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Phenotypic and molecular detection of metallo- β -lactamase genes of *Salmonella enterica* strains isolated from poultry meat

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ABSTRACT

The present study was carried out to detect *Salmonella enterica* in meat samples of commercial boilers (CB) and the spent hens (SH) in Ardabil, Iran. Metallo- β -lactamase (MBL) enzyme produced by *Salmonella enterica* strains isolated from poultry meat samples were detected by both biochemical and molecular methods. The study included 20 positive samples for *Salmonella enterica* from boilers (CB) and spent hens (SH). The prevalence of *Salmonella enterica* for CB was 22% (11/50) and for SH was 18% (9/50). The antibiotic susceptibility testing for both CB and SH showed maximum number of *Salmonella enterica* isolates were resistant against Augmentin (30 μ g), Cotrimaxazole (25 μ g) and Tetracycline (25 μ g) and susceptible against Ofloxacin (5 μ g) and Gentamicin (10 μ g). Screening phenotypic confirmatory test for Metallo- β -lactamase (MBL) for CB, (n= 11, 100%) were positive for MBL while for SH (n= 8, 88.88%) samples were positive for MBL. The results showed that MBL positive *Salmonella enterica* isolates from CB and SH meat samples contained gene *bla_{VIM}* (n=11, 57.89%), *bla_{IMP}* (n=6, 31.57%) and *bla_{SPM-I}* (n=2, 10.52%). Since *Salmonella* infections in poultry are high due to large demand and antibiotic resistance to strains, so the purpose of the current study is to focus on the detection of MBL enzyme produced by *Salmonella enterica* isolates.

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Introduction

The advancement in the occurrence and spread of drug resistance in microbial community has impacted negatively to discoveries of medicine. Although antibiotics have saved innumerable lives from mortality and morbidity, however microbial resistance is posing a threat to these discoveries. The rise of Gram-negative carbapenem-resistant bacteria in hospital and communities is an alarming progress in the field of disease control (Bush and Jacoby, 2010; Chika et al, 2014; Walsh, et al, 2005). This threat foretells the jeopardy to public health with potent antibiotics that are used against serious infections.

Metallo- β -lactamases (MBLs) are broad spectrum zinc enzymes that are able to inactivate most clinically useful beta-lactam antibiotics specifically Carbapenems which are considered as the most effective antibiotics against *Salmonella enterica* (Crowder et al, 2006; Bahar et al, 2010). Carbapenem resistance occurs because of decrease in antibiotics absorption due to lack of an outer membrane porin, as oprD, exclusion from the cell by efflux pump, decrease in outer membrane permeability and production of MBL (Boroumand et al, 2012).

MBLs also divided into several families as follows: IMP, VIM, SPM, GIM, SIM, DIM, AIM, KHM, NDM and KPC. Most of them, if not all, genes encoding IMP, VIM and SPM types as well

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as GIM are found as gene cassettes in class 1 integrons, although IMP MBL genes are also found on class 3 integrons (Liakopoulos et al, 2013; Rizek et al, 2014). The VIM, IMP and SPM types are the most clinically significant carbapenemases which encoded by *bla_{IMP}*, *bla_{VIM}* and *bla_{SPM}* genes (Liakopoulos et al, 2013; Moosavian and Rahimzadeh, 2015).

Microbes become resistant to antimicrobial drugs after selective pressure of antibiotics and mutation. Intensive efforts for the control of infection and epidemiological issues are required for multidrug resistance in microbes and their potential spread to the environment. Antibiotics application to the production of livestock and poultry has led to the emergence of drug resistant bacteria in veterinary community (Ejikeugwe et al, 2017).

Multidrug-resistant (MDR) *Salmonella* strains are characterized by high levels of antibiotic resistance that are often attributed to the acquisition of specific mechanisms (Brunell et al, 2017). *Salmonella* is one the leading pathogens related to food poisoning and causes major bacterial diseases in poultry resulting in significant losses (Rostango et al, 2006). The integron gene is one of the main multidrug resistance genes in *Salmonella* (Hu and Li, 2009). *Salmonella* has been reported to become increasingly resistant to antimicrobials involving β-lactamases and other associated mechanisms (Gonzalez-Sanz et al, 2009; Revathi et al, 1998).

