Bacterial Pathogens in the Food Industry: Antibiotic Resistance and Virulence Factors of Salmonella enterica Strains Isolated from Food Chain Links

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Abstract: Salmonella is one of the most important foodborne pathogens. Fifty-three strains of Salmonella deposited in the Culture Collection of Industrial Microorganisms—Microbiological Resources Center (IAFB) were identified using molecular and proteomic analyses. Moreover, the genetic similarity of the tested strains was determined using the PFGE method. Main virulence genes were identified, and phenotypical antibiotic susceptibility profiles and prevalence of resistance genes were analyzed. Subsequently, the occurrence of the main mechanisms of β-lactam resistance was determined. Virulence genes, invA, fimA, and stn were identified in all tested strains. Phenotypic tests, including 28 antibiotics, showed that 50.9% of the strains were MDR. The tet genes associated with tetracyclines resistance were the most frequently identified genes. Concerning the genes associated with ESBL-producing Salmonella, no resistance to the TEM and CTX-M type was identified, and only two strains (KKP 1597 and KKP 1610) showed resistance to SHV. No strains exhibited AmpC-type resistance but for six Salmonella strains, the efflux-related resistance of PSE-1 was presented. The high number of resistant strains in combination with multiple ARGs in Salmonella indicates the possible overuse of antibiotics. Our results showed that it is necessary to monitor antimicrobial resistance profiles in all food chain links constantly and to implement a policy of proper antibiotic stewardship to contain or at least significantly limit the further acquisition of antibiotic resistance among Salmonella strains.

Keywords: Salmonella; foodborne pathogens; virulence factors; antibiotic resistance; food safety

1. Introduction

Salmonella is a Gram-negative, facultatively anaerobic, non-spore-forming bacteria of the Enterobacteriaceae family [1–3], including only two species: Salmonella enterica and Salmonella bongori [2]. Despite reports of the isolation of a third species of Salmonella called S. subterranea [4], newly released analyses have suggested that it is ultimately assigned to a different cluster, and thus, it has been reclassified to the species Atlantibacter subterranea [5].

S. enterica has six subspecies, namely, Salmonella enterica subspp. enterica, Salmonella enterica subsp. salamae, Salmonella enterica subsp. arizonae, Salmonella enterica subsp. diarizone,
Salmonella enterica subsp. houtenae, and Salmonella enterica subsp. indica [6]. The vast majority (about 99%) of Salmonella strains that cause infections in humans or other warm-blooded animals belong to the species S. enterica [7], which due to the wide variety, has been divided into groups and serological types and currently includes 2659 serovars [8]. The main reservoir of S. enterica subsp. enterica are breeding animals such as poultry, pigs, and cattle [9]. In humans, S. Typhi is responsible for systematic infections and typhoid fever, whereas paratyphoid is caused by the S. enterica of the Paratyphi A, Paratyphi B, or Paratyphi C serovars [10]. Other serovars, such as S. Enteritidis or S. Typhimurium, both in humans and animals, are associated with non-typhoidal salmonellosis [11,12].

Salmonella is one of the main causes of food poisoning resulting from the consumption of contaminated food and water [2,13,14]. It has been estimated that Salmonella causes 115 million human infections and 370,000 deaths per year globally [8]. According to the European Union One Health 2020 Zoonoses Report published in December 2021 by the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) [15], salmonellosis is the second most commonly reported foodborne gastrointestinal infection in humans after campylobacteriosis and is an important cause of foodborne outbreaks in European Union Member States (EU MS) and non-MS countries. According to the above report, in 2020, the EU had the lowest number of reported cases of salmonellosis since 2007. It was probably related to both the COVID-19 pandemic and the withdrawal of the United Kingdom from the EU structures. According to ECDC, in 2020, 52,701 cases of salmonellosis were confirmed in the EU Member States, which corresponds to an EU reporting rate of 13.7 per 100,000 population. A similar trend in the occurrence of salmonellosis was observed in 2016–2020. S. Enteritidis, S. Typhimurium, monophasic S. Typhimurium (1,4,[5],12:i:–), S. Infantis, and S. Derby were the most frequently isolated Salmonella serovars from hospitalized patients. In total, 22 MS reported 694 foodborne outbreaks of Salmonella in 2020, which caused 3686 diseases, 812 hospitalizations, and 7 deaths. Salmonella caused nearly a quarter (22.5%) of all foodborne outbreaks in 2020.

The cause of the foodborne salmonellosis epidemic with strong evidence was eggs and egg products, pork and products thereof, and bakery products. In 2021, Poland itself reported to the Rapid Alert System for Food and Feed (RASFF) Systems 176 cases of Salmonella in food and feed (in 2020: 89 notifications). In Poland, 8269 cases were recorded, including 7975 food poisonings caused by Salmonella (in 2020: 5468 cases, including 5300 food poisonings). However, it should be emphasized that in 2021, the number of cases of all infectious diseases, except for COVID-19, was lower than in previous years due to, inter alia, limited social contacts [16,17].

Antibiotic resistance (AR) has rapidly evolved in the last few decades to become one of the greatest public health threats of the XXI century nowadays. The widespread use of antibiotics, especially the broad-spectrum ones, has contributed to the development of specialist drug defense strategies by bacterial pathogens [18,19]. The mechanisms of AR are then disseminated in the environment, for example, through horizontal gene transfer (HGT) between bacteria and by lysogenic bacteriophages (temperate phages) [18–20]. The World Health Organization (WHO) notes that Salmonella is one of the microorganisms in which some resistant serovars have emerged, affecting the food chain [21]. According to Commission Implementing Decision, 2013/652/EU, which applied from 1 January 2014 until December 2020, monitoring of AMR in Salmonella was mandatory in the major domestically produced animal populations and their derived meat. Specific monitoring of extended-spectrum β-lactamas (ESBLs), AmpC- and carbapenemase-producing Salmonella was also required [22]. The analysis of AMR in Salmonella isolates from hospitalized humans included dominant serovars corresponding to those found in animal species [22,23]. WHO is strengthening the capacities of national and regional laboratories in the surveillance of foodborne pathogens as well as promoting the integrated surveillance of antimicrobial resistance (AMR) of bacterial pathogens in the food chain [21].

Thus, considering the above, our research aimed to determine the antibiotic resistance profile of Salmonella strains isolated from different food chain links.
2. Materials and Methods

2.1. Taxonomic Identification of the Salmonella Strains

A total of 53 *Salmonella* strains used in this study were originally isolated from different food chain links (i.e., animals and animal breeding rooms, food production lines, food products, and hospitalized patients). The strains have been isolated since the 1980s and deposited in the Culture Collection of Industrial Microorganisms—Microbiological Resources Center (IAFB). The belonging of the isolated strains to the *Salmonella* genus was confirmed by amplification of the 16S rRNA gene region. Bacterial DNA was isolated using a commercial DNeasy PowerFood Microbial Kit (Qiagen, GmbH, Hilden, Germany) and amplified with 16S–F (5′–AGAGTTTGATCCTGGCTCAG–3′) and 16S–R (5′–ACGGCTACCTTGTTACGACT–3′) primers [24]. The PCR conditions for the gene amplification were as follows: 2 min of initial denaturation at 95 °C, followed by 35 amplification cycles of denaturation at 94 °C for 30 s, hybridization at 51 °C for 35 s, and extension step at 72 °C for 1 min, ending with a final extension period of 72 °C for 10 min (SimpliAmp™ Thermal Cycler, Applied Biosystems™, ThermoFisher Scientific, Waltham, MA, USA). The amplicons were separated by electrophoresis on 2% agarose gel containing the SimplySafe™ interfering compound (5 µL/100 mL; EURx, Gdansk, Poland). To estimate the size of the amplicons, 5 µL of a DNA Ladder in the range of 100–3000 bp was used (A&A Biotechnology, Gdansk, Poland). Electrophoresis was carried out at 110 V for 60 min using the Sub-Cell GT Horizontal Electrophoresis System (Bio–Rad, Madrid, Spain). The bands were visualized using the GeneFlash Network Bio Imaging System (Syngene, Wales, UK). Sequencing was outsourced to Genomed S.A. company (Poland). Raw sequences were analyzed using BLASTn (NCBI) and deposited in the GenBank database. Moreover, taxonomic identification of bacterial strains was performed using proteomic profiles generated by MALDI–TOF–MS (Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry) analysis (Shimadzu Biotech, Manchester, UK).

2.2. Subtyping Salmonella Strains Using Pulsed-Field Gel Electrophoresis (PFGE)

PFGE was performed according to the international PulseNet CDC guidelines [25] and using the XbaI restriction enzyme. PFGE was performed using a CHEF DR–III PFGE system (Bio–Rad Laboratories, Inc., Hercules, CA, USA), and the following parameters were applied: separation on a 1% agarose gel (Pulsed Field Certified Agarose, Bio–Rad) in 0.5 M Tris–Borate–EDTA (TBE) buffer at 14 °C for 20 h (pulse times of 2.2–63.8 s). The gels were stained with 0.5 µg/mL of ethidium bromide for 15 min and photographed under UV transillumination using a QuantityOne (BioRad, Madrid, Spain) software and GelDoc 2000 (BioRad, Madrid, Spain) system. The banding patterns were analyzed with bionumerics Gel Compar II 6.5 software (Applied Maths, Sint–Martens–Latem, Belgium) using the Dice coefficient and the UPGMA (Unweighted Pair-Group Method with Arithmetic mean) algorithm. A position tolerance of 1% was adopted for the generation of a dendrogram. *Salmonella* strains with more than 95% similarity were clustered together as identical.

