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PREVALENCE, ANTIMICROBIAL RESISTANCE PROFILE AND COMPARISON OF METHODS FOR THE ISOLATION OF SALMONELLA IN CHICKEN LIVER FROM ARGENTINA

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Abstract

This study was conducted to estimate the apparent prevalence of *Salmonella* spp. in chicken livers obtained from markets in Entre Ríos, Argentina, using two culture methods (preenrichment and direct selective agar plating). We also determined the antimicrobial resistance of *Salmonella* isolated strains and evaluated the performance of the two culture methods and selective-differential plating media used for *Salmonella* isolation. Of 666 chicken livers studied, 32 organs (4.8%) related to 4 poultry slaughterhouse companies were positive for *Salmonella sp.* using one or two culture methods. Fifty *Salmonella* strains were isolated from the positive liver samples and were typed into 3 serovars: *S.* ser. Schwarzengrund (78%), *S.* ser. Enteritidis (18 %), and *S.* ser. Typhimurium 4(%). More than one *Salmonella* serovar was found in livers belonging to two chicken slaughterhouse companies. All strains were susceptible to all antibiotics tested, with the exception of erythromycin (100% resistant) and streptomycin (22% intermediate sensitivity).. Overall, 32 (4.80%) and 3 (0.45%) of the chicken liver samples were positive for *Salmonella* sp. in pre-enrichment method and direct selective agar plating method, respectively; these percentages were significantly different (P=0.0001; kappa= 0.16). There was also a statistical difference in relative accuracy, sensitivity and negative predictive value between the pre-enrichment method and the direct selective agar plating method; the first had greater values for these parameters than the direct selective agar plating method. These parameters were statistically different between MacConkey agar (MCA) and modified lysine iron (MLIA) in the two culture methods; the second had greater values than MCA for both culture methods. This study shows that even though serovars that are important for public health were isolated, the prevalence of *Salmonella* sp. is low in chicken livers from Entre Rios, Argentina. The isolated strains do not have multiresistance patterns. Furthermore, the preenrichment method and MLIA

are superior to the direct selective agar plating method and MCA for *Salmonella* sp. isolation from chicken liver samples, respectively.

1. Introduction

Commercially grown chickens process large amounts of feed. The liver carries out a large number of important digestive, metabolic and excretory activities, all of which have a significant role on the health and productivity in poultry (Dutta, 2010). Because it is rich in iron, zinc and some vitamins, chicken liver is a food source and it is used in different recipes, such as rice, soup or sauces (Ministry of Agriculture, Livestock and Fisheries of the Republic of Argentina , 2014). Giblets (edible viscera) includes the chicken liver, heart, gizzard and neck (Argentine Food Code, 2017).

Giblets (predominately the liver) may be contaminated with bacteria, for example *Salmonella* (Molla & Mesfin, 2003; El-Aziz, 2013), which presents an important global public health problem, with substantial morbidity, as well as having a significant economic impact (Sharma & Carlson, 2000). Since the liver is the predominate organ involved, it is the preferred organ to culture to detect bacterial contamination (Gast, 2013). Poultry meat and its derivatives are among the food products that cause the most concern to public health authorities, because of the associated risks of bacterial food poisoning. Contamination with *Salmonella* in poultry products can occur at any step along the food chain, which consists of production, processing, distribution, retail marketing, handling and preparation (Dookeran, Baccus-Taylor, Akingbala, Tameru, & Lammerding, 2012). The modernization of chicken farms and globalization of the bird breeding trade have also have played a role in the bacterial contamination of poultry products (Velge, Cloeckeart, & Barrow., 2005).

Due to their widespread use, numerous bacteriological culture media (selective enrichment broths and selective agar plates) are used to monitor *Salmonella* in food and food ingredients. The media may contain inhibitors to stop or delay the growth of non-target

organisms, have particular substrates that only the target bacteria can degrade, or that confer a particular color to the growing colonies (Manafi, 2000). However, the process of isolating *Salmonella* spp. is to some extend prone to failure. Depending on the type of competitive bacteria, detection of occasional colonies of *Salmonella* may be easier if the appropriate plating medium is used. Unfortunately, the composition of the flora is never known in advance; therefore, the appropriate plating medium may not be used for culture (Busse, 1995). Numerous agar media are available for the isolation of salmonellae so, at least, two different media, preferable with dissimilar indicator systems for differentiating this bacteria from other organisms, should be used (Gast, 2013).

