Diagnostic Microbiology and Infectious Disease 95 (2019) 93–98

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Antimicrobial Susceptibility Studies

Emergent multidrug-resistant nontyphoidal *Salmonella* serovars isolated from poultry in Brazil coharboring $bla_{CTX\text{-}M\text{-}2}$ and *qnr*B or $bla_{CMY\text{-}2}$ in large plasmids

Rafael Antonio Casarin Penha Filho [a,*], Joseane Cristina Ferreira [b], Ana Maria Iba Kanashiro [c], Angelo Berchieri Junior [a], Ana Lúcia da Costa Darini [b]

[a] School of Agricultural and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, SP, Brazil, 14884-900
[b] School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (USP), Ribeirão Preto, SP, Brazil, 14040-903
[c] Instituto Biológico de Descalvado, Descalvado, SP, Brazil, 13690-000

ARTICLE INFO

Article history:
Received 22 October 2018
Received in revised form 1 March 2019
Accepted 4 April 2019
Available online 29 April 2019

Keywords:
Foodborne
Salmonellosis
Infection
Antimicrobial resistance

ABSTRACT

The number of foodborne gastroenteritis caused by nontyphoidal *Salmonella* (NTS) worldwide is estimated to be 80.3 million each year. Currently, antimicrobial-resistant NTS disseminated in the animal environment increases the risk of aggravated foodborne outbreaks. Poultry are important source of foodborne NTS infections. This study was conducted to evaluate the phenotypic and genotypic characteristics of 83 NTS isolates from poultry, classified within 36 different serovars. The most prevalent serovar was *S.* Schwarzengrund (10/83), from which 8/10 were multidrug resistant (MDR). The antimicrobial susceptibility testing showed a total of 18 MDR isolates, from which 8/18 coharbored $bla_{CTX\text{-}M\text{-}2}$ and *qnr*B5. The genes *qnr*B5, $bla_{CTX\text{-}M\text{-}2}$, *qnr*B2, or $bla_{CMY\text{-}2}$ were also found alone in other MDR isolates. All resistance genes were harbored in large plasmids, ranging from 30 to 270 kb. The pCoIE replicon was present in 8 MDR isolates; however it was not associated with resistance. IS*CR1* and class I integron structures were always associated with $bla_{CTX\text{-}M\text{-}2}$.

1. Introduction

Currently, more than 2600 different serovars of *Salmonella* spp. have been identified (Issenhuth-Jeanjean et al., 2014); however, ??a restrict number has been frequently involved in foodborne infections, such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium (*S.* Typhimurium) or *S.* Enteritidis (Poirel et al., 2012). Nontyphoidal *Salmonella* (NTS) infections are caused by flagellated, non–host-specific serovars and frequently occur as a zoonosis. The outcome of infection can be characterized by different symptoms, ranging from mild, self-limiting diarrhea to a severe invasive septicemic infection, depending on different factors, such as infecting serovar, bacterial inoculum, age of host, genes present in *Salmonella* Pathogenicity Islands, invasiveness, and host adaptation of the isolate (Mead et al., 1999). NTS infections in humans are underdiagnosed in many countries; however, it is estimated that 1.4 million infections, 15,000 hospitalizations, and approximately 400 deaths occurs annually only in the United States of America (Voetsch et al., 2004). Globally, the estimated number of infections reaches 93 million (Majowicz et al., 2010).

The antimicrobial resistance reported in NTS isolated from food-producing animals has become an increasing concern (Scott, 2003). It has been estimated that 86% of total number of NTS infections worldwide are foodborne. Eggs and poultry meat have been frequently associated to outbreaks in humans (Ford et al., 2018; Majowicz et al., 2010).

During the last 15 years, resistance genes that were commonly found in commensal bacteria causing nosocomial infections have emerged in pathogenic enterobacteria, such as *Salmonella* sp. (Liebana et al., 2013). In the present days, the emergence of antimicrobial resistance among different NTS serovars, especially to fluoroquinolones and third-generation cephalosporins, is a growing concern for public health because of the reduced drugs options for treatment available for human or animal health (Aboufaycal et al., 2007). The prophylactic usage of antimicrobial drugs contributed to the emergence and maintenance of resistant bacteria in the animal environment, and this procedure may increase the risks of successful establishment of resistant NTS strains in commercial farms (Liebana et al., 2013; Tollefson and Miller, 2000).

