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The Isolation of Antibiotic-Resistant *Salmonella* from Intestine and Liver of Poultry in Shiraz Province of Iran

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Abstract: The aims of this study were to isolation of *Salmonella* from poultry farms of Shiraz province of Iran and determination of their susceptibility against common antibiotics. One hundred and ninety two samples were harvested from intestine and liver of chickens and were aseptically cultured in enrichment and selective media. Out of 192 samples, 30 *Salmonella* were isolated. Four different serogroups were found among 30 *Salmonella* isolates. Strains of serogroup D1, which accounted for 70% of total isolates, were the most common isolates. PCR products of all isolated *Salmonella* showed a predicted 284 bp amplified DNA fragment of *inv*A gene. All of 30 *Salmonella* strains were susceptible to the antimicrobial effect of Cephalothin, Tylosin, Colistin, Ciprofloxacin, Enrofloxacin, Gentamicin, Chloramphenicol, Cephalotin and Cefotaxime. But 20.7% of *Salmonella* strains were resistant to Trimethoprim, Nalidixic acid, Flumequine, Tetracycline and Neomycin, 24.2% to Streptomycin, 34.5% to Kanamycin and 13.8% to Amikacin.

Key words: *Salmonella*, antibiogram, poultry, Iran, antibiotic

Introduction

Salmonella serotypes are important zoonotic pathogens in humans and animals (Winokur *et al*., 2000). Salmonella serotypes cause a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia (Bennasar *et al*., 2000). Food-borne diseases caused by Salmonella serotypes occur at high frequently in industrialized nations and developing countries and represent an important public health problem worldwide (White *et al*., 2001; Lampel *et al*., 2000). Salmonella are among the major bacterial pathogens of poultry in the all world and most Salmonella infection in humans result from the ingestion of contaminated poultry (Carli *et al*., 2001).

The administration of antimicrobial agents in chickens creates selection pressure that favors the survival of antibiotic resistant pathogens. Resistance of Salmonella to commonly used antimicrobials is increasing both in the Veterinary and public health sectors and has emerged as a global problem (Molla *et al*., 2003).

The aim of present work was to determine the antibiotic-resistance of Salmonella strains isolated from chickens in Shiraz area.

Materials and Methods

Isolation: One hundred and ninety two samples were harvested from intestine and liver of chickens in poultry farms of Shiraz, a city in south of Iran. Samples were aseptically cultured in to selenite F broth (Merck) with sterile swab and incubated at 37°C for 18-24 hours. Subsequently, a loopful of each broth was streaked on to *Salmonella* - *Shigella* agar (Merck) and xylose lysine deoxycholate agar (Merck) for further incubation at 37°C for 24 hrs. Non fermenting lactose and negative urease bacteria had selected and were transferred to nutrient agar slant (Merck) and incubated at 37°C for 24 hrs prior serological screening using *Salmonella* antisera (Difco).

Polymerase Chain Reaction (PCR): Extraction of DNA was performed by boiling for 10 min and centrifuged at 6000 rpm for 5min. *Salmonella* specific primers, S139 and S141 (Rahn *et al*., 1992) which used in this study have respectively the following nucleotide sequence based on the *invA* gene of *Salmonella* 5´ – GTG AAA TTA TCG CCA CGT TCG GGC AA – 3´and 5´ – TCA TCG CAC CGT CAA AGG AAC C –3´. DNA amplification was performed based on the protocol of Rahn *et al*. (1992). Amplified products were resolved in 1.2% agarose gel stained with ethidium bromide. A current of 120 V was applied to each gel. A 100 bp DNA ladder (Fermentas) was used as a marker for determining the molecular weight of PCR product.

Disk diffusion method: The disk diffusion method was performed to determine susceptibility of the *Salmonella* isolates based on the NCCLS 1996 protocol. The bacterial suspension turbidity adjusted to McFarland standard number 0.5, in Mueller Hinton broth (Merck) and cultured fluently over the entire surface of Muller

