fmvz - unesp
Faculdade de Medicina Veterinária e Zootecnia
Campus de Botucatu

PERFIL MOLECULAR E RESISTÊNCIA A ANTIMICROBIANOS DE *Salmonella* ISOLADA EM LINFONODOS MESENTÉRICOS DE SUÍNOS

FÁBIO SOSSAI POSSEBON

Botucatu – SP
Fevereiro de 2016

UNIVERSIDADE ESTADUAL PAULISTA
FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

PERFIL MOLECULAR E RESISTÊNCIA A ANTIMICROBIANOS DE *Salmonella* ISOLADA EM LINFONODOS MESENTÉRICOS DE SUÍNOS

FÁBIO SOSSAI POSSEBON

Dissertação apresentada junto ao Programa de Pós-Graduação em Medicina Veterinária para obtenção do título de mestre.

Orientador: Prof. Ass. Dr. José Paes de Almeida Nogueira Pinto

Fevereiro/2016

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Possebon, Fábio Sossai.
Perfil molecular e resistência a antimicrobianos de Salmonella isolada em linfonodos mesentéricos de suínos / Fábio Sossai Possebon. - Botucatu, 2016

Dissertação (mestrado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina Veterinária e Zootecnia
Orientador: José Paes de Almeida Nogueira Pinto
Capes: 50505009

1. Suíno - Doenças. 2. Anti-infecciosos. 3. Drogas - Resistência em microorganismos. 4. Antibióticos. 5. Salmonella.

Palavras-chave: Epidemiologia; MDR; MLST; Salmonella; Suínos.

Nome do autor: Fábio Sossai Possebon

Título: PERFIL MOLECULAR E RESISTÊNCIA A ANTIMICROBIANOS DE *Salmonella* ISOLADA EM LINFONODOS MESENTÉRICOS DE SUÍNOS

COMISSÃO EXAMINADORA

Prof. Dr. José Paes de Almeida Nogueira Pinto
Presidente e Orientador
Departamento de Higiene Veterinária e Saúde Pública
FMVZ – UNESP Botucatu

Prof. Dr. Luís Augusto Nero
Membro
Departamento de Veterinária
Universidade Federal de Viçosa

Prof. Dr. Luciano dos Santos Bersot
Membro
Departamento de Ciências Veterinárias
Universidade Federal do Paraná – Campus Palotina

Data do exame: 22/02/2016

Agradecimentos

A minha família por nunca medir esforços para me propiciar uma educação de qualidade.

A minha namorada Sil, pela paciência e companheirismo em todos os momentos.

Aos meus amigos, por se tornarem uma grande segunda família, parte fundamental para meu desenvolvimento.

Ao meu orientador Prof. Paes, por ter sido um exemplo de profissional, de caráter, e a cima de tudo um grande amigo.

Ao Professor Ricardo (Curan) pelos valiosos conselhos e por ter sido um grande parceiro profissional e amigo.

Aos Professores que me avaliaram nas bancas de qualificação e defesa: Professora Vera, Professor Pantoja, Professor Luciano e Professor Nero por terem contribuído imensamente, não somente para o projeto de pesquisa, mas também para minha formação como pesquisador.

Aos profissionais dos frigoríficos e do Serviço de Inspeção Federal, por sempre estarem dispostos a ajudar de prontidão.

A Cefar® por fornecer os discos de antibiograma.

A Dra. Monique e Dra. Sueli do Instituto Adolfo Lutz, por terem possibilitado a sorotipagem das cepas.

Ao Professor João Pessoa, Camila e toda equipe do LDM, por terem possibilitado a realização do MLST e contribuído com vasto conhecimento na área de biologia molecular.

A toda equipe do SOAP e do IBTEC pelo apoio e amizade.

A CAPES por conceder a bolsa de mestrado.

Lista de quadros

Lista de Figuras

Sumário

Resumo

O presente trabalho avaliou 250 animais de 25 lotes distintos abatidos em 4 estabelecimentos do estado de São Paulo para a presença de *Salmonella* nos linfonodos mesentéricos, e caracterizou o perfil de resistência das cepas isoladas aos principais antibióticos. O patógeno estava presente em 36,4% das amostras e 72% dos lotes analisados. Dos 91 isolados, 67 foram sorotipados, e os principais sorovares encontrados foram *S*. Typhimurium (n=13), *S.* 1.4,5,12:i:- (n=12) *S.* Infantis (n=12) e *S.* Havana (n=11). Os compostos com menor eficácia frente aos isolados foram estreptomicina e tetraciclina (68,1% de resistência) ampicilina e sulfonamidas (62,6%), cloranfenicol (56,0%), trimetoprim-sulfametoxazol (41,8%) e ácido nalidíxico (40,7%). Os mais efetivos foram aztreonam e cefoxitina, (ambos com 3,3% de resistência) e cefepima e ceftriaxona, (ambos com 7,7%). Cepas multidrogas resistentes (MDR) corresponderam a 70,3% dos isolados. Oito cepas foram submetidas ao MLST: quatro *S.* Typhimurium e uma *S.* 1.4,5,12:i:- todas pertencentes ao ST 19, duas *S.* Infantis pertenceram ao ST 32 e uma *S.* Derby, pertencente ao ST 40. A grande prevalência do patógeno nos animais analisados, com altas taxas de resistência aos antibióticos e pertencentes a grupos genéticos frequentemente associados a surtos e doenças em humanos demonstram que a cadeia produtiva da carne suína é uma fonte de contaminação em potencial nos casos de salmonelose, sendo necessárias medidas preventivas eficazes para o controle do patógeno e diminuição do risco de veiculação de patógenos por alimentos.

Abstract

This study evaluated 250 animals of 25 different batches processed in four slaughterhouses in São Paulo state - Brazil for the presence of *Salmonella* in the mesenteric lymph nodes, and characterized the resistance profile for the main antibiotics. The pathogen was present in 36.4% of samples and 72% of the analyzed batches. Of the 91 isolates, 67 were serotyped, and the main serovars found were *S*. Typhimurium (n = 13), *S*. 1.4,5,12:i- (n = 12) *S*. Infantis (n = 12) and *S*. Havana (n = 11). The compounds with less efficacy were streptomycin and tetracycline (68.1% resistant) ampicillin and sulphonamides (62.6%), chloramphenicol (56.0%), trimethoprim-sulfamethoxazole (41.8%), and nalidixic acid (40.7%). The most effective were cephalothin and aztreonam, (both with 3.3% resistant) and ceftriaxone and cefepime (both with 7.7%). Multidrug-resistant strains (MDR) accounted for 70.3% of the isolates. Eight strains were submitted to MLST: Four *S*. Typhimurium and one *S*.1.4,5,12:i:- all belonging to the ST 19, two *S*. Infantis, belonging to the ST 32 and one *S*. Derby, belonging to ST 40. The high prevalence of the pathogen in the analyzed animals, with high rates of resistance to antibiotics and belonging to genetic groups that are often associated with outbreaks and disease in humans, shows that the production chain of pork is a potential source of contamination in salmonellosis cases, with the necessity of effective preventive measures for pathogen control and lower the risk of foodborne diseases transmission.

Capítulo 1

Introdução

A carne suína representa uma importante fonte de nutrientes para a população, apresentando elevados níveis de proteínas de alto valor nutricional, aminoácidos essenciais, vitaminas do complexo B e minerais (KAUFFMAN, 2001). Atualmente é a mais consumida no mundo, sendo que sua produção ultrapassa 100 milhões de toneladas/ano. No cenário mundial, o Brasil é o quarto maior produtor e exportador do segmento, tendo abatido em 2014, se considerarmos os somente os estabelecimentos com inspeção oficial, mais de 32 milhões de animais e apresentando um consumo per capita de 14,60 Kg/habitante (ABPA, 2015).

Neste contexto, a preocupação com a segurança alimentar se faz necessária para assegurar o fornecimento de produtos livres de patógenos. Dentre os principais micro-organismos causadores dessas enfermidades, pode-se destacar *Salmonella*. Dados parciais de 2014 revelaram que nos Estados Unidos, este foi o patógeno de maior incidência e responsável pelo maior número de óbitos em humanos dentre os veiculados por alimentos contaminados (CRIM et al., 2015). Por apresentar sintomas relativamente comuns nos indivíduos afetados e por falta de empenho ou condição por parte dos órgãos médicos e de vigilância epidemiológica, sua ocorrência é subnotificada, mas calcula-se que nos EUA 1,4 milhões de pessoas são acometidas anualmente e os custos diretos com medicamentos e internações atingem em torno de 2,6 bilhões de dólares (MEAD et al., 2010).

O gênero *Salmonella* compreende bacilos Gram-negativos, não formadores de esporos, pertencentes à família Enterobacteriaceae. São ainda micro-organismos anaeróbios facultativos, capazes de utilizar citrato como única fonte de carbono, quase sempre produzem gás a partir da glicose e raramente fermentam lactose ou sacarose. Em sua maioria, são móveis, pois possuem flagelos peritríquios (FRANCO; LANDGRAF, 1996).

Hald et al. (2004) destacaram o consumo de carne suína como um importante fator na ocorrência da veiculação de patógenos por alimentos. Dentre as carnes vermelhas, a suína é a mais frequentemente associada à *Salmonella*, estando o

patógeno intimamente ligado à cadeia produtiva do animal, da fazenda ao varejo, tendo sido detectado em diversas etapas e insumos envolvidos na produção (SANCHEZ et al., 2007; ZHENG; BONDE; SØRENSEN, 2007; LITTLE et al., 2008; YANG et al., 2010). Dados da literatura revelaram que na Alemanha, 20% dos casos de salmonelose foram associados ao consumo deste tipo de carne (STEINBACH; HARTUNG, 1999). Em um estudo de caso-controle para estimar os fatores de riscos associados à salmonelose, Mughini-Gras et al. (2014) afirmaram que 39,9% dos casos esporádicos da doença em ambiente doméstico estavam associados ao consumo de carne suína na Holanda.

Lo Fo Wong et al. (2002) relataram que a introdução de animais positivos no frigorífico e subsequente transmissão para animais negativos durante o processamento, estavam relacionados com a alta prevalência do micro-organismo no produto final, ressaltando fatores importantes como o estresse do animal na chegada ao abatedouro, influenciando na excreção de *Salmonella* por portadores e o manejo e fluxograma de abate, como pontos que possibilitavam a contaminação dos demais animais.

Revisão da literatura

- Prevalência e caracterização dos sorovares de *Salmonella* em suínos

Trabalhos em diversos países reportaram a prevalência de *Salmonella* nas diferentes etapas da cadeia produtiva da carne suína, utilizando-se de diferentes metodologias e reportando diferentes números. Sanchez et al. (2007) ao realizarem uma metanálise de artigos que relataram a prevalência da bactéria em diversas unidades de produção de suínos na Europa, durante o período de 1990 até 2005, encontraram valores médios de 59% de propriedades positivas e 17% de animais positivos. Foram também determinados no trabalho os principais pontos responsáveis pelos resultados de prevalências divergentes dentre as diversas pesquisas, sendo destacados: o método microbiológico utilizado, o número de amostras analisadas e o país onde o estudo foi realizado como os fatores mais significativamente envolvidos em tal variação.

Ao compararem a sensibilidade do uso de “pools” de *swabs* de carcaças com a pesquisa individualizada para *Salmonella*, utilizando um total de 18.984 amostras obtidas em abatedouros na Dinamarca, Sørensen et al. (2007) reportaram uma baixa prevalência de resultados positivos (1,4%) quando utilizaram 5 animais aleatoriamente selecionados por dia, amostrados individualmente. Quando 5 animais foram amostrados em *pool*, tal prevalência aumentou para 4,1%.

