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An Evaluation of the Pathogenic Potential, and the Antimicrobial Resistance, of *Salmonella* Strains Isolated from Mussels

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Abstract: *Salmonella* spp. and antimicrobial resistant microorganisms are two of the most important health issues worldwide. In the present study, strains naturally isolated from mussels harvested in Galicia (one of the main production areas in the world), were genetically characterized attending to the presence of virulence and antimicrobial resistance genes. Additionally, the antimicrobial profile was also determined phenotypically. Strains presenting several virulence genes were isolated but lacked all the antimicrobial resistance genes analyzed. The fact that some of these strains presented multidrug resistance, highlighted the possibility of bearing different genes than those analyzed, or resistance based on completely different mechanisms. The current study highlights the importance of constant surveillance in order to improve the safety of foods.

Keywords: *Salmonella* spp.; virulence genes; antibiotic resistance; mussel

1. Introduction

*Salmonella* spp. is one of the most important foodborne pathogens worldwide, as highlighted by figures of salmonellosis in Europe, for which 52,702 confirmed cases were reported in 2020. In Spain, 3526 human salmonellosis were reported that year. At the distribution level, the highest number of *Salmonella*-positive samples was reported to be from meat products intended to be cooked (broiler, turkey and even pig and bovine meat) [1]. Regardless of these figures, *Salmonella* spp. is specifically regulated by the European Regulation 2073/2005 in a plethora of foodstuffs, including live mollusks [2]. In line with this Regulation, is the fact that the European Food Safety Authority (EFSA) reported that 2.01% of the outbreaks linked to the consumption of fish and fishery products were caused by *Salmonella* spp. [3]. In addition to this, another major health problem is the increased resistance of the microorganisms to antibiotics [4]. It was estimated that, every year, antimicrobial resistant microorganisms (ARMs) cause more than 23,000 deaths in the United States [5]. It has been previously reported that *Salmonella* spp. can be multidrug resistant (MDR) to antibiotics, thus becoming an even more important threat [6]. The EFSA data on antimicrobial resistance in 2018/2019, reported a 25.4% MDR in isolates of *Salmonella* spp. of human origin. Regarding the isolates from the food production chain, approximately 38% of the strains obtained from chicken, turkey and swine farms were MDR, but the highest number of MDR was found in pig carcasses, from which 43.3% of the *Salmonella* spp. recovered were MDR [7].
Spain is the third largest mussel producer worldwide, and Galicia accounts for 98% of the Spanish production [8]. In this region, the production has been developed in five different Rias: Vigo, Pontevedra, Arousa, Ares-Betanzos and Muros-Noia, but mainly in Arousa [9–11]. Previous studies have already reported the presence of pathogenic bacteria in this economically important mussel production area. These studies included pathogenic <i>Vibrio</i> and <i>Campylobacter</i> species, as well as Shiga toxin-producing <i>Escherichia coli</i>, thus highlighting the importance of screening for additional microorganisms, which can pose a risk for consumers [12–14]. In addition to these studies, <i>Salmonella</i> spp. was also detected and characterized in this area more than 10 years ago. All these facts highlight the importance of analyzing this food product [15,16]. For these reasons, the aim of the present study is to determine the presence of virulence genes in <i>Salmonella</i> spp. Strains, naturally isolated from mussels harvested in the Galician Rias (NW Spain), determine their antimicrobial resistance profiles, and compare these, if possible, with the results observed in previous studies conducted in the same geographical area and in other parts of the world.

2. Materials and Methods

2.1. Sampling

Mussel samples, harvested in the Rias of Arousa and Vigo (Figure 1), were analyzed from 2012 to 2016. The samples were collected, as previously reported [12]. Briefly, the mussels were collected from the rafts, placed in sterile bags and transported under refrigeration to the laboratory. Upon arrival, they were washed with tap water, opened and collected under aseptic conditions (dead or broken mussels were discarded). Twenty-five grams of mussel (tissue and liquor), obtained from a minimum of 15 individuals, were weighted and diluted 1/10 in 225 mL of buffered peptone water (BPW, BioMérieux, S.A., Marcy l’Etoile, France), homogenized for 90 s and incubated at 37 °C for 18 ± 2 h. Once the incubation was completed, the DNA was extracted and analyzed by qPCR, as described below.

![Figure 1. Geographical area for mussel sample collection.](image-url)
supernatant. The pellet was resuspended in 50 μL of Lysis Buffer plus 5 μL of proteinase K solution (Sigma-Aldrich, St. Louis, MO, USA), and homogenized by vortexing. Then, the samples were incubated at 95 °C for 15 min. After incubation, the samples were left at room temperature for 3 min and were centrifuged for 1 min at 14,000 rpm. Finally, 250 μL of milli-Q water was added and the samples were centrifuged for 1 min at 14,000 rpm. All extracts were stored at −80 °C until qPCR analysis.

2.3. Screening of Salmonella spp. by qPCR in Mussel Samples

The detection of Salmonella spp. was performed with the MicroSEQ® (Applied Biosystems, Foster City, CA, USA) kit following the supplier’s instructions. The DNA extraction from the pre-enriched cultures was performed, as described in M & M 2.3, and 5 μL of DNA was used as a template. The reactions were performed in a final reaction volume of 20 μL, and run in a 7500 Fast Real Time PCR System Thermal Cycler (Applied Biosystems, Foster City, CA, USA) with the following thermal profile: 95 °C for 5 min (Hot-Start) followed by 40 cycles of denaturation at 95 °C for 5 s and annealing–extension at 60 °C for 30 s. Positive qPCR results were confirmed streaking the pre-enrichment culture on CHROMID® Salmonella ELITE agar, and incubated at 37 °C for 18 ± 2 h. Typical colonies were subcultured on triptic soy agar (TSA), incubated at 37 °C for 18 ± 2 h and then biochemically identified with API20E (BioMérieux, S.A., Marcy l’Etoile, France).