2.3. Detection of Virulence Genes in Salmonella Strains

Salmonella strains were tested for six virulent genes (*invA*, *fimA*, *stn*, *spvC*, *spvR*, and *rck*) using PCR with sets of specific primer pairs (Table 1). Detailed parameters of individual PCR reactions are presented in Table S1 (Supplementary Materials). Amplicons were separated by electrophoresis, as described in Section 2.1. To estimate the size of the amplicons, a DNA Ladder in the range of 100–1000 bp was used (A&A Biotechnology, Gdansk, Poland).
Table 1. The primer pairs used for detection of virulence factors in Salmonella strains.

| Target Gene | Primer Sequences 5'-3' | Annealing Temperature | Product Size | Reference |
|-------------|------------------------|-----------------------|--------------|-----------|
| invA        | F-GTGAATTATCGCACGTCCGA R-TCATCGCAGGTTCGAAAAA | 63 °C | 284 bp | [26] |
| fimA        | F-CCTTTCTCCATCGTCCTGAA R-TGGTGTTATCTGCCTGACCA | 56 °C | 260 bp | [27] |
| stn         | F-CTTTGGTCGTAAAATAAGGC G-CTGCCCAGAGACATCTT | 56 °C | 260 bp | [28] |
| spoC        | F-ACCTGGTCCAACCAAAATCCGGA R-TCGTCCTGATTTTCGCCACCATCA | 63 °C | 571 bp | [29] |
| spoR        | F-CAGGTCTTCTAGTATGGCA R-TTCTGGGGGAAATGGTCAGT | 56 °C | 310 bp | [30] |
| rck         | F-CTGACCACCCATCCGTGT R-GTAACCGACACCAACGTT | 56 °C | 479 bp | [31] |

2.4. Antimicrobial Sensitivity Testing

Salmonella strains were tested in vitro for their susceptibility to 28 antimicrobial agents (Oxoid, Hampshire, United Kingdom). Antimicrobial susceptibility tests were performed using a Kirby–Bauer disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [32] and Clinical and Laboratory Standards Institute (CLSI) [33] standards on Mueller–Hinton agar (Merck). The plates were incubated at 37 °C for 18 ± 2 h. The following antimicrobial agents belonging to eight different classes were tested: (1) penicillins: ampicillin (AMP, 10 µg), sulbactam/ampicillin (SAM, 20 µg), amoxicillin/clavulanic acid (AMC, 30 µg), piperacillin (PRL, 30 µg), piperacillin/tazobactam (TZP, 36 µg), ticarcillin/clavulanic acid (TTC, 85 µg); (2) cephalosporins: cefepime (FEP, 30 µg), ceftaxime (CTX, 5 µg), ceftaroline (CPT, 5 µg), ceftazidime (CAZ, 10 µg), ceftazidime/avibactam (CZA, 14 µg), ceftolozane/tazobactam (CZA, 40 µg), ceftriaxone (CRO, 30 µg); (3) carbapenems: ertapenem (ETP, 10 µg), imipenem (IMP, 10 µg), meropenem (MEM, 10 µg); (4) monobactams: aztreonam (ATM, 30 µg); (5) fluoroquinolones: ciprofloxacin (CIP, 5 µg), pefloxacin (PEF, 5 µg), levofloxacin (LEV, 5 µg), moxifloxacin (MXF, 5 µg), ofloxacin (OFX, 5 µg), norfloxacin (NOR, 10 µg); (6) aminoglycosides: amikacin (AK, 30 µg), gentamycin (CN, 30 µg), tobramycin (TOB, 10 µg); (7) phenicols: chloramphenicol (C, 30 µg), and (8) sulfonamides: sulfamethoxazole/trimethoprim (SXT, 25 µg). The tests were made in triplicate, and the mean diameter of the inhibitory zones was calculated. Susceptibility of the isolates to antimicrobial agents was categorized (as susceptible or resistant) by measurement of the inhibition zone, according to interpretive criteria that adhered to the EUCAST guidelines. Escherichia coli ATCC 25922 was used as the reference strain. Salmonella strains resistant to three or more different antimicrobial classes were categorized as multidrug-resistant (MDR) isolates.

Multiple antibiotic resistance (MAR) phenotypes were recorded for Salmonella strains showing resistance to more than two antibiotics, and the MAR index [34] was calculated as:

\[
MAR = \frac{\text{Number of resistance to antibiotics}}{\text{Total number of antibiotics tested}}
\]  

2.5. Determination of Antibiotics Resistance Profile of Salmonella Strains

Mueller–Hinton agar was used to culture the Salmonella strains overnight at 37 °C. Bacterial DNA was isolated using a commercial DNeasy PowerFood Microbial Kit (Qi-agen, GmbH, Hilden, Germany). The presence of twenty-five resistance genes (strA/strB, aadA, aadB, aacC, floR, cat1, cat2, mcr1, mcr2, mcr3, mcr4, mcr5, aphA1-IAB, aphA1, aphA2, tetA, tetB, tetC, sul1, sul2, sul3, dfrA1, dfrA10, and dfrA12) were analyzed using specific primer pairs by conventional PCR reaction. The primer pairs sequences and PCR product size are shown in Table 2. Detailed parameters of individual PCR reactions are presented in Table S1 (Supplementary Materials). Amplicons were separated by electrophoresis, as described in Section 2.1. To estimate the size of the amplicons, a DNA Ladder in the range of 100–1000 bp or 100–3000 bp was used (A&A Biotechnology, Gdansk, Poland). Escherichia coli ATCC 25922 was used as the negative control.
Table 2. The primer pairs and gene targets used for the detection of antimicrobial resistance in *Salmonella* strains.

