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Full Length Research Paper

Isolation of *Staphylococcus* spp. genera from broiler breeder flocks in East Azerbaijan Province of Iran: Prevalence and antimicrobial susceptibility

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***Staphylococcus* spp. are Gram-positive cocci present on the skin and mucous membranes of humans and animals, and also as environmental contaminants. The aim of this study was to investigate the prevalence and antimicrobial susceptibility of *Staphylococcus* spp. genera from broiler breeder flocks in East Azerbaijan province of Iran. In this study, 14 broiler breeder flocks were studied for existence of *Staphylococcus* infections. In this flocks, initially detected by attention to the clinical symptoms such as laminitis, arthritis specially hock and foot joints and secondarily by necropsy. Then we selected 6 samples from each flock and these samples were cultured in media and antibiogram were carried out to determine the antibiotic susceptibility. The results of our study showed that of 14 farms, 12 farms (85.71%) were positive and 2 farms (14.29%) were negative from existence of the staphylococcal infections. In general, 84.7% of isolated species was susceptible to selected antibiotics, 5.6% was moderate and 9.7% was resistant. Results showed that outbreak of staphylococcal infections in poultry is high and because of its epidemiological importance, measures must be taken in this field to minimize the zoonotic disease transmissible from poultry to humans.**

Key words: *Staphylococcus aureus*, broiler breeder, antibiotic resistance, Iran.

INTRODUCTION

Staphylococcus spp. are Gram-positive cocci present on the skin and mucous membranes of humans and animals, and also as environmental contaminants. Most staphylococci occur as commensals; however, *Staphylococcus aureus* strains produce various toxins and enzymes that cause disease in humans (Lowy, 1998). The targets of infection are the skin, soft tissue, tissues of the respiratory system, bones, joints, and the endovascular system, and infections may be life-threatening in persons with multiple risk factors (Musher et al., 1994). Treating *S. aureus* infection has been complicated by the emergence of drug-resistant strains. For instance, methicillin-resistant *S. aureus* (MRSA) infection is now a serious public health problem in both hospitals and the community (Moran et al., 2006). Spread of these drug-resistant strains occurs mainly from colonized MRSA or from infected person to person. But food has also been implicated as a source of spread in one outbreak case of blood and wound infections in hospitalized patients in a university hospital in The Netherlands (Kluytmans et al., 1995). In addition to humans, livestock such as swine, cattle, and chickens may also contract *S. aureus* infection and develop mastitis, arthritis, septicemia, and so forth (Quin et al., 2002). During slaughter, sources of microbiological contamination on carcass may come from the hide or gastrointestinal tract of the animal or from the slaughter plant environment, including facilities and personnel. Therefore, monitoring carcasses and the slaughter plant environments for specific microorganisms affecting public health is important. In 2000, Taiwan launched a program similar to the Nationwide Microbiological Baseline Data Collection Program designed by the U.S. Food Safety

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Table 1. Results obtained from different biochemical tests.

Genera	Coagulase test	Hemolysis	Pigmented colonies	Mannitol salt agar	Maltose
Staphylococcus aureus	+	+	+	+	+
S. intermedius	+	+	-	(d)	±
S. hyicus	(d)	-	-	-	-
S. epidermidis	-	(d)	-	-	+
S. saprophyticus	-	-	(d)	(d)	+
S. aureus ssp. anaerobious	+	+	-	0	+
S. capare	-	(d)	-	(d)	(d)
S. gallinarum	-	(d)	(d)	+	+
S. arlettae	-	-	+	+	+
S. lentus	-	-	(d)	+	(d)
S. equorum	-	(d)	-	+	(d)
S. simulans	-	(d)	-	+	±
S. delphini	0	+	-	(+)	+
S. chromogenes	-	-	+	(d)	(d)

d: 11-89% positive, +: 90% and more positive, -: 90% and more negative, 0: unknown.

and Inspection Service (FSIS) (U.S. Department of Agriculture, 1996). *S. aureus* is one of many pathogenic microorganisms screened in this program because enterotoxin producing *S. aureus* will cause food poisoning in humans (U.S. Department of Agriculture, 1996). The prevalence and concentration of *S. aureus* in pork carcasses (2000 to 2003) and chicken carcass rinse fluids (2000 to 2002) from Taiwan were reported previously (Chen et al., 2004; Yeh et al., 2005, 2004). However, data for the following years have not been reported. Additionally, the antibiotic susceptibility of the *S. aureus* strains collected from these carcasses has not been determined. Antimicrobial agents used in therapy and as feed supplements to promote growth in food animals may increase the spread of drug-resistant bacteria and the transfer of drug-resistant bacteria from animals to humans (Chaslus-Dancla et al., 2000; Salauze et al., 1990). This study reports the prevalence of *S. aureus* isolated from broiler breeder carcasses during 2010 in Iran.

