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Prevalence, serovar, and antimicrobial resistance of *Salmonella* isolated from meat and minced meat used for production smoked sausage

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Abstract. The objective of this study was to research the prevalence, serovars, and antimicrobial resistance profiles of *Salmonella* isolated from meat and minced meat used for the production of fermented sausage. A total of 116 samples were tested, and among them, 20 (17.2%) were positive. *Salmonella* was detected in 3 (10.3%) beef samples, 5 (19.2%) pork samples, and 6 (20.7%) poultry samples. In minced meat, the *Salmonella* prevalence was 18.8%. *Salmonella enterica* serovar Agama (5.2%) was the most commonly identified serovar, followed by *S. Enteritidis* (4.3%), *S. Typhimurium* (3.4%), *S. Infantis* (2.6%), and *S. Lindenburg* (1.7%). Most of the serovars identified in the present study are recognized as frequent causes of human salmonellosis. Thus, the presence of these serovars means foods with these meats are a likely source of human infections. We found the *Salmonella* isolates exhibited high rates of resistance to antimicrobials tetracycline, ampicillin, streptomycin, and ciprofloxacin. The highest level of resistance was to tetracycline (75%), followed by resistance to ampicillin (50%), streptomycin (30%), ciprofloxacin (20%), gentamicin (20%), and neomycin (10%). The high-level resistance observed for some of the serovars calls for concern. *Salmonella* with multidrug resistance in meat used to produce fermented sausages is considered a high additional risk for human health.

1. Introduction

Fermented sausages are high-value traditional products. Fermented sausages and air-dried meats are at additional risk of containing bacterial hazards, as in most cases, they are not subject to the heating process. Fermented meat products result from a complex microbiological activity that mainly consists of lactic fermentation and several characteristic biochemical changes triggered by lactic acid bacteria. These bacteria play a crucial role in this fermentation: acidification, while they can also have proteolytic and lipolytic activities [1].

Meat used for the production of fermented sausages can contain residual levels of antimicrobial compounds [2]. The presence of high levels of antibiotic residues in raw meat used to produce raw smoked sausage is a call for concern. Research by Kjeldgaard et al. shows that even minimal residual amounts of antibiotics at the level legally allowed in raw materials negatively affect starter cultures [2]. Fermented sausages prepared from meat with residual concentrations of antibiotics at or close to deemed acceptable levels can lead to full or partial fermentation failures, and therefore to the production of an unsafe product. Outbreaks of severe, sausage-borne gastrointestinal infections caused by verocytotoxic (Shiga toxicogenic) *Escherichia coli* (VTEC/STEC), *Salmonella*, and *Listeria monocytogenes* occur



regularly [3]. Food-borne diseases caused by antibiotic-resistant bacteria are an important public health problem worldwide. The use of antimicrobial drugs in agriculture or the food industry can contribute to the formation of resistance in strains (including genotypic resistance), which leads to a relative increase in the number of resistant bacteria in microbial communities. *Salmonella* with multiple antibiotic resistance (AR) presents a considerable threat to public health and food safety [4].

This work aimed to detect *Salmonella* contamination of raw and minced meat used in the production of fermented sausages and determine the bacterial isolates' antimicrobial susceptibility.

2. Materials and Methods

2.1 Samples

The objects researched were meat (pork, beef, poultry) and minced meat used to produce raw smoked fermented sausages obtained from a meat processing enterprise in the central region of Russia. Samples were collected from June 2020 to December 2020.

2.2. Isolation and identification of *Salmonella*

The sample analyses were carried out according to the modified standard ISO 6579 [5]. Pre-enrichment was done using Buffered Peptone Water (BPW) (Merck, Germany). A homogenized sample (25 g) was added to 225 ml of BPW. After homogenization with a Stomacher (AES, France), the bag was then closed and incubated for 18 h at 37°C. Then, 0.1 ml aliquots of cultures were incubated in 10 ml of Rappaport-Vassiliadis *Salmonella* Enrichment Broth (RVS) (Merck, Germany) at 41.5°C and 10 ml Muller-Kauffmann Tetrathionate Novobiocin Broth (MKTT) (Merck, Germany) at 37°C for 24 h. Loopfuls of RVS and MKTT cultures were streaked onto Xylose-Lysine-Tergitol 4 (XLT4) selective agar plates (Merck, Germany) and chromogenic Brilliance *Salmonella* agar (Oxoid, United Kingdom), then incubated at 37°C for 24 h. Presumptive colonies were subjected to further analysis using biochemical tests. Biochemical identification was performed using a RapID ONE System (Remel, USA).