2.4. Genetic Characterization of the Isolates

2.4.1. Screening for Virulence Genes

A total of five virulence genes were screened (Table 1). These were: invA (first gene in an operon which is thought to trigger the internalization of Salmonella spp.); hilA (important in the regulation of the Type III secretion system); sopB (encodes for Salmonella outer protein B, part of the Type III secretion system); pefA (plasmid encoded fimbriae) and spvC, encoded in a virulence plasmid, whose expression helps the replication of Salmonella in the host cell reticuloendothelial system, especially in cells of the liver and spleen [17]. The detection of these genes was performed, as previously described [18–22].

| Primers | Target Gene | Sequence (5′-3′) | Reference |
|---------|-------------|------------------|-----------|
| invA-f  | invA        | GTG AAA TTA TCG CCA CGT TCG GGC AA TCA TCG CAC CGT CAA AGG AAC C | [19] |
| invA-r  |             |                  |           |
| spvC-1  | spvC        | CGG AAA TAC CAT CTA CAA ATA CCG AAA CCC ATA CTT ACT CTG | [18] |
| spvC-2  |             |                  |           |
| pefA1   | pefA        | TGT TTC CGG GCT GCT GCT | [20] |
| pefA2   |             |                  |           |
| hilA DS | hilA        | CGG AAG CTT ATT TGC GCC ATG CCT AGG TAG GCA TGG ATC CCC GCC GGC GAG ATT GTG | [21] |
| hilA US |             |                  |           |
| sopB PRSB1 | sopB   | CCA CCG TTC TGG GTA AAC AAG AC AGG ATT GAG CTC TCT TGG GCA T | [22] |
| sopB PRSB2 |             |                  |           |
| armA-f  | armA        | TAT GGG GGT CTT ACT ATG CCT TCT | TAT GGG GGT CTT ACT ATG CCT TCT | [23] |
| armA-r  |             |                  |           |
| rmtA-f  | rmtA        | TCT GCC ATT CCC TCC TTC TCC TTC TCT GGC TCC | TCT GCC ATT CCC TCT TTC TCC TTC TCT GGC TCC | [22] |
| rmtA-r  |             |                  |           |
| rmtB-f  | rmtB        | TCA AGC ATG CCC TCA CCT C | GCC GGG CAA AGG TAA AAT CC | [23] |
| rmtB-r  |             |                  |           |
| rmtC-f  | rmtC        | CTC AGA TCT GAC CCA ACA AAG A AGG ATT GAG C | CTC AGA TCT GAC CCA ACA AAG | [24] |
| rmtC-r  |             |                  |           |
| rmtD-f  | rmtD        | CTG TTT GAA GCC AGC GGA AAG C | GCG CCT CCC TCC ATT CCC GGT AAT AG | [22] |
| rmtD-r  |             |                  |           |
| npmA-f  | npmA        | CTC AAA GGA ACA AAG ACG G GA A CA TG C GCA CAA ACT C | ATA AAA CCG GCA GCC GTG | [24] |
| npmA-r  |             |                  |           |
| CTX-M-15-F1 | blac  | GAA TTT TGA CTC TGG GGG | |
| CTX-M-15-F2 |             |                  |           |
2.4.2. Screening for Antibiotic Resistance Genes

In addition to these virulence factors, genes implicated in the resistance to aminoglycosides and β-lactams were also screened. The selected genes were *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD* and *npmA* for the aminoglycosides, while *blaCTX-M-15* was targeted to assess resistance to β-lactams. The detection of all these genes was performed, as previously described [23,24]. A detailed list of the primers used is provided in Table 1. Additionally, the specific thermal profiles for each genetic target is provided in Table 2. All primers were purchased from Sigma-Aldrich (St. Louis, MO, USA).

### Table 2. Thermal profiles set for each genetic target.

| Gene  | Hot-Start Temperatures | Cycles | Denaturalization | Hybridization | Extension | Final Extension | Fragment Size (bp) |
|-------|------------------------|--------|------------------|---------------|-----------|-----------------|-------------------|
| invA  | 94 °C/5 min            | 30     | 93 °C/1 min      | 42 °C/1 min   | 72 °C/2 min| 72 °C/4 min     | 284               |
| specC | 94 °C/5 min            | 25     | 94 °C/55 s       | 55 °C/55 s    | 72 °C/55 s| 72 °C/10 min    | 700               |
| pefA  | 94 °C/3 min            | 30     | 94 °C/1 min      | 65 °C/1 min   | 72 °C/1 min| 72 °C/10 min    | 1348              |
| hilA  | 94 °C/5 min            | 30     | 94 °C/1 min      | 55 °C/1 min   | 72 °C/2 min| 72 °C/10 min    | 1348              |
| sopB  | 94 °C/5 min            | 30     | 94 °C/1 min      | 55 °C/1 min   | 72 °C/2 min| 72 °C/10 min    | 1348              |
| armA  | 94 °C/5 min            | 30     | 94 °C/1 min      | 55 °C/1 min   | 72 °C/2 min| 72 °C/10 min    | 1348              |
| rmtA  | 635                    |        |                  |               |           |                 | 1348              |
| rmtB  | 459                    |        |                  |               |           |                 | 1348              |
| rmtC  | 752                    |        |                  |               |           |                 | 1348              |
| rmtD  | 375                    |        |                  |               |           |                 | 1348              |
| npmA  | 641                    |        |                  |               |           |                 | 1348              |
| *blaCTX-M-15* | 483 |    |                  |               |           |                 | 1348              |

2.5. PCR Tests for Isolate Characterization

The detection of the different virulence and antimicrobial resistance genes was performed by PCR in a final reaction volume of 25 µL, with a final primer concentration of 1000 nM and 2 µL of template. To this end, a PTC-200 (MJ Research, Waltham, MA, USA) thermocycler was used, programed with the specific thermal profile detailed in Table 2.

After amplification, the fragments were visualized after electrophoresis in a 1.8 % agarose gel (Promega, Madison, WI, USA) prepared with 1 X Tris-Borate buffer (TBE 1 X, Tris-base 89 mM, boric acid 89 mM, 2 mM EDTA, pH 8.3), to which 5 µL of a 1 % ethidium bromide solution (PanReac-AppliChem, Barcelona, Spain) was added. A total of 5 µL of the PCR product was mixed with another 5 µL of acridine orange loading buffer (PanReac-AppliChem, Barcelona, Spain), and fully loaded in the gel. Additionally, 2 µL of 100 bp DNA ladder (Nippon Genetics Europe, Düren, Germany) was mixed with 8 µL of loading buffer. The electrophoresis was performed in a kuroGel Midi Plus 15 (VWR, Radnor, PA, USA) cuvette with a Model 300 V programable power source (VWR, Radnor, PA, USA), and the fragments were migrated for 1 h and 30 min at 60 V after what was visualized under UV light with a Benchtop UV transilluminator (UVP, Upland, CA, USA).