| Target Gene/ Antibiotic | Resistance Mechanism                  | Primer Sequences 5′-3′ | Annealing Temperature | Product Size | Reference |
|-------------------------|---------------------------------------|------------------------|-----------------------|--------------|-----------|
| *strA*/*strB* streptomycin | Aminoglicoside phosphotransferase     | F–ATGGTGAGCCTAAACTCT   | 63 °C                 | 891 bp       | [35]      |
|                         |                                       | R–CTCTCAGTGAGAACAAA    |                       |              |           |
| *aadA* streptomycin     | Streptomycin adenyltransferase        | F–GTGGATGGCGGCTAAAGCC | 63 °C                 | 525 bp       | [36]      |
|                         |                                       | R–AAATGCCCAGTCGCCAGCG  |                       |              |           |
| *aadB* gentamicin       | Aminoglicoside transferase            | F–GAGGAGTTGGACTATGGTT  | 60 °C                 | 208 bp       | [35]      |
|                         |                                       | R–CTTCATCGGATAGTAAAG   |                       |              |           |
| *aacC* gentamicin       | Aminoglicoside acetyltransferase      | F–GGCGCCGATCAAAGATTTATTCGAGA | 58 °C              | 448 bp       | [37]      |
|                         |                                       | R–CCATTCGATGCGAAGGAAAG   |                       |              |           |
| *floF* florfenicol      | Efflux                                | F–CACGTTGACCTCTATATGG  | 61 °C                 | 888 bp       | [7]       |
|                         |                                       | R–ATGCGAAGAGTAAGCGCAGAC |                       |              |           |
| *floR* chloramphenicol  | Efflux                                | F–AACCCGCCCTCTGGATCAAGTCAA | 60 °C             | 548 bp       | [38]      |
|                         |                                       | R–CAAATCACAGCCCCAGCTGTATC |                       |              |           |
| *cat1* chloramphenicol  | Choloramphenicol acetyltransferase    | F–CCTTATAACCAAGCCGTTCGAGA | 56 °C             | 491 bp       | [38]      |
|                         |                                       | R–TCACAGACGGCAGTACAGAC |                       |              |           |
| *cat2* chloramphenicol  | Choloramphenicol acetyltransferase    | F–CCGGATTGACCTGAATACCT  | 56 °C                 | 456 bp       | [38]      |
|                         |                                       | R–TCACGATCAGCATGAAGAC   |                       |              |           |
| *mcr1* colistin         | Phosphoetanolamine transferase        | F–AGTCCGTTGTCTTCTGTCGC | 58 °C                 | 320 bp       | [39]      |
|                         |                                       | R–AGATCCTTGCTCTGGCTTG   |                       |              |           |
| *mcr2* colistin         | Phosphoetanolamine transferase        | F–CAAGTGGTGGGTCGCAAGTT  | 58 °C                 | 715 bp       | [39]      |
|                         |                                       | R–TCTAGCCCCAAGCAGTACC  |                       |              |           |
| *mcr3* colistin         | Phosphoetanolamin transferase         | F–AAAATGATGTCGCCTTATAG  | 58 °C                 | 929 bp       | [39]      |
|                         |                                       | R–AATGAGATGTTGACATTTTT  |                       |              |           |
| *mcr4* colistin         | Phosphoetanolamine transferase        | F–TCACCTTCCATCTGGGCTTG  | 58 °C                 | 1116 bp      | [39]      |
|                         |                                       | R–TTGGTCATGACATCTTTTG   |                       |              |           |
| *mcr5* colistin         | Phosphoetanolamine transferase        | F–ATGGGTTGTCTGCAATTAC  | 58 °C                 | 1644 bp      | [39]      |
|                         |                                       | R–TCATTGCTGTCTGCTTCTTG  |                       |              |           |
| *aphA*II-1AB kanamycin  | Aminoglicoside phosphotransferase     | F–AAACCTTGGCTGAGGCG    | 55 °C                 | 461 bp       | [40]      |
|                         |                                       | R–CAAGCCTATCCATCGGTTGA  |                       |              |           |
| Target Gene/ Antibiotic | Resistance Mechanism | Primer Sequences 5'-3' | Annealing Temperature | Product Size | Reference |
|------------------------|----------------------|-------------------------|-----------------------|--------------|-----------|
| **aphA1 neomycin**     | Aminoglicoside phosphotransferase | F–ATGGGCTCGCGATAATGTC R–CTCACCGAGGCAGTTCCAT | 60 °C | 634 bp | [7] |
| **aphA2 neomycin**     | Aminoglicoside phosphotransferase | F–GATTTGAAACAGATGGGATTC R–CCATGATGGATACCTTCTCG | 60 °C | 347 bp | [7] |
| **tetA tetracycline**  | Efflux                | F–GCTACATCCGTGGCGCTTC R–CATAGATCGGCGTGAAGAGG | 56 °C | 210 bp | [38] |
| **tetB tetracycline**  | Efflux                | F–TTGGTGGGCAAGTTTG T–GTAATGGGCAATAACACCG | 53 °C | 659 bp | [38] |
| **tetC tetracycline**  | Efflux                | F–CTTGGAGAGCCTTCAACCAG R–ATGGTCTGATCTACTACAGC | 56 °C | 417 bp | [38] |
| **sul1 sulfamethoxazole** | Dihydropteroate synthase inhibitor | F–CGGGCGTGGCTACCTGAACG R–GCCGATCGGGCTGAAGTTCCG | 66 °C | 433 bp | [35] |
| **sul2 sulfamethoxazole** | Dihydropteroate synthase inhibitor | F–CGGGATCGTCAACATAACCT R–TGTTGGGGAAGTCTACACGC | 66 °C | 721 bp | [35] |
| **sul3 sulfamethoxazole** | Dihydropteroate synthase inhibitor | F–GGGAGCCGCTTCCAGTAAT R–TCCGGAACCTGCAATATTA | 57 °C | 500 bp | [7] |
| **dfrA1 trimethoprim** | Dihydrofolate reductase | F–CAATGGCCTGGTGGTGGGAC R–CCGGGCTGATGTTATTTGG | 62 °C | 253 bp | [41] |
| **dfrA10 trimethoprim** | Dihydrofolate reductase | F–TCAAGGCACATCTCAGGinan R–ATCTATTTGCAACTACACC | 59 °C | 433 bp | [41] |
| **dfrA12 trimethoprim** | Dihydrofolate reductase | F–TTCGCAGACTCAGTGGG R–CGGGTGGAGAAGCTGGAAT | 63 °C | 330 bp | [41] |
2.6. Screening for Phenotypic and Genotypic Detection of β-lactamases-Producing Salmonella Strains

In the last stage of the research, the phenotypic and genotypic assessment of the ability to produce β-lactamases by Salmonella strains was carried out. Phenotypic detection of ESBL-producing Salmonella was performed by the double-disc synergy test (DDST) on Mueller–Hinton agar (Merck) with amoxicillin/clavulanic acid (AMC, 30 µg), ceferozone (FEP, 30 µg), cefotaxime (CTX, 30 µg), and ceftazidime (CAZ, 30 µg) disks (Oxoid, Hampshire, UK). Samples were considered to be ESBL-positive when the inhibition zone around cefotaxime or ceftazidime increased toward the central disk with AMC [42]. Moreover, for the detection of ESBL- and carbapenemases-producing Salmonella, commercial selective media were used: CHROMagar ESBL and CHROMagar mSuperCARBA, respectively (Graso Biotech, Starogard Gdanski, Poland).

The presence of five βla genes (blaTEM, blaCTX-M, blaSHV, blaCMY-2, and blaPSE-1) related to resistance to β-lactams were analyzed using specific primer pairs by conventional PCR reaction. The primer pairs sequences and predicted PCR product size are shown in Table 3. Detailed parameters of individual PCR reactions are presented in Table S1 (Supplementary Materials). Amplicons were separated by electrophoresis, as described in Section 2.1. To estimate the size of the amplicons, a DNA Ladder in the range of 100–1000 bp was used (A&A Biotechnology, Gdansk, Poland). Escherichia coli ATCC 25922 was used as the negative control.

### Table 3. Primers used for detection of target β-lactamases-related genes in Salmonella strains.

| Target Gene | Resistance Mechanism | Primer Sequences 5′-3′ | Annealing Temperature | Product Size | Reference |
|-------------|----------------------|------------------------|-----------------------|--------------|-----------|
| blaTEM      | TEM-type ESBL        | F–ATGAGTATTCAACATTTCCG R–CTGACAGTTACCAATGCTTA | 55 °C | 867 bp | [43]       |
| blaCTX-M    | CTX-type ESBL        | F–CGCTTTGCGATGTGCAG R–ACCCGGATATCGTTGGT | 60 °C | 585 bp | [44]       |
| blaSHV      | SHV-type ESBL        | F–AGGATTGACTGCTTTTTTG R–ATTGTGCTGATTTTCGCTTG | 55 °C | 393 bp | [45]       |
| blaCMY-2    | AmpC                 | F–GACACGCCCTTTCTCCACA R–TGACACAGGAAGGCTACGTA | 55 °C | 1000 bp | [45]       |
| blaPSE-1    | Efflux               | F–GCAAGTACGGCAGGAATCA R–GAGCTAGATAGATGCTACAAA | 60 °C | 422 bp | [46]       |

3. Results and Discussion

3.1. Source of Isolation and Taxonomic Identification of the Salmonella Strains

Salmonella strains deposited in the Culture Collection of Industrial Microorganisms—Microbiological Resources Center (IAFB) were used in this study. Strains were classified into the Salmonella genus based on biochemical features. A panel of 53 strains isolated from different food chain links: animals and animal breeding rooms (ABR, n = 9), food production lines (FPL, n = 3), food products (FP, n = 38), and hospitalized patients (HP, n = 3) was analyzed. The taxonomic affiliation of all strains to the genus Salmonella was confirmed either by molecular methods (amplification of the 16S rRNA gene region) or by the analysis of proteomic profiles (using MALDI–TOF–MS). All nucleotide sequences of the strains have been deposited in the GenBank database (Table 4).
Table 4. Source of isolation and taxonomic identification of the *Salmonella* strains.

| Bacterial Strain Number | Year of Isolation | Source of Isolation | Bacteria Identification Acc. to MALDI-TOF MS | Bacteria Identification Acc. to 16S rRNA Sequencing | GenBank Accession Number |
|-------------------------|-------------------|---------------------|---------------------------------------------|----------------------------------------------------|--------------------------|
| KKP 996                 | 1981              | HP/fecal sample     | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON627842                |
| KKP 997                 | 1981              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | MW046052                |
| KKP 998                 | 1991              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764274                |
| KKP 999                 | 1991              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON627845                |
| KKP 1000                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON312999                |
| KKP 1001                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | MW332255                |
| KKP 1002                | 2005              | FP                  | *Salmonella sp.*                           | *Salmonella enterica* subsp. *enterica*            | ON340716                |
| KKP 1003                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON756138                |
| KKP 1004                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON627844                |
| KKP 1005                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON627847                |
| KKP 1006                | 2005              | FP                  | *Salmonella sp.*                           | *Salmonella enterica* subsp. *enterica*            | ON764251                |
| KKP 1007                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON627846                |
| KKP 1008                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON340717                |
| KKP 1009                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764277                |
| KKP 1010                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764279                |
| KKP 1039                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764252                |
| KKP 1040                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764280                |
| KKP 1041                | 2005              | FP                  | *Salmonella sp.*                           | *Salmonella enterica* subsp. *enterica*            | ON764253                |
| KKP 1042                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON798424                |
| KKP 1043                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764281                |
| KKP 1044                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764287                |
| KKP 1045                | 2005              | FP                  | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764254                |
| KKP 1113                | 2005              | FP/halvah           | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON775567                |
| KKP 1169                | 2006              | FP/sesame seeds     | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764259                |
| KKP 1193                | 1987              | HP/fecal sample     | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764258                |
| KKP 1213                | 2009              | FP/caraway seeds    | *Salmonella enterica* subsp. *enterica*     | *Salmonella enterica* subsp. *enterica*            | ON764805                |
Table 4. Cont.

