

PHENOTYPIC CHARACTERIZATIONS AND ANTIMICROBIALS RESISTANCE OF *SALMONELLA* STRAINS ISOLATED FROM PIGS FROM FATTENING FARMS

CARACTERIZAREA FENOTIPICĂ ȘI REZISTENȚA LA SUBSTANȚE ANTIMICROBIENE A SALMONELELOR IZOLATE DE LA PORCI DIN ÎNGRĂȘĂTORII

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ABSTRACT | REZUMAT

Infections produced by *Salmonella* are a current issue, being among the first causes of collective food poisoning, represent a real public health danger. Economically, they have crucial importance due to losses produced both in animals and in humans. Approximately 70% of pork carcasses that are found contaminated in slaughterhouses come from clinical health animals which were carriers. It is considered that between 5% and 30% of pork carcasses can be *Salmonella* contaminate. In this study, the samples come from 8 fattening farms from the Western part of Romania. In these farms were described digestive disorders, reducing daily gain food and non-uniformity of animals. *Salmonella* strains belonging to *enterica* and *choleraesuis* species were isolated from pigs at different ages, from 4 days of age to 19 weeks of age. All *Salmonella* strains present multiple resistance at tested antimicrobials.

Keywords: *Salmonella*, pig, antimicrobials, resistance

Infecțiile produse de salmonele reprezintă o problemă curentă, fiind printre primele cauze ale toxiinfecțiilor alimentare la om, reprezentând un real pericol asupra sănătății publice. Din punct de vedere economic, prezintă o importanță deosebită din cauza pierderilor atât la animale, cât și la oameni. Aproximativ 70% din carcasele de porc contaminate din abatoare provin de la animale sănătoase, dar care erau purtătoare. Se consideră că între 5 și 30% din carcasele de porc pot fi contaminate cu salmonele. În acest studiu au fost preluate probe provenite din 8 ferme de porci din partea de vest a României. În aceste ferme au fost descrise probleme digestive, reducerea sporului zilnic și neuniformitate a animalelor. De la porci cu vârste diferite, de la 4 zile de viață la 19 săptămâni, au fost izolate și identificate tulpini de *Salmonella enterica* și *choleraesuis*. Toate tulpinile de *Salmonella* au prezentat rezistență multiplă la antibiotice.

Cuvinte cheie: *Salmonella*, porci, substanțe antimicrobiene, rezistență

The *Salmonella* infection may be clinically inapparent or may manifest as sepsis, enterocolitis or pulmonary alterations, rarely as meningoencephalitis or abortion (2, 10, 17, 18).

To prevent contaminated carcasses to enter the food chain, sustained efforts are being made, on one hand, to keep the pig herds free of *Salmonella* and, on the other hand, to monitor all processing stages from the slaughterhouse with the HACCP system, so that, in the end, carcasses or sliced meat to be most assuredly free of *Salmonella* (12, 13).

Keeping pig farms free of *Salmonella* is accomplished by respecting the biosecurity in farms, especially the control of rodents, as rats have an important role in preserving *Salmonella* in these farms. Epidemiological surveillance is also important (10, 14, 18).

Within the One Health context, a recent review

conducted in our country (18) was highlighted the importance of the integrated approach of the worrying antimicrobial resistance spreading of zoonotic bacteria via the food chain, including *Salmonella* spp.

In this regard, some investigations pointed out the occurrence of the pig origin multidrug-resistant (MDR) *Salmonella* spp., both at the slaughtering and processing units' level (19), as well as in finished products (20). However, for the understanding of the complex puzzle of the antimicrobial resistance phenomenon, additional studies from different stages of pig production are still required.

The study's aim was to make a phenotypical characterization of some strains of *Salmonella* isolated from pigs and an evaluation of their behaviour towards antimicrobial substances, knowing that the resistance phenomenon is common in *Salmonella* isolated strains.

Bacteriological examinations were accomplished in the research lab of Infectious Diseases and Preventive Medicine department of the Faculty of Veterinary Medicine from Timisoara.

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Table 1

The samples analysed for *Salmonella* sp detection

No crt.	Farm	Age (weeks)	Sample type	Number of samples
1.	F 1	< 1	organs	3
2.	F 2	7	organs	5
3.	F 3	7	organs	2
4.	F 4	8	organs	2
5.	F 5	9	organs	7
6.	F 6	9	organs	3
7.	F 7	12	organs	5
8.	F 8	19	faeces	10
Total				37

MATERIALS AND METHODS

The research of this paper was made over a 6 month. There were processed samples derived from 8 intensive growing system fattening farms from the western part of the country, where digestive disorders, reducing daily weight gain and non-uniformity were reported. The samples were sent to the Department of Infectious Diseases and Preventive Medicine to establish the aetiology of the digestive disorders. Several types of samples (organs and faeces) were taken from several age groups (Table 1).

