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(Article begins on next page)

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Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: A research for methicillin resistant *Staphylococcus aureus* (MRSA)

G. Pesavento, B. Ducci, N. Comodo, A. Lo Nostro *

Department of Public Health, Food Safety Laboratory, University of Florence, Viale Morgagni 48, 50132 Florence, Italy

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a risk factor for patients in general population and particularly in immunocompromised patients. As a matter of fact, it can produce serious infections that may then evolve in septicaemia. However, transmission of MRSA from food to people can represent a serious problem only for immunocompromised people. Vancomycin is the elective antimicrobial commonly used in case of MRSA infection, but *S. aureus* strains with reduced sensibility to vancomycin are emerging worldwide. We isolated 42 strains of *S. aureus* from 176 samples of raw meat (poultry, pork and beef) during a one-year survey. Each strain was tested against twelve antimicrobial to verify antibiotic resistance. We found no evidence of methicillin-, teicoplanin- or vancomycin-resistance, but a lot of multiresistant microorganisms, i.e. resistant to three or more antibiotics. These results confirm the hypothesis that antibiotics resistance is present not only in nosocomial bacteria, but also in community environments microorganisms.
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Keywords: Staphylococcus aureus; Antimicrobial resistance; Food; Meat

1. Introduction

In the recent decades, antimicrobial resistance or/and sensitivity in bacteria have been considered as a major problem in Public Health. Colonization of patients with methicillin-resistance or/and reduced vancomycin-sensitivity in *Staphylococcus aureus* is a crucial factor in health care (Bradley, 1999; Brun-Buisson, 1998). Genes expressing antibiotic resistance in *S. aureus* can be either in chromosomic or in plasmidic DNA. In the case of plasmidic resistance, *S. aureus* cannot conjugate with another cell because of the lack of the "sexual pilus", that is usually present in Gram negative bacteria. In such a condition, fagic transduction or membrane binding are necessary (Courvalin, 1994).

The first evidence of *S. aureus* resistance to penicillin appeared in 1941, only after 2 years since its introduction in clinical therapy. Penicillin resistance is plasmidic, and therefore it spread out very quickly to several other strains, with the result that in the 1980s approximately 90% of *S. aureus*, isolated from patients, were resistant. Unlikely, methicillin-resistance is chromosomic, and therefore its diffusion is slower than the former, but it keeps going.

Because of the extended use and misuse of antibiotics (e.g. in agriculture, stock-farming and in the treatment of human diseases), the number of bacteria that are resistant to antimicrobial agents is rapidly increasing. Isolates from food have shown, in the last decade, a considerable increase in resistance against most antibiotics (Valsangiacomo, Dolina, Peduzzi, & Jäggli, 2000; Yücel, Citak, & Onder, 2005) and also against methicillin (Kitai et al., 2005).

In humans, the prevalence of methicillin-resistant *S. aureus* (MRSA) largely depends on the region, site of

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* Corresponding author. Tel.: +39 55 4598 530; fax: +39 55 4598 935.
E-mail address: antonella.lonostro@unifi.it (A. Lo Nostro).

infection, and whether the infection was nosocomial or community onset (Acco, Ferreira, Henriques, & Tondo, 2003). The prevalence of MRSA isolated from nosocomial infections was greater than that from community infections, and spanned from 1.8% in Switzerland and 2% in The Netherlands, to 73.8% in Hong Kong (Diekema et al., 2001; Joo Lee et al., 2001; Naimi et al., 2001; Salmenlinna & Vuopio-Varkila, 2