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Characterization of *Salmonella* species from pork meat in Tolima, Colombia¤

Caracterización de las especies de Salmonella en carne porcina en Tolima, Colombia

Caracterização de espécies de Salmonella a partir de carne de porco em Tolima, Colômbia

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Summary

Background: *Salmonella* is a Gram-negative bacterium and the principal cause of human gastroenteritis that originates from the consumption of animal products. **Objective:** to determine serotype and antibiotic resistance of *Salmonella* spp. isolated from pork meat and environmental samples in 6 slaughterhouses and 14 butcheries in Tolima, Colombia. **Methods:** slaughterhouses and butcheries were selected depending on their slaughter capacity and compliance with good manufacturing practices. Samples (n = 507) were taken from carcasses, the environment, and fomites (*i.e.*, surfaces of knives, hooks, floor, siphons, work surfaces, and transport trucks), then cultured in *Salmonella* selective media. Following this, the isolated *Salmonella* spp. was identified using a conventional biochemical test and genus antiserum (Poli A + Vi). The Kauffman-Whyte scheme was used for serotyping and the agar diffusion method (Kirby-Bauer) was used to determine antibiotic sensitivity. **Results:** Manhattan, Derby, Typhimurium, Javiana, Muenster, Hvittingfoss, Sinsfort, Kattbus, and Saint Paul serotypes of *Salmonella* were isolated from both pork meat and environmental samples, being Derby the most common serotype. *Salmonella* isolates showed antibiotic multiresistance mainly to tetracycline, lincomycin and nalidixic acid. **Conclusions:** several *Salmonella* serotypes are present in slaughterhouses and meat samples from butcheries, and they show similar antibiotic resistance patterns. This work represents the first report on *Salmonella* serotypes in slaughterhouses and pork meat from butcheries in Tolima, Colombia.

Keywords: *antibiotic resistance, enterobacteria, isolates, serotyping.*

Resumen

Antecedentes: *Salmonella* es una bacteria Gram-negativa y la principal causa de gastroenteritis en humanos transmitida por consumo de alimentos de origen animal. **Objetivo:** determinar el serotipo y la resistencia

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antibiótica de *Salmonella* spp. aisladas de carne porcina y muestras ambientales en 6 plantas de beneficio y 14 expendios en Tolima, Colombia. **Métodos:** las plantas de beneficio y los expendios fueron seleccionados dependiendo del volumen de animales sacrificados y el establecimiento de las buenas prácticas de manufactura. Las muestras (n = 507) fueron tomadas de las carcasas, ambiente y fómites (*i.e.* superficie de cuchillos, ganchos, piso, sifones, mesones y camiones de transporte), cultivadas en medios de cultivo selectivos para *Salmonella.* Las *Salmonella* spp., aisladas fueron identificadas mediante purnas bioquímicas convencionales y antisuero de género (Poli A + Vi). La sero-tipificación fue llevada a cabo a través del esquema Kauffman-Whyte y la sensibilidad antibiótica a través del método de difusión en agar (Kirby-Bauer). **Resultados:** fueron aislados los serotipos de *Salmonella* Manhattan, Derby, Typhimurium, Javiana, Muenster, Hvittingfoss, Sinsfort, Kattbus y Saint Paul tanto de carne porcina como muestras ambientales, siendo el serotipo Derby el más frecuente. Los aislamientos mostraron patrones de multi-resistencia antibiótica, principalmente a tetraciclina, lincomicina y ácido nalidíxico. **Conclusiones:** diversos serotipos de *Salmonella* fueron aislados de muestras de plantas de beneficio y expendios de carne porcina y estos demostraron patrones similares de resistencia antibiótica. Este trabajo representa el primer reporte de serotipos de *Salmonella* en plantas de beneficio y expendios de carne porcina en Tolima, Colombia.

Palabras clave: *aislamientos, enterobacterias, resistencia a antibióticos, serotipificación*.

