

ISOLATION AND IDENTIFICATION OF SOME PATHOGENIC STRAINS FROM RAW AND PROCESSED MEAT SAMPLES

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Abstract

Food poisonings can be lethal situations for digestive tractus, caused by ingestions of food contaminated with microbial germs or with theirs metabolic products. In this paper there were determinate bacteria from Salmonella genus, on the following types of samples, harvested from slaughterhouses and outlets: poultry and bird organs (thighs, gizzard and hearts and bird carcass), raw pig meat (pork neck, pork ribs and pork ham), processed pig meat (mincemeat and sausages). From analysed samples there were isolated: S. enteritidis, S. infantis, S. enterica, S. tennessee, S. saintpaul, S. bredeney, S. typhirium and S. muenchen. 50% of these bacteria were found in processed pork meat samples, followed by poultry and pork meat (raw meat), with 25% each. The isolation and identification technique was done with horizontal method, following these steps: pre-enrichment in unselective liquid mediums, enriching in selective liquid mediums, isolation and identification, identity confirmations.

Key words: isolation, identification, meat sample, pathogenic bacteria.

INTRODUCTION

The presence of pathogenic germs, in raw meat and meat products, favors the development of diseases at consumers (Apostu, 2006). Salmonella sp. frequently contaminate the animal carcass, and because of that, they produce food poisonings, which are on top list in most of the countries (Bărzoi, 2002). Salmonella sp. belong to the group of severely pathogenic germs (Apostu, 2004).

Getting ill may be caused by a single serotype or multiple serotypes associated with each other (Tudor, 2002).

As a consequence of some factors as: import of food products, transportation of food products in improper conditions, resistance of bacteria in the external environment, it has come to the situation in which other products, that normally are not favorable environment for salmonella, develops contamination with Salmonella sp. (Ulea, 2017).

In order to keep under control these risk situations, it is necessary to standardize the identification methods, by laboratory analysis, according to ISO regulations (Dan, 2001).

MATERIALS AND METHODS

In order to establish the frequency of food products contamination with different types of Salmonella, there were collected and analyzed samples in 2017 and 2018, as follows: 367 samples of poultry and bird organs, 244 samples of raw pig meat, 648 samples of processed pig meat, during 2017 and 412 samples of poultry and bird organs, 294 samples of raw pig meat, 673 samples of processed pig meat, during 2018.

The work technique for Salmonella sp. isolation and identification, by horizontal method, assume the following steps:

- Stage I - Pre-enrichment in unselective liquid mediums.

The Salmonella bacteria may be in low number and often accompanied by a large number of Enterobacteriaceae or other bacteria species. Pre-enrichment is necessary to discover the low number of Salmonella sp. or modified Salmonella sp.

Buffered peptone water is inoculated along with sample, then incubated at $37^{\circ}\text{C} \pm 10^{\circ}\text{C}$ for 18 ± 2 h.

- Stage II - Enriching in selective liquid mediums.

The Rappaport-Vassiliadis soybean medium (RVS broth) and tetrathionate/novobiocin Muller-Kauffmann broth (MKTn broth) are inoculated with bacteria culture medium obtained in buffered peptone water. The RVS broth is incubated at $41.5 \pm 1^\circ\text{C}$ for 24 ± 3 hours and the MKTn broth at $37 \pm 1^\circ\text{C}$ for 24 ± 3 hours.

- Stage III - Isolation and identification.

From the culture mediums obtained, two solid selective mediums are inoculated: xylose-lysine-deoxycholate agar (XLD agar (Figure 1) and Rambach agar (Figure 2).

They are incubated at $37 \pm 1^\circ\text{C}$ and examined after 24 ± 3 h.

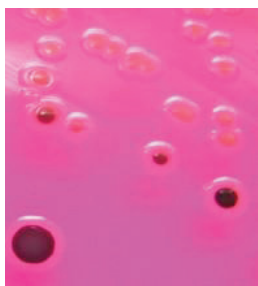


Figure 1. *Salmonella* colonies on X.L.D Agar



Figure 2. Typical *Salmonella* (red) colonies on Rambach Agar

- Stage IV - Identity confirmation.

Isolated colonies, presumed as *Salmonella* sp., are confirmed by biochemical and serological tests.