In addition, selected *S. aureus* strains from the previous years and the present study were assayed for antibiotic susceptibility. The antibiotic resistance profile of *S. aureus* isolated from livestock carcasses would provide important public health information.

The aim of this study was to investigate the prevalence and antimicrobial susceptibility of *Staphylococcus* spp. genera from broiler breeder flocks in East Azerbaijan province of Iran.

MATERIALS AND METHODS

In this study, 14 broiler breeder flocks were studied for existence of *Staphylococcus* infections. In this flocks, initially detected by attention to the clinical symptoms such as laminitis, arthritis specially hock and foot joints and secondarily by necropsy. Then we selected 6 samples from each flock and these samples were cultured in media and antibiogram were carried out to determine the antibiotic susceptibility.

For sampling, carcasses which are fresh samples were obtained from liver, heart and lungs and in stale carcasses from joints and bone marrow. Initially the surface of the tissue was cauterized and then sampling was carried out by Hans near the flame. Then samples were implanted into the blood agar medium and cultured as linear-5-region method then incubated at 37˚C for 24-48 h. Then colonies were assayed from shape, color and size. Finally the biochemical test such as catalase, oxidase, coagulase and OF was carried out to prove the genera (Table 1).

For antimicrobial resistance we used from mueller hinton agar medium and discs dipped with considered antibiotic were located in the plates.

Table 2. Results obtained from antibiogram test.

Antibiotic	No. of sensitive	No. of moderate	No. of resistant
Amoxicillin	12	0	0
Enrofloxacin	12	0	0
gentamycin	8	4	0
Ampicillin	12	0	0
Tetracycline	5	0	7
Trimethoprim	12	0	0

RESULTS AND DISCUSSION

The results of our study showed that of 14 farms, 12 farms (85.71%) were positive and 2 farms (14.29%) were negative from existence of the staphylococcal infections. The result of antibiogram test is shown in Table 2.

Table 3. Results obtained from treatment with Amoxicillin.

Capacity	Farm	Week 10		Week 11		Week 12		Week 13		Week 14		Week 15		Week 16		Week 17		Week 18		Week 19		Week 20		Week 21		Week 22		Week 23		Sum	
		Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated	Losses	Eliminated
25000	1	1	5	2	9	3	24	6	35	4	30	1	8	1	6	2	12	3	18	4	32	2	14	1	9	0	5	0	1	30	208
20000	2	0	3	0	6	1	18	4	28	4	24	0	7	0	3	4	13	2	19	5	27	1	10	0	4	0	2	0	0	21	164
25000	3	2	7	0	11	3	23	7	38	5	31	2	9	2	5	0	9	4	21	3	25	3	12	2	8	1	4	0	2	34	205
30000	4	3	6	3	14	4	20	5	34	6	29	3	11	2	7	1	10	3	16	4	28	4	15	2	10	2	5	0	2	42	207
30000	5	1	8	2	10	3	19	3	29	7	38	4	18	1	6	2	14	5	22	7	34	3	17	1	9	1	6	0	1	40	231
20000	6	1	5	0	8	2	17	3	24	5	29	2	9	1	5	2	18	5	25	6	26	2	14	2	5	1	3	0	1	32	189
20000	7	0	4	1	7	5	20	6	32	7	34	3	12	1	4	2	14	3	21	3	28	3	11	1	7	1	6	0	3	36	203
25000	8	0	7	2	8	2	19	3	24	5	28	1	9	1	5	2	16	3	26	4	29	2	14	1	6	2	3	0	4	28	198
20000	9	0	4	1	9	2	17	4	31	4	26	3	8	2	6	2	12	3	26	6	27	1	9	2	7	0	6	0	3	30	191
25000	10	1	6	2	11	2	18	4	25	7	31	4	12	3	8	2	15	3	18	4	30	3	17	2	12	2	7	0	4	39	214
30000	11	1	6	2	11	3	20	3	25	6	30	3	14	1	7	2	16	4	18	5	23	3	19	1	10	1	7	0	2	35	208
20000	12	1	7	2	9	2	19	3	22	5	25	2	11	1	6	2	19	5	28	6	29	2	12	2	6	1	4	0	2	34	199
Mean of losses and eliminates																														33.42	201.42

In general, 84.7% of isolated species were susceptible to selected antibiotics, 5.6% were moderate and 9.7% were resistant.

Then we start to treatment of flocks by Amoxicillin for 6 days at the dose of 30 mg/kg and results have been showed in the Table 3.