Resumo

Antecedentes: a *Salmonella* é uma bactéria Gram-negativa e causa principal de gastroenterite humana transmitida por consumo de produtos de origem animal. **Objetivo:** determinar o serotipo e de resistência a antibióticos de *Salmonella* spp. isolada a partir de carne de porco e amostras ambientais em 6 frigoríficos e lojas 14 açougues em Tolima, Colômbia. **Métodos:** abatedouros e açougues foram selecionados de acordo com o volume de animais sacrificados eo estabelecimento de boas práticas de manufatura. As amostras (n = 507) foram retirados de cadáveres, meio ambiente e fômites (ou seja, superfícies de facas, ganchos, piso, sifões, mésons e caminhões de transporte), cultivadas em meios de cultura de *Salmonella* seletiva e a isolada *Salmonella* spp., foram identificados usando e anti-soro de teste género bioquímica convencional (Poli A + Vi). A sorotipagem foi realizada utilizando esquema de Kauffman-Whyte e teste de sensibilidade aos antibióticos foi feito pelo método de difusão em ágar (Kirby-Bauer). **Resultados:** "o" sorotipos de *Salmonella* de Manhattan,= o surotipos de *Salmonella* Derby, Typhimurium, Javiana, Muenster, Hvittingfoss, Sinsfort, Kattbus e Saint Paul foram isolados de ambas as carnes de porco e amostras ambientais, sendo o sorotipo Derby o mais frequente. Os isolados de *Salmonella* apresentaram padrões de multirresistência aos antibióticos, principalmente para tetraciclina, lincomicina e ácido nalidíxico. **Conclusões:** vários sorotipos de *Salmonella* estão presentes em matadouros e lojas amostras de carne de porco de talho e mostram padrões similares de resistência a antibióticos. Este trabalho representa o primeiro relato de sorotipos de *Salmonella* em abatedouros e açougues de carne de porco em Tolima, Colômbia.

Palavras chave: *enterobacteria, isolamentos, resistência aos antibióticos, sorotipagem*.

Introduction

Salmonellosis is the principal gastroenteritis etiology in humans associated with consumption of food from animal origin, where the most frequently isolated serovar in ill humans and pigs is *S.* Typhimurium (Torpdahl *et al*., 2006). This pathogen is the most common cause of acute infectious gastroenteritis in Colombia (Bustos *et al.,* 2008).

In spite of the well-known role of *Samonella* as a foodborne disease (FBD), it is difficult to estimate FBD incidence due to the lack of a strict link with food (Flint *et al*., 2005) and a number of limitations including: 1) people do not always seek medical aid when they become infected; 2) physicians do not always request a stool culture in suspicious cases; 3) not all positive cases are reported and shared in databases; and 4) there are differences in health-care seeking behaviors among differing age groups (Kumar *et al*., 2009; Meneses, 2010). To address these difficulties, the world health organization (WHO) developed surveillance programs for FBD such as *Salm-Surv* for salmonellosis (Petersen *et al*., 2002) as well as sentinel sites for other bacteria including *Salmonella*, *Shigella*, and *Brucella* (Flint *et al*., 2005).

Salmonellosis is considered a major health concern worldwide; it is estimated that 95% of these particular infections are FBDs (Xia *et al*., 2009).

Human infections by *Salmonella* serovars have been reported in several countries, including Colombia (Ashbolt *et al.,* 2004; Durango *et al*., 2004; Voetsch *et al*., 2004; Vaillant *et al*., 2005). In the United States, an estimated 56.8% of salmonellosis cases in humans might be attributable to pigs (BIOHAZ, 2012). Arguello *et al*. (2013b) demonstrated a 40.9% *Salmonella* prevalence in Danish pig herds, measured at the slaughterhouse. However, overall prevalence in Danish pork was reported to be as low as 1.2% (Alban *et al*., 2012). Similarly, Arguello *et al*. (2012) showed 39% *Salmonella* prevalence in pig carcasses in Spain. Bolton *et a*l. (2013) reported 36.8% prevalence in the UK, and Methner *et al*. (2011) reported 13.8% in Germany. Arcos *et al*. (2013) demonstrated 4.3% *Salmonella* prevalence at slaughterhouses and retail market in Tolima, Colombia.