| Bacterial Strain Number | Year of Isolation | Source of Isolation | Bacteria Identification Acc. to MALDI-TOF MS | Bacteria Identification Acc. to 16S rRNA Sequencing | GenBank Accession Number |
|-------------------------|-------------------|---------------------|---------------------------------------------|---------------------------------------------------|--------------------------|
| KKP 1217                | 2009              | FP/coriander        | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON764807                |
| KKP 1514                | 2009              | FPL/pump filter     | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON756136                |
| KKP 1597                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON461374                |
| KKP 1608                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON312943                |
| KKP 1610                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON313000                |
| KKP 1611                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON764857                |
| KKP 1612                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON764858                |
| KKP 1613                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON766359                |
| KKP 1614                | 2009              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON312941                |
| KKP 1636                | 2010              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON773156                |
| KKP 1761                | 2010              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON798425                |
| KKP 1762                | 2010              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON340720                |
| KKP 1763                | 2010              | FP                  | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON773159                |
| KKP 1775                | 1997              | HP/fecal sample     | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON832663                |
| KKP 1776                | 1995              | ABR/poultry         | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON461376                |
| KKP 3078                | 2019              | FP/confectionery industry | Salmonella enterica subsp. enterica       | Salmonella enterica subsp. enterica               | MW034593                |
| KKP 3079                | 2019              | FPL/conveyor belt   | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | MW033548                |
| KKP 3080                | 2019              | FP/confectionery industry | Salmonella enterica subsp. enterica       | Salmonella enterica subsp. enterica               | MW033536                |
| KKP 3081                | 2019              | FPL/production tank | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | MW033602                |
| KKP 3814                | 2016              | ABR/henhouse        | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON732733                |
| KKP 3815                | 2016              | ABR/henhouse        | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON732742                |
| KKP 3816                | 2016              | ABR/henhouse        | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON756119                |
| KKP 3817                | 2016              | ABR/henhouse        | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON756120                |
| KKP 3818                | 2016              | ABR/henhouse        | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON756135                |
| KKP 3819                | 2018              | ABR/poultry         | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON732745                |
| KKP 3820                | 2018              | ABR/poultry         | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON732744                |
| KKP 3821                | 2018              | ABR/poultry         | Salmonella enterica subsp. enterica         | Salmonella enterica subsp. enterica               | ON732827                |

Abbreviations: ABR—animals and animal breeding rooms; FPL—food production lines; FP—food products; HP—hospitalized patients.
Genetic identification (16S rRNA amplification) of most *Salmonella* strains coincided with proteomic identification. For three strains, the identification with the use of the MALDI–TOF–MS allowed us to obtain the result of belonging to the genus of bacterial isolates. These three *Salmonella* strains (KKP 1002, KKP 1006, and KKP 1041) were isolated from food products (specific origin unknown). During the heat treatment of food, bacterial cells could be damaged, which could affect the identification result based on protein profiles.

### 3.2. Subtyping *Salmonella* Strains Using Pulsed-Field Gel Electrophoresis (PFGE)

Pulsed-field gel electrophoresis (PFGE) was used to assess the genetic similarity of the *Salmonella* strains. For 7 *Salmonella* strains, including KKP 996, KKP 1001, KKP 1003, KKP 1004, KKP 1040, KKP 1043, and KKP 1514, the restriction pattern in PFGE was not obtained. Isolates that clustered >95% were considered the same clones (Figure 1). Genotyping of *Salmonella* strains by PFGE showed a relatively high diversity of isolates. Only a few tested strains had the same restriction pattern. Strains with identical restriction patterns are marked in red boxes (Figure 1).

### 3.3. Detection of Virulence Genes in *Salmonella* Strains

*Salmonella* encodes numerous genes such as *invA*, *fimA*, *str*, *spvC*, *spvR*, and *rck* involved in bacterial pathogenicity (Table 5) [47]. In our study, the presence of *invA* gene in all tested *Salmonella* strains was confirmed. *invA* located on pathogenicity island 1 (SPI-1, *Salmonella* Pathogenicity Islands 1) has been extensively studied for its ability to promote the virulence of *Salmonella* [47,48]. SPI-1 is required to invade host intestinal epithelium cells (the *invA* gene is involved in this process) [49], induce an inflammatory reaction, and disrupt the host’s epithelial barrier [19,50]. The *fimA* and *str* genes were also present in all tested strains. The *fimA* gene encodes the FimA protein, which is necessary for the assembly of type I fimbriae in *Salmonella* [51,52]. The fimbriae are *Salmonella* filamentous surface structures that contribute to the colonization of the host’s epithelium cells [47]. The *str* gene encodes *Salmonella* enterotoxin, mainly associated with *S. Typhi*, *S. Typhimurium*, and *S. Enteritidis* serovar infections [53]. Clinically, the *str* gene is a biomarker differentiating enterotoxic *S. enterica* strains from most *S. bongori* strains and other rods from the *Enterobacteriaceae* family [47,53,54]. In *Salmonella* strains, the *str* gene exhibits high nucleotide sequence homology but limited similarity to its corresponding gene in other closely related enteric bacteria. Detection of the *str* gene has been reported to be effective in detecting more than 50 strains of *S. enterica* and two strains of *S. bongori* without cross-reactivity to other more common intestinal strains [54]. The presence of the *spvC* and *spvR* genes was confirmed in 13 (24.5%) tested *Salmonella* strains. Moreover, in these strains, sequence of the *rck* gene was also detected. The *spvC* gene, present in plasmids and/or chromosomes, enhances the systemic proliferation of the bacterial pathogen and contributes to its replication outside the small intestine. Together with the *invA* and *ssel* (located on the SPI-2), *spvC* facilitates the prediction of the overall pathogenicity, invasiveness, and replication potential of *Salmonella* [55]. The *spv* gene product—SpvR is a regulator of the *spv*ABCD system, which is essential for systemic virulence [47]. The *spv* gene also encoded resistance to macrophage damage while the plasmid-borne Rck outer membrane protein (product of *rck* gene) confers resistance to complement killing [56]. In addition, The Rck protein has the ability to promote bacterial invasion of mammalian cells [57]. The expression of the *rck* gene is regulated by SdiA, a quorum sensing (QS) regulator, which is activated by acyl homoserine lactones (AHL) produced by other bacteria strains [58]. In our study, the presence of the *rck* gene was found in 20 (37.7%) tested *Salmonella* strains.
3.3. Detection of Virulence Genes in Salmonella Strains

Salmonella encodes numerous genes such as invA, fimA, stn, spvC, spvR, and rck involved in bacterial pathogenicity (Table 5) [47]. In our study, the presence of invA gene in all tested Salmonella strains was confirmed. invA located on pathogenicity island 1 (SPI-1, Salmonella Pathogenicity Islands 1) has been extensively studied for its ability to promote the virulence of Salmonella [47,48]. SPI-1 is required to invade host intestinal...
### Table 5. Detection of virulence markers in *Salmonella* strains.

| *Salmonella* Strain Number | invA | fimA | stn | spvC | spvR | rck |
|--------------------------|------|------|-----|------|------|-----|
| KKP 996                  | +    | +    | +   | +    | +    | +   |
| KKP 997                  | +    | +    | +   | -    | -    | -   |
| KKP 998                  | +    | +    | +   | -    | -    | +   |
| KKP 999                  | +    | +    | +   | -    | -    | +   |
| KKP 1000                 | +    | +    | +   | -    | -    | +   |
| KKP 1001                 | +    | +    | +   | -    | -    | +   |
| KKP 1002                 | +    | +    | +   | -    | -    | -   |
| KKP 1003                 | +    | +    | +   | -    | -    | -   |
| KKP 1004                 | +    | +    | +   | -    | -    | -   |
| KKP 1005                 | +    | +    | +   | -    | -    | +   |
| KKP 1006                 | +    | +    | +   | -    | -    | -   |
| KKP 1007                 | +    | +    | +   | -    | -    | -   |
| KKP 1008                 | +    | +    | +   | -    | -    | -   |
| KKP 1009                 | +    | +    | +   | -    | -    | -   |
| KKP 1010                 | +    | +    | +   | -    | -    | -   |
| KKP 1039                 | +    | +    | +   | -    | -    | -   |
| KKP 1040                 | +    | +    | +   | -    | -    | -   |
| KKP 1041                 | +    | +    | +   | -    | -    | -   |
| KKP 1042                 | +    | +    | +   | -    | -    | -   |
| KKP 1043                 | +    | +    | +   | -    | -    | -   |
| KKP 1044                 | +    | +    | +   | -    | -    | -   |
| KKP 1045                 | +    | +    | +   | -    | -    | -   |
| KKP 1113                 | +    | +    | +   | -    | -    | -   |
| KKP 1169                 | +    | +    | +   | -    | -    | -   |
| KKP 1193                 | +    | +    | +   | -    | -    | -   |
| KKP 1213                 | +    | +    | +   | -    | -    | +   |
| KKP 1217                 | +    | +    | +   | -    | -    | -   |
| KKP 1514                 | +    | +    | +   | -    | -    | +   |
| KKP 1597                 | +    | +    | +   | -    | -    | -   |
| KKP 1608                 | +    | +    | +   | -    | -    | -   |
| KKP 1610                 | +    | +    | +   | -    | -    | -   |
| KKP 1611                 | +    | +    | +   | -    | -    | -   |
| KKP 1612                 | +    | +    | +   | -    | -    | -   |
| KKP 1613                 | +    | +    | +   | -    | -    | -   |
| KKP 1614                 | +    | +    | +   | -    | -    | -   |
| KKP 1636                 | +    | +    | +   | +    | -    | -   |
| KKP 1761                 | +    | +    | +   | -    | -    | +   |
| KKP 1762                 | +    | +    | +   | -    | -    | -   |
| KKP 1763                 | +    | +    | +   | -    | -    | -   |
Table 5. Cont.

