UNIVERSIDADE ESTADUAL PAULISTA
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FMVZ – UNESP – CAMPUS DE BOTUCATU - SP

RESISTÊNCIA ANTIMICROBIANA E PREVALÊNCIA DE SOROVARES DE *SALMONELLA* SPP. ISOLADOS DE FEZES E LINFONODOS DE SUINOS

JOÃO BOSCO PEREIRA GUERRA FILHO

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JOÃO BOSCO PEREIRA GUERRA FILHO

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

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Resumo

O objetivo do presente trabalho foi avaliar a prevalência de *Salmonella* spp. e seu perfil de resistência a antibióticos em suínos abatidos em frigoríficos sob inspeção federal localizados no interior do estado de São Paulo. Para tanto foram utilizados diferentes tipos de amostras, tais como fezes e linfonodos mediastínicos, mesentéricos e submandibulares, sendo 50 amostras de cada tipo, possibilitando avaliar a relevância do tipo de material analisado em relação ao real status do animal frente à contaminação pelo patógeno. Com base nas amostras positivas foi realizada a sorotipagem das cepas e teste de resistência aos antibióticos. A prevalência do patógeno foi de 10% no total das amostras (20/200), sendo os maiores percentuais de positividade encontrados nos linfonodos submandibulares com 20% de positivos (10/50) e mesentéricos com 18% (9/50) e os menores valores encontrados nas fezes com 2% de positivos (1/50) e linfonodos mediastínicos com nenhuma amostra positiva. Os sorovares predominantes foram *S*. Typhimurium com 55% das amostras (11/20), seguido de *S*. enterica subspécie enterica 4,5,12:i:- com 35% (7/20) e os sorovares *S*. Brandenburg e *S*. Derby com 5% (1/20) cada. Todas as amostras isoladas apresentarem resistência frente a pelo menos um dos antimicrobianos testados, sendo que 90% (18/20) apresentaram resistência a pelo menos 4 drogas simultaneamente e 15% (3/20) foram enquadradas como multi drogas resistentes. Os maiores índices de resistência foram encontrados para Ciprofloxacina e Tetraciclina com 90% de resistentes (18/20) cada, seguido de Ácido Nalidíxico com 80% (16/20), Sulfonamidas com 75% (15/20), Cloranfenicol e Estreptomicina com 70% (14/20) cada, Sulfametoxazole-Trimetroprim com 65% (13/20), Ampicilina com 25% (5/20), Cefotaxime com 10% (2/10) e Ceftriaxona e Gentamicina com 5% (1/20) cada. As amostras resistentes à Ciprofloxacina foram testadas para a presença da enzima ESBL, sendo 100% delas consideradas negativas.

Palavras-chave: *Salmonella* spp., linfonodos, fezes, alimentos, suínos, sorotipagem, resistência a antibióticos, ESBL.

Abstract

The objective of this study was to evaluate the prevalence of *Salmonella* spp. and their antibiotic resistance profiles in swine slaughtered in abattoirs under federal inspection located in the state of São Paulo. For both types of samples, such as feces and mediastinal, mesenteric and submandibular lymph nodes, 50 samples of each type being possible to evaluate the relevance of the type of material analyzed in relation to the actual status of the animal against the contamination by the pathogen were used. Based on positive samples, serotyping of the strains and antibiotic resistance test was performed. The prevalence of the pathogen was 10% of total samples (20/200) wich the highest positivity found in the submandibular lymph nodes with 20% positive (10/50) and mesenteric with 18% (9/50) and lower found in the feces with 2% positive (1/50) and mediastinal lymph nodes with no positive sample. The predominant serotypes were S.Typhimurium with 55% of the samples (11/20) followed by S. enterica subspecies enterica 4,5,12: i: -, 35% (7/20) and the serotypes S. Brandenburg and S. derby 5% (1/20) each. All isolates were resistant at least one of the antimicrobials tested, among them 90% (18/20) showed resistance to at least four drugs simultaneously and 15% (3/20) were classified as multi drug resistant. The highest rates were found for Ciprofloxacin and Tetracycline resistance with 90% (18/20) each, followed by Nalidixic Acid with 80% (16/20), Sulfonamides 75% (15/20), Chloramphenicol and Streptomycin with 70% (14/20) each, Trimethoprim-Sulfamethoxazole 65% (13/20), Ampicillin 25% (5/20), Cefotaxime 10% (2/10) and Ceftriaxone and Gentaminicine with 5% (1/20) each. Resistant to Ciprofloxacin samples were tested for the presence of ESBL enzyme, 100% of them considered negative.

Keywords: *Salmonella*, lymph nodes, feces, food, pork, serotyping, antibiotic resistance, ESBL.

Capítulo 1

1 Introdução

As enfermidades transmitidas por alimentos sempre ofereceram grandes riscos a toda população mundial, sendo que apesar de todo o incremento tecnológico destinado à produção de alimentos de origem animal visar à prevenção e erradicação dessas doenças, o aumento do volume de produção e da comercialização destes produtos em escala global tem contribuído para a ocorrência delas e surgimento de situações de risco à saúde pública (CARRASCO et al. 2012).

Neste cenário, a salmonelose tem relevante destaque, sendo a enfermidade de origem alimentar de maior frequência no mundo, sendo caracterizada por gastroenterite causada pela ingestão de alimentos contaminados por *Salmonella* spp. Nas últimas décadas o sorovar *S*. Enteritidis tem sido um dos principais associados a este quadro, porém *S*. Typhimuirum também possui um papel de destaque, gerando grande preocupação dos pesquisadores (CDC, 2006; BOLLAERTS et al., 2008).

Entre diversos fatores associados à presença deste patógeno nos alimentos, destaca-se a contaminação inicial de produtos de origem animal desde as etapas primárias de produção até a comercialização do produto final, incluindo, durante todos esses processos, o risco de contaminação cruzada a partir de outros alimentos, fator que deveria ser controlado durante os processos industriais.

Além da grande ocorrência desta doença no homem a partir de produtos de origem animal, observa-se o surgimento de resistência aos antimicrobianos utilizados para o combate ao patógeno, sendo que vários princípios ativos comumente usados em tratamentos veterinários são comuns aos utilizados em tratamentos de pessoas (GOMEZ-LAGUNA et al., 2011). Nas fazendas de produção também é considerável o grande uso de antimicrobianos como forma de tratamento e estratégias de manejo tais como uso de probióticos visando otimizar a nutrição dos animais (GOMES - NEVES et al. 2012).

Neste contexto, a carne suína apresenta notável importância na perpetuação destes riscos, uma vez que as etapas de produção primária e

transporte destes animais oferecem potencial risco para a contaminação do animal e durante o processamento industrial também se observam etapas onde pode haver a contaminação do alimento.

Ressalta-se o risco de transmissão por este tipo de alimento devido a falhas no preparo, resultando em produtos mal cozidos, fator comumente observado neste tipo de produto. Registre-se também que existem diversos produtos e derivados a partir de carne suína que não sofrem tratamento térmico durante sua produção e nem para o consumo como salames e mortadelas.