Although *Salmonella* species have been isolated from commercial pig farms (Fierro *et al*., unpublished data) and pork meat at slaughterhouses and butcheries in Tolima (Arcos *et al*., 2013) limited information is available to establish a clear picture of pig health status in this region. Currently, the main risk factors associated with *Salmonella* incidence in pig farms are the lack of pest controls and sow replacement programs (Henao *et al*., 2012). Few studies have addressed antibiotic resistance of *Salmonella* isolates from pig farms in Tolima (Fierro *et al*., 2011).

The aim of this study was to conduct a preliminary characterization of *Salmonella* species isolated from pig carcasses and environmental samples at slaughterhouses and butcheries in Tolima.

Materials and methods

Ethical considerations

The Ethics Committee of the Research Center of Universidad del Tolima approved this study in January 16, 2011.

Population

Six slaughterhouses and 14 pork butcheries of the 32 present in Tolima were included in the study based on their routine slaughter volume and use of good manufacturing practices (GMP). The Colombian institute for drug and food surveillance (INVIMA) supported the study. Carcasses and environmental samples were collected at the slaughterhouse after slaughter and again after delivery to the butcheries. Trucks and shop environments were also sampled. Collected samples were submitted to the Laboratorio de Diagnóstico Veterinario at Universidad del Tolima.

Sample size

Six Tolima municipalities (Chaparral, Fresno, Guamo, Ibagué, Líbano and Mariquita) were selected taking into account the number of slaughtered pigs per week (>80). Based on 0% *Salmonella* prevalence in Ibagué slaughterhouses, with a 95% confidence level and 5% expected prevalence (Pabón, 1978), sample size was calculated using the following equation (Thrusfield, 2007):

$$n = \frac{z^2 \times p \times q}{d^2}$$

Where:

z^2: prefixed confidence coefficient ($1{,}96^2$ for a 95% confidence).

p: expected prevalence (in this study, 5% = 0.05).

q: 1- p.

d: Accuracy (in this study, 5%).

$$n = \frac{(1.96)^2 \times 0.05 \times (1 - 0.05)}{(0.05)^2} = \frac{3.8416 \times 0.05 \times 0.95}{0.025} = 72.99$$

The calculated minimum number of samples was 73. A total of 507 samples were taken in this study.

Sample collection

Destructive and non-destructive sampling methods were used on the surface of pig carcasses at slaughterhouses and butcher shops. A non-destructive method was used for carcass skin with sterile swabs (3M Manufacturing Company, Saint Paul, MN, USA),

which were hydrated with buffered peptone water for initial dilution and pre-enrichment step. A 10x10 cm area was calculated in each carcass and three vertical and horizontal smears were made from cheeks, abdomen and leg. In addition, 100 g of pork meat was sampled from the abdomen and throat of each carcass. These were cut with sterile scalpels (destructive method) and placed in individual sterile and hermetic bags. Environmental samples were collected from knives, work surfaces, floors, siphons and trucks using commercial swab sponges (EnviroSponge™ / HydroSponge™, Biotrace International®, South Africa). All samples were kept refrigerated until analysis.

Salmonella isolation

Salmonella species were isolated following standard international guidelines (ISO 6579, 2002). Briefly, samples were incubated in buffered peptone water as a pre-enrichment step at 37 °C for 24 hours, followed by a selective enrichment step in two media: tetrathionate broth at 37 °C (Müller-Kauffmann) and Rappaport Vassiliadis at 42 ºC. Bacterial samples were cultured in SS agar (*Salmonella-Shigella*, Oxoid, Germany), XLT_4 agar (Xylose Lysine Tergitol-4, Oxoid, Germany), and XLD agar (Xylosa Lysine Desoxycholate, Oxoid, Germany). Suspected bacterial colonies were cultured in McConkey agar and Trypticase soy agar (TSA) and confirmed as *Salmonella* spp. by using Poli A-I + Vi antiserum (Difco® 222641, USA). In addition, *Salmonella* isolates were confirmed by typical biochemical tests through an API ® 20E test (Biomereux, France).

Serotyping

Salmonella isolates were serotyped using the Kauffman-White scheme (Brenner, 1998) for O and H antigens with commercial antiserum (Difco, Becton, Dickinson and Company, Sparks, MD). Serotyping was done based on the antigenic description by Grimont & Weill (2007) as well as nomenclature described by Tindal *et al*. (2005) and the judicial commission of the international committee on systematics of prokaryotes (JCICSP, 2005).