The antimicrobials are used by the poultry industry to enhance growth and feed efficiency and to reduce bacterial disease. Antibiotic residues in food of animal origin produces a potential threat for direct toxicity in humans (cancer, allergic reaction, etc.), and low levels of antibiotic exposure may result in the alteration of microflora and the possibility of antibiotic resistance (Ahaduzzaman, Hassan, Alam, Islam & Uddin .., 2014; Hassan et al., 2014). During the past few decades, there has been a growing public health concern over the worldwide emergence of antibiotic-resistant strains of a number of pathogenic bacteria, including *Salmonella* (Capita &Alonso-Calleja, 2013). Although most cases of human salmonellosis are self-limiting and typically resolved in five to seven days without antimicrobial treatment, antibiotic therapy may be necessary for severe cases, extraintestinal disease or if a person is immunocompromised (Berrang et al., 2009). In this scenario, *Salmonella* strains that are resistant to antimicrobials are especially threatening because they may compromise the effective treatment of human salmonellosis. People infected with antibiotic-resistant strains are more likely to suffer an adverse health event such as prolonged illness, increased severity of illness, hospitalization or

death than those infected with susceptible strains (Cook et al., 2009). Recently, the World Health Organization (2017) published a global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics**,** where *Salmonella* spp. fluoroquinolone-resistant is considered a priority 2 (high). Therefore, the monitoring of antimicrobial resistance is essential for providing information on the magnitude of, and trends in, resistance, and to plan and monitor the effect of targeted interventions.

In Argentina, the National Zoonotic Disease Control Program of the Ministry of Health, created in 2011, has incorporated salmonellosis into the main zoonotic diseases of the country (Ministry of Health, 2011). Different studies in this country show the importance of *Salmonella* ser. Enteritidis (SE) and *S.* ser. Typhimurium (ST) in child and adult gastroenteritis (Mancini, 2013,Torres, Sánchez & González ., 2016). No studies have yet been conducted on chicken livers in Argentina, identifying *Salmonella* prevalence and serovars and their antibiotic resistant profile. Therefore, the present work was conducted to 1) estimate the apparent prevalence of *Salmonella* spp. on chicken livers obtained from markets in Entre Ríos, Argentina, using two culture methods, 2) evaluate the performance of two culture methods and selective-differential plating media used for *Salmonella* isolation, and 3) determine the antimicrobial resistance of *Salmonella* isolated from chicken liver.

2. Materials and Methods

2.1. Sampling

Since no previous studies had been performed, a sample size of 666 chicken livers was determined, with an absolute error of 5%, a 99% confidence level, and an expected prevalence of

50%. Frozen chicken livers or giblets were collected from different retail markets associated with 9 poultry slaughter companies in 3 counties (Colón, Gualeguaychú, Uruguay) of Entre Rios, Argentina, between October 2015 and May 2016. Samples were labeled and transported in ice chests to the INTA Laboratory of Poultry Health (Concepción del Uruguay, Entre Ríos) for *Salmonella* sp. culture.

2.2. Salmonella spp. isolation and identification

Frozen chicken livers and giblets were thawed either in the refrigerator (2-8°C) for 18-24 hours or at room temperature (22-25ºC) for 4 to 5 hours before analysis. In the case of the giblets, the liver was separated from the heart, gizzard and neck using sterile procedures. Aseptic techniques were followed for all steps to avoid contamination. Once thawed, the imprint (direct selective agar plating) method was used for *Salmonella* isolation by direct pressure of the cut surface of a liver sample onto MacConkey agar (MCA; Acumedia, Michigan, US) and modified lysine iron agar (MLIA). The plates were streaked with a sterile loop, then incubated at 35 ± 2ºC for 18-24 h. The composition of MLIA (g/l) was lysine iron agar 34.5 g (Acumedia), bile salt N°3 1.5 g (Britania, Buenos Aires, Argentina), lactose 10 g (Anedra, Holland), sucrose 10 g (Biopack, Buenos Aires, Argentina), sodium thiosulfate 6.76 g (Biopack), and ferric ammonium citrate 0.3 g (Carlo Erba, Milano, Italy). If there was no bacterial growth, the plates were then reincubated for a total time of 48 hours. Additionally, 1- 2 g of each liver was weighed separately and placed in a sterile tube, in which buffered peptone water (Acumedia) was added 1:10 (weight/volume), homogenized using a vortex mixer and then incubated at 35± 2°C for 18- 24 h. (preenrichment method). One milliliter of incubated broth was then transferred to 10 mL of tetrathionate broth base (Acumedia), in addition to 20 mL/L of iodine potassium iodide solution (6 g of iodine -