| Salmonella Strain Number | Virulence Genes |
|--------------------------|----------------|
|                          | invA | fimA | stn | spvC | spvR | rck |
| KKP 1775                 | +    | +    | +   | +    | +    | +   |
| KKP 1776                 | +    | +    | +   | +    | +    | +   |
| KKP 3078                 | +    | +    | +   | +    | +    | +   |
| KKP 3079                 | +    | +    | +   | –    | –    | –   |
| KKP 3080                 | +    | +    | +   | –    | –    | –   |
| KKP 3081                 | +    | +    | +   | –    | –    | –   |
| KKP 3814                 | +    | +    | +   | +    | +    | +   |
| KKP 3815                 | +    | +    | +   | +    | +    | +   |
| KKP 3816                 | +    | +    | +   | +    | +    | +   |
| KKP 3817                 | +    | +    | +   | +    | +    | +   |
| KKP 3818                 | +    | +    | +   | +    | +    | +   |
| KKP 3819                 | +    | +    | +   | +    | +    | +   |
| KKP 3820                 | +    | +    | +   | +    | +    | +   |
| KKP 3821                 | +    | +    | +   | –    | –    | –   |

The presence of virulence genes in the Salmonella genome has been studied by many research groups, but the results are inconsistent. The invA gene was present in all tested Salmonella strains, according to some studies [56,59]. Other authors reported that the invA gene was present in 66% [47] and 91% [60] of the tested strains. A Salmonella virulence genes profile similar to the results obtained by our team was reported by Deguenon et al. [61], who confirmed the presence of the invA, fimA, and stn in all Salmonella strains, while the spvC and spvR sequences were found in only 10% and 20% of the tested strains, respectively. In turn, Bolton et al. [56] determined the prevalence of the rck gene in Salmonella at the level of 62.1% (18/29). In other studies [62], including ESBL-producing Salmonella, the presence of the rck gene was not confirmed in any of the strains.

3.4. Antibiotic Resistance Profiles in Salmonella Strains

Antibiotics are usually used in the treatment of infections of bacterial etiology, and their widespread use in recent decades has led to a huge problem related to the antibiotic resistance of bacterial pathogens [63–66]. β-lactam antibiotics constitute the most numerous and most frequently used group of antibiotics [67,68]. This group includes four main subgroups: penicillins, cephalosporins, carbapenems, and monobactams [69]. The mechanism of action of β-lactams consists in interfering with the synthesis of the cell wall and inhibiting the formation of bridges connecting the peptidoglycan subunits. β-lactam antibiotics block the activity of the enzymes, including transpeptidases and carboxypeptidases, which are involved in the synthesis of peptidoglycan in the bacterial cell wall [68,70,71]. Fluoroquinolones (fluorinated quinolones, FQ) are commonly used in salmonellosis therapy [72,73], and their activity is associated with the inhibition of DNA synthesis by blocking topoisomerases II, DNA gyrase, and topoisomerase IV [74–76]. Another group of antibiotics used in the treatment of salmonellosis is aminoglycosides that bind to the 30S ribosome subunit, which leads to a disturbance in the reading of genetic information and inhibition of bacterial protein synthesis [77,78]. The mechanism of phenicol action also consists in inhibiting the synthesis of bacterial proteins but as a result of binding to the large (50S) ribosome subunit [79,80]. The last group of antibiotics tested in our study was sulfonamides. Sulfonamides are structural analogs of para-aminobenzoic acid (PABA) that inhibit the synthesis of folic acid and, indirectly, nucleic acids in bacterial cells [81–83].
In our study, *Salmonella* strains were tested for susceptibility to twenty-eight antimicrobial agents belonging to eight different classes (Table 6). Among the tested strains, seven (13.2%) showed no phenotype resistance to any of the tested antibiotics. All strains were sensitive to meropenem (carbapenem) and levofloxacin (fluoroquinolone). In this study, most of the *Salmonella* strains showed a MAR (Multiple Antibiotic Resistance) index lower than 0.3, whereas one of the strains (*S. enterica* strain KKP 998) showed a MAR index above 0.5 (MAR index = 0.61).

**Table 6.** Phenotype resistance of *Salmonella* strains.

| *Salmonella* Strain Number | Antibiotic Resistance Pattern | MAR Index | MDR |
|---------------------------|------------------------------|-----------|-----|
| KKP 996                   | no resistance *              | -         |     |
| KKP 997                   | no resistance *              | -         |     |
| KKP 998                   | AMC-TTC-FEP-CTX-CPT-CAZ-CT-CRO-ETP-IMP-ATM-PER-MXF-OFX-AK-CN-TOB | 0.61      | +   |
| KKP 999                   | PRL-CPT-CAZ-CRO-PEF-MXF-C   | 0.25      | +   |
| KKP 1000                  | AMP-SAM-AMC-PRL-TTC-CPT-CN-TOB-C | 0.32      | +   |
| KKP 1001                  | CPT-CT-AK-CN-TOB            | 0.18      |     |
| KKP 1002                  | PRL-CPT-ATM-CIP-PEF-MXF-NOR-CN | 0.29      | +   |
| KKP 1003                  | CN-TOB                      | 0.07      |     |
| KKP 1004                  | AMC-TZP-TTC-FEP-CTX-CPT-CT-MXF-OFX-AK-TOB | 0.39      | +   |
| KKP 1005                  | CPT-AK                      | 0.07      |     |
| KKP 1006                  | CPT-AK                      | 0.07      |     |
| KKP 1007                  | AMC-TTC-CPT-CT-CRO-MXF-AK-CN-TOB-SXT | 0.36      | +   |
| KKP 1008                  | no resistance *             | -         |     |
| KKP 1009                  | CPT-CIP-MXF-CN              | 0.14      | +   |
| KKP 1010                  | CPT-PEF-OFX-AK-SXT          | 0.18      | +   |
| KKP 1039                  | MXF-AK-TOB                  | 0.11      |     |
| KKP 1040                  | no resistance *             | -         |     |
| KKP 1041                  | CPT-AK                      | 0.07      |     |
| KKP 1042                  | CPT                         | -         |     |
| KKP 1043                  | CPT-ETP-CN-TOB              | 0.14      | +   |
| KKP 1044                  | AMC-PRL-TZP-TTC-CPT-CT-MXF-IMP-MXF-AK-CN-TOB | 0.39      | +   |
| KKP 1045                  | CPT-MXF                     | 0.07      |     |
| KKP 1113                  | AK                          | -         |     |
| KKP 1169                  | no resistance *             | -         |     |
| KKP 1193                  | CT-CN-TOB                   | 0.11      |     |
| KKP 1213                  | PRL-TZP-CPT-CT-CRO-ETP-OFX-AK-CN-TOB | 0.36      | +   |
| KKP 1217                  | CPT-CN-TOB                  | 0.11      |     |
| KKP 1514                  | CPT-CT-CRO-ETP-ATM-CIP-MXF-AK-CN-TOB | 0.36      | +   |
| KKP 1597                  | CPT-ETP-CIP-MXF-AK-CN-TOB   | 0.25      | +   |
| KKP 1608                  | no resistance *             | -         |     |
| KKP 1610                  | FEP-AK                      | 0.07      |     |
| KKP 1611                  | CPT-AK                      | 0.11      |     |
Table 6. Cont.

