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Antibiotic Resistance Pattern and Biofilm Formation Ability of Clinically Isolates of *Salmonella enterica* Serotype *typhimurium*

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Background: The emergence of antimicrobial-resistant bacteria with biofilm formation ability may be a major threat to public health and food safety and sanitation.
Objectives: The aim of this study was to determine antibiotic resistance patterns and biofilm production characteristics of *Salmonella typhimurium* isolated from different species of birds.
Materials and Methods: The antibiotic resistance patterns of 38 pre-identified isolates were screened by standard Kirby-Bauer disc-diffusion method performed on Mueller–Hinton agar to a panel of 17 antibiotics. The extent of biofilm formation was measured by Microtiter plate (MTP)-based systems.
Results: The highest antimicrobial resistance was detected against nalidixic acid (97%), followed by doxycycline (86%), colistin (84%), streptomycin (84%) and tetracycline (84%). All isolates were sensitive to amikacin (100%) and 97% and 95% of the isolates were sensitive to ceftazidime and ceftriaxone, respectively. Twenty one different antibiotic resistance patterns were observed among *S. typhimurium* isolates. According to the results of the microtitre plate biofilm assay, there was a wide variation in biofilm forming ability among *S. typhimurium* isolates. Most of the isolates (60.52%) were not capable of producing biofilm, while 26.31%, 7.89%, and 5.26% isolates were weak, strong and moderate biofilm producers, respectively.
Conclusions: It was concluded that nearly all *S. typhimurium* isolates revealed a high multiple antibiotic resistant with low biofilm forming capabilities which proposed low association between biofilm formation and antibiotic resistance of a major food important pathogen.

Keywords: Drug Resistance; Biofilms; *Salmonella typhimurium*

1. Background

The genus of *Salmonella* is a major cause of food borne disease. Humans is getting sick through consumption of contaminated food mainly meat, poultry, eggs and vegetables. Nearly all Salmonella serovars belongs to *S. enterica* subsp. *enterica* and the most frequent throughout the world are *S. enterica* serovar typhimurium and *S. enterica* serovar enteritidis. *Salmonella* spp. Infection causes four different clinical manifestations which non-typhoid salmonellosis is more frequent in industrialized countries (1, 2).

The resistant phenomenon of microorganisms to antibiotic is a serious threat to public health. Different serotypes of *Salmonella* show a relatively high antimicrobial resistant (3) and *S. typhimurium* is one of the serotypes that have showed the highest average antimicrobial resistance (1).

The problem of antibiotic resistance becomes more serious when the issue of biofilm formation ability in these bacteria is also considered. Biofilm is defined as organized communities of bacteria which adhere to wet surface and begin to excrete a slimy substance which known as Extracellular Polymeric Substances (EPS) allowing them to develop complex three-dimensional, resilient and attached communities to all kinds of material (4). Bacterial biofilms are responsible for many foodborne disease outbreaks originated from food factories in the absence of regular sanitation regimens (5). While some virulence-associated characteristics of *Salmonella*, such as resistance to chemical disinfectants and antibiotics have been well studied, there are little data available that describe bacterial adhesion, biofilm formation on abiotic surfaces, the correlation between the antibiotic resistance and biofilm formation ability of bacteria.

2. Objectives

The primary aim of this study was to determine the antibiotic resistance pattern and biofilm formation of 38 clinical isolates of *S. typhimurium* as important foodborne pathogen isolated from different species of birds.

3. Materials and Methods

3.1. Bacterial Isolates

Pre-identified clinical isolates of *S. enterica* Serovar typhimurium (n = 38) were obtained from the Faculty of Veterinary Medicine, Urmia University, Iran. The isolates had previously been isolated from different avian species and identified by molecular typing to be representative of the *S. typhimurium* (2). All isolated bacteria were preserved on cryogenic beads (Mast CryobankTM, UK) at -70°C. A culture of *Escherichia coli* ATCC 25922 acquired from the culture collection of the Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Urmia University, Iran used as a control strain in antimicrobial susceptibility test.

3.2. Preparation of Bacterial Cultures

To resuscitate the bacteria, one bead from every cryogenic tube was placed in 10 mL Luria Bertani (LB) Broth, Miller (BiomarkTM, India) and incubated statically for 24 hours at 37 ± 1°C. Prior to the experiment, the bacterial cultures were reactivated by two subcultures LB broth. Bacterial suspensions were prepared according to Moradi et al. 2011 (6).