Antibiotic resistance

Antibiotic resistance was assessed with the Kirby-Bauer method (agar diffusion) to determine sensitivity against several antimicrobials (ampicillin, amoxicillin, apramycin, ciprofloxacin, chloramphenicol, cephalexin, enrofloxacin, gentamicin, kanamycin, lincomycin, nalidixic acid, neomycin, nitrofuranoine, tetracycline, and sulfametoxazole/trimethoprim). A bacterial suspension in Mueller-Hinton agar (Oxoid, Germany) was calibrated according to 0.5 McFarland scale of turbidity. The 2005 protocol of the national committee for clinical laboratory standards (NCCLS) was used to interpret bacterial growth inhibition on plate at 37 ºC for 24 h.

Results

Isolation of Salmonella species

Salmonella spp. was isolated from 25 out of 507 samples (4.9%) including 421 from carcasses and 86 from environments. *Salmonella* prevalence in carcasses was 3.32% (14/421) whereas prevalence in environmental samples was 12.79% (11/86). *Salmonella* isolated from carcasses included 10 from meat (71.4%) and 4 from smears of carcass surfaces (28.6%). *Salmonella* isolated from environmental samples included 3 from knives (27.2%), 3 from hooks (27.2%), 3 from siphons (27.2%), and 2 from floors (18.4%). In summary, *Salmonella* isolates were found in meat (40%), carcass smears (16%), knives (12%), hooks (12%), siphons (12%) and floors (8%).

Serotyping

The identified *Salmonella* serotypes were *Samonella* ser. Manhattan (2/25; 8%), *Salmonella* ser. Derby (9/25; 36%), *Salmonella* ser. Typhimurium (3/25; 12%), *Salmonella* ser. Javiana (1/25; 4%), *Salmonella* ser. Muenster (5/25; 20%), *Salmonella* ser. Hvittingfoss (2/25; 8%), *Salmonella* ser. Sinsfort (1/25; 4%), *Salmonella* ser. Kattbus (1/25; 4%), and *Salmonella* ser. Saint Paul (1/25; 4%; Table 1).

Antibiotic resistance

The majority of *Salmonella* species isolated in this study were resistant to more than 2 antibiotics (60%). Regardless of its origin, *Salmonella* isolates

showed antibiotic multi-resistance to lincomycin (25/25; 100%), tetracycline (19/25; 76%), nalidixic acid, and neomycin (Table 1). Only one *Salmonella* isolate showed resistance to both amoxicillin and ampicillin.

Table 1. Serotypes and antibiotic resistance profiles of *Salmonella* strains (n = 25) isolated from butcher's shops (BS) and slaughterhouses (S) in Tolima, Colombia.