Com base no exposto, o presente estudo tem como finalidade investigar a prevalência deste patógeno em linfonodos de suínos, importante indicativo de contaminação de carcaças e consequentemente dos produtos finais, e também analisar os sorovares associados a esta contaminação e sua susceptibilidade frente aos principais princípios ativos de antimicrobianos utilizados neste sistema.

2 Revisão de Literatura

2.1 Classificação do gênero *Salmonella*

Os micro-organismos do gênero *Salmonella* pertencem à família Enterobacteriaceae, sendo divididas em 3 espécies, *Salmonella subterranea*, S. *bongori* e S. *enterica*, sendo esta última a de maior interesse em medicina veterinária. Possuem atualmente 2610 sorotipos descritos, seguindo o esquema proposto por Kauffman e White, sendo os mesmos determinados pelos antígenos somáticos (O), flagelares (H) e capsulares (LPSN, 2014).

São pequenos bacilos Gram-negativos, sem capacidade de formação de esporos, anaeróbios facultativos e em sua grande maioria móveis, possuindo flagelos peritríquios. São capazes de utilizar citrato como única fonte de carbono, produzem gás a partir da glicose e a grande maioria não fermenta lactose ou sacarose (CLIVER, 1990; FRANCO & LANDGRAF, 2005).

Em termos gerais, pode-se afirmar que *Salmonella* é um micro-organismo amplamente difundido na natureza, tendo como o principal reservatório o trato intestinal de aves, anfíbios, répteis e mamíferos incluindo o homem (BARROS et al., 2002).

2.2 Salmonelose

As enfermidades transmitidas por alimentos (ETA) são definidas como qualquer doença causada pela ingestão de água ou alimentos contaminados, sendo hoje conhecidos mais de 250 tipos, incluindo quadros causados por toxinas naturais dos alimentos como, por exemplo, cogumelos venenosos e toxinas de algas e peixes, até contaminação do alimento por produtos químicos como metais pesados, porem na sua grande maioria são causadas pela contaminação do alimento por vírus e bactérias (BRASIL, 2014).

Neste cenário, a salmonelose representa uma importante ETA, estando presente em países em desenvolvimento e também nos desenvolvidos, representando um sério risco à saúde pública em todo o mundo (European Food Safety Authority (EFSA, 2012).

Em 2008, na União Europeia, foram registrados 131.468 casos humanos confirmados devido a esta bactéria, sendo a segunda maior causa de ETA, ficando atrás somente da campilobcteriose. Embora tenha diminuído o índice de casos em países deste continente, em diversos outros do mundo a tendência tem aumentado nos últimos anos, o que gera preocupação e necessidade de práticas para controle, tratamento e erradicação dessa enfermidade (*CARRASCO* et al., 2012).

Os sorovares *S.* Enteritidis e *S.* Typhimurium são reportados como aqueles de maior adaptação ao ser humano, sendo que na União Européia e EUA são os organismos dois isolados com maior frequência (Centers for Desease Control) (CDC, 2006).

Nos Estados Unidos são estimados anualmente em torno de 1,4 milhões de casos humanos de salmonelose, sendo que destes, 95% têm origem alimentar, culminando em mais de 500 mortes (MEAD et al. 1999, BARBER et al., 2002).

Na Austrália a ocorrência dos sorovares é variável, sendo que em 2008 *S*. Typhimuirum foi o mais comumente relatado, e S. Enteritidis foi atribuído como o maior causador da doença no homem, mesmo não sendo uma doença endêmica naquele pais (YATES, 2011).

No Brasil, de 2000 a 2013, foram notificados 8871 surtos de ETA, dos quais *Salmonella* foi o agente causador em 1522 deles, sendo que do total de surtos notificados, 4,26% estiveram associados à carne suína e seus derivados (BRASIL, 2014).

No contexto geral, *S*. Enteritidis está mais associada ao consumo de produtos de aves, como carnes e ovos e *S*. Typhimurium ligado a uma série de animais de produção, como suínos e bovinos (COGAN & HUMPHREY, 2003).

Assim, considera-se que a doença de origem alimentar mais ocorrente no mundo seja a salmonelose (D'AOUST et al, 2001; BOLLAERTS et al., 2008), sendo que de 10 a 15% das ocorrências de gastroenterites agudas são causadas por esta enfermidade (JAY, 2005).

Os casos de gastroenterites causadas por *Salmonella* spp. podem levar de distúrbios intestinais leves, dores abdominais, calafrios, vômitos, desidratação e dores de cabeça a sintomas mais graves como disenteria, mas na maioria dos casos não há necessidade de hospitalização, o que leva ao não isolamento do agente patogênico causador, gerando uma ocorrência subestimada desse patógeno na população humana (SANTOS et al., 2002).

O aumento da distribuição global de alimentos e do movimento de pessoas facilita a propagação deste agente, possibilitando assim a introdução de novos sorotipos de *Salmonella* em países importadores de animais e alimentos (CARRASCO et al. 2012).

As salmonelas podem resistir meses no ambiente, mas são susceptíveis à luz solar e aos desinfetantes mais comuns como fenóis, clorados e iodados (OLIVEIRA et al., 2002). A contaminação pode ocorrer através de material fecal durante o abate, afetando os produtos de origem animal. O micro-organismo pode se multiplicar facilmente e alcançar a dose infectante para diferentes condições de sistema imune do hospedeiro. Usualmente considera-se que a enfermidade ocorra após a ingestão de 10^6 UFC de salmonella/g de alimento, mas há relatados de casos com doses infectantes a partir de 10-100 células (COGAN, 2002).

As células do patógeno têm seu ótimo crescimento entre 35 e 43°C, sendo o máximo de 49°C, podendo este ser evitado pela refrigeração do alimento abaixo de 5°C ou pela manutenção dos mesmos acima de 63°C, embora temperaturas acima de 55°C já sejam consideradas seguras (ICMSF, 1996).

Outros fatores intrínsecos do alimento interferem diretamente no crescimento das salmonelas, como o valor de atividade de água (a_w), sendo seu mínimo de 0,94 e ótimo de 0,99, mas podendo sobreviver até mais de um ano em alimentos com valores baixos, tais como o chocolate por exemplo. O pH ótimo para o crescimento do patógeno é de 7 a 7,5 mas o espectro aceitável para isso é amplo, variando de 3,8 a 9,5 (ICMSF, 1996).

A incorreta execução dos procedimentos de higienização e desinfecção de equipamentos está entre as principais causas de contaminação de produtos alimentares dentro do setor industrial (JESSEN & LAMMERT, 2003).

De acordo com a literatura, o ambiente doméstico também tem importante atuação na propagação das ETA. Nos países da Europa, Austrália, Nova Zelândia, EUA e Canadá, até 87% dos surtos de origem alimentar têm relação com o preparo doméstico (ASSELT et al., 2008). Foi constatado que o cenário doméstico de manipulação de alimentos está intimamente relacionado a mais de 50% dos surtos de origem alimentar na Holanda, Alemanha e Espanha (BEUMER, BLOOMFIELD, EXNER, FARA & SCOTT, 1998; SCOTT, 1996). No Brasil, dos 4.577 surtos notificados no período de 1999 a 2008, 45,2% aconteceram em ambiente doméstico (BRASIL, 2008).