3.3. Antimicrobial Susceptibility Tests

Antimicrobial susceptibility pattern of isolates to a panel of 17 antibiotics were screened by standard Kirby-Bauer disc-diffusion method performed on Mueller–Hinton agar (Merck, Darmstadt, Germany). The plates were incubate at 35 ± 1°C for 18 hours, as described by Clinical and Laboratory Standards Institute guidelines (7). The commercially antibiotics disks (Padtan Teb, Iran) were used in this study were included Amikacin (AMK; 30 μg), Ampicillin (AMP; 10 μg), Chloramphenicol (C; 30 μg), Ceftazidime (CAZ; 30 μg), Cephalothin (CF; 30 μg), Ciprofloxacin (CIP; 5 μg), Colistin (COL; 10 μg), Ceftriaxone (CRO; 30 μg), Doxycycline (DOX; 30 μg), Enrofloxacin (ENR; 5 μg), Gentamicin (GMC; 10 μg), Kanamycin (K; 30 μg), Nalidixic acid (NA; 30 μg), Norfloxacin (NOR; 10 μg), Streptomycin (STR; 10 μg), Sulfamethoxazole, Trimethoprim (SXT; 25 μg) and Tetracycline (TE; 30 μg). Tested antibiotics were selected because of their common use in veterinary practice and hospitals. After incubation at 35 ± 1°C for 18 hours, zone-size interpretation were measured in mm and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediate or resistant. The *E. coli* ATCC 25922 was used as control in all assays. An isolate was defined as resistant if it was resistant to one of the antibiotics tested, whereas isolates resistant to two or more antimicrobials were classified as Multiple Antibiotic Resistant (MAR) (1). A MAR index for an isolate is also calculated as: Number of antibiotics to which isolate was resistant/Total number of antibiotics against which isolate was tested (8).

3.4. Biofilm Assay

The procedure was performed by Microtiter plate (MTP)-based systems using 24-well flat-bottomed polystyrene microtiter plate (Maxwell) according to procedure described by Lizcano et al. (9) and Xu et al. (10) with some modifications. An amount of 200 μL bacterial suspension with OD600 = 0.1 was inoculated directly to each well filled previously with 1800 μL of LB broth, using four wells per isolates to reach a suspension with 7 log CFU/mL per well. Plates were wrapped with parafilm and incubated at 37 ± 1°C for 24 hours. To determine the planktonic cell growth, the suspended cells were collected and optical absorbance was measured at 620 nm (G). Then, the plates were washed three times by phosphate buffered saline (PBS) (Sigma-Aldrich) and allowed to air-dry for 20 minutes. In the next step, biofilms were stained with 2 mL of 1% w/v crystal violet (CV) for 30 minutes, washed twice with taped water to remove excess stain and then allowed to air-dry for 30 minutes. Biofilm was quantified by eluting CV with 2 mL 95% v/v ethanol and determining the optical absorbance of the eluted dye at 540 nm ($CV_{adherence}$). The negative control was used to eliminate the background staining from the CV-stained cells ($CV_{control}$).

The extent of biofilm formation was determined by applying Equation 1: (11)

$$\text{SBF} = \frac{CV_{adherence} - CV_{control}}{G} \quad (1)$$

Where SBF is the specific biofilm formation, $CV_{adherence}$ is the OD 540 nm of stained attached bacteria, $CV_{control}$ is the OD 540 nm of stained control wells and G is the OD 620 nm of cells growth in suspended culture.

Microscopic examination of the wells contained the crystal violet stained biofilms was directly carried out under oil immersion with transmitted light using an Olympus CH40 system microscope after cutting the base of the wells.

3.5. Statistical Analysis

All experiments were carried out in triplicate and repeated in two independent sets of experiments. Statistical comparison of antibiotic resistance and biofilm formation rates among serovars was analyzed by the Chi-square test using GraphPad Prism version 5.00 (San Diego, USA).