The presence of metallo-β-lactamase was studied in other strains of bacteria i.e. *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, which cause resistance to antibiotics, (Yong et al, 2009; Ghangosha et al, 2015). This study is aimed at screening the presence of metallo-β-lactamase genes in *Salmonella enterica* in poultry meat samples, as well as assessing them on molecular and phenotypic basis.

Material and Methods

Samples collection and processing

A total of 100 meat samples positive for *Salmonella enterica* (50 samples of CB and 50 samples of SH) were collected from Ardabil, Iran. Samples were transported to the Microbiology Lab for microbiological analysis. Part of each

collected meat sample (0.5 g) was placed into test tube containing 6 ml buffered Peptone and the tubes were covered with cotton wool and incubated at 37°C for 24 hrs.

After incubation, 50 µl of overnight grown culture was used to spread on already prepared and solidified Brilliant green agar (BG) (LabM, UK) and XLD. Plates were incubated at 37°C for 24 hours after labeling properly with sample number. If there are single colonies which caused the color of the medium to change from yellow to red/pink were sub-cultivated onto nutrient agar and incubated overnight for 24 hours at 37°C. Then it was passed in differential culture media to perform biochemical tests.

Antibiotics sensitivity for Salmonella isolates

Disk diffusion antibiotic sensitivity test was used in determining sensitivity of the *Salmonella enterica* isolates obtained from different meat samples to various antibiotics at different concentrations. Augmentin (30 µg), Ofloxacin (5 µg), Gentamicin (10 µg), Nalidixic acid (30 µg), Nitrofurantoin (200 µg), Cotrimaxazole (25 µg) and Tetracycline (25 µg) were used to determine sensitivity pattern.

Serotyping for potential Salmonella strains

Serotyping was performed on Muller-Hinton agar by obtaining pure cultures of *Salmonella* (RS, 2003). Agglutination was tested on a clean glass slide using Polyvalent O and H antisera. According to the Kauffmann-White scheme, slide cohesion tests were used to examine and study the serotypes in *Salmonella* isolates from the poultry meat (Brenner et al, 2000; Schrader et al, 2008).

Screening for the presence of Metallo-β-lactamases (MBL)

The susceptibility of the test isolates to the carbapenems was evaluated by the Kirby-Bauer disk diffusion method as per the CLSI criteria (Gautam et al, 2013; Clinical Laboratory Standard Institute, 2011). MBL-producing isolates was suspected when the test organism(s) was resistant to any of the carbapenems (imipenem and meropenem). As per the CLSI criteria, isolates showing inhibition zone diameter (IZD) of ≤23mm were suspected to produce MBL enzymes and these isolates were subjected to phenotypic confirmation test (Chika et al, 2014; Clinical

Laboratory Standard Institute, 2011). For *Salmonella enterica* isolates obtained from commercial boilers (CB) and the spent hens (SH), 20 samples each were suspected to produce MBL enzymes and subsequently subjected to phenotypic detection of MBL as described below.

Phenotypic detection of MBL

MBL production was phenotypically evaluated by the inhibition-based assay using EDTA (Chika et al, 2014). The test bacteria isolates (adjusted to 0.5 MacFarland turbidity standards) were aseptically swabbed on MH agar plates, and imipenem (10µg) and meropenem (10µg) disks impregnated with EDTA (0.5µg) was aseptically placed on the MH agar plates. Supplementary imipenem (10µg) and meropenem (10µg) disks without EDTA were also placed alongside antibiotic disks impregnated with EDTA. Antibiotic disks were placed 20mm apart, and all the plates were incubated at 30 °C for 24hrs. A difference of ≥7mm between the zones of inhibition of any of the carbapenem disks tested alone compared to disks with EDTA infers MBL production phenotypically (Chika et al, 2014; Chika et al, 2016).

DNA extraction

The DNA extraction was carried out using commercial DNA kit, Fermentas genomic DNA purification kit (Fermentas, USA). The manufacturer's instructions were carefully followed at each step. First of all, lysis buffer 400 µl was added to bacterial broth cultures (200 µl). Then 600 µl of chloroform was added and the suspension was inverted for about 3 to 5 times. Centrifuge was performed afterwards for 2 minutes at 12,800 g. The supernatant obtained as a result of centrifugation was removed. NaCl solution (100 µl) was added and afterwards 70% cold ethanol was added in the quantity 300 µl. The mixture was placed for 10 minutes at -20 °C. Centrifugation was performed again at 12,800 g for three to four minutes. The DNA pellet was obtained that was air-dried and then dissolved in 100 µl TE buffer and saved for further use in the refrigerator at 4 °C.

