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## Differences in antimicrobial resistance of *Salmonella* spp. isolated from humans, animals and food products in Kazakhstan

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**ABSTRACT:** The aims of this study were twofold: 1. to isolate *Salmonella* spp. from different sources in Kazakhstan, to determine their serovars and resistance profiles and 2. to evaluate similarities in antimicrobial susceptibility of *Salmonella* spp. isolated from humans, animals and food products. From the 10 212 samples tested *Salmonella* spp. were isolated in 47 cases. The predominant serovar isolated from humans and food products was *S. Enteritidis*. Although different animal species were tested the *Salmonella* spp. were isolated mainly from chickens and ducks. *S. Enteritidis* and *S. Typhimurium* were the most prevalent serovars in raw poultry meat. The most frequent resistances were those to nalidixic acid, ampicillin and tetracycline. Human isolates demonstrated lower resistance compared to animal and food isolates. The genes encoding antimicrobial resistance in human isolates in most cases were absent except for some isolates which harboured *tet*, *sul*, *stre* and *bla* TEM genes encoding resistance to the oldest antimicrobial classes. The situation in animals and food products was less beneficial as besides different genes, integrons associated with the horizontal gene transfer were detected. The findings suggest that antimicrobials in animal sector should be used more strictly, paying attention to critically important antimicrobials for humans and possible horizontal transfer of pathogens and their genetic determinants.

**Keywords:** *Salmonella* Paratyphi, One Health, salmonellosis, zoonoses

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## INTRODUCTION

*Salmonella* spp. are one of the most frequently isolated foodborne pathogens. It is a major worldwide public health concern, accounting for 93.8 million foodborne illnesses and 155,000 deaths per year (Eng et al., 2015). Gastroenteritis is the most common manifestation of *Salmonella* infection, followed by bacteraemia and enteric fever (Majowicz et al. 2010). Non-typhoidal *Salmonella enterica* infection, caused by different serovars from contaminated food, is an important cause of both sporadic gastroenteritis and outbreaks internationally. In the European Union, over 91,000 human cases are reported each year (EFSA, 2018). In Australia, the incidence of infection due to *Salmonella* spp. in the community is estimated to be 185 infections per 100,000 population per year (Kirk et al., 2014). Enteric fever that is caused by *Salmonella enterica* Typhi causes illness in south-central Asia, Southeast Asia, and southern Africa by more than 100 cases per 100,000 person-years (Marks et al., 2017). It is also prevalent in many other parts of the world. For instance, approximately 200 to 300 cases of *S. Typhi* were reported in the United States each year (Lynch et al, 2009).

Epidemiological data and incidence cases of salmonellosis are registered in many countries including the USA, EU, China, Australia, India and others while some countries still are white spots in the map according to the prevalence of salmonellosis (Eng et al., 2015). In Kazakhstan the incidence of diagnosed salmonellosis in 1999 and 2000 was about 3500-4000 cases per year, while typhoid fever incidence was about 30-110 per year (WHO 2001).

Main factors associated with *Salmonella* outbreaks include incomplete cooking of food products, improper storage and direct contact with raw ingredients (Lynch et al. 2006). The food products that are predominantly associated with the outbreaks include animal products such as milk, poultry and eggs, as well as food products such as chocolate and peanut butter (Eng et al., 2015). Animals are the main carriers and reservoir of non-typhoid *Salmonella*. The transmission of *Salmonella* usually occurs through gastrointestinal tract by ingestion of food or water contaminated with infected animals. Direct contact with infected animals also constitutes a risk of infection (Swanson et al., 2007). The worldwide incidence rate of *Salmonella* infection is high as the strains can be found naturally in the environment and in both domestic and wild animals including cats, dogs, am-

phibians, reptiles and rodents (Eng et al., 2015). The diversity of possible reservoirs of infection results in significant challenges for public health authorities to control the infections (Swanson et al. 2007; Dione et al. 2011).

*Salmonella* resistance to at least 3 antibiotics started to be reported in 1960s (Dyson et al. 2019). Since then, the frequency of resistant isolates has increased in many countries; however, the spectrum of resistance or the rate of multidrug resistant isolates depends on country, source of isolation and *Salmonella* serovar. For instance, in the USA *Salmonella* spp. recovered from poultry were resistant to streptomycin (30.9%), gentamicin (12.6%), sulfadimethoxine (20.9%), tetracycline (13.9%), and trimethoprim-sulfamethoxazole combination (8.6%). Among these isolates, 20% were multi-drug resistant, showing resistance to three or more antibiotics; noteworthy is that 67% of *S. Heidelberg* and 54% of *S. Kentucky* isolates have shown resistance to five or more antibiotics (Liljebjelke et al., 2017). In China high rates of resistant *salmonellae* from chicken were found to sulfisoxazole (76.1%), tetracycline (75.3%), ampicillin (48.0%), and ofloxacin (44.7%). Notably, antimicrobial susceptibility tests have identified resistance to polymyxin B (2.0%) and imipenem (0.5%) (Zhang et al., 2018). In Poland the resistance of salmonella isolated from non-meat food products was low with the highest prevalence to nalidixic acid (35.2%), sulphonamides (6.6%), ampicillin (4.9%), amoxicillin/clavulanic acid (2.5%), streptomycin, cefoxitin, gentamicin and tetracycline (1.6%) (Mąka et al., 2015). Although an official data about salmonellosis prevalence in Kazakhstan is collected, there is no current systematic data about the situation of antimicrobial resistance of the strains that are prevalent in humans and in other sources including animals, food products or feedstuffs. Thus, the aims of this study were twofold: 1. to isolate *Salmonella* spp. strains from different sources in Kazakhstan, to determine their serovars and resistance profiles and 2. to evaluate similarities among antimicrobial susceptibility of *Salmonella* spp. isolated from humans, animals and food products.

