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Amoxicillin / Clavulanic Acid and Cefotaxime Resistance in *Salmonella* Minnesota and *Salmonella* Heidelberg from Broiler Chickens

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Abstract
This study investigated the resistance of various *Salmonella* strains to beta-lactam antibiotics. *Salmonella* Minnesota (36 strains) and *Salmonella* Heidelberg (24 strains) were isolated from broiler chickens and carcasses by the Disk Diffusion Test and resistance genes *bla*CTX-M-8, *bla*ACC-1 and *bla*CMY-2 were detected by PCR. Of the 60 strains tested, 80% were resistant to at least one antibiotic. Specifically, 66.7% were resistant to amoxicillin/clavulanic acid and 75% were resistant to cefotaxime. Among the amoxicillin/clavulanic acid resistant strains, the *bla*CMY-2 gene was detected in 40%, *bla*ACC-1 in 37.5% and *bla*CTX-M-8 in 7.5%. Among the cefotaxime resistant strains, we detected the genes *bla*CTX-M-8 in 13.3%, *bla*ACC-1 in 33.3%, and *bla*CMY-2 in 31.1%. The presence of cefotaxime- and amoxicillin/clavulanic acid-resistant *Salmonella* in poultry, and the prevalence of extended spectrum betalactamases and AmpC-betalactamases in these strains are of huge concern to public health and economy.

Introduction

Europe leads the list of rigorous buyers of imported poultry meat. Through the Rapid Alert System for Food and Feed (RASFF) protocol published by the European Commission on Food Safety and Health, there was a significant increase in notifications regarding poultry products from Brazil during 2013 and 2014 due to the presence of *Salmonella*. In particular, *Salmonella* Heidelberg was the most prevalent serotype in Brazilian chicken meat in 2013 (RASFF, 2014, 2015). Recent studies in Brazil have also shown an increased rate of infection in chickens by *Salmonella* Heidelberg and Minnesota (Cardoso *et al*., 2015; Voss-Rech *et al*., 2015), which was credited to improved measures controlling for Enteritidis and Typhimurium serotypes, thereby increasing the frequency of other serotypes.

A study conducted in Brazil from 2003-2012 found changes over time in the dynamics of *Salmonella* spp. serotypes isolated from animals (including birds), food, and humans. Specifically, there was a decrease in detection of Enteritidis and Typhimurium serotypes, but an increase (starting in 2008) in Minnesota, Mbandaka, Senftenberg, Agona, Schwarzengrund, Infantis, and Panama serotypes (ANVISA, 2012). Similarly, the Agriculture, Livestock and Food Supply Ministry reported an increase in *Salmonella*

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Minnesota percentage in broiler chickens from 0.96% in 2004-2008 to 9.38% in 2009-2010 (Freitas, 2011). Among the *Salmonella* spp. serotypes that can infect humans, Heidelberg seems to be the most invasive and capable of causing diseases with greater severity than other paratyphoid serotypes. Heidelberg is also among the most commonly isolated serotypes in both birds (and humans in Canada and USA), and is one of the five main serotypes associated with human salmonellosis (CDC, 2008; 2014; FDA, 2010; PHAC, 2014).

Consumption of poultry products – mainly chicken meat – has often been associated with salmonellosis (WHO, 2016). *Salmonella* spp. can enter the food chain from animal production and technological processing through cross-contamination and product commercialization. The use of antimicrobials in the majority of human salmonellosis cases is not recommended, but in systemic infections, such as those caused by *Salmonella* Heidelberg, drugs such as third generation cephalosporins are commonly used (Hoffmann *et al*., 2014). A study conducted in the Netherlands from 1999-2013 years, revealed that among 200 *Salmonella* Heidelberg strains isolated from human infections, animal production, and poultry meat, 23.5% were resistant to extended-spectrum cephalosporins (Liakopoulos *et al*., 2016). Resistance has alarming implications on food-borne transmission and public health, and has been reported in several countries (Hoffmann *et al*., 2014; Liakopoulos *et al*., 2016).