RESULTS AND DISCUSSIONS

There were collected and analyzed food samples, in order to establish the frequency of food products contamination with different types of *Salmonella*. In Table 1 and Figure 3,

there are presented the tested products type and the number of samples, for 2017 and 2018.

Table 1. Sample category and number of samples collected in 2017 and 2018

Tested product type	Number of samples	
	2017	2018
Poultry and bird organs	367	412
Raw pig meat	244	294
Processed pig meat	648	673
Total number of samples	1259	1379

In 2017, there were collected 367 samples of poultry and bird organs, 244 samples of raw pig meat, 648 samples of processed pig meat

In 2018, there were collected 412 samples of poultry and bird organs, 294 samples of raw pig meat and 673 samples of processed pig meat.

In 2017, from a total of 1259 collected samples there were found 7 types of *Salmonella*: *S. enterica* – 2 samples; *S. enteritidis* – 3 samples; *S. bredeney* – 2 samples; *S. saintpaul* - 3 samples; *S. ifantis* – 1 sample; *S. tennessee* – 3 samples, *S. typhimurium* – 5 samples.

In 2018, from a total of 1379 collected samples there were found 8 types of *Salmonella*: *S. enterica* – 2 samples; *S. enteritidis* – 2 samples; *S. bredeney* – 1 sample; *S. saintpaul* - 3 samples; *S. ifantis* – 1 sample; *S. tennessee* – 2 samples, *S. typhimurium* – 3 samples, *S. muenchen* – 1 sample.

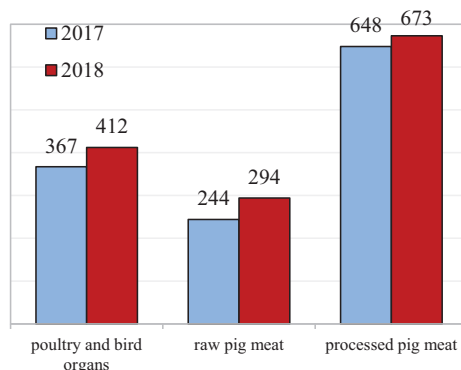


Figure 3. Sample category and total number of samples

In Table 2 and Figure 4 there are presented the number of samples for each the category of poultry and bird organs: thighs - 128 samples, gizzard and hearts – 110 samples and bird carcass – 129 samples, for 2017 and thighs -

153 samples, gizzard and hearts – 127 samples and bird carcass – 132 samples in 2018.

Table 2. Sample category and number of samples collected in 2017 and 2018, for poultry and bird organs

Sample category	Number of samples	
	2017	2018
<i>Chicken thighs</i>	128	153
<i>Poultry gizzard and hearts</i>	110	127
<i>Bird carcass</i>	129	132
Total number of samples	367	412

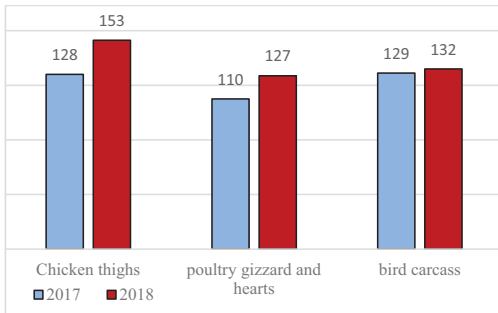


Figure 4. Number of samples for poultry and bird organs

In Table 3 and Figure 5 and 6 there are presented the number and percent of samples found contaminated with different types of *Salmonella* sp. in poultry and bird organs.

Table 3. Occurrence percent of different types of *Salmonella* sp. in poultry and bird organs

Sample category	Stereotype	Salmonella sp.			
		2017		2018	
		No.	%	No.	%
<i>Chicken thighs</i>	<i>S. enteritidis</i>	1	0.78	1	0.65
	<i>S. infantis</i>	1	0.78	1	0.65
	<i>S. enterica</i>	1	0.78	1	0.65
<i>Poultry gizzard and hearts</i>	<i>S. tennessee</i>	1	0.90	-	-
<i>Bird carcass</i>	<i>S. enteritidis</i>	1	0.77	1	0.75
		5		4	

In 2017, from a total of 367 tested samples, there were isolated 5 *Salmonella* types: *S. enterica*; *S. enteritidis*; *S. infantis*; *S. tennessee*, founded in 5 samples (1.36%).