Molecular detection of *bla_{VIM}*, *bla_{IMP}* and *bla_{SPM-1}* genes

PCR amplification for the detection of MBL genes (*bla_{VIM}*, *bla_{IMP}* and *bla_{SPM-1}*) was performed

using specific primers (Table 1) from Sinaclone, Iran. PCR was performed according to the method reported by Senda et al. (1996) (Senda et al, 1996). PCR mixture (25 µL) consisted of 1 µL DNA, 0.24 Mm dNTPs, forward and reverse primers 5pm and Taq polymerase (1 unit) (Qiao et al, 2017).

Table 1- Nucleotide sequences of primers used for detection of metallo-beta lactamase genes.

Gene	Primers Sequence (5'-3')
<i>bla_{VIM}</i>	Forward 5'- AGTGGTGAGTATCCGACAG-3' Reverse 5'- ATGAAAGTGCCTGGAGAC-3'
<i>bla_{IMP}</i>	Forward 5'- CTACCGCAGCAGAGTCTTTG-3' Reverse 5'- AACCAGTTTTGCCTTACCAT-3'
<i>bla_{SPM-1}</i>	Forward 5'- GCGTTTTGTTGTTGCTC -3' Reverse 5'-TTGGGGATGTGAGACTAC-3'

Results

The prevalence of *Salmonella enterica* in CB samples was 22% (11/50) and for SH samples was 18% (9/50). In the current study, 100 meat samples (50 samples from CB and 50 samples from SH) were collected from Ardabil, which were positive for *Salmonella enterica*. Sensitivity of the *Salmonella enterica* isolates obtained from different meat samples to various antibiotics at different concentrations was determined by disk diffusion antibiotic sensitivity test shown in Fig.1.

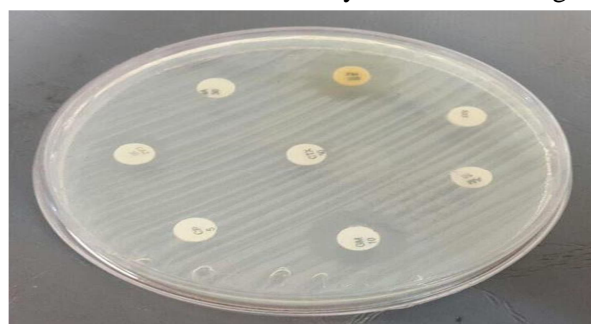


Fig. 1- Antibacterial activity assay against *Salmonella* strains by the disk diffusion method.

In case of both CB and SH, maximum number of *Salmonella enterica* isolates were found to be resistant against Augmentin (30 µg) while resistance against Cotrimaxazole (25 µg) and Tetracycline (25 µg) was found to be at second and third number respectively (Table2). Maximum number of *Salmonella enterica* isolates of both CB and SH were found to be susceptible against Ofloxacin (5 µg) and Gentamicin (10 µg)

(Table 2) indicating that these two drugs were other antibiotic. effective to kill *Salmonella enterica* than any

Table 2- Antimicrobial susceptibility of *Salmonella enterica* isolates

Antibiotics (μg)	Commercial boilers (CB) (n=50)			Spent hens (SH) (n=50)		
	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Augmentin (30 μg)	0(0)	5(10)	45(90)	0(0)	3(6)	47(94)
Ofloxacin (5 μg)	34(68)	3(6)	13(26)	37(74)	3(6)	10(20)
Gentamicin (10 μg)	25(50)	5(10)	20(40)	28(56)	2(4)	20(40)
Nalidixic acid (30 μg)	0(0)	20(40)	30(60)	0(0)	15(30)	35(70)
Nitrofurantoin (200 μg)	10(20)	15(30)	25(50)	8(16)	12(24)	30(60)
Cotrimoxazole (25 μg)	2(4)	5(10)	43(86)	1(2)	7(14)	42(84)
Tetracycline (25 μg)	5(10)	10(20)	35(70)	0(0)	12(24)	38(76)