2.3 Serological identification

The *Salmonella* isolates were serotyped by slide agglutination using polyvalent O and H antisera (Petsal, Russia) following the Kauffmann-White scheme [6].

2.4 Antibiotic susceptibility testing

Six different antibiotics were used in this study, namely, ciprofloxacin (5 µg), streptomycin (300 µg), gentamicin (10 µg), amoxicillin (20 µg), neomycin (30 µg), and tetracycline (30 µg) (Oxoid, United Kingdom). Antimicrobial susceptibility tests were performed on Mueller-Hinton agar (Oxoid, UK) using the disc diffusion technique. The surface of the Mueller-Hinton agar plate was evenly inoculated with the culture using a sterile cotton swab. The antibiotic discs were applied from a disc dispenser (Oxoid, UK) onto the surface of the inoculated agar. After 18-24 h of incubation, the diameter of growth inhibition around the discs was measured and interpreted as sensitive or resistant according to the Clinical and Laboratory Standards Institute [7].

3. Result and Discussion

3.1 *Salmonella* contamination of raw meat and minced used for the production of raw smoked sausage

In the current study, 116 samples of meat and minced meat used to produce raw smoked sausage were assessed for contamination by *Salmonella*, and the overall prevalence was 16.7%. *Salmonella* was detected in 3 (10.3%) beef samples, 5 (19.2%) pork samples, and 6 (20.7%) poultry samples. The prevalence of minced meat contaminated with *Salmonella* was 18.8%. A summary of the prevalence of *Salmonella* from meat samples and minced meat is presented in Figure 1.

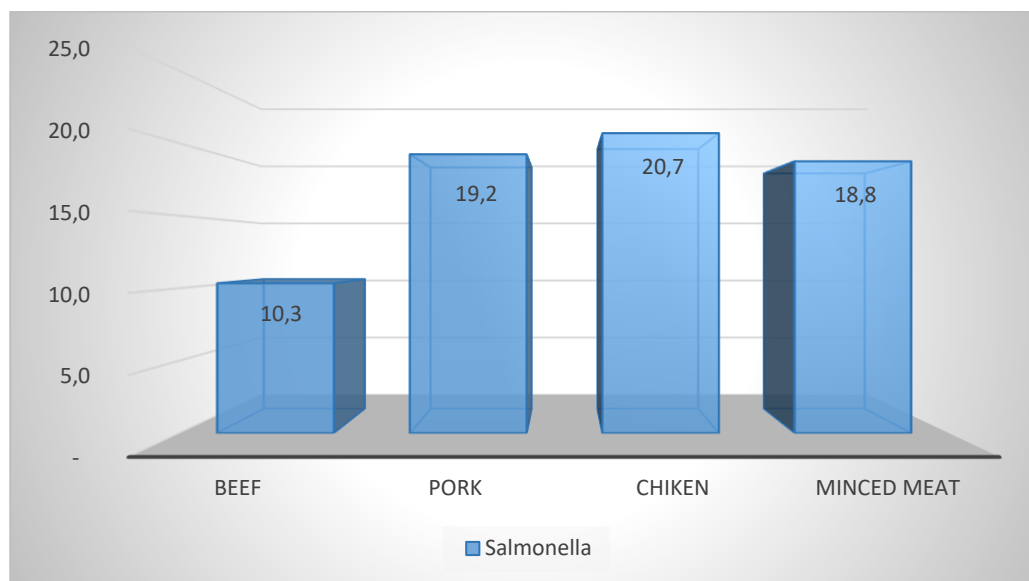


Figure 1. Prevalence of *Salmonella* in meat and minced meat used for the production of raw smoked sausage [%]

Salmonellosis is the most common and challenging to eradicate the zoonotic bacterial infection in the world that affects both animals and humans. According to the Federal Service Russian for Surveillance on Consumer Rights Protection and Human Wellbeing, by the end of 2018, more than 50 outbreaks of group morbidity associated with food consumption in public catering and trade were registered in Russia [8].

The presence of *Salmonella* in raw smoked sausage is a significant risk to consumer health. Sausage manufacturers commonly inoculate sausage meat with lactic-acid-producing bacteria to control the fermentation process. The fermentation induces pH reduction, and the reduction in water activity during maturation contributes to the inactivation of *Salmonella* spp. in the raw ingredient mix.