Source	Sample	*Salmonella* serotype	Kirby Bauer Test*	
			R	S
S1	Knife	Manhattan	10,12	1,2,3,4,5,6,7,8,9,11,13,14,15
	Hooks	Manhattan	10,12,14	1,2,3,4,5,6,7,8,9,11,13,15
	Meat (neck)	Derby	10,12,14	1,2,3,4,5,6,7,8,9,11,13,15
	Carcass swab	Derby	10,12,14	1,2,3,4,5,6,7,8,9,11,13,15
S2	Knife	Derby	10,12,14	1,2,3,4,5,6,7,8,9,11,13,15
	Hook	Derby	10,14	1,2,3,4,5,6,7,8,9,11,12,13,15
	Floor	Derby	10,14	1,2,3,4,5,6,7,8,9,11,12,13,15
	Siphon	Derby	3,8,10,14	1,2,4,5,6,7,9,11,12,13,15
BS1	Meat (leg)	Derby	10,12,14	1,2,3,4,5,6,7,8,9,11,13,15
	Carcass swab	Derby	10,14	1,2,3,4,5,6,7,8,9,11,12,13,15
S3	Knife	Derby	3,9,10,12,14	1,2,4,5,6,7,8,11,13,15
S4	Hook	Typhimurium	1,2,4,5,6,7,10,11,12,14	3,8,9,13,15
	Meat (hip)	Typhimurium	10,14	1,2,3,4,5,6,7,8,9,11,12,13,15
	Meat (neck)	Javiana	7,10,12,13	1,2,3,4,5,6,8,9,11,12,15
BS2	Carcass swab	Muenster	8,10,11,12,13,14,15	1,2,3,4,5,6,7,9
	Carcass swab	Muenster	10,11,14,15	1,2,3,4,5,6,7,8,9,12,13
BS3	Carcass swab	Hvittingfoss	10,12	1,2,3,4,5,6,7,8,9,11,13,14,15
	Carcass swab	Hvittingfoss	10	1,2,3,4,5,6,7,8,9,11,12,13,14,15
BS4	Siphon	Sinstorf	10,11	1,2,3,4,5,6,7,8,9,12,13,14,15
BS5	Carcass swab	Kattbus	10,11,14	1,2,3,4,5,6,7,8,9,12,13,15
	Siphon	Saint Paul	9,10,12,14	1,2,3,4,5,6,7,8, 11,13,15
BS6	Meat (leg)	Muenster	10,11,12,13,14,15	1,2,3,4,5,6,7,8,9
	Carcass swab	Muenster	10,11,14	1,2,3,4,5,6,7,8,9,12,13,15
	Carcass swab	Muenster	10,11	1,2,3,4,5,6,7,8,9,12,13,14,15
	Floor	Typhimurium	10,11,14	1,2,3,4,5,6,7,8,9,12,13,14,15

*R: Resistant, S: Sensitive, 1: amoxicillin, 2: ampicillin, 3: apramycin, 4: ciprofloxacin, 5: chloramphenicol, 6: cephalexin; 7: enrofloxacin, 8: gentamicin, 9: kanamycin, 10: lincomycin, 11: nalidixic acid, 12: neomycin, 13: nitrofuranoine, 14: tetracycline, 15: sulfamethoxazole/trimethoprim.

Discussion

Salmonella serotypes Derby, Typhimurium, Heidelberg, Worthington, and Mbandaka are the most common serotypes in swine worldwide (Davies *et al.,* 1997; Mueller-Doblies *et al*., 2013). *S*. Typhimurium and *S*. Derby are the main serotypes isolated from pigs at slaughterhouses in the European Union and

the United States (Anonymous, 2008). In this study, *S*. Derby was the main isolated serotype (36%) from pork. Similar results were reported in Italy (Piras *et al.,* 2011; 47%) and France (40.5%; Bouvet *et al*., 2003), where *S*. Typhimurium (27%) was the second serotype in this country—it was slightly higher (36.9%) in Germany (Methner *et al*., 2011). In contrast, studies by Yang *et al*. (2013) in China reported *S*. Enteritidis as the main serotype isolated from pork origin and they isolated only one *S.* Derby out of 31 *Salmonella* serotypes. De Busser *et al*. (2011) also reported *S.* Typhimurium as the main isolate (58.7%), whereas *S.* Derby had a prevalence of 8.3% in Belgium. Recently, Arguello *et al.* (2013b) reported 64.4% and 4.9% *S.* Typhimurium and *S.* Derby prevalence in Denmark, respectively.

Salmonella Muenster and *S.* Typhimurium were the second and third more frequent serotypes isolated in this study, respectively, which is in agreement with Prendergast *et al*. (2012) who reported several serotypes in Ireland, including *S.* Typhimurium, *S.* Infantis, *S.* Derby*, S.* Virchow, and *S.* Livingstone (two strains isolated from porcine carcass swabs could not be fully typed and therefore were referred to as *S.* Unnamed). Similarly, Botteldoorn *et al*. (2003), Bolton *et al*. (2013), Delhalle *et al*. (2009), McDowell *et al*. (2007), Meneses (2010), Arguello *et al*. (2012), Arguello *et al*. (2013a), and Mueller-Doblies *et al*. (2013) found *S*. Typhimurium, *S.* Derby, *S*. Rissen, *S*. Muenster, and *S*. Javiana in pork samples from other countries. In this study, *S.* Saint Paul was isolated in 4% of the samples (1/25), in contrast with reports by Kikuvi *et al*. (2010) indicating this serotype was predominant (64.2%) in Kenya. Other serotypes such as *S.* Manhattan, *S*. Javiana, *S*. Hvittingfoss*, S.* Sinsfort, and *S*. Kattbus, have been previously reported in Germany (Methner *et al*., 2011). Our results indicate that the main *Salmonella* serotypes isolated from pork meat and environmental samples have similarities to those from North America and European countries, and differ from those originating in Africa.

