



Detection of coagulase-positive *Staphylococcus* and its antibiotics resistance profile, recovered from cattle carcasses of the state of Paraná, Brazil

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Abstract

Staphylococcus sp. is a commensal bacterium in humans. Nonetheless, it is considered as an opportunistic microbe, being frequently associated with food poisoning outbreaks and antimicrobial resistance. The purpose of this work was to evaluate the presence of coagulase-positive *Staphylococcus* sp. in 100 cattle carcasses slaughtered in a slaughterhouse in the northwestern region of Paraná, Brazil, and to identify its *in vitro* resistance profile against different antimicrobials. Samples were collected through swabs from the slaughtered cattle carcasses, and then were forwarded to the Preventive Veterinary Medicine laboratory, at University of Paranese (UNIPAR). From the 100 samples, 18 (18%) recorded growth of microorganisms, and 16 samples of these were selected for the antimicrobial assay, according to the standard count for coagulase-positive *Staphylococcus*. Results of antibiogram showed that; 56.25% of the samples were resistant to Vancomycin, 50% to Penicillin, 31.25% to Tetracycline and Ampicillin, 18.75% to Erythromycin and Enrofloxacin, 12.5% to Oxacillin, 6.25% to Gentamicin and Clindamycin, whereas, only Cephalothin did not show resistance to any of the samples tested. Current study reported that antibiotic resistant strains (ARS) of coagulase-positive *Staphylococcus* sp. were present in the collected samples; and could have been originated from the food handlers, the utensils, the environment, or even from the animal itself. Accordingly, such results showed that it is important to stimulate the good hygiene and sanitary conditions of the food handlers, making them aware of their responsibility towards single health. Moreover, the indiscriminate use of antimicrobials may be responsible for the increase in the antibiotic resistant *S. aureus*.

Keywords: Food safety, Staphylococci, meat inspection, microbiological quality, antimicrobial resistance

1. Introduction

Brazil was a large producer and supplier of food to the international markets, with a prominent position in the production and exportation of beef (IBGE, 2015). The expectation is that at 2020, the national production of meat will meet the demand of almost 50% of the global markets. Large part of the world's population will be consuming this food item (Brasil, 2015). Accordingly, there is a growing concern for the consumption of a product that offers both safety and quality. Ferreira *et al.*, (2007) reported that food considered fit for human consumption must comply with high quality standards and identities in the hygienic-sanitary conditions.

Beef has intrinsic characteristics such as; its chemical composition, high water activity and pH near neutrality, and was therefore an excellent medium for microbial multiplication. When not controlled, such microorganisms could cause significant economic and health losses to the consumers (Palma, 2010; Peretti and Araújo, 2010; Ferreira and Simm, 2012).

Chesca *et al.*, (2001); Brasil. (2012) stated that microorganisms were widely distributed inside slaughterhouses. The muscle of live animals was sterile, so its contamination by microbes took place during bleeding, skinning and evisceration, as well as by handlers, utensils and by the surrounding environment itself.

Meat can be contaminated by several microorganisms such as bacteria. These bacteria might cause diseases and problems to the public health, since this food was most frequently related to Food borne illnesses (FBIs) (Greig and Ravel, 2009; Oliveira *et al.*, 2010). In Brazil, expenses with hospitalization due to FBIs reached approximately 45 million Brazilian reais per year; whereas in the United States, the same expenses might reach up to

150 billion Dollars yearly (The price, 2010). Earlier, Trabulsi and Alterthum, (2008) reported that presence of bacteria in products from an animal origin might lead to food poisoning cases, due to the capacity of some microorganisms to produce enterotoxins such as in *Staphylococcus* sp.

Species of the genus *Staphylococcus* were Gram-positive cocci, non-motile, facultative anaerobe and catalase-positive (Quinn, 2005; Baker *et al.*, 2008). Presence of *Staphylococcus aureus* (*S. aureus*) in food might indicate inappropriate hygienic conditions and/or ineffective processing. Such contamination by *S. aureus* most probably had originated from inappropriate human handling, as this bacterium was a commensal in healthy individuals inhabiting skin, mouth, nasal fossae and the gut (Franco and Landgraf, 2006; Forsythe, 2013). *S. aureus* presented properties allowing its fast colonization, posterior invasion through small lesions on the skin and mucosa, and therefore handling of food was one of its main transmission routes (Lu *et al.*, 2005). *S. aureus* was one of the most common pathogens in several types of infections affecting humans and animals (ANVISA, 2015). It was also considered as one of the most frequently associated bacterium to food poisoning, and presented resistance to several antimicrobials; therefore, treatment of infected people became harder (Traber, 2008).