The constant selective pressure by antibiotic usage increases the prevalence rates of resistance genes in the environment, especially those harbored in mobile genetic elements (MGE), such as plasmids. Furthermore, antimicrobials also increase the risk of transfer of the MGE from commensal microbiota to pathogenic enterobacteria, such as *Salmonella* (Nordmann et al., 2012). Plasmid-mediated quinolone resistance (PMQR) is frequently reported in NTS (Ford et al., 2018; Garcia-Fernandez et al., 2009); moreover, plasmids up to 50 kb carrying

* Corresponding author. Tel.: +55-16-3209-7359.
E-mail address: rafaelpenha12@yahoo.com.br (R.A.C. Penha Filho).

https://doi.org/10.1016/j.diagmicrobio.2019.04.003
0732-8893/

qnr genes have been shown to be transferable to *E. coli* and *Salmonella* sp. (Garcia-Fernandez et al., 2009).

The maintenance and dissemination of resistance genes by enterobacteria in the animal environment have been attributed mainly to commensal *E. coli*; however, antimicrobial resistant isolates of *Salmonella* sp. have been increasingly reported (Liebana et al., 2013). Previous works reported the presence of resistance plasmids carrying different CTX-M genes and $bla_{CTX\text{-}M\text{-}2}$ inserted into the chromosome of *E. coli* isolated from the microbiota of apparently healthy food-producing chickens (Ferreira et al., 2014; Hirai et al., 2013). In this study, NTS isolates from 6 different poultry farms in Brazil were serotyped to evaluate the prevalence of serovars, and the antimicrobial susceptibility was deeply investigated. The resistance genes to β-lactams and quinolones were determined. Furthermore, the phenotype and genotype of resistant isolates were characterized, including the epidemiological and molecular attributes, multidrug resistance (MDR) profiles, PFGE types of the isolates, genetic environment of resistance genes, and plasmid types involved in the dissemination of resistance.

2. Materials and methods

2.1. Bacterial isolates

Between 2009 and 2012, 83 *Salmonella enterica* isolates were isolated from broiler chickens from 6 different farms and 1 slaughterhouse located in the State of Sao Paulo or Goias, Brazil (Table 1). The distance among farms ranged from 200 to 1000 km. Isolates were characterized with biochemical tests using API 20E strips (bioMérieux, France) and serotyped at Adolfo Lutz Institute, SP, Brazil.

2.2. Antimicrobial susceptibility testing

All *Salmonella* isolates were submitted to antimicrobial susceptibility test using the disk diffusion method, and the results were analyzed according to Clinical and Laboratory Standards Institute (CLSI) documents M100-S22 and M31-A2. Fifteen antibiotics were tested, including beta-lactams amoxicillin-clavulanic acid (AMC), cefotaxime (CTX), ceftazidime (CAZ), ceftiofur (EFT), cefoxitin (FOX), cefepime (FEP), aztreonam (ATM), and ertapenem (ETP) and nonlactam antibiotics nalidixic acid (NAL), ciprofloxacin (CIP), enrofloxacin (ENR), tetracycline (TET), trimethoprim-sulfamethoxazole (SXT), chloramphenicol (CHL), and florfenicol (FFC). The isolates were classified as MDR when not susceptible to at least 3 classes of antimicrobial drugs, as previously defined (Magiorakos et al., 2012).

Table 1
Prevalence of *Salmonella* serovars isolated from chicken.