## MATERIALS AND METHODS

### Place and sampling

Bacteriological investigations were performed at the Institute of Applied Biotechnology, Kostanay University during 2018-2019. The aim was to obtain wide spectrum of *Salmonella* isolates prevalent in Ka-

zakhstan. Animal carcasses and raw meat including poultry, lamb, beef were collected in supermarkets and markets at a retail level. Clinical and pathological material of animals was collected at slaughterhouses and animal farms. *Salmonella* spp. strains already isolated from humans (in hospitals and other health-care facilities) were delivered to the department of Microbiology of Kostanay University as isolates without the data about their concrete source (name of the patient) of isolation. Ready to use, raw and frozen food products were obtained at retail markets and supermarkets. In total 10212 samples were tested from which 1006 samples were from live animals, animal carcasses and milk, 383 food samples and 8823 human samples.

### Isolation and identification of *Salmonella*

Isolation of *Salmonella* was performed according to EN ISO 6579-1 (ISO, 2017) procedure for *Salmonella* detection. Xylose Lysine Deoxycholate (XLD) agar and Salmonella Shigella (SS) Agar (Oxoid, UK) were used as plating media after the enrichment procedure. Isolates obtained from health-care facilities were re-cultivated on XLD agar.

Identification of the isolates was performed using classical biochemical testing including fermentation of carbohydrates, production of hydrogen sulfide, indole, production of lysine decarboxylase, urease, oxidase, catalase, MR-VP as well as other conventional tests.

Serotyping was performed using slide agglutination test with sera to O and H antigens (Petsal, Russia) as well as Wellcolex Colour salmonella serogroup

identification latex test (Remel, UK) according to the manufacturer's instructions.

### Susceptibility testing

Antimicrobial susceptibility testing was performed using the disc diffusion method according to Kirby-Bauer (Hudzicki, 2009). The following discs with antimicrobials ( $\mu\text{g}$ ) (Thermo Fisher, UK) were used: ampicillin (10), cefoperazone (75), cefoxitin (30), streptomycin (10), kanamycin (30), gentamicin (10), tetracycline (30), doxycycline (30), chloramphenicol (30), ciprofloxacin (5), nalidixic acid (30), gemifloxacin (5), enrofloxacin (5), ofloxacin (5) and sulfamethoxazole-trimethoprim (25). Interpretation of the results was carried out using CLSI clinical breakpoints (CLSI, 2019) set for *Salmonella* except for ofloxacin, gemifloxacin and nalidixic acid for which breakpoints set for Enterobacteriaceae were used.

### Detection of the genes encoding antimicrobial resistance

Molecular testing of the isolates was performed at the Microbiology and Virology Institute, Lithuanian University of Health Sciences. DNA material for molecular testing was obtained after bacterial lysis according to the extraction protocol prepared by the EU Community Reference Laboratory for Antimicrobial Resistance with slight modifications (Ruzauskas et al., 2014). Detection of the genes encoding antimicrobial resistance was performed by PCR. Annealing temperatures and oligonucleotides used are presented in Table 1.

**Table 1.** Antimicrobial resistance genes tested and oligonucleotide primers used in the study

| Primer name            | Sequence (5'-3')          | Size, bp and t (°C) | Target gene        | Source               |
|------------------------|---------------------------|---------------------|--------------------|----------------------|
| <i>bla</i> TEM-F       | GAGTATTCAACATTTTCGT       | 857 (50)            | <i>tem</i>         | Maynard et al., 2003 |
| <i>bla</i> TEM-R       | ACCAATGCTTAATCAGTGA       |                     |                    |                      |
| <i>bla</i> SHV-F       | TCGCCTGTGTATTATCTCCC      | 768 (60)            | <i>shv</i>         | Ojdana et al., 2014  |
| <i>bla</i> SHV-R       | CGCAGATAAATCACCACAATG     |                     |                    |                      |
| <i>oxa</i> 1-F         | TCAACAAATCGCCAGAGAAG      | 276 (55)            | <i>oxa</i> group I | Bert et al., 2002    |
| <i>oxa</i> 1-R         | TCCACACCAGAAAACCAG        |                     |                    |                      |
| <i>oxa</i> group 5-F   | AGCCGCATATTTAGTTCTAG      | 644 (56)            | <i>oxa</i> group V | Bert et al., 2002    |
| <i>oxa</i> group 5-R   | ACCTCAGTTCCTTTCTCTAC      |                     |                    |                      |
| <i>ctx</i> M-F         | ATGTGCAGYACCAGTAARGT      | 593 (50)            | <i>ctx</i> M       | Pagani et al., 2003  |
| <i>ctx</i> M-R         | TGGGTRAARTARGTSACCAGA     |                     |                    |                      |
| <i>ctx</i> M group 2-F | ATGATGACTCAGAGCATTTCGCCGC | 876 (56)            | <i>ctx</i> M2      | Celenza et al., 2006 |
| <i>ctx</i> M group 2-R | TCAGAAACCGTGGGTTACGATTTT  |                     |                    |                      |
| <i>cm</i> y2-F         | GCACTTAGCCACCTATACGGCAG   | 758 (58)            | <i>cm</i> y        | Hasman et al., 2005  |
| <i>cm</i> y2-R         | GCTTTTCAAGAATGCGCCAGG     |                     |                    |                      |