Na Itália, Piras et al. (2011) relataram uma prevalência de animais positivos na ordem de 30,5% quando eram analisados os linfonodos mesentéricos, 16,4% quando a pesquisa era realizada em fezes e conteúdo intestinal, e 14,1% quando foram utilizados *swabs* de carcaças e fígado. Foi realizada ainda a pesquisa do patógeno nas superfícies do abatedouro, sendo positivas 38,0% das que não estavam em contato direto com a carne, e 35% das que tinham contato direto com a mesma. Os pesquisadores reportaram ainda que de 31 animais identificados como portadores do patógeno (presença nos linfonodos e/ou conteúdo intestinal) somente em oito foi isolada *Salmonella* na carcaça. Foram determinados oito diferentes sorovares no trabalho, sendo *S.* Derby e *S.* Typhimurium os mais prevalentes (42,7% e 23,3%, respectivamente).

Em ambas as situações, deve-se destacar o considerável aumento de resultados positivos quando a pesquisa utilizava linfonodos ou conteúdo intestinal como unidade analítica ao invés de *swabs* de carcaças

Pesquisas brasileiras também reportaram a presença do patógeno nos animais abatidos. Silva et al. (2009) relataram que no estado do Mato Grosso foram detectados 16,6% dos animais positivos para o patógeno quando pesquisados linfonodos mesentéricos e tonsilas. Os principais sorovares isolados foram novamente *S.* Derby (16%) e *S.* Typhimurium (14%), seguidos por *S.* London e *S.* Give (ambos 12%).

Em trabalho semelhante, realizado no Rio Grande do Sul, Bessa et al. (2004) encontraram uma maior prevalência do patógeno, da ordem de 55,6% de positivos. Tal trabalho também se utilizou da análise de linfonodos mesentéricos e conteúdo intestinal para a pesquisa de *Salmonella*. Destacaram ainda que a prevalência de animais positivos nos linfonodos foi de 17,6% e nas fezes 18,6%. Os sorovares que

se destacaram como mais isolados foram *S.* Typhimurium (24,3%), *S.* Agona (19,9%), *S.* Derby (13,2%) e *S.* Bredeney (12,0%).

A pesquisa de *Salmonella* nos linfonodos mesentéricos é comumente utilizada para indicar o *status* do animal como portador deste patógeno (BAHNSON et al., 2006) e quando a amostragem é realizada a partir do conteúdo intestinal, pode demonstrar seu caráter excretor (DAVIES et al., 1998). Autores relatam que a infecção dos animais pela bactéria ocorre predominantemente por via oral e os linfonodos mesentéricos atuam primariamente como uma barreira, porém posteriormente se tornam reservatórios do patógeno, que pode sobreviver intracelularmente nos macrófagos e neutrófilos até que alterações fisiológicas desencadeadas pelo stress, como a liberação de catecolaminas e alterações da motilidade intestinal, propiciem sua liberação nas fezes em poucas horas (STRAW et al., 2012).

- Resistência a antimicrobianos de cepas de *Salmonella* isoladas de suínos

Sendo a salmonelose uma enfermidade transmitida primariamente por alimentos, com destaque para os de origem animal, a ocorrência de micro-organismos resistentes a antimicrobianos nos produtos alimentícios originários de tais fontes pode ser considerada um grave problema de saúde pública, agravado se forem consideradas as drogas que são concomitantemente utilizadas no tratamento dos animais e dos seres humanos (THAI et al., 2012).

Apesar de promover uma infecção auto limitante na maior parte dos casos, sem necessidade de antibioticoterapia, por vezes, *Salmonella* não tifoidal pode causar enfermidades mais severas, principalmente quando ocorre o acometimento de outros sistemas além do trato gastrintestinal. Em tais situações, o uso de fármacos se faz necessário para salvaguardar a vida do indivíduo acometido (ANGULO et al., 2000; PARRY; THRELFALL, 2008). Estudos relataram o isolamento de diversas cepas de *Salmonella* resistentes aos principais grupos de antimicrobianos utilizados tanto na produção animal, quanto no tratamento de seres humanos.

Gomez-Laguna et al. (2011) determinaram o perfil de resistência a antimicrobianos em cepas de *Salmonella* isoladas em suínos que foram criados em sistema *free-range* na Espanha, e destacaram como os compostos com maior incidência de resistência: estreptomicina (46%), tetraciclina (30%), sulfonamidas (25%) e ampicilina (23%). Revelaram ainda que 36% das cepas que apresentaram algum tipo de resistência, foram concomitantemente resistentes a 4 ou mais compostos antimicrobianos, sendo consideradas pelos autores como multidrogas resistentes (MDR).

Durante estudo similar realizado no Vietnã, porém contemplando carne de origem avícola e suína, Thai et al. (2012) determinaram que 78,4% dos isolados eram resistentes a pelo menos um antimicrobiano. Os compostos menos efetivos foram tetraciclina (58,5%), sulfonamidas (58,1%), estreptomicina (47,3%) ampicilina (39,8%), cloranfenicol (37.3%), trimetoprim (34.0%), e ácido nalidíxico (27.8%). Um total de 23,2% dos isolados foi classificado como MDRs, sendo que 8,3% foram resistentes a mais de 9 antibióticos.

Dados brasileiros revelaram perfis semelhantes de resistência. Castagna et al. (2001) ao analisarem amostras de suínos provenientes do Rio Grande do Sul, destacaram como principais compostos aos quais as cepas do patógeno apresentaram resistência: sulfonamidas (83,9%), tetraciclina (37,4%), cotrimoxazol (25,2%), ampicilina (20,2%), cloranfenicol (16,1%), estreptomicina (14,1%), ácido nalidíxico (10,1%). As amostras MDR representaram 24,2% do total de isolados.

Weiss et al. (2002), também em estudos no Rio Grande do Sul, utilizando-se de fezes, *swabs* retais, e linfonodos mesentéricos, isolaram cepas de *Salmonella* resistentes a sulfonamidas (97,8%) estreptomicina (82,6%), tetraciclina (36,9%) e sulfazotrim (15,2%).

O isolamento de *Salmonella* resistente aos antimicrobianos em suínos é relativamente comum, e o uso inadequado de antibióticos na cadeia produtiva dos animais, seja com finalidade terapêutica, como medidas profiláticas, ou como promotores de crescimento, está relacionado com tal ocorrência (ANGULO et al., 2000; STRAHILEVITZ et al., 2009). Destaca-se ainda o grande número de cepas

resistentes aos compostos convencionalmente utilizados no tratamento da salmonelose em humanos, como a ampicilina e cotrimoxazol.

-Determinação do perfil molecular pela técnica de MLST (*MultiLocus sequence typing)*

A determinação das fontes de contaminação dos animais por *Salmonella*, bem como a distribuição do patógeno através dos rebanhos é de fundamental importância para compreender sua epidemiologia e traçar medidas efetivas de controle, porém a grande variabilidade de sorovares isolados na produção de suínos, associados com a grande quantidade de vetores e portadores assintomáticos dificultam substancialmente a determinação da origem da bactéria (KICH et al., 2007). Por mais de sete décadas a sorotipagem das cepas de *Salmonella,* baseada na determinação dos epítopos dos antígenos capsulares (O) e flagelares (H), tem sido a principal ferramenta para estudar a epidemiologia do patógeno. Tal classificação é amplamente utilizada pela comunidade científica e órgãos de saúde pública, e sua nomenclatura normalmente é associada aos quadros clínicos e espécies acometidas, ou a região onde foi isolado primariamente (por exemplo: *S.* Choleraesuis, *S.* Abortusequi, *S.* London, *S.* Dublin). Recentemente, a nomenclatura de novos sorovares de *Salmonella* foi padronizada de modo a contemplar sua fórmula antigênica (como *S.* 1,4,5,12:i:-) (GRIMONT; WEILL, 2008). Todavia tal classificação não leva em conta a perspectiva evolutiva dos isolados, agrupando por vezes cepas com pouca similaridade genética e em outras não conseguindo agrupar micro-organismos provenientes de linhagens semelhantes (ACHTMAN et al., 2012).

Diversas alternativas têm sido desenvolvidas, visando estreitar as classificações para *Salmonella* e outros micro-organismos. Técnicas como o PFGE (*Pulse field gel electrophoresis)* e MLVA (*MultiLocus Variable number of tandem repeats Analysis)* se mostraram valiosas para estudar as similaridades ou diferenças de cepas isoladas em surtos ou regiões, porém não são capazes de determinar a relação evolutiva das cepas em relação a outros isolados (ACHTMAN et al., 2012). Métodos que utilizam a análise de partes conservadas do genoma

bacteriano, que sofreriam pouca ou nenhuma pressão de seleção genética, foram desenvolvidos para estimar tal relação e determinar clones semelhantes e as arvores evolutivas dos patógenos, possibilitando um estudo epidemiológico mais aprofundado. Ao final da década de 80, foi desenvolvido o MLEE (*MultiLocus Enzyme Electrophoresis*, onde se inferiu a presença de diferentes alelos de um mesmo gene através da verificação da migração de proteínas em um gel de eletroforese (SELANDER et al., 1986). Posteriormente, com o advento da análise genômica e métodos de sequenciamento de DNA, foi desenvolvida a técnica de MLST (*MultiLocus sequence typing)*.

O MLST para *Salmonella* consiste no sequenciamento de sete genes conservados do genoma bacteriano, visando determinar qual alelo de cada gene específico está presente na amostra, atribuindo um número para cada alelo pesquisado. Desta forma, cada amostra recebe uma sequência de sete números, os quais irão determinar sua *sequence type* (ST). Os diferentes alelos para cada gene e os ST já catalogados são disponibilizados online no site http://mlst.warwick.ac.uk/mlst/dbs/Senterica e este banco de dados pode ser alimentado por diversas instituições de pesquisa, possibilitando-se a comparação dos diferentes isolados em diversas fontes e regiões geográficas (JOLLEY; MAIDEN, 2014).

Almeida et al. (2013) pesquisaram o perfil molecular de *S.* Infantis obtidas no estado de São Paulo ao longo de 25 anos, isoladas em pacientes humanos e alimentos, e descobriram que todas as cepas avaliadas através do MLST (n=16) pertenciam ao ST32. Os autores afirmaram que este seria o principal perfil clonal para o sorovar em questão, e juntamente com a PFGE, concluíram que o mesmo subtipo do patógeno estava presente na maior parte dos pacientes e alimentos estudados.

Cai et al. (2016) demonstraram que cepas de *Salmonella* com perfis semelhantes foram isoladas em abatedouros de suinos e mercados varejistas na China, utilizado também as técnicas de MLST e PFGE. Destacaram ainda que os mesmos perfis são isolados frequentemente em surtos de salmonelose por órgãos de saúde pública, demonstrando um possível elo epidemiológico para a doença.

Em um estudo similar na Alemanha, porém contemplando o sorovar *S.* Derby, Hauser et al. (2011) também conseguiram detectar um subtipo prevalente do patógeno circulante nos suínos, sua carne e em humanos, ressaltando a relevância deste ciclo de transmissão.

Assim, tendo em vista o papel dos suínos como fonte de proteína animal e, paradoxalmente, a sua participação como veículos de *Salmonella* ao homem, torna-se importante avaliar sob nossas condições, a prevalência do patógeno nos rebanhos aqui criados, bem como o perfil de resistência das cepas isoladas a alguns antimicrobianos empregados na criação animal e na terapêutica humana, além da circulação do patógeno dentre os diferentes núcleos de produção.

Objetivos

- Determinar a prevalência de *Salmonella* em linfonodos mesentéricos de suínos abatidos em frigoríficos com inspeção oficial no estado de São Paulo, além de caracterizar seus sorovares.

- Determinar a resistência das cepas isoladas aos principais compostos antimicrobianos.

- Determinar o perfil molecular de cepas isoladas através da técnica de *MultiLocus Sequence Typing* (MLST) e comparar os perfis encontrados com as diferentes origens dos lotes analisados e dados da literatura.

Referências

ABPA. **Relatório Anual - Carne Suína**. Disponível em: <http://abpa-br.com.br/files/RelatorioAnual_UBABEF_2015_DIGITAL.pdf>.