The use of Beta-lactams in animal feed as a growth performance additive is banned in Brazil (Brasil, 2009). Extended-spectrum beta-lactam resistance is usually due to intracellular production of extended spectrum betalactamases (ESBL). The most frequently encountered ESBLs belong to the TEM, SHV and CTX-M groups, in which their encoding genes are found in plasmids (which normally harbor other genes that confer resistance to aminoglycosides, chloramphenicol, sulfonamides, trimethoprim, and tetracycline) (Cánton *et al*., 2012). CTX-M enzymes are able to hydrolyze third generation cephalosporins (Bonnet, 2008). AmpC-betalactamases are enzymes encoded by genes with a chromosomal origin called *ampC* (Hanson, 2003) and have also been detected in *Salmonella* spp. that lack this chromosomal gene. Enzyme production in this bacterium is mediated by plasmid genes (Pérez-Pérez and Hanson, 2002).

The objective of this work is to characterize antimicrobial resistance of *Salmonella* Minnesota and Heidelberg strains isolated from live chickens and carcasses against amoxicillin/clavulanic acid and cefotaxime. We will do so using the Disk Diffusion Test, followed by the detection of resistance genes using Polymerase Chain Reaction (PCR).

Materials and Methods

Samples

60 *Salmonella enterica* strains (36 Minnesota and 24 Heidelberg serotypes) were studied in isolates from nine live chickens and 51 slaughtered chickens from slaughterhouses with Federal Inspection Service located in the South and West-Center regions of Brazil. Isolation occurred from 2012-2013 according to U.S. FDA Bacteriological Analytical Manual (Hammack *et al*., 2014). Strains were serotyped at the Enterobacteria Laboratory (Department of Bacteriology) in Oswaldo Cruz Foundation in Rio de Janeiro State (IOC, FIOCRUZ, RJ, Brazil).

Disk Diffusion Test

The susceptibility of samples to antimicrobial agents Amoxicillin/Clavulanic Acid (20/10 µg) and Cefotaxime (30 µg) was evaluated by the Disk Diffusion Test according to previously established methods (Bauer *et al*., 1966), following resistance parameters provided by CLSI (2013).

Polymerase Chain Reaction (PCR)

PCR was performed, following DNA thermal extraction, for genotypic resistance characterization. Specific primer pairs were used to detect ESBL (which confer resistance to third generation Cephalosporins) and AmpC-betalactamases (which confer resistance to Beta-Lactams, except Carbapenem and are resistant to Clavulanic Acid) (Table 1).

Each reaction contained 29.25 µL of PCR water, 5.0 µL of DMSO, 5.0 µL of 10X Buffer, 2.0 µL 50 mM $MgCl_2$, 2.5 µL of each primer (10 pmol/µL; Invitrogen), 1.0 µL of 10 mM DNTP, 0.25 µL 5 U/µL Taq Polymerase (Ludwig Biotec), and 2.5 µL of DNA (final volume of 50 µL). PCR reactions included denaturation at 94°C for 10 minutes, 35 cycles at 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 60 seconds, with a final extension at 72°C for 10 minutes (Nadjar *et al*., 2000; Decré *et al*., 2002; Jouini *et al*., 2007). Positive controls used were *Salmonella* Heidelberg (IOC 200/14), and

Klebsiella pneumoniae (CCBH 3589) provided by Enterobacteria Laboratory (Department of Bacteriology) in Oswaldo Cruz Institute Foundation in Rio de Janeiro State, Brazil.