|                   |                            |          |                    |                              |
|-------------------|----------------------------|----------|--------------------|------------------------------|
| <i>PER-1-F</i>    | ATGAATGTCATTATAAAAGCT      | 927 (48) | <i>per</i>         | Celenza et al., 2006         |
| <i>PER-1-R</i>    | TTAATTTGGGCTTAGGG          |          |                    |                              |
| <i>PER-2-F</i>    | ATGAATGTCATCACAAAATG       | 927 (49) |                    | Celenza et al., 2006         |
| <i>PER-2-R</i>    | TCAATCCGGACTIONACT         |          |                    |                              |
| <i>tetA-F</i>     | GTGAAACCCAACATACCCC        | 888 (55) | <i>tetA</i>        | Maynard et al., 2003         |
| <i>tetA-R</i>     | GAAGGCAAGCAGGATGTAG        |          |                    |                              |
| <i>tetB-F</i>     | CCTTATCATGCCAGTCTTGC       | 774 (55) | <i>tetB</i>        | Maynard et al., 2003         |
| <i>tetB-R</i>     | ACTGCCGTTTTTTTCGCC         |          |                    |                              |
| <i>aadB-F</i>     | ATGGACACAACGCAGGTCGC       | 534 (55) | <i>aadB</i>        | Asadollahi et al., 2012      |
| <i>aadB-R</i>     | TTAGGCCGCATATCGCGACC       |          |                    |                              |
| <i>aadA-F</i>     | GTGGATGGCGGCCTGAAGCC       | 528 (68) | <i>aadA</i>        | Asadollahi et al., 2012      |
| <i>aadA-R</i>     | AATGCCCAGTCGGCAGCG         |          |                    |                              |
| <i>rmtB-F</i>     | ATGAACATCAACGATGCCCT       | 769 (55) | <i>rmtB</i>        | Yan et al., 2004             |
| <i>rmtB-R</i>     | CCTTCTGATTGGCTTATCCA       |          |                    |                              |
| <i>armA-F</i>     | CAAATGGATAAGAATGATGTT      | 774 (55) | <i>armA</i>        | Galimand et al., 2003        |
| <i>armA-R</i>     | TTATTTCTGAAATCCACT         |          |                    |                              |
| <i>aphA1-F</i>    | AAACGTCTTGCTCGAGGC         | 500 (55) | <i>aphA1</i>       | Frana et al., 2001           |
| <i>aphA1-R</i>    | CAAACCGTTATTCATTCGTGA      |          |                    |                              |
| <i>aacA4-F</i>    | ATGACTGACATGACCTTGCG       | 487 (55) | <i>aacA4</i>       | Odumosu et al., 2015         |
| <i>aacA4-R</i>    | TTAGGCATCACTGCGTGTTCG      |          |                    |                              |
| <i>aac(3)II-F</i> | TGAAACGCTGACGGAGCCTC       | 369 (65) | <i>aac(3)II</i>    | Sandvang et al., 2009        |
| <i>aac(3)II-R</i> | GTCCAACAG GTAGCACTGAG      |          |                    |                              |
| <i>strA-F</i>     | CCTGGTGATAACGGCAATTC       | 546 (55) | <i>strA</i>        | Lanz et al., 2003            |
| <i>strA-R</i>     | CCAATCGCAGATAGAAGGC        |          |                    |                              |
| <i>strB-F</i>     | ATCGTCAAGGGATTGAAACC       | 509 (55) | <i>strB</i>        | Lanz et al., 2003            |
| <i>strB-R</i>     | GGATCGTAGAACATATTGGC       |          |                    |                              |
| <i>catII-F</i>    | ACACTTTGCCCTTTATCGTC       | 495 (55) | <i>catII</i>       | Vassort-Bruneau et al., 1996 |
| <i>catII-R</i>    | TGAAAGCCATCACATACTGC       |          |                    |                              |