ACHTMAN, M.; WAIN, J.; WEILL, F.X.; NAIR, S.; ZHOU, Z.; SANGAL, V.; KRAULAND, M. G.; HALE, J. L.; HARBOTTLE, H.; UESBECK, A.; DOUGAN, G.; HARRISON, L. H.; BRISSE, S. Multilocus Sequence Typing as a Replacement for Serotyping in *Salmonella enterica*. **PLoS Pathog**, v. 8, n. 6, p. e1002776, 2012. Disponível em: <http://dx.doi.org/10.1371%2Fjournal.ppat.1002776>.

ALMEIDA, F.; PITONDO-SILVA, A.; OLIVEIRA, M. A.; FALCÃO, J. P. Molecular epidemiology and virulence markers of *Salmonella* Infantis isolated over 25 years in São Paulo State, Brazil. **Infection, Genetics and Evolution**, v. 19, p. 145–151, 2013.

ANGULO, F. J.; JOHNSON, K. R.; TAUXE, R. V; COHEN, M. L. Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. **Microbial drug resistance (Larchmont, NY)**, v. 6, n. 1, p. 77, 2000.

BAHNSON, P. B.; DAMMAN, D. J.; ISAACSON, R. E.; MILLER, G. Y.; WEIGEL, R. M.; TROUTT, H. F. Prevalence and serovars of *Salmonella enterica* isolated from ileocolic lymph nodes of market pigs reared in selected Midwest US swine herds. **Journal of Swine Health and Production.**, v. 14, n. 4, p. 182–188, 2006.

BESSA, M. C.; COSTA, M. da; CARDOSO, M. Prevalência de *Salmonella sp*. em suínos abatidos em frigoríficos do Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 24, n. 2, p. 80–84, 2004.

CAI, Y.; TAO, J.; JIAO, Y.; FEI, X.; ZHOU, L.; WANG, Y.; ZHENG, H.; PAN, Z.; JIAO, X. Phenotypic characteristics and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail markets in Yangzhou, China. **International Journal of Food Microbiology**, 2016.

CASTAGNA, S. M. F.; BESSA, M. C.; CARVALHO, D. A.; CARDOSO, M.; COSTA,

M. Resistência a antimicrobianos de amostras de *Salmonella sp*. isoladas de suínos abatidos no estado do Rio Grande do Sul. **Arquivo da Faculdade de Veterinária da UFRGS, Porto Alegre**, v. 29, p. 44–49, 2001.

CRIM, S. M.; GRIFFIN, P. M.; TAUXE, R.; MARDER, E. P.; GILLISS, D.; CRONQUIST, A. B.; CARTTER, M.; TOBIN-D'ANGELO, M.; BLYTHE, D.; SMITH, K. Preliminary Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food—Foodborne Diseases Active Surveillance Network, 10 US Sites, 2006–2014. **MMWR. Morbidity and mortality weekly report**, v. 64, n. 18, p. 495–499, 2015.

DAVIES, P. R.; BOVEE, F. G.; FUNK, J. A.; MORROW, W. E.; JONES, F. T.; DEEN, J. Isolation of *Salmonella* serotypes from feces of pigs raised in a multiple-site production system. **Journal of the American Veterinary Medical Association**, v. 212, n. 12, p. 1925, 1998.

FRANCO, B. D. G. M.; LANDGRAF, M. **Microbiologia dos alimentos**. São Paulo: Atheneu, 1996.

GOMEZ-LAGUNA, J.; HERNANDEZ, M.; CREUS, E.; ECHEITA, A.; OTAL, J.; HERRERA-LEON, S.; ASTORGA, R. J. Prevalence and Antimicrobial Susceptibility of *Salmonella* Infections in Free-Range Pigs. **Veterinary Journal**, v. 190, n. 1, p. 176–178, 2011. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20932784>.

GRIMONT, P.; WEILL, F.-X. Antigenic formulae of the *Salmonella* servovars. **WHO Collaborating Centre for Reference and Research on *Salmonella***, p. 1–167, 2008. Disponível em: <http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089\npapers2://publication/uuid/CA3447A0-61BF-4D62-9181-C9BA78AF0312>.

HALD, T.; VOSE, D.; WEGENER, H. C.; KOUPEEV, T. A Bayesian Approach to Quantify the Contribution of Animal-Food Sources to Human Salmonellosis. **Risk Analysis**, v. 24, n. 1, p. 255–269, 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15028016>.

HAUSER, E.; HEBNER, F.; TIETZE, E.; HELMUTH, R.; JUNKER, E.; PRAGER, R.; SCHROETER, A.; RABSCH, W.; FRUTH, A.; MALORNY, B. Diversity of *Salmonella enterica* serovar Derby isolated from pig, pork and humans in Germany. **International Journal of Food Microbiology**, v. 151, n. 2, p. 141–149, 2011.

JOLLEY, K. A.; MAIDEN, M. C. J. Using multilocus sequence typing to study bacterial variation: prospects in the genomic era. **Future microbiology**, v. 9, n. 5, p. 623–30, 2014. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/24957089>.

KAUFFMAN, R. G. Meat Composition. **Meat Science and Applications**, p. 711, 2001.

KICH, J. D.; COLDEBELLA, A.; MORES, N.; FRATAMICO, P. M.; CALL, J. E.; LUCHANSKY, I. Rastreabilidade da *Salmonella* do crescimento ao abate de suínos. In: CONGRESSO DA ASSOCIAÇÃO BRASILEIRA DE VETERINÁRIOS ESPECIALISTAS EM SUÍNOS (ABRAVES), **Anais**...2007.

LITTLE, C. L.; RICHARDSON, J. F.; OWEN, R. J.; DE PINNA, E.; THRELFALL, E. J. *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: Prevalence, characterization and antimicrobial resistance pattern, 2003-2005. **Food Microbiology**, v. 25, n. 3, p. 538–543, 2008.

LO FO WONG, D. M. A.; HALD, T.; VAN DER WOLF, P. J.; SWANENBURG, M. Epidemiology and control measures for *Salmonella* in pigs and pork. **Livestock Production Science**, v. 76, n. 3, p. 215–222, 2002.

MEAD, G.; LAMMERDING, A. M.; COX, N.; DOYLE, M. P.; HUMBERT, F.; KULIKOVSKIY, A.; PANIN, A.; DO NASCIMENTO, V. P.; WIERUP, M. Scientific and technical factors affecting the setting of *Salmonella* criteria for raw poultry: a global perspective. **Journal of Food Protection**, v. 73, n. 8, p. 1566–1590, 2010.

MUGHINI-GRAS, L.; ENSERINK, R.; FRIESEMA, I.; HECK, M.; VAN DUYNHOVEN, Y.; VAN PELT, W. Risk factors for human salmonellosis originating from pigs, cattle, broiler chickens and egg laying hens: a combined case-control and source attribution analysis. **PloS one**, v. 9, n. 2, p. e87933, 2 jan. 2014. Disponível em:

<http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0087933>. Acesso em: 23 out. 2015.

PARRY, C. M.; THRELFALL, E. J. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. **Current Opinion in Infectious Diseases**, v. 21, n. 5, p. 531–538, 2008.

PIRAS, F.; BROWN, D. J.; MELONI, D.; MUREDDU, A.; MAZZETTE, R. Investigation of *Salmonella enterica* in Sardinian slaughter pigs: prevalence, serotype and genotype characterization. **International Journal of Food Microbiology**, v. 151, n. 2, p. 201–9, 2011. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/21940060>.

SANCHEZ, J.; DOHOO, I. R.; CHRISTENSEN, J.; RAJIC, A. Factors Influencing the Prevalence of *Salmonella* Spp. in Swine Farms: A Meta-Analysis Approach. **Preventive Veterinary Medicine**, v. 81, n. 1-3, p. 148–177, 2007. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/17498826>.

SELANDER, R. K.; CAUGANT, D. A.; OCHMAN, H.; MUSSER, J. M.; GILMOUR, M. N.; WHITTAM, T. S. Methods of multilocus enzyme electrophoresis for bacterial population genetics and systematics. **Applied and Environmental Microbiology**, v. 51, n. 5, p. 873, 1986.

SILVA, M. C. da; FARIA, G. S.; PAULA, D. A. J. de; MARTINS, R. P.; JUNIOR, J. G. C.; KICH, J. D.; COLODEL, E. M.; NAKAZATO, L.; DUTRA, V. Prevalência de *Salmonella sp.* em suínos abatidos no Estado do Mato Grosso. **Ciência Rural**, v. 39, p. 266–268, 2009.

SØRENSEN, L. L.; WACHMANN, H.; ALBAN, L. Estimation of *Salmonella* prevalence on individual-level based upon pooled swab samples from swine carcasses. **Veterinary Microbiology**, v. 119, n. 2-4, p. 213, 2007.

STEINBACH, G.; HARTUNG, M. Attempt to estimate the share of human *Salmonella* infections, which are attributable to *Salmonella* originating from swine]. **Berliner und Münchener Tierärztliche Wochenschrift**, v. 112, n. 8, p. 296, 1999.

STRAHILEVITZ, J.; JACOBY, G. A.; HOOPER, D. C.; ROBICSEK, A. Plasmid-Mediated Quinolone Resistance: A Multifaceted Threat. **Clinical Microbiology Reviews**, v. 22, n. 4, p. 664–689, 2009. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/19822894>.

STRAW, B. E.; D´ALLAIRE, S.; MENGENLING, W. L.; TAYLOR, D. J. **Diseases of swine**. 10th. ed. [s.l.] Wiley-Blackwell, 2012.

THAI, T. H.; HIRAI, T.; LAN, N. T.; YAMAGUCHI, R. Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. **International Journal of Food Microbiology**, v. 156, n. 2, p. 147–151, 2012.

WEISS, L. H. N.; NONIG, R. B.; CARDOSO, M.; DA COSTA, M. Ocorrência de *Salmonella sp* em suínos de terminação no Rio Grande do Sul. **Pesquisa Veterinária Brasileira**, v. 22, n. 3, p. 104–108, 2002.

YANG, B.; QU, D.; ZHANG, X.; SHEN, J.; CUI, S.; SHI, Y.; XI, M.; SHENG, M.; ZHI, S.; MENG, J. Prevalence and Characterization of *Salmonella* Serovars in Retail Meats of Marketplace in Shaanxi, China. **International Journal of Food Microbiology**, v. 141, n. 1-2, p. 63–72, 2010. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/20493570>.

ZHENG, D. M.; BONDE, M.; SØRENSEN, J. T. Associations between the proportion of *Salmonella* seropositive slaughter pigs and the presence of herd level risk factors for introduction and transmission of *Salmonella* in 34 Danish organic, outdoor (non-organic) and indoor finishing-pig farms. **Livestock Science**, v. 106, n. 2, p. 189–199, 2007.

Capítulo 2

Artigo a ser submetido para a revista Journal of Food Protection, ISSN 0362-028X

***Salmonella* in pig's mesenteric lymph nodes**

Multilocus sequence typing and antimicrobial resistance characterization of *Salmonella* isolates from mesenteric lymph nodes of swine.

Possebon, F.S.[1*]; Yamatogi, R. S.[1]; Pinto, J.P.A.N.[1]

[1]Department of Veterinary Hygiene and Public Health, School of Veterinary Medicine and Animal Science, Univ Estadual Paulista – UNESP. Distrito de Rubião Júnior, s/n, Botucatu, SP, Brazil. Postal Code 18.618-970.

Keywords: *Salmonella*, pigs, MLST, MDR, epidemiology.

*Corresponding author: Fábio Sossai Possebon. Department of Veterinary Hygiene and Public Health, School of Veterinary Medicine and Animal Science, UNESP – Univ Estadual Paulista, Caixa Postal 572, 18618-970, Botucatu, São Paulo, Brazil. Telephone: +55-14-38802258, e-mail: fabio.cid@fmvz.unesp.br

Introduction

Pork stands as an important protein source for the population, being the most consumed meat in the world, with its production surpassing 100 million tons/year. Brazil stands in the fourth position in the global production and exportation ranking, and slaughtered in 2014 more than 32 million swine under federal inspection (*1*).