Screening of Metallo-β-lactamases (MBL) and phenotypic detection

The *Salmonella enterica* isolates were suspected to produce MBL when these were resistant to either imipenem or meropenem. The *Salmonella enterica* isolates showing inhibition zone diameter (IZD) of ≤ 23 mm were suspected to produce MBL enzymes and these isolates were subjected to phenotypic confirmation test (Fig. 2, Table 3). For *Salmonella enterica* isolates obtained from commercial boilers (CB) and the spent hens (SH), 20 samples each were suspected to produce MBL enzymes and subsequently subjected to phenotypic detection of MBL (Table 3). MBL production was phenotypically evaluated by the inhibition-based assay using EDTA. A difference of ≥ 7 mm between the zones of inhibition of any of the carbapenem disks tested alone compared to disks with EDTA inferred phenotypically MBL positive.

In case of commercial boilers and spent hens *Salmonella* positive samples 11(22%) and 9(18%) respectively were suspected to be MBL positive too. Out of these, CB samples were 100% (11/11) MBL positive while SH samples gave 88.88% (8/9) MBL positive result (Table 3).

Table 3- MBL detection in *Salmonella enterica* isolates

Organism	Host positive for <i>Salmonella</i>	Suspected MBL producers n (%)	MBL Positive n (%)	MBL Negative n (%)
<i>Salmonella enterica</i>	Commercial boilers(CB) (n=20)	11(22%)	11(100%)	0
<i>Salmonella enterica</i>	Spent hens (SH) (n=20)	9(18%)	8(88.88%)	1(0.1%)

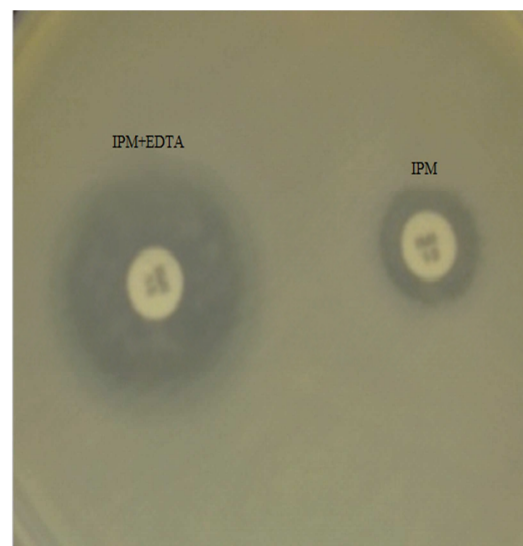


Fig. 2- Imipenem disk test for *Salmonella enterica* isolates.

Serotypes detected

Out of the 20 positive samples of *Salmonella*, two serotypes were detected of which 15 were *Salmonella enteritidis* and 5 serovar *Salmonella typhimorium*.

bla_{VIM}, *bla_{IMP}* and *bla_{SPM-1}* genes molecular detection

The *bla_{VIM}*, *bla_{IMP}* and *bla_{SPM-1}* genes were detected in MBL phenotypically positive samples by PCR based amplification detection method. The results showed that *S. enterica* isolates from commercial boilers meat samples and spent hens contained meat samples contained gene *bla_{VIM}* (11, 57.89%), *bla_{IMP}* (6, 31.57%) and *bla_{SPM-1}* (2, 10.52%) (Fig. 3, Table 4).

Table 4- *bla_{VIM}*, *bla_{IMP}* and *bla_{SPM-1}* genes were detected in MBL phenotypically positive samples

MBL Positive n (%)	Total samples positive for salmonella	<i>bla_{VIM}</i> genes obtained by PCR	<i>bla_{IMP}</i> genes obtained by PCR	<i>bla_{SPM-1}</i> genes obtained by PCR
19	20	<i>bla_{VIM}</i> 57.89% n=11	31.57% n=6	<i>bla_{SPM-1}</i> 5.60% n=2