| <i>cmlA-F</i>     | TTGCAACAGTACGTGACAT        | 293 (55) | <i>cmlA</i>        | Keyes et al., 2000           |
| <i>cmlA-R</i>     | ACACAACGTGTACAACCAG        |          |                    |                              |
| <i>sul1-F</i>     | TTCGGCATTCTGAATCTCAC       | 822 (55) | <i>sul1-F</i>      | Christabel et al., 2012      |
| <i>sul1-R</i>     | ATGATCTAACCCCTCGGTCTC      |          |                    |                              |
| <i>sul2-F</i>     | CGGCATCGTCAACATAACC        | 722 (50) | <i>sul2-F</i>      | Pereten et al., 2003         |
| <i>sul2-R</i>     | GTGTGCGGATGAAGTCAG         |          |                    |                              |
| <i>sul3-F</i>     | GAGCAAGATTTTTGGAATCG       | 792 (51) | <i>sul3-F</i>      | Pereten et al., 2003         |
| <i>sul3-R</i>     | CATCTGCAGCTAACCTAGGGCTTTGA |          |                    |                              |
| <i>dfr1-F</i>     | ACGGATCCTGGCTGTTGGTTGGACGC | 254 (55) | <i>dfr1</i>        | Gibreel et al., 1998         |
| <i>dfr1-R</i>     | CGGAATTCACCTTCCGGCTCGATGC  |          |                    |                              |
| <i>dfr5-F</i>     | GCBAAGGDGARCAGCT           | 394 (44) | <i>dfr5</i>        | Seputiene et al., 2010       |
| <i>dfr5-R</i>     | TTMCCAYATTGATAGC           |          |                    |                              |
| <i>dfrA7-F</i>    | AAAATTTCAATTGATTTCTGCA     | 471 (44) | <i>dfr7</i>        | Navia et al., 2003           |
| <i>dfrA7-R</i>    | TTAGCCTTTTTTCCAAATCT       |          |                    |                              |
| <i>mcr1-F</i>     | CGGTCACTCCGTTTGTTC         | 309 (58) | <i>mcr1</i>        | Liu et al., 2015             |
| <i>mcr1-R</i>     | CTTGGTCGGTCTGTAGGG         |          |                    |                              |
| <i>mcr2-F</i>     | TGTTGCTTGTGCCGATTGGA       | 567 (58) | <i>mcr2</i>        | Xavier et al., 2016          |
| <i>mcr2-R</i>     | AGATGGTATTGTTGGTTGCTG      |          |                    |                              |
| <i>qnrA-F</i>     | ATTTCTCACGCCAGGATTTG       | 516 (53) | <i>qnrA</i>        | Robicsek et al., 2006        |
| <i>qnrA-R</i>     | GATCGGCAAAGGTTAGGTCA       |          |                    |                              |
| <i>qnrB-F</i>     | GATCGTGAAAGCCAGAAAGG       | 469 (53) | <i>qnrB</i>        | Robicsek et al., 2006        |
| <i>qnrB-R</i>     | ACGATGCCTGGTAGTTGTCC       |          |                    |                              |
| <i>qnrS-F</i>     | ACGACATTCGTCAACTGCAA       | 417 (53) | <i>qnrS</i>        | Robicsek et al., 2006        |
| <i>qnrS-R</i>     | TAAATTGGCACCCTGTAGGC       |          |                    |                              |
| <i>qepA-F</i>     | CAGTGGACATAAGCCTGTTC       | 218 (60) | <i>qepA</i>        | Liu et al., 2008             |
| <i>qepA-R</i>     | CCCAGGCATAGACTGTA          |          |                    |                              |
| <i>teg1-F</i>     | TTATTGCTGGGATTAGGC         | 164 (55) | integrase I class  | Chen et al., 2013            |
| <i>teg1-R</i>     | ACGGCTACCCTCTGTTATC        |          |                    |                              |
| <i>teg2-F</i>     | ACGACATTCGTCAACTGCAA       | 233 (50) | integrase II class | Goldstein, 2001              |
| <i>teg2-R</i>     | TAAATTGGCACCCTGTAGGC       |          |                    |                              |