Salmonella spp. tem sido o patógeno mais implicado em surtos de enfermidades transmitidas por alimentos dentre os micro-organismos patogênicos introduzidos nas residências por pessoas, animais de estimação, insetos, águas de abastecimento e ar (REDMOND & GRIFFIN 2003; BARKER et al., 2003).

Embora seja expressiva, a participação do ambiente doméstico na ocorrência de surtos de enfermidades transmitidas por alimentos é subestimada pelas autoridades de saúde pública devido à ocorrência, em geral, de sintomas brandos que não levam à necessidade de apoio médico além de acometerem poucos indivíduos e de maneira não coletiva (WELKER et al., 2010).

2.3 Salmonelose vinculada à carne suína

A carne suína é um importante alimento para o consumo humano, oferecendo uma ótima quantidade de nutrientes, entre eles aminoácidos essenciais, vitaminas do complexo B, minerais e proteínas de alto valor nutritivo (KAUFFMAN, 2001). Atualmente é a carne produzida em maior volume no mundo, chegando a uma quantidade anual superior a 100 milhões de toneladas em 2013, sendo o país de maior produção a China, ultrapassando 50 milhões de toneladas (ABIPECS, 2013). O Brasil ocupa uma expressiva

posição entre os países produtores de carne suína, chegando a marca de mais de 3 milhões de toneladas em 2013, considerando somente o abate de animais em estabelecimentos sob inspeção federal, o que o coloca na 4ª colocação mundial entre os produtores e em 1° entre os exportadores (ABIPECS, 2014).

Em todo o mundo o consumo per capta de carne suína atingiu valores superiores a 60 kg em alguns países, sendo que no Brasil este índice supera os 14 kg (ABIPECS, 2013), o que ressalta a importância de sua inocuidade quando de sua colocação junto ao mercado consumidor.

A ocorrência de salmonelose pode estar associada ao consumo deste alimento, sendo que há o potencial risco de contaminação do animal pela bactéria em diversas etapas da cadeia produtiva, desde sua produção através de alimentos e água contaminados oferecidos aos animais (NAYAK et al., 2003). A aglomeração gerada durante o transporte e baias de descanso no frigorifico também são consideradas fonte importantes de infecção por *Salmonella* antes do abate (HURD et.al., 2001; SWANENBURG et al., 2001).

A ocorrência da bactéria é grande mesmo em rebanhos animais de países de primeiro mundo como os constituintes de União Européia, onde foi observada a prevalência em 28,2% dos animais de fazendas de reprodução e 33,% em fazendas da produção suína (EFSA, 2010), sendo que em Portugal esses valores chegam a 45,5% e 43,3% nas respectivas fazendas(EFSA, 2010, GOMES - NEVES et al. 2012).

Em dados sobre a ocorrência do patógeno na Europa, publicados entre 1990 e 2005, a prevalência da bactéria ocorreu em média em 59% das propriedades e 17% dos animais (SANCHEZ et al., 2007).

Em Portugal, a prevalência em suínos pesquisados em frigoríficos foi de 17,6% a partir de amostragens escalonadas de linfonodos mesentéricos, superfícies de carcaças, carne e *swabs*, sendo os principais sorovares encontrados *S*. Thyphimurium (53,3%), *S*. Derby (18,3%), *S*. Rissen (6,6%), *S*. Mbandaka (5%), *S*. London (5%), *S*. Give(3,3%), *S*. Enteritidis (1,6%) e S. Sandiego (1,6%)(GOMES - NEVES et al., 2012).

No Vietnã, segundo maior produtor de suínos da Ásia (ABIPECS 2014), a prevalência foi de 49,8% para amostras de *swabs* e 34,8% para amostras de linfonodos, em um total de 178 suínos analisados, sendo *S*. Derby (50.0%) e *S*. Typhimurium (27,4%) os sorovares mais frequentes (ELLERBROEK et al., 2010).

Diversos casos de salmonelose são relatados devido ao consumo de carne suína na Dinamarca e Alemanha, sendo que a estimativa de valores varia de 15 a 20% dos casos humanos relatados (BORCH et al., 1996; BERENDS et al., 1998)

Na Holanda, o número de casos de salmonelose ultrapassa 35.000 por ano (Haavelar et al. 2011), sendo que deste total, de 15 a 20% estão associados ao consumo de carne e produtos à base de suínos (VAN PELT et al., 2008).

No Brasil também são diversos os surtos e relatos de pesquisadores em relação à ocorrência desta patógeno associado à carne suína. No estado do Mato Grosso foram detectados 16,6% de animais positivos quando foram realizadas pesquisas nos linfonodos mesentéricos e tonsilas, sendo os principais sorovares Derby (16%) e Typhimurium (14%), seguidos de London e Give (12% em ambos) (SANCHEZ et al., 2009).

Em trabalho semelhante realizado no Rio Grande do Sul, a prevalência de animais positivos foi de 55,66% quando foram testados linfonodos mesentéricos, fezes e conteúdo intestinal, sendo que associados aos órgãos linfóides a prevalência foi de 17,6% e nas fezes de 18,6%, sendo os principais sorovares Typhimurium (24,3%), Agona (19,9%), Derby (13,2%) e Bredeney (12,0%) (BESSA, COSTA e CARDOSO, 2004).

Também no Brasil, no estado de Santa Catarina, observou-se a prevalência de 90% de animais positivos para amostras de *swabs* do assoalho da laringe e 67% para linfonodos mesentéricos, sendo os principais sorovaresTyphimurium (50,7%), Panama (28,5%), Derby (6,3%), Senftenberg (5,4%), Mbandaka (4,6%), Infantis (1,5%), Houtenae (0,7%), Montevideo (0,5%) e *Salmonella* sp. (1,9%) (KICH et al., 2011).

Devido à associação entre a infecção dos animais ser predominantemente por via oral, os linfonodos mesentéricos acabam atuando como uma barreira primária a este patógeno; porém, posteriormente, tais animais acabam se tornando reservatórios do patógeno o que propicia sua liberação ao ambiente (STRAW et al., 2012). Assim é comumente adotada a pratica de isolamento de *Salmonella* a partir de linfonodos para indicar seu status de portador (BAHNSON et al., 2006), visto que a análise do conteúdo intestinal pode revelar na verdade seu caráter excretor (DAVIES et al., 2008). Na literatura também se observa a utilização de linfonodos armazenados sob congelamento, onde a estocagem de até -70°C por 14 dias não interferiu na pesquisa do patógeno quando comparada a grupos controle (BAHNSON et al. 2006). Mesmo com a adoção de estratégias para a redução de contaminação nas granjas suínas, com a utilização do sistema “all-in-all-out”, por exemplo, é preciso a adoção de procedimentos adequados nas etapas seguintes como transporte ao frigorifico e baias de espera (LO FO WONG et al., 2003), e também durantes as etapas ligadas ao próprio fluxograma de abate, como a escalda de carcaças.