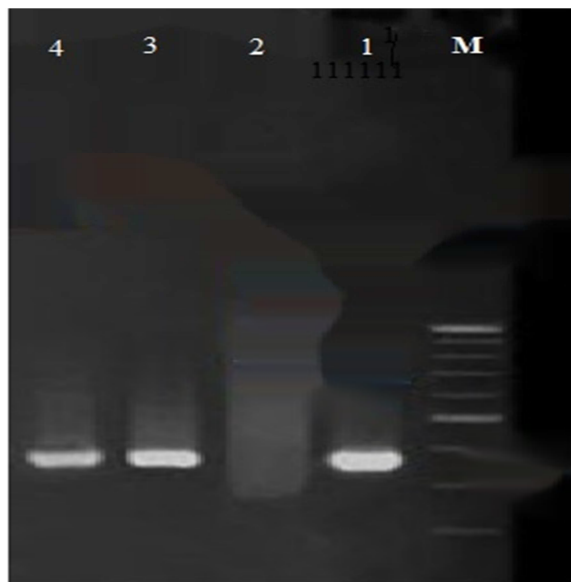


Fig. 3- PCR for detection of *bla_{VIM}* gene in *Salmonella enterica* isolates.

M: DNA size marker; Lane 1: Positive control *bla_{VIM}*; Lane 2: Negative control; Lane 3-4: PCR product of *bla_{VIM}* gene (261 bp).

Discussion

Resistance to antibiotics in non-hospital environments is a growing concern that has risked the efficacy of potential antibiotics. *Salmonella* species are notorious gram negative bacteria responsible for a number of infections that are becoming resistant to available antibiotics. *S. enterica* in meat samples is major health concern and in current research meat samples of commercial boilers (CB) and the spent hens (SH) from Ardabil, the city of Ancient Iran were studied for the detection of *S. enterica*. Metallo-β-lactamase (MBL) enzyme produced by *S. enterica* strains isolated from poultry meat samples were detected by both biochemical and molecular methods. The 50 positive samples each of CB and SH for *S. enterica* were included in the current research. The prevalence of *S. enterica* for CB was 22% (11/50) and for SH was 18% (9/50).

The isolated *S. enterica* strains were subjected to antibiotic susceptibility testing and for both CB and SH, maximum number of *S. enterica* isolates were found to be resistant against Augmentin (30 µg) while resistance against Cotrimaxazole (25 µg) and Tetracycline (25 µg) was found to be at second and third number respectively. Maximum number of *S. enterica* isolates from both CB and SH were found to be susceptible against Ofloxacin (5 µg) and Gentamicin (10 µg) indicating that these two drugs were effective to kill *S. enterica* than any other antibiotic. This is in agreement with Ezekiel et al. (2011) who showed that *Salmonella* found in poultry meat was found to be 100% resistant to augmentin (Ezekiel et al, 2011). In another study, tetracycline was found to be 90%–100% resistant against *Salmonella* (Sakaridis et al, 2011). Miranda et al. (2008) in a study also reported the development of resistant strains of *Salmonella* of poultry meat (Miranda et al, 2008). The rising numbers of resistant *Salmonella* strains in poultry can progress towards an epidemic (Velge et al, 2005; White et al, 2001). In addition to poultry resistant *Salmonella* strains are also found in cow meat and milk which showed resistance to Gentamicin, Nitrofurantoin, Cotrimaxazole and Tetracycline (Addis et al, 2011).

Extensive research has been done on serotypes in *Salmonella* and a variety of serotypes including Enteritidis, Typhimurium, Derby, Anatum and

Albany have been implicated in Salmonellosis of the poultry. Antibiotic-resistant rate in *Salmonella* isolates identified in humans is steadily increasing in recent years. They also in antibiotic susceptibility test have shown multiple resistances in most isolated samples. Regarding the importance of *Salmonella* contamination in poultry breeding and its impact on public health and increasing antibiotic resistance of *Salmonella* (Graziani et al, 2008), serotyping and investigation of antibiotic resistance pattern of isolates was performed in this study that *S. enteritidis* was the dominant serotype by a high ratio 75%.