**Table 2.** Characteristics of *Salmonella* isolated from humans in Kazakhstan

| Isolate number | Serovar               | Phenotypic Resistance                  | Genotypic Resistance              | Integrans   |
|----------------|-----------------------|--|-----------------------------------|-------------|
| 733            | <i>S. Tshiongwe</i>   | CIP                                    |                                   | -           |
| 735            | <i>S. Enteritidis</i> | NAL, CIP                               |                                   | -           |
| 737            | <i>S. Blegdam</i>     |  |                                   | -           |
| 739            | <i>S. Blegdam</i>     |  |                                   | -           |
| 990            | <i>S. Enteritidis</i> | NAL, ENR                               |                                   | -           |
| 991            | <i>S. Enteritidis</i> | CIP                                    |                                   | -           |
| 993            | <i>S. Enteritidis</i> | TET                                    |                                   | -           |
| 994            | <i>S. Enteritidis</i> | STR, DOX                               | <i>strB</i>                       | -           |
| 998            | <i>S. Enteritidis</i> | ENR, NAL, CIP                          |                                   | -           |
| 999            | <i>S. Enteritidis</i> | NAL, ENR, CIP, GEMI,                   |                                   | -           |
| 1106           | <i>S. Enteritidis</i> | NAL, CIP                               |                                   | -           |
| 1107           | <i>S. Enteritidis</i> |  |                                   | -           |
| 1108           | <i>S. Enteritidis</i> |  |                                   | -           |
| 1109           | <i>S. Enteritidis</i> |  |                                   | -           |
| 1110           | <i>S. Enteritidis</i> |  |                                   | -           |
| 1111           | <i>S. Enteritidis</i> | NAL, CIP                               |                                   | -           |
| 1112           | <i>S. Enteritidis</i> |  |                                   | -           |
| 1113           | <i>S. Enteritidis</i> | TET                                    | <i>tet B</i>                      | -           |
| 1205           | <i>S. Enteritidis</i> | NAL, ENR, CIP, GEMI                    |                                   | -           |
| 1206           | <i>S. Enteritidis</i> | AMP, TET, DOX, NAL, ENR, CIP, OFX, SXT | <i>blaTEM, tet A, tet B, sul3</i> | + (class I) |
| 1280           | IIIc 4p               | NAL, ENR, CIP                          |                                   | -           |

NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; OFX - ofloxacin; STRE - streptomycin; SXT - sulfamethoxazole/trimethoprim

**Table 3.** Characteristics of *Salmonella* isolated from animals in Kazakhstan

| Serovar               | Source of Isolation | Phenotypic Resistance                        | Genotypic resistance                   | Integrans   |
|-----------------------|---------------------|--|--|-------------|
| <i>S. Typhimurium</i> | duck                | STR, KAN, ENR                                |  | -           |
| <i>S. Typhimurium</i> | duck                | STR, CHL, TET, ENR,                          |  | -           |
| <i>S. Typhimurium</i> | chicken             |  |  | -           |
| <i>S. Typhimurium</i> | chicken             |  |  | -           |
| <i>S. Paratyphi C</i> | chicken             | CHL, TET, DOX, NAL, CIP, SXT                 | <i>tetA, sul3, cmlA, catII</i>         | + (class I) |
| <i>S. Enteritidis</i> | chicken             | NAL, CIP                                     |  | -           |
| <i>S. Enteritidis</i> | chicken             | NAL, CIP                                     |  | -           |
| <i>S. Enteritidis</i> | chicken             | AMP, STR, SXT                                | <i>Bla TEM, aadA, dfr1</i>             | -           |
| <i>S. Enteritidis</i> | chicken             | STR, TET, DOX, ENR, CIP, GEMI, NAL, SXT      | <i>tet A, sul 2, dfr1, strA, str B</i> | -           |
| <i>S. Enteritidis</i> | chicken             | ENR, NAL, CIP                                |  | -           |
| <i>S. Enteritidis</i> | chicken             | AMP, STR, ENR                                | <i>bla TEM, aacA4,</i>                 | -           |
| <i>S. Enteritidis</i> | chicken             | NAL, ENR, CIP                                |  | -           |
| <i>S. Moscow</i>      | chicken             | NAL, ENR, CIP                                |  | -           |
| <i>S. Paratyphi C</i> | chicken (imported)  | FOX, STR, TET, DOX, NAL, ENR, CIP, OFX, GEMI | <i>aadA, tet A, qnr B</i>              | + (class I) |

NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; OFX - ofloxacin; SXT - sulfamethoxazole/trimethoprim; CHL - chloramphenicol; STR - streptomycin; KAN - kanamycin

**Table 4.** Characteristics of *Salmonella* isolated from food products in Kazakhstan

| Serovar                   | Source of isolation            | Phenotypic Resistance                                  | Genotypic resistance                        | Integrans             |
|---------------------------|--------------------------------|--|---|-----------------------|
| <i>S. Enteritidis</i>     | Roasted chicken drumstics      |  |   | -                     |
| <i>S. Enteritidis</i>     | Roasted chicken drumstics      | NAL, ENR, CIP  |   | -                     |
| <i>S. Enteritidis</i>     | Sausage                        | NAL, ENR, CIP  |   | -                     |
| <i>S. Enteritidis</i>     | Cake                           |  |   | -                     |
| <i>S. Enteritidis</i>     | Ice-cream                      | NAL, ENR, CIP  |   | -                     |
| <i>S. Enteritidis</i>     | Cake                           | NAL, ENR, CIP  |   | -                     |
| <i>S. Enteritidis</i>     | cake                           |  | tet B                                       | -                     |
| <i>S. Enteritidis</i>     | Refrigerated chicken drumstics | AMP, STR, KAN, TET, DOX, ENR                           |   | -                     |
| <i>S. Enteritidis</i>     | Sausage                        | AMP, STR, KAN, CHL, DOX, ENR                           |   | -                     |
| <i>S. Enteritidis</i>     | Refrigerated chicken drumstics | AMP,STR, KAN, CN, TET, DOX                             |   | -                     |
| <i>Salmonella</i> group C | Force meat                     | AMP, STR, CHL, TET, DOX, NAL, ENR, CIP, OFX, GEMI, SXT | aadB, tet A, tet B, sul3, catII, qnrA       | + (Class I)           |
| <i>S. Tennessee</i>       | Meat in paste                  | AMP, CFP, KAN, CHL, TET, DOX, NAL, ENR, CIP, SXT       | ctxM, aphA1, aadA, tet A, sul3, cmlA, catII | + (Class I, Class II) |

NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; CN - gentamicin; OFX - ofloxacin; SXT - sulfamethoxazole/trimethoprim; CHL - chloramphenicol; STR - streptomycin; KAN - kanamycin; CFP - cefpodoxime

### Data analysis

Occurrence of multi-resistant isolates was calculated by dividing the number of isolates resistant to at least 3 antimicrobial classes by the total number of obtained isolates. Statistical analysis was performed using IBM SPSS Statistics package, version 20.

Comparison between categorical variables was calculated using the chi-square test. Results were considered statistically significant if  $P \leq 0.05$ .

### RESULTS

From total 10 212 samples tested (1006 samples from live animals, animal carcasses and milk; 383 food and 8823 human samples) *Salmonella* were isolated in 47 cases (0.46%). Twenty one (21) isolates (0.24%) were obtained from humans, fourteen (14) (1.39%) - from live or slaughtered animals and twelve (12) isolates (3.13 %) - from ready to use, raw or refrigerated food products (Tables 2-4).

As it could be seen from Table 2, the predominant *Salmonella* serovar isolated from humans was *S. Enteritidis* (81% from all isolates). Susceptibility varied among the strains: seven isolates were susceptible to all tested antimicrobials while a single isolate was resistant to eight (8) of the tested antimicrobials. The most frequent resistance was towards nalidixic acid, fluoroquinolones and tetracyclines. The prevalence of the genes encoding resistance was low. Integrans

were detected in a single multi-resistant isolate of *S. Enteritidis*.

Although different animal species were tested the *Salmonella* were isolated only from chickens and ducks (Table 3). The serovar variety in poultry was higher to those of humans. The predominant serovar were *S. Typhimurium* and *S. Enteritidis*. Two isolates were identified as *S. Paratyphi* C. One of those isolates was resistant to tetracyclines, nalidixic acid, ciprofloxacin, chloramphenicol, sulfonamides and trimethoprim and harboured class I integrans while the other - to 2d generation cephalosporins, streptomycin, tetracyclines and all fluoroquinolones tested. Two (2) strains out of fourteen (14) isolated from animals were susceptible to all antimicrobials tested while six (6) isolates were multi-resistant.

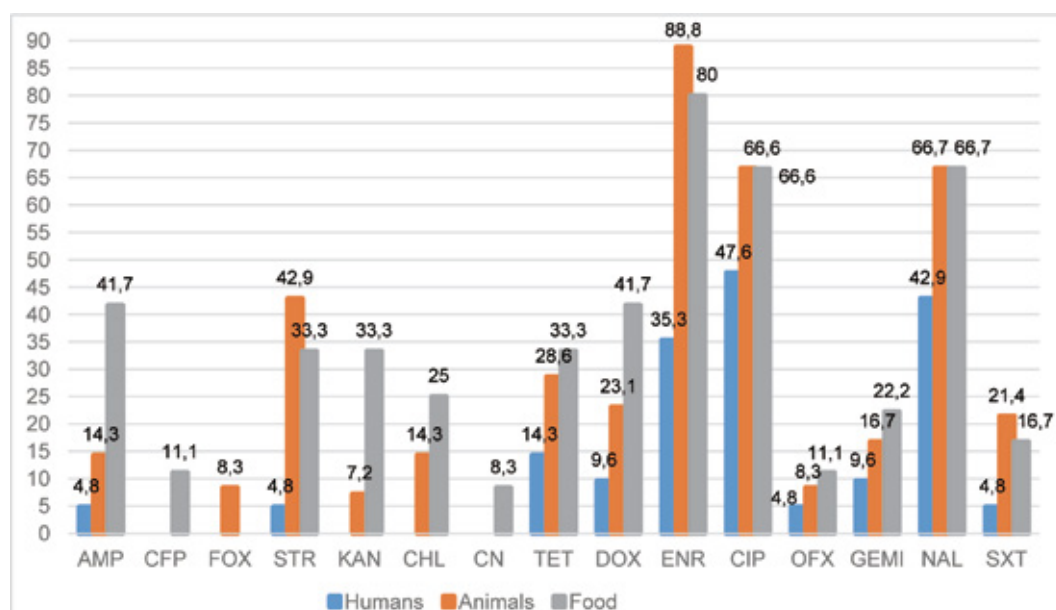
*S. Enteritidis* was the predominant serovar in food products (81% from all isolates) however, two other serovars- (*Salmonella* group C and *S. Tennessee*) had much wider spectrum of resistance. Five (5) isolates out of twelve (12) of food origin were multi-resistant, two (2) strains were susceptible to all antimicrobials and four (4) had similar resistance patterns being resistant only to (fluoro)quinolones. The isolate of *S. Tennessee* was a single strain harboured integrans of both I and II class.

