

Microbiology

Susceptibility of Salmonella Strains to Antibiotics and R-Plasmids Profiling

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ABSTRACT. At present, gram-negative bacteria start to take up leading position in common structure of zoonotic infections, displacing other infections to the sideline. This study was conducted to investigate the occurrence of *Salmonella* sp., to determine their susceptibility to antibiotics and plasmid profiling. In this research, the material for bacteriological investigation was taken out of tracheae of the chickens from two poultry farms. A total of 72 *Salmonella* sp. were isolated from samples, from which 48 strains were serotyped as *S. typhimurium* and 24 strains – as *S. enteritidis*. It has been established that isolated salmonella strains are characterized by high resistance to antibiotics of broad spectrum. Their antimicrobial susceptibility was tested to 9 antibiotics. All salmonella strains were found to be resistant to penicillin, ampicillin, erythromycin, whereas, they were susceptible to streptomycin, chloramphenicol and slightly susceptible to kanamycin and gentamicin. Plasmids determining resistance to streptomycin, chloramphenicol, tetracycline, gentamicin, carbenicillin, kanamycin and belonging to the incompatibility groups T, I, M, N were revealed by the methods of elimination and conjunction. *Salmonella* strains contain also plasmids of group IncPI having wide spectrum of hosts. © 2014 Bull. Georg. Natl. Acad. Sci.

Key words: *Salmonella*, antibiotic resistance, R-plasmid

Salmonella sp. is a gram negative rod shaped, motile and facultative anaerobe bacteria in the family of *Enterobacteriaceae*. Chicken and poultry products have been implicated as one of the major sources of *Salmonella* infections in human. *Salmonella* infections still occur at high frequencies in industrialized nations and developing countries as well. Infections due to *Salmonella* serotypes continue to be a major public health concerned [1, 2]. The US Department of

Agriculture's Food safety Inspection Service (USDA-FSIS) estimated that in 2007 poultry products accounted for approximately 60% of the food-borne illnesses originating from *Salmonella* [3]. Currently, the control strategies of salmonellosis besides vaccines, antibiotics are mainly applied for disease treatment and prevention. However, due to the continual clinical using of antibiotics in large amount, antimicrobial drug resistance is an increasing prob-

Table 1. The results of antibiotic susceptibility of Salmonella strains

Strains	Antibiotics								
	Pc	Ap	Em	Km	Cm	Sm	Tc	Cb	Gm
<i>Salmonella typhimurium 1</i>	R	R	R	M	S	S	M	M	M
<i>Salmonella typhimurium 2</i>	R	R	R	M	S	S	M	M	M
<i>Salmonella typhimurium 3</i>	R	R	R	M	S	S	M	M	M
<i>Salmonella typhimurium 4</i>	R	R	R	M	S	S	M	M	M
<i>Salmonella typhimurium 5</i>	R	R	R	M	S	S	M	M	M
<i>Salmonella typhimurium 6</i>	R	R	R	M	S	S	M	M	M
<i>Salmonella enteritidis A</i>	R	R	R	M	S	R	M	R	R
<i>Salmonella enteritidis B</i>	R	R	R	M	S	R	M	R	R

lem. High resistance to drugs of salmonella strains is often conditioned by the presence of resistance factors (R-plasmids) to antibiotics in bacterial cells. This is an important peculiarity of the species which create substantial difficulties in the treatment of infections caused by them. Consequently the detection of salmonella drug resistance is very important to prevent salmonellosis in chicken [4].

Our aim was to serotype isolated salmonella strains from chickens, test their antimicrobial resistance, classification of the detected plasmids and determination of their phenotypic characters.

Materials and methods

Bacterial isolates. 72 salmonella strains were collected from chickens of two poultry farms, Kaspi region, in 2012-2013. Material for bacteriological investigation was taken from trachea of the chicken by sterile tampon. It was inoculated on sugar broth, bloody agar, Mueller medium and was incubated at 37°C for 18-24 h. Suspect salmonella colonies were confirmed according to morphological, cultural, biochemical and serological properties, after which they were serotyped.