Serovar	No. of isolates (%)	Serovar	No. of isolates (%)
S. Schwarzengrund	10/83 (12%)	*S.* 4,5,12:R	1/83 (1.2%)
S. Infantis	7/83 (8.5%)	*S.* Belem	1/83 (1.2%)
S. 6,7:Z10	6/83 (7.3%)	*S.* Hadar	1/83 (1.2%)
S. Mbandaka	5/83 (6.0%)	*S.* Heidelberg	1/83 (1.2%)
S. Newport	5/83 (6.0%)	*S.* 6.7:R:-	1/83 (1.2%)
S. Havana	4/83 (4.9%)	*S.* Albany	1/83 (1.2%)
S. Senftenberg	4/83 (4.9%)	*S.* Anatum	1/83 (1.2%)
S. 6,8:CH	3/83 (3.6%)	*S.* Agona	1/83 (1.2%)
S. Brandenburg	3/83 (3.6%)	*S.* Minnesota	1/83 (1.2%)
S. Tennessee	3/83 (3.6%)	*S.* O:13,23:I	1/83 (1.2%)
S. Braenderup	2/83 (2.4%)	*S.* O:16,14,24,25:Y	1/83 (1.2%)
S. Cerro	2/83 (2.4%)	*S.* O:3,10	1/83 (1.2%)
S. Corvallis	2/83 (2.4%)	*S.* O:9,12:- 1,5	1/83 (1.2%)
S. Enteritidis	2/83 (2.4%)	*S.* Orion	1/83 (1.2%)
S. Kentucky	2/83 (2.4%)	*S.* Paratyphi B	1/83 (1.2%)
S. Montevideo	2/83 (2.4%)	*S.* Saintpaul	1/83 (1.2%)
S. Typhimurium	2/83 (2.4%)	*S.* San Diego	1/83 (1.2%)
S. 4,12:I	1/83 (1.2%)	*S.* Worthinghton	1/83 (1.2%)

2.3. Screening and identification of β-lactamase genes

Cefotaxime-resistant isolates were tested to investigate the production of ESBL using the double disk synergy test with cefotaxime, ceftazidime, and amoxicillin associated to clavulanic acid (Oxoid, UK) and further tested by PCR for detection of β-lactamase–encoding genes bla_{TEM}, bla_{SHV}, bla_{OXA}, $bla_{CTX\text{-}M}$, and *amp*C, as previously described (Bernabeu et al., 2012; Perez-Perez and Hanson, 2002; Woodford et al., 2006). After PCR, the amplicons were sequenced on both strands using the automatic sequencer ABI 3100 (Applied Biosystems, UK). Results were compared with sequences available at databases from http://www.lahey.org/studies/ and http://blast.ncbi.nlm.nih.gov/Blast.cgi.

2.4. Genetic environment of bla genes

The characterization of the genetic environment of upstream and downstream flanking regions from the *bla* genes was performed by PCR, as previously described, to evaluate the presence of MGE associated to the resistance gene (Dhanji et al., 2010). Briefly, reverse or forward primers of the resistance gene were used in combination with primers for the MGE, ISCR1, and class 1 integron structures.

2.5. Detection of PMQR genes

The resistance to quinolones and fluoroquinolones associated to the presence of PMQR-encoding genes *qnr*A, *qnr*B, *qnr*S, and *aac*(6′)-Ib-cr was investigated by PCR in MDR *Salmonella* sp. isolates using previously described methods (Cattoir et al., 2007).

2.6. Pulsed field gel electrophoresis (PFGE)

PFGE type of all ESBL-producing or positive PMQR *Salmonella* isolates was determined according to the protocol developed by the Centers for Disease Control and Prevention (http://www.cdc.gov/pulsenet/protocols.htm). Profiles were analyzed with BioNumerics fingerprinting Software (AppliedMaths, Belgium). Classification of isolates within the same PFGE-type considered a minimum Dice similarity index of 85%.

2.7. Plasmid replicon typing

Plasmids present in MDR isolates were characterized by the PCR-based replicon typing (PBRT) method, as described previously (Carattoli et al., 2005). Additionally, ColE plasmids were searched in all isolates showing resistance to fluoroquinolones (Garcia-Fernandez et al., 2009). Nontypeable plasmids by PBRT were designated as NT in Table 2.

2.8. Characterization of the resistance plasmids

The location of ESBL-, AmpC-, or PMQR-encoding genes in the respective carrying plasmids were characterized by *S1*-PFGE followed by Southern blot and hybridization with specific probes, as described previously (Sambrook et al., 1989). The specific DNA probes were obtained with AlkPhos Direct Labeling and Detection System with CDP-Star (GE Healthcare, UK), according to manufacturer instructions.