Although some studies have shown that serotypes isolated from pig farms, slaughterhouses and those detected in infected animals are unrelated or completely different (Hurd *et al.,* 2001), there is an emerging concern that the transport method and carcass reception at butcheries could increase the contamination rate or bacterial isolation from carcasses or environmental samples. In this regard, our study found two *Salmonella* isolates in carcasses at slaughterhouses, whereas 10 *Salmonella* isolates were detected at butcheries (Table 1), thus, although we cannot exclude the possibility of contamination during transport or carcass handling, contamination of pork carcasses seems to be more probable during handling at butcher shops given that transport trucks were negative to *Salmonella*.

Antibiotic, multi-resistant pattern by *Salmonella* isolates has been reported from pig samples worldwide (Herrera-León *et al*., 2007; Nwachukwu *et al*., 2010; Liu *et al*., 2011; Yang *et al*., 2013). In this study, tetracycline resistance was high, similar to reports of *Salmonella* isolates from pork in another countries (Aragaw *et al*., 2007; Meneses, 2010; Nwachukwu *et al*., 2010; Mueller-Doblies *et al*., 2013; Xia *et al*., 2013; Yang *et al*., 2013). In contrast, resistance to this antibiotic was lower than 10% in other studies (Kikuvi *et al*., 2010). Similarly, all *Salmonella* isolates showed resistance to lincomycin, despite few reports describe resistance to this antibiotic (Arroyo and Arroyo 1995; Fierro *et al.,* 2011). De Geeter *et al*., (1976) reported that the use of lincomycin in pig diets did not affect the spread of *S.* Typhimurium through the feces. In addition, high resistance to clindamycin—a lincosamide antibiotic—has been reported in *Salmonella* spp. strains (Harakeh *et al.,* 2005; Thakur and Bajaj, 2006). Crossed resistance has been demonstrated through *linF* gene expression (Achard *et al.*, 2005), which could partially explain the high resistance observed by the strains in the present study.

Antibiotic resistance by *Salmonella* isolates to nalidixic acid was also high (32%). Other researchers have reported similar results (35.8%; McDowell *et al*., 2007). Our results appear to be higher than values reported by Mueller-Doblies *et al*. (2013), who found less than 9% *Salmonella* isolates resistant to this antibiotic in Great Britain and 2.1% in Ireland (McDowell *et al.,* 2007). In contrast, Nwachukwu *et al*. (2010) reported 87.5% resistance in Nigeria, and Yang *et al*. (2013) found 77.6% resistance to nalidixic acid in China.

Differences were also found for chloramphenicol, gentamycin, and ampicillin resistance. Values were

lower (4%) in the present study compared to those reported by Nwachukwu *et al*. (2010), Piras *et al*. (2011), Mueller-Doblies *et al*. (2013), and Yang *et al*. (2013).

Salmonella isolates were resistant to sulfamethoxazole/trimethoprim (12%). Mueller-Doblies *et al.* (2013) and Yang *et al.* (2013) reported higher resistance to both antibiotics (47.6% and 83.6%, respectively). On the other hand, *Salmonella* showed no resistance to ciprofloxacin, kanamycin, and apramycin, in contrast to what has been reported by Yang *et al*. (2003; 24.3%) and McDowell *et al*. (2007; 1.6%).

In conclusion, Derby, Muenster, and Typhimurium serotypes are the predominant *Salmonella* isolates from slaughterhouses and butcher's shops in Tolima. These serotypes were isolated from carcasses as well as from equipment used at pork butcheries. *Salmonella* isolates showed multi-resistance to various antibiotics and this finding constitutes an alert signal and an important issue that needs to be addressed by the national institute of surveillance of drugs and foods (INVIMA) which regulates the use of antimicrobial agents in the pig production chain.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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