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Detection of Antibiotic Resistance Bacterial Isolates from Retail Chickens and Eggs in Azerbaijan

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Abstract

Microbial Resistance to antibiotics is on the rise, in part because of inappropriate use of antibiotics in human medicine but also because of practices in the agricultural industry. Intensive animal production involves giving chicken large quantities of antibiotics to promote growth and prevent infection. These uses promote the selection of antibiotic resistance in bacterial populations. The resistant bacteria from agricultural environments may be transmitted to humans, in whom they cause disease that cannot be treated by conventional antibiotics. The objective of this study was to investigate the antibiotic resistance of bacteria isolated from commercial broiler chicken farms and village chicken farms.

Keywords: Antibiotics, resistance, animal products

Introduction

In modern food animal production, antimicrobial agents are used in one of four different ways. *Therapy:* treatment of infections in clinically sick animals, preferably with a bacteriological diagnosis. *Metaphylactics:* treatment of clinically healthy animals belonging to the same flock or pen as animals with clinical symptoms. In this way infections may be treated before they become clinically visible and the entire treatment period thereby shortened (Aarestrup, 2005; Hao; Cheng et al, 2014). In modern productions systems this is the only way to treat large broiler flocks with water medication. *Prophylactics:* treatment of healthy animals to prevent disease in a period where they are stressed (e.g. medicated early weaning) (Munita and Arias, 2016). This use of antimicrobial agents may indicate management problems, and is in most countries not legal or considered imprudent. *Growth promotion:* continuous inclusion of antimicrobial agents in animal feed to improve growth (Blair et al,

2015). Antimicrobial agents are medicines used to treat infections caused by bacteria in particular (Nathan and Cars, 2014; Lushniak, 2014). They are essential to both human and animal health, but in recent years, some bacteria have demonstrated full or partial resistance to various antimicrobial agents (Aslam et.al, 2018; Lye et al, 2012; Molbak, 2004). This phenomenon, called antimicrobial resistance (AMR), is rising concern for both public and animal health (Nathan and Cars, 2014; Bonnie and Marshall, 2011). Many of the actions implemented to improve animal health depend on the availability and appropriate use of quality veterinary medicines, and notably antimicrobial agents (Nathan and Cars, 2014). Animal health is a key component of policies to improve animal welfare, food security and food safety (ISO 6887; Littmann and Viens, 2015).The OIE believes it is vital to enable adequate access to effective antimicrobial agents to treat animal diseases, but emphasises the need to regulate that access through the intervention of well-trained veterinarians, whose ethics are ensured by national Veterinary Statutory Bodies as laid down by law (Nathan and Cars, 2014). Veterinary practice Antibiotics not only administered prophylactically but subtherapeutic doses are administered routinely via feed to increase feeding efficiency such as rate of weight gain in poultry subtherapeutic doses of antibiotics in feed promotes bacterial resistance by decimating the susceptible population of micro-organisms normally present in animals, selecting drug resistant strains (Cavalieri et al, 2005; O'Neill, 2016).

If drug-resistant bacteria spread to humans through the consumption of animal products, resistant bacteria could colonize their new hosts transferring antibiotic resistance to the normal drug-susceptible bacteria already present in the human gut flora (Blair et al, 2015; Randall et al, 2013).

The primary aim of this study was to estimate the proportion of isolates resistant to specified antimicrobials amongst *E. coli, Salmonella spp., Enterococcus spp.* isolated from the gut of Azerbaijan meat chickens.

Materials and Method

The samples were collected between August 2018 and January 2019. The methods followed for this study are in line with recommendations from the OIE Chapter 6.7 "Harmonisation of national antimicrobial resistance surveillance and monitoring programmes" (OIE Standards. 2015; OIE. 2018; Veldman et al, 2017) and ISO 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media (ISO, 2014; ISO, 2017; Singer et al, 2016). Chicken samples and eggs were collected from retail markets and the bacterial cultural were obtained from the chicken samples and eggs (ISO, 2018; ISO/TS 17728; Sohail et al, 2016).

Whole chickens were putted sterile plastic bags and added 1000ml sterile isotonic solution and washed, shaken 15 minutes. 1ml suspension was taken and resuspend the particles (1:10-1:1000) and each dilution (100mikrolitr) streaked direct onto SBA agar. The agar plates were incubated at 37°C for 24 h. CFUs were counted simple tick methods and multiply the dilution quantity and founded average numbers of CFUs (ISO 17604; Singer et al, 2016).

