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Identification and antimicrobial susceptibilities of 21 strains of *Salmonella* from poultry

Yanhong Wang[1]*, Huijun Shao[1*], hualu Sun[1], Bai Wu[1], Jie Zhu[1], Wei Hang[1], Yan Liu[1] and Feng Xue[2]

[1]Key Laboratory of Animal Infectious Diseases of Ministry of Agriculture, College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu 225009, China.
[2]Animal, Plant & Food Inspection Center, Jiangsu Entry-exit Inspection and Quarantine Bureau of P.R.C., Nanjing 210001, China.

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A total of 21 *Salmonella* strains were isolated from clinical cases in Yangzhou, China, and the surrounding areas from Feb 2011 to Dec 2011. All isolates were screened by PCR amplification for InvA gene, which is specific to the *Salmonella* genus. Thus, twenty-one *Salmonella* were identified from the specimens. The isolates belonged to 6 serovars of *Salmonella*. Both *Salmonella typhimurium* and *Salmonella pullorum* were the dominant serovars isolated. Results of susceptibility test show that a large percentage of *Salmonella* were resistant to doxycycline (90.5%), polymyxin B (85.7%), streptomycin (85.7%), lomefloxacin (85.7%), and nalidixic acid (81.0%). The high resistance among these strains from infection animals suggested that essential measure such as infection control and drug rational use need to be taken.

Key words: *Salmonella*, isolation, identification, serovar, resistance.

INTRODUCTION

Salmonella is a heterogeneous species of Gram-negative enterobacteria and is a commensal of the intestine of many animal species. *Salmonella* a pathogen of significant importance in poultry, can cause septicemia and enteritis. *Salmonella* includes more than 2500 different serotypes (Dunkley et al., 2009; Foley and Lynne, 2008; Popoff et al., 2001). *Salmonella pullorum* and *Salmonella typhimurium* are the most prevalent serovars in the worldwide domestic birds (chichen, duck, geese), and may cause significant disease or death in poultry (Pan et al., 2010; Tizard, 2004). Contaminated poultry products are important sources of human *Salmonella* infections. Moreover, *S. typhimurium* is the most frequently reported serovars associated with human food-borne illnesses (Fearnley et al., 2011). Serovars *S. typhimurium* is most prevalent in geese and ducks. Occurrence of the same *Salmonella* serovars in humans, animals and food might indicate their epidemiological links (Hoszowski et al., 2012; He et al., 2011; Pan et al., 2010).

Antibiotic-resistant *Salmonella* are becoming increasingly of concern due to indiscriminate use of antibiotics in animal feeds as growth promoters and therapeutic agents, is a further threat to human and animal health (Forshell and Wierup, 2006). Therefore, resistant strains make it more difficult to treat patients with severe infections. For instance, *S. typhimurium* DT104 is a high rate of resistance to particular antimicrobials among veterinary isolates in Europe, North America, and Asia (Chiu et

*Corresponding authors. E-mail: wyh7405@163.com, shaohuijunlll@126.com. Tel: 86-514-87979036. Fax: 86-514-87972218.

al., 2006; Futagawa-Saito et al., 2008; Gebreyes et al., 2004; Graziani et al., 2008; Hur et al., 2011; USDA, 2006). The present study was carried out to determine the prevalence of *Salmonella* serovars in bird infections including chickens, ducks, and geese. All isolates were also examined for antibiotic resistance.

MATERIALS AND METHODS

Bacterial strains of isolation and culture conditions.

Individual clinical specimens, including liver, ovaries and other organs, were collected from 45 cases of poultry affected by Salmonellosis in Yangzhou and the surrounding areas from February 2011 to December 2011. Most of the animal sampled showed typical clinical signs including septicemia and enteritis. The strains were isolated from the sample, and grown on blood agar (LB containing 5% [v/v] defibrinated sheep's blood) at 35°C for 24 h. The subcultures bacteria were streaked onto Luria-Bertani (LB) agar (Oxoid) and MacConkey agar (Shanghai China Academy Of Sciences Shanghai Hexapod Technology Development Co., Ltd.) and incubated at 37°C for 24 h.

Amplification of InvA gene

The InvA gene of the isolated strains was used for *Salmonella* PCR detection, and amplified enzymatically with the forward primer-5′GTGAAATTATCGCCACGTTCGGGCAA3′ and reverse primer -5'TCATCGCACCGTCAAAGGAACC-3' according to Rahn (1992) and Malorny (2003). A negative control tube with *Escherichia coli* S11701 identified and a positive control tube with *Salmonella* S6702 confirmed by Key Laboratory of Animal Infectious Diseases of Ministry of Agriculture, Yangzhou University, China, DNA were included on each occasion. PCR was performed with the initial denaturation of 3 min at 95°C, followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 58°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 8 min. The expected size of amplicon was approximately 284 bp, and visualized by agarose gel electrophoresis on a 1% agarose gel.