| Salmonella Strain Number | Antibiotic Resistance Pattern | MAR Index | MDR |
|--------------------------|--------------------------------|-----------|-----|
| KKP 1612                 | AMC-TTC-CPT-CRO-IMP-PEF-MXF-AK-CN-TOB | 0.36      | +   |
| KKP 1613                 | AK                              | -         |     |
| KKP 1614                 | no resistance *                 | -         |     |
| KKP 1636                 | PRL-CRO-PEF-MXF-AK-CN-TOB       | 0.25      | +   |
| KKP 1761                 | CT-CRO-PEF-MXF-NOR-AK-CN-TOB    | 0.29      | +   |
| KKP 1762                 | AMC-CPT-CT-CRO-MXF-AK-CN-TOB    | 0.29      | +   |
| KKP 1763                 | CN                              | -         |     |
| KKP 1775                 | PRL-TZP-FEP-CPT-CT-MXF-CN       | 0.25      | +   |
| KKP 1776                 | TTC-FEP-AK-TOB                  | 0.14      | +   |
| KKP 3078                 | CPT-MXF-CN                      | 0.11      | +   |
| KKP 3079                 | PRL-CPT-CT-CRO-AK-CN-TOB       | 0.25      | +   |
| KKP 3080                 | AMC-FEP-CTX-CPT-CT-CRO-MXF-OFX-AK-CN-TOB | 0.39   | +   |
| KKP 3081                 | TZP-TTC-FEP-PEF-MXF-AK-CN-TOB   | 0.29      | +   |
| KKP 3814                 | AK                              | -         |     |
| KKP 3815                 | AMC-CPT-CT-CRO-CIP-PEF-MXF-AK-CN | 0.32      | +   |
| KKP 3816                 | CPT-AK                          | 0.07      |     |
| KKP 3817                 | CPT-ETP-ATM-CIP-MXF-CN          | 0.21      | +   |
| KKP 3818                 | AK                              | -         |     |
| KKP 3819                 | PEF                             | -         |     |
| KKP 3820                 | AMP-SAM-PRL-TTC-CPT-AK          | 0.25      | +   |
| KKP 3821                 | FEP-CTX-CPT-CAZ-CZA-CT-CRO-ETP-ATM-PEF-AK-TOB | 0.43 | +   |

* means no resistance to the tested antibiotics. Notes: AMP—ampicillin; SAM—sulbactam/ampicillin; AMC—amoxicillin/clavulanic acid; PRL—piperacillin; TZP—piperacillin/tazobactam; TTC—ticarcillin/clavulanic acid; FEP—cefepime; CTX—ceftaxime; CPT—ceftaroline; CAZ—ceftazidime; CZA—ceftazidime/avibactam; CT—ceftolozane/tazobactam; CRO—ceftroxime; ETP—ertapenem; IMP—imipenem; ATM—aztreonam; CIP—ciprofloxacin; PEF—pelofoxacin; MXF—moxifloxacin; OFX—ofloxacin; NOR—norfoxacin; AK—amikacin; CN—gentamycin; TOB—tobramycin; C—chloramphenicol; SXT—sulphamethoxazole/trimethoprim. Abbreviations: MAR—Multiple Antibiotic Resistance; MDR—Multi-Drug Resistant strain.

Moreover, a high prevalence of MAR was observed amongst the strains; 50.9% (27/53) of the isolates were MDR (Multi-Drug Resistant). Salmonella enterica strain KKP 998 (isolated from food product) exhibited the most extensive resistance profile to 17 antibiotics (AMC-TTC-FEP-CTX-CPT-CAZ-CT-CRO-ETP-IMP-PEF-MXF-OFX-AK-CN-TOB), belonging to 6 different classes of antibiotics (penicillins, cephalosporins, carbapenems, monobactams, fluoroquinolones, and aminoglycosides). Extensive resistance profiles were also exhibited by S. enterica strains KKP 3821, KKP 1004, KKP 1044, and KKP 3080. S. enterica strain KKP 3281 (isolated from animal breeding rooms) was resistant to 12 antimicrobials (FEP-CTX-CPT-CAZ-CZA-CT-CRO-ETP-IMP-PEF-TOB) from 5 different classes of antibiotics (cephalosporins, carbapenems, monobactams, fluoroquinolones, and aminoglycosides), while the remaining three strains (isolated from food products) showed resistance to the 11 tested antibiotics. Some antibiotics were completely ineffective against tested bacteria (unpublished data). S. enterica strains KKP 1000 and KKP 3820 showed full growth with ampicillin, piperacillin, and chloramphenicol discs. Discs with sulphamethoxazole/trimethoprim (cotrimoxazole) did not inhibit the growth of S. enterica strains KKP 1007 and KKP 1010. In the case of S. enterica strain, KKP 3821 zones of growth inhibition were observed for five antibiotics (cefotaxime, ceftazidime, ceftazidime/avibactam, ceftolozane/tazobactam, and aztreonam). Moreover, as many as 14 strains of Salmonella...
(26.4%) were resistant to all tested antibiotics from the aminoglycosides class (i.e., amikacin, gentamycin, and tobramycin) (Table 6). *Salmonella* strains showed the highest resistance to antibiotics from the aminoglycoside class (Table 7). Against amikacin, gentamicin, and tobramycin, phenotypic resistance was exhibited by 31 (58.5%), 26 (49.1%), and 24 (45.3%) strains, respectively. Ceftaroline, belonging to the class of broad-spectrum cephalosporins, was effective against the smallest number of strains tested. Thirty-two of the tested *Salmonella* strains (60.4%) were resistant to this antibiotic.

Table 7. Prevalence of phenotypic antibiotic resistance in *Salmonella* strains.

| Antimicrobial Class | Antimicrobial Agent | Number of Resistant Strains | Percentage of Resistant Strains (%) |
|---------------------|---------------------|-----------------------------|------------------------------------|
| Penicillins         | ampicillin          | 2                           | 3.8                                |
|                     | sulbactam/ampicillin| 2                           | 3.8                                |
|                     | amoxicillin/clavulanic acid | 9 | 17.0                          |
|                     | piperacillin        | 9                           | 17.0                                |
|                     | piperacillin/tazobactam | 5 | 9.4                           |
|                     | ticarcillin/clavulanic acid | 9 | 17.0                         |
| Cephalosporins      | cefepime            | 8                           | 15.1                                |
|                     | cefotaxime          | 4                           | 7.6                                |
|                     | ceftaroline         | 32                          | 60.4                                |
|                     | ceftazidime         | 3                           | 5.7                                |
|                     | ceftazidime/avibactam | 1 | 1.9                           |
|                     | ceftolozane/tazobactam | 14 | 26.4                         |
|                     | ceftriaxone         | 14                          | 26.4                                |
| Carbapenems         | ertapenem           | 7                           | 13.2                                |
|                     | imipenem            | 3                           | 5.7                                |
|                     | meropenem           | 0                           | 0.0                                |
| Monobactams         | aztreonam           | 5                           | 9.4                                |
| Fluoroquinolones    | ciprofloxacin       | 6                           | 11.3                                |
|                     | pefloxacin          | 11                          | 20.8                                |
|                     | levofloxacin        | 0                           | 0.0                                |
|                     | moxifloxacin        | 20                          | 37.7                                |
|                     | ofloxacin           | 6                           | 11.3                                |
|                     | norfloxacin         | 2                           | 3.8                                |
| Aminoglycosides     | amikacin            | 31                          | 58.5                                |
|                     | gentamycin          | 26                          | 49.1                                |
|                     | tobramycin          | 24                          | 45.3                                |
| Phenicols           | chloramphenicol     | 3                           | 5.7                                |
| Sulfonamides        | sulphamethoxazole/trimethoprim | 2 | 3.8                         |
large percentage of MDR strains, i.e., they are insensitive to at least one antibiotic from at least three groups of antibacterial drugs used in the treatment of infections caused by *Salmonella* [84]. The results of studies published by Pławińska-Czarnak et al. [7] also confirm a high percentage (53.8%) of MDR *Salmonella* strains that showed resistance to β-lactams, aminoglycosides, cephalosporins, fluoroquinolones, sulfonamides, and tetracyclines. The high resistance to fifth-generation cephalosporins (ceftaroline), which are used in the treatment of severe bacterial infections, seems to be of concern. Among the tested *Salmonella* strains, as many as 60.4% were resistant to ceftaroline. Compared to the early-generation cephalosporins, ceftaroline has better stability to β-lactamases. However, it is inactivated by several classes of these enzymes and, thus, is not recommended for the treatment of ESBL-positive Gram-negative bacteria infections, as well as infections caused by bacteria producing metallo-β-lactamases or AmpC-type cephalosporinases [85]. The presence of a high percentage of strains resistant to the fifth generation of cephalosporins is an alarming situation, given the risk of transferring resistance genes in the environment. Cefaroline is the drug of choice among cephalosporins and is active against multidrug-resistant *Staphylococcus aureus*, including MRSA, VRSA, and VISA [85,86]. Another class of antibiotics used in severe *Salmonella* infections is the sulfonamides; however, in this case, only 3.8% of the strains showed resistance. There was also no high percentage of strains resistant to carbapenems, which are used if ciprofloxacin and third-generation cephalosporin fail. In the study by Marin et al. [87], all isolated *Salmonella* strains showed resistance to at least one antibiotic, and 72% were MDR strains, with gentamicin–colistin and gentamicin–colistin–ampicillin being the most frequently observed resistance patterns. In a study conducted in China [88], 50.4% of the *Salmonella* isolates mostly originated from food products that were MDR. In total, 73% of the MDR *Salmonella* strains were resistant to tetracycline, 67% to ampicillin, and 59% to doxycycline. Our research shows a similar share of multidrug-resistant *Salmonella* strains (50.9%); however, significantly fewer of them were resistant to ampicillin (3.8%). Results obtained in another study carried out in Brazil [42] indicated that the highest percentage of *Salmonella* strains originated from broiler processing plants that were resistant to nalidixic acid and tetracycline. Strains resistant to meropenem, imipenem, and ciprofloxacin were not detected, while resistance to imipenem and ciprofloxacin was observed in 5.7% and 11.3% of *Salmonella* strains, respectively.