The eggs were putted sterile plastic bags and added 100ml sterile isotonic solution and washed, shaken 15 minutes. 1ml suspension was taken and resuspend the particles (1:10-1:1000) and each dilution(100mikrolitr) streaked direct onto SBA agar. The agar plates were incubated at 37°C for 24 h. CFUs were counted simple tick methods and multiply the dilution quantity and founded average numbers of CFUs (Holmes et al, 2016; ISO, 17604; Nathan and Cars, 2014).

100mikl yolk and white from eggs were suspended sterile isotonic solution different tubes and resuspend the particles (1:10-1:1000) and each dilution(100mikrolitr) streaked direct onto SBA agars. CFUs were counted simple tick methods and multiply the dilution quantities and founded average numbers of CFUs (ISO 6579-1:2017; ISO 6887; ISO 6887-1:2017; ISO 7218).

Enterococcus isolation and typing

The prepared sample was shaken to resuspend the particles, and then streaked direct onto SBA agar. The agar plates were incubated at 37°C for 24 h. From a pure subculture from the original colony, bacteria were harvested for storage at -20°C on cryo-beads in two separate, identical containers labelled with the sample code and the laboratory reference number (ISO 6887-1:2017; Lynch et al, 2013; Murray, 1990).

E.coli isolation and typing

E.coli was isolated using the ISO 16649-1:2018 for *E.coli spp.* using SBA, Endo agar and Bismuth sulfite agar and incubated at 37°C for 24h (Dodani, 2018; ISO 16649-1:2018; Islam et al, 2016).

The prepared sample was shaken to resuspend the particles, and then streaked direct from SBA onto Endo agar which achieved both bacterial isolation and type confirmation. The agar plates were incubated at 37°C for 18h and then one clone was selected and subcultured onto Bismuth sulfite agar. *E. coli* isolation was confirmed using an indole test. From a pure subculture from the original colony, bacteria were harvested for storage at -20°C on cryo-beads in two separate, identical

containers labelled with the sample code and the laboratory reference number (ISO 16649-1:2018; Kalia et al, 2014).

Salmonella spp. isolation and typing

Salmonella was isolated using the method ISO 6579:2002 for *Salmonella spp.* using SBA, Endo agar and Bismuth sulfite agar and incubated at 37°C for 24h. Bacterial isolates were harvested for storage at -20°C on cryo-beads in two separate, identical containers labelled with the sample code and the laboratory reference number (ISO 6579-1:2017;Wellington et al, 2013).

Each isolated and identified colonies have been submitted to an antibiogram test carried out by the disk diffusion method, as recommended by the National Committee for Clinical Laboratory Standards (Bonnie and Marshall, 2011).

Antimicrobial susceptibility for the isolates was determined by the broth microdilution method either on veterinary reference card panels according to the manufacturers' guidelines or in-house panels prepared according to Clinical and Laboratory Standards Institute (CLSI) standards. Isolates were subjected to analysis using both Clinical Breakpoints and Epidemiological Cut-off Values (ECOFF) (Liu et al, 2010).

Isolates were screened for the following antibiotics and doses: ceftriaxone (CFX) 30 mg, gentamicin (GEN) 10 mg, tetracycline (TET) 30 mg, erythromycin (ERI) 15 mg, amoxicillin (AX) 25 mg. More this concentration considered as antibiotic resistant bacteria (Bonnie and Marshall, 2011; Singer et al, 2016; Smith et al, 2007; Singer et al, 2016).

Results

Different antimicrobial resistance patterns were identified from all chicken samples. 8 bacterial strains (4 *Enterococcus spp.*, 2 *E.coli,* 2 *Salmonella spp.*) were isolated from the chicken of the different poultry farms (Table 1). Total bacterial counts (colony forming units-CFU) were underestimation which was given ISO standards. SB, HG, SN, VC conventional names were given to poultry farms. *Enterococcus spp.* was detected from SB, HG, SN and VC, *E.coli* was identified from SN and VC, *Salmonella spp*. was sensed HG and VC. *Enterococcus spp., E.coli*. and *Salmonella spp.* were isolated in VC.

Table 1. Isolated bacterial strains from the chicken of the different poultry farms

	Enterococcus spp.	*E.coli*	*Salmonella spp.*
SB	+	-	-
Hg	+	-	+
Sn	+	+	-
Vc	+	+	+

In order to determine resistances, all strains were tested susceptibility to 5 antibiotics. The results are summarized in Table 2.