In this context, concern about food safety is necessary to ensure the supply of pathogen-free products thereby preventing the occurrence of foodborne pathogens. Among the main microorganisms associated with these diseases, we can highlight *Salmonella*, being the most incident and responsible for the highest number of deaths in the United States (*19*).

Hald et al. (*25*) highlights the consumption of pork as an important factor in food-associated pathogens. Among the red meats, pork is the most frequently associated with *Salmonella*, and is closely related to its production chain, from farm to fork, being detected in several steps of production and raw materials (*35*, *42*, *54*, *56*).

The isolation of *Salmonella* in mesenteric lymph nodes of pigs is used to evaluate the carrier status of the animal (*6*). The infection by these bacteria occurs primarily orally, with mesenteric lymph nodes acting as barriers, but later becoming pathogen reservoirs, which can survive intracellularly in macrophages and neutrophils until physiological changes triggered by stress, such as the release of catecholamines and changes in intestinal motility, propitiate its fecal release in few hours (*47*). This can lead to the contamination of the industrial environment and retail

market products if slaughterhouse steps fails to prevent cross-contamination (*37*, *41*).

Other important concern about salmonellosis transmitted by animal origin products is the occurrence of antibiotics resistant strains, exacerbated if the drugs that are concomitantly used in the treatment of animals and humans are considered (*49*)

Although promotes self-limiting infection in most cases, and do not require treatment with antibiotics, sometimes nontyphoidal *Salmonella* can cause more severe illnesses, especially when there is impairment of more systems than the gastrointestinal tract. In these situations, the use of drugs is necessary to safeguard the life of the affected individual (*4*, *40*). Several studies have reported the isolation of *Salmonella* strains that are resistant to the main groups of antimicrobials, used both in animal production and the treatment of humans (*14*, *23*, *49*, *53*). Ampicillin, sulfonamides, streptomycin and tetracycline are highlighted as ineffective drugs for many of these strains, and special attention is given to a high prevalence of multi drugs resistant (MDR), when four or more compounds are ineffective (*40*).

The determination of the contamination sources, as well as the distribution of the pathogen through livestock is of fundamental importance to understand its epidemiology and design effective control measures, however the great variability of serotypes isolated in swine production, associated with the large number of vectors and asymptomatic carriers, substantially hinder the determination of bacterial origin (*33*). For this reason, several alternatives have been developed, to help with a better discrimination of Salmonella serotypes and other microorganisms.

Among then, MLST is highlighted as an interesting tool to evaluate the genetic diversity and genealogy of the isolates, and provide substantial epidemiological and evolutionary data. This technique is based on sequencing seven housekeeping genes in the bacterial genome, to determine which allele of each particular gene is present in the sample, assigning a number to each one studied. Thus, each sample receives a sequence of seven numbers, which will determine its sequence type (ST). The alleles for each gene, and the cataloged STs are available online at http://mlst.warwick.ac.uk/mlst/dbs/Senterica and this database can be supplied by many research institutions, allowing the comparison of different isolates from different sources and geographical regions (*31*). *Salmonella* MLST data in Brazil is currently scarce, even more when related to swine isolates, and can provide relevant information about the epidemiology of the pathogen.

The aim of this study was evaluate the prevalence of *Salmonella* in pigs slaughtered under official inspection in São Paulo state – Brazil, and determinate the resistance of the isolates to the main antibiotics compounds of animal production and human therapeutics, besides the MLST characterization of the main serovars found.

Materials and Methods

- Samples collection

Mesenteric lymph nodes of 250 pigs were collected from 25 different batches of animals slaughtered in four plants with official inspection from São Paulo state, which were individually packaged in sterile plastic bags, and sent in cool boxes with ice to the laboratory. The animals were randomly selected and distributed throughout

the industrial batch processing, respecting an interval of at least three animals among the chosen. Each batch was raised in a different farm, and they were originated from 22 cities of 4 states in south and southeast of Brazil (figure 1). The sample size was estimated with the help of OpenEpi software (*48*), assuming a confidence interval of 95% and a 5% confidence limit. The population parameter was estimated based on the data from the Brazilian Institute of Geography and Statistics (IBGE) for livestock production, considering the number of animals slaughtered in the state of São Paulo in 2013 (1.7 million animals) (*30*) and an estimated prevalence for *Salmonella* of 20% in mesenteric lymph nodes (*8*, *44*).

- Samples processing

The surface of the lymph nodes was cleaned with alcohol 70° GL and individual samples were sliced in petri dishes with a scalpel, both sterile, in laminar flow hood. Material portions of 10g were obtained from each animal.

- *Salmonella* research

The isolation of *Salmonella* occurred in accordance with the methodology recommended by Andrews et al. (*3*). For each 10g samples of lymph node was added 90 mL of buffered peptone water 1% (BPW 1% - Oxoid). After homogenization in stomacher (400 circulator - Seaward - UK) the mix was incubated at 36ºC for 18 – 24 hours, for the pre-enrichment step. Posteriorly, 0.1 mL and 1.0 mL of the mix was transferred to 10 mL of the selective enrichment broths Rappaport-Vassiliadis (RV - Difco) and Tetrathionate (TT - Oxoid) respectively, being incubated at 42ºC for 18 – 24h. Both culture media were then striated at the surface of petri dishes with

Bismute Sulphite agar (BS - Oxoid) and Xylose Lysine Deoxycholate agar (XLD - Oxoid), which were incubated at 36ºC for 18-24h. Typical colonies were peaked in Triple Sugar Iron (TSI - Oxoid) and Lysine Iron Agar (LIA - Oxoid), being both incubated at 36ºC for 18-24h. At least, the polyvalent antiserum O and H (Probac) was used to confirm the characteristic samples in biochemical tests.

The isolates were sent on nutrient agar (Difco) to the Enteropathogens Laboratory of the Instituto Adolfo Lutz for confirmation and determination of serotype with agglutination of specific *Salmonella* antisera produced by the institution according to the standardized methodology. The serotype was defined by the characterization of its somatic and flagellar antigens according to the Kauffmann-White-LeMinor scheme (*24*).

- Antibiotics resistance determination

The antimicrobials were chosen based on the Clinical Laboratory Standards Institute (CLSI) guidelines (*17*), using the agar diffusion method as recommended by Kirby & Bauer (*7*). The strains were suspended in sterile saline (0.85% NaCl) to obtain turbidity compatible with the 0.5 McFarland scale level (approximately 1.5 x 10^8 CFU / mL). The bacterial suspension was seeded on the surface of Mueller-Hinton agar plates (Oxoid), with the aid of a sterile swab, and to these were added antibiogram discs of the compounds to be tested. The plates were incubated at 35 °C for 18h. After this period, the inhibition zones were read and the results were interpreted as resistant (R), intermediate resistant (R) or susceptible (S) according to the CLSI Manual, thus allowing to infer the effectiveness of antimicrobials. The

chosen compounds were ampicillin (10 µg), cefepime (30 µg), cefotaxime (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), ceftriaxone (30 µg), meropenem (10 µg), aztreonam (30 µg), amikacin (30 µg), gentamicin (10 µg), streptomycin (10 µg), nalidixic acid (30 µg), sulfonamides (300 µg), trimethoprim-sulfamethoxazole (01.25 / 23.75 µg), chloramphenicol (30 µg), and tetracycline (30 µg) (*17*). All resistant or intermediate resistant strains were considered non-susceptible to the referred antibiotic (*36*).

- MLST

MLST was performed using seven conserved housekeeping genes (aroC, dnaN, hemD, hisD, purE, sucA and thrA). The detailed protocol of the MLST procedure, including the housekeeping gene's amplification primers and the annealing temperatures, is available in the MLST database (http://mlst.warwick.ac.uk/mlst/dbs/Senterica. All amplifications were performed in a total volume of 50 µL as described by Souza et al. (*46*). A sample of the complete mix, without the DNA, was used as a negative control in all runs.

- Statistical analysis

The prevalence and 95% confidence limits (CL95%) of *Salmonella* were calculated considering the 250 animals and posteriorly the 25 batches of the study, being considered positive the batches with one or more positive results. The calculations were made with the PROC FREQ procedure of the Statistical Analysis Software – SAS 9.4 (*43*).

Results and Discussion

Salmonella was isolated in 91 samples, resulting in a prevalence of 36.4% (CL95% 30.4 – 43.4). Bessa et al. found 37.3% (CL95% 32.4 – 43.4%) of positives (*8*), and Silva et al. (*44*) reported a lower prevalence, of 19.4% in similar studies. This differences can be associated to several factors, but some can be highlighted, such as the kind of production cycle (when animals can be subjected to different handling stress due to the mixture of different batches and ages), the number of animals transported at the same time in the trucks and the waiting time in the slaughterhouse stalls (*9*, *44*). If we take into consideration batches when the pathogen was isolated in at least one sample, 72% (18/25, CL95% 50.6 – 87.9%) of then were positive.

For the 91 isolates, 67 were serotyped, revealing 9 different serovars (table 1). The main serovars were *S.* Typhimurium (n=13) and its monophasic variant *Salmonella enterica subsp. enterica* 1.4,5,12:i:- (n=12), followed by *S.* Infantis (n=12) and *S.* Havana (n=11).

S. Typhimurium was isolated in two batches from slaughterhouse 1, one batch from slaughterhouse 3, and 2 batches from slaughterhouse 4 (total of 5 batches from 3 slaughterhouses). Its isolation is constantly related to animal origin food, specially pork meat, being a main serovars involved in human outbreaks and standing as one of the most prevalent worldwide (*5*, *9*, *27*, *38*, *51*). Monophasic strains of this serovar, such as *S.* 1.4,5,12:i:- had gained notoriety in the last years,

being considered an important emergent pathogen (*21*). Its isolation had raised substantially and several outbreaks had been reported (*5*, *12*, *28*, *38*). In our research, *S.* 1.4,5,12:i:- was recovered from two batches in slaughterhouse 1 and three batches in slaughterhouse 3 (total of five batches in 2 slaughterhouses).

S. Infantis is also closely related to pork production (*16*, *20*). It was isolated in two batches from slaughterhouse 1 and two batches from slaughterhouse 4 (total of 4 batches in 2 slaughterhouses). Although this serovar is considered to have less virulence factors than Typhimurium (*52*), its association to human salmonellosis is frequent (*2*, *16*, *20*).

S. Havana is less associated with pork production chain, with few relates of its isolation in swine (*14*, *55*). In this research, all isolates were from only two batches, raised in the same city and processed in slaughterhouse 1. The possibility that these isolates represents a local circulating strain must be considered.

Among the analyzed antibiotics, streptomycin and tetracycline were the less effective, followed by ampicillin and sulfonamides and chloramphenicol (table 1). The isolation of resistant strains to this antibiotic classes are of concern for the scientific community and public health organs, as this drugs are widely used both in human and animal therapeutics (*29*). Several studies have reported the isolation of similar resistance patterns (*23*, *32*, *38*, *49*, *55*). The most effective drugs were aztreonam and cefoxitin (table 1). The selective pressure made by the intense use of antibiotics through the years can be associated with the resistance to the earlier developed drugs, in comparison to the most recent compounds (*55*).

Most of the isolates were MDR (64/91 – 70.3%). Two isolates were resistant to ten antimicrobials simultaneously, six strains were resistant to nine antimicrobials

and eleven were resistant to eight strains. This could be associated with the presence of plasmids and integrons, which can transmit clusters of several resistance genes simultaneously from strain to strain, and have been studied and reported in swine isolates with elevated MDR rates (*10*, *22*, *45*).