2.6. Antimicrobial Resistance Test

The antimicrobial resistance tests were performed on a VITEK®2 (BioMérieux, S.A., Marcy l’Etoile, France) using the AST-N244 cards (ceflotin; cefditoren; nitrofurantoin; tobramycin; fosfomycin; ampicillin; amoxicillin/clavulanic acid; piperacillin/tazobactam; cefuroxime; cefuroxime/axetil; cefoxitin; cefotaxim; ceftazidime; cefepime; imipenem; amikacin; gentamicin; nalidixic acid; ciprofloxacin; tigecycline; and trimethoprim/sulfamethoxazole). The classification as sensitive (S), intermediate (I) or resistant (R) was based on the criteria determined by the “Clinical and Laboratory Standards Institute” [25].

3. Results and Discussion

3.1. Prevalence and Serotypes of Salmonella enterica

Over the period of study, a total of 27 *Salmonella* spp. isolates were recovered from the mussel samples. A detailed list of the strains isolated, along with the sampling site, is pro-
vided in Table 3. Martinez et al. previously evaluated the presence of foodborne pathogens by PCR, in bivalve mollusks from the Ria de Arousa, over a period of 18 months [26]. In the mentioned study, 22 mussel sample batches were negative for invA, thus negative for Salmonella spp. The low prevalence of Salmonella in this type of product, along with the small number of batches tested, can justify these results. In line with this observation, Lozano-Leon et al. reported 19 Salmonella positive samples from 5907 mussel batches, representing a prevalence of 0.3% [27]. This prevalence contrasts with that observed in other countries where, for instance, Zahlı et al. found a prevalence of Salmonella of 19.15% in mussels collected from Moroccan markets; likewise, Mannas et al. and Setti et al. found a Salmonella prevalence of 15.4% and 10% respectively, in mussel samples collected from the Atlantic coastline of Morocco [28–30]. Although an attempt was made to establish a correlation between the environmental factors, such as rainfall and fecal contamination with the prevalence of Salmonella, the results were contradictory [29,30].

Table 3. Salmonella strains isolated in the present study.

| Code   | Strain           | Year of Isolation | Origin       |
|--------|------------------|-------------------|--------------|
| AMC 28 | S. montevideo    | 2012              | Ria de Arousa|
| AMC 90 | S. rissen        | 2014              | Ria de Arousa|
| AMC 92 | Salmonella spp.  | 2014              | Ria de Arousa|
| AMC 93 | Salmonella spp.  | 2014              | Ria de Arousa|
| AMC 200| S. wentworth     | 2014              | Ria de Arousa|
| AMC 238| S. typhimurium   | 2015              | Ria de Arousa|
| AMC 239| S. rissen        | 2015              | Ria de Arousa|
| AMC 240| S. rissen        | 2015              | Ria de Arousa|
| AMC 256| Salmonella spp.  | 2015              | Ria de Arousa|
| AMC 257| S. offa          | 2015              | Ria de Arousa|
| AMC 265| S. montevideo    | 2015              | Ria de Arousa|
| AMC 266| S. senftenberg   | 2015              | Ria de Arousa|
| AMC 267| S. senftenberg   | 2015              | Ria de Arousa|
| AMC 268| S. typhimurium   | 2015              | Ria de Arousa|
| AMC 270| S. agona         | 2015              | Ria de Vigo  |
| AMC 281| Salmonella spp.  | 2015              | Ria de Arousa|
| AMC 287| Salmonella spp.  | 2015              | Ria de Arousa|
| AMC 288| Salmonella spp.  | 2015              | Ria de Arousa|
| AMC 289| S. senftenberg   | 2015              | Ria de Arousa|
| AMC 290| Salmonella spp.  | 2015              | Ria de Arousa|
| AMC 291| S. typhimurium   | 2015              | Ria de Arousa|
| AMC 294| S. typhimurium   | 2015              | Ria de Arousa|
| AMC 299| S. typhimurium   | 2015              | Ria de Arousa|
| AMC 300| Salmonella spp.  | 2015              | Ria de Vigo  |
| AMC 301| S. bredeney      | 2015              | Ria de Arousa|
| AMC 303| Salmonella spp.  | 2016              | Ria de Vigo  |
| AMC 327| S. liverpool     | 2016              | Ria de Vigo  |

*Serotype information obtained from Lozano-Leon et al. [27] in this reference additional molecular information may be obtained for the designated strains.

S. typhimurium was the most prevalent serotype (4/27), followed by S. rissen (3/27) and S. senftenberg (3/27). The information available about the serotypes present in mussel samples is limited, but the presence of S. senftenberg in mussels has to be pointed out as this serotype was associated with the persistent contamination in high saline environments in mussel facilities, between 1998 and 2002 [31]. Curiously, Setti et al. detected the presence of this serotype in the seawater and sediment of Morocco’s Atlantic coast, but not in mussels [29]. Previous studies carried out in the Rias of Vigo, Pontevedra, Arousa and Muros-Noia, between 1998 and 2002, in mussels, seawater and mollusk depuration plants, found that S. senftenberg was the most prevalent Salmonella serotype [15,32]. In addition to this, the isolates of this serotype were higher in the Ria de Arousa in comparison to the other Rias. In the present study, all the isolates of S. senftenberg were from Ria de Arousa. The
data from both previous studies mentioned, and the fact that this serotype is still isolated in mussel samples from Ria de Arousa, seem to indicate that this serotype can be endemic to this area. The *S. senftenberg* isolates characterized by Martínez-Urtaza et al., presented a rugose morphotype, which is associated with a high capacity to produce biofilm [31,33]. This can explain the persistence of these strains in mussel production plants, and that they are a permanent source of contamination in Ria de Arousa. Likewise, the fact that this serotype is isolated in marine samples from other parts of the world, seems to indicate a high adaptation of this serotype to high salt concentrations.