Table 1. Pair of primers for the study of AmpC-betalactamases and Extended Spectrum Betalactamase (ESBL) in strains of *Salmonella* Minnesota and *Salmonella* Heidelberg

Resistance genes	Primers
*bla*CMY-2-F	5'-ATG ATG AAA AAA TCG TTA TGC-3'
*bla*CMY-2-R	5'-TTG CAG CTT TTC AAG AAT GCG-3'
*bla*ACC-1-F	5'-CAC CGA AGC CGT TAG TTG AT-3'
*bla*ACC-1-R	5'-AAG TGG GTT CGC TGA GTA AA-3'
*bla*CTX-Mgp-8-F	5'-TGA TGA GAC ATC GCG TTA AG-3'
*bla*CTX-Mgp-8-R	5'-TAA CCG TCG GTG ACG ATT TT-3'

*bla*CMY-2 – CMY-2 betalactamase (AmpC-betalactamase group); *bla*ACC-1 – ACC-1 betalactamase (AmpC-betalactamase group); *bla*CTX-Mgp-8 – CTX-M-8 betalactamase (ESBL group); F – Forward; R – Reverse.

Statistical analysis

Data collected were subjected to non-parametric test (Test G: Williams) using Bioestat 5.3 program (Ayres *et al*., 2007).

Results

We observed resistance to at least one of the two antimicrobials tested in 80% (48/60) of the *Salmonella* strains, with 66.7% (40/60) resistant to Amoxicillin/Clavulanic Acid, and 75% (45/60) resistant to Cefotaxime (Table 2). 23 out of the 40 strains that yielded resistance to Amoxicillin/Clavulanic Acid were Heidelberg serotype (all isolated from carcasses) while the remaining 17 strains were Minnesota serotype. Of these 17 Minnesota serotypes, four were isolated from live chickens while 13 were from carcasses. 22 out of the 45 *Salmonella* strains resistant to Cefotaxime were Heidelberg serotype (isolated from carcasses) and 23 were Minnesota serotype (six were isolated from live chickens and 17 were from carcasses). Resistance to both antimicrobials was observed in 61.7% (37/60) of the *Salmonella* strains and sensitivity to both antimicrobials was observed in 20% (12/60) of them.

Table 2. Phenotypic profile of *Salmonella* Minnesota and Heidelberg by the Disk Diffusion Test of Amoxicillin/Clavulanic Acid and Cefotaxime

Serotypes*	Sources	AMC R*	CTX R*	AMC E CTX R*	Total of Resistant Samples	Total of Samples
Salmonella Minnesot	Live chickens**	1,6% (1/60)	5% (3/60)	5% (3/60)	7 (11,7%)	9
	Carcasses**	1,6% (1/60)	8,3% (5/60)	20% (12/60)	18 (30%)	27
Salmonella Heidelberg	Carcasses	1,6% (1/60)	0% (0/60)	36,6% (22/60)	23 (38,3%)	24
Total		3	8	37	48 (80%)	60

* Test G: Williams, $P < 0.05$; ** Test G: Williams, $P > 0.05$; AMC: Amoxicillin/Clavulanic Acid; CTX: Cefotaxime; R – Resistant.

With respect to *Salmonella* Minnesota, the resistance profiles were similar between *Salmonella* from live chicken versus carcass. Regarding the resistance of *Salmonella* Minnesota and *Salmonella* Heidelberg to the antimicrobials (Amoxicillin/Clavulanic Acid, and Cefotaxime), there was a significant difference ($P < 0.05$) between the observed proportions, with the highest proportion of resistance found for Amoxicillin/Clavulanic Acid and Cefotaxime together, and the lowest for Amoxicillin/Clavulanic Acid alone (Table 2).

After PCR was performed, of the 40 amoxicillin/clavulanic acid resistant *Salmonella* strains, *bla*CTX-M-8 gene was detected in 7.5% (3/40), *bla*CMY-2 gene in 40% (16/40) and *bla*ACC-1 gene in 37.5% (15/40) (Table 3). Three *bla*CTX-M-8 positive strains were Minnesota serotype, one isolated from live chicken and two from carcasses. Among *bla*CMY-2 positive strains, two were Heidelberg serotype from carcasses and 14 were Minnesota serotype, with

four from live chicken and ten from carcasses. The *bla*ACC-1 gene was found in 15 strains of *Salmonella* Minnesota, three of which were isolated from live chickens and 12 isolated from carcasses.