Anedra, Chile-; 5 g of potassium iodide -Laboratorios Olivieri, Argentina-; 20 mL of demineralized water), brilliant green 0.1% (Sigma, Steinheim, Germany), and 40 mg/mL of novobiocin (Sigma), and incubated at 35 ± 2°C for 18-24 h. Afterwards, a loopful of tetrathionate broth was streaked on MCA (Acumedia) and MLIA, and incubated at 35 ± 2°C for 18-24 h.–Following the same procedure, if there was no bacterial growth the plates were then reincubated for a total time of 48 hours.

Two presumed *Salmonella* colonies on each selective-differential agar plate were biochemically confirmed using triple-sugar iron agar (Britania), lysine iron agar (Acumedia), Simmons citrate (Oxoid, Basingtoke, UK), sulfide indole motility medium (Britania), phenylalanine agar (Hi-Media, Bombay, India) and ortho-nitrophenyl-β-galactoside (ONPG) test (Britania). All *Salmonella* isolations were preserved on nutritive (Acumedia) slant agar until serotyping, which was carried out according to the White-Kauffmann-Le Minor scheme, using somatic (AgO) and flagellar (AgH) antigens (Grimont &Weill, 2007). A liver was considered positive to *Salmonella* sp. when this bacterium was isolated from at least one colony on a selective-differential agar plate.

2. 3. Analysis of performance criteria of methods and differential-selective agars in poultry liver samples

The performance of the two culture methods and differential-selective agars were evaluated for relative accuracy (RAc), sensitivity (RSe), specificity (RSp), positive predictive value (RPPV), negative predictive value (RNPV), and agreement (Kappa coefficient and

McNemar's test) in chicken liver samples. For this study, relative true positive is defined as a sample where *Salmonella* sp. is detected in at least one differential selective agar. Relative true negative is defined as a sample where *Salmonella* spp. was not detected in any differential-selective agar. Kappa coefficients were summarized, according to Dawson and Trap (2004), as excellent agreement (0.93 to 1.00), very good agreement (0.81 to 0.92), good agreement (0.61 to 0.80), fair agreement (0.41 to 0.60), slight agreement (0.21 to 0.40), poor agreement (0.01 to 0.20), and no agreement (<0.01). McNemar's test was calculated using a chi-square approximation at $P \leq 0.05$ (GraphPad Software, 2017).

2.4. Antimicrobial sensitivity testing

The antibiotic susceptibility test was performed by the standard disk diffusion method using Mueller-Hinton agar (Difco™, Sparks, MD, USA) and the results were interpreted in accordance with the criteria of the National Committee for Clinical Laboratory Standards (2013, 2015). The isolates were screened for resistance to the following antibiotics: amikacin (30 µg); ampicillin (10 µg); amoxicillin/clavulanic acid (30 µg); fosfomycin (50 µg); colistin (10 µg); tetracycline (30 µg); florfenicol (30 µg); enrofloxacin (10 µg); gentamicin (10 µg); erythromycin (15 µg); suphamethoxazole/trimethoprim (25 µg); doxycycline (30 µg); neomycin (30 µg); cephalothin (30 µg); norfloxacin (10 µg); amoxicillin (10 µg); kanamycin (30 µg); ciprofloxacin (5 µg); cefixime (5 µg); cefotaxime (30 µg); cefoxitin (30 µg); ceftazidime (30 µg); chloramphenicol (30 µg); imipenem (10 µg), nalidixic acid (30 µg), streptomycin (10 µg), tigecycline (15 µg), azithromycin (15 µg), cefpodoxime (19 µg); and fosfomycin/tylosin (160 µg/40 µg). All the antibiotic disks except fosfomycin/tylosin (FOSBAC PLUS T-BEDSON™,

Britania) were purchased from Oxoid. The zone diameter breakpoint used for fosfomycin/tylosin was the same as fosfomycin.