Antibiotic resistant *S. aureus* was a global issue and control of this bacterium was of utmost importance and greater challenge, referred to the inappropriate use of antimicrobial drugs by humans and animals (Caddick *et al.*, 2006; ANVISA, 2015).

Several studies on the antimicrobial sensitivity profile have been carried out worldwide; however, results varied according to the environment and regions where these bacterial strains have been isolated. Some of these strains

were resistant to antimicrobials (Aarestrup *et al.*, 2008).

The aims of this article were to detect the presence of *Staphylococcus* sp. in cattle carcasses slaughtered in a slaughterhouse in the northwestern region of the state of Paraná, and to observe its antibiotics resistance profile.

2. Materials and methods

2.1. Study area and collection of samples

This study was performed in a cattle and pig slaughterhouse inspected by the State Inspection Service of Paraná (SIP/POA), located in the northwestern region of the state. A total of 100 bovine carcasses were used in this study. At the collection dates, the cattle carcasses were randomly selected between male and female, with ages ranging between 14 months and 6 years, of different breeds. The Animal Transit Slip (Guia de Trânsito Animal - GTA) was used only to know the source of the animals. Cattles used in this research originated from the cities of Alto Piquiri, Brasilândia do Sul, Cafezal do Sul, Iporã, Perobal, Pérola, Umuarama and Xambrê.

The microbial samples were collected using sterile swabs within a 2 cm² area in the external intercostal region of the carcasses, throughout their right and left sides. These swabs were stored in test tubes containing sterile peptone water; stored in thermal boxes under refrigeration, and then forwarded to the Preventive Veterinary Medicine Laboratory at Universidade Paranaense (UNIPAR).

2.2. Microbiological assays

All samples were analyzed according to the experimental procedures proposed by Silva *et al.*, (1997), where the direct plate count was the selected methodology for research on *S. aureus*. First,

samples were sown on Petri plates containing Baird-Parker agar medium supplemented with Potassium tellurite and egg yolk (direct dilution to 10⁻¹, 10⁻² and 10⁻³). Inoculated plates were then incubated in an oven at 37°C for 48 h. After incubation, plates containing between 20-200 typical colonies were selected for counting. The characteristic black colonies; 2-3 mm in diameter, bright, with opaque ring, surrounded by a transparent clear halo, standing out over the opacity of the medium were selected and examined for Gram staining, catalase and coagulase assays.

Disk diffusion test was performed for the antibiogram, as proposed by the Clinical Laboratory Standards Institute (CLSI, 2018). The following antibiotics were used: Ampicillin (AMP, 10), Cephalothin (CFL, 30), Enrofloxacin (ENO, 05), Erythromycin (ERI, 15), Gentamicin (GEN, 10), Oxacillin (OXA, 1), Penicillin G (PEN, 10), Tetracycline (TET, 30), and Vancomycin (VAN, 30). They were selected according to previous works of Freitas *et al.*, (2005); Perlin *et al.*, (2015); Salaberry *et al.*, (2016). Plates were incubated at 37°C for 24 h and then inhibition zones formed around the disks were inspected. The inhibition zones were measured using a caliper in millimeters (mm) and then were divided into three categories; sensitive, intermediary and resistant.

3. Results

From the 100 microbial samples analyzed, 18 % recorded bacterial growths in at least one dilution (Table 1). From the 18 samples which presented microbial growth, 16 (88.88%) of them were characterized as coagulase-positive *Staphylococcus*, they were positive also for the catalase test. All of the 16 samples were used for the antibiograms; 87.5% of these samples showed resistance to at least one antibacterial agent.

Table 1. Growth of coagulase positive *S. aureus* isolates in 10^{-1} , 10^{-2} , and 10^{-3} dilutions, recovered from 16 samples of cattle carcasses

Sample no.	10^{-1} cells/ml	10^{-2} cells/ml	10^{-3} cells/ml
41		4	
43		3	
44		3	1
47		6	
51	1		
52	4	12	173
53	2	2	
58	2	3	
61**	> 200	> 200	> 200
68	6	2	
69	4	1	
70 a***	8		2
70 b***	6		4
71**	> 200	> 200	> 200
72	4		
73	3	3	
74		2	
75**	> 200	> 200	> 200
78	1		

*The other samples did not present growth in any dilution.