*S. typhimurium* was also one of the most prevalent serotypes isolated from the Galician marine environments, in previous studies conducted by Martínez-Urtaza et al. [15,16]. The presence of this serotype in mussels, represents an important concern for public health since, together with *S. enteritidis*, it is one of the most pathogenic serotypes of *Salmonella*. Factors, such as densely populated areas and inefficient wastewater treatments, can be related to the prevalence of *Salmonella* in the marine environment. In line with this hypothesis, a study carried out on the coast of Colombia established a relationship between inefficient wastewater treatment systems and the presence of *Salmonella* in beach waters [34]. *S. typhimurium* is the second serotype most commonly associate with human salmonellosis in the European Union, and therefore highly populated coastal regions can suffer a potential contamination with human pathogenic *Salmonella* serotypes [35]. On the other hand, Galicia is an important farming region with a large number of cattle, swine and poultry farms. These farms can contaminate the nearby aquifers and rivers with bacterial pathogens that can end up in the sea. It is worth noting that the serotype Senftenberg was also isolated from Galician poultry farms, between 2011–2015 [36]. Finally, it is also important to mention that *S. agona* was isolated in this study, and also showed a high prevalence of molluscan shellfish by Martínez-Urtaza et al. [15]. Isolates belonging to this serotype have been characterized by their high capacity to form biofilms, which can partly explain why this serotype can be a permanent source of contamination [37].

### 3.2. Genetic Characterization of the Isolates

#### 3.2.1. Screening for Virulence Genes

All the strains isolated were positive for *invA* (100%); 7 were positive for *hilA* and *sopB* (25.9%), 6 for *spvC* (22.2%) and only 1 was positive for *pefA* (3.7%). The strains analyzed can be classified into 7 different groups, attending to the virulence genes presented, which would be: P1 (*invA*); P2 (*invA/hilA/sopB*); P3 (*invA/spvC*); P4 (*invA/hilA/spvC*); P5 (*invA/hilA/sopB/spvC/pefA*); P6 (*invA/sopB*) and P7 (*invA/hilA/sopB/spvC*), as depicted in Figure 2 and summarized in Table 4, and only one isolated (*S. typhimurium* 268) presented all the virulence genes analyzed. It is not surprising that all the strains were positive for *invA*, as this gene has been extensively used for the specific detection of *Salmonella* spp. by a wide variety of DNA amplification methods, being also selected for interlaboratory validation studies [38–40]. However, in the present study, only 25.9% of the isolates were positive for the main virulence transcriptional regulator of *Salmonella* Pathogenicity Island 1 (SPI-1) *hilA*, and 59.3% of isolates did not present any gene other than *invA* [41]. A study previously conducted by Campioni et al., reported higher percentages of *sopB* and *spvC*. It is noteworthy that the study focused on *spvB*, but these two genes belong to the same operon, *spvRABCD* [42]. These differences can be explained either by a difference in the pathogenic potential of the strains, or directly by the fact that these genes can be conserved in certain serovars (Campioni’s study was restricted to *Salmonella enteritidis*). In this sense, Lamas et al. observed that *spvC* was present in only 44.8% of the *Salmonella* strains isolated from Galician poultry farms. However, that gene was mainly present in *S. typhimurium* and *S. enterica* subsp *arizonae*, in which this gene is chromosomally encoded, but almost absent in the other serotypes of *S. enterica* subsp. *enterica* [43]. Ammar et al., like Campioni, analyzed the strains related with human disease, and obtained high percentages of *hilA* (88%), *sopB* (41.2%) and *pefA* (41.2%); the only discrepancy among them was for *spvC*, as only 5.9% of Ammar’s strains possessed the gene [44]. The results obtained in the present...
work are aligned with those reported by Gharieb et al., who indicated the percentages of 10%, 30% and 16.7–30% for pefA, hilA and sopB respectively [45].

Attending to the geographical distribution, it is worth commenting that 25.9% of the isolates that presented more than one virulence gene belonged to the Ria of Arousa, while 14.8% belonged to the Ria of Vigo. Of particular importance is the fact that P7 (all 5 virulence genes) was obtained from a sample harvested in Vigo, and P5 (4 virulence genes) belonged to a sample harvested in Arousa. Caution must be taken when extracting conclusions related to the abundance as, due to the fact that certain areas are more productive, more samples were analyzed. In line with this, in previous studies, a bias existed towards the Ria of Arousa, due to a higher number of samples harvested and analyzed from this area, as a consequence of having a higher production [12,13]. The current study adds to the others previously published, informing the presence of different pathogens in this economically important area, and highlighting the importance of depuration for the safety of the consumers.

3.2.2. Screening for Antimicrobial Resistance Genes

Regarding the presence of antimicrobial resistance genes, none of the seven genetic targets selected were detected in any of the isolates obtained. The fact that none of the genes screened were positive, but the isolates presented phenotypic resistance (see below) to many aminoglycosides, suggests the presence of additional mechanisms of resistance to these compounds, or the presence of other genes rather than those screened, such as blαSHV, blαTEM or other blα, instead of M-15 in ESBL strains or aacC for the resistance of aminoglycosides [46–48].

Figure 2. Virulence profiles obtained for the 27 isolates analyzed, based on the presence of the genes invA, hilA sopB, pefA and spvC.
Table 4. Summary of the antibiotic resistance and virulence genes for each of the strains isolated.

| Strain                  | AMC Code | Area | Strain | AMC Code | Area |
|-------------------------|----------|------|--------|----------|------|
| S. senftenberg          | 28       | A    | S. senftenberg | 28       | A    |
| S. senftenberg          | 26       | V    | S. montevideo  | 256      | A    |
| S. rissen               | 265      | A    | S. rissen     | 265      | A    |
| S. agona                | 289      | A    | S. wentworth  | 267      | V    |
| S. wentworth            | 290      | A    | S. bredeney   | 230      | V    |
| S. liverpool            | 291      | V    | S. bredeney   | 230      | V    |
| S. bredeney             | 291      | V    | S. liverpool  | 230      | V    |
| S. offa                 | 270      | V    | S. offa       | 270      | V    |
| S. montevideo           | 256      | A    | S. montevideo | 256      | A    |
| S. rissen               | 265      | A    | S. monterey   | 267      | V    |
| S. montevideo           | 256      | A    | S. montevideo | 256      | A    |
| S. rissen               | 265      | A    | S. rissen     | 265      | A    |

Antibiotics: ciprofloxacin; FOF: fosfomycin; NIT: nitrofurantoin; SXT: trimethoprim-sulfamethoxazole; AMK: amikacin; TGC: tigecycline and TZP: piperacillin-tazobactam.