S. aureus has been tested in meat and poultry products to assess microbiological safety, sanitation conditions during processing, and storage quality of products (Tompkin, 1983). *S. aureus* is a frequent etiological agent of food poisoning (Halpin-Dohnalek and Marth, 1989; Lo´pez et al., 1993; Jablonski and Bohach, 1997). Its presence in poultry (Waldroup, 1996) emphasizes the need for laboratory surveillance for this bacterial pathogen. However, not all *S. aureus* strains present in processed poultry carcasses are a cause for public health concern. The animal strains make a very small contribution to human food poisoning (Ha´jek and Marsa´lek, 1971; Shiozawa et al., 1980; Parker, 1983; Isigidi et al., 1990).

On the other hand, the *Staphylococcus. aureus* isolates from human sources may be considered the most dangerous strains of public health significance (Isigidi et al., 1992). In fact, poultry meat has been frequently associated with food borne illness in which initial contamination is traceable to food handlers (Halpin-Dohnalek and Marth, 1989).

In one study carried out by Mulders et al. (2010) revealed that a total of 405 broilers were sampled upon their arrival at the slaughterhouse, of which 6.9% were positive. These broilers originated from 40 Dutch slaughter flocks of which 35.0% were positive. MRSA contamination in the different compartments of slaughterhouses increased during the production day, from 8% to 35%. Of the 119 MRSA isolates, predominantly livestock-associated MRSA ST398 was found, although 27.7% belonged to ST9 (spa type t1430).

Riddell (1980) stated that rickets and arthritis/osteomyelitis due to infection with *Staphylococcus aureus* were significant problems only in one flock each, while rotated tibia caused

significant losses in three flocks. Long bone distortion was the major cause of economic loss and occurred in all flocks. Possible causes of long bone distortion are briefly discussed.

Alfonso and Barnes (2006) demonstrate that Neonatal staphylococcal osteomyelitis should be considered when recently placed turkey flocks experience increased mortality, especially if theydevelop severe swelling and inflammation of toes following trimming and have enlarged swollen feet, tendons, or joints.

Lin et al. (2009) reported that all 207 strains were sensitive to nitrofurantoin and vancomycin. Over 50% were resistant to clindamycin (MIC that inhibited 90% of strains [MIC90] = 32 microg/ml) and tetracycline (MIC90 = 64 microg/ml). The percentages resistant to methicillin (oxacillin), chloramphenicol, erythromycin, and tylosin were 19.4% (40 of 207), 18.8% (39 of 207), 23.2% (48 of 207), and 20.8% (43 of 207) with MIC90s of 8, 64, > or = 64, and > or = 128 microg/ml, respectively. The methicillin-resistant *S. aureus* (MRSA) strains exhibited resistance to more antibiotics than did the methicillin-susceptible strains, and 87.5% (35 of 40) of the MRSA strains carried the mecA gene sequence. Since MRSA infections have become a public health concern in both communities and hospitals, testing for the presence of MRSA in animal carcasses during slaughtering operations are warranted.

Also, Hanson et al. (2011) state that *S. aureus* strains were isolated from 27 of 165 samples, giving an overall prevalence of 16.4%. Turkey, pork, chicken, and beef had individual *S. aureus* prevalence rates of 19.4, 18.2, 17.8, and 6.9%, respectively. Two isolates of MRSA were isolated from pork, giving an overall prevalence of 1.2%. One MRSA isolate was positive for the PVL gene. Common spa types included t034, t337, t008, and t002. These results suggest that MRSA is present on low numbers of retail meat in Iowa.

In one other research de Boer et al. (2009) showed that MRSA strains were isolated from 264 (11.9%) of 2217 samples analyzed. Isolation percentages for the meat species were: beef (10.6%), veal (15.2%), lamb and mutton (6.2%), pork (10.7%), chicken (16.0%), turkey (35.3%), fowl (3.4%) and game (2.2%). The majority (85%) of the isolated strains belonged to spa-types of pulsed-field gel electrophoresis (PFGE) non-typeable (NT)-MRSA, corresponding to the multilocus sequence type ST398, a type also recently isolated in the Netherlands from pigs. However, a smaller part of these strains were found to be of other ST's, possibly of human origin.

Further studies are needed to elucidate transmission routes of MRSA in relation to meat and other foods and to provide the tools for preventing the spread of MRSA. At present the high prevalence of MRSA in meat has not been shown to contribute significantly to the dissemination of MRSA to humans and the possible health hazard for consumers of the presence of MRSA in foods should be further elucidated.

Conclusion

With comparison of above mentioned results it has been revealed that outbreak of staphylococcal infections in poultry is high and because of its epidemiological importance, measures must be taken in this field to minimize the zoonotic disease transmissible from poultry to humans.

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