The isolates screened, the most common resistance was observed against gentamicin, amoxicillin and erythromycin. Although 62.5% isolates were resistance to ceftriaxone and 62.5% isolates were resistance to tetracycline. *Enterococcus spp (SN), E.coli (SN), Enterococcus spp.(VC), E.coli(VC)* were perform resistance to ceftriaxone but *Enterococcus spp (SB)* and both *Salmonella spp.* strains were perform sensitivity to ceftriaxone. *Enterococcus spp (SB), Enterococcus spp(HG), Salmonella spp.(HG), Salmonella spp.(VC)* were perform resistance but *Enterococcus spp (SN), E.coli (SN), E.coli (VC) were perform sensitivity* to tetracycline. *Salmonella spp (VC)* resistance is increasing against ceftriaxone (Table 2).

Enterococcus spp. achieve the most common resistance to antibiotics. *Salmonella spp (VC)* perform resistance against gentamicin, tetracycline, amoxicillin and erythromycin but both isolates of *Salmonella spp* perform the sensitivity against ceftriaxone. *E.coli* spread resistance to gentamicin, amoxicillin and erythromycin but thoughtful to tetracycline (Table 3).

Discussion

The results show that *Enterococcus spp* are commonly found in the poultry farms but CFU level is underestimation. Antibiotic resistance *Enterococcus spp* were isolated from broiler chickens in terms of distribution among farms. Our results showed that *Enterococcus spp* strains were resistant to amoxicillin, gentamicin, erythromycin. Though the enterococci in this study showed acquired resistance traits to a number of antibiotics, they did generally not show resistance to the clinically relevant antibiotic tetracycline and ceftriaxone, especially among the *Enterococcus spp (HG), Enterococcus spp (SB)* strains. Most challenging are strains that have acquired multiple antibiotic resistance, especially resistance to erythromycin, amoxicillin and gentamicin. It is difficult to assess the impact of antibiotic-resistant

Table 2. The results of susceptibility testing

Antibiotics	Conventional names of poultry farms	Isolated bacteria	Concentration (mg)							
Gentamicin			8	16	32	64	128	256	512	1024
	SB	*Enterococcus spp*	R	R	R	S	S	S	S	S
	SN	*Enterococcus spp*	R	R	R	R	R	R	S	S
		E.coli	R	R	R	R	R	R	S	S
	HG	*Enterococcus spp*	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	R	R	R
	VC	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	R	R	R
Ceftriaxone			0.01	0.1	5	10	30	60	120	240
	SB	*Enterococcus spp*	R	R	R	R	S	S	S	S
	SN	*Enterococcus spp*	R	R	R	R	R	S	S	S
		E.coli	R	R	R	R	R	R	R	R
	HG	*Enterococcus spp*	R	R	R	R	R	R	R	R
		Salmonella spp.	S	S	S	S	S	S	S	S
	VC	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	R	R	S	S
		Salmonella spp.	R	R	S	S	S	S	S	S

Tetracycline			0.01	0.1	5	10	30	60	120	240
	SB	*Enterococcus spp*	R	R	R	R	R	R	R	R
	SN	*Enterococcus spp*	R	R	S	S	S	S	S	S
		E.coli	R	R	R	S	S	S	S	S
	HG	*Enterococcus spp*	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	S	S	S
	VC	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	S	S	S	S
		Salmonella spp.	R	R	R	R	R	S	S	S
Amoxicillin			0.01	0.1	5	10	30	60	120	240
	SB	*Enterococcus spp*	R	R	R	R	R	R	R	R
	SN	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	R	R	R	R
	HG	*Enterococcus spp*	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	R	R	R
	VC	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	R	R	R
Erythromycin			0.01	0.1	5	10	30	60	120	240
	SB	*Enterococcus spp*	R	R	R	R	R	R	R	R
	SN	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	R	R	R	R
	HG	*Enterococcus spp*	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	R	R	R
	VC	*Enterococcus spp*	R	R	R	R	R	R	R	R
		E.coli	R	R	R	R	R	R	R	R
		Salmonella spp.	R	R	R	R	R	R	R	R