Table 3. Presence of resistance genes using PCR in *Salmonella* Minnesota and Heidelberg strains resistant to Amoxicillin/Clavulanic Acid and/or Cefotaxime (assessed using the Disk Diffusion Test)

Samples	Antimicrobials Resistance genes	Amoxicillin/Clavulanic Acid			Cefotaxime			Amoxicillin/Clavulanic Acid + Cefotaxime*		
		*bla*CMY-2	*bla*ACC-1	*bla*CTX-M-8	*bla*CMY-2	*bla*ACC-1	*bla*CTX-M8	*bla*CMY-2*	*bla*ACC-1*	*bla*CTX- M-8*
Salmonella Minnesota	Live chickens*	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/3)	33,3% (1/3)	66,7% (2/3)	100% (3/3)	66,7% (2/3)	33,3% (1/3)
	Carcasses*	100% (1/1)	100% (1/1)	0% (0/1)	0% (0/5)	20% (1/5)	20% (1/5)	75% (9/12)	91,6% (11/12)	16,6% (2/12)
Salmonella Heidelberg	Carcasses	0% (0/1)	0% (0/1)	0% (0/1)	0% (0/0)	0% (0/0)	0% (0/0)	9,1% (2/22)	0% (0/22)	0% (0/22)
Total		2	2	0	0	2	3	14	13	3

*Test G: Williams, $P < 0.05$; *bla*CMY-2 – CMY-2 betalactamase (AmpC-betalactamase group); *bla*ACC-1 – ACC-1 betalactamase (AmpC-betalactamase group); *bla*CTX-M-8 – CTX-M-8 betalactamase (ESBL group).

Among the 45 Cefotaxime resistant strains, the *bla*CTX-M-8 gene was detected in 13.3% (6/45), *bla*CMY-2 in 31.1% (14/45) and *bla*ACC-1 in 33.3% (15/45). These *bla*CTX-M-8 genes were detected in six Minnesota strains, three of which were isolated from live chickens and three from carcasses. Among *bla*CMY-2 positive strains, two were Heidelberg serotype isolated from carcasses and 12 were Minnesota serotype (three of which were isolated from live chicken and nine from carcasses). The *bla*ACC-1 gene was also found in 15 Minnesota strains resistant to Cefotaxime, three of which were isolated from live chicken and 12 from carcasses.

Considering the antimicrobial with greater resistance rate, a comparison was made between the frequency of detection of the resistance gene according to *Salmonella* Minnesota origin (live chicken vs carcass). We found that the highest frequency was for CMY-2 in live chicken, followed by ACC-1 in carcass, and finally, CTX-M-8 in carcass (Table 3).

Discussion

From all the samples we studied, 80% demonstrated resistance to at least one of the antimicrobials tested. 66.7% were resistant to Amoxicillin/Clavulanic Acid and 75% were resistant to Cefotaxime. Other studies have found similar resistance frequencies in *Salmonella* spp. strains, such as Cortez *et al*. (2006) who found 72.4% resistance to Cefotaxime and 55.2% to Amoxicillin/Clavulanic Acid from poultry slaughterhouses isolates in São Paulo state in 2003 and 2004. In contrast, Minharro *et al*. (2015) observed 100% Amoxicillin/Clavulanic Acid resistance after analyzing *Salmonella* strains isolated from carcasses, livers, and hearts of slaughtered chickens in Tocantins state in 2011 and 2012. These studies, together with our results, reflect the high rate of resistance to beta-lactams in strains of *Samonella* spp. in Brazil today.