Biochemical tests and *Salmonella* serovars

The ability to ferment glucose, mannitol, lactose, sucrose, adonitol, and maltose in the presence of omithine decarboxylase, lysine decarboxylase, and urease activity, and the ability to produce indole, diacetyl, and hydrogen sulfide were performed in conventional tube media for *Enterobacteriaceae* (Hangzhou Microbial Reagent Co., Ltd, China) at 35°C for 24 h. *Salmonella* serovars was identified by slide agglutination according to the Kauffmann and white scheme.

Antimicrobial susceptibility tests

Antimicrobial susceptibility tests were done on pure, 1-day-old cultures of all the *Salmonella* isolates, using the Kirby-Bauer disk diffusion test and antimicrobial susceptibility slip (Hangzhou Microbial Reagent Co., Ltd, China), according to the Clinical and Laboratory Standards Institute (CLSI, 2006) standards.

Strains were evaluated as susceptible, or resistant, and multidrug resistance (MDR) defined as resistance to four or more antibiotics. The following antimicrobials were tested: amikacin (30 µg), kanamycin (30 µg), gentamycin (10 µg), ceftriaxone sodium (30 µg), doxycycline (30 µg), streptomycin (10 µg), levofloxacin (5 µg), norxacin (10 µg), furazolidone (300 µg), polymyxin B (300 IU), sulfamethoxazolum-trimethoprimum (23/75 µg), lomefloxacin (10 µg), chloramphenicol (30 µg), and nalidixic acid (30 µg).

RESULTS

Bacterial strains of isolation and Cultural and physiological features

Of the samples, 46.7% (21 of 45) of salmonellosis cases tested positive for *Salmonella* using the culture isolation method. The 21 *Salmonella* isolates belonged to 6 different serovars (Table 1). The predominant serovars were *S. typhimurium* (9/21), and *S. pullorum* (7/21). Other serovars isolated were *Salmonella paratyphi B* (2/21), *Salmonella enteritidis* (1/21), *Salmonella* d*erby* (1/21) and *Salmonella typhisuis* (1/21). In this study, all *Salmonella* isolates was able to ferment glucose, mannitol, but could not ferment lactose, sucrose, and adonitol and were positive to omithine decarboxylase, lysine decarboxylase, and negative to urease activity, indole, and V-P. Furthermore, variation in the biochemical characterization among maltose and hydrogen sulfide of 21 isolates was found (Table 1).

Salmonella identification

All isolates tested positive using the culture method and positive control 284 bp band on electrophoresis (Figure1).

Antibiotic resistance profiles of isolates

A large percentage of *Salmonella* were resistant to doxycycline (90.5%), polymyxin B (85.7%), streptomycin (85.7%), lomefloxacin (85.7%), and nalidixic acid (81.0%). In addition, resistance rates of the field strains to gentamycin, norxacin, furazolidone, levofloxacin, sulfamethoxazolum - trimethoprimum, ceftriaxone, kanamycin, amikacin and chloramphenicol were 52.4, 52.4, 38.1, 33.3, 28.6, 28.6, 23.8, 19.1 and 19.1%, respectively. All the strains were found to be resistant to two or more of the 14 antibiotics tested (Table 2), most (19/21) of isolates had a MDR. A majority (13/21) were co-resistant to lomefloxacin, nalidixic acid, streptomycin, polymyxin B, and doxycycline. Moreover, S2 was resistant to 13 antibiotics except for furazolidone.

DISCUSSION

Salmonellosis is an important cause of disease and death in poultry and contaminated poultry products pose a significant health hazard to humans (Tizard, 2004). In this study, 21 *Salmonella* had been isolated form diseased or

Table 1. Summary of 21 isolates.

Strain	Collection date	Animal species	Maltose	Hydrogen sulfide	Serovar
S1	24/2/2011	Chicken	−	−	*S. pullorum*
S2	24/2/2011	Chicken	−	−	*S. paratyphi B*
S3	24/2/2011	Chicken	−	−	*S. pullorum*
S4	26/2/2011	Chicken	−	−	*S. Pullorum*
S5	4/3/2011	Geese	+	+	*S. typhimurium*
S6	16/3/2011	Geese	+	+	*S. paratyphi B*
S7	26/3/2011	Chicken	−	+	*S. pullorum*
S8	26/3/2011	Chicken	−	−	*S.typhisuis*
S9	4/5/2011	Duck	+	+	*S. dublin*
S10	21/8/2011	Geese	+	+	*S. typhimurium*
S11	5/9/2011	Duck	+	+	*S. typhimurium*
S12	5/9/2011	Hen	−	−	*S. pullorum*
S13	27/9/2011	Chicken	+	+	*S. pullorum*
S14	13/9/2011	Chicken	−	−	*S. pullorum*
S15	14/9/2011	Geese	+	+	*S. typhimurium*
S16	24/9/2011	Geese	+	+	*S. typhimurium*
S17	26/9/2011	Geese	+	+	*S. typhimurium*
S18	11/10/2011	Geese	+	+	*S. typhimurium*
S19	11/11/2011	Geese	+	+	*S. typhimurium*
S20	21/12/2011	Geese	+	+	*S. typhimurium*
S21	31/12/2011	Geese	+	+	*S. enteritidis*