3. Results

3.1. Prevalence of Salmonella enterica serovars

The 83 *Salmonella enterica* isolates from commercial poultry were serotyped and classified among 36 different serovars. The prevalence of each serovar isolated from chickens is presented in Table 1. *S.* Schwarzengrund was the most prevalent serovar found (10/83; 12%) followed by *S.* Infantis (7/83; 8.5%). *S.* Enteritidis and *S.* Typhimurium

Table 2
Phenotypic and genotypic characteristics of MDR *Salmonella* isolates from chickens.

Isolate n.	Serovar	State / local	Antibiotic resistance	PFGE	Resistance gene	Genetic environment	Plasmid	
							Replicon	Size (kb)
24,736	Schwarzengrund	GO / Farm 1	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	A	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A	280
24,737	Newport	GO / Farm 1	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	B	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A	280
24,739	Schwarzengrund	GO / Farm 1	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	A	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A pColE	280
24,740	Schwarzengrund	GO / Farm 3	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	A	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A	280
24,742	Schwarzengrund	GO / Farm 4	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	A	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A	280
24,743	Newport	GO / Farm 2	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	B	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A	280
24,745	Schwarzengrund	GO / Farm 2	CIP ENR,NAL,TET,SXT	A	qnrB5	-	HI2A pColE	270
24,748	Schwarzengrund	GO / Farm 1	CTX,CAZ,CTF,FEP,ATM,TET,SXT	A	CTX-M-2	ISCR1 + Int class I	HI2A	280
24,749	Schwarzengrund	GO / Farm 1	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	A	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A pColE	280
24,752	Newport	GO / Farm 1	CTX,CAZ,CTF,FEP,ATM,CIP,TET,SXT	B	CTX-M-2	ISCR1 + Int class I	HI2A pColE	280
24,757	Schwarzengrund	GO / Farm 2	CTX,CAZ,CTF,FEP,ATM,CIP ENR,NAL,TET,SXT	A	CTX-M-2, qnrB5	ISCR1 + Int class I	HI2A pColE	280
A04	Albany	SP / Slaughterhouse A	CIP ENR,NAL,TET,SXT	D	NI	NI	NI	NI
A28	Albany	SP / Slaughterhouse A	FOX,CIP,ENR,NAL TET,SXT	D	NI	NI	NI	NI
A14	Worthinghton	SP / Slaughterhouse A	CIP,NAL,TET,CHL	E	NI	NI	NI	NI
B02	Senftenberg	SP / Slaughterhouse B	FOX,CIP,ENR,NAL,SXT	F	qnrB2	ND	HI2A pColE	280
B39	Kentucky	SP / Slaughterhouse B	FOX,CIP, NAL,TET,SXT	C	qnrB2	ND	pColE	30
B41	Kentucky	SP /Slaughterhouse B	FOX,CIP,ENR,NAL,TET SXT	C	qnrB2	ND	pColE	30
81	Heidelberg	SP / Farm 5	CTX,CAZ,FOX,NAL,CIP, LEV,GEN,TET,SXT,CHL	G	CMY-2	ND	I1	150

CTX = cefotaxime; CAZ = ceftazidime; CFT = ceftiofur; FEP = cefepime; FOX = cefoxitin; ATM = aztreonam; NAL = nalidixic acid; CIP ciprofloxacin; LEV = levofloxacin; ENR = enrofloxacin; TET = tetracycline; GEN = gentamicin; CHL = chloramphenicol; SXT = trimethoprim-sulfamethoxazole; NI: not identified; ND = not determined. The replicon type and the size of the plasmids that carry resistance genes are underlined.

had the same prevalence rates (2/83; 2.4%) among *Salmonella* isolates evaluated.