Screening phenotypic confirmatory test used for detection of Metallo- β -lactamase (MBL) enzyme for CB, (n= 11, 22%) were positive for MBL while for SH (n= 9, 18%) samples were positive for MBL. The results showed that MBL positive *S. enterica* isolates from CB and SH meat samples contained gene *bla_{VIM}* (11, 57.89%), *bla_{IMP}* (6, 31.57%) and *bla_{SPM-1}* (2, 10.52%) of MBL. This evidence has been supported in a study by Murphy et al., (2003) where MBL gene SPM-1 from *Pseudomonas aeruginosa* (48-1997A) was cloned (Murphy et al, 2003). The results showed that it had ORF (open reading frame) homologous with *S. enterica* serovar Typhimurium suggesting that its origin was *Salmonella* sp. Another similar study done by C. Rizek et al., (2014) have also identified carbapenemase genes *bla_{SPM}* and *bla_{VIM}* in carbapenem-resistant *Pseudomonas aeruginosa* isolates which further supports the results of this study (Rizek et al, 2014).

Similarly, another member of the Enterobacteriaceae family, *Klebsiella* isolated from non-hospital environments was reported to produce MBL suggesting the evidence for multi-drug resistance being spread to other sources (Ejikeugwu et al, 2017). Thus *Salmonella* has a significant potential to become increasingly resistant towards a range of antimicrobials. Research is needed to further evaluate the different strains of *Salmonella* that prevail in the meat samples as it can lead to the increased transfer and spread of MBL-producing bacteria in the food chain. This will help the scientific community to plan strategies accordingly.

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References

- Addis, Z., Kebede, N., Sisay, Z., Alemayehu, H., Wubetie, A., and Kassa, T. (2011). Prevalence and antimicrobial resistance of *Salmonella* isolated from lactating cows and in contact humans in dairy farms of Addis Ababa: a cross sectional study, BMC Infect Dis. 11, 222.
- Bush, K. and Jacoby, G.A. (2010). Updated functional classification of beta-lactamases. Antimicrob Agents Chemother, 54(3), 969-76.
- Bahar, M.A., Jamali, S. and Samadikuchaksaraei, A. (2010). Imipenem-resistant *Pseudomonas aeruginosa* strains carry metallo-beta-lactamase gene *bla(VIM)* in a level I Iranian burn hospital. Burns, 36(6), 826-30.
- Boroumand, M. A., Anvari, M. S., and Habibi, E., Detection of vim- and ipm-type metallo-beta-lactamases in *Pseudomonas aeruginosa* clinical isolates. Arch Iran Med, 2012. 15(11): p. 670-3.
- Brenner, F. W., Villar, R. G., Angulo, F. J., Tauxe, R., and Swaminathan, B. (2000). *Salmonella* nomenclature. J Clin Microbiol, 38(7), 2465-7.
- Brunelle, B.W., Bearson, B.L. and Allen, H.K. (2017). Prevalence, evolution, and dissemination of antibiotic-resistance in *Salmonella*. Foodborne Pathog Ant Res. Om V. Singh, 331-348.
- Chika, E., Chijioke, E., Ifeanyichukwu, I., Jerry, O., Stanley, E., Emmanuel, U., Carissa, D. and Charles, E. (2016). Antibigram and Detection of Metallo-Beta-Lactamase (MBL) positive *Escherichia coli* isolates from abattoir. Nat Sci, 14(11), 65-69.
- Chika, E., Malachy, U., Ifeanyichukwu, I., Peter, E., Thaddeus, G. and Charles, E. (2014). Phenotypic detection of metallo- β -lactamase (MBL) enzyme in Enugu, Southeast Nigeria. Am J Biol chem pharm Sci, 2(2), 1-6.
- Clinical Laboratory Standard Institute, C. (2011). Performance standards for antimicrobial disk susceptibility test. Fifteenth informational supplement, CLSI document M100-S15, Wayne, USA.
- Crowder, M.W., Spencer, J., and Vila, A. J. (2006). Metallo-beta-lactamases: novel weaponry for antibiotic resistance in bacteria. Acc Chem Res, 39(10), 721-8.
- Ejikeugwu, C. D.C., Eluu, S., Oguejiofor, B., Ezeador, C., Ogene, L., Iroha, I. (2017). Isolation and Phenotypic Detection of Metallo-Beta-Lactamase (MBL)-Producing *Klebsiella* Species from Cow Anal Swabs. Glob J Pharmaceu Sci, 2(3).
- Ezekiel, C.N., Olarinmoye, A.O., Jnr, J.O., Olaoye, O.B. and Edun, A.O. (2011). Distribution, antibiogram and multidrug resistance in Enterobacteriaceae from commercial poultry feeds in Nigeria. Afr j microbiol res, 5(3), 294-301.
- Gautam, V., Singhal, L., Arora, S., Jha, C. and Ray, P. (2013). Reliability of Kirby-Bauer disk diffusion method for detecting carbapenem resistance in *Acinetobacter baumannii*-calcoaceticus complex isolates. Antimicrob Agents Chemother, 57(4), 2003-4.
- Ghamgosha, M., Shahrekizahedani, S., Kafizadeh, F., Bameri, Z., Taheri, R. A. and Farnoosh, G. (2015). Metallo-beta-Lactamase VIM-1, SPM-1, and IMP-1 Genes Among Clinical *Pseudomonas aeruginosa* Species Isolated in Zahedan, Iran. Jundishapur J Microbiol, 8(4), e17489.
- González-Sanz, R., Herrera-Leon, S., De la Fuente, M., Arroyo, M., and Echeita, M. A. (2009). Emergence of extended-spectrum β -lactamases and AmpC-type β -lactamases in human *Salmonella* isolated in Spain from 2001 to 2005. Journal of antimicrobial chemotherapy, 64(6), 1181-1186.
- Graziani, C., Busani, L., Dionisi, A.M., Lucarelli, C., Owczarek, S., Ricci, A., Mancin, M., Caprioli, A. and Luzzi, I. (2008).