Eight strains were selected for the molecular typing with MLST (Table 2). The five chosen *S.* Typhimurium strains were classified as ST 19. This is the main ST for this serovar, corresponding up to 74% of the isolates, and its ST complex (isolates with similar STs) represented 93% of *S.* Typhimurium strains in MLST database (*34*). This ST was isolated in different countries from many sources, including diseased humans (*15*, *18*, *39*, *50*). The *S.* 1.4,5,12:i:- sample was also characterized as ST 19. The sharing of the same ST by both serovars is related to their genetic similarity (*11*), and reinforces the importance of the epidemiological surveillance for both classical and monophasic variants of *S.* Typhimurium.

The two *S.* Infantis samples analyzed belonged to ST 32. Its isolation is also reported in humans and animal sources (*2*, *15*, *16*). The same occurred for the *S.* Derby isolate evaluated (ST 40) (*13*, *26*). Both STs of these strains stands among the most frequently reported in literature, suggesting that they are the main MLST profiles for these serovars.

The high prevalence of *Salmonella* in swine mesenteric lymph nodes, with the presence of STs which are clearly related to human gastroenteritis, isolated from bathes of distinct origins and slaughtered in different plants, associated with the high levels of antibiotics resistance, is an alarming fact as regards public health, indicating that swine production chain fails to control this pathogen dissemination, being a potential source for the occurrence of salmonellosis. Efforts must be spent to mince

the chance of *Salmonella* transmission by pork products and prevent new infections and outbreaks.

Acknowledgments

The authors would like to thank CAPES for the financial support and Instituto Adolfo Lutz for the serotyping of the strains.

References

1. ABPA. 2015. Relatório Anual - Carne Suína. Associação Brasileira de Proteína Animal.
2. Almeida, F., A. Pitondo-Silva, M. A. Oliveira, and J. P. Falcão. 2013. Molecular epidemiology and virulence markers of *Salmonella* Infantis isolated over 25years in São Paulo State, Brazil. *Infection, Genetics and Evolution*. Elsevier B.V. 19:145–151.
3. Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana. 2014. *Salmonella* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md. Gaithersburg, Md.
4. Angulo, F. J., K. R. Johnson, R. V Tauxe, and M. L. Cohen. 2000. Origins and consequences of antimicrobial-resistant nontyphoidal *Salmonella*: implications for the use of fluoroquinolones in food animals. *Microbial drug resistance (Larchmont, NY)* 6:77.
5. Arnedo-Pena, A., S. Sabater-Vidal, S. Herrera-León, J. B. Bellido-Blasco, E. Silvestre-Silvestre, N. Meseguer-Ferrer, A. Yague-Muñoz, M. Gil-Fortuño, A. Romeu-García, and R. Moreno-Muñoz. 2016. An outbreak of monophasic and biphasic *Salmonella* Typhimurium, and *Salmonella* Derby associated with the consumption of dried pork sausage in Castellon (Spain). *Enfermedades infecciosas y microbiologia clinica*.
6. Bahnson, P. B., D. J. Damman, R. E. Isaacson, G. Y. Miller, R. M. Weigel, and H. F. Troutt. 2006. Prevalence and serovars of *Salmonella enterica* isolated from ileocolic lymph nodes of market pigs reared in selected Midwest US swine herds. *Journal of Swine Health and Production.* 14:182–188.

7. Bauer, A. W., W. M. Kirby, J. C. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology* 45:493–496.
8. Bessa, M. C., M. da Costa, and M. Cardoso. 2004. Prevalência de *Salmonella sp*. em suínos abatidos em frigoríficos do Rio Grande do Sul. *Pesquisa Veterinária Brasileira*. SciELO Brasil 24:80–84.
9. Bonardi, S., I. Alpigiani, I. Bruini, E. Barilli, F. Brindani, M. Morganti, P. Cavallini, L. Bolzoni, and S. Pongolini. 2016. Detection of *Salmonella enterica* in pigs at slaughter and comparison with human isolates in Italy. *International Journal of Food Microbiology* 218:44–50.
10. Boonkhot, P., P. Tadee, and P. Yamsakul. 2015. Class 1 integrons characterization and multilocus sequence typing of *Salmonella* spp . from swine production chains in Chiang Mai and Lamphun provinces , Thailand. *Japanese Journal of Veterinary Research* 63:83–94.
11. Bugarel, M., S. A. Granier, E. Bonin, M. L. Vignaud, S. Roussel, P. Fach, and A. Brisabois. 2012. Genetic diversity in monophasic (1,4,[5],12:i:- and 1,4,[5],12:-:1,2) and in non-motile (1,4,[5],12:-:-) variants of *Salmonella enterica* S. Typhimurium. *Food Research International* 45:1016–1024.
12. Burns, A. M., G. Duffy, D. Walsh, B. K. Tiwari, J. Grant, P. G. Lawlor, and G. E. Gardiner. 2016. Survival characteristics of monophasic *Salmonella* Typhimurium 4,[5],12:i:- strains derived from pig feed ingredients and compound feed. *Food Control* 64:105–114.
13. Cai, Y., J. Tao, Y. Jiao, X. Fei, L. Zhou, Y. Wang, H. Zheng, Z. Pan, and X. Jiao. 2016. Phenotypic characteristics and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail markets in Yangzhou, China. *International Journal of Food Microbiology*. Elsevier 222:56–64.
14. Castagna, S. M. F., M. C. Bessa, D. A. Carvalho, M. Cardoso, and M. Costa. 2001. Resistência a antimicrobianos de amostras de *Salmonella sp.* isoladas de suínos abatidos no estado do Rio Grande do Sul. *Arquivo da Faculdade de Veterinária da UFRGS, Porto Alegre* 29:44–49.
15. Chang, Y. C., J. Scaria, M. Ibraham, S. Doiphode, Y.-F. Chang, A. Sultan, and

H. O. Mohammed. 2015. Distribution and factors associated with *Salmonella enterica* genotypes in a diverse population of humans and animals in Qatar using multi-locus sequence typing (MLST). *Journal of infection and public health*.

16. Chironna, M., S. Tafuri, M. S. Gallone, A. Sallustio, D. Martinelli, R. Prato, and C. Germinario. 2014. Outbreak of *Salmonella* infantis gastroenteritis among people who had eaten at a hash house in southern Italy. *Public health* 128:438–43.
17. CLSI. 2013. Performance standards for antimicrobial susceptibility testing M100-S23. Clincal Laboratory and Standards Institute, Wayne PA.
18. Cordeiro, N. F., L. Yim, L. Betancor, D. Cejas, V. García-Fulgueiras, M. I. Mota, G. Varela, L. Anzalone, G. Algorta, G. Gutkind, J. A. Ayala, J. A. Chabalgoity, and R. Vignoli. 2013. Identification of the first blaCMY-2 gene in *Salmonella enterica* serovar Typhimurium isolates obtained from cases of paediatric diarrhoea illness detected in South America. *Journal of Global Antimicrobial Resistance* 1:143–148.
19. Crim, S. M., P. M. Griffin, R. Tauxe, E. P. Marder, D. Gilliss, A. B. Cronquist, M. Cartter, M. Tobin-D'Angelo, D. Blythe, and K. Smith. 2015. Preliminary Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food—Foodborne Diseases Active Surveillance Network, 10 US Sites, 2006–2014. *MMWR. Morbidity and mortality weekly report* 64:495–499.
20. Dionisi, A. M., C. Lucarelli, I. Benedetti, S. Owczarek, and I. Luzzi. 2011. Molecular characterisation of multidrug-resistant *Salmonella enterica* serotype Infantis from humans, animals and the environment in Italy. *International journal of antimicrobial agents* 38:384–9.
21. European Food Safety Authority. 2010. Scientific Opinion on monitoring and assessment of the public health risk of "*Salmonella* Typhimurium-like" strains. *EFSA* 8:1–48.
22. García-Fierro, R., I. Montero, M. Bances, M. Á. González-Hevia, and M. R. Rodicio. 2015. Antimicrobial Drug Resistance and Molecular Typing of *Salmonella enterica* Serovar Rissen from Different Sources. *Microbial Drug*

Resistance 00:150821134512001.

23. Gomez-Laguna, J., M. Hernandez, E. Creus, A. Echeita, J. Otal, S. Herrera-Leon, and R. J. Astorga. 2011. Prevalence and antimicrobial susceptibility of *Salmonella* infections in free-range pigs. *Veterinary Journal*, 2010/10/12 ed. 190:176–178.
24. Grimont, P., and F.-X. Weill. 2008. Antigenic formulae of the *Salmonella* servovars. *WHO Collaborating Centre for Reference and Research on Salmonella* 1–167.
25. Hald, T., D. Vose, H. C. Wegener, and T. Koupeev. 2004. A Bayesian approach to quantify the contribution of animal-food sources to human salmonellosis. *Risk Analysis*, 2004/03/19 ed. 24:255–269.
26. Hauser, E., F. Hebner, E. Tietze, R. Helmuth, E. Junker, R. Prager, A. Schroeter, W. Rabsch, A. Fruth, and B. Malorny. 2011. Diversity of *Salmonella enterica* serovar Derby isolated from pig, pork and humans in Germany. *International Journal of Food Microbiology*. Elsevier B.V. 151:141–149.
27. He, X., Xu, X. Li, K. Liu, B. Yue, T. 2016. Identification of *Salmonella enterica* Typhimurium and variants using a novel multiplex PCR assay. *Food Control* 65:152–159.
28. Hopkins, K. L., M. Kirchner, B. Guerra, S. A. Granier, C. Lucarelli, M. C. Porrero, A. Jakubczak, E. J. Threlfall, and D. J. Mevius. 2010. Multiresistant *Salmonella enterica* serovar 4,[5],12:i:- in Europe: a new pandemic strain? European Centre for Disease Prevention and Control (ECDC) - Health Comunication Unit.
29. Hsueh, P. R., L. J. Teng, S. P. Tseng, C. F. Chang, J. H. Wan, J. J. Yan, C. M. Lee, Y. C. Chuang, W. K. Huang, D. Yang, J. M. Shyr, K. W. Yu, L. S. Wang, J. J. Lu, W. C. Ko, J. J. Wu, F. Y. Chang, Y. C. Yang, Y. J. Lau, Y. C. Liu, C. Y. Liu, S. W. Ho, and K. T. Luh. 2004. Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and Choleraesuis from Pigs to Humans, Taiwan. *Emerging Infectious Diseases* 10:60–68.
30. IBGE. 2014. Relatório da produção de carne suína no estado de São Paulo. *Estatística da produção pecuária*.

31. Jolley, K. A., and M. C. J. Maiden. 2014. Using multilocus sequence typing to study bacterial variation: prospects in the genomic era. *Future microbiology* 9:623–30.

32. Kich, J. D., A. Coldebella, N. Morés, M. G. Nogueira, M. Cardoso, P. M. Fratamico, J. E. Call, P. Fedorka-Cray, and J. B. Luchansky. 2011. Prevalence, distribution, and molecular characterization of *Salmonella* recovered from swine finishing herds and a slaughter facility in Santa Catarina, Brazil. *International journal of food microbiology* 151:307–13.

33. Kich, J., N. Mores, I. A. Piffer, A. Coldebella, A. Amaral, L. Ramminger, and M. Cardoso. 2005. Fatores associados à soroprevalência de *Salmonella* em rebanhos comerciais de suínos. *Ciência Rural* 35:398–405.

34. Lan, R., P. R. Reeves, and S. Octavia. 2009. Population structure, origins and evolution of major *Salmonella enterica* clones. *Infection, Genetics and Evolution* 9:996–1005.

35. Little, C. L., J. F. Richardson, R. J. Owen, E. de Pinna, and E. J. Threlfall. 2008. *Campylobacter* and *Salmonella* in raw red meats in the United Kingdom: Prevalence, characterization and antimicrobial resistance pattern, 2003-2005. *Food Microbiology* 25:538–543.

36. Magiorakos, a. P., a. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, a. Vatopoulos, J. T. Weber, and D. L. Monnet. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 18:268–281.

37. Morrow, W. E., P. R. Davies, T. See, J. Eisemann, K. Zering, S. Kihlstrom, and K. Karli. 1999. Prevalence of *Salmonella* spp in the feces on farm and ceca at slaughter for a cohort of finishing pigs. *Production Epidemiology* 155–157.