dead birds. From our results, *S. typhimurium* (8/10) was the most prevalent serovars isolated from infected geese. Pan et al. (2010) examined 505 geese faecal samples which were collected between 2008 and 2009 for *Salmonella* species in China. They reported that 54 samples (10.7%) form healthy geese were positive for *Salmonella*, *S. typhimurium* (18.5), the predominant serovars. Trawinska et al. (2008) found that the serovars *S. typhimurium* (44.8%) were predominant in the isolates of geese in 2001-2005 in Poland. However, few reports are available on the prevalence of *Salmonella* serovars isolated from infected geese. Our results suggest that *S. typhimurium* is a singnificant source of pathogens in geese. On the other hand, *S. pullorum* (7/9) was the predominant serovars in chickens which is in agreement with Pan et al. (2010) who reported that *S. pullorum* (84.9%) was the predominant *Salmonella* serovar isolated from chickens in Eastern China. *S. pullorum*, the causative agent of Pullorum disease is the most prevalent host-adapted pathogen in China (Pan et al., 2009). In this study, Other serovars such as *S. paratyphi B*(2/21), *S. enteritidis* (1/21), *S. derby* (1/21) and *S. typhisuis* (1/21) were also isolated form diseased domestic birds such duck and chicken.

Salmonella isolated from infected animal cases (chickens, ducks, and geese) were resistant to doxycycline (90.5%), polymyxin B (85.7%), streptomycin (85.7%), lomefloxacin (85.7%), nalidixic acid (81.0%), gentamycin (52.4%) and norxacin (52.4%). However, Pan et al. (2010) observed that *Salmonella* species isolated from faecal samples of healthy domestic animals (chickens, ducks, geese and pigs) were resistant to nalidixic acid (48.8%), and streptomycin (38.3%). The higher resistance rates of *Salmonella* strains isolated in this study suggests that the strains might have originated from treatments where antimicrobials have been utilized. A low level of resistance to chloramphenicol and furazolidone was found which may be due to their banned use for some time in domestic animals.

This study demonstrated that *S. typhimurium* is the common serovars in geese and a majority of isolates was resistant to multi-drug. *S. typhimurium* is also the major cause of human salmonellosis via contaminated food, becoming increasingly multi-drug resistance (Torpdahl et al., 2013; Cavallaro et al., 2011; Wójcik et al., 2012). Therefore measures to reduce *Salmonella* infection and antimicrobial usage are important to animals.

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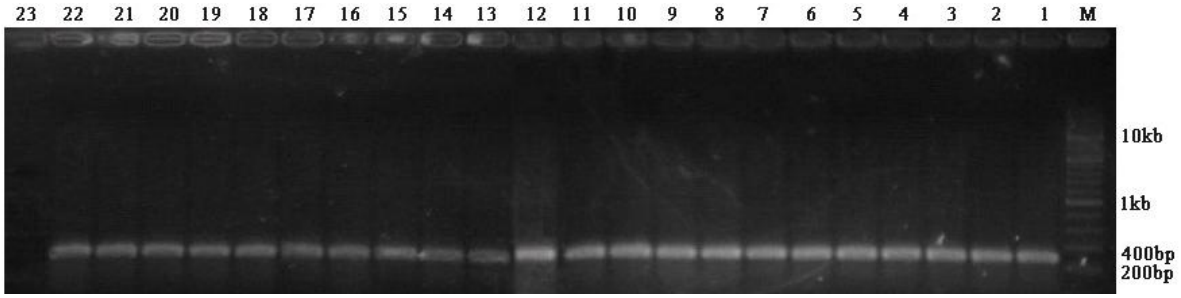

Figure 1. PCR product of invA gene. M, 200bp maker; lane 1-21, 21strains isolated from clinical case; lane 22, positive control; lane 23, negative control.

Table 2. Antimicrobial resistance rates of *Salmonella* isolates.

Antimicrobial	Number (%) of resistant isolate	Antimicrobial	Number (%) of resistant isolate
Doxycycline	19(90.5)	Furazolidone	8(38.1)
Polymyxin B	18(85.7)	Levofloxacin	7(33.3)
Streptomycin	18(85.7)	Sulfamethoxazolum-trimethoprimum	6(28.6)
Lomefloxacin	18(85.7)	Ceftriaxone	6(28.6)
Nalidixic acid	17(81.0)	Kanamycin	5(23.8)
Gentamycin	11(52.4)	Amikacin	4(19.1)
Norxacin	11(52.4)	Chloramphenicol	4(19.1)

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