4. Results

4.1. Antimicrobial Resistance Profiles

Table 1 shows the percentages of *S. typhimurium* isolates resistant to different antibiotics used in the antimicrobial susceptibility test. Among 17 antimicrobial agents used for screening the antimicrobial resistance

of the isolates, the highest number of isolates was resistant to nalidixic acid (97%), followed by of doxycycline (86%) and colistin (84%), streptomycin (84%) and tetracycline (84%). All Isolates were sensitive to amikacin and 97% and 95% of isolates were sensitive to ceftazidime and ceftriaxon, respectively.

Among all tested isolates, 35 of 38 isolates (92%) were resistance to two or more antibiotics and 3 of 38 isolates (8%) were susceptible to at least one antibiotic. The MAR patterns of isolates are shown in Table 2. Among the 17 antibiotics tested, 21 different resistance patterns were observed in *S. typhimurium* isolates. The maximal MAR index (0.58) was detected in 2 isolates (ST35 and ST36). The most common resistance pattern was COL, DOX, K, NA, STR, SXT, T which displayed by five isolates.

A significant relationship was observed between the resistance of the bacterium and the antibiotics category. The resistance ratios to antibiotics belongs to aminoglycosides (except of amikacin), tetracyclines and polymyxin, were found to be extremely high, compared to antibiotics belongs to third-generation cephalosporins which exhibited sharp sensitive pattern (Table 1).

Table 1. Resistance of *S. typhimurium* Isolates to Different Antibiotics Used in the Antimicrobial Susceptibility Test [a]

Antibiotics	Resistant	Intermediate	Sensitive
Amikacin (AMK)	0	0	100
Ampicillin (AMP)	11	21	68
Chloramphenicol (C)	26	21	53
Ceftazidime (CAZ)	0	3	97
Cephalothin (CF)	26	32	42
Ciprofloxacin (CIP)	0	8	92
Colistin (COL)	84	16	0
Ceftriaxone (CRO)	0	5	95
Doxycycline (DOX)	86	11	3
Enrofloxacin (ENR)	16	81	3
Gentamicin (GMC)	0	11	89
Kanamycin (K)	63	5	32
Nalidixic acid (NA)	97	3	0
Norfloxacin (NOR)	0	18	82
Streptomycin (STR)	84	8	8
Sulfamethoxazole – trimethoprim (SXT)	76	0	24
Tetracycline (TE)	84	0	16

[a] Data are presented as %.

Table 2. Different Antibiotic Resistance Patterns of *S. typhimurium* Isolates

Pattern	Isolate No.	Antibiotic Resistant Pattern [a]	MAR Index [b]	No. of Isolate
1	ST33	DOX	0.05	1
2	ST17, ST30	NA	0.05	2
3	ST32, ST37	COL/NA	0.11	2
4	ST8	DOX/NA/STR/TE	0.23	1
5	ST9	COL/NA/STR/TE	0.23	1
6	ST2	DOX/K/NA/STR/TE	0.29	1
7	ST6	COL/DOX/NA/STR/SXT	0.29	1
8	ST5	COL/DOX/K/NA/STR/TE	0.35	1
9	ST3, ST12, ST19, ST23	COL/DOX/NA/STR/SXT/TE	0.35	4
10	ST1, ST10, ST22, ST24, ST28	COL/ DOX/ K/ NA/ STR/ SXT/ TE	0.41	5
11	ST4	C/COL/DOX/NA/STR/SXT/TE	0.41	1
12	ST21	C/DOX/K/NA/STR/SXT/TE	0.41	1
13	ST25	AMP/CF/COL/DOX/NA/SXT/TE	0.41	1
14	ST7, ST15, ST16, ST20	C/COL/DOX/K/NA/STR/SXT/TE	0.47	4
15	ST14, ST18, ST27, ST38	CF/COL/DOX/K/NA/ STR/SXT/TE	0.47	4
16	ST13	C/CF/COL/DOX/K/NA/STR/SXT/TE	0.52	1
17	ST14	AMP/C/COL/DOX/K/NA/STR/SXT/TE	0.52	1
18	ST26, ST29, ST34	CF/COL/DOX/ENR/K/NA/STR/SXT/TE	0.52	3
19	ST31	C/COL/DOX/ENR/K/NA/STR/SXT/TE	0.52	1
20	ST35	AMP/C/COL/DOX/ENR/K/NA/STR/SXT/TE	0.58	1
21	ST36	AMP/CF/COL/DOX/ENR/K/NA/STR/SXT/TE	0.58	1