Virulence Genes: invA, spec, pefA, hilA, espB, arsA, ctmA, ctmB, ctmC, ctaD, npmA, blaCTX-M-15.

*“S”: Sensitive; “R”: Resistant; “I”: Intermediate and “N”: Not tested. *“+”: positive result for the corresponding gene. *Serotype information obtained from Lozano-Leon et al. [27]. In this reference, additional molecular information can be obtained for the designated strains AMP: ampicillin; AMC: Amoxicillin/clavulanic acid; CEF: cefotaxime; CTX: cefotaxime; CSM: cefuroxime; FOX: ceftoxitin; CDN: cefditoren; CTX: cefotaxime; CAZ: ceftazidime; FEP: cephepine; ETP: ertapenem; IPM: imipenem; GEN: gentamicin; TOB: tobramycin; NAL: nalidixic acid; CIP: ciprofloxacin; FOF: fosfomycin; NIT: nitrofurantoin; SXT: trimethoprim-sulfamethoxazole; AMK: amikacin; TGC: tigecycline and TZP: piperacillin-tazobactam.
3.3. Antimicrobial Resistance

All 27 isolated strains presented MDR, as all them were resistant to at least 4 antimicrobials. Furthermore, 11 were resistant to 5 antimicrobials, and 3 to 9 of the drugs tested. None of the isolates were sensitive to all of the antimicrobials tested. All the isolates were resistant to cefuroxime and cefuroxime/axetil, a second generation cephalosporin. Similar to previous presentations, all 27 strains can be classified into 8 different profiles attending to their antimicrobial resistance characteristics. This would be: P1 (cefuroxime, cefuroxime/axetil, cefalotin, gentamicin and amikacin); P2 (cefalotin, cefuroxime, cefuroxime/axetil, cefoxitin, gentamicin and tobramycin); P3 (ampicillin, cefuroxime, cefuroxime/axetil, cefoxitin, gentamicin and amikacin); P4 (ampicillin, cefuroxime, cefuroxime/axetil, cefoxitin, gentamicin, trimethoprim/sulfamethoxazole and amikacin); P5 (ampicillin, cefalotin, cefuroxime, cefuroxime/axetil, cefoxitin, gentamicin, tobramycin, nalidixic acid and ciprofloxacin); P6 (ampicillin, cefuroxime, cefuroxime/axetil and trimethoprim/sulfamethoxazole); P7 (ampicillin, cefalotin, cefuroxime, cefuroxime/axetil, cefoxitin, gentamicin, tobramycin and trimethoprim/sulfamethoxazole) and P8 (ampicillin, amoxicillin/clavulanic acid, cefuroxime, cefuroxime/axetil, cefoxitin, cefotaxime, gentamicin, trimethoprim/sulfamethoxazole and amikacin). Most of the isolates tested belonged to P1, as depicted in Figure 3 and summarized in Table 4.

Figure 3. Antibiotic resistance profiles obtained from the 27 isolates.

Previous studies, conducted by Martinez-Urtaza et al., on Salmonella isolated in the same area of study, indicated a higher incidence of strains resistant to certain antibiotics, such as ceftazidime, or in agreement with the findings reported in the present work, such as the incidence in amoxicillin/clavulanic acid resistance [49]. However, it is worth commenting that further serotyping information would be needed for a better comparison, as the cited study only focused on S. senftenberg, and variability in the resistance profiles
can exist linked to specific serovars. Martinez-Urtaza et al. also evaluated the resistance profile of *S. typhimurium* isolates from bivalve mollusks, from 1998 to 2002 [16]. They found that 69.6% of isolates were sensitive to all the antimicrobials tested while, in the present study, the 4 *S. typhimurium* presented multidrug resistance. These results follow the trend of the last few years, in which an increase in resistance has been observed in non-typhoidal *Salmonella* [50]. For example, Giacometti et al. observed an increased trend in the antimicrobial resistance profile of *S. typhimurium* strains isolated from bivalve mollusks collected from Ferrara, Italy, between 2001 and 2017 [51]. In Spain, the same type of trend was observed in strains of *S. enterica* isolated from chicken samples, in which the average number of resistances increased from 3.98% in 1993 to 5.00% in 2006, and an increased incidence in the resistance to cephalothin, enrofloxacin and tetracycline was also observed [52].

It is worth noting that resistance among these isolates was not limited to one type of antibiotic. As it can be observed in pattern 8 of Figure 3, three isolates were resistant to β-lactams (ampicillin, cefalotin, cefuroxime, cefuroxime/axetil and cefoxitin), aminoglycosides (gentamicin and tobramycin) and quinolones (nalidixic acid and ciprofloxacin), covering three different classes of antibiotics. It is also important to note that isolates within this resistance profile, namely AMC 270 and AMC 281, belonged to the virulence profiles 6 ([*invA*/*sopB]*) and 7 ([*invA*/*spvC*/*hilA*/*sopB]*) respectively, thus presenting additional virulence factors, and possibly posing an added risk.

Giacometti et al. reported low resistance to third generation cephalosporins and quinolones on isolates of *S. typhimurium*. These finding were in line to those reported in the present study. Contrary to their results, 96.3% of the isolates, included in the current study, were resistant to gentamicin, while Giacometti et al. only observed 1.9% [51]. These results also differ from those observed by Zahli et al., who did not report any isolate resistant to gentamicin from mollusks from the North Coast of Morocco [28]. Additionally, Martinez-Urtaza et al. found only one isolate of *S. typhimurium* that was resistant to gentamicin, among the ones studied from marine environments between 1998 and 2002 [16]. Interestingly, the high resistance to gentamicin has not been observed in *Salmonella* isolated from other points of the food chain in Galicia. Lamas et al. found that all the *Salmonella* isolated from poultry farms from 2011–2015 were sensitive to gentamicin [36]. In the EU report on the antimicrobial resistance in zoonotic bacteria in 2015, the data from Spain showed resistance to gentamicin in 3.8% and 6.3% of isolates of *Salmonella* from humans and calves, respectively [53].