Table 3 Poultry farms and isolated bacteria

Conventional names of poultry farms	Isolated bacteria	Gentamicin	Ceftriaxone	Tetracycline	Amoxicillin	Erythromycin
SB	*Enterococcus spp*	R	**S**	R	R	R
SN	*Enterococcus spp*	R	R	**S**	R	R
	E.coli	R	R	**S**	R	R
HG	*Enterococcus spp*	R	R	R	R	R
	Salmonella spp.	R	**S**	R	R	R
VC	*Enterococcus spp*	R	R	R	R	R
	E.coli	R	R	**S**	R	R
	Salmonella spp.	R	**S**	R	R	R

enterococci from foods on potential human pathogenicity. For the reason that antibiotic resistance alone cannot explain the virulence of enterococci. In order to become pathogenic, they need to express virulence traits associated with adhesion, translocation, and evasion of immune responses and cause pathological changes. It is clear that in the hospital environment, antibiotics may influence selection of pathogenic enterococci, which may lead to infections or superinfections (Molbak, 2004). Eaton and Gasson showed that the incidence of virulence factors was highest among clinical enterococcal isolates, followed in decreasing order by food strains and starter strains, suggesting that the food and starter strains have a lower potential for pathogenicity (CLSI, 2012).

E.coli and Salmonella spp isolates were recovered from VC and HG poultry farms. Obtaining such bacteria of the chicken samples from retail markets were due to the bad safety practice, which followed poultry farms. Similar antibiotic resistance patterns were observed in different farms, indicating that certain isolates were clonal. Clonal isolates could also play an important role in the emergence of antibiotic-resistant *Escherichia coli* strains. Forgetta and et al came same conclusion for *Escherichia fergusonii* (Davies and Davies, 2010) High levels of resistance to gentamicin, amoxicillin and erythromycin could be due to extensive use of this antibiotics over the years since its introduction to feed animals. This finding differed from that of Rooklidge (Pena-Miller et al, 2013; Rooklidge, 2004). The observation in the present study of isolates resistant to amoxicillin is in contrast with that reported by Smith et al. (Viktória et al, 2018; Smith, 2007) in *E. coli* isolated from poultry. Co-resistances were observed in the vast majority of strains this is due to co-selection with other resistance factors.

Two Salmonella isolates displayed resistance to the gentamicin, tetracycline, amoxicillin, erythromycin, but both strains of salmonella were susceptible to ceftriaxone. Of particular importance is the isolation of tetracycline and erythromycin resistant salmonella. The latter can cause severe illness. The ability of bacteria to acquire antibiotic-resistance genes and subsequently spread them to many different bacterial species is well known (Hall, 1997).

Our finding of the two salmonella isolates and *E.coli* from two brands of chicken samples from two grocery stores demonstrates the potential for the contamination of food during handling and processing. However, the contamination of retail meats with resistant salmonella mainly reflects carriage of the organism by poultry farms; intervention strategies should therefore focus principally on reducing the number of pathogens present on farms and in slaughterhouses.

Variations in husbandry practices among farms may play a role in the antibiotic resistance profile of isolates, because a significant difference was noticed between different farms.

Conclusions

Antibiotic resistance is one of the most serious threats to human health today (World Health Organization.2014). We should seek to increase our knowledge regarding the extent of the AMR issue. Ecological and environmental aspects of the issue need not be ignored; all the elements of “one health” should be part of the control policy. Alternative strategies may also play a fruitful role, especially in developing countries. Current global interest indicates that AMR is not an unheeded issue anymore. Although this attention is not itself adequate to combat AMR, a global code of conducts implementing all the options of action against AMR might eliminate in the future (Roca et al, 2015). The creation of new antibiotics targeting the growing threat of multidrug resistance is a goal that remains "alarmingly elusive” (Merelli et al, 2013). Alternatives to antibiotics such as probiotics and lytic bacteriophages can help to decrease the burden of AMR globally. The threat of resistance will always accompany any new drug introduced for clinical use. The only possible, sustainable solution is to keep pace with it. This will involve introducing profound changes in the use of these drugs, including stewardship programs for rational use and improve targeted therapy. Furthermore, implementation of adequate preventive measures such as vaccines and faster diagnostic tools as well as improving hygiene and reducing the use of antibiotics in animals, will be the only way for preserving the usefulness of antibiotics for future generations and ensure a healthy future for the world’s population. Finally, it is of critical importance to acquire a more comprehensive understanding of the molecular, evolutionary and ecological mechanisms governing the spread of antibiotic resistance. It is time to distinguish the exact costs of antibiotic use in agricultural practice in terms of antibiotic resistance and its significances on the sustainability of susceptible bacterial flora in the environment and to act accordingly.

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