[a] Amikacin (AMK; 30 μg), Ampicillin (AMP; 10 μg), Chloramphenicol (C; 30 μg), Ceftazidime (CAZ; 30 μg), Cephalothin (CF; 30 μg), Ciprofloxacin (CIP; 5 μg), Colistin (COL; 10 μg), Ceftriaxone (CRO; 30 μg), Doxycycline (DOX; 30 μg), Enrofloxacin (ENR; 5 μg), Gentamicin (GMC; 10 μg), Kanamycin (K; 30 μg), Nalidixic acid (NA; 30 μg), Norfloxacin (NOR; 10 μg), Streptomycin (STR; 10 μg), Sulfamethoxazole – trimethoprim (SXT; 25 μg) and Tetracycline (TE; 30 μg).
[b] Multiple Antibiotic Resistant.

Table 3. Specific Biofilm Formation (SBF) Values of Different *S. typhimurium* Isolates in Microtiter Plate Assay [a]

Isolate No.	SBF	Isolate No.	SBF	Isolate No.	SBF
ST1	0.80 (M)	ST14	1.44 (S)	ST27	0.29 (N)
ST2	0.36 (W)	ST15	0.08 (N)	ST28	0.35 (W)
ST3	1.83 (S)	ST16	0.05 (N)	ST29	0.07 (N)
ST4	0.10 (N)	ST17	0.05 (N)	ST30	0.07 (N)
ST5	0.37 (W)	ST18	0.68 (W)	ST31	0.23 (N)
ST6	0.10 (N)	ST19	0.09 (N)	ST32	0.25 (N)
ST7	0.44 (W)	ST20	0.04 (N)	ST33	0.03 (N)
ST8	0.42 (W)	ST21	0.19 (N)	ST34	0.29 (N)
ST9	0.61 (W)	ST22	0.83 (M)	ST35	0.35 (W)
ST10	0.63 (W)	ST23	0.08 (N)	ST36	0.39 (W)
ST11	0.09 (N)	ST24	1.79 (S)	ST37	0.06 (N)
ST12	0.07 (N)	ST25	0.10 (N)	ST38	0.09 (N)
ST13	0.13 (N)	ST26	0.21 (N)		

[a] Abbreviations: S, strong ability; M, moderate ability; W, weak ability; N, negative ability.

4.2. Biofilm Formation Ability

Based on the SBFs obtained, isolates were classified into the following categories according to the formula 1: strong (S) (SBF $\geq$ 1.10), moderate (M) (0.70 - 1.09), weak (W) (0.35 - 0.69) and negative (N) (SBF < 0.35) (11). As expected, there was a wide variation in biofilm forming ability among isolates. Most of the isolates (60.52%) were not capable of producing biofilm, while 26.31%, 7.89%, and 5.26% isolates were weak, strong and moderate biofilm producers, respectively (Table 3). Isolates including ST1, ST3, ST14, ST22 and ST24 had a relatively higher biofilm-forming capacity than other isolates (Table 3).

5. Discussion

In the present study, antibiotic resistance patterns and biofilm formation ability of 38 *S. typhimurium* isolates of avian origin were examined to acquire fundamental data that could help evolving a strategy for control and prevention in food processing plants. *S. typhimurium* is among the major zoonotic pathogens with a high occurrence of antibacterial resistance (12). This invasive serotype poses health hazard to human by eating different contaminated raw meat and poultry products (13). Different antibiotic resistance profiles of *Salmonella* spp. have reported by several authors from poultry meat products (1, 3, 14). In the present study, a large number of isolates showed resistance to multiple antibiotics (Table 2). The finding of 21 different resistance patterns from 38 isolates indicates a very heterogeneous *S. typhimurium* population which reflects a relatively serious public health problem and need strict regulation for the use of antimicrobials in the agriculture and human health care sectors.

Expanded-spectrum aminoglycosides (AG) resistance to *S. typhimurium* in food animal may be acquired by humans via food borne sources and appears to pose a risk of AG resistance in humans (15). All tested antibiotics in this work are those which commonly prescribed to treat animal disease in Iran. The relatively high level of resistance to AG could due to a reflection of misuse or abuse of these antibiotics in the farms (Table 1).

