-	-	-	0
	Bacillus spp.	-	-	-	-	1	-	-	-	-	-	0
	Citrobacter	2	3	1	2	3	1	2	1	1	-	0
Tylosin *****	*Enterobacter*	2	6	2	3	2	4	-	3	1	1	4.16
	Escherichia	16	8	9	5	13	6	15	10	7	9	9.2
	Klebsiella	1	1	2	-	1	-	-	-	-	-	0

Bold lines indicate breakpoints for resistance according to: *Aarestrup *et al*., (2000) and White *et al*., (2003); **White *et al*., (2000); ***National Committee for Clinical Laboratory Standards (NCCLS) Guidelines (Chicago Department of Public Health, 1998); ****Aarestrup *et al*., (2000); *****White *et al*., (2003)

Fig. 1: The number of isolated resistant bacteria from eggs and day-old chicks to the used antibiotics

chick's gut prior to feeding, but in our study no micrococci were seen. Facultative anaerobes include members of Entero-bacteriaceae such as *E. coli*, *Citrobacter spp.*, *Proteus spp.* and *Klebsiella spp.* which are frequently present but in lower numbers. Smaller numbers of other organisms such as the aerobe, *Pseudomonas spp.* and yeasts may be found throughout the gut from time to time (Clarke and Bauchop, 1977; Board and Fuller, 1994). Our results were in general agreement with mentioned surveys.

Since faecal contamination is thought to be a major cause of egg contamination by *Salmonella*, it is not surprising that other members of the Enterobacteriaceae, particularly *E. coli*, can also be isolated from eggs. Between 0.5 and 6% of eggs from normal hens contain *E. coli*. Thus, hatched chicks may already have *E. coli*-infected yolk sacs leading to neonatal mortality. However, other organisms including *Proteus spp.* and enterococci may also be involved, suggesting involvement of the gut flora (Board and Fuller, 1994). Our results showed that about 3.5% of eggs were infected with *E. coli*, but 81.65% of chicks showed contamination with this micro-organism.

The results obtained in this paper confirm the finding of Board and Fuller (1994) that the coliforms are the only organisms normally present in the chicks gut flora. In the study of Nazer and Safari (1994), isolates of bacteria comprising *E. coli* (37.64%), *Klebsiella spp.* (14.11%), *Bacillus spp.* (2.35%) were cultured from dead-in shell chick. In our study *E. coli* was found in larger numbers throughout the alimentary tract of chicks (68.04%) that has similar range of bacterium found by Smith, (1965) and Rajaian *et al.*, (2002). Other major differences between isolated bacteria from two groups are that the eggs had an incidence of *Proteus spp.*, *Pseudomonas spp.*, *Staphylococcus spp.* and *Streptococcus spp.*, while the chicks did not have them. These results indicate poor nest hygiene which could have provided an opportunity for contamination the eggs with fecal organisms. Also under the conditions employed for incubating the eggs, coliforms would be preferentially selected in favour of other microorganisms. The absence of any *Salmonella* among both groups has to be noted in this study.

The sensitivity test performed showed the presence of resistant bacteria (Fig. 1). The finding of chloramphenicol resistance in eggs (2.17%) and chicks (15.97%) was not

expected, because this antibiotic must not be used in poultry production in Iran. Also this resistance pattern to other antibiotics (except of furazolidone) that have not been used in tested farm was seen. Illegally use of this antibiotic in poultry farms and transmission of resistant bacteria via wild birds, workers, equipments and also through feed could be the reason. The occurrence of enrofloxacin resistance among chicks' isolated bacteria was higher (37.5%) than that observed among the same bacteria of eggs (26.08%). Antibiotic susceptibility data from the present study demonstrated that erythro-mycin resistance in isolated bacteria from chicks and eggs from the same broiler breeder were similar. All of other isolated bacteria from eggs and chicks showed high resistance to furazolidone except of *Proteus spp.* and *Pseudomonas spp*. The reason of this resistance might be due to use of this antibiotic in feed of broiler breeders or other environmental possibilities. The occurrence of trimethoprim and tylosin resistance in isolated bacteria from chicks was very low compared to that observed among bacteria from eggs (Fig. 1). These antibiotics were not used in the tested farm.

In conclusion, the results indicate that antibacterial-resistant bacteria might be transmitted to human by the consumption of eggs containing such multi-resistant bacteria and that the use of antibiotics common both in human and animal care should be avoided. To diminish bacterial contami-nation rates in eggs, it's critical that risk reduction strategies are used throughout the food chain. These strategies include on-farm practices that reduce bacteria carriage, increased hygiene at hatchery, setter and also in retail level, continued implemen-tation of HACCP systems and increased consumer education efforts.

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