3.2. Antimicrobial susceptibility

The percentage of resistance to the antibiotics tested among all 83 isolates was as follows: AMC (13.5%), CTX (13.5%), CAZ (13.5%), EFT (13.5%), FOX (6%), FEP (12%), AZT (14.5%), NAL (41%), CIP (52%), ENR (31%), TET (28%), SXT (20.5%), and CHL (1.2%). No isolate showed resistance to FFC and ETP. Among 83 isolates studied, 18 (21.7%) showed MDR phenotype. The serovars of *Salmonella* sp. showing MDR phenotype were *S.* Schwarzengrund (8/18), *S.* Newport, (3/18), *S.* Kentucky (2/18), *S.* Albany (2/18), *S.* Worthinghton (1/18), *S.* Senftenberg (1/18), and *S.* Heidelberg (1/18), as detailed in Table 2.

3.3. Characterization of β-lactamase genes and determination of genetic environment

The PCR revealed that 10/18 (56%) MDR isolates carried $bla_{\text{CTX-M-2}}$. Furthermore, the IS*CR1* element was found immediately upstream from the $bla_{\text{CTX-M-2}}$ gene. This arrangement was confirmed in all 10 isolates harboring $bla_{\text{CTX-M-2}}$ by PCR (size ~2.9 kb). The genetic environment of $bla_{\text{CTX-M-2}}$ revealed the *sul1* and integrase (*intI*) genes (7 kb amplicon) upstream from the resistance gene, confirming the association of $bla_{\text{CTX-M-2}}$ gene and IS*CR1* with a class 1 integron.

The phenotypic production of *amp*C β-lactamase was found in 1 *S.* Heidelberg isolate. The PCR and sequencing confirmed and showed the presence of $bla_{\text{CMY-2}}$ gene in this isolate. The genetic environment of this gene could not be determined.

3.4. Prevalence of PMQR genes in MDR isolates

PMQR-encoding genes were found in 12/18 MDR isolates (67%): 7 *S.* Schwarzengrund, 2 *S.* Newport, 2 *S.* Kentucky, and 1 *S.* Senftenberg. The sequencing of PCR amplicons revealed that 9 isolates carried *qnr*B5 gene (7 *S.* Schwarzengrund and 2 *S.* Newport) and 3 carried *qnr*B2. The genes *qnr*A, *qnr*S, and *aac*(6′)-Ib-cr were not found (Table 2).

3.5. PFGE-type of resistant NTS isolates

All 18 MDR *Salmonella* isolates were successfully typed by PFGE and were grouped into 7 PFGE types designated as A, B, C, D, E, F, and G. The classification of the isolates is available in Table 2, and the dendrogram of isolates from the same serovar is shown in Fig. 2. Among the isolates, 8 *S.* Schwarzengrund isolated from 4 different farms belonged to the same PFGE type A. The 3 MDR *S.* Newport isolates from 2 different farms were classified as PFGE type B. The 2 *S.* Kentucky isolates from the same slaughterhouse belonged to PFGE type C. The 2 *S.* Albany isolated at the same slaughterhouse belonged to PFGE type D, the *S.* Worthinghton was classified as PFGE type E, *S.* Senftenberg was classified as PFGE type F, and *S.* Heidelberg (CMY-2 producer) was classified as PFGE type G.

3.6. Analysis of resistance plasmids and resistance gene location

The results of S1-PFGE and Southern blot hybridization revealed that *qnr*B5 and $bla_{\text{CTX-M-2}}$ genes were co-located in the same plasmid in 80% (8/10) of the ESBL-producing isolates (Fig. 1). The replicons carrying ESBL and/or PMQR genes in 12 MDR isolates were characterized as HI2A by PBRT method; however, hybridization showed that resistance genes were located in large plasmids, with sizes that ranged from 30 to 270 kb. One *S.* Heidelberg isolate (n. 81) carried the $bla_{\text{CMY-2}}$ gene harbored in the Inc. I1 plasmid with approximately 150 kb. The replicon pColE was identified in 4 *S.* Schwarzengrund isolates (n. 24,739, n. 24,749, n. 24,745, and n. 24,757), 2 *S.* Kentucky isolates (B39 and B41), and 1 *S.* Senftenberg isolate (B02). The hybridization results of the plasmid sizes carrying the resistance genes are shown in Fig. 1 and Table 2.