Fluoroquinolones and third generation cephalosporins are among the relatively effective drugs used for treatment of salmonellosis (16). All isolates showed a relatively high degree of sensitivity to ciprofloxacin (92%) and ceftriaxone (95%), (commonly used fluoroquinolones and cephalosporins antibiotics), which may reflect restricted use of them in avian. This finding is in accordance with those reported from Vietnam (17, 18) and is in contrast to report from Turkey (19).

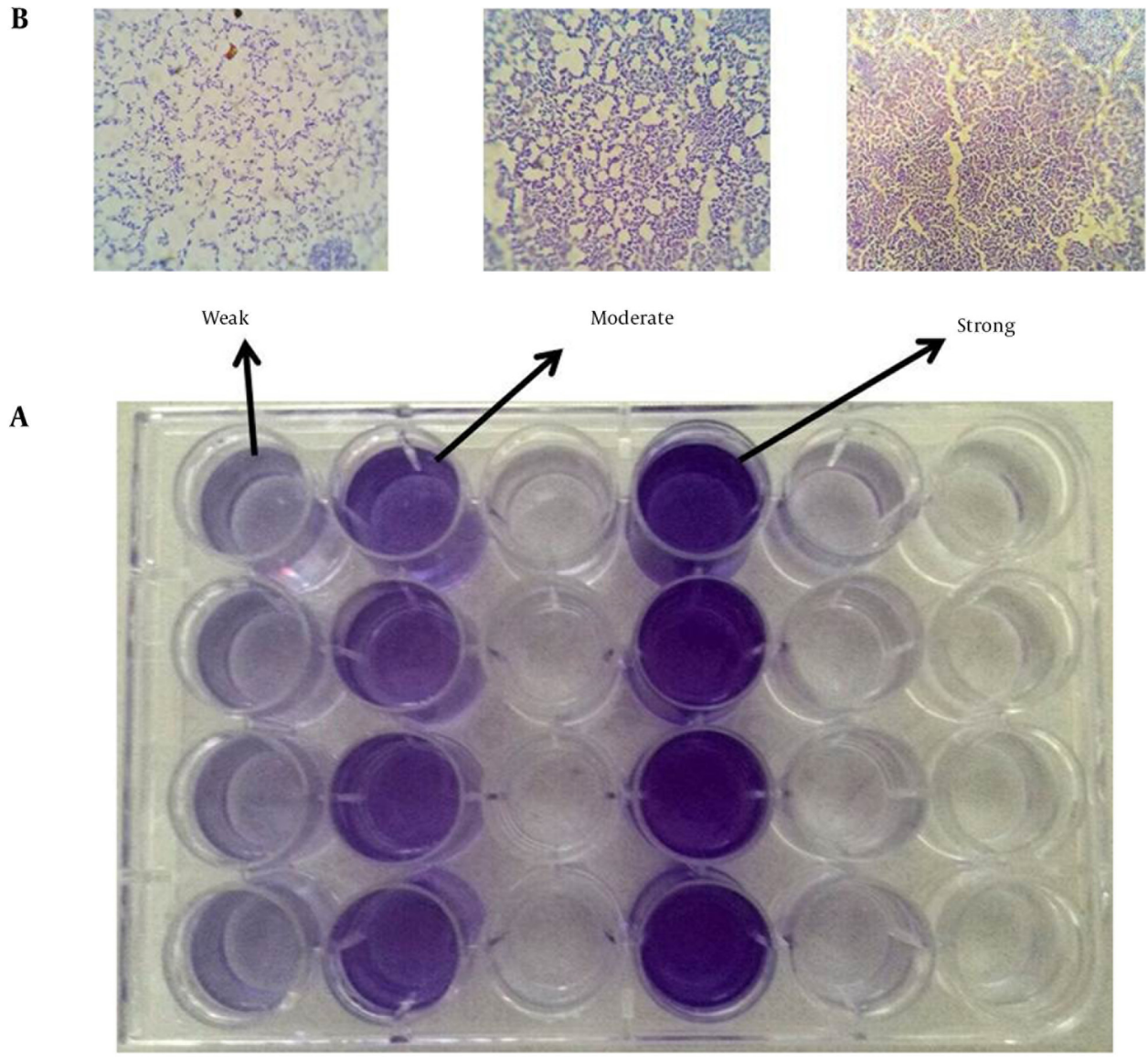
Tetracyclines are among the most commonly used antimicrobial drugs in animal husbandry. In this study, along with aminoglycoside resistance, resistance to tetracyclines is somewhat common. The incidence of tetracyclines resistance among *Salmonella* is variable with respect to the country of isolation and can be prevented by the correct use of prescribed antibiotics and good hygiene and infection control (20). The results of the present study are similar to the findings by Mazengia et al.