High resistance to second-generation cephalosporins was observed in this study. In humans, cefuroxime is the fourth most consumed antibiotic per inhabitant in Galicia [54]. This usage could be partly responsible for the high resistance observed in this pathogen; all the isolates were resistant to cefuroxime. This group of antimicrobials are widely used in animal production in Spain, which can cause an environmental pressure that selects bacteria resistant to these antibiotics [55]. Finally, 37.0% of the isolates were resistant to ampicillin. This is one of the most common resistances observed in *Salmonella* [53]. Penicillins are the most widely used group of antibiotics in animal production, which makes these resistances common in foodborne pathogens [55].

Finally, it is also important to highlight the data on intermediate resistances. These were observed for amoxicillin/clavulanic acid, ceftazidime, cefepime, nitrofurantoin and piperacillin-tazobactam. The WHO has classified these substances as critically important antimicrobials (CIAs), with the exception of nitrofurantoin [56]. Of particular interest is the antimicrobial profile of *Salmonella* AMC 92, which, in addition to the resistance to nine different antimicrobials, also presented three intermediate resistances to ceftazidime, cefotaxime and piperacillin-tazobactam. All isolates were sensitive to: cefditoren, ceftazidime, cefepime, ertapenem, imipenem, fosfomycin, nitrofurantoin, tigecycline and piperacilnine/tazobactam; thus, these antimicrobials still represent a reliable way to treat possible infections associated with these bacteria.
4. Conclusions

*S. typhimurium* and *S. senftenberg* are serotypes commonly isolated from mussels and the latter seems to be endemic to the Ría de Arousa. Several strains isolated from mussel samples were positive for more than 1 virulence gene. All isolates were negative for the antimicrobial resistance genes tested, but presented phenotypic resistance to more than 4 antibiotics. Even though the multiresistance seems widespread, the strains under study were still susceptible to another 9 different antibiotics, which can represent the future first line treatment. The authors would like to highlight that depuration procedures are efficient in eliminating *Salmonella* spp. from mussels, and, if detected in this product, it would most likely be the result of a post-depuration contamination rather than the inefficacy of the treatment.

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**References**

1. EFSA; ECDC. The European Union One Health 2020 Zoonoses Report. *EFSA J.* 2021, 19, e6971.

2. European Commission Regulation (EC) No 2073/2005. *Microbiological Criteria for Foodstuffs*; European Union: Brussels, Belgium, 2005.

3. EFSA. Foodborne Outbreaks Reported in 2019. Available online: https://app.powerbi.com/view?r=eyJrIjoiY2FmNmUzYWEtZ

4. Garrido-Maestu, A.; Lozano-León, A.; Rodriguez-Souto, R.R.; Vieites-Maneiro, R.; Chapela, M.-J.; Cabado, A.G. Presence of pathogenic *Vibrio* species in fresh mussels harvested in the southern Rias of Galicia (NW Spain). *Food Control* 2016, 59, 799–765. [CrossRef]

5. Costas-Rodríguez, Soto, R.R., Garrido-Maestu, A.; Pastoriza-Fontan, A.; Lozano-León, A. Investigation and characterization of Shiga toxin-producing *Escherichia coli* present in mussels from harvesting areas in Galician southern Rias (NW Spain). *J. Food Saf.* 2017, 37, e12367. [CrossRef]

6. Chen, S.; Zhao, S.; Schroeder, C.M.; Lu, R.; Yang, H.; McDermott, P.F.; Ayers, S.; Meng, J. Characterization of Multiple-Antimicrobial-Resistant *Salmonella* Serovars Isolated from Retail Meats. *Appl. Environ. Microbiol.* 2004, 70, 1–7. [CrossRef]

7. EFSA; ECDC. The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria. *EFSA J.* 2021, 18.

8. Ferreira, M.; Lago, J.; Vieites, J.M.; Cabado, A.G. Chapter 8: World Production of Bivalve Mollusks and Socioeconomic Facts Related to *Teh Impact of Marine Biotoxins, in Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection* (Chapter 8: World Production of Bivalve Mollusks and Socioeconomic Facts Related to *Teh Impact of Marine Biotoxins, in Seafood and Freshwater Toxins: Pharmacology, Physiology, and Detection*). The case of the floating raft culture in the Galician mussel sector. Taylor & Francis Group: Abingdon, UK, 2014; ISBN 978-1-4665-0514-8.

9. Caballero-Miguez, G.; Garza Gil, M.D.; Varela Lafuente, M.M. The institutional foundations of economic performance of mussel production: The Spanish case of the Galician floating raft culture. *Mar. Policy* 2009, 33, 288–296. [CrossRef]

10. Caballero-Miguez, G.; Garza-Gil, M.D.; Varela-Lafuente, M.M. Legal change, property rights system and institutional stability: The case of the floating raft culture in the Galician mussel sector. *Ocean Coast. Manag.* 2012, 55, 84–93. [CrossRef]

11. Costas-Rodríguez, M.; Lavilla, I.; Bendicho, C. Classification of cultivated mussels from Galicia (Northwest Spain) with European protected designation of origin using trace element fingerprint and chemometric analysis. *Anal. Chim. Acta* 2010, 664, 121–128. [CrossRef]

12. Rodríguez-Souto, R.R., Garrido-Maestu, A.; Pastoriza-Fontan, A.; Lozano-León, A. Investigation and characterization of Shiga toxin-producing *Escherichia coli* present in mussels from harvesting areas in Galician southern Rias (NW Spain). *J. Food Saf.* 2017, 59, 709–765. [CrossRef]

13. Garrido-Maestu, A.; Lozano-León, A.; Rodríguez-Souto, R.R.; Vieites-Maneiro, R.; Chapela, M.-J.; Cabado, A.G. Presence of pathogenic *Vibrio* species in fresh mussels harvested in the southern Rias of Galicia (NW Spain). *Food Control* 2016, 59, 799–765. [CrossRef]

14. Lozano-León, A.; Rodríguez-Souto, R.R.; González-Escalona, N.; Lloto-Taboada, J.; Iglesias-Canle, J.; Álvarez-Castro, A.; Garrido-Maestu, A. Detection, molecular characterization, and antimicrobial susceptibility of *Campylobacter* spp. isolated from shellfish. *Microb. Risk Anal.* 2021, 18, 100176. [CrossRef]
15. Martinez-Urtaza, J.; Saco, M.; Hernandez-Cordova, G.; Lozano, A.; Garcia-Martin, O.; Espinosa, J. Identification of Salmonella Serovars Isolated from Live Molluscan Shellfish and Their Significance in the Marine Environment. J. Food Prot. 2003, 66, 226–232. [CrossRef]