** Samples 61, 71 and 75 were not considered since they present more than 200 typical colonies.

*** Sample 70 was divided into two plates (70a/70b), forming a total of 16 positive samples for coagulase-positive *Staphylococcus*

Only 12.5% of the samples did not present any resistance. However, one or more antibacterials were classified as intermediaries, as it was not possible to conclude if the tested strain was sensitive or resistant to this concerned drug. Carcasses in this study were not assessed after shipping of the product. This point could increase the proliferation of *S. aureus* in beef, since during shipping the meat will be in direct contact with the handler to be transported, and also be in contact with the shipping vehicle, and finally with the consumer's hand who will receive the meat.

Such factors might lead to rapid multiplication of the microorganism, as well as the production of enterotoxins. These factors might pose risks to public health, since the bacterium was

capable of producing enterotoxins that cause food poisoning in humans. Even if the meat received thermal treatment before consumption, there was also a risk of food poisoning since the toxin produced by *S. aureus* was thermo-stable, thus it could not be inactivated.

The following results regarding the antibiogram have been recorded: Vancomycin was the drug which presented the highest resistance (56.25%) among the samples, followed by Penicillin G (50%), Tetracycline and Ampicillin (31.15%), Erythromycin and Enrofloxacin (18.75%), Oxacillin (12.50%), and Gentamicin and Clindamycin (6.25%). Cephalothin was 100% sensitive in all samples of *S. aureus* tested (Table 2).

Table 2. Antibiograms performed on 16 samples of coagulase positive *S. aureus* isolates recovered from cattle carcasses

Antibiotics	Sensitive (%)	Intermediate (%)	Resistant (%)	Total (%)
Ampicillin	68.75	0	31.25	100
Cephalothin	100	0	0	100
Clindamycin	87.5	6.25	6.25	100
Enrofloxacin	68.75	12.5	18.75	100
Erythromycin	56.25	25	18.75	100
Gentamicin	93.75	0	6.25	100
Oxacillin	81.25	6.25	12.5	100
Penicillin G	50	0	50	100
Tetracycline	56.25	12.5	31.25	100
Vancomycin	18.75	25	56.25	100

*Antibiotics listed alphabetically, and results were expressed in percentage of *S. aureus* isolates resistant to each drug tested

4. Discussion

Several pathogenic microorganisms can be transmitted through food from animal sources including beef. Among such microorganisms, it is important to emphasize that *S. aureus* was the main source of outbreaks of food borne illnesses in the world. This species originated from handlers bearing enterotoxigenic strains, with the nasal fossae being the main reservoir of such bacterium. Therefore, food handlers were important links in the epidemiological outbreaks of food borne illness (Tigre and Borelly, 2011).

In a similar study of Perlin *et al.*, (2015) performed in Paraná, meat sausages registered in the municipal inspection were analyzed. From a total of 31 samples collected among dry, fresh, cooked and/or smoked products, 22.58% were non-compliant for *S. aureus* counts, which should not be greater than 5×10^2 cells/g according to Brasil. (2001). Highest prevalence however was observed in cooked and/or smoked products (57.14%). Marchi, (2006) previously confirmed the presence of *S. aureus* in 8 (26.7%) of the 30 samples of ground beef. Similarly, Arçari, (2011) observed the presence of *S. aureus* in 80% of the ground beef samples, collected from supermarkets in the city of Vitória, the state of Espírito Santo. In the previous studies of

Perlin *et al.*, (2015); Marchi, (2006); Arçari *et al.*, (2011), the authors reported high contamination percentages of beef by *S. aureus*. It is important to mention that this product had been examined through all slaughtering, refrigeration, storage, industrialization and shipping processes until reaching its final destination. During these stages, multiplication of the bacteria was very quick. Studies which assessed ground beef presented higher values for the presence of *S. aureus*, since the utensils and equipment used for grinding the beef were frequently incorrectly sanitized. This posed the risk of the food become in contact with other microbes already installed in the grinding machine, which accelerated their growth. In industrialized products, contamination might also take place due to failure in good manufacturing practices of the product such as; poor sanitation of equipment and utensils, as well as of handlers of this food.