(2014) (20), which showed that tetracycline resistance was observed most frequently in 106 *Salmonella* isolates from various raw poultry samples obtained from retail markets of Seattle, WA.

In a similar study by Agarwal et al. (2011) (21), different extent of biofilm formation by *Salmonella* was observed among different serotypes. 151 strains of *Salmonella* consisting of reference and environmental isolates from diverse sources belonging to 69 serotypes were screened for biofilm production. Majority of strains (57.61%) were found to be moderate biofilm producers (21).

As demonstrated in Figure 1 microscopic observations of *Salmonella* biofilms growing on the surface of polystyrene microtiter plate tended to confirm SBFs results (Table 3). Different studies were conducted to compare the ability of different *Salmonella* serovars to produce biofilm on different surface (21, 22). Various factors (growth medium, incubation period, fixation of adhered cells and staining) affect development of Salmonella biofilm on microtiter plate (23). According to those studies, the source of isolates (from humans, animals or food) did not affect the biofilm formation.

Figure 1. A, Different Biofilm Types (Weak, Moderate and Strong) of *S. typhimurium* Isolates Grown in LB Broth at 37 ± 1°C on 24 Well Polystyrene Microtiter Plates; B, Micrographs of Different Biofilm Producing Bacteria



Bacteria stained with crystal violet and observed under a ×1000 objective.

As shown in the Table 1, three isolates (8%) were relatively susceptible to at least one antibiotic, but none of them showed high biofilm formation properties on polystyrene microtiter plate. On the other hand, most of the MAR bacteria used in this study did not reveal high biofilm formation ability and only 5 of them showed strong or moderate biofilm formation properties. The current study results are in contrast to the findings by Kim and Wei (2007) (24) which indicated that strong biofilm formers were also multidrug resistance bacteria.

In conclusion, the results of this study clearly showed that all *S. typhimurium* isolates revealed a high multiple antibiotic resistances with low capacity of biofilm production on polystyrene microtiter plates according to microtiter plate (MTP)-based systems. In this study, high prevalence of multiple antibiotic resistant found in the *S. typhimurium* isolates, indicates a serious need for implementing antibiotics surveillance program. Moreover, it suggests that legislation should be implemented by the authorities to enforce more prudent use of antibiotics in human and veterinary medicines.

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Authors' Contributions

Study concept and design and contributed to the development of the protocol: Hadi Ghasemmahdi, Hossein Tajik, Rojan Modaresi. Analysis and interpretation of data: Karim Mardani. Drafting of the manuscript: Mehran Moradi, Armen Badali, and Mahdi Dilmaghani. Statistical analysis: karim Mardani.