Quando o animal está contaminado pela bactéria em seu trato gastrointestinal há um risco potencial de contaminação cruzada para o restante da carcaça em diversas etapas do fluxograma do abate de suínos (CARRASCO, et al., 2012). As principais maneiras são através de contaminação cruzada por fezes e conteúdo de estomago e laringe, mas a contaminação ambiental através de superfícies de trabalho, utensílios e manipuladores também desempenha um importante papel no risco de contaminação da carcaça (CARRASCO et al., 2012). O índice de contaminação cruzada de carcaças suínas durante o processo de abate chega a 29% (BOTTELDOORN et al., 2003) e até 30% em estudos de vários autores (CARRASCO et al., 2012). Em estudos realizados na Holanda, o nível de contaminação da carcaça devido ao processo industrial atinge valores de até 69% (DUGGAN et al., 2010).

Durante a linha industrial do frigorifico de suínos, há relevante associação entre a prevalência de *Salmonella* nas carcaças destes animais e as etapas de pré-abate e pré-evisceração, tendo sido observado que a fase de

limpeza da carcaça através da escalda estava diretamente associada à presença da bactéria na carcaça ao final do abate (LETELLIER et al., 2009). Deve-se destacar que a escalda deve ser realizada com água a no mínimo 62°C, sendo este uma etapa crítica para a contaminação de outras carcaças pelo patógeno (CARRASCO et al., 2012).

A etapa de evisceração da carcaça também é de suma importância na contaminação cruzada por este patógeno, destacando-se as etapas de abertura do abdome e retirada do cólon onde podem ocorrer perfurações, que posteriormente irão expor a carcaça durante as etapas de manipulação, assim como as superfícies, equipamentos e manipuladores em contato com ela (DUGGAN et al., 2010).

Após o abate, as etapas seguintes também conferem importante atenção à qualidade microbiológica da carne suína, entre eles a preparação e armazenamento no varejo devido à atuação de fatores como manipulação, tempo e temperatura (LO FO WONG et al., 2003); por isso devem ser tomados cuidados específicos para que não se propicie a situação para proliferação bacteriana nessas etapas, principalmente quando há manipulação de grandes quantidades de carne crua e de diferentes espécies (CARRASCO et al., 2012).

Em resumo, devem ser tomados cuidados não somente em todas as etapas da cadeia produtiva, mas também durante a comercialização, bem como o preparo culinário, para que a carne suína e seus sub-produtos não venham a se contaminar por *Salmonella* e se torne veículo do patógeno ao homem.

2.4 Resistência a antimicrobianos

Dado o notável risco de transmissão do agente a seres humanos, principalmente por via alimentar, ressalta-se o perigo relacionado ao surgimento de bactérias, principalmente aquelas encontradas em animais, que possuem resistência aos antimicrobianos comumente utilizados na pecuária, mas que também podem ser responsáveis por infecções humanas, tornando a resistência a essas drogas um importante fator de risco à saúde pública.

Embora na maioria dos casos a infecção pelo patógeno possa ser auto-limitante, em alguns casos podem ocorrer manifestações mais severas, principalmente quando há ocorrência em pacientes imuno-comprometidos, o que pode levar à contaminação através da corrente sanguínea ou também quando se dissemina por tecidos adjacentes além do inicialmente infectado, geralmente o trato gastro-intestinal, levando a gastroenterites severas (EFSA, 2012). Nesses casos se faz necessário o uso de drogas para um tratamento efetivo e respaldo a integridade da vida do indivíduo (ANGULO et al., 2000).

O tratamento preconizado para salmonelose em humanos é baseado na administração de antibióticos do grupo das fluorquinolonas e quinolonas para adultos e cefalosporinas de terceira geração para crianças (LESSER & MILLER, 2005; EFSA, 2012). Em casos de pacientes com endocartidites ou infecção endovascular, preconiza-se tratamento a base de cloranfenicol (LESSER & MILLER, 2005). Diversos estudos, porém, têm apontado o isolamento de diversas cepas de *Salmonella* resistentes aos principais fármacos adotados nessas praticas terapêuticas, tanto de caráter veterinário quanto humano (EFSA, 2012).

Tem sido relatada a ocorrência de resistência aos antimicrobianos em amostras obtidas de suínos em 16 países pertencentes à União Européia, e em outros 14 países quando investigados amostras de carne suína (EFSA, 2012).

Em pesquisa realizada em suínos criados no sistema “free-range” na Espanha, a partir das cepas de *Salmonella* isoladas, observou-se o perfil de resistência, sendo a maior ocorrência deste fenômeno relacionado à Estreptomicina (46%), seguida de Tetraciclina (30%), Sulfonamidas (25%) e Ampicilina (23%), sendo que 36% das cepas apresentaram a característica de multidrogas resistentes (MDR), por apresentarem resistência a 4 ou mais compostos microbianos concomitantemente (GOMEZ-LAGUNA et al., 2011).

Durante estudo similar realizado no Vietnã, no isolamento de cepas de *Salmonella* a partir de carne de aves e suínos observou-se resistência a pelo menos um antimicrobiano em 78,4% das estirpes, sendo que as mesmas mostraram uma maior resistência à Tetraciclina (58,5%), seguida de Sulfonamidas (58,1%), Estreptomicina (47,3%), Ampicilina (39,8%),

Cloranfenicol (37,3%), Trimethroprim (34,0%) e Ácido Nalidíxico (27,8%), sendo que do total, 23,2% foram caracterizadas como multi drogas resistentes (MDR) e 8,3% apresentando resistência concomitante a mais de 9 antimicrobianos (THAI et al., 2012).

Em pesquisas realizadas no Brasil foram observadas semelhanças no perfil de resistência das cepas isoladas. Segundo CASTAGNA et al., 2001, ao analisarem cepas de *Salmonella* isoladas a partir de suínos provenientes de granjas no Rio Grande do Sul, o maior índice de resistência foi para Sulfonamidas (83,9%), seguida por Tetraclina (37,4%), Cotrimoxazol (25,2%), Ampicilina (20,2%), Cloranfenicol (16,1%), Estreptromicina(14,1%) e Ácido Nalidíxico (10,1%), sendo que do total das cepas estudadas, 24,2% eram MDR.

Em trabalho semelhante, também no estado do Rio Grande do Sul, a partir de amostras de fezes, linfonodos mesentéricos e material coletado através de zaragatoas em retos suínos, isolaram-se cepas de *Salmonella* resistentes a Sulfonamidas (97,8%), Estreptomicina (82,6%), Tetraciclina (36,9%) e Sulfazotrim (15,2%) (WEISS et al., 2002).

Em enterobactérias também se observa a ocorrência de espécies que produzem um grupo de enzimas capazes de hidrolisar o anel beta lactâmico de penicilinas, cefalosporinas e monobactâmicos, sendo a principal delas a BetaLactamase de Espectro Ampliado (ESBL), resultando em inativação do antibiótico, interferindo negativamente na terapêutica aplicada com embasamento nessas drogas (SOUSA JUNIOR et al. 2004).