3. Results

3.1. Apparent prevalence of *Salmonella* in chicken liver obtained from markets in Entre Ríos, Argentina.

Of the 666 poultry livers studied by two culture methods, 32 organs (4.8%) positive for *Salmonella* sp. were from 4 poultry slaughterhouse companies. *Salmonella* sp was detected in either one or two culture methods. However, 24 *Salmonella* sp. positive samples were found in liver obtained from markets associated with one chicken slaughterhouse company (Table 1).

Fifty *Salmonella* strains were isolated from the positive liver samples and were typed into 3 serovars: *S.* ser. Schwarzengrund (78%), SE (18%), and ST (4%). Forty-seven strains were isolated from preenrichment and 3 strains from direct selective agar plating method. More than one *Salmonella* serovar was found in livers from two chicken slaughterhouse companies, while only one serovar was found in livers from the other two chicken slaughterhouse companies (Table 2).

3.2. Performance of two *Salmonella* culture methods and selective-differential plating

Because of the absence of false positive samples, the RSp and RPPV for the two culture methods and the two plating media in preenrichment method and MLIA in direct selective agar plating method was 1. However, no samples were positive for *Salmonella* sp using MCA in direct selective agar plating method, so RSp was 1, and RPPV was indeterminate for this media and method.

Overall, 32 (4.80%) and 3 (0.45%) of the 666 chicken liver samples were positive for *Salmonella* sp. in the preenrichment method and direct selective agar plating method, respectively; these percentages were significantly different ($P < 0.05$). Although the three liver samples, which were *Salmonella* sp. positive by direct selective agar plating method, were also positive by the preenrichment method, the agreement between the two methods was poor. There was also a statistical difference in RAc, RSe and RNPV between preenrichment method and direct selective agar plating method. Preenrichment had greater values for these parameters than the direct selective agar plating method (Table 3).

The results of RAc, RSe, RNPV and the agreement (McNemar's test and Kappa coefficient) calculation for the selective-differential media in the two culture methods used are shown in Table 4. RAc, RSe and RNPV were statistically different between MCA and MLIA in the two culture methods; the second had greater values for these parameters than MCA for both culture methods. Overall, 15 (2.25%) and 32 (4.80%) samples yielded *Salmonella* sp. on MCA and MLIA for preenrichment method, respectively; these percentages were significantly different ($P< 0.05$). Since 15 liver samples were *Salmonella* sp. positive by MCA and MLIA, the agreement between the two methods was good. On the other hand, overall 0 (0%) and 3 (0.45%) samples yielded *Salmonella* sp. on MCA and MLIA for direct selective agar plating method, respectively; these percentages were not significantly different ($P >0.05$) and there was no agreement between MCA and MLIA.

3.3. Antimicrobial resistance of *Salmonella* isolated from chicken liver

From 50 *Salmonella* isolates studied, all strains were susceptible to all antibiotics tested with the exception of erythromycin and streptomycin. All *Salmonella* strains were resistant to

erythromycin, and only 11 isolates (22%), which belong to *S.* Schwazengrund, showed an intermediate sensitivity to streptomycin.

4. Discussion

In the present study, 666 samples of chicken liver were acquired from different retail markets in order to calculate the *Salmonella* spp. prevalence. There is no previously published data for the apparent prevalence of *Salmonella* sp. in chicken livers from Entre Rios and Argentina. The study revealed a prevalence rate of 4.8%. It is known that the microbial status of offal, such as livers, is an indicator of slaughterhouse hygiene practices (Kramer, Frost, Bolton, & Wareing, 2000). Varying *Salmonella* sp. incidence rates in chicken livers (Molla &Mesfin, 2003; Rodrigo, Adesiyun, Asgarali, & Swanston, 2006; Abdellah, Fouzia, Abdelkader, Rachida & Mouloud, 2009; El-Aziz, 2013) were reported between 1% and 40.0% in other countries (Morocco, Ethiopia, Egypt and Trinidad). The variation of *Salmonella* contamination in chicken livers can be due to differences in sampling techniques, number of samples studied, distribution of salmonellae in a lot examined, the detection methods employed, and cross-contamination during production, processing, distribution and retail marketing (Abu-Ruwaida, Sawaya, Dashti, Murad, & Al-Othman, 1994; Bryan &Doyle, 1995; Dominguez, Gomenz & Zumalacarregui, 2002; Harrison, Griffith, Tennant & Peters, 2001; Rusul, Khair, Radu, Cheah & Yassin, 1996; Russell & Walker, 1997; Uyttendaele, Debevere, Lips & Neyts, 1998).