In 2018, from a total of 412 tested samples, there were isolated 3 *Salmonella* types: *S.*

enterica; *S. enteritidis*; *S. infantis*, founded in 4 samples (0.97%).

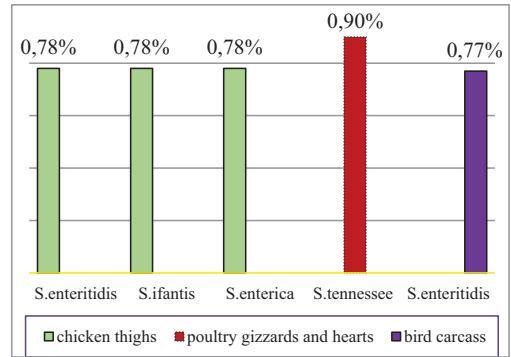


Figure 5. Occurrence percent of different types of *Salmonella* sp. in poultry and bird organs, in 2017

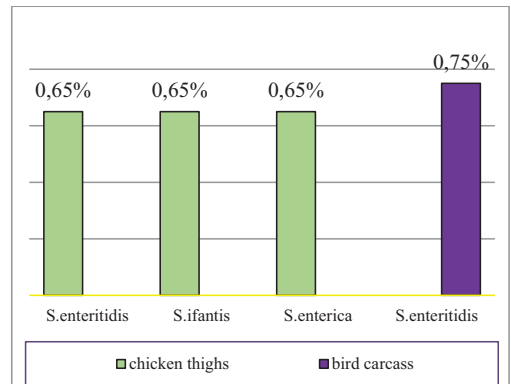


Figure 6. Occurrence percent of different types of *Salmonella* sp. in poultry and bird organs, in 2018

In Table 4 and Figure 7 there are presented the number of samples for each category of raw pork meat: pork neck – 83 samples, pork ribs – 90 samples and pork ham – 71 samples, in 2017 pork neck – 110 samples, pork ribs – 68 samples and pork ham – 116 samples, in 2018

Table 4. Sample category and number of samples collected in 2017 and 2018, for raw pork meat

Sample category	Number of samples	
	2017	2018
<i>Pork neck,</i>	83	110
<i>Pork ribs</i>	90	68
<i>Pork leg</i>	71	116
Total number of samples	244	294

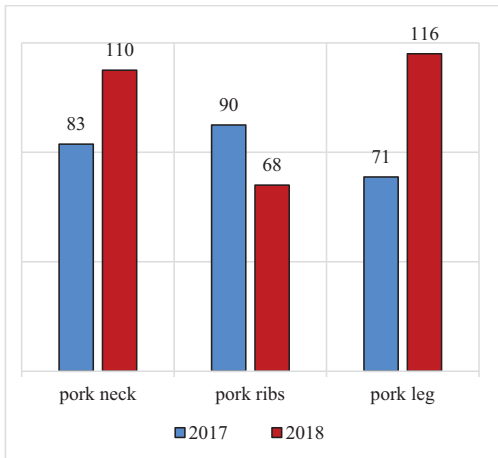


Figure 7. Number of samples, for raw pig meat

In Table 5 and Figure 8 and 9 there are presented the number and percent of samples found contaminated with different types of Salmonella sp. in raw pork meat

Table 5. Occurrence percent of different types of Salmonella sp. in in raw pork meat

Sample category	Stereotype	Salmonella sp.			
		2017		2018	
		No.	%	No.	%
Pork neck	<i>S. tennessee</i>	1	1.20	1	0.90
	<i>S. bredeney</i>	1	1.11	-	-
Pork ribs	<i>S. typhimurium</i>	1	1.11	1	1.47
	<i>S. saintpaul</i>	1	1.41	1	0.86
Pork leg	<i>S. muenchen</i>	-	-	1	0.86
		4		4	

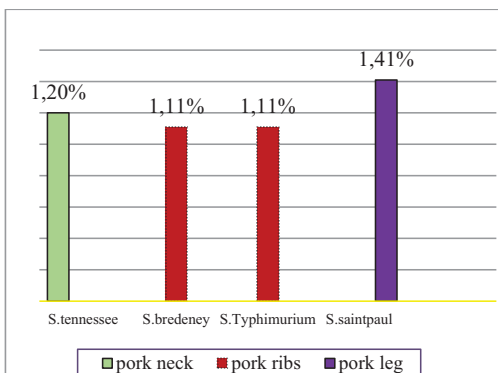


Figure 8. Occurrence percent of different types of Salmonella sp. in in raw pork meat, in 2017

