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Araştırma Makalesi ***Research Article***

Antimicrobial Resistance Profiles of the Two Porcine *Salmonella* Typhimurium Isolates

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Abstract

The aim of the study is to detect the presence of the Salmonella species in swine with diarrhea, and to investigate their antimicrobial resistance and extended spectrum beta lactamase (ESBL) and/or AmpC β-lactamase production. For this purpose, stool samples from three commercial pig farms in Istanbul and Tekirdag were collected and processed for Salmonella isolation by culture and isolates were identified by biochemical activity tests. *Salmonella* isolates were confirmed by PCR then serotyped. Antimicrobial resistance and ESBL and AmpC production of the isolates were determined according to the Clinical and Laboratory Standards Institute (CLSI) standard. In the study, two hundred and thirty eight stool samples were examined. Salmonella spp. were obtained from 2 samples, and the isolation rate was determined as 0.8%. Both of the isolates were defined as *Salmonella enterica* subsp. *enterica* serovar Typhimurium (serotype 1, 4, [5], 12: I: 1, 2) by serotyping. Both of them were resistant to cefaclor, cloxacillin and lincomycin (100%). Multidrug resistance (resistance ≥3 antimicrobials) observed in all isolates. ESBL and AmpC production were not detected in any of the isolates. To our knowledge, this is the first report of the isolation of *S.* Typhimurium in pigs with diarrhea in Turkey. This study also represents the first report of multi-drug resistant *S.* Typhimurium isolates from pig stools in Turkey.

Introduction

Salmonella infections occur in both animals and humans, worldwide. In industrialized countries, non-typhoid *Salmonella* types are an important cause of bacterial gastroenteritis (Van den Brandhof et al., 2003). The most common clinical manifestations of salmonellosis in animals are gastrointestinal disorders; followed by acute septicemia, abortion, arthritis and respiratory disease (Davies, 2004).

Salmonella enterica subsp. *enterica* serovar Typhimurium can cause infections in humans and animals. Most human cases are food borne; however, the bacteria can be transmitted through direct contact and indirectly from the contaminated environment (Rice et al., 2003). *S.* Typhimurium is a common cause of salmonellosis in many countries (Davies, 2004; Mmolawa et al., 2002) and it was reported that the predominant serotype in Europe (van Pelt et al., 2003). In addition, it was indicated that *S.* Typhimurium strains were the most important serotypes that frequently causes food-related and widespread infections in Turkey (Erdem et al., 2005).

The infected pigs with *Salmonella* types, shed the bacteria through faeces that was caused by contamination of slaughterhouses and might be created a potential health risk for humans (Swanenburg et al., 2001).

It was a known fact that, infections caused by antimicrobial resistant bacteria in humans and animals has been increasing lately (Arias and Murray, 2015;

Bagcigil et al., 2007; Yılmaz et al., 2015). It was suggested that the increase usage of cephalosporins in human and veterinary medicine might be the main cause of extended-spectrum β-lactamase (ESBL) and AmpC β-lactamase producing bacteria colonization. β-lactamase enzymes are mainly plays an important role in the Enterobacteriaceae, and β-lactamase resistance could be transferred among other bacteria via plasmids (Li et al., 2007).

Presence of multi-drug resistance (resistance to ≥3 antimicrobials) were created a potential risk for the treatment of the infections and restrain the drug choice (Van Boxstael et al., 2012). The drug resistance rate, varies between different serotypes, some *S.* Typhimurium strains have been reported resistant to 10 or more antimicrobial agents (Poppe et al., 2002).

The aim of the study is to detect the presence of the Salmonella species in swine, and to investigate their antimicrobial resistance and ESBL and/or AmpC β-lactamase production.

Materials and Methods

Stool samples were taken from 238 pigs with diarrhea from three different farms in Çorlu (Tekirdağ), Ayazma and Arnavutköy (Istanbul). The stool samples were collected into sterile sample tubes and transported to the laboratory in the cold chain.