The frequency of antimicrobial resistance in *Sal-*

*monella* isolates obtained from different sources is presented in Fig 1.

The most frequent resistance among *Salmonella* isolates was demonstrated to fluoro(quinolones), ampicillin, streptomycin and tetracycline. Resistance to critically important antimicrobials for humans was also detected and varied depending on the source of isolation. Human isolates demonstrated lower resistance compared to animal and food isolates. Food iso-

lates were more frequently resistant to antimicrobials compared to animal isolates except for streptomycin and enrofloxacin which were more frequently non effective against animal isolates and for nalidixic acid for which the number of resistant isolates from animals and foods had the same range. The correlation between the strains isolated from different sources regarding their resistances to separate antimicrobials is presented in Table 5.



NAL - nalidixic acid; CIP - ciprofloxacin; ENR - enrofloxacin; TET - tetracycline, DOX - doxycycline; GEMI - gemifloxacin; AMP - ampicillin; CN - gentamicin; OFX - ofloxacin; SXT - sulfamethoxazole/trimethoprim; CHL - chloramphenicol; STR - streptomycin; KAN - kanamycin; FOX - cefoxitin; CFP - cefpodoxime

**Fig 1.** Antimicrobial resistance (% of resistant isolates) of *Salmonella* isolated from humans, animals and food products

**Table 5.** Statistical differences among resistances in *Salmonella* isolates from different sources

| Antibiotics                       | Human vs Animals |    |              | Human vs Food |    |              | Animals vs Food |    |           |
|-----------------------------------|------------------|----|--------------|---------------|----|--------------|-----------------|----|-----------|
|                                   | Value            | df | P - value    | Value         | df | P - value    | Value           | df | P - value |
| Ampicillin                        | 0.972            | 1  | 0.324        | 6.991         | 1  | <b>0.008</b> | 2.462           | 1  | 0.117     |
| Cefoperazone                      | constant         |    |              | 2.414         | 1  | 0.120        | 1.400           | 1  | 0.237     |
| Cefoxitin                         | 1.805            | 1  | 0.179        | constant      |    |              | 0.788           | 1  | 0.375     |
| Streptomycin                      | 10.862           | 1  | <b>0.001</b> | 7.966         | 1  | <b>0.005</b> | 0.248           | 1  | 0.619     |
| Kanamycin                         | 1.544            | 1  | 0.214        | 7.966         | 1  | <b>0.005</b> | 2.854           | 1  | 0.091     |
| Gentamicin                        | constant         |    |              | 1.805         | 1  | 0.179        | 1.213           | 1  | 0.271     |
| Levomycetin                       | 3.182            | 1  | 0.074        | 5.775         | 1  | <b>0.016</b> | 0.478           | 1  | 0.490     |
| Tetracycline                      | 2.146            | 1  | 0.143        | 2.910         | 1  | 0.088        | 0.069           | 1  | 0.793     |
| Doxycycline                       | 1.176            | 1  | 0.278        | 4.721         | 1  | <b>0.030</b> | 0.991           | 1  | 0.319     |
| Enrofloxacin                      | 6.801            | 1  | <b>0.009</b> | 5.040         | 1  | <b>0.025</b> | 0.281           | 1  | 0.596     |
| Ciprofloxacin                     | 0.638            | 1  | 0.424        | 0.524         | 1  | 0.469        | 0.000           | 1  | 1.000     |
| Ofloxacin                         | 0.171            | 1  | 0.679        | 0.408         | 1  | 0.523        | 0.046           | 1  | 0.830     |
| Gemifloxacin                      | 0.366            | 1  | 0.545        | 0.879         | 1  | 0.348        | 0.103           | 1  | 0.748     |
| Nalidixic acid                    | 1.733            | 1  | 0.188        | 1.429         | 1  | 0.232        | 0.000           | 1  | 1.000     |
| Sulfamethoxazole/<br>trimethoprim | 2.305            | 1  | 0.129        | 1.310         | 1  | 0.252        | 0.094           | 1  | 0.759     |



## DISCUSSION

This study describes the prevalence of *Salmonella* spp. in different sources in Kazakhstan. Up to date only the data from humans were collected by national and international responsible institutions within the country. Salmonellosis is one of the leading causes of foodborne infections in humans with animals being reservoirs of *Salmonella*. *Salmonella* colonizes the gastrointestinal tract of food animals (Andino et al., 2015) and is shed via feces (Narváez-Bravo et al., 2013). Although the clinical manifestation of salmonellosis in different food-producing animal species can be unequally expressed, all animal species can be a source of foodborne infection (Chaney et al., 2017; Evangelopoulou et al., 2015; Pande et al., 2016).