Our study identified three serovars found in chicken liver: *S.* ser. Schwarzengrund, SE and ST; the first being the most prevalent serotype. Other studies related to chicken livers from giblets found ST, *S.* ser. Braenderup, *S.* ser. Kiambu, *S.* ser. Newport, *S.* ser. Montevideo and/or *S.* ser. Heidelberg (Molla &Mesfin, 2003; Rodrigo, Adesiyun, Asgarali, & Swanston, 2006

Abdellah, Fouzia, Abdelkader, Rachida & Mouloud, 2009; El-Aziz, 2013). *S.* ser. Schwarzengrund has been isolated from infectious in human and from animal sources (Bangtrakulnonth et al., 2004; Centers for Disease Control and Prevention, 2013;). This serovar is frequently associated with poultry production such as chickens (Chen, Hwang, Wang, Shih & Tsen, 2011), and laying hens (Poppe, Irwin, Messier, Finley, & Oggel, 1991, Soria et al. 2017). On the other hand, the isolation of ST and SE in our study indicates the public health significance of these serovar in contaminated chicken meat and meat products. This risk may be high if chicken meat or giblets are consumed undercooked or there is cross-contamination in the kitchen with *Salmonella* during meal preparation (D'Aoust, 1989; Scott, 1996; Uyttendaele, Debevere, Lips & Neyts,1998).

Multi-drug resistant *Salmonella*, which are resistant to two or more classes of antimicrobials, can be frequently encountered and may reduce the effectiveness of treatments (Frye &Fedorka-Cray, 2007). In reference of antibiotic resistance in *Salmonella*, it is focused mostly on the general increase in number of resistant strains, multiresistant spread of resistant clones or resistance genes and diminishing efficacy of some antimicrobial classes recently introduced into medical and veterinary practice (Chiu et al., 2002; Wray & Wray, 2000). Most of the trends were not identified in the present study, where all *Salmonella* isolates were only found to be resistant to erythromycin. Similar results were reported for SE and ST isolated from raw chicken meat at retail markets (Thung et al., 2016). Resistance to erythromycin (macrolide antibiotic) and penicillin has been reported as the most common resistance profile in retail meat products (Sallam, Mohammed, Hassan, & Tamura, 2014). Although, this could be due to improper usage or overuse of a particular antimicrobial causing resistance to occur, it is known that Gram-negative bacilli, as *Salmonella* sp., are usually intrinsically resistant to macrolide

antibiotics (Chambers, 2006; Nakajima, 1999). On the other hand, low levels of intermediate resistances to streptomycin (22%) were observed. Similar results were reported for SE and ST strains recovered from retail chicken meats (Yang et al., 2010; Thung et al., 2016).

In reference to detection method, the relative effectiveness of two culture methods and differential-selective agars for the recovery of *Salmonella* sp. from naturally contaminated chicken liver was compared in this work. We found a poor agreement between the two culture methods, and that the preenrichment method has a higher RSe, RAc and RNPV than a direct selective agar plating method. Although Valentin-Bon, Brackett, Seo, Hammack and Andrews (2003) used different selective plating media; they found that preenrichment method provided greater sensitivity for SE isolation in contaminated egg slurries than the direct selective agar plating method. It is well known that pathogens have to compete with indigenous microflora, which can interfere at the time of testing. Sequential enrichment in nonselective and selective media allows enhancing detection, recovery of sublethally injured *Salmonella* and also increase the number of cells (van Schothorst &van Leusden 1975; Chen, Anantheswaran & Knabel, 2001; Stephenson, Satchell, Allen & Andrews, 1991). *Salmonella* can be present in chicken liver in such small numbers that they cannot be detected by the direct method. Furthermore, the preenrichment method studied the presence of *Salmonella* sp. in the entire organ (internal and external), while the direct method studied only considers the internal part of livers. The results reported here are consistent with those studies regarding the use of preenrichment and direct enrichment for the recovery of foodborne pathogens (D'Aoust Sewell & Warburton, 1992; Hammack, Amaguaña, June, Sherrod, & Andrews, 1999; Powrie & Nakai, 1986; Stephenson, Satchell, Allen & Andrews, 1991).