38. Mueller-Doblies, D., K. Speed, and R. H. Davies. 2013. A retrospective analysis of *Salmonella* serovars isolated from pigs in Great Britain between 1994 and 2010. *Preventive veterinary medicine* 110:447–55.

39. Murgia, M., B. Bouchrif, M. Timinouni, A. Al-Qahtani, M. N. Al-Ahdal, P. Cappuccinelli, S. Rubino, and B. Paglietti. 2015. Antibiotic resistance determinants and genetic analysis of *Salmonella enterica* isolated from food in Morocco. *International journal of food microbiology* 215:31–9.

40. Parry, C. M., and E. J. Threlfall. 2008. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. *Current Opinion in Infectious Diseases* 21:531–538.

41. Piras, F., D. J. Brown, D. Meloni, A. Mureddu, and R. Mazzette. 2011. Investigation of *Salmonella enterica* in Sardinian slaughter pigs: prevalence, serotype and genotype characterization. *International Journal of Food Microbiology* 151:201–9.

42. Sanchez, J., I. R. Dohoo, J. Christensen, and A. Rajic. 2007. Factors influencing the prevalence of *Salmonella* spp. in swine farms: a meta-analysis approach. *Preventive Veterinary Medicine*, 2007/05/15 ed. 81:148–177.

43. SAS. 2011. SAS/STAT 9.3 User's Guide. *User's Guide. SAS Institute Inc., Cary, NC*.

44. Silva, M. C. da, G. S. Faria, D. A. J. de Paula, R. P. Martins, J. G. C. JUNIOR, J. D. Kich, E. M. Colodel, L. Nakazato, and V. Dutra. 2009. Prevalência de *Salmonella sp*. em suínos abatidos no Estado do Mato Grosso. *Ciência Rural*. SciELO Brasil 39:266–268.

45. Sinwat, N., S. Angkittitrakul, and R. Chuanchuen. 2015. Characterization of Antimicrobial Resistance in *Salmonella enterica* Isolated from Pork, Chicken Meat, and Humans in Northeastern Thailand. *Foodborne Pathogens and Disease* 12:759–765.

46. Souza, R. A., A. Pitondo-Silva, D. P. Falcão, J. P. Falcão. 2010. Evaluation of four molecular typing methodologies as tools for determining taxonomy relations and for identifying species among *Yersinia* isolates. *Journal of Microbiological Methods* 82:141–150.

47. Straw, B. E., S. D´Allaire, W. L. Mengenling, and D. J. Taylor. 2012. Diseases of Swine, 10th ed. Wiley-Blackwell.

48. Sullivan, K. M., A. Dean, and M. M. Soe. 2009. OpenEpi: a web-based

epidemiologic and statistical calculator for public health. *Public Health Reports*.

49. Thai, T. H., T. Hirai, N. T. Lan, and R. Yamaguchi. 2012. Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. *International Journal of Food Microbiology* 156:147–151.
50. Torpdahl, M., M. N. Skov, D. Sandvang, and D. L. Baggesen. 2005. Genotypic characterization of *Salmonella* by multilocus sequence typing, pulsed-field gel electrophoresis and amplified fragment length polymorphism. *Journal of Microbiological Methods* 63:173–184.
51. Van Boxstael, S., K. Dierick, X. Van Huffel, M. Uyttendaele, D. Berkvens, L. Herman, S. Bertrand, C. Wildemauwe, B. Catry, P. Butaye, and H. Imberechts. 2012. Comparison of antimicrobial resistance patterns and phage types of *Salmonella* Typhimurium isolated from pigs, pork and humans in Belgium between 2001 and 2006. *Food Research International* 45:913–918.
52. Volf, J., H. Havlickova, H. Hradecka, P. Ondrackova, J. Matiasovic, M. Faldyna, and I. Rychlik. 2010. Epidemiology and interaction of *Salmonella* enterica serovar Derby, Infantis and Typhimurium with porcine alveolar macrophages. *Veterinary microbiology* 146:105–10.
53. Weiss, L. H. N., R. B. Nonig, M. Cardoso, and M. da Costa. 2002. Ocorrência de *Salmonella sp* em suínos de terminação no Rio Grande do Sul. *Pesquisa Veterinária Brasileira* 22:104–108.
54. Yang, B., D. Qu, X. Zhang, J. Shen, S. Cui, Y. Shi, M. Xi, M. Sheng, S. Zhi, and J. Meng. 2010. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *International Journal of Food Microbiology*, 2010/05/25 ed. 141:63–72.
55. Yin, M., B. Yang, Y. Wu, L. Wang, H. Wu, T. Zhang, and G. Tuohetaribayi. 2016. Prevalence and characterization of *Salmonella enterica* serovar in retail meats in market place in Uighur, Xinjiang, China. *Food Control* 64:165–172.
56. Zheng, D. M., M. Bonde, and J. T. Sørensen. 2007. Associations between the proportion of *Salmonella* seropositive slaughter pigs and the presence of herd level risk factors for introduction and transmission of *Salmonella* in 34 Danish

organic, outdoor (non-organic) and indoor finishing-pig farms. *Livestock Science* 106:189–199.

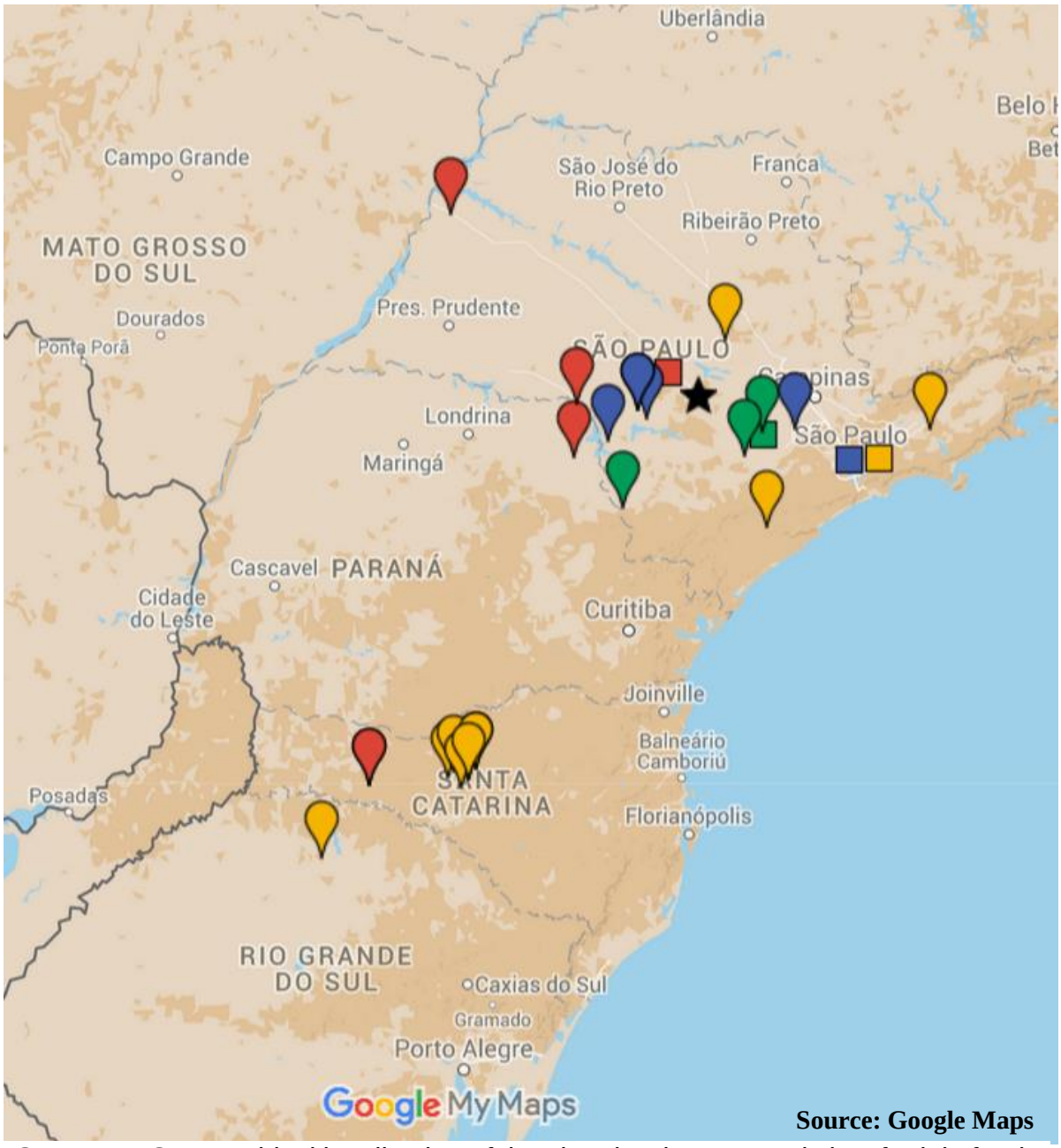

FIGURE 1 – Geographical localization of the slaughterhouses and city of origin for the swine batches evaluated for Salmonella presence in the mesenteric lymph nodes.

- Squares indicate the slaughterhouses localizations, balloons indicate the origin cities of the batches and the star indicates the laboratory location (Botucatu City, SP)

- Markers (squares and balloons) with similar colors indicates that the batch was processed in the respective slaughterhouse

Table 1 – Number of samples and antibiotic resistant strains for the serovars isolated in mesenteric lymph nodes of swine in São Paulo state - Brazil

Serovar	n of isolates	amp	cpm	ctx	cfo	caz	cro	mer	atm	ami	gen	est	nal	sul	sut	clo	tet	MDR*
S. Typhimurium	13	8	1	2	0	1	0	2	1	1	3	7	8	8	7	8	9	9
S.I.4,5,12:i:-	12	7	2	2	0	3	1	2	0	2	4	7	2	8	11	5	12	10
S. Infantis	12	7	0	1	0	1	3	4	0	4	2	6	2	4	3	6	6	5
S. Havana	11	3	1	1	1	2	1	2	0	1	2	7	0	3	0	3	0	4
S. Panamá	7	7	0	0	0	0	0	2	0	3	0	5	7	7	7	7	7	7
S. Derby	6	1	1	1	0	1	0	4	1	4	0	6	0	2	1	0	6	5
S. Bovismorbificans	4	4	0	1	0	0	1	2	0	0	0	4	0	4	2	3	4	4
S. Coeln	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S. Newport	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Not serotyped	24	20	2	2	2	1	1	1	1	3	8	19	18	21	7	19	18	20
Total	91	57	7	10	3	9	7	19	3	18	19	62	37	57	38	51	62	64

amp = ampicillin (10 µg), cpm = cefepime (30 µg), ctx = cefotaxime (30 µg), cfo = cefoxitin (30 µg), caz = ceftazidime (30 µg), cro = ceftriaxone (30 µg), mer = meropenem (10 µg), atm = aztreonam (30 µg), ami = amikacin (30 µg), gen = gentamicin (10 µg), est = streptomycin (10 µg), nal = nalidixic acid (30 µg), sul = sulfonamides (300 µg), sut = trimethoprim-sulfamethoxazole (01.25 / 23.75 µg), clo = chloramphenicol (30 µg), tet = tetracycline (30 µg)

*Multi Drug Resistant

Table 2 – City of origin, ST characterization and antibiotic resistance pattern of *Salmonella* strains isolated from swine mesenteric lymph nodes submitted to MLST analysis.