From the 40 Amoxicillin/Clavulanic Acid resistant *Salmonella* strains identified by the Disk Diffusion Test, the *bla*CMY-2 gene was detected in 40% (16/40). The presence of this gene in Amoxicillin/Clavulanic Acid resistant *Salmonella* Heidelberg isolated from chickens or derived products has been reported in Canada and United States, relating it to outbreaks induced by *Salmonella* Heidelberg in humans (Andrysiak *et al*., 2008; Folster *et al*., 2011). It was demonstrated, as in our study, that 31.1% of cefotaxime resistant strains and 40% of Amoxicillin/Clavulanic Acid resistant strains

had the *bla*CMY-2 gene, implying the involvement of AmpC betalactamases which promotes resistance to third-generation cephalosporins and Beta-lactam + betalactamases inhibitor (Clavulanic Acid) combinations. It also shows the gene emergence, which may be a consequence of the use of these drugs in poultry farms in different parts of the world. Recently, an increase of extended-spectrum cephalosporins resistance in *Salmonella* Heidelberg in the Netherlands was attributed to frequent occurrence of strains carrying the IncI1/ST12 plasmid encoding *bla*CMY-2 gene in production animals and poultry products imported from Brazil (Liakopoulos *et al*., 2016). Another gene from the AmpC betalactamase family, *bla*ACC-1, was detected in our study in 35.4% (17/48) of Amoxicillin/Clavulanic Acid and/or Cefotaxime resistant samples. The presence of this gene in samples resistant to Cephalosporins and Penicillins resistant samples has also been reported by other authors in Tunisia and India (Ktari *et al*., 2009; Gokul *et al*., 2010).

The CTX-M gene has already been detected in different *Salmonella* spp. serotypes in many countries. In Brazil, Silva *et al*. (2013) analyzed 93 *Salmonella* Schwarzengrund and Agona strains isolated from different stages of the poultry production cycle in 2008 and 2009, and observed the presence of the CTX-M-2 gene in 14% of the samples. Fernandes *et al*. (2009) studied 153 *Salmonella* Typhimurium strains isolated from humans and animals between 2003 and 2004 and detected the presence of the *bla*CTX-M gene in 6.5% of the samples. In general, ESBL confer resistance to third generation Cephalosporins and are inhibited by betalactamase inhibitors, whereas AmpC-betalactamases are not inhibited (Alvarez *et al*., 2004). This was observed in this study, where almost all isolates with the presence of *bla*ACC-1 and *bla*CMY-2 were resistant to Amoxicillin with Clavulanic Acid, a betalactamase inhibitor. With the increased prevalence of ESBL-producing bacteria, rapid and accurate identification has proved to be increasingly important clinically. Despite the variety of available methods, ESBL identification by conventional phenotypic methods is difficult in routine laboratory tests due to the large number of betalactamases variants in association to these ESBL with AmpC and in association with metallo-betalactamases or to outer membrane permeability modification (Grimm *et al*., 2004; Drieux *et al*., 2008). Thus, genotypic determination has the potential to identify the resistance-causing gene and translate this into clinically useful information that may assist in improving diagnostic practice and salmonellosis treatment.

The detection of *bla*CTX-M-8, *bla*CMY-2, and *bla*ACC-1 resistance genes in *Salmonella* Minnesota and *Salmonella* Heidelberg highlights the diversity of resistance genes against antimicrobials. The possibility of plasmids transmission of these genes to other serotypes or other bacterial species generates a need for more comprehensive studies, considering that the Heidelberg serotype plays an important role in zoonosis.

In this study, samples were obtained from slaughterhouses and poultry farms of south and west-center regions of Brazil, and the *Salmonella* Minnesota serotype corresponded to 60% of the studied strains. Voss-Rech *et al*. (2015) detected this serotype presence in 40.24% of the strains when analyzing 82 *Salmonella* spp. strains isolated from poultry farms within the same regions and showed the highest occurrence of the Minnesota serotype in Mato Grosso do Sul state. Our findings reinforce the prevalence of this serotype in these regions, characterized as centers of poultry export.

Conclusion

The presence of Amoxicillin/Clavulanic Acid and/or Cefotaxime resistant *Salmonella* Heidelberg and *Salmonella* Minnesota in both live chicken and carcass indicates the serotypes' potential as sources of antibacterial resistance transmission, with possible implications both for collective health and the Brazilian poultry export trade. Measures that establish the appropriate use of antibiotics in animal production in an effort to prevent the development of drug-resistant *Salmonella* strains are of vital importance. These efforts can reduce the impact on public health and consolidate the Brazilian market in the international scene by adding value to the product and making it more competitive.