Vanderlinde *et al.*, (1998) assessed the microbiological quality of cattle carcasses and observed that the *S. aureus* population increased by 27.7%, after the carcasses had been refrigerated for 24h. In Australia, Phillips *et al.*, (2001) also assessed the microbiological quality of cattle carcasses and observed that 24.3% of the 1275 samples assessed after 12h of refrigeration, contained *S. aureus*. In the

current study, the percentage of coagulase-positive *Staphylococcus* detected was 18%; however, this study was performed on carcasses selected directly from the slaughter line after skinning. Carcasses were not assessed after refrigeration, and therefore the percentage of contamination by coagulase-positive *S. aureus* could be higher, as multiplication of these bacteria was not interrupted during refrigeration, it only becomes slower. Moreover, in the study of Rosina and Monego, (2013), beef samples collected from the supermarkets showed the presence of *S. aureus* in 95% of these analyzed samples.

S. aureus samples isolated from food showed characteristics of virulence and resistance to several antibiotics routinely used in the treatment of diseases (Freitas *et al.*, 2005). This pathogen was considered important due to the combination of its virulence through toxins production, its invasive ability, and its antibiotic resistance characters (Caddick *et al.*, 2006; Souza, 2011). No other bacterial species with similar virulence against the human body presented such flexibility to survive and resist antibacterial therapy (Souza, 2011). Bacterial antibiotic resistance mechanisms were mediated by the acquisition of genes that code for enzymes initially known as penicillinases (currently referred to as beta-lactamases) (ANVISA, 2015). This caused the dissemination of bacteria resistant to beta-lactame antibiotics, which were previously efficient in treating infections caused by *S. aureus* (Aarestrup *et al.*, 2008).

An important landmark in Staphylococcal treatment was the appearance of *S. aureus* strains resistant to Methicillin (MRSA). According to the data released at 2010 by the Food and Drug Association (FDA), approximately 70% of the bacteria causing hospital infections were resistant to at least one of the drugs used for treating these infections, especially methicillin (Barret, 2005).

Classic MRSA strains showed resistance to other groups of drugs used in their treatments such as; Clindamycin, Erythromycin and Tetracycline (Ito *et al.*, 2003). Such data were in accordance with our current results, suggesting that our strains might be MRSA ones.

Cruvinel *et al.*, (2011) in a similar study on patients hospitalized in the intensive care unit (ICU) in the Federal District, Brazil, recovered bacterial isolates from nasal samples which revealed that 95% of the patients were infected by *S. aureus*. The antibiogram of these *S. aureus* isolates revealed their resistance to Ampicillin, Tetracycline, Gentamicin, Erythromycin and Clindamycin. In another earlier study carried out in Rio de Janeiro by Junqueira *et al.* (2009), they detected *S. aureus* strains resistant to Penicillin and Ampicillin. These results were in accordance to our study, showing that certain antibiotics were not effective in the treatment of Staphylococcal infections. Results obtained in the aforementioned papers were related to each other's and also to our study, since coagulase-positive *Staphylococcus* strains isolated from products of animal origin such as beef, could be originated from the animal itself and not only from the food handlers. This might be due to possible sanitary problems in the animal production cycle causing recurrent bacterial infections, with fewer possibilities of successful treatments due to antibiotics resistance. Current study treating with coagulase-positive *Staphylococcus* strains recovered from cattle carcasses, was extremely important to inform and warn health professionals, scientific and academic communities, researchers, and the societies in general of the risks that such bacteria could bring to human health.

Conclusion

Presence of bacteria at the first stages of meat production process, in addition to emergence of antibiotics resistant strains, were issues that need to

be considered and addressed to identify and solve failures in the meat industry processes. Most workers at slaughterhouses do not have enough knowledge to understand that they can save lives by simply washing their hands. Despite the difficulty in eliminating coagulase-positive *Staphylococcus* from food; due to its presence in the natural microbiota of both animals and humans; however, contamination can be avoided when good hygiene practices were adopted throughout the meat production chain until it reaches the consumer. It is the mission of veterinarians to inform, teach and make the workers involved in meat production aware of such facts.

Conflict of interests

The authors declare that there is no conflict of interests

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