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed on 8 Salmonella isolates by the indicator disc method (method of paper discs) and the method of 2-fold serial dilutions in liquid medium. The following antibiotics were used: penicillin (Pc), ampicillin (Ap), erythromycin (Em), streptomycin (Sm), tetracycline (Tc), kanamycin (Km), chloramphenicol (Cm), carbenicillin (Cb), gentamicin (Gm). Resistance was determined accord-

ing to reference zone diameter interpretive standards.

Plasmid profiling. The plasmids revealed in salmonella strains were classified according to the system of *E. coli* strains applying tester plasmids. Also the system of *P. aeruginosa* PAO tester strains were used. Elimination of the resistance markers was conducted by the replica method. For identification of plasmid incompatibility groups, *P. aeruginosa* and *E. coli* strains with tester plasmids were applied.

Results and Discussion

72 Salmonella strains were isolated and identified from two poultry farms. From all the strains 48 strains were identified to be *S. typhimurium* and 24 strains – *S. enteritidis*.

Eight salmonella strains out of 72 were chosen to test for their susceptibility to antibiotics. All of the strains showed resistance to Pc, Ap, Em. The strains were susceptible to Cm and slightly susceptible to Km and Tc. While *S. typhimurium* isolates were susceptible to Sm, *S. enteritidis* were resistant to this antibiotic. *S. typhimurium* strains were found to be slightly susceptible to Cb and Gm, but *S. enteritidis* strains showed resistance to them (Table 1).

In order to study distribution of R-plasmids in Salmonella strains, all 72 strains were exposed to elimination by acridine orange. 50% of strains lost resistance markers. Eight strains of salmonella, which carried resistance markers, were chosen to find out the nature of genetic control of high drug resistance of Salmonella strains. It was established that all tested strains of salmonella contain conjugative R-plasmids. They contain genes, which determine resistance to

Table 2. Plasmids belonging to *E. coli* incompatibility group, found in salmonella strains

Sampling site	Plasmid	Characteristic	Incompatibility group	Mol. wt (MDa)	Frequency of conjunctive transfer
Poultry farms	pJ 100	Km Sm Tra+	J	40.0	10 ⁻⁴
	pM 101	Sm Tc Tra+	M	30.0	10 ⁻³
	pN 102	Sm Tc Km Tra+	N	62.0	10 ⁻⁴
	pT 103	Km Tb Tra+	T	58.0	10 ⁻³
	pJ 15	Km Sm Tra+	J	40.0	10 ⁻³
	pM 16	Sm Tc Tra+	M	30.0	10 ⁻⁴
	pN 17	Sm Tc Km Tra+	N	60.0	10 ⁻³
	pT 18	Km Tc Tra+	T	50.0	10 ⁻³

streptomycin (72%), chloramphenicol (62%), tetracycline (81%), gentamicin (50%), carbenicillin (60%) and kanamycin (70%).

The resistance of salmonella strains to Pc, Ap and Em might be determined by chromosomal genes, as obtained transconjugantes transmission of resistance determinants to the mentioned antibiotics was not observed.

Obtained results indicate that salmonella strains which contain plasmids, belong both to *E. coli* incompatibility group and *P. aeruginosa* incompatibility group.

Characterizing conjugative plasmids isolated from salmonella strains in the poultry farms, should be mentioned that plasmids with molecular weights 40.0 and 30.0 MDa carry resistance to streptomycin, kanamycin, tetracycline and belong to the J, M incompatibility group. They are indicated as pJ 100 and pM 101, respectively. Plasmids with molecular weight 62.0 and 58.0 MDa relatively belong to the incompatibility group N and T and are indicated as pN 102, pT 103, respectively.

Conjunctive plasmids with molecular weights

40.0; 30.0; 60.0 and 50.0 MDa, belong to the incompatibility groups J, M, N, T, respectively. They are indicated as pJ 15, pM 16, pN 17, pT 18, respectively (Table 2).