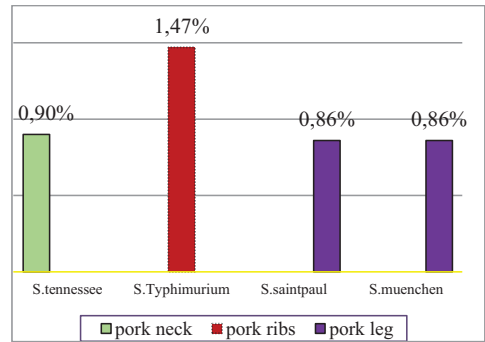


Figure 9. Occurrence percent of different types of Salmonella sp. in in raw pork meat, in 2018

In 2017, from a total of 244 tested samples, there were isolated 4 Salmonella types: *S. bredeney*; *S. saintpaul*, *S.typhimurium*, *S. tennessee*), founded in 4 samples (1.64 %).

In 2018, from a total of 294 tested samples, there were isolated 4 Salmonella types: *S. tennessee*, *S. saintpaul*, *S. typhimurium*, *S. muenchen*, founded in 4 samples (1.36 %).

In Table 6 and Figure 10 there are presented and the number of samples for each category of processed pig meat: mincemeat – 331 samples and sausages – 317 samples, in 2017 and mincemeat – 350 samples and sausages – 323 samples, in 2018.

Table 6. Sample category and number of samples collected in 2017 and 2018, for processed pig meat

Sample category	Number of samples	
	2017	2018
<i>Pork - Mincemeat</i>	331	350
<i>Pork sausages</i>	317	323
Total	648	673

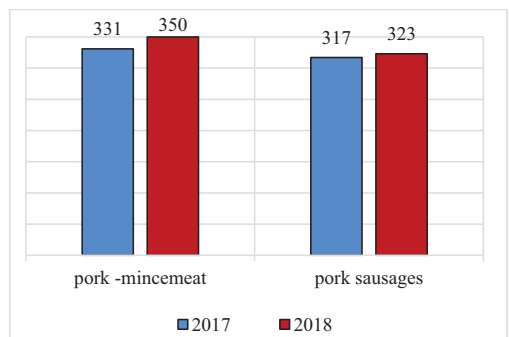


Figure 10. Number of samples, for processed pig meat

In Table 7 and Figures 11 and 12 there are presented the number and percent of samples found contaminated with different types of Salmonella sp. in processed pork meat

Table 7. Occurrence percent of different types of Salmonella sp. in processed pig meat

Sample category	Salmonella sp.				
	Stereotype	2017		2018	
		No.	%	No.	%
Pork - Mince meat	<i>S. tennessee</i>	1	0.30	1	0.29
	<i>S. enterica</i>	1	0.30	1	0.29
	<i>S. typhimurium</i>	2	0.60	1	0.29
	<i>S. saintpaul</i>	1	0.30	1	0.29
Pork sausages	<i>S. bredeney</i>	1	0.32	1	0.31
	<i>S. typhimurium</i>	2	0.63	1	0.31
	<i>S. saintpaul</i>	1	0.32	1	0.31
	<i>S. muenchen</i>	-	-	1	0.31
		9		8	

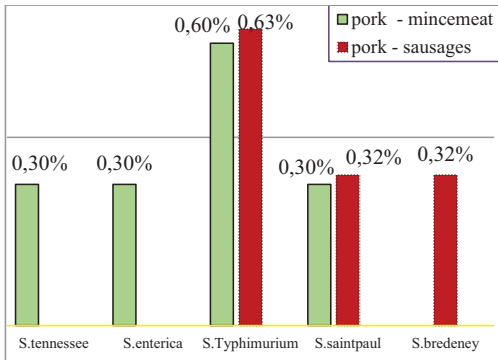


Figure 11. Occurrence percent of different types of Salmonella sp. in processed pig meat, in 2017