Esses dados evidenciam a necessidade de adoção de práticas corretas para utilização de antimicrobianos, principalmente quanto ao uso de probióticos como ferramenta para melhoria da nutrição animal, mas também quanto às terapêuticas antimicrobianas, incluindo a aplicação de doses e posologias corretas, alternância entre os princípios ativos, prudência e diagnósticos corretos e pesquisa de compostos modernos.

3 Objetivos

O presente trabalho tem como objetivos:

a) Pesquisar a presença de *Salmonella* spp. em fezes, linfonodos mesentéricos, mediastínicos e submandibulares de suínos abatidos em frigoríficos sob inspeção federal no interior do Estado de São Paulo e determinar seus sorovares.

b) Determinar o perfil de resistência aos principais antimicrobianos utilizados no tratamento da salmonelose.

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Capítulo 2

Trabalho a ser enviado para a revista **Journal Food Protection**

(Normas para publicação especificadas no Anexo I)

RESISTÊNCIA A ANTIMICROBIANOS E PREVALÊNCIA DE SOROVARES DE *SALMONELLA* SPP. ISOLADOS DE FEZES E LINFONODOS DE SUINOS

[Prevalence, serotype and antimicrobial resistance patterns of *Salmonella* spp. serovars isolated in feces and different porcine lymph nodes.]

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Prevalence, serotype and antimicrobial resistance patterns of *Salmonella* spp. serovars isolated in feces and different porcine lymph nodes.

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Keywords: *Salmonella* spp, lymph nodes, feces, swine, antibiotic resistance, ESBL.

Abstract

Salmonellosis is a food desease transmited by bacteria of the genus *Salmonella*, and the food from pigs are potentially important in their occurrence. In recent decades, strains of this genus have shown resistance to several antimicrobials used in human and animal therapy, with serious risks to public health. Were researched *Salmonella* spp. from feces, mediastinal, mesenteric and mandibular lymph nodes, 50 samples each, obtained from pigs of abattoirs in São Paulo state, Brazil. Positive samples were serotyped at the Adolfo Lutz Institute. The serotypes were tested for antibiotic resistance and the resistant to ciprofloxacin tested for the Expanded Spectrum Beta Lactamase enzyme. The prevalence was 10% (20/200) in total, with 20% (10/50) to submandibular lymph nodes, 18% (9/50) to mesenteric lymph nodes, 2% (1/50)to feces and 0% (0/50) to mediastinal lymph nodes. The serovars found were S. typhimurium, 55% (11/20), S. enteric subspecies enterica 4,5,12: i: - ,35% (7/20) and S. Brandenburg and S. Derby with 5% (1/20) each. All samples showed resistance to at least one antimicrobial, 90% (18/20) were resistant to four drugs simultaneously, and 15% (3/20) were multidrug-resistant. Resistance ratios were Ciprofloxacin and Tetracycline 90% (18/20) each, Nalidixic Acid 80% (16/20), sulfonamides 75% (15/20), Chloramphenicol and Streptomycin 70% (14/20) each, Sulfametoxazole- trimethoprim 65% (13/20), Ampicillin 25% (5/20), Cefotaxime10% (2/10), ceftriaxone and gentamicin 5% (1/20) each. The samples were 100% negative for ESBL. The study confirmed the important role of pigs in the epidemiological chain of the agent as well as the high antimicrobial resistance rates, reinforcing the need for careful use of these drugs and a constant vigilance on the emergence of multidrug-resistant strains.

Introduction

Foodborne deseases always offered great risks to world population and despite the attention given to the prevention and eradication of these diseases, the increase in production volume and marketing of these products on a global scale, have contributed to their occurrence, resulting on serious problems related to public health (*7*).

In this scenery, salmonellosis has been highlighted as the foodborne illness most frequent in the world (*5*), reaching 131,468 annual human cases reported in the European Union (*7*) and 45,828 in the United States (*10*), even if the occurrence of mild symptoms still lead to an underestimated notification (*26*).

In Brazil, from 2000 to 2013, were reported 8871 foodborne deseases outbreaks which *Salmonella* was the causative agent in 1522 of them, and in the total of reported outbreaks, 4.26% were associated with swine meat and its derivatives. In South and Southeast states larger notifications were recorded, while the lowest values of the other not reflect a low incidence but an underestimated notification (*6*). In studies conducted in these states, where are the largest producers of pigs in the country, (*7*) found a prevalence of 55,66% and 90% when the pathogen was researched in lymph nodes and larynx floor respectively of animals in Rio Grande do Sul (*4*) and 67% in lymph nodes, in Santa Catarina (*21*).

The disease in humans is characterized by gastroenteritis and acquired by eating food contaminated by the agent. In recent decades the serovar S. Enteritidis has been the higher associated to that illness, but S. Typhimuirum has also highlighted, generating great concern (*10, 5*).

Pork has considerable important in the transmission of *Salmonella* and may be contaminated from primary production and transport of animals until the industrial processing (*18*), as in the

steps of pre-slaughter and pre-evisceration, especially during scalding (*24, 7*), and the opening of the abdomen and withdrawing colon (*13*).

Due to the association between animal infection is predominantly oral, mesenteric and submandibular lymph nodes works as a primary barrier to this pathogen, retaining them, but infected animals are becoming reservoirs with the possibility to release the agent to the ambient (*29*). Therefore, the isolation of *Salmonella* from lymph nodes indicates the pathogen carrier status (*3*) and the analysis of intestinal contents can only reveal his excretory character to the ambient (*12*).

In most of the human cases the pathogen infection is self-limiting but in some cases the manifestations may be more severe, especially in immunocompromised patients (*15*). The recommended treatment for salmonellosis in humans is based on the administration of antibiotics from the group of fluoroquinolones and quinolones for adults, third-generation cephalosporins for children (*23, 15*) and chloramphenicol in cases of patients with endocartidites or endovascular infection (*23*). However, several studies have pointed out the isolation of several strains of *Salmonella* resistant to the main drugs in these therapeutic practices, adopted in both veterinary and human protocols (*15*).

In the European Union the records of occurrence of antimicrobial resistance in 16 countries for samples obtained from pigs, and 14 for pork samples (*15*). In Spain, from serovars of *Salmonella* spp., observed resistance to streptomycin (46%), tetracycline (30%), sulfonamides (25%) and ampicillin (23%), and 36% of serovars showed the characteristic of multidrug-resistant (MDR) (*19*).

During a similar study conducted in Vietnam, from strains isolated from poultry and pork meat, there was resistance to at least one antimicrobial in 78.4% of serovars, with 23.2% MDR and 8.3% with concurrent resistance for 9 more antimicrobials (*31*).

In Brazil, a study conducted in Rio Grande do Sul, serovars of *Salmonella* spp. isolated from pigs showed resistance to sulfonamides (83.9%) tetraclin (37.4%), cotrimoxazole (25.2%), ampicillin (20.2%), chloramphenicol (16.1%), streptromycin (14 , 1%), and nalidixic acid (10.1%), 24.2% were MDR (*8*).