References

Alvarez M, Tran JH, Chow N & Jacoby GA. 2004. Epidemiology of conjugative plasmid-mediated AmpC β-lactamases in the United States. Antimicrobial Agents and Chemotherapy, 48: 533-537. DOI: 10.1128/AAC.48.2.533-537.2004

Andrysiak AK, Olson AB, Tracz DM, Dore K, Irwin R, Ng LK & Gilmour MW. 2008. Genetic characterization of clinical and agri-food isolates of multi drug resistant *Salmonella enterica* serovar Heidelberg from Canada. BMC Microbiology, 8: 89. DOI: 10.1186/1471-2180-8-89

ANVISA 2012. Relatório de pesquisa em vigilância sanitária de alimentos. Programa nacional de monitoramento da prevalência e da resistência bacteriana em frango. Agência Nacional de Vigilância Sanitária, Brasília, Brasil.

Ayres M, Ayres Júnior M, Ayres DL & Santos ADA. 2007. 5th Ed. Aplicações estatísticas nas áreas das ciências bio-médicas. Instituto Mamirauá. Belém. 364 Pages.

Bauer AW, Kirby WM, Sherris JC & Turck M. 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45: 493-496.

Brasil 2009. Instrução Normativa SDA nº 26, de 09 de julho de 2009. Regulamento Técnico para a fabricação, o controle de qualidade, a comercialização e o emprego de produtos antimicrobianos de uso veterinário. Ministério da Agricultura, Pecuária e Abastecimento.

Cantón R, González-Alba JM & Galán JC. 2012. CTX-M enzymes: origin and diffusion. Frontiers in Microbiology, 3:110. DOI: 10.3389/fmicb.2012.00110

Cardoso A, Kanashiro A, Stoppa G, Castro A, Luciano R & Tessari E. 2015. Ocorrência de *Salmonella* spp. em carcaças de frango provenientes de abatedouros do estado de São Paulo, Brasil, no período de 2000 a 2010. Revista Científica Eletrônica de Medicina Veterinária, 24: 1-12.

CDC. 2008. Salmonella surveillance: annual summary, 2006. Centers for Disease Control and Prevention. Department of Health and Human Services, Atlanta.

CDC. 2014. National Antimicrobial Resistance Monitoring System: enteric bacteria. 2012 Human isolates final report. Centers for Disease Control and Prevention. Department of Health and Human Services, Atlanta.

CLSI. 2013. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From animals; Approved Standard. 4th. VET01-A4. 33(7), 94 Pages.

Cortez ALL, Carvalho ACFB, Ikuno AA, Bürger KP & Vidal-Martins AMC. 2006. Resistência antimicrobiana de cepas de *Salmonella* spp. isoladas de abatedouros de aves. Arquivos do Instituto Biológico, 73: 157-163.

Decré D, Verdet C, Raskine L, Blanchard H, Burghoffer B, Philippon A, Sanson-Le-Pors MJ, Petit JC & Arlet G. 2002. Characterization of CMY-type β-lactamases in clinical strains of *Proteus mirabilis* and *Klebsiella pneumoniae* isolated in four hospitals in the Paris area. Journal of Antimicrobial Chemotherapy, 50: 681-688. DOI: 10.1093/jac/dkf193

Drieux L, Brossier F, Sougakoff W & Jarlier V. 2008. Phenotypic detection of extended-spectrum β-lactamase production in Enterobacteriaceae: review and bench guide. Clinical Microbiology and Infection, 14: 90-103. DOI: 10.1111/j.1469-0691.2007.01846.x

FDA. 2010. National Antimicrobial Resistance Monitoring System-enteric bacteria (NARMS): 2007 executive report. U.S. Food and Drug Administration. Department of Health and Human Services, Rockville.