However, antibiotics used as growth promoters or to treat disease in livestock can eventually end up in meat, and starter cultures sensitive to antibiotics will not acidify the sausage meat effectively [2]. The complete or partial fermentation process failures allow survival of pathogens capable of causing severe food-borne infections, including *Salmonella*.

The prevalence of *Salmonella* in the minced meat for the production of fermented sausages was 18.8%. In a similar study, Piras et al. reported that *Salmonella* prevalence was 24% in ground meat for fermented sausage and products at the end of acidification. The pathogen was also detected in a sausage sample at the end of ripening (2%) [9]. Our result on *Salmonella* prevalence in samples of raw minced meat (18.8%) was higher than those reported by other authors who observed prevalences between 0.3 and 4.3 % [10,11].

Contamination of minced meat with *Salmonella* is still considered a significant problem in food hygiene. Despite substantial improvements in technology and hygienic practices employed in all stages of meat production, salmonellosis remains an intransigent threat to human health. Therefore, the resulting data will be hugely beneficial for future risk assessment in the production of fermented sausages.

3.2 Serological identification of *Salmonella*

From all samples, five different serovars were identified among 20 *S. enterica* isolates (Table 1). *S. Agama* (5.2%) was the most commonly identified serovar, followed by *S. Enteritidis* (4.3%), *S. Typhimurium* (3.4%), *S. Infantis* (2.6%) and *S. Lindenburg* (2 %).

The identified serovars from beef were *S. Enteritidis*, *S. Typhimurium*, and *S. Agama*. *Salmonella* isolates from pork were *S. Typhimurium* (7.7%), *S. Infantis* (7.7%), and *S. Lindenburg* (3.8%). The isolated *Salmonella* serovars from chicken were *S. Agama* and *S. Enteritidis* (6.7% each), *S. Typhimurium* (3.3%), *S. Infantis* (3.8%).

The identified *Salmonella* serovars from minced meat were *S. Agama* (9.3%), *S. Enteritidis* (6.3%), and *S. Lindenburg* (3.1%).

Table 1. Prevalence of *Salmonella* serovars in samples of raw meats and minced meat used for production raw smoked sausage

Serovars (antigenic formula)	Beef (n=29)	Pork (n=26)	Poultry (n=29)	Minced meat (n=32)	Total (n=116)
<i>S. Enteritidis</i>	1 (3.4)	0	2 (6.9)	2 (6.3)	5 (4.3)
<i>S. Typhimurium</i>	1 (3.4)	2 (7.7)	1 (3.4)	0	4 (3.4)
<i>S. Lindenburg</i>	0	1 (3.8)	0	1 (3.1)	2 (1.7)
<i>S. Agama</i>	1(3.4)	0	2 (6.9)	3 (9.4)	6 (5.2)
<i>S. Infantis</i>	0	2 (7.7)	1 (3.4)	0	3 (2.6)
Total <i>Salmonella</i> isolates, no (%)	3 (10.3)	5 (19.2)	6 (20.7)	6 (18.8)	20 (17.2)

S. Agama, *S. Enteritidis*, and *S. Typhimurium* were the most prevalent serovars identified in the current study. The prevalence of different *Salmonella* serovars in meat products has been investigated in many countries. *Salmonella enterica* ser. 6.7:d:- (29%), *S. Agama* (28%), and *S. Typhimurium* (16%) were the three most prevalent serovars in retail meat and meat products in China [12].

S. Derby, *S. Typhimurium*, and *S. Enteritidis* are frequently identified worldwide, and the latter is one of the most common serovars associated with human salmonellosis [13,14]. This serovar also accounted for 4.3 % of the isolates recovered from meat and minced meat in the current study.

S. Agama was isolated from all the meat types except pork in this study. *S. enterica* *Agama* was initially isolated and named after the rainbow lizard (*Agama Agama*) in West Africa. A study by Ahmed et al. showed that *Salmonella* *Agama* was obtained from the poultry environment (feed and water), dead birds (liver, spleen, and ovarian follicle), and seemingly healthy birds (cloaca swabs). Also, one of the predominant serovars in the study by Ahmed et al. was *S. Agama* - 28%. It was isolated from all the poultry farms in North Central Nigeria [15]. In our study, *S. Agama* was also detected in poultry but in beef too. The presence of *S. Agama* in the samples of minced meat is most likely from samples of poultry and beef.

S. Infantis has been one of the most frequent serovars in many countries. *S. Infantis* has been isolated from humans, animals, and vegetables, meats (e.g., broiler and chicken) [16,17].