In enterobacteria also observed the occurrence of species that produces a group of enzymes that hydrolyzes the beta lactam ring of antimicrobials, the main one being the Expanded Spectrum Beta Lactamase (ESBL), resulting in inactivation of penicillins, cephalosporins and monobactams (*28*).

Based on the above, this study aimed to investigate the presence of *Salmonella* spp. in feces, mesenteric lymph nodes, mediastinal and submandibular pigs and characterize their serovars, from this, determine the resistance profile to the main antimicrobials used in medical and veterinary field.

Material and methods

Samples were taken randomly from different animals, 50 feces samlpes and 150 lymph nodes of pigs, with no apparent gross lesions, being 50 mediastinal, 50 mesenteric and 50 submandibular. All animals were in the finishing phase (150-180 days), and the samples were obtained in slaughterhouses lines under Federal Inspection Service in the State of São Paulo, Brazil(*22*).

Feces were collected in the evisceration and inspection tables, using sterile universal bottles collectors of 50 mL. The lymph nodes were removed from carcasses and placed in plastic bags.

The samples were kept at refrigerator temperature (4-8 ° C) and sent to the Veterinary Hygiene and Public Health department FMVZ - UNESP / Botucatu, where were stored at -20 ° C until the realization of the diagnostic tests.

For the isolation of *Salmonella* spp., the samples were placed under refrigeration (4-8 ° C) for 24 hours before the start of the procedures for de-icing material. Feces samples were fractionated 1g of each sample in sterile plastic bags individually using sterile toothpicks and a precision weighing-machine. Samples of lymph nodes were externally disinfected with alcohol 70 ° GL and then fractionated to obtain 1 g of sample with petri plate and bistoury with sterile disposable blade, stored in sterile plastic and weighed on a precision weighing-machine.

To sterile plastic bags containing 1g of sample each was added 9 ml of buffered peptone water 1% (BPW) and then held manual homogenization of the mixture, then incubating the bags in an incubator for 24h at 35 ° C.

Subsequently was made the traditional sequence for isolation of *Salmonella* spp., transferring a 100µL aliquot of the mixture to a test-tube containing 10 ml of Rappaport-Vassiliadis broth (RV) and 1000µL to a tube containing 10 ml of Tetrathionate broth (TT), selective broth for this bacterium. TT tubes were incubated in an incubator at 35 ° C for 18 to 24 hours and RV in a water bath at 42 ° C for 18 to 24 hours. After this period, a heave of each tube was seeded on Petri dishes containing agar Xylose Lysine Deoxycholate (XLD) and Petri dishes containing agar Bismuth Sulfite (BS), being incubated for 24 hours at 35 ° C in an incubator. The characteristic colonies of *Salmonella* spp. were striated to tubes containing triple sugar iron agar (TSI) and lysine iron agar (LIA) for preliminary biochemical tests, which are incubated in a incubator for 18 to 24 h at 35 ° C. In suspected samples were held the remaining biochemical tests: production of indole, Methyl Red reaction and Voges-Proskauer, citrate utilization, urease production, utilization of glucose and lactose, observation of the movement and production of phenylalanine deaminase. The samples with typical biochemical characteristics were confirmed by agglutination test in polyvalent antiserum specific for *Salmonella* spp. The entire methodology for the isolation of *Salmonella* spp. was performed according to the recommendations of Andrews et al. (*2*).

Positive samples were then picked into brain heart infusion broth (BHI) and then re-isolated on plates of agar XLD and BS for purification. After that they were transferred to agar stock to be sent to the Adolfo Lutz Institute for the realization of serotyping.

For the antimicrobial resistance profile test, after serotyping, *Salmonella* spp colonies were suspended in BHI broth and, if necessary, added sterile saline solution (1% NaCl) to obtain turbidity compatible with 0.5 McFarland scale. The solution was seeded on plates containing Mueller Hinton agar using a sterile swab and added antibiogram discs of selected compounds for the study of the resistance profile. The antimicrobial agents tested were those indicated by Clinical and Laboratory Standards Institute (CSLI) as the basis of treatment for human contamination caused by Enterobacteriaceae, which are: Nalidixic acid (NAL), Amikacin (AMI), Ampicillin (AMP), Aztreonam (AZT), Cefepime (CPM), Cefotaxime (CTX), Cefoxitin (CFO), Ceftazidime (CAZ), Ceftriaxone (CRO), ciprofloxacin (CIP), Chloramphenicol (CLO), Streptomycin (EST), Gentamicin (GEN), Meropenem (MER), Sulfonamides (SUL), Trimethoprim-Sulfametoxazole (SUT) and Tetracyclin (TET) (*11*).

Thereafter the plates were incubated at 36 ° C for 18-24h in an incubator so that it could be carried out observation and measurement of growth inhibition halos, thus enabling the effectiveness of antimicrobial inferred from the reference values (*11*). For classified as intermediate resistance values were considered as resistant if the sample (*9*).

The serotypes that were resistant to ciprofloxacin were tested for presence of ESBL enzyme by the method of double disk diffusion to check synergism between the antimicrobial Clavulanic acid in relation to Aztreonam, Ceftazidime, Ceftriaxone and Cefotaxime (*11*).

For this, the samples were re-isolated and plated on Mueller Hinton agar plates similarly to resistance test, but in each was added to the center of plate an antibiogram disc of Clavulanic acid and with a millimeter ruler the antibiogram discs of Aztreonam, Ceftazidime, Ceftriaxone and Cefetaxime were arranged at a distance of 25 mm from the central disk, each directed to a

quadrant equidistantly from each other, forming a zone of analysis between the central disc and four antibiotic to be observed (*11*).

Confirmation of positive sample was made by observing the formation of a secondary inhibition halo formed by synergism of the tested antibiotics with the initial intersection of the inhibition zones of each antimicrobial (*11*).

For statistical analysis, the binomial dependent variable was subjected to logistic regression analysis using the PROC LOGISTIC of the Statistical Analysis Software (SAS 9.2, Inst. Inc., Cary, NC, USA). The results are shown as a percentage. When necessary, data were contrasted by the least square difference PROC GLM. For all analyzes, we adopted the significance level of 5% ($P < 0.05$).

Results

The prevalence of *Salmonella* spp. was 10% of total samples (20/200), with the highest rates found in the submandibular lymph nodes with 20% of positive samples (10/50), followed by mesenteric with 18% (9/50) and the lowest prevalence found in feces samples with 2% (1/50). In Mediastinal lymph nodes was not isolated any strain of the pathogen. About the type of sample, mesenteric and submandibular lymph nodes showed statistical similarity ($p > 0.05$) and were considered superior compared to samples of feces and mediastinal lymph nodes when analyzed for prevalence ($p < 0.05$).

Among the serotypes identified, the most prevalent was S. Typhimurium with 55% of the samples (11/20), followed by S. enterica subspecies enterica 4,5,12: i: - 35% (7/20) and S . brandenburg and S. derby with 5% (1/20) each.