Because our goal was to isolate only the *Salmonella*, we used pre-enrichment and enrichment culture media, recommended by SR EN ISO 6579/A1 standard - Amendment 1 – Annex D: *Salmonella* sp detection from faeces and environmental samples from the primary production stage (20).

The incubation of cultures was made at different temperatures, 37°C and 42°C for 24 hours and, when necessary, the time was prolonged to 48 hours. The strains' identification was based on morphological, cultural and biochemical features on the following media: Rappaport Vasiliadis broth, peptone water, Levine, MIU, SS, TSI, MacConkey, XLD and Chromagar *Salmonella*. The phenotypical characterization and taxonomic classification were made with the API 20E gallery (bio Merieux, France).

The antimicrobials' sensitivity was tested using the Kirby-Bauer method from the primary culture or subcultures. This lab method is flexible and rather cheap (23).

RESULTS AND DISCUSSIONS

The isolation and identification of *Salmonella* strains from faeces samples were performed according to the ISO standard of isolation method for *Salmonella* (24). This method facilitates the isolation of *Salmonella* strains using culture media that inhibit the growth of other *Enterobacteriaceae* that would complicate the bacteriological examination.

After incubating the samples for 24 hours at 37°C in peptone water, all broth showed turbidity.

A colour change was shown in the Rappaport-Vasiliadis broth, from dark blue to light blue or yellow in all 10 tested samples, which would mean, according to literature (24), *Salmonella* multiplication in the culture media.

Another stage of the bacteriological exam was replicating on XLD medium, on which there were observed typical pink and black-centred *Salmonella* colonies ("cat-eye colonies").

On SS agar, out of 10 Rappaport-Vasiliadis broth samples with modified aspect, 8 presented colourless, black-centred colonies, which would be *Salmonella*, according to the literature (7, 24).

In addition to these, there were other types of pink colonies of *Escherichia* and *Enterobacter*. They were ignored in the study.

Passages were made from the *Salmonella*-looking colonies on other media – MIU and TSI – to reveal other biochemical features to better differentiate colonies with similar aspects.

Salmonella formed violet colonies on CHROMagar being easily differentiated from other enterobacteria.

On Rambach medium, *Salmonella* formed pink/red colonies, while most coliforms form blue colonies.

The final identification was made with the API 20E kit. Out of the 37 samples that grew on culture media and that could have been *Salmonella*, 26 strains could be defined as *Salmonella* genus, 8 strains isolated from faeces and 18 strains isolated from organs; all the other samples were defined as *Proteus spp*, *Enterobacter spp* and *E. coli*.

After the bacteriological examination, out of the 10 faeces samples, 8 were positive for *Salmonella* and out of 27 organ samples, 18 were positive for *Salmonella*.

Using special media and the API 20E test, it was possible to confirm the 26 strains of *Salmonella* and to determine their species. There were identified *S. enterica* and *S. choleraesuis* species. *S. enterica* was isolated from faeces samples of 19 weeks old pigs.

From the literature, it is known that the isolation of *Salmonella* from pigs younger than 2 weeks is much less frequent than in fatteners (6). A study made by Asai et al. (2000) showed the greatest percent of *Salmonella* in fattening pigs (17.3%) and in suckling piglets (4.5%) (2).