Serovar	Slaughterhouse	City/State	ST	Number of resistant antibiotic	Resistance Pattern
Typhimurium	1	Quatiguá/PR	19	8	amp clo ctx mer nal sul sut tet
Typhimurium	1	Ourinhos/SP	19	5	clo nal sul sut tet
Typhimurium	3	Boituva/SP	19	1	ctx
Typhimurium	4	Jambeiro/SP	19	6	amp clo est nal sul tet
S.1.4,5,12:i:-	1	Quatiguá/PR	19	10	amp caz clo cpm cro ctx est sul sut tet
Infantis	1	Xavantina/SC	32	4	amp clo sut tet
Infantis	4	Jambeiro/SP	32	5	ami caz est gen mer
Derby	2	Arantu/SP	40	4	atm est mer tet

amp = ampicillin (10 µg), cpm = cefepime (30 µg), ctx = cefotaxime (30 µg), caz = ceftazidime (30 µg), mer = meropenem (10 µg), atm = aztreonam (30 µg), ami = amikacin (30 µg), gen = gentamicin (10 µg), est = streptomycin (10 µg), nal = nalidixic acid (30 µg), sul = sulfonamides (300 µg), sut = trimethoprim-sulfamethoxazole (01.25 / 23.75 µg), clo = chloramphenicol (30 µg), tet = tetracycline (30 µg).

Journal of Food Protection® Instruction for Authors

Disponível em: https://www.foodprotection.org/upl/downloads/library/journal-of-food-protection-instructions-for-authors.pdf

SCOPE OF THE JOURNAL

The Journal of Food Protection® (JFP) is an international monthly scientific journal in the English language published by the International Association for Food Protection (IAFP). JFP is intended for publication of research and review articles on all aspects of food protection and safety. Major emphases of JFP are placed on studies dealing with (i) causes (primarily microbial, microbially-derived toxins, food-related toxicants, and allergens) and pre- and post-harvest control of all forms of foodborne illness as well as risk assessment; (ii) contaminants (e.g., feces, insects, rodents) and their control in raw foods and in foods during processing, distribution, preparation, and service to consumers; (iii) causes of food spoilage and its control through processing; (iv) food fermentations and food-related probiotics; and (v) microbiological food quality and methods to assay microbiological food quality.

Manuscripts of a sensitive nature. Bioterrorism and food security are of major concern to all involved in food production, processing, evaluation and distribution including members of IAFP. Manuscripts dealing with sensitive issues are expected to approach the subject from a preventative stance and not provide a how to guide. A review policy is used in the evaluation of manuscripts submitted for publication in journals printed by IAFP to minimize the possibility that their contents may be used to pose a food security threat.

Suitability of publication. Prospective authors with questions about the suitability of their research are invited to request an opinion from the Scientific Editors.

HOW TO SUBMIT MANUSCRIPTS

Submit manuscripts online at http://foodprotection.allentrack. net. Instructions for online submission and a sample manuscript for formatting purposes are available at that site. Corresponding authors will be required to complete an online Assignment of Copyright form after their manuscript has been reviewed and revised. All material dealing with affairs of the Association, book reviews, or news and events of interest to Members is published in Food Protection Trends (FPT).

Such material should be sent directly to Donna Bahun, FPT Production Editor at dbahun@foodprotection.org.

TYPES OF PAPERS

Research papers. Research papers report the results of original research which have not been published elsewhere. If the research has in part been previously reported, such as on a Web site, in a thesis or dissertation, or in another journal, this must be disclosed in the author's letter of submission. The journal will consider for publication research reports, which due to government regulations, have previously appeared on Web sites. A research paper usually consists of 10–12 double-spaced typewritten pages of text, the reference list, tables and figures. Research papers deal with its subject in some depth.

Research notes. A research note is a short paper that describes observations made in a limited area of investigation. Negative results are sometimes best reported in the form of a research note. However, the research note should not be used as a vehicle for reporting results of inferior research. A research note usually consists of nine or fewer double-spaced typewritten pages of text and appropriate figures and tables. The author must specify that a manuscript is submitted as a research note so it can be properly evaluated during the review process. Journal of Food Protection® Instruction for Authors

Review and mini-review papers. Review papers are scholarly summaries of the literature that synthesize the current state of knowledge. While review papers covering any aspect of food protection or safety can be submitted for consideration, those papers that critically evaluate emerging, neglected or 'hot' topics in which there have been important recent advances are particularly encouraged. The journal also publishes mini-reviews. These papers focus on a narrower aspect of food safety and are generally under 8,000 words in length (including text and references). All review papers should include a title page, abstract, introduction, main text with appropriate headings and subheadings (paragraph lead-ins), conclusions, acknowledgments (optional) and references. Use of summary tables and figures is also encouraged. Letter to the Editor. JFP invites

Letters to the Editor. Letters commenting on articles printed in this publication are subject to review from the Scientific Editors before acceptance. Letters to the Editor are limited to no more than 5 double-spaced pages. The author

of the article that is the focus of the letter is provided the opportunity to respond to the comments. This response is sent back to the author of the letter who is then given the option to continue with the publication process or to withdraw the Letter to the Editor. If withdrawn, neither the Letter to the Editor nor the author's response will be published. If not withdrawn, both the Letter to the Editor and the author's response will be published in their entirety. Please send all Letters to the Editor to the Administrative Editor at the address below.

PREPARATION OF MANUSCRIPTS

All parts of manuscripts must be typed fully double-spaced, at least 10-pt. type including references, tables, table captions, footnotes, and figure legends. Manuscripts must be in Word, WordPerfect or text formats. Page margins on all sides must be at least 1 in. (2.5 cm) wide. Lines on each page must be numbered to facilitate review of papers; but final revised manuscripts must NOT have line numbers. Number all pages, including tables and figures. JFP uses American conventions of spelling and punctuation. Manuscripts are divided into sections, which must be arranged in the following order: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, References, Figure legends, Tables, and Figures. Except for the introduction, all of these sections have separate headings, which should appear in the manuscript worded exactly as above. Subheadings take the form of paragraph lead-ins. Paragraph lead-ins should be boldface, indented, and run in with the text, separated by a period. Third-order subheadings will not be accepted. JFP follows many of the recommendations for manuscript preparation in the ASM Style Manual, 2nd ed., 1991, published by the American Society for Microbiology. Authors will find useful guidance concerning scientific nomenclature, abbreviations, numbers and measurement, English, references, tables, and figures, as well as a helpful bibliography. For further reference, see Scientific Style and Format: The CBE Manual, 6th ed., Cambridge University Press, 1994; and The Chicago Manual of Style, 15th ed., University of Chicago Press, 2003; and the bibliographies in these guides.

ORGANIZATION OF RESEARCH PAPERS AND RESEARCH NOTES

Title page. Type double-spaced on a separate page. At the top provide a running head indicating the topic of the paper. Then list the full title of the paper; the names of all authors; and name and address of the institution(s) or organization(s) where the work was done. Do not use trade names in titles. When authors are affiliated with more than one department or unit within an institution or with more than one institution, superscript numbers are used to indicate each author's address. Above the footnotes, supply three to five key words, indicating the principal topics of the paper, to be used for indexing. Footnotes are used to give the present addresses of authors who are no longer at the institution(s) where the work was done. A footnote asterisk (*) must be placed after the name of the author to whom correspondence about the paper and proofs are to be sent. The telephone, fax, and E-mail numbers of this author are placed in the footnote of the author for correspondence. No manuscript text appears on the title page. Statements regarding institutional practices are not allowed in any part of the manuscript. Statements disclaiming endorsement or approval of the views reflected in the manuscript should be in the Acknowledgment section.

Abstract. An abstract of no more than 2,000 characters, including spaces, must be placed on the second page of the paper to summarize the principal points of the study. The abstract does not contain references, figures, or tables. Abstracts are reprinted separately by abstracting services and therefore must be meaningful without reference to the body of the paper.

Introduction. The introductory section has no title and begins on the page following the abstract. It provides the reader with sufficient background information to evaluate the results of the research. An extensive review of the literature is not needed. The introduction also gives the rationale for and objectives of the study that is being reported.

Materials and Methods. Sufficient information must be provided so that another researcher can repeat the experiments that are described in the paper. If reference is made to a method published elsewhere in a journal or document that may not be readily available to most readers, then details of the method are to be included. If a published method is modified, such modification(s) must be described. Sources (company, city, state, or country) of unusual chemicals, bacterial strains,

reagents, and equipment must be identified. Delete registered and trademarks when given with trade names.

Availability of Materials. By publishing in the journal, the authors agree that, subject to requirements or limitations imposed by national or international laws or regulations, or institutional policies, any DNAs, viruses, microbial strains, mutant animal strains, cell lines, antibodies, and similar materials described in the article are available from a national collection or will be made available in a timely fashion, at reasonable cost, and in limited quantities to members of the scientific community for noncommercial purposes. The authors agree that they have the authority to comply with this policy either directly or by means of material transfer agreements through the owner.

Microarray Data. Where appropriate, complete microarray data must be deposited in a public database such as GEO, ArrayExpress, or CIBEX and must be accessible without restrict-ion from the date of publication. The accession number must be included in the paper before publication and be accompanied by the Web site address of the databank.

Results. The Results section provides information by means of text, tables, and figures. Results and Discussion may be combined, or there may be a separate Discussion section. If a Discussion section is to be included, place extensive interpretations of results in the Discussion section. Tables and figures must be numbered in the order in which they are mentioned in the text. All tables and figures must be cited in the text. Tables and figures reporting results should not be cited in the Materials and Methods section.

Discussion. Do not extensively repeat the introduction or Results sections. Provide an interpretation of the results in relation to known information. Conclusions should be included in this section.

Acknowledgments. Acknowledge financial and personal assistance (sources other than your institution) and any potential conflict of interest. Additionally, disclaimers of product endorsement or disclaimers of the views reflected by the manuscript are appropriate here.

References. Number and order the references alphabetically by the last names of the authors between and within each reference. Order references chronologically only when all authors' names are the same. Only the first author's name and initials are inverted. All references must be cited in the text by italicized

numbers in parentheses, with a space between the numbers of the references: (3, 7, 22). Journal names are italicized and abbreviated according to the style of BIOSIS. References may be made to papers that are in press, i.e., that have been accepted for publication. References for papers that have not been accepted for publication should be listed by the authors' names, as submitted for publication. Tables and figures follow the references (see preparation of figures section). Examples of different types of references are given below.

Paper in a journal

Farakos, S. M. S., D. Schaffner, and J. F. Frank. 2014. Predicting survival of Salmonella in low-water activity foods: An analysis of literature data. J. Food Prot. 77:1448–1461.

Paper or chapter in a book

Stopforth, J. D., J. N. Sofos, and F. F. Busta. 2005. Sorbic acid and sorbates, p. 49–90. In P. M. Davidson, J. N. Sofos and A. L. Branen (ed.), Antimicrobials in Foods, 3rd Ed. CRC Taylor and Francis, Boca Raton, FL.

Book by author(s)

Pitt, J. I., and A. D. Hocking. 1997. Fungi and food spoilage. Blackie Academic and Professional, London.

Book by editor(s)

Doyle, M. P., and R. L. Buchanan (ed.). 2012. Food microbiology: fundamentals and frontiers, 4th Ed. ASM Press, Washington, D.C.

Patent

Hussong, R. V., E. H. Marth, and D. G. Vakaleris. January 1964. Manufacture of cottage cheese. U.S. patent 3,117,870.

Publication with no identifiable author or editor

Anonymous. 2001. Real decree 3-484/2000 (12 January 2001) on the hygiene of ready-to-eat foods. BOE no. 11. Boletín Oficial de Estado, Madrid, Spain.

Electronic mail

E-mail messages should include the name of the person who sent the message, the date, the subject, the sender's E-mail address, and availability (if appropriate). Notaro, J. 13 June 1994. Banned in the USA [E-mail:jnotaro@ukans.edu]. Available from: the author at Smith@odo.msoe.edu. If the subject is not available, the message should be listed as a Personal Communication. Sofos, J. N. 3 January 2001. Personal communication [E-mail: john.sofos@colostate.edu].