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References

1. Alvarez-Fernandez E, Alonso-Calleja C, Garcia-Fernandez C, Capita R. Prevalence and antimicrobial resistance of Salmonella serotypes isolated from poultry in Spain: comparison between 1993 and 2006. *Int J Food Microbiol.* 2012;**153**(3):281–7.
2. Dilmaghani M, Ahmadi M, Zahraei-Salehi T, Talebi A. Detection of Salmonella enterica Serovar Typhimurium from Avians Using Multiplex-PCR. *VRF.* 2012;**2**(3):157–65.
3. Mayrhofer S, Paulsen P, Smulders FJ, Hilbert F. Antimicrobial resistance profile of five major food-borne pathogens isolated from beef, pork and poultry. *Int J Food Microbiol.* 2004;**97**(1):23–9.
4. Simoes M, Simoes LC, Vieira MJ. A review of current and emergent biofilm control strategies. *LWT - Food Science and Technology.* 2010;**43**(4):573–83.
5. Shi X, Zhu X. Biofilm formation and food safety in food industries. *Trends Food Sci Tech.* 2009;**20**(9):407–13.
6. Moradi M, Tajik H, Razavi Rohani SM, Oromiehie AR. Effectiveness of Zataria multiflora Boiss essential oil and grape seed extract impregnated chitosan film on ready-to-eat mortadella-type sausages during refrigerated storage. *J Sci Food Agric.* 2011;**91**(15):2850–7.
7. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. In: Approved standard, editor. USA: 2013.
8. Krumperman PH. Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. *Appl Environ Microbiol.* 1983;**46**(1):165–70.
9. Lizcano A, Chin T, Sauer K, Tuomanen EI, Orihuela CJ. Early biofilm formation on microtiter plates is not correlated with the invasive disease potential of Streptococcus pneumoniae. *Microb Pathog.* 2010;**48**(3-4):124–30.
10. Xu HUA, Lee H-Y, Ahn J. Characteristics of Biofilm Formation by Selected Foodborne Pathogens. *Journal of Food Safety.* 2011;**31**(1):91–7.
11. Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, Blanco J, et al. Measurement of biofilm formation by clinical isolates of Escherichia coli is method-dependent. *J Appl Microbiol.* 2008;**105**(2):585–90.
12. Torpdahl M, Lauderdale TL, Liang SY, Li I, Wei SH, Chiou CS. Human isolates of Salmonella enterica serovar Typhimurium from Taiwan displayed significantly higher levels of antimicrobial resistance than those from Denmark. *Int J Food Microbiol.* 2013;**161**(2):69–75.
13. Soltan Dallal MM, Sharifi Yazdi MK, Mirzaei N, Kalantar E. Prevalence of Salmonella spp. in Packed and Unpacked Red Meat and Chicken in South of Tehran. *Jundishapur J Microbiol.* 2014;**7**(4).
14. Singh RP, Yadav AS, Tripathi V, Singh RP. Antimicrobial resistance profile of Salmonella present in poultry and poultry environment in north India. *Food Control.* 2013;**33**(2):545–8.
15. Folster JP, Rickert R, Barzilay EJ, Whichard JM. Identification of the aminoglycoside resistance determinants armA and rmtC among non-Typhi Salmonella isolates from humans in the United States. *Antimicrob Agents Chemother.* 2009;**53**(10):4563–4.
16. Su LH, Chiu CH, Chu C, Ou JT. Antimicrobial resistance in nontyphoid Salmonella serotypes: a global challenge. *Clin Infect Dis.* 2004;**39**(4):546–51.
17. Thai TH, Hirai T, Lan NT, Yamaguchi R. Antibiotic resistance profiles of Salmonella serovars isolated from retail pork and chicken meat in North Vietnam. *Int J Food Microbiol.* 2012;**156**(2):147–51.
18. Vo AT, van Duijkeren E, Gaastra W, Fluit AC. Antimicrobial resistance, class 1 integrons, and genomic island 1 in Salmonella isolates from Vietnam. *PLoS One.* 2010;**5**(2).
19. Arslan S, Eyi A. Occurrence and antimicrobial resistance profiles of Salmonella species in retail meat products. *J Food Prot.* 2010;**73**(9):1613–7.
20. Mazengia E, Samadpour M, Hill HW, Greeson K, Tenney K, Liao G, et al. Prevalence, concentrations, and antibiotic sensitivities of Salmonella serovars in poultry from retail establishments in Seattle, Washington. *J Food Prot.* 2014;**77**(6):885–93.
21. Agarwal R, Singh S, Bhilegaonkar K, Singh V. Optimization of microtitre plate assay for the testing of biofilm formation ability in different Salmonella serotypes. *Int Food Res J.* 2011;**18**:1493–8.
22. Majtan J, Majtanova L, Xu M, Majtan V. In vitro effect of subinhibitory concentrations of antibiotics on biofilm formation by clinical strains of Salmonella enterica serovar Typhimurium isolated in Slovakia. *J Appl Microbiol.* 2008;**104**(5):1294–301.
23. Stepanovic S, Cirkovic I, Ranin L, Svabic-Vlahovic M. Biofilm formation by Salmonella spp. and Listeria monocytogenes on plastic surface. *Lett Appl Microbiol.* 2004;**38**(5):428–32.
24. Kim SH, Wei CI. Biofilm formation by multidrug-resistant Salmonella enterica serotype typhimurium phage type DT104 and other pathogens. *J Food Prot.* 2007;**70**(1):22–9.