16. Martinez-Urtaza, J.; Liebana, E.; Garcia-Migura, L.; Perez-Pin, P.; Perez-Piñeiro, P.; Saco, M. Characterization of Salmonella enterica serovar Typhimurium from marine environments in coastal waters of Galicia (Spain). Appl. Environ. Microbiol. 2004, 70, 4030–4034. [CrossRef]

17. Van Asten, A.J.A.M.; Van Dijk, J.E. Distribution of “classic” virulence factors among Salmonella spp. FEMS Immunol. Med. Microbiol. 2005, 44, 251–259. [CrossRef] [PubMed]

18. Swamy, S.C.; Barnhart, H.M.; Lee, M.D.; Dreesen, D.W. Virulence determinants invA and spvC in Salmonellae isolated from poultry products, wastewater, and human sources. Appl. Environ. Microbiol. 1996, 62, 3768–3771. [CrossRef]

19. Rahn, K.; De Grandis, S.A.; Clarke, R.C.; McEwen, S.A.; Galan, J.E.; Ginocchio, C.; Curtiss, R.; Gyles, C.L. Amplification of an invA gene sequence of Salmonella Typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. Mol. Cell. Probes 1992, 6, 271–279. [CrossRef]

20. Murugkar, H.V.; Rahman, H.; Dutta, P.K. Distribution of virulence genes in Salmonella serovars isolated from man & animals. Indian J. Med. Res. 2003, 117, 66–70.

21. Cardona-Castro, N.; Restrepo-Pineda, E.; Correa-Ochoa, M. Detection of hilA gene sequences in serovars of Salmonella enterica subspecies enterica. Mem. Inst. Oswaldo Cruz 2002, 97, 1153–1156. [CrossRef]

22. Rahman, H. Prevalence & phenotypic expression of sopB gene among clinical isolates of Salmonella enterica. Indian J. Med. Res. 2006, 123, 83.

23. Fritsche, T.R.; Castanheira, M.; Miller, G.H.; Jones, R.N.; Armstrong, E.S. Detection of methyltransferases conferring high-level resistance to aminoglycosides in Enterobacteriaceae from Europe, North America, and Latin America. Antimicrob. Agents Chemother. 2008, 52, 1843–1845. [CrossRef]

24. Leflon-Guibout, V.; Jurand, C.; Bonacorsi, S.; Espinasse, F.; Guelfi, M.C.; Duportail, F.; Heym, B.; Bingen, E.; Nicolas-Chanoin, M.H. Emergence and spread, of three clonally related virulent isolates of CTX-M-15-producing Escherichia coli with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital. Antimicrob. Agents Chemother. 2004, 48, 3736–3742. [CrossRef] [PubMed]

25. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 9th ed.; CLSI: Pittsburgh, PA, USA, 2012; Volume 32, ISBN 156287839.

26. Martínez, O.; Rodríguez-Calleja, J.M.; Santos, J.A.; Ótero, A.; García-López, M.L. Foodborne and indicator bacteria in farmed mussel shellfish before and after depuration. J. Food Prot. 2009, 72, 1443–1449. [CrossRef] [PubMed]

27. Lozano-León, A.; García-Omil, C.; Dalama, J.; Rodríguez-Souto, R.; Martínez-Urtaza, J.; González-Escalona, N. Detection of colistin resistance mcr-1 gene in Salmonella enterica serovar Rissen isolated from mussels, Spain, 2012 to 2016. Eurosurveillance 2019, 24, 1–5. [CrossRef] [PubMed]

28. Zahli, R.; Sølværi, J.; Abrini, J.; Copa-Patiño, J.L.; Nadia, A.; Scheu, A.-K.; Nadia, S.S. Prevalence, typing and antimicrobial resistance of Salmonella isolates from commercial shellfish in the North coast of Morocco. World J. Microbiol. Biotechnol. 2021, 37, 170. [CrossRef]

29. Setti, I.; Rodriguez-Castro, A.; Pata, M.P.; Cadarso-Suárez, C.; Yakoubei, B.; Bensmael, L.; Moukrim, A.; Martínez-Urtaza, J. Characteristics and dynamics of Salmonella contamination along the coast of agadir, Morocco. Appl. Environ. Microbiol. 2009, 75, 7700–7707. [CrossRef] [PubMed]

30. Mannas, H.; Mimouni, R.; Chauouy, N.; Hamadi, F.; Martínez-Urtaza, J. Occurrence of Vibrio and Salmonella species in mussels (Mytilus galloprovincialis) collected along the Moroccan Atlantic coast. Springerplus 2014, 3, 1–11. [CrossRef] [PubMed]

31. Martínez-Urtaza, J.; Peiteado, J.; Lozano-León, A.; García-Martín, O. Detection of Salmonella Senftenberg Associated with High Saline Environments in Mussel Processing Facilities. J. Food Prot. 2004, 67, 256–263. [CrossRef]

32. Martínez-Urtaza, J.; Saco, M.; De Novoa, J.; Perez-Piñeiro, P.; Peiteado, J.; Lozano-León, A.; García-Martín, O. Influence of Environmental Factors and Human Activity on the Presence of Salmonella Serovars in a Marine Environment. Appl. Environ. Microbiol. 2004, 70, 2089–2097. [CrossRef] [PubMed]

33. Obe, T.; Nannapaneni, R.; Sharma, C.S.; Kiess, A. Homologous stress adaptation, antibiotic resistance, and biofilm forming ability of Salmonella enterica serovar Heidelberg ATCC8326 on different food-contact surfaces following exposure to sublethal chlorine concentrations 1. Poult. Sci. 2018, 97, 951–961. [CrossRef]

34. Soto-Varela, Z.E.; Rosado-Porto, D.; Bolívar-Attoni, H.J.; González, C.P.; Pantoja, B.G.; Alvarado, D.E.; Anfuso, G. Preliminary microbiological coastal water quality determination along the department of atlántico (Colombia): Relationships with beach characteristics. J. Mar. Sci. Eng. 2021, 9, 122. [CrossRef]

35. EFSA; ECDC. The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016. EFS A J. 2017, 15, e00577. [CrossRef]

36. Lamas, A.; Fernandez-No, I.C.; Miranda, J.M.; Vázquez, B.; Cepeda, A.; Franco, C.M. Prevalence, molecular characterization and antimicrobial resistance of Salmonella serovars isolated from northwestern Spanish broiler flocks (2011–2015). Poult. Sci. 2016, 95, 2097–2105. [CrossRef] [PubMed]