4. Discussion

In this study, we noticed an increased isolation of other NTS serovars rather than *S.* Enteritidis and *S.* Typhimurium, which together represented only 9.7% of the isolates from poultry (Table 1), and none of these presented MDR profile. In the USA, the National Veterinary Services Laboratories reported that the most frequent serovar isolated in 2012 from clinical nonhuman sources was *S.* Typhimurium and those from nonclinical nonhuman sources were *S.* Kentucky (14.9%) followed by *S.* Enteritidis (13.2%) (Centers for Disease Control and Prevention (CDC) et al., 2015); however, in 2016, most isolated serovars

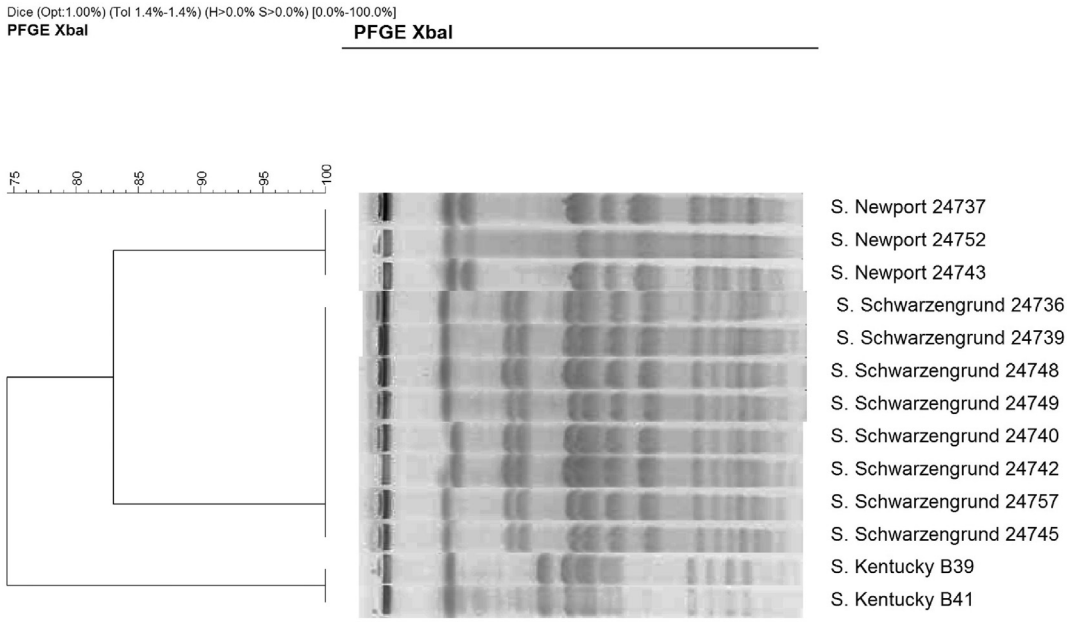

Fig. 1. Dendrogram of genomic similarity obtained by *Xba*I-PFGE of 19 ESBL and/or *qnr*-producing *Salmonella* isolates.

in these ranks were serovar 4,[5],12:i:- (14.75%) and *S.* Senftenberg (13%), respectively (Morningstar et al., 2016). Although the CDC *Salmonella* Annual Report shows *S.* Enteritidis as the most isolated serovar from human clinical samples, the second serovar in 2016 rank was *S.* Newport, surpassing *S.* Typhimurium in the last years (Centers for Disease Control and Prevention (CDC), 2016). Different factors may be involved in the prevalence variation of *Salmonella* serovars isolated from poultry. During the last decade, specific vaccines and biosecurity measures have been applied aiming specifically to control *S.* Enteritidis and *S.* Typhimurium in commercial poultry farms worldwide, including in Brazil, which may have contributed to decrease the prevalence of these 2 serovars, and consequently, the emergence of others may have occurred. However, it should also be considered the possibility that some serovars have higher capacity to acquire MGE involved in the antimicrobial resistance, which may contribute for their emergence in certain niches and environments. The most prevalent serovar found in poultry from Brazil was *S.* Schwarzengrund (12%; 10/83); furthermore, high rates of MDR could be noticed among these isolates, reaching 90% (9/10). This characteristic may have contributed to the successful establishment and dissemination of this serovar in the animal environment and different flocks in Goias State. Moreover, these isolates belonged to the same PFGE-type A and all carried *qnr*B5 or $bla_{\text{CTX-M-2}}$ or both genes harbored in a large plasmid of 280 kb (Table 2). Although little is known about *S.* Schwarzengrund molecular epidemiology, there is evidence that the stable carriage of MGE involved in antimicrobial resistance may cause the emergence of novel MDR *Salmonella* serovars, reaching or transcending the epidemiological relevance of *S.* Enteritidis and *S.* Typhimurium for animal and public health in some countries (Bai et al., 2016).