According to the latest report released by EFSA and ECDC [22], in the years 2019–2020 in the UE, there was a high percentage of *Salmonella* resistant to ampicillin, sulfonamides, and tetracyclines isolated from hospitalized patients. Zoonotic isolates showed moderate to very high resistance to these antibiotics. A very high percentage of FQ-resistant strains was observed in zoonotic isolates. *Salmonella* isolates from patients showed moderate resistance to ciprofloxacin. High resistance to third-generation cephalosporins has been observed neither for zoonotic strains nor those isolated from patients. In our study, none of the strains originated from hospitalized patients showed resistance to ampicillin and sulfonamides, but *S. enterica* KKP 996 and KKP 1193 strains (66% of strains isolated from hospitalized patients) showed genotypic resistance to tetracyclines (Table 8). Low percentage of FQ-resistant strains was observed amongst zoonotic isolates. Similar to the data collected in the EFSA/ECDC report, resistance to cefotaxime, ceftriaxone, and ceftazidime did not occur frequently (7.6%, 26.4%, and 5.7%, respectively) (Table 7). According to the EFSA/ECDC report, 25.4% of the strains isolated from patients were multidrug resistant. A significantly higher percentage of MDR strains was observed in *Salmonella* strains isolated from animals: 53.6% from broiler carcasses, 43.3% from pigs, and 23.1% from calves [22]. The above report [22] indicates the main etiological factors of *Salmonella* infections and underlines that special caution should be exercised regarding contact with raw materials and food of animal origin. Our outcomes confirmed that food is a common source of multidrug-resistant pathogenic bacteria (47.4% (18/38) MDR strains from food products and 55.6% (5/9) MDR strains from animals or animal breeding rooms).
Table 8. Distribution of AMR-related genes in relation to antibiotic resistance patterns in *Salmonella* strains.

| *Salmonella* Strain Number | Phenotypic Antibiotic Resistance Pattern | Genotypic Antibiotic Resistance Profile |
|-----------------------------|----------------------------------------|----------------------------------------|
| KKP 996                     | no resistance *                        | *flo*<sub>F</sub>, *tet*<sub>C</sub>    |
| KKP 997                     | no resistance *                        | *tet*<sub>C</sub>                       |
| KKP 998                     | AMC-TTC-FEP-CTX-CPT-CAZ-CT-CRO-ETP-ATM-PEF-MXF-OFX-AK-CN-TOB | *strA*/strB, *flo*<sub>F</sub>, *aphA*1, *tet*<sub>C</sub>, sul1 |
| KKP 999                     | PRL-CPT-CAZ-CRO-PEF-MXF-C              | *aad*<sub>A</sub>, *flo*<sub>R</sub>, sul1 |
| KKP 1000                    | AMP-SAM-AMC-PRL-TTC-CPT-CN-TOB-C       | *aad*<sub>A</sub>, *flo*<sub>F</sub>, *flo*<sub>R</sub>, *tet*<sub>A</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1001                    | CPT-CT-AK-CN-TOB                       | *flo*<sub>F</sub>, *tet*<sub>A</sub>    |
| KKP 1002                    | PRL-CPT-ATM-CIP-PEF-MXF-NOR-CN         | *tet*<sub>A</sub>, *tet*<sub>C</sub>    |
| KKP 1003                    | CN-TOB                                 | ND **                                   |
| KKP 1004                    | AMC-TZP-TTC-FEP-CTX-CPT-CT-MXF-OFX-AK-TOB | *aad*<sub>A</sub>, *flo*<sub>R</sub>, *tet*<sub>B</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1005                    | CPT-AK                                 | *flo*<sub>R</sub>, *tet*<sub>B</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1006                    | CPT-AK                                 | *tet*<sub>C</sub>, sul1                |
| KKP 1007                    | AMC-TTC-CPT-CT-CRO-MXF-AK-CN-TOB-SXT  | *ada*<sub>A</sub>, *flo*<sub>R</sub>, *tet*<sub>B</sub>, sul1 |
| KKP 1008                    | no resistance *                        | *tet*<sub>B</sub>, *tet*<sub>C</sub>    |
| KKP 1009                    | CPT-CIP-MXF-CN                         | *tet*<sub>B</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1010                    | CPT-PEF-OFX-AK-SXT                     | *strA*/strB, *aad*<sub>A</sub>, *tet*<sub>A</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1039                    | MXF-AK-TOB                             | *tet*<sub>B</sub>, *tet*<sub>C</sub>    |
| KKP 1040                    | no resistance *                        | ND **                                   |
| KKP 1041                    | CPT-AK                                 | *ada*<sub>A</sub>, *tet*<sub>B</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1042                    | CPT                                    | *strA*/strB, *tet*<sub>B</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1043                    | CPT-ETP-CN-TOB                         | *tet*<sub>C</sub>, sul1                |
| KKP 1044                    | AMC-PRL-TZP-TTC-CPT-CRO-IMP-MXF-AK-CN-TOB | *flo*<sub>F</sub>, *tet*<sub>C</sub>    |
| KKP 1045                    | CPT-MXF                                | *tet*<sub>B</sub>, *tet*<sub>C</sub>, sul1 |
| KKP 1113                    | AK                                     | ND **                                   |
| KKP 1169                    | no resistance *                        | *strA*/strB, *tet*<sub>C</sub>, sul1, sul2 |
| KKP 1193                    | CT-CN-TOB                              | *tet*<sub>C</sub>, sul1                |
| KKP 1213                    | PRL-TZP-CPT-CT-CRO-ETP-OFX-AK-CN-TOB   | *tet*<sub>B</sub>                       |
| KKP 1217                    | CPF-CN-TOB                             | *tet*<sub>B</sub>, sul1                |
| KKP 1514                    | CPT-CT-CRO-ETP-ATM-CIP-MXF-AK-CN-TOB   | *tet*<sub>B</sub>, *tet*<sub>C</sub>    |
| KKP 1597                    | CPT-ETP-CIP-MXF-AK-CN-TOB              | *tet*<sub>A</sub>, *tet*<sub>B</sub> |
| KKP 1608                    | no resistance *                        | *flo*<sub>F</sub>                       |
| KKP 1610                    | FEP-AK                                 | sul1                                    |
| KKP 1611                    | CPT-AK                                 | ND **                                   |
| KKP 1612                    | AMC-TTC-CPT-CRO-IMP-PEF-MXF-AK-CN-TOB  | *tet*<sub>C</sub>                       |
| KKP 1613                    | AK                                     | *tet*<sub>C</sub>                       |
| KKP 1614                    | no resistance *                        | *tet*<sub>C</sub>                       |
| KKP 1636                    | PRL-CRO-PEF-MXF-AK-CN-TOB              | *tet*<sub>A</sub>, *tet*<sub>B</sub>, *tet*<sub>C</sub> |
| KKP 1761                    | CT-CRO-PEF-MXF-NOR-AK-CN-TOB           | *tet*<sub>B</sub>, *tet*<sub>C</sub>    |
| KKP 1762                    | AMC-CPT-CT-CRO-MXF-AK-CN-TOB           | *tet*<sub>B</sub>                       |
| KKP 1763                    | CN                                     | *tet*<sub>B</sub>, *tet*<sub>C</sub>    |
Table 8. Cont.

| Salmonella Strain Number | Phenotypic Antibiotic Resistance Pattern | Genotypic Antibiotic Resistance Profile |
|--------------------------|----------------------------------------|----------------------------------------|
| KKP 1775                 | PRL-TZP-FEP-CPT-CT-MXF-CN              | ND **                                  |
| KKP 1776                 | TTC-FEP-AK-TOB                         | tetC                                   |
| KKP 3078                 | CPT-MXF-CN                             | tetB                                   |
| KKP 3079                 | PRL-CPT-CT-CRO-AK-CN-CN-TOB            | tetA, tetC                             |
| KKP 3080                 | AMC-FEP-CTX-CPT-MXF-OFX-AK-CN-TOB      | floR, tetA, tetC                       |
| KKP 3081                 | TZP-TTC-FEP-PER-MXF-AK-CN-CN-TOB       | tetA, tetB, tetC                       |
| KKP 3814                 | AK                                      | tetB                                   |
| KKP 3815                 | AMC-CPT-CT-CRO-CIP-PER-MXF-AK-CN-CN-TOB| tetB                                   |
| KKP 3816                 | CPT-ETP-ATM-CIP-MXF-CN-CN-TOB          | tetB                                   |
| KKP 3818                 | AK                                      | tetB                                   |
| KKP 3819                 | PEF                                      | floR, tetC, sul1                       |
| KKP 3820                 | AMP-SAM-PRL-TTC-CPT-CT-CR-ETP-ATM-PER-TOB|aadA, floR, aphA1-IAB, sul1 |
| KKP 3821                 | FEP-CTX-CPT-CAZ-CT-CRO-ETP-ATM-PER-TOB | ND **                                  |