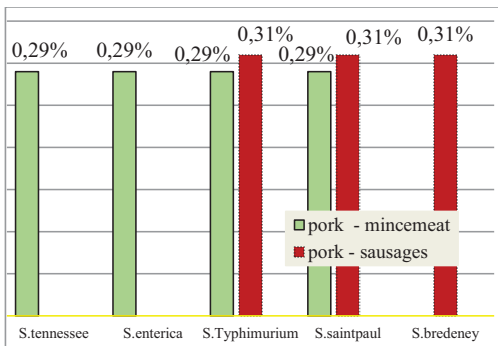


Figure 12. Occurrence percent of different types of Salmonella sp. in processed pig meat, in 2018

In 2017, from a total of 648 tested samples, there were isolated 5 Salmonella types: *S. enterica*, *S. bredeney*, *S. saintpaul*, *S. typhimurium*, *S. tennessee*, founded in 9 samples (1.39 %).

In 2018, from a total of 673 tested samples, there were isolated 6 Salmonella types: *S. tennessee*, *S. enterica*, *S. saintpaul*, *S. typhimurium*, *S. bredeney*, *S. muenchen*, founded in 8 samples (1.19 %).

It can be observed that the dominant types of Salmonella in poultry and bird organs were *S. enteritidis* and *S. enterica*, while in raw pork meat and processed pork meat the dominant types of Salmonella were *S. saintpaul*, *S. typhimurium* and *S. tennessee*.

In 2017, the most of the samples with Salmonella sp. were found in processed pork meat (50%), followed by poultry and bird organs (28%) and raw pork meat (22%). In 2018, 50% of Salmonella bacteria were also found in processed pork meat samples, followed by poultry and pork meat (raw meat), with 25% each (Figures 13 and 14).

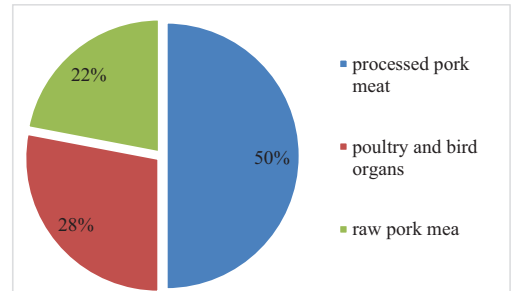


Figure 13. The percent of contamination depending on product type samples, in 2017

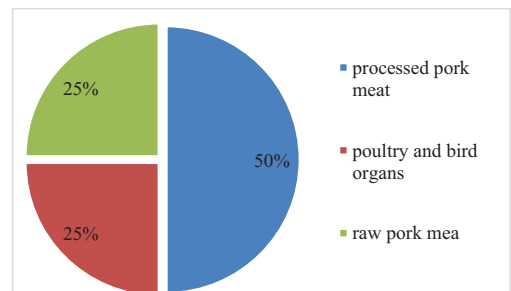


Figure 14. The percent of contamination depending on product type samples, in 2018

CONCLUSIONS

In 2017 and 2018, from collected samples there were isolated different types of Salmonella: *S. enterica*; *S. enteritidis*; *S. bredeney*; *S. saintpaul*; *S. infantis*; *S. tennessee*, *S. typhimurium*. In 2018 there was isolated one more: *S. muenchen*.

Both in 2017 and 2018, it can be observed that the most of these contaminated samples, were isolated in processed pig meat (50%), followed by poultry meat (28% and 25% respectively) and raw pig meat (22% and 25% respectively).

The dominant types of Salmonella in poultry and bird organs were *S. enteritidis* and *S. enterica*, while in raw pork meat and processed pork meat the dominant types of Salmonella were *S. saintpaul*, *S. typhimurium* and *S. tennessee*.

The presence of Salmonella sp. in meat is favored by failure to comply the transport conditions, animals stressing at slaughterhouse, failure to comply the slaughtering technology and programs of hygiene and disinfection.

Food products can be contaminated at all stages of processing, including in the consumer phase. Potential contamination sources are: ingredients, manufacturing procedures, thermal processing (heating or cooling techniques), advanced processing techniques, packaging methods.

In processing industry and especially in slaughterhouses it is imperative to implement

and strictly follow the HACCP and hygiene good practice guidelines.

In order to reduce the market penetration of products contaminated with Salmonella, another important objective is to check the carcasses and raw materials at the reception stage.

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