Fernandes SA, Paterson DL, Ghilardi-Rodrigues ÂC, Adams-Haduch JM, Tavechio AT & Doi Y. 2009. CTX-M-2–producing *Salmonella* Typhimurium isolated from pediatric patients and poultry in Brazil. Microbial Drug Resistance, 15: 317-321. DOI: 10.1089/mdr.2009.0050

Folster JP, Pecic G, McCullough A, Rickert R, Whichard JM. 2011. Characterization of *bla*CMY-encoding plasmids among *Salmonella* isolated in the United States in 2007. Foodborne Pathogens and Disease, 8: 1289-1294. DOI: 10.1089/fpd.2011.0944

Freitas J. 2011. Evolução de sorovares Modelo de Banco de Cepas. Seminário Internacional de Salmonellose Aviária. Rio de Janeiro.

Gokul BN, Menezes GA & Harish BN. 2010. ACC-1 β-lactamase–producing *Salmonella enterica* serovar Typhi, India. Emerging Infectious Diseases, 16: 1170-1171. DOI: 10.3201/eid1607.091643

Grimm V, Ezaki S, Susa M, Knabbe C, Schmid RD & Bachmann TT. 2004. Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance.

Journal of Clinical Microbiology, 42: 3766-3774. DOI: 10.1128/JCM.42.8.3766-3774.2004

Hammack T, Andrews WH & Jacobson A. 2014. USFDA bacteriological analytical manual. http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm#Prep Accessed on January 29. 2017.

Hanson ND. 2003. AmpC β-lactamases: what do we need to know for the future? Journal of Antimicrobial Chemotherapy, 52: 2-4. DOI: 10.1093/jac/dkg284

Hoffmann M, Zhao S, Pettengill J, Luo Y, Monday SR, Abbott J, Ayers SL, Cinar HN, Muruvanda T, Li C, Allard MW, Whichard J, Meng J, Brown EW & McDermott PF. 2014. Comparative genomic analysis and virulence differences in closely related *Salmonella enterica* serotype Heidelberg isolates from humans, retail meats, and animals. Genome Biology and Evolution, 6: 1046–1068. DOI: 10.1093/gbe/evu079

Jouini A, Vinué L, Slama KB, Sáenz Y, Klibi N, Hammami S, Boudabous A & Torres C. 2007. Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia. Journal of Antimicrobial Chemotherapy 60: 1137-1141. DOI: 10.1093/jac/dkm316

Ktari S, Arlet G, Verdet C, Jaoua S, Kachrid A, Redjeb SB, Mahjoubi-Rhimi F & Hammami A. 2009. Molecular epidemiology and genetic environment of acquired *bla*ACC-1 in *Salmonella enterica* serotype Livingstone causing a large nosocomial outbreak in Tunisia. Microbial Drug Resistance, 15: 279-286. DOI: 10.1089/mdr.2009.0035

Liakopoulos A, Geurts Y, Dierikx CM, Brouwer MSM, Kant A, Wit B, Heymans R, Pelt W & Mevius DJ. 2016. Extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar heidelberg strains, the netherlands. Emerging Infectious Diseases, 22: 1257-1261. DOI: 10.3201/eid2207.151377

Mendonça N, Manageiro V, Robin F, Salgado MJ, Ferreira E, Caniça M & Bonnet R. 2008. The Lys234Arg substitution in the enzyme SHV-72 is a determinant for resistance to clavulanic acid inhibition. Antimicrobial Agents and Chemotherapy, 52: 1806-1811. DOI: 10.1128/AAC.01381-07

Minharro S, Nascimento CA, Galletti JP, Merisse TJ, Feitosa ACF, Santos HD, Dias FEF, Santana ES, Baldani CD & Andrade MA. 2015. Antimicrobial susceptibility of *Salmonella* serovars isolated from edible offal and carcasses of slaughtered poultry in the state of Tocantins, Brazil. Semina: Ciências Agrárias, 36: 2661-2670.