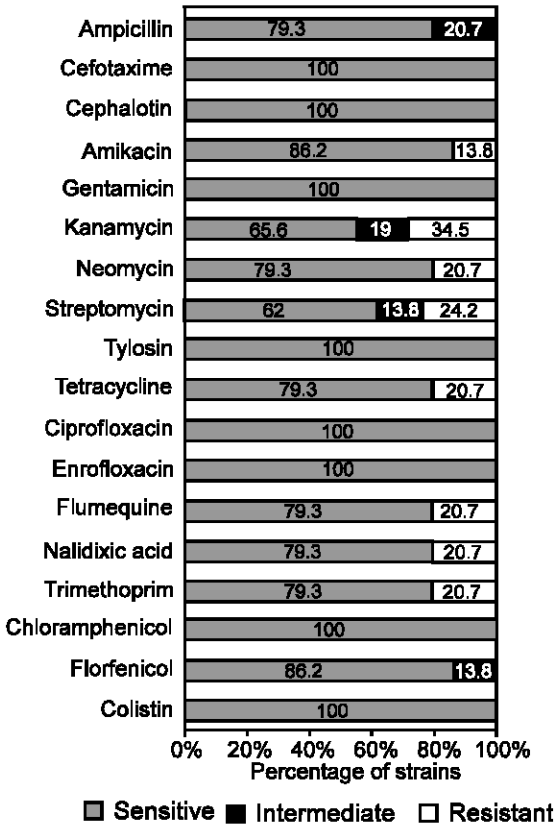

Fig. 1: Antibiotic susceptibility of isolated *Salmonella* from chicken in Shiraz province of Iran

Hinton agar (Merck) with sterile cotton swab. Commercial antibiotic disks containing single concentrations of each antibiotic were then placed on to the inoculated plate surface. The zone of inhibition of growth around each disk after overnight incubation at 37°C, were measured in millimeters. The zone diameter was interpreted using a zone size interpretation chart (Lorian, 1996). The antibiotic and their concentration were as follow: Amikacin 30µg, Ampicillin 10µg, Cefotaxime 30µg, Cephalotin 30µg, Chloramphenicol 30µg, Ciprofloxacin 5µg, Colistin 10µg, Kanamycin 30µg, Neomycin 30µg, Streptomycin 10µg, Trimethoprim 5µg, Nalidixic-acid 30µg, Gentamycin 10µg, Tetracycline 30µg, Florfenicol 30µg, Enrofloxacin 5µg, Flumequin 5µg, Tylosin 30µg (Quinn *et al.*, 1994).

Results

Out of 192 samples, 30 *Salmonella* were isolated. Four different serogroups were found among 30 *Salmonella* isolates. Strains of serogroup D1, which accounted for 70% of total isolates, were the most common isolates. Strains in serogroup C1 were the second most common serotypes (20%) and other strains were belonged to serogroup C2 (6.6%) and serogroup B (3.3%). PCR products of all isolated *Salmonella* showed a predicted 284 bp amplified DNA fragment of *inv*A gene.

All of 30 *Salmonella* strains were susceptible to the antimicrobial effect of Cephalothin, Tylosin, Colistin, Ciprofloxacin, Enrofloxacin, Gentamicin, Chloramphenicol, Cephalotin and Cefotaxime. But 20.7% of *Salmonella* strains were resistant to Trimethoprim, Nalidixic acid, Flumequine, Tetracycline and Neomycin, 24.2% to Streptomycin, 34.5% to Kanamycin and 13.8% to Amikacin (Fig.1).

Discussion

The results showed that *Salmonella* infection is about 15.6 in intestine and liver of broilers in poultry farms and serogroup D1 with 70% frequency is dominant in Shiraz area. The present study supports the ability of these specific primer sets to confirm the isolates as *Salmonella*. The ability of *Salmonella* specific primers to detect and confirmation *Salmonella* species accurately is primarily due to the primer sequences that are selected from the gene *invA* of *S.typhimurium*. *Salmonella invA* gene code for a protein in inner membrane of bacteria which is necessary for invasion of epithelial cells (Darwin *et al.*, 1999).

All strains of serogroup C2 and 76.2% Strains of serogroup D1 were susceptible to all antibiotics but strains of serogroup C1 which contain 20% of total isolates were resistant to majority of antibiotics. The antimicrobial resistance pattern of the *Salmonella* strains isolated from chickens indicate that a large proportion of the strains were resistant to a variety of drugs tested. At all, 20.6% of *Salmonella* strains exhibited multiple resistances to more than eight antibiotics. *Salmonella* are among those most known to carry plasmids, which encode for drug resistance. This implies that widespread use of antimicrobials in animals or humans may cause an increase in the frequency of occurrence of bacteria resistant to other antimicrobials as the R plasmid may encode resistance to additional antimicrobial agents (Molla *et al.*, 2003). Integrons are a special case of multidrug resistance. Epidemiological studies of *enterobacteriaceae* in France and Netherlands showed that over half of the isolates tested carried an integrons. Multidrug resistance can also often be caused by reduced uptake or the expression of porins and changes in the cell, which cause reduced uptake or expression of efflux pumps (Fluit *et al.*, 2001).

Efforts are needed to reduce the prevalence of resistant *Salmonella* in broiler chickens, including the adoption of guidelines for the prudent use of antimicrobial agents in animals used for food and a reduction in the number of pathogens present on farms.

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