- Antimicrobial resistance in *Salmonella enterica* serovar Typhimurium from human and animal sources in Italy. *Veterinary microbiology*, 128(3-4), 414-418.
- Hu, X. and Li, G. (2009). Advances in the relationship between integrons and drug resistance of bacteria. *World Notes on Antibiotics*, 30, 255-263.
- Liakopoulos, A., Mavroidi, A., Katsifas, E. A., Theodosiou, A., Karagouni, A. D., Miriagou, V. and Petinaki, E. (2013). Carbapenemase-producing *Pseudomonas aeruginosa* from central Greece: molecular epidemiology and genetic analysis of class I integrons. *BMC Infect Dis*, 13, 505.
- Miranda, J. M., Vázquez, B. I., Fente, C. A., Barros-Velázquez, J., Cepeda, A., and Franco, C.M. (2008). Evolution of resistance in poultry intestinal *Escherichia coli* during three commonly used antimicrobial therapeutic treatments in poultry. *Poultry sci*, 87(8), 1643-1648.
- Moosavian, M. and Rahimzadeh, M. (2015). Molecular detection of metallo-beta-lactamase genes, bla IMP-1, bla VIM-2 and bla SPM-1 in imipenem resistant *Pseudomonas aeruginosa* isolated from clinical specimens in teaching hospitals of Ahvaz, Iran. *Iran J Microbiol*, 7(1), 2-6.
- Murphy, T.A., Simm, A. M., Toleman, M. A., Jones, R. N., and Walsh, T. R. (2003). Biochemical characterization of the acquired metallo-β-lactamase SPM-1 from *Pseudomonas aeruginosa*. *Antimicrobial agents and chemotherapy*, 47(2), 582-587.
- Qiao, J., et al., (2017). Characterization of extended-spectrum beta-lactamases (ESBLs)-producing *Salmonella* in retail raw chicken carcasses. *Int J Food Microbiol*, 248, 72-81.
- Revathi, G., Shannon, K. P., Stapleton, P. D., Jain, B. K. and French, G. L. (1998). An outbreak of extended-spectrum, β-lactamase-producing *Salmonella* senftenberg in a burns ward. *Journal of Hospital Infection*, 40(4), 295-302.
- Rizek, C., Fu, L., dos Santos, L.C., Leite, G., Ramos, J., Rossi, F., and Costa, S. F. (2014). Characterization of carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates, carrying multiple genes coding for this antibiotic resistance. *Ann Clin Microbiol Antimicrob*, 13, 43.
- Rostagno, M.H., Wesley, I.V., Trampel, D.W., and Hurd, H.S. (2006). *Salmonella* prevalence in market-age turkeys on-farm and at slaughter. *Poultry science*, 85(10), 1838-1842.
- RS, H. (2003). A global *Salmonella* surveillance and laboratory support project of the World Health Organization: Laboratory Protocols (Isolation of *Salmonella*) 4.
- Sakaridis, I., Soultos, N., Iossifidou, E., Koidis, P., and Ambrosiadis, I. (2011). Prevalence and antimicrobial resistance of *Salmonella* serovars from chicken carcasses in northern Greece. *J Food Saf*, 31(2), 203-210.
- Schrader, K.N., Fernandez-Castro, A., Cheung, W. K., Crandall, C. M., and Abbott, S.L. (2008). Evaluation of commercial antisera for *Salmonella* serotyping. *J Clin Microbiol*, 46(2), 685-8.
- Senda, K., Arakawa, Y., Ichiyama, S., Nakashima, K., Ito, H., Ohsuka, S., Shimokata, K., Kato, N. and Ohta, M. (1996). PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. *J clin microbiol*, 34(12), 2909-2913.
- Velge, P., Cloeckert, A. and Barrow, P. (2005). Emergence of *Salmonella* epidemics: The problems related to *Salmonella enterica* serotyp Enteritidis and multiple antibiotic resistance in other major serotypes. *Vet res*, 36(3), 267-288.
- Walsh, T. R., Toleman, M. A., Poirel, L., and Nordmann, P. (2005). Metallo-β-lactamases: the quiet before the storm? *Clin microbiol rev*, 18(2), 306-325.
- White, D.G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P.F., McDermott, S., Wagner, D.D. and Meng, J. (2001). The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *New Eng j med*, 345(16), 1147-1154.
- Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K. and Walsh, T. R. (2009). Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob Agents Chemother*, 53(12), 5046-54.