Web pages

Include author, date, title, availability information, and accession date, if needed. Anonymous. 19 February 2000. Avis du Centre national de reference des Listeria de l'Institut Pasteur [press release]. Available at: http://www.agriculture.gouv.fr/actu/doss/com190200.htm. U.S. Food and Drug Administration. 1999. Guidance for industry: reducing microbial food safety hazards for sprouted seeds. Docket no. 99D-4488. Available at: http://vm.cfsan.gov/~dms/sprougd1. html. Accessed 17 July 1999. Wang, S. L., and G. C. L. Chu. 2001. Evaluation of modified atmosphere packaging systems for retaining freshness of Ontario's fruit and vegetables. Available at: http://gov. on.ca/OMAFREA.../archives/researchfund/ofpdocs/fp4041.html. Accessed 9 November 2001.

Full-text articles obtained from an online source

For journals without volume and page information, a document number may be used:

Harrison, C. L., P. Q. Schmidt, and J. D. Jones. 2 January 1992. Aspirin compared with acetoaminophen for relief of headache. Online J. Therap. [serial online]. Doc. no. 1.

For journals with volume and page information, include same information as print journals as well as availability information and accession date:

Friedman, S. A. January 1988. Preeclampsia: a review of the role of prostaglandins. Obstet. Gynecol. [serial online] 71:22–37. Available from: BRS Information Technologies, McLean VA. Accessed 15 December 1990.

ORGANIZATION OF REVIEW, MINI-REVIEW OR GENERAL INTEREST PAPERS

Review, mini-review or general interest papers must have a title page and an abstract as described in the section on research papers. The remainder of the text begins with an introductory statement and then is divided into appropriate sections with headings and subheadings. An acknowledgment section may come at the end of the text, followed by the references, as described for a research paper. Authors are encouraged to cite appropriate recent review papers in lieu of discussing numerous older papers.

PREPARATION OF TABLES

If submitting tables, the format must be XLS or DOC. Each table, comprising the title, body, and footnotes, must be typed double-spaced on a page separate from the text, following the Figure Legend or References. Number tables consecutively as cited in the text. The title is brief but fully descriptive of the information in the table. Headings and subheadings must be concise; abbreviations are used. Use no vertical rules and only three full horizontal rules: under the title, under the box heads, and at the bottom of the table. Use italic superscript letters for footnotes. Like data in columns reads down, not across. A well-organized table should be understandable without extensive reference to the text.

PREPARATION OF FIGURES

Type figure legends double-spaced in a list on a page separate from the figures. The figure legend should be placed within the manuscript file following the References. Number each consecutively as cited in the text. All illustrations, both line drawings and halftones (e.g., photographs), must be submitted in electronic format, preferably in separate files. Figures should not be less than 85 mm wide and should not be framed with a box. Figures containing multiple components (e.g., 1A, 1B, 1C, etc.) should be mounted together on the same page with appropriate labels. Place the figure number on the upper-right corner of the page. Data presented in figures must not be repeated in tables.

Photographs can be printed in color, but there is an additional cost to the author. Color quotes will be provided to author after acceptance of the manuscript.

Embed fonts when using Photoshop, CorelDraw, Illustrator and other graphics programs. If you do not embed your fonts, and we do not have them in our library, your figure will not convert to PDF. The preferred formats for electronic figures are TIF, EPS, JPG or PDF. The following native application file formats are also acceptable for final figures: Adobe Photoshop, Adobe Acrobat, Illustrator, Macromedia FreeHand, Corel Draw, Canvas, PowerPoint, Word and Excel. If you have other software, you should scan your figures and submit as TIF files. The resolution required for halftone and color images is 300 dots per inch (dpi); line is usually good at 300 dpi, but if there are fine lines and screens, figures should be scanned at 600 dpi. Please note that images that are in JPG or GIF format are normally 72 dpi and not acceptable for printing. Digital color files must be submitted in CMYK mode.

SUPPLEMENTAL MATERIALS

Supplemental material may be provided with a submitted manuscript at the discretion of the author(s). The Journal does not use supplemental material in making a peer-review decision, but rather this material is provided as a service to readers if they wish to access raw data for alternative analyses. Access to such supplemental material will be the responsibility of the author(s), who must provide a web link in the manuscript and maintain this web link for ready access by interested parties.

COMMON ABBREVIATIONS Frequently used acceptable abbreviations are given below. For further details on abbreviations, see the current edition of the ASM Style Manual. Note that a period is used with some but not all abbreviations. Abbreviations of non-SI units (e.g., atm) must be followed by the corresponding converted quantity and SI unit in parentheses: 1 atm = 101.29 kPa. (Exception: lb/in2 .)

ångström, Å atmosphere, atm base pairs, bp British thermal unit, BTU calorie, cal centimeter, cm CFU (never spelled out: colony-forming units) cubic centimeter, cm3 day (no abbreviation) degree Celsius, °C degree Fahrenheit, °F diameter, diam enzyme-linked immunosorbent assay, ELISA equivalent weight, equiv wt fluid ounce, fl oz foot (feet), ft gallon, gal gram, g gravity, g hour(s), h inch, in. international unit, IU intramuscular, i.m. intraperitoneal, i.p. intravenous, i.v. kilocalorie, kcal kilogram, kg lux, lx meter, m microequivalent, μeq microgram, μg microliter, μl

micrometer, µm micromole, µmol milliequivalent, meq milligram, mg milliliter, ml millimeter, mm millimolar, mM minute(s), min molar, M mole, mol most probable number, MPN nanometer, nm normal, N number, no. parts per billion, ppb parts per million, ppm percent, % PCR (never spelled out: polymerase chain reaction) pound, lb pounds per square inch, lb in2 revolutions per minute, rpm second, s species (singular), sp. species (plural), spp. specific activity, sp. act UV (never spelled out: ultraviolet) volume, vol weight, wt

POLICY ON COMMERCIALISM

Manuscripts submitted for consideration for publication in JFP are not to be used as a platform for commercialism or the promotion of branded products or services. References to branded products or services, except as may be warranted by scientific merit and research data or as are necessary for the understanding, evaluation and replication of the work described are to be avoided. In general, the trade name of a product should be used only once in a manuscript and that is in the "Materials and Methods" section. In addition, evaluation of scientific merit is not possible with strict proprietary secrecy. Authors must reveal the basis for the activity or mechanism of a proprietary product so that reviewers may gauge its plausibility. The excessive use of brand names, product names, logos or trade names, failure to substantiate performance claims, and the failure to objectively discuss alternative methods, processes, products and equipment may be considered indicators of commercialism. Disclosure and acknowledgment of both funding sources and any conflicts of interest by the authors is encouraged. Restricting commercialism benefits the authors and the audience of JFP. The Scientific Editor shall in his or her sole discretion, determine whether a submitted manuscript violates this policy on commercialism.

REVIEW PROCEDURE

Authors of manuscripts submitted for consideration to be published in JFP are notified by E-mail after the manuscripts have passed an initial quality check. Authors can monitor the status of their papers by logging on to http://foodprotection.allentrack.net. Authors are responsible for their login ID and password throughout the review process. The manuscript number assigned must be included in all future correspondence and on the revised manuscript for

identification. Manuscripts are accepted for publication only after they have been reviewed by two or more members of the Editorial Board or by others with the requisite expertise. After review, the manuscript is returned to the author for revision in accord with suggestions made by the reviewers and the Editor. Authors can hasten publication of their papers by submitting well-written manuscripts conforming to JFP style and by revising and returning manuscripts promptly. If, after review of a manuscript is completed, the author chooses to withdraw rather than to revise the paper, the Scientific Editor must be notified promptly. If the author does not respond within two months after a reviewed paper is returned, the paper will be considered withdrawn. Authors are notified by E-mail when a manuscript has or has not been accepted for publication. Page proofs of accepted manuscripts are sent to the author for correction within 4-6 weeks after acceptance. They should be proofread carefully according to the instructions attached and returned within four days. Authors will be charged for major revisions to their manuscripts.

Membership in the Association is not a prerequisite for accept-ance of a manuscript for publication. Non-member scientists are invited to submit papers for consideration for publication. The Scientific Editors assume that the corresponding author has received proper clearance from his or her organization and from co-authors for review and publication of the paper. It is also assumed that the paper is not being considered for publication in any other journal or publication.

Authors are responsible for the scientific accuracy of their papers. JFP assumes no responsibility for errors made, including those that may be made in the copy-editing process, or conclusions reached by authors. Papers accepted for publication become the copyrighted property of JFP and IAFP. No part of the publication maybe reproduced or transmitted in any form, or by any means, electronic or mechanical, including photocopy, recording, or any information storage and retrieval system, except in limited quantities for the non-commercial purposes of scientific or educational advancement, without permission in writing from the Administrative Editor. Full-text articles may not be released on other Web sites (including PubMed) until 12-months after publication. In the case of authors whose research was supported by NIH or another funding agency with an open access mandate, the authors may deposit the final accepted manuscript on PubMed Central or a similar site as required by the agency for public release 12-months after publication. Permission to post the published article on other Web sites (intranet and

extranet only) must be obtained through IAFP Copyright Clearance Center at copyright.com after the 12-month embargo period has expired.

PLAGIARISM POLICY

The Journal of Food Protection does not allow any form of plagiarism. Plagiarism is considered to be a serious breach of scientific ethics by the Journal. Incidents of plagiarism in a manuscript or published paper whether detected or reported, will be dealt with severely in accordance with the International Association for Food Protection Policy on Plagiarism (http://www.foodprotection.org/upl/downloads/library/policyon-plagiarism.pdf).

International Association of Food Protection is a member of CrossCheck, a service offered by CrossRef and powered by iThenticate software. iThenticate is a plagiarism screening service that verifies the originality of content submitted before publication. iThenticate checks submissions against millions of published research papers, and billions of web content. Authors, researchers and freelancers can also use iThenticate to screen their work before submission by visiting www.ithenticate.com.

MANUSCRIPT SERVICE FEES

A page charge of US$90 per printed page for Members of IAFP, or US$120 per printed page for nonmembers for publication of all research papers and notes, and US$45 per printed page of all submitted review, mini-review and general interest papers will be assessed. Review, minireview papers invited by one of the Scientific Editors are exempt from the manuscript service charge. An open access option of $2,500 for IAFP Members and $3,000 for non-members is available for authors who would like their article available with immediate, unrestricted open access upon publication.

Organizations and institutions commonly accept the manuscript service charge as a necessary cost of conducting research and communicating the results. An exemption from payment of the page charge will be made only under extenuating circumstances that must be described by the author(s) when it is first submitted.

Authors will be informed of the actual cost of the manuscript service fee upon receipt of proofs of the paper. Arrangements for payment of the manuscript service fee must be made at that time.

REPRINTS

Journal of Food Protection® uses an online system to provide authors with the option to purchase paper reprints.

Corresponding authors will receive an E-mail two weeks prior to publication containing a unique URL that will link to a web portal where the reprint order can be placed.

Corresponding authors will be provided with a complimentary PDF of the published article by E-mail one to two weeks prior to publication. This PDF may be forwarded to co-authors.

After publication, individual articles published in Journal of Food Protection® and its predecessor, the Journal of Milk, Food and Technology, from 1967 to the current issue are available online at http://www.ingentaconnect.com/content/iafp/jfp. Contact the Association's Order Processing Department to order earlier articles published in the Journal of Milk and Food Technology and its predecessor, the Journal of Milk Technology.

INDEXES

The Journal of Food Protection® is indexed in Agricola, Barbour Index, Biobase, Biological Abstracts, BIOSIS Preview/BIOSIS, Chemical Abstracts, Current Contents, Dairy Science Abstracts, EBSCO Discovery, EMCare, FSTA, Google Scholar, IC Journals Master List, Index Medicus, Microbiological Abstracts (CAS), Microsoft Academic Search, OCLC/World Cat Discovery Services, ProQuest, Pubmed/Medline, Scopus and Science Citation Index.

CORRESPONDING ADDRESS

Journal of Food Protection® Attn: Didi Loynachan, Administrative Editor 6200 Aurora Avenue, Suite 200W Des Moines, IA 50322-2864, USA Phone: +1 515.276.3344 Fax: +1 515.276.8655 E-mail: dloynachan@foodprotection.org