Media for efficient isolation of salmonellae should be selective and have a reliable indicator system to reveal the presence of *Salmonella* colonies. These properties should be stable during the time of storage and use of the media (Rappold & Bolderdijk, 1979). In this work, best results were obtained with MLIA. Edwards and Fife (1961) introduced this medium to detect lysine decarboxylation and H_2S production of *Arizona* spp. Novobiocin, bile salts, lactose, and sucrose were added to enhance the selectivity and differentiation capacity of lysine iron agar. Differentiation of Enterobacteriaceae on MLIA is based on a color change from purple to yellow if lysine is not decarboxylated and lactose or sucrose or both are fermented. Typical salmonellae decarboxylate lysine and do not ferment lactose or sucrose; the purple color of the medium is maintained. H_2S-positive salmonellae grow with black centered colonies. The United States Department of Agriculture (USDA) lists DMLIA as part of the differentiation and confirmation *Salmonella* sp. steps starting with streaking to Brilliant Green Sulfa agar and either DMLIA or Xylose- Lysine Tergitol™ 4 agar (United States Department of Agriculture, 2017). Rappold and Bolderdijk (1979) found that MLIA increased *Salmonella* sp. isolation from mixed cultures of different Enterobacteriaceae compare to other selective plating media (Brilliant Green agar, Deoxycholate Lactose agar, *Salmonella*-Shigella agar, Hektoen Enteric Agar, and Xylose- Lysine Deoxycholate agar).

5. Conclusion

This study shows that, although serovars that have importance in public health were isolated, the prevalence of *Salmonella* sp. is low in chicken livers from Entre Rios, Argentina. Due to all *Salmonella* strains were susceptible to most antibiotics tested with the exception of erythromycin, multi-resistance is not a problem in theses strains, but it is inadvisable to use

erythromycin to treat salmonellosis. Furthermore, the preenrichment method and MLIA are superior to the direct selective agar plating method and MCA for the recovery of *Salmonella* sp. from chicken liver samples, respectively.

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Table 1. *Salmonella* sp. positive chicken liver samples obtained from markets related to nine chicken slaughterhouse companies in Entre Rios, Argentina by two culture methods.

Chicken slaughterhouse companies	*Salmonella* isolation	
	No. of livers	% positive (number of livers)
A	74	0 (0)
B	74	1.35 (1)
C	74	0 (0)
D	74	1.35 (1)
E	74	0 (0)
F	74	0 (0)
G	74	0 (0)
H	74	8.11 (6)
I	74	32.43 (24)
Total	666	4.80 (32)

Table 2. Serovars contamination in liver samples obtained from markets related to 4 *Salmonella*-positive chicken slaughterhouse companies in Entre Rios, Argentina.

Chicken slaughterhouse companies	*Salmonella* positive liver samples	
	No. serovars isolated	Serovars isolated (No. positive samples)
B	1	Enteritidis (1)
D	1	Typhimurium (1)
H	2	Schwazengrund (4), Enteritidis (2)
I	2	Schwazengrund (22), Enteritidis (2)
Total	3	Schwazengrund (26), Enteritidis (5), Typhimurium (1)

Table 3. Relativity accuracy (RAc), sensitivity (RSe), negative predictive value (RNPV), and agreement (Kappa coefficient and McNemar's test) for *Salmonella* sp. isolation in poultry liver samples collected from markets of Entre Ríos, Argentina, by pre-enrichment and direct selective agar plating methods. Confidence intervals are in parenthesis.

Method	RAc	RSe	RNPV	P value[1]	Kappa coefficient
Pre-enrichment	1.00[a] (1.00 – 1.00)	1.00[a] (0.91 – 1.00)	1.00[a] (1.00-1.00)	0.0001	0.16 (0.00 - 0.46)
Direct plating	0.96[b] (0.94 - 0.97)	0.09[b] (0.02 - 0.22)	0.96[b] (0.94-0.97)		

[1] Determined with McNemar´s chi-square test for paired samples.