As shown in Table 2, the presence of both MGE IS*CR1* and class I integron was associated with a 280 kb plasmid in all *S.* Schwarzengrund and *S.* Newport isolates that harbored $bla_{\text{CTX-M-2}}$ (ESBL gene) and *qnr*B5 (PMQR gene). The sum of the sizes of both resistance genes (*qnr*B5 and $bla_{\text{CTX-M-2}}$) associated to IS*CR*1 and class I integron reached 10 kb. However, the *S.* Schwarzengrund isolate from farm 2, which carried only *qnr*B5 (n. 24,745), harbored this resistance gene in a plasmid of 270 kb without the MGE IS*CR1* and class I integron. These findings may suggest a possible participation of both MGE, class I integron and IS*CR*1, in the mobility and insertion of $bla_{\text{CTX-M-2}}$ into the large nontypeable resistance plasmids found in all isolates from farm 2. Class I integron structure (*IntI1*) was previously found associated with the dissemination of resistance genes in *Salmonella* isolates from human patients in Brazil. This structure was found in 55 *Salmonella enterica* isolates and was associated to different resistance genes, from which *addA1* was most frequently found, whereas $bla_{\text{CTX-M-8}}$ and $bla_{\text{CTX-M-9}}$ were found in 1 and 6 isolates, respectively (Villa et al., 2012). In the present work, from 83 *Salmonella* isolates evaluated, 10 harbored $bla_{\text{CTX-M-2}}$ gene, and the class 1 integron was associated with all these isolates. These findings suggest not only that $bla_{\text{CTX-M-2}}$ was disseminated in these farms but also that the MGE IS*CR1* and class I integron or the whole resistance plasmids were disseminated, considering the similarity among the plasmids isolated from different poultry farms.

The $bla_{\text{CMY-2}}$ gene was previously found in *S.* Heidelberg isolated from humans in Brazil; however, the resistance plasmid was not determined nor evaluated for an epidemiological evaluation (Villa et al., 2012). *S.* Heidelberg, frequently associated with invasive infections, is considered one of the most important MDR serotypes related with CMY-2 production in both poultry and humans in North America. The transmission of this gene in both human and poultry has been associated with diverse plasmid families but mainly *Inc*I1 and *Inc*A/C (Antunes et al., 2016). However, $bla_{\text{CMY-2}}$ has been reported in Brazil with low frequency. In this study, *S.* Heidelberg characterized as MDR also carried $bla_{\text{CMY-2}}$ harbored in *Inc*I1 plasmid; these findings suggest a potential risk of dissemination and successful establishment of this resistant isolate among poultry and humans, as previously reported with other MDR serovar (Bai et al., 2016). Especially considering the critical resistance profile of the *S.* Heidelberg isolate producing CMY-2 β-lactamase, which was only susceptible to carbapenem among all tested antimicrobials, as shown in Table 2. Moreover, the fact that the gene was harbored in a *Inc*I1 plasmid of 150 kb should also be considered an important factor contributing to the dissemination of this resistance gene in the field once this replicon is frequently associated with antimicrobial resistance in different enterobacteria, including *Salmonella* and *E. coli* (García-Fernández et al., 2008).