Most of the *Salmonella* serovars identified in the present study are recognized as frequent causes of human salmonellosis. Thus, these serovars in meat products show these foods are likely sources of human infections.

3.3 Antibiotic susceptibility testing

In general, a high percentage of resistance to the tested antimicrobials was observed across all the serovars. Even though all isolates of *S. Enteritidis* were susceptible to gentamicin, streptomycin, and neomycin, they were resistant to tetracycline 60%, ampicillin (40%), and ciprofloxacin 20%. Around 25% of *S. Typhimurium* strains were resistant to gentamicin and neomycin, 50% were resistant to streptomycin, and 100% were resistant to tetracycline. The increase in antibiotic-resistant *Salmonella* is a significant concern worldwide.

Table 2. Antimicrobial resistance of *Salmonella* serovars isolated from raw meats and minced meat used for production fermentation sausage (AMC – ampicillin, GEN – gentamicin, TE – tetracycline, CIP – ciprofloxacin, STR – streptomycin, NEO – neomycin)

	AMC no (%)	GEN, no (%)	TE no (%)	CIP no (%)	STR no (%)	NEO no (%)
<i>S. Enteritidis</i>	2 (40)	0	3 (60)	1(20)	0	0
<i>S. Typhimurium</i>	3 (75)	1(25)	4(100)	0	2(50)	1(25)
<i>S. Lindenburg</i>	1 (50)	0	2(100)	0	1(50)	0
<i>S. Agama</i>	3 (50)	2 (33)	4(67)	3(50)	2(33)	1(17)
<i>S. Infantis</i>	1 (33)	1(33)	2(67)	1(33)	1(33)	0
Total	10(50)	4(20)	15(75)	4(20)	6(30)	2(10)

All the isolates showed high resistance to ciprofloxacin (20-50%) except for *S. Typhimurium* and *Salmonella* Lindenburg, which were susceptible to ciprofloxacin.

Resistance to two and more antibiotics was common in most isolates from samples in the current study. *S. Agama* was one of the most prevalent serovars in this study and showed a high level of resistance to most of the commonly used antimicrobials.

In a similar study, a total of 62% of *Salmonella* isolates exhibited resistance to at least one antimicrobial drug. In another study, resistance to sulfamethoxazole (38%), ampicillin (24%), nalidixic acid (24%), ciprofloxacin (24%), and tetracycline (19%) was identified the most frequently [18]. Notably, in our study, the highest level of resistance against most antibiotics was shown among *S. Agama*, which exhibited increased resistance to tetracycline (67%), ampicillin (50%), ciprofloxacin (50%), streptomycin (33%), gentamicin (33%) and neomycin (17%). The high level of resistance to most of the antimicrobials tested in this study, especially ciprofloxacin, is problematic because fluoroquinolones are used strategically to treat salmonellosis. This resistance can be caused by indiscriminate use of antimicrobials at recommended doses or at subtherapeutic doses in feed as growth promoters and chemotherapeutic agents to control epizootics on the farms [15].

The widespread overuse and misuse of antimicrobial agents in food animal production have contributed to the development of antimicrobial-resistant pathogens such as *Salmonella* that has emerged as a major health problem worldwide.

4. Conclusion

Residual antibiotics in meat can reduce the proliferation of starter culture in raw sausage fermentation and thus disrupt the fermentation process for raw smoked sausage. Still, these concentrations do not affect the survival or even multiplication of antibiotic-resistant pathogens. Therefore, the presence of pathogenic bacteria, such as *Salmonella*, in meat and minced meat used to produce smoked sausages pose a threat to consumers. Our study found *S. Agama*, *S. Typhimurium*, *S. Lindenburg*, *S. Enteritidis*, and *S. Infantis* in meat and minced meat. Worryingly, most *Salmonella* serovars identified in the present study are recognized as frequent causes of human salmonellosis. In cases of improperly fermented meat products, for example, due to the presence of antibiotics in meat, these serovars can survive and cause human disease. Also, in the present study, we found that *Salmonella* isolates exhibited high rates of resistance to antimicrobials tetracycline, ampicillin, streptomycin, and ciprofloxacin. The high-level resistance observed for some of the *Salmonella* serovars isolated in this study calls for concern. Our findings indicate that it is imperative to continue monitoring *Salmonella*'s prevalence and researching antimicrobial resistance in meat and minced meat used to produce raw smoked sausage.

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