The highest resistance rates were found for Ciprofloxacin (CIP) and tetracycline (TET), with 90% (18/20) resistant strains each, followed by nalidixic acid (NAL) 80% (16/20), sulfonamides (SUL) 75% (15/20), Chloramphenicol (CLO) and streptomycin (EST) 70% (14/20) each, Trimethoprim-

sulfamethoxazole (SUT) 65% (13/20) ampicillin (AMP) 25 % (5/20), cefotaxime (CTX) 10% (2/10) and ceftriaxone (CRO) and Gentaminicina (GEN) 5% (1/20) each (FIGURE 1).

The serovar S. typhimurium showed resistance to several antimicrobial, especially in relation to Tetracycline, 100% of the samples (n = 11), followed by Ciprofloxacin with 90.91% (n = 10), Nalidixic acid, sulfonamides and chloramphenicol with 72.73% (n = 8) each, streptomycin 63.64% (n = 7), Trimethoprim-sulfamethoxazole with 54.55% (n = 6), ampicillin 36.36% (n = 4) and Gentamicin and Cefotaxime and with 9.09% (n = 1) each (TABLE 1).

The serovar S. enterica subspecies enterica 4,5,12: i: - showed 100% (n = 7) of resistance to antimicrobial Nalidixic acid, sulfamethoxazole Trimethoprim-, sulfonamides, tetracycline and Estreptromicina. For other antimicrobial the values were 85.71% (n = 6) for ciprofloxacin, 71.43% (n = 5) for Chloramphenicol and 14.71% (n = 1) to ampicillin, gentamicin and cefotaxime (TABLE 1).

The single isolated S. serovar Brandenburg shows resistance only front ciprofloxacin and Ceftriaxone, or 11% of the antibiotics tested (Table 1). The single isolate of S. serovar Derby was resistant to ampicillin, Trimethoprim-Sulfamethoxazole, sulfonamides, Estreptromicina, tetracycline, chloramphenicol and ciprofloxacin, or 41% of the antibiotics tested (TABLE 1).

Among the isolated serovars, all were resistant to at least one of the antimicrobial tested, with 90% of them (n = 18) showed resistance to at least 4 antimicrobial simultaneously (TABLE 2). Considering the standards determined by CLSI for MDR samples, simultaneously resistant to ampicillin, chloramphenicol, streptomicyn, Trimethoprim-Sulfamethoxazole and Tetracycline, 15% (n = 3) of the samples showed this pattern (TABLE 2).

There were no positive results for the presence of ESBL enzyme.

Discussion

The results of the proposed work had a prevalence of 10% for *Salmonella*, a value common to the literature as 16.6% in the state of Mato Grosso, Brazil (*25, 27*), and 17.6% in Portugal (*18*) but differing in other works as 55.66% in the state of Rio Grande do Sul, Brazil (*4*), 67% in the state of Santa Catarina, Brazil (*21*), 34.8% in Vietnam (*14*) and 33% in the European Union (*15*).

Considering the type of sample analyzed (lymph node or feces), the materials that had a higher prevalence were the submandibular and mesenteric lymph nodes compared with feces and mediastinal lymph nodes. This fact supports the idea that the health status of a farm has a reliable result when analyzed from lymph node samples compared to feces samples (*12, 29*), associated with the fact that the presence of the pathogen in the feces indicate the excretory character of the animal instead of the real carrier status (*29, 3*).

The differences observed in the positivity for the pathogen on the different types of lymph nodes may be related to anatomical position of them. The highest positivity was observed in the mesenteric, associated with the gastrointestinal tract drainage and submandibular which coprophagic habit of the pigs given to act as an initial barrier to pathogens. The infection can not develop, but the animals start to act as pathogen reservoirs (*3, 29*). On lymph nodes where that exposure is not observed, no positive samples were detected, as is the case of mediastinal lymph nodes.

In relation to those serovars, the high percentage of S. Typhimurium observed at work reflects the increasing occurrence of this serovar in the global scenery (*7*). In United States he occupied the 1st position among the most reported serovars by the year 2011, becoming the second, losing position S. Enteritidis (*10*). The high incidence of this serovar is also observed in the European Union (*15*) and Brazil (*21*).

It should also be given special attention to the occurrence of 35% (7/20) of S. enterica subspecies enterica 4,5,12: i-, a serovar with similar characteristics to S. Typhimurium, characterized by minor differences in the flagellar phase (*30*). It is now considered one of the main serovars isolated from pigs in Europe (*15*), Brazil (*20*) and the United States, where their notifications increased 351% between 2001 and 2011, from 20 ° to 5 ° more reported, reflecting in large part also changes in reporting practices (*10*).

The serovars S. Brandenburg and S. Derby were found in only one sample each and these values were similar to those observed in epidemiological studies in other countries (*21, 7, 31*).

Our data support the intimate association between swine and the pathogen. On the other hand, as regards the resistance of the strains to the tested drugs was observed similarity of our values (FIGURE 1) for resistance profiles as tetracycline (96.5%), and nalidixic acid (95.5%) (*20*), sulfonamides (97.8%) and streptomycin (82.6%) (*32*), however in similar work are described contrasting values to certain in this, as resistance rates for Streptomycin with (46 to 47.3%), tetracycline (30 -58.5%), sulfonamides (25 to 58.1%) and acid Nalidixic (27.8%) (*19, 31*). The high resistance rates opposite Ciprofloxacin (FIGURE 1) are similar to those found in Spain (97.1%) (*15*) but differ in some of the literature, where lower values were found, between 0 and 3.7% (*20, 9*), and in other European countries ranging from 0 to 16.1% (*15*), whereas Nalidixic Acid, Ciprofloxacin and Ceftazidime are the antimicrobials indicated for therapy in cases of severe clinical conditions in man (*23*).

The resistance profile seen in S. typhimurium is different from those presented in the literature, where resistance to the antimicrobials Ciprofloxacin, Nalidixic acid and tetracycline were respectively 0.3%, 1.7% and 26.8% reported in samples of United States (*9*), however, were similar in serovars isolated from feces and swine carcasses in Brazil, where the resistance to nalidixic acid was 64.3% and Tetracycline 96.4% (*20*), also in Estonia where the index resistance against the Ampicilin, sulfonamides and tetracycline ranged from 70 to 90% (*32*).

The serovar S. enterica subspecies enterica 4,5,12: i: - presented different from those reported in the United States, where the antimicrobials Trimethoprim-sulfamethoxazole and Chloramphenicol showed no resistant strains; Nalidixic acid showed 0.8% of resistant, gentamicin 2.5%, streptomycin and Ampicilin 28.8% each and Tetracyclin 33.1% (*9*).

S. Brandenburg showed resistance values similar to those notifications in the United States, where the resistance of serovar front of 3 active ingredients was 0.5% (*9*).

The resistance profile presented by S. Derby was positive for 41% of antimicrobial tested, in relation to this serovar, in Germany was observed resistance in 28.6% of the samples for Ampicillin, 38.1% for Sulfonamides, 23 5% for tetracycline, 9.5% for Ciprofloxacin and 2.4% for chloramphenicol (*15*).