In salmonella strains from both two farms were found R-plasmids which belong to IncPI incompatibility group, having molecular weights 30.0 and 35.0 MDa and determine the resistance to tetracycline, kanamycin, carbenicillin and gentamicin; they are indicated as pR 1, pR2, pR 5 and pR 6, respectively (Table 3).

In conclusion, our study identified different *Salmonella* spp. among chicken farms in one region of Georgia. This information might be helpful for tracking the sources of food-borne infections and designing preventive measures against salmonella infection, especially that caused by *S. typhimurium* and *S. enteritidis* which are agg-transmitted pathogens of poultry and also have human health implications through consumption of contaminated poultry products. High resistance of salmonella strains to antibiotics revealed in this study emphasises the need for more attention to this area.

Table 3. Plasmids belonging to *P. aeruginosa* incompatibility group, found in salmonella strains

Sampling site	Plasmid	Characteristic	Incompatibility group	Mol. wt (MDa)	Frequency of conjunctive transfer
Poultry farms	pR 1	Km Cb Tc Gm Tra+	IncPI	30.0	10 ⁻⁴
	pR 2	Km Cb Tc Gm Tra+	IncPI	35.0	10 ⁻⁴
	pR 5	Km Cb Tc Gm Tra+	IncPI	30.0	10 ⁻⁴
	pR 6	Km Cb Tc Gm Tra+	IncPI	35.0	10 ⁻⁴

მიკრობიოლოგია

Salmonella-ს ეპიდემიოლოგიური შტამების მგრძობელობა ანტიბიოტიკების მიმართ და R-პლაზმიდების პროფილის განსაზღვრა

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(წარმოდგენილია აკადემიის წევრის თ. საღუნაშვილის მიერ)

წინამდებარე ნაშრომში მოყვანილია მონაცემები 2012-2013 წლებში, კასპის რაიონის ორი ფერმერული მეურნეობიდან გამოყოფილი სალმონელას შტამების მგრძობელობის შესახებ ანტიბიოტიკების მიმართ. ნაჩვენებია პოლირეზისტენტული ფორმების ფართო გავრცელება. განსაკუთრებული რეზისტენტობით ხასიათდებიან გენტამიცინის, კარბენიცილინის და კანამიცინის მიმართ. დადგენილია, რომ შტამების 50% შეიცავს კონიუგაციურ R-პლაზმიდებს, რომლებიც შეუთავსებლობის სხვადასხვა ფგუფებს მიეკუთვნებიან. ეს პლაზმიდები განაპირობებენ მდგრადობას სტრეპტომიცინის, ქლორამფენიკოლის, ტეტრაციკლინის, გენტამიცინის, კარბენიცილინის და კანამიცინის მიმართ. მათი შეუთავსებლობის ფგუფებია T, J, M, N. მოლეკულური მასები 40.0; 62.0; 58.0; 30.0; 60.0; 50.0 MDa. სალმონელას ეპიდემიოლოგიურ შტამებში გამოვლენილი იქნა პლაზმიდები, რომლებიც მიეკუთვნებიან IncP შეუთავსებლობის ფგუფს. მათი მოლეკულური მასებია 30.0; 35.0 MDa. ჩატარებული გამოკვლევების საფუძველზე დადგენილია, რომ კონიუგაციური R-პლაზმიდები მონაწილეობენ ეპიდემიოლოგიური შტამების ფორმირებაში.

REFERENCES:

1. C.G. de Freitas, A. P. Santana, P. H. Caldeira da Silva, et al. (2010), International Journal of Food Microbiology, **139**, 1-2: 15-22.
2. E. Álvarez-Fernández, C. Alonso-Calleja, C. García-Fernández, R. Capita (2012), International Journal of Food Microbiology, **153**, 3: 281-287.
3. M. Hensel (2004), International Journal of Medical Microbiology, **294**, 2-3: 95-102.
4. R. Zhang, C.T. Zhang (2006), Microbes and Infection, **8**, 6: 1613-1622.

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