The culture was conducted to the standard of *Salmonella* isolation from stool samples. Biochemical tests were carried out by Api 20 E (Bio Merieux, France) (Ertaş et al., 2014; Holt et al., 1994; Türkyılmaz et al., 2007). Confirmation of the genus *Salmonella* was performed by genus-specific PCR according to Rahn et al. (1992), and Shanmugasamy et al. (2011). *S. enteritidis* strain (KUEN 349) was used as the positive control. The serotyping of the isolates was carried out on the Veterinary Control and Research Institute, *Salmonella* Reference Laboratory, Etlik, Ankara.

Antimicrobial resistance of the isolates was determined by the Kirby-Bauer disc diffusion method according to the Clinical Laboratory Standards Institute (CLSI) manual (2013). The tested antibiotics are; ampicillin (AMP 10 µg), amoxicillin (AML 10 µg), amoxicillin/clavulonic acid (AMC 10 µg), cefaclor (CEC 30 µg), cephalexin (CL 30 µg), cefoperazone (CFP 75 µg), ceftiofur (CFT 30 µg), chloramphenicol (C 30 µg), ciprofloxacin (CIP 5 µg), cloxacillin (OB 6 µg), enrofloxacin (ENR 5 µg), gentamycin (GN 5 µg), kanamycin (K 30 µg), lincomycin (MY 2 µg), nalidixic acid (NA 30 µg), nitrofurantoin (F 300 µg), streptomycin (S 10 µg), sulbactam/ampicillim (SAM 20 µg) and sulfamethoxazole**/**trimethoprim (SXT 25 µg).

ESBL screening and confirmatory tests of the isolates were evaluated according to the guidelines of the CLSI by Kirby-Bauer disc diffusion tests. The susceptibility was tested against cefotaxime (30 µg), ceftriaxone (30 µg), ceftazidim (30 µg), cefpodoxim (10 µg), aztreonam (30 µg) (HiMedia) in screening tests. The diameter of the zone of inhibition was measured and interpreted according to ESBL screening test limits in CLSI (2013). The production of phenotypic AmpC β-lactamase was determined by resistance to cefoxitine (30 µg) (≤14 mm) and susceptibility to cefepime (30 µg) (≥18 mm) (CLSI, 2013; EFSA, 2011; Giske et al., 2012).

Results

Salmonella spp. isolated in 2 of the 238 samples, the isolation rate was determined as 0.8%. Both of the isolates were obtained from the samples collected to the same source. Additionally, isolates were verified as *Salmonella* by PCR (Figure 1) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (serotype 1, 4, [5], 12: I: 1, 2) by serotyping.

Multiple antibiotic resistance determined in the both of the isolates. Antimicrobial resistance patterns of the isolates were shown in the Table 1. The productions of phenotypic ESBL and AmpC β-lactamase have not been detected in any of the isolates. The results of the screening tests were shown in Table 2.

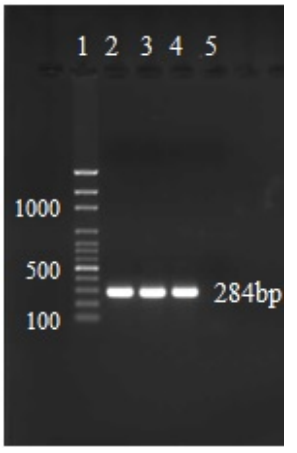

Figure 1. Agarose gel electrophoresis image of PCR products.

Lane 1; 100 bp molecular weight marker, lane 2; positive sample 1, lane 3; positive sample 2, lane 4; positive control, lane 5; negative control.

Table 1. Antimicrobial resistance patterns of the isolates.

	Resistance Pattern		
Isolate 1	CEC	OB	MY
Isolate 2	CEC	OB	MY

CEC: Cefaclor, OB: Cloxacillin, MY: Lincomysin

Table 2. Phenotypic ESBL and AmpC β-lactamase assessments of the isolates.

	ESBL Screening Test				Result	AmpC Definition Test			Result
	Inhibition Zone Diameter (mm)					Inhibition Zone Diameter (mm)			
	CTX	**CTR**	**CAZ**	**CPD**		**CTX**	**CX**	**CAZ**	
Isolate 1	26	24	24	23	-	26	22	22	-
Isolate 2	28	29	24	22	-	26	21	21	-

CTX: Cefotaxime, CTR: Ceftriaxone, CAZ: Ceftazidime, CPD: Cefpodoxime, CX: Cefoxitin, -: Negative

Discussion

Salmonella infections may observe in all of the domestic animals and humans. The young ones, pregnant and lactating animals are the most susceptible proportion of the populations. Enteric disease is the most common clinical manifestation, but a wide range of clinical signs, which include acute septicaemia, abortion, arthritis and respiratory disease, may be seen. (Baggesen et al., 2000; Boyen et al., 2008).

There were a limited number of studies carried out on bacterial diseases in pigs in Turkey (Bağcıgil et al., 2013; Metiner et al., 2007; Metiner et al., 2013). Likewise, during the literature search at the planning stage of the current study, has not been encountered any sources for the investigation of Salmonella shedding in pigs raised in Turkey. However, there have been some studies on colonization of *Salmonella* spp. in cattle, sheep and poultry (Aydın et al., 2001; Genç, 2002; Genç and Otlu, 2005).

In the worldwide, *Salmonella* spp. isolation rate from swine were reported between 4.3% and 27.5% (Bolton et al., 2013; Li et al., 2013; Vieira-Pinto et al., 2006; White et al., 2004). In the current study, isolation rate was much lower than the rates of the previous studies. It was thought that the low isolation rate of *Salmonella* types could be based on limited number of swine population in Turkey.

The multidrug-resistance of the *Salmonella* types reduces the therapeutic options in cases of invasive infections in both human and animals. Rajic et al. (2004), reported that was observed of resistance for chloramphenicol (4.7%), ampicillin (7.8%), kanamycin (11.8%), sulphamethoxazole (21.1%), streptomycin (25.5%) and tetracycline (38.8%). Wright et al. (2005), was reported that multi-drug resistant *S*. Typhimurium was isolated from an epidemic. Sisak et al. (2006), indicated that the multi-drug resistance in pig isolates, and the isolates were resistant to amoxicillin/clavulonic acid, sulphamethoxazole/trimethoprim, nalidixic acid and enrofloxacin. De Jong et al. (2014), reported that the rate of multi-drug resistance in pig as 39.7%. In the current study, two *Salmonella* isolates had multi-drug resistance with cefaclor, cloxacillin and lincomycin.

It was emphasized that the widespread use of the antibiotics in animals may lead to the increase of the colonization of resistant bacteria such as ESBL and AmpC β-lactamase producers and the resistant strains may spread to the society (Davies, 2004). It was reported that ESBL and AmpC β-lactamases can be transmitted between the bacteria with their transferable genetic elements and clinical over use of the β-lactam antibiotics may cause to a significant public health risk (Frye and Fedorka-Cray, 2007; Li et al., 2007). In a study that was carried out in the USA, indicated that the ceftiofur resistance in *Salmonalla* strains were 9.8% in cats, 19.2% in horses and 20.8% in dogs (Frye and Fedorka-Cray, 2007). De Jong et al. (2014) reported that two of 128 *S.* Typhimurium isolates were producing ESBL while two of them were producing AmpC β-lactamase. In the current study, any of the isolates were produced ESBL and AmpC β-lactamase.

In conclusion, in the current study, isolation rate was much lower than the rates of the previous studies. It

was thought that the reason for this result, there were a limited swine population in Turkey, compared to other countries. On the other hand, both of the isolates (100%) had multi-drug resistance to the antimicrobials. When the transmission risk rather low, the results were suggested that if the infections caused by this species happened, it would be difficult to treat.

To our knowledge, this is the first report of the isolation of *S.* Typhimurium in pigs with diarrhea in Turkey. This study also represents the first report of multi-drug resistant *S.* Typhimurium isolates from pigs' stools in Turkey.

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