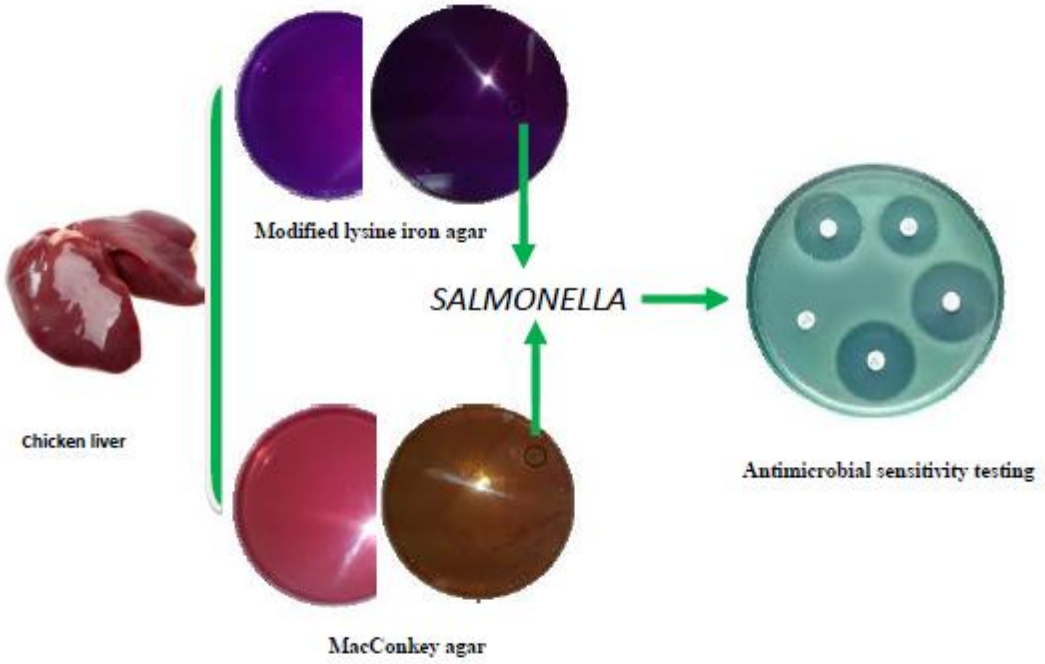
[a,b] Values followed by different letters in the same column are significantly different ($P < 0.05$).

Table 4. Relativity accuracy (RAc), sensitivity (RSe), negative predictive value (RNPV), and agreement (Kappa coefficient and McNemar's test) for selective-differential plating media used in *Salmonella* sp. isolation from poultry liver samples collected from markets of Entre Ríos, Argentina, by pre-enrichment and direct selective agar plating methods. Confidence intervals are in parenthesis. MacConkey agar: MCA; and modified lysine iron agar: MLIA.

Method	Plating media	No. of positive samples	RAc	RSe	RNPV	P value[1]	Kappa coefficient
Pre-enrichment	MCA	15	0.97[a] (0.96-0.98)	0.47[a] (0.31-0.64)	0,97[a] (0.96-0.98)	0.0001	0.63 (0.45-0.80)
	MLIA	32	1[b] (1.00 – 1.00)	1[b] (0.91 – 1.00)	1[b] (1.00-1.00)		
Direct plating	MC	0	1[a] (1.00-1.00)	0[a] (0.00-0.53)	1[a] (0.99-1.00)		
	MLIA	3	1[a] (0.99-1.00)	1[b] (0.47-1.00)	1[a] (1.00-1.00)	0.2482	0 (0.00-1.00)

[1] Determined with McNemar´s chi-square test for paired samples.

[a,b] Values followed by different letters in the same column for each method are significantly different ($P \leq 0.05$).



Graphical abstract

Highlights

-The prevalence of *Salmonella* sp. is low in chicken livers from Entre Rios, Argentina.

- Multi-resistance is not a problem in *Salmonella* strains isolated from chicken livers.

- The preenrichment method is superior to the direct selective plating method.

-The MLIA is superior to MCA for *Salmonella* sp. isolation from chicken liver.

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