PMQR in *Salmonella* and *E. coli* is frequently associated with the presence of *qnr* family of genes harbored in pColE replicons (Garcia-Fernandez et al., 2009). Our analysis of the plasmid replicons carried by each *Salmonella enterica* isolate showed that pColE plasmids were co-resident with HI2A replicon in isolates of *S.* Schwarzengrund (n. 24,739, n. 24,745, n. 24,749, and n. 24,757), *S.* Newport (n. 24,752), and *S.* Senftenberg (n. B02). However, as demonstrated in Fig. 1 and

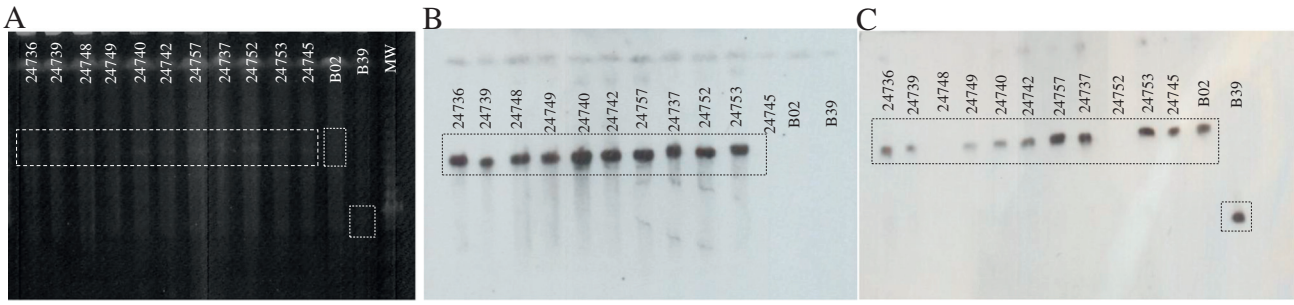

Fig. 2. Plasmid location of $bla_{CTX\text{-}M\text{-}2}$ and *qnr*B genes using probe hybridization. (A) PFGE profiles of plasmids from MDR *Salmonella* isolates after digestion with *S1* nuclease enzyme. (B) Southern blot and hybridization with $bla_{CTX\text{-}M\text{-}2}$ probe after *S1*-PFGE. (C) Southern blot and hybridization with probe for *qnr*B genes after *S1*-PFGE. MW: molecular weight marker.

detailed in Table 2, the hybridization showed that the *qnr* genes were not present in pColE replicons but in the same plasmids that harbored the ESBL-producing genes, Inc HI2A (Fig. 1). The *qnr*B5 gene was the most frequent, found in 9 isolates, from which 8 were also ESBL isolates. Considering that fluoroquinolones alongside with cephalosporins are the most important options to treat *Salmonella* infections, the association of PMQR and ESBL genes found in 8 MDR isolates is highly threatening and demonstrates the potential of any of these isolates to successfully disseminate in the animal environment. PMQR genes were found alone or associated to ESBL genes in 4 different *Salmonella* serovars: *S*. Schwarzengrund, *S*. Kentucky, *S*. Senftenberg, and *S*. Newport, always in large plasmids with approximately 280 kb.

The PFGE analysis of the *S*. Newport, *S*. Schwarzengrund, and *S*. Kentucky revealed that MDR isolates were clonally disseminated among different farms at the serovar level and at the pulsotype level with 100% similarity (Fig. 2). Considering the long distance between the location of each farm, the dissemination of these bacteria may have occurred both vertically, through contaminated eggs or horizontally, spread by common visitors and vehicles circulating in these sites. However, it is difficult to track the initial point of the dissemination considering the epidemiological complexity of *Salmonella* sp., which may involve even wild animals and birds.

Plasmids carrying resistance genes in commensal *E. coli* were shown to be successfully transferred *in vivo* to pathogenic bacteria, including *Salmonella* (Canton and Ruiz-Garbajosa, 2011). Although it is difficult to show under field conditions, the dissemination of resistance and exchange of genetic material between commensal and pathogenic bacteria may possibly be involved in the increased resistance rate found in *Salmonella*, especially if genes are harbored in plasmids. In the present work, we show the evaluation of resistance in *Salmonella* isolates from poultry and demonstrate the high level of resistance attributed to the presence of resistance genes in conjugative plasmids.

Conflict of interest statement

None to declare.

Acknowledgments

We would like to thank São Paulo Research Foundation (FAPESP) for the constant support for our research (grant no. 2014/14494-8). Rafael Penha Filho (FAPESP grants 2016/12293-0 and 2017/00620-0).

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