Nadjar D, Rouveau M, Verdet C, Donay JL, Herrmann JL, Lagrange PH, Philippon A & Arlet G. 2000. Outbreak of *Klebsiella pneumoniae* producing transferable AmpC-type β-lactamase (ACC-1) originating from *Hafnia alvei*. FEMS Microbiology Letters, 187: 35-40. DOI: 10.1111/j.1574-6968.2000.tb09133.x

Pérez-Pérez FJ & Hanson ND. 2002. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. Journal of Clinical Microbiology, 40: 2153-2162. DOI: 10.1128/JCM.40.6.2153-2162.2002

PHAC. 2014. Annual summary 2012. National Enteric Surveillance Program. Public Health Agency of Canada.

RASFF. 2014. The Rapid Alert System for Food and Feed. 2013 annual report. European Commission-Health and Food Safety. https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2013.pdf Accessed on January 31. 2017.

RASFF. 2015. The Rapid Alert System for Food and Feed. 2014 annual report. European Commission-Health and Food Safety. https://ec.europa.eu/food/sites/food/files/safety/docs/rasff_annual_report_2014.pdf Accessed on January 31. 2017.

Silva KC, Fontes LC, Moreno AM, Astolfi-Ferreira CS, Ferreira AJP & Lincopan N. 2013. Emergence of extended-spectrum-β-lactamase CTX-M-2-producing *Salmonella enterica* serovars Schwarzengrund and Agona in poultry farms. Antimicrobial Agents and Chemotherapy, 57: 3458-3459. DOI: 10.1128/AAC.05992-11

Voss-Rech D, Vaz CS, Alves L, Coldebella A, Leão JA, Rodrigues DP & Back A. 2015. A temporal study of *Salmonella enterica* serotypes from broiler farms in Brazil. Poultry Science, 94: 433-441. DOI: 10.3382/ps/peu081

WHO. 2016. *Salmonella* (non-typhoidal). Fact sheet Nº 139. http://www.who.int/mediacentre/factsheets/fs139/en/ Accessed on January 31. 2017.

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بررسی مقاومت به آموکسی‌سیلین/ کلاولانیک اسید و سفوتاکسیم در سویه‌های *سالمونلا مینوستا* و *سالمونلا هایدلبرگ* جداسازی شده از جوجه‌های گوشتی

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چکیده

در این مطالعه مقومت سویه‌های مختلف باکتری *سالمونلا* به آنتی‌بیوتیک‌های بتا- لاکتون مورد مطالعه قرار گرفت. *سالمونلا مینوستا* (۳۶ سویه) و *سالمونلا هایدلبرگ* (۲۴ سویه) از جوجه‌های گوشتی و لاشه‌ی آن‌ها از طریق روش انتشار دیسک جداسازی شد و ژن‌های مقاوم blaCTX-M-8، blaACC-1 و blaCMY-2 بوسیله پی‌سی‌آر شناسایی شد. از ۶۰ سویه‌ی مورد بررسی، ۸۰٪ حداقل به یکی از آنتی‌بیوتیک‌ها مقاوم بودند. مشخصا، ۶۶/۷٪ به آموکسی‌سیلین/ کلاولانیک اسید و ۷۵٪ به سفوتاکسیم مقاوم بودند. در بین سویه‌های مقاوم به آموکسی‌سیلین/ کلاولانیک اسید، ژن blaCMY-2 در ۴۰٪، ژن blaACC-1 در ۳۷/۵٪، و ژن blaCTX-M-8 در ۷/۵٪ تشخیص داده شد. در بین سویه‌های مقاوم به سفوتاکسیم، ما ژن blaCTX-M-8 را در ۱۳/۳٪، ژن blaACC-1 را در ۳۳/۳٪، و ژن blaCMY-2 را در ۳۱/۱٪ تشخیص دادیم. وجود سویه‌ای *سالمونلای* مقاوم به سفوتاکسیم و آموکسی‌سیلین/ کلاولانیک اسید، و بتالاکتامازها در این سویه‌ها از نظر بهداشت و سلامت عمومی و اقتصادی مهم هستند.

کلمات کلیدی
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