37. Diez-García, M.; Capita, R.; Alonso-Calleja, C. Influence of serotype on the growth kinetics and the ability to form biofilms of Salmonella isolates from poultry. Food Microbiol. 2012, 31, 173–180. [CrossRef]
38. Cheng, C.M.; Lin, W.; Van, K.T.; Phan, L.; Tran, N.N.; Farmer, D. Rapid Detection of Salmonella in Foods Using Real-Time PCR. J. Food Prot. 2008, 71, 2436–2441. [CrossRef] [PubMed]

39. Hara-Kudo, Y.; Yoshino, M.; Kojima, T.; Ikedo, M. Loop-mediated isothermal amplification for the rapid detection of Salmonella. FEMS Microbiol. Lett. 2005, 253, 155–161. [CrossRef] [PubMed]

40. Malorny, B.; Cook, N.; D’Agostino, M.; De Medici, D.; Croci, L.; Abdulmawjood, A.; Fach, P.; Karpiskova, R.; Aymeric, T.; Kwaitek, K.; et al. Multicenter validation of PCR-based method for detection of Salmonella in chicken and pig samples. J. AOAC Int. 2004, 87, 861–866. [CrossRef]

41. Boddicker, J.D.; Knosp, B.M.; Jones, B.D. Transcription of the Salmonella invasion gene activator, hilA, requires HilD activation in the absence of negative regulators. J. Bacteriol. 2003, 185, 525–533. [CrossRef]

42. Campioni, F.; Moratto Bergamini, A.M.; Falcão, J.P. Genetic diversity, virulence genes and antimicrobial resistance of Salmonella Enteritidis isolated from foods and humans over a 24-year period in Brazil. Food Microbiol. 2012, 32, 254–264. [CrossRef]

43. Libby, S.J.; Lesnick, M.; Hasegawa, P.; Kurth, M.; Belcher, C.; Fierer, J.; Guiney, D.G. Characterization of the spr locus in Salmonella enterica serovar Arizona. Infect. Immun. 2002, 70, 3290–3294. [CrossRef]

44. Ammar, A.M.; Mohamed, A.A.; El-Hamid, M.I.A.; El-Azzouny, M.M. Virulence genotypes of clinical Salmonella serovars from broilers in Egypt. J. Infect. Dev. Ctries. 2016, 10, 337–346. [CrossRef]

45. Gharieb, R.M.; Tartor, Y.H.; Khedr, M.H.E. Non-Typhoidal Salmonella in poultry meat and diarrhoeic patients: Prevalence, antibiogram, virulotyping, molecular detection and sequencing of class I integrons in multidrug resistant strains. Gut Pathog. 2015, 7, 34. [CrossRef]

46. Ho, P.L.; Wong, R.C.; Lo, S.W.; Chow, K.H.; Wong, S.S.; Que, T.L. Genetic identity of aminoglycoside-resistance genes in Escherichia coli isolates from human and animal sources. J. Med. Microbiol. 2010, 59, 702–707. [CrossRef] [PubMed]

47. Cloeckaert, A.; Praud, K.; Doublet, B.; Bertini, A.; Carattoli, A.; Butaye, P.; Imberechts, H.; Bertrand, S.; Collard, J.M.; Arlet, G.; et al. Dissemination of an extended-spectrum-β-lactamase blaTEM-52 gene-carrying IncI1 plasmid in various Salmonella enterica serovars isolated from poultry and humans in Belgium and France between 2001 and 2005. Antimicrob. Agents Chemother. 2007, 51, 1872–1875. [CrossRef]

48. Wachino, J.I.; Shibayama, K.; Kurokawa, H.; Kimura, K.; Yamane, K.; Suzuki, S.; Shibata, N.; Ike, Y.; Arakawa, Y. Novel plasmid-mediated 16S RNA methyltransferase, NpmA, found in a clinically isolated Escherichia coli strain resistant to structurally diverse aminoglycosides. Antimicrob. Agents Chemother. 2007, 51, 4401–4409. [CrossRef] [PubMed]

49. Martinez-Urtaza, J.; Liebana, E. Use of pulsed-field gel electrophoresis to characterize the genetic diversity and clonal persistence of Salmonella Senftenberg in mussel processing facilities. Int. J. Food Microbiol. 2005, 105, 153–163. [CrossRef] [PubMed]

50. Michael, G.B.; Schwarz, S. Antimicrobial resistance in zoonotic nontyphoidal Salmonella: An alarming trend? Clin. Microbiol. Infect. 2016, 22, 968–974. [CrossRef] [PubMed]

51. Giacometti, F.; Pezzi, A.; Galletti, G.; Tamba, M.; Merialdi, G.; Piva, S.; Serraino, A.; Rubini, S. Antimicrobial resistance patterns in Salmonella enterica subsp. enterica and Escherichia coli isolated from bivalve molluscs and marine environment. Food Control 2021, 121, 107590. [CrossRef]

52. Álvarez-Fernández, E.; Alonso-Calleja, C.; García-Fernández, C.; Capita, R. Prevalence and antimicrobial resistance of Salmonella serotypes isolated from poultry in Spain: Comparison between 1993 and 2006. Int. J. Food Microbiol. 2012, 153, 281–287. [CrossRef]

53. EFSA. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. EFSA J. 2017, 15, e04694. [CrossRef]

54. Prioritarianas, T. Boletín Epidemiológico de Galicia; Galician Healthcare Service: Galicia, Spain, 2018; Volume 27, pp. 1–23.

55. European Medicines Agency. Sales of Veterinary Antimicrobial Agents in 31 European Countries in 2018: Trends from 2010–2018; European Medicines Agency: Amsterdam, The Netherlands, 2019; ISBN 9789291550685.

56. Collignon, P.C.; Conly, J.M.; Amendont, A.; McEwen, S.A.; Aidara-Kane, A.; Griffin, P.M.; Agerso, Y.; Dang Ninh, T.; Donadio-Godoy, P.; Fedorka-Cray, P.; et al. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies to Control Antimicrobial Resistance from Food Animal Production. Clin. Infect. Dis. 2016, 63, 1087–1093. [CrossRef]