* means no resistance to the tested antibiotics | ** ND means: no resistance genes were detected. Notes: AMP—ampicillin; SAM—sulbactam/ampicillin; AMC—amoxicillin/clavulanic acid; PRL—piperacillin; TZP—piperacillin/tazobactam; TTC—ticarcillin/clavulanic acid; FEP—cefepime; CTX—cefotaxime; CPT—ceftazidime; CZA—ceftazidime/avibactam; CT—ceftolozane/tazobactam; CRO—ceftaxoxime; ETP—ertapenem; IMP—imipenem; ATM—aztreonam; CIP—ciprofloxacin; PER—pemfloxacn; MXF—moxifloxacin; OFX—ofloxacin; NOR—norfloxacin; AK—amikacin; CN—gentamycin; TOB—tobramycin; C—chloramphenicol; SXT—sulphamethoxazole/trimethoprim

3.5. Genotypic Resistance Profiles in Salmonella Strains

A genotypic resistance profile was determined for a panel of Salmonella strains using 25 primer pairs. Salmonella strains belonging to one clone in PFGE (Figure 1) did not show the same virulence profiles. The aadB and aacC genes encoding resistance to gentamicin (an aminoglycoside antibiotic) were not identified in any of the strains. There was also no presence of mcr1, mcr2, mcr3, and mcr4 genes, encoding resistance to colistin, belonging to peptide antibiotics, and dfrA1, dfrA10 and dfrA12 genes associated with resistance to trimethoprim (dihydrofolate reductase inhibitor). Regarding the genes encoding chloramphenicol resistance, cat1 and cat2 were not found in any of the tested strains. However, the presence of the third chloramphenicol resistance gene (floR) was confirmed in 7 (13.2%) of the tested strains. Phenotypic resistance to chloramphenicol was confirmed only in three Salmonella strains—KKP 999, KKP 1000, and KKP 3820 (Table 8). Among the two tested genes of resistance to neomycin (aminoglycoside antibiotic), the aphA1 was present in only one (1.9%) Salmonella strain (KKP 998), whereas aphA2 was not detected in any of the strains. In turn, the genes encoding resistance to sulfamethoxazole were also tested in the Salmonella strains, and out of the three tested genes (sul1, sul2, and sul3), no sul3 gene was found in any of the strains. Moreover, in seven Salmonella strains (i.e., KKP 1003, KKP 1040, KKP 1113, KKP 1611, KKP 1775, KKP 3816, and 3821), none of the tested resistance genes was identified. Importantly, only S. enterica strain KKP 1040 showed phenotypical sensitivity to all tested antibiotics with the simultaneous absence of all tested resistance genes.

The highest percentage of resistant strains was found for tetracycline, where 10 (18.9%), 23 (43.4%), and 31 (58.5%) Salmonella strains contained the tetA, tetB, and tetC genes, respectively (Table 9). A high percentage of Salmonella strains resistant to tetracyclines is consistent with the data from the EFSA and ECDC report [22] from 2022. A relatively high percentage of Salmonella strains (35.8%) contained the sul1 gene, encoding resistance.
to sulfamethoxazole, although only two (3.8%) *Salmonella* strains showed phenotypic resistance to sulfamethoxazole with an inhibitor (trimethoprim) (Table 7).

### Table 9. Prevalence of genotypic antibiotic resistance in *Salmonella* strains.

| Antibiotic     | Target Gene       | Number of Resistant Strains (n = 53) | Percentage of Resistant Strains (%) |
|----------------|-------------------|-------------------------------------|-----------------------------------|
| streptomycin   | strA,strB         | 4                                   | 7.6                               |
|                | aadA              | 7                                   | 13.2                              |
| florfenicol    | floF              | 7                                   | 13.2                              |
| chloramphenicol| floR              | 7                                   | 13.2                              |
| kanamycin      | aphA-IAB          | 1                                   | 1.9                               |
| neomycin       | aphA1             | 1                                   | 1.9                               |
| tetracycline   | tetA              | 10                                  | 18.9                              |
|                | tetB              | 23                                  | 43.4                              |
|                | tetC              | 31                                  | 58.5                              |
| sulfamethoxazole| sul1              | 19                                  | 35.8                              |
|                | sul2              | 1                                   | 1.9                               |

### 3.6. Screening for Phenotypic and Genotypic Detection of β-lactamases-Producing *Salmonella* Strains

Since the phenotype sensitivity to antibiotics can be conferred by several different antibiotic resistance genes (ARGs), in the last step of our research, the presence of the main mechanisms of β-lactam resistance (phenotypically and genotypically expressed) in *Salmonella* was determined. In *Salmonella*, as in other bacteria from the *Enterobacteriaceae* family, the main mechanism of resistance to β-lactam antibiotics are β-lactamases encoded by *bla* genes [7,89,90]. Many different β-lactamases have been described, but β-lactamases of the TEM type (named after the patient Temoneira), CTX-M type (active on cefotaxime, first isolated at Munich), and SHV type (sulfhydryl reagent variable) predominate in *Salmonella* [89,91–93]. They belong to β-lactamases with a broad spectrum of substrate activity (ESBL). ESBL enzymes inactivate cephalosporins and first-, second-, and third-generation penicillins [7,89]. They are not active against carbapenems [94]. ESBL genes of the TEM and CTX-M types were not identified among our strains. CTX-M enzymes are active against cephalosporins and monobactams and are currently of great epidemiological and clinical importance [7]. The SHV-type ESBL gene was identified in two isolates—*S. enterica* strains KKP 1597 and KKP 1610 isolated from food products (Table 4). The presence of the ESBL mechanism was not confirmed phenotypically; therefore, it is likely that the *bla*SHV gene associated with SHV-type ESBL resistance in *S. enterica* KKP 1597 and KKP 1610 strains may be inactive. According to the literature, the presence of *bla*SHV is often associated with the *Enterobacteriaceae* family in nosocomial infections [7]. The presence of *bla*SHV in *Salmonella* strains isolated from hospitalized patients was not confirmed in our study. Another group of β-lactamases is AmpC, which confers resistance to all β-lactam antibiotics except fourth-generation cephalosporins and carbapenems [95,96]. Contrary to ESBL, the AmpC group is not sensitive to β-lactam inhibitors such as clavulanic acid, sulbactam, and tazobactam [95]. The mechanism of AmpC can be encoded by genes located on chromosomes or plasmids [96]. It has been shown that in *Salmonella*, resistance to broad-spectrum cephalosporins is often associated with *bla*CMV–2 gene [97]. In our study, none of the strains exhibited a resistance mechanism to AmpC-type β-lactamases. Another gene encoding resistance to β-lactam antibiotics is the *bla*PSE-1 gene located on the first-class integron [7,89]. Moreover, the presence of the *bla*PSE–1 gene associated with the PSE-1 drug efflux mechanism was identified in six *Salmonella* strains, including KKP 1000, KKP 1004,
KKP 1005, KKP 1007 isolated from food products, and KKP 3819 and KKP 3820 isolated from poultry. Moreover, no carbapenemase-producing *Salmonella* strains were detected among the tested isolates.

According to the EFSA and ECDC report, the percentage of ESBL and AmpC-producing *Salmonella* strains ranged from very low to low (animal isolates) and very low among isolates obtained from hospitalized patients. Carbapenemase-producing isolates were not detected in any of the zoonotic *Salmonella* strains, while in 2019–2020, among isolates from humans, only three carbapenemase-producing *Salmonella* strains were detected [22]. The results from the above-mentioned report are comparable to our study and confirm the low percentage of *Salmonella* strains with resistance mechanisms.

4. Conclusions

*Salmonella* isolates show phenotypic resistance to many antibiotics and encode numerous genes associated with antimicrobial resistance. The high number of resistant *Salmonella* strains (isolated both at the end of the 20th century and in recent years) in combination with multiple ARGs indicates the possible irrational/unjustified use of antibiotics for many years. The problem of the development of ESBL or AmpC resistance mechanisms in *Salmonella* strains resulting from both our research and European reports is not alarming yet; however, it is necessary to constantly monitor antimicrobial resistance profiles in all food chain links and to implement a policy of rational antibiotic stewardship (AMS), which may stop or at least significantly limit the further acquisition of antibiotic resistance among *Salmonella* strains. A significant reduction in the use of antibiotics in animal husbandry may limit the transfer of antibiotic resistance genes through food. The development of new, alternative antibacterial agents also represents a relevant approach. One concept that recurs due to the growth of MDR strains is the use of strictly lytic bacteriophages. Currently, phage therapy is an experimental treatment aimed at eradicating bacterial strains for which antibiotic therapy does not bring the expected results. The use of specific bacteriophages in the food industry in the EU countries is not approved for use yet, unlike, for example, in the USA or Canada, where commercial preparations based on phage cocktails against foodborne pathogens for food products are applied.

**Supplementary Materials:** The following are available online at https://www.mdpi.com/article/10.3390/pathogens11111323/s1, Table S1: The PCR conditions for amplification of virulence markers, AMR-related and β-lactamases-related genes in *Salmonella* strains.

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