Knowledge of the prevalence and diversity of *Salmonella* serovars in animals and food can provide important information necessary to develop preventive measures and strategies at different stages of the food chain such as application hazard analysis and critical control programs in meat production industries to ensure food safety (Tietjen and Fung, 1995; Gutema et al., 2019). According to this study the most prevalent serovar of *Salmonella* in Kazakhstan was *S. Enteritidis* which was the most frequently detected both in humans (81%) and food products (83%). According to the World Health Organization, *S. Enteritidis* is one of the most frequently isolated *Salmonella* serovars (along with *S. Typhimurium*) from countries involved in the Global Foodborne Infections Network (Hendriksen et al., 2011). The other serovars isolated from humans included *S. Blegdam* and *S. Tshiongwe*. *S. Blegdam* which is genetically similar to *S. Enteritidis* was isolated for the first time in the USA in the middle of last century from a patient suffering with the enteric type of fever and extensive erythema during the fifth week after onset (Holt and Newton, 1948). There is a lack of data about its prevalence during past decades, although *S. Blegdam* was recently isolated from broilers in Egypt (Ammar et al., 2019). *S. Tshiongwe* was described as a pathogenic serovar for both humans and animals and was also isolated from foods (Thong et al., 2004; Mshelbwala et al., 2017). This study demonstrated that the variety of serovars prevalent in animals in Kazakhstan was not wide; besides *S. Enteritidis*, which prevalence was 50% of all serovars, *S. Typhimurium* (29%) was also common. This serovar was recovered from poultry. The disease caused by *S. Typhimurium* is of public health significance, as it is associated with food poisoning in humans (Dar et al., 2017). The other highly pathogenic to man serovar

*S. Paratyphi C* was isolated from chicken carcasses in two cases. This serovar is associated with human disease although the carriers can be different animal species (ECDC, 2019). Although paratyphoid fever is a rare disease in Europe and the USA it is still prevalent in Asia (Ekdahl et al., 2005).

Interesting data was also obtained according to the antimicrobial susceptibility testing: the isolates from humans were less frequently resistant to different antimicrobials comparing to animal and particularly food isolates, although it is known that salmonellosis is a foodborne human infection. All of the isolates obtained from humans, *in vitro* were susceptible to chloramphenicol, cephalosporins and aminoglycosides (except one isolate which was resistant to streptomycin), while the isolates from animals and foods had different susceptibility patterns. Although aminoglycosides are poorly effective against salmonellosis *in vivo* (Kihlstrom and Andaker, 1985), these antimicrobials are good indicators testing *in vitro* when comparing differences in antimicrobial susceptibility of different isolates, particularly isolated from different sectors. As it is important to understand the chain of salmonellosis “from farm to table” the data obtained in this study is interestingly enough demonstrating quite big differences among susceptibility in salmonella isolates from animals, foods and humans. The most frequent resistance in the isolates irrespective from the source of isolation was towards (fluoro)quinolones, aminopenicillins, streptomycin, tetracyclines and sulphamethoxazole/trimethoprim. These antimicrobials are frequently used both in humans and animals. Comparing the resistance between animal and food isolates the differences were less obvious except for beta-lactams and kanamycin with the highest resistance frequency being in food isolates. Isolates from animals had the highest resistance towards enrofloxacin compared to isolates from other sources. This is probably associated with the frequent use of enrofloxacin for animal treatment in Kazakhstan as it is known that broad use of fluoroquinolones has been followed by emergence of resistance to this class of antimicrobials (Hooper, 2001). Although all food products from which *Salmonella* were isolated contained ingredients of animal origin (meat, milk products or eggs) only a part of the products were originated from Kazakhstan while some of them were imported (data are not presented). Resistance to 3d generation cephalosporins was detected only in the isolates from foods but not from humans and animals, meaning that *salmonellae* circulating in Kazakh-

stan are quite specific and isolated. This fact is also proved by the low overall prevalence of *Salmonella* and by the poor variety of serovars circulating within the country. Imported food however, may change the situation in case of foodborne infections appear. The current situation regarding antimicrobial resistance in humans is beneficial with possibility to treat salmonellosis with antimicrobials of the 1<sup>st</sup> choice. The best known genes encoding antimicrobial resistance in human isolates mostly were absent except for some isolates which harboured *tet*, *sul*, *stre* and *bla*TEM genes encoding resistance to the oldest antibiotic classes. The situation in animals and food products was less beneficial as besides different genes, integrons associated with the horizontal gene transfer were detected more frequently. These findings suggest that antimicrobials in animal sector should be used more strictly particularly paying attention to critically important antimicrobials for humans and possible horizontal transfer of pathogens and their genetic determinants between environment (animals, food ingredients) and

humans. Since multi-resistant highly pathogenic to humans serovars, as *S. Paratyphi C*, were detected in animal carcasses, monitoring of *Salmonella* prevalence in animals should be implemented on a regular basis and measures for *Salmonella* eradication should be foreseen.

In conclusion, food import is one of the key factors changing the *Salmonella* variety and its antimicrobial resistance patterns in Kazakhstan. Usage of enrofloxacin in veterinary medicine however, is the factor of increasing salmonella resistance to fluoroquinolones that may have negative impact on treatment of salmonellosis infections.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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