The amount of MDR samples presented in this study corroborates those found in the literature, where pigs analyzed in Vietnam, showed 23.2% of the samples with this profile (*31*) and in a similar study conducted in Brazil the rate was 24.2% (*8*). In Europe are reported various levels of MDR samples obtained from pigs, ranging from 13.6% in Estonia to 59.4% in Ireland (*15*).

The high antimicrobial resistance rates can be attributed, among other reasons, to the frequent and continuous use in animal production (*18, 21*), and the reservoir profile often observed in this species, through some lymph nodes, can contribute to the emergence of MDR variants (*17*).

In our study were not detected strains with ESBL enzyme, and the European Union it was observed in 0.6% of the samples isolated from pork, when tested for cefotaxime and 0.5% for Ceftazidime (*16*).

In this study we conclude that *Salmonella* spp. continues to present significant prevalence rates in pigs and foodborne deseases notifications throughout the global scenery, with S.

typhimurium still the most reported even with the adoption of control measures implemented over several decades (*7*) but also increasing the occurrence of other strains as S. enterica subspecies enterica 4,5,12: i: - (*10*). The high resistance rates found in this study reveals that despite regional differences for resistance to some antimicrobials, the above indices reveal important health care public regarding the use of these drugs in livestock, both in therapy and in nutritional performance enhancing strategies, and especially in cases of use in therapy in man.

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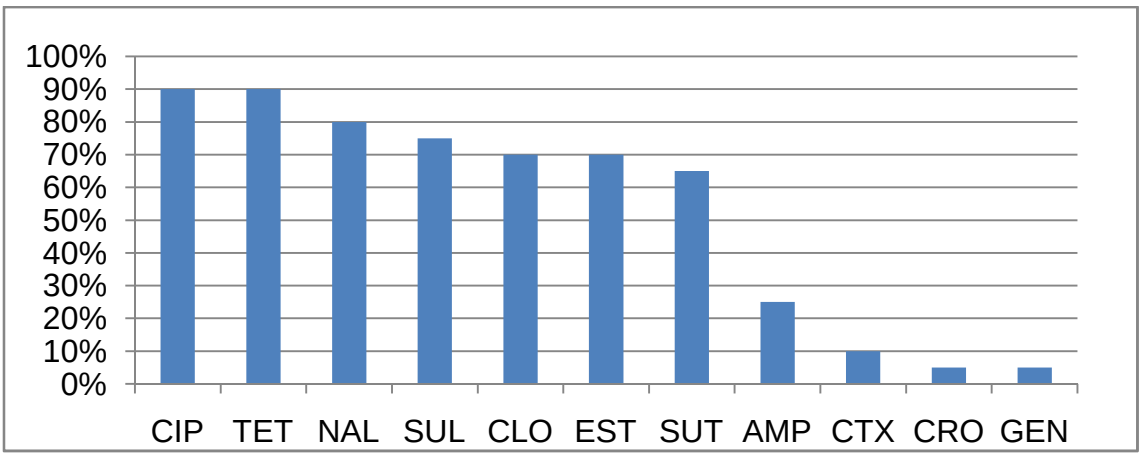
Tables and Figures

FIGURE 1. Antimicrobial resistance in relation to isolated positive samples.



Ciprofloxacin (CIP), tetracycline (TET), Nalidixic acid (NAL), sulfonamides (SUL), chloramphenicol (CLO), Streptomycin (EST), sulfamethoxazole Trimethoprim-(SUT), ampicillin (AMP), cefotaxime (CTX), Ceftriaxone (CRO), Gentaminicina (GEN).

TABLE 1. *Salmonella* serovars of resistance profile isolated from lymph nodes and faeces of pigs against the tested antimicrobials

Antimicrobial	Serovars (N=20)			
	Thyphimurium	Enterica subsepcies enterica 4,5,12:i:-	Brandenburg	Derby
	N=11	N=7	N=1	N=1
NAL	8(72,73)	7(100,0)	0	0
AMP	4(36,36)	1(14,29)	0	1(100,0)
SUT	6(54,55)	7(100,0)	0	1(100,0)
GEN	1(9,09)	1(14,29)	0	0
SUL	8(72,73)	7(100,0)	0	1(100,0)
EST	7(63,64)	7(100,0)	0	1(100,0)
TET	11(100,0)	7(100,0)	0	1(100,0)
CIP	10(90,91)	6(85,71)	1(100,0)	1(100,0)
CLO	8(72,73)	5(71,43)	0	1(100,0)
CRO	0	0	1(100,0)	0
CTX	1(9,09)	1(14,29)	0	0

N (%) - Number of samples and percentage in relation to specified serovar. Ciprofloxacin (CIP), tetracycline (TET), Nalidixic acid (NAL), sulfonamides (SUL), chloramphenicol (CLO), Streptomycin (EST), Trimethoprim-sulfamethoxazole (SUT), ampicillin (AMP), cefotaxime (CTX), Ceftriaxone (CRO), Gentaminicina (GEN).

TABLE 2. resistance profile of the different serotypes of *Salmonella* front of antimicrobials.

Serovar	Antimicrobial
S. Typhimurium	CIP
S. Brandenburg	CIP, CRO
*S.*Typhimurium	NAL,CIP, CLO, TET
S. Typhimurium	CIP, SUT, SUL, TET
S. Typhimurium	NAL, CIP, CLO, EST, TET
S. Typhimurium	NAL, CTX, CIP, CLO, TET
S. Typhimurium	NAL, AMP, CIP, SUL, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL,CIP, SUT, SUL, EST, TET
S. Typhimurium	AMP, CLO, SUT, SUL, EST, TET
S. Typhimurium	AMP,CIP, CLO, SUL, SUT, EST, TET
S. Derby	AMP,CIP, CLO, EST,SUL, SUT, TET
S. Typhimurium	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Typhimurium	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Typhimurium	NAL, CIP, CLO, SUT, SUL, EST, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL, AMP, SUT, GEN, SUL, EST, TET
S. Enterica subespécie enterica 4,5,12:i:-	NAL, CTX, CIP, CLO, SUT, SUL, EST, TET

Ciprofloxacin (CIP), tetracycline (TET), Nalidixic acid (NAL), sulfonamides (SUL), chloramphenicol (CLO), streptomycin (EST), Trimethoprim-sulfamethoxazole (SUT), ampicillin (AMP), cefotaxime (CTX), ceftriaxone (CRO), Gentamicin (GEN)

Anexo 1 – Normas para publicação no periódico Journal of Food Protection

Journal of Food Protection®
Instruction for Authors

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.

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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.

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 or 'hot' topics in which there have been important recent advances are particularly encouraged. Such 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.

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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.

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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

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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.

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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.

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ångström, Å
atmosphere, atm
base pairs, bp
British thermal unit, BTU
calorie, cal
centimeter, cm
CFU (never spelled out: colony-forming units)
cubic centimeter, cm^3
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 in^2
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

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