تشخیص فنوتیپ و مولکولی ژنهای متالوبتالاکتاماز در گونه های سالمونلا انتریکا جدا شده از گوشت مرغ

سیامک قضائی^{*۱}

چکیده

مطالعه حاضر برای شناسایی سالمونلا انتریکا در نمونه های گوشت جوجه گوشتی (CB) و مرغ های گوشتی (SH) در شهرستان اردبیل انجام شد. آنزیم متالو-بتا-لاکتاماز (MBL) تولید شده توسط سویه های سالمونلا انتریکا جدا شده از نمونه های گوشت مرغ توسط روش های بیوشیمیایی و مولکولی شناسایی شد. این مطالعه شامل ۲۰ نمونه مثبت برای سالمونلا انتریکا از نمونه های گوشت جوجه کبابی (CB) و مرغ گوشتی (SH) بود. شیوع سالمونلا انتریکا برای CB ۲۲٪ (50/11) و برای SH برابر ۱۸٪ (۹/۵۰) بود. آزمایشات حساسیت آنتی بیوتیکی برای هر دو CB و SH نشان داد که تعداد زیادی از جدایه های سالمونلا انتریکا در برابر آگومنتین (۳۰ میکروگرم)، کوتریموکسازول (۲۵ میلی گرم) و تتراسیکلین (۲۵ میکروگرم) مقاوم بوده و نسبت به افلوکساسین (۵ میکروگرم) و جنتامایسین (۱۰ میکروگرم) حساسیت دارند. غربالگری تست تأیید فنوتیپی برای متالو بتالاکتاماز (MBL) برای CB، n=11، 100٪ (مثبت بود، در حالی که برای SH 8/88.88٪) مثبت بود. نتایج نشان داد که جدایه های MBL مثبت سالمونلا انتریکا از نمونه های گوشت جوجه گوشتی (CB) و گوشت مرغ (SH) دارای ژن های blaVIM (n=6، 57.89%)، blaIMP (n=11، 31.57%) و blaSPM-1 (n=2، 10.52%) بود. از آنجائیکه میزان آلودگی در گوشت طیور به دلیل تقاضای بالا و درمان آنتی بیوتیکی، مقاومت سویه ها به آن بالاست، هدف از مطالعه حاضر، تمرکز بر شناسایی آنزیم MBL تولید شده توسط جدایه های سالمونلا انتریکا است.

واژگان کلیدی: سالمونلا انتریکا، متالوبتالاکتاماز، PCR، حساسیت آنتی بیوتیکی، آنزیم.

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