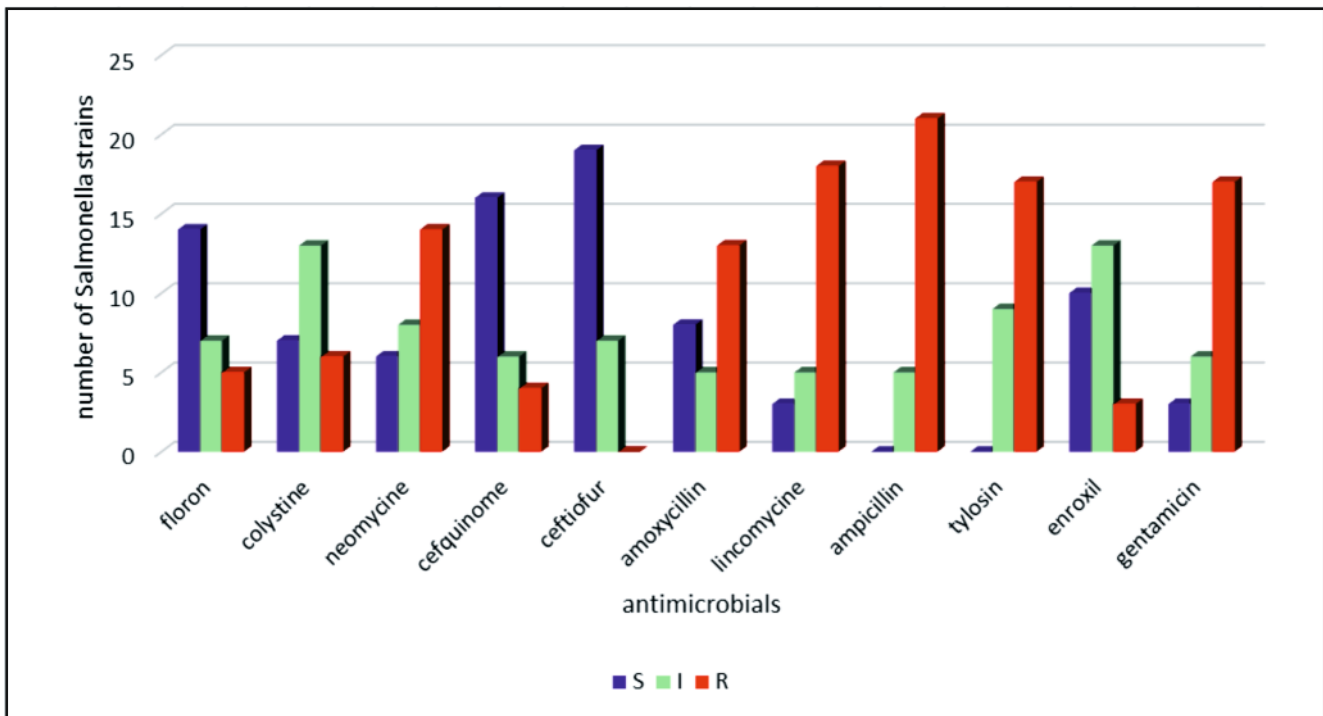


Fig. 1. Results of antimicrobial sensitivity testing of *Salmonella* strains

Our research in suckling piglets revealed 2 positive samples out of 3 tested.

Our serological research demonstrated the presence of anti-*Salmonella* antibodies in all age categories (5, 15, 16). Weaned piglets are the most frequently affected by *Salmonella* infection, while fat pigs and adults are only occasionally affected. In suckling piglets, the clinically manifested disease due to *S. choleraesuis* is less frequent, the reason why is not fully elucidated. It seems this protection is offered by maternal antibodies via colostrum. The disease may appear in this category in the herds that were free of *Salmonella* infection (1, 4, 8). The great number of *Salmonella* strains isolated from the tested samples allowed the diagnosis of digestive disorders, the infection with *S. choleraesuis* and *S. enterica*. When testing the antimicrobial sensitivity, we observed a various behaviour of isolated strains, with sensitive, moderately sensitive and resistant strains (Fig. 1).

The resistance at microbials phenomena was observe to more antimicrobials. The greatest resistance was shown to lincomycin and ampicillin, by 78.5%, at tylosin and gentamicin by 64.2% and at amoxycillin, by 50%. It is worth mentioning that these antibiotics were often used in farms to treat all kinds of diseases, including digestive disorders. Ceftiofur and cefquinome, third and fourth-generation cephalosporins are recommended in treating systemic infections in pigs (3). Also, these betalactamines are often used in human medicine, being the first choice in treating enterobacteria infections. In Spain, multi-resistance was

found in 55% of *S. enterica* strains isolated in 2015 (7). Even though gastroenteritis produced by non-typhoid *S. enterica* is self-limited and usually doesn't require treatment, bacteremia still appears in over 5% of pigs and is often deadly and antibiotic therapy is needed. The results obtained in this study showed that 35.7% of tested strains were resistant to quinolones. Cephalosporins had the greatest sensitivity, 57.1% to cefquinome, 71.4% to ceftiofur and 57.1% to florfenicol. As they are quite expensive, these antibiotics are not commonly used in farms with many animals to treat. Even though the number of enroxil resistant strains was small, 7 strains were moderately sensitive, which meaning the resistance is going to appear also for this chemotherapeutic. Similar results were obtained by other researchers, too (8, 9). The excessive usage of antibiotic treatments administered to sows had as a result the selection of multi-resistant *Salmonella* strains in suckling piglets, in this age group we observed resistance to 5 antimicrobials out of 11 tested. The resistance phenomenon was observed in all tested farms, in all age groups. The greatest resistance was observed in the age group of 9 weeks, in F5 and F6 farms to 7 antimicrobials out of 11 tested.

CONCLUSIONS

The results obtained by investigation of *Salmonella* strains isolated from different samples (organs and faeces) from piglets of different ages show the following: (a) the age of piglets from which these strains

were isolated varied between suckling piglets of 4 days and fatteners of 19 weeks; (b) Two species of *Salmonella* were isolated: *S. enterica* and *S. choleraesuis*. For *S. enterica* more tests are necessary (serotyping) to be able to determine the subspecies, if it is zoonotic or not; (c) Multiple resistance to antimicrobials was observed in all 14 tested strains of *Salmonella*; (d) cephalosporins and florfenicol had the best results as antimicrobials, but they are expensive, so generally, they are avoided. The least efficient antibiotics were those used frequently in pig farms to treat various diseases (ampicillin, neomycin, lincomycin, gentamicin). To avoid the apparition of multi-resistant *Salmonella* strains in pig farms, proper management of infections is necessary, performing antibiograms and monitoring the effectiveness to establish the degree of contamination with *Salmonella*.

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