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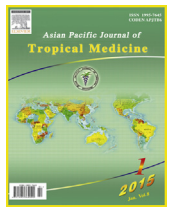

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Some pathogenic characters of paratyphoid *Salmonella enterica* strains isolated from poultry

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ABSTRACT

Objective: To investigate some pathogenic characters of *Salmonella enterica* strains isolated from poultry.

Methods: Twenty-three genetically distinct *Salmonella enterica* strains, of different serovars and pulsotype, were examined for virulence traits. Resistance to gastric acid environment was estimated by measuring the percentage of survived bacterial cells after exposure for 2 h to a synthetic gastric juice. Strains were analyzed with PCR for the presence of the following virulence genes: *mgtC* and *rhuM* located on SPI-3, *sopB* and *pipB* located on SPI-5, *Salmonella* virulence plasmid (*spv*) R (*spvR*), *spvB* and *spvC* located on *Salmonella* plasmid virulence and *sodCI*, *sopE*, and *gipA* located on prophage. Finally, resistance to 21 antibiotics was tested with Kirby–Bauer method.

Results: A percentage of 82.60% of strains were resistant to gastric environment after induction and 60.87% of the strains exhibited constitutive resistance too. Nineteen different virulence profiles were detected. The phage related genes *sodCI* and *sopE* and the plasmid mediated operon *spvR, spvB and spvC* (*spvRBC*) were detected in 82.60%, 47.82% and 52.17% of strains, respectively. Typhimurium and Enteritidis strains showed the highest number of virulence genes. Twenty-one different antibiotic resistance profiles were obtained and two isolates (Typhimurium and Enteritidis) resulted sensible to all the tested molecules. The ampicillin, streptomycin, sulfonamide and tetracycline resistance profile was detected in seven isolates (30.43%).

Conclusion: Our results show that paratyphoid *Salmonella* strains with several characters of pathogenicity, that may be cause of severe pathology in animals and humans, are circulating among poultry.

1. Introduction

Salmonellosis is one of the most important zoonosis worldwide. The preferential route of transmission from animals to humans is through contaminated food or foodstuffs and, in particular, eggs, egg products and poultry meat are the primary source of infection for humans [1].

More than 2600 *Salmonella* serovars exist and all may be pathogenic for humans and animals, at least as cause of intestinal disorders [2]. However, only a limited number of serovars, mainly Typhimurium and Enteritidis, are most frequently associated to human infections [1].

Almost all salmonellae have salmonella pathogenicity island-1 (SPI-1) and salmonella pathogenicity island-2 (SPI-2), that include genes encoding for factors for intestinal and systemic infections, respectively [2,3].

However, more genes, less conserved in the genus *Salmonella*, determine the pathogenicity of this bacterium [4,5]. Salmonella pathogenicity island-3 (SPI-3) is involved in intracellular proliferation and Mg^{2+} uptake, and it contributes to systemic dissemination. Salmonella pathogenicity island-5 (SPI-5) has genes encoding for effector proteins for SPI-1 and SPI-2 and they are important to the development of intestinal symptoms and for intracellular surviving [4].

Virulence genes could be transferred between salmonellae by bacteriophage. In particular, many virulence factors carried on

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prophages have been described, for serovars Typhimurium and Enteritidis, like sopE and gipA involved in intestinal colonization, and sodCI, an enzyme that protects salmonellae from the "oxidative burst" in macrophage environment [6,7]. Moreover, many non-typhoid *Salmonella* strains associated with extra-intestinal infections in humans and animals carry an additional locus termed *Salmonella* virulence plasmid (*spv*), located on *Salmonella* virulence plasmid [8].

Resistance to antimicrobials influences the infection development. This could be related to different factors: treatment failure and consequent persistence of the infection, disruption of the normal competitive gut flora and, moreover, it is demonstrated that antibiotics can directly improve the bacterial virulence [9].

The aim of the present study was to investigate some pathogenic characters of *Salmonella enterica* (*S. enterica*) strains isolated from poultry, in particular: 1) resistance to gastric acid environment, 2) presence of virulence genes of SPI-3, SPI-5, plasmids and prophages, 3) antibiotic resistance.

2. Material and methods

2.1. Bacterial strains

Forty-four *S. enterica* strains isolated from 2010 to 2014 were selected for the study. All strains were isolated from asymptomatic poultry during routine investigations. The isolates included: 19 *S.* ser. Enteritidis, 13 *S.* ser. Typhimurium, 8 *S.* ser. Infantis, 3 *S.* ser. Typhimurium monophasic variant and 1 *S.* ser. Thompson.

Isolates were screened with pulsed field gel electrophoresis, following the protocol reported by other authors [10], and only the strains belonging to different pulsotypes were further analyzed in order to avoid isolates redundancy.

2.2. Resistance to gastric environment

Constitutive and inducible gastric acid resistance was evaluated following the protocol previously described by Xia *et al* [11]. Briefly, *S. enterica* isolates were grown at 37 °C overnight in LB-MOPS (Luria Bertani broth, plus morpholinepropanesulfonic acid, 100 mmol/L, pH 8.0) and LB-MES (Luria Bertani broth, plus morpholineethanesulfonic acid, 100 mmol/L, pH 5.5) broths to evaluate the constitutive and inducible resistance, respectively. Cultures were diluted 1:200 in synthetic gastric juice (8.3 g proteose–peptone, 3.5 g glucose, 2.05 g NaCl, 0.6 g KH_2PO_4, 0.11 g $CaCl_2$, 0.37 g KCl, 0.05 g porcine bile, 0.1 g lysozyme, 13.3 mg, ultrapure water 1 L; pH was adjusted to 3.0 with 6 mol/L HCl) and incubated at 37 °C in water bath for 2 h. Viable cell counts were determined before and after incubation by plating serial dilutions in PBS (pH 7.2) on LB agar. Results were expressed as the percentage of survived cells after synthetic gastric juice challenge. Three replicates were done for each strain. The minimum percentage of survived cells to consider a strain resistant was fixed to 1%.

2.3. Presence of virulence genes

DNA was extracted from overnight cultures of each isolate using the DNeasy Blood and Tissue Kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions and stored at 4 °C until used as template for PCR assays.

The presence of *spvR*, *spvB*, *spvC*, *sodCI*, *mgtC*, *sopE*, *sopB*, *pipB*, *rhuM* and *gipA* genes was evaluated. Table 1 shows target genes, their location and amplification products size. Single PCR was executed for each gene, following protocols reported by other authors [7,12–15].

2.4. Antibiotic resistance

Resistance to 22 antibiotics was evaluated by the standard disk diffusion method of Kirby–Bauer, on Mueller Hinton Agar (Oxoid, Basingstoke, UK), as describe in Clinical and Laboratory Standards Institute (CLSI) manual [16]. The following antibiotics were employed (Oxoid): amoxycillin–clavulanic acid (30 μg), ampicillin (10 μg), amikacin (30 μg), cephalothin (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), colistin (10 μg), enrofloxacin (5 μg), florfenicol (30 μg), gentamycin (10 μg), kanamycin (30 μg), nalidixic acid (2 μg), nitrofurantoin (300 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (25 μg), sulfonamide (300 μg), tetracycline (30 μg), tigecycline (15 μg), tobramycin (10 μg), trimethoprim (5 μg).

Results were interpreted following European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint tables and, where not possible, according to National Committee for Clinical Laboratory Standards (NCCLS) indications [17,18].

3. Results

3.1. Bacterial strains

Twenty-three *Salmonella* strains were selected on the basis of the pulsed field gel electrophoresis results. Ten different pulsotypes were obtained for ser. Typhimurium, 9 for ser. Enteritidis, 2 for ser. Typhimurium monophasic variant, and 1 for ser. Infantis.

3.2. Resistance to gastric environment

Four strains (17.39%), one *S.* ser. Thompson and three *S.* ser. Enteritis, resulted sensible to the gastric acid environment, both before and after induction. Five strains (21.74%) were resistant only after induction. The remaining 14 strains (60.87%) showed both constitutive and induced resistance. Table 2 reports the results obtained for each analyzed strain, in relation with virulence genes and antibiotic resistance profiles.

3.3. Presence of virulence genes

The genes *spvR*, *spvB* and *spvC* (*spvRBC*) were always found in association. The gene *sodCI* was detected in 19/23 (82.60%) strains, *mgtC* in 13/23 (56.52%), *spvRBC* in 12/23 (52.17%) strains, *sopE* in 11, *sopB* in 11 and *pipB* in 11/23 (47.82%) strains, *rhuM* in 4/23 (17.39%) strains and *gipA* in 2/23 (8.69%) strains.

Table 3 shows the distribution of the virulence genes among the analyzed serovars. In particular, the *S.* ser. Infantis isolate did not show any investigated genes. *S.* ser. Thompson strains had only *sopB* and *pipB* genes. The genes *sopE* and *spvRBC* were detected in Enteritis and Typhimurium strains. *gipA* was observed only in Typhimurium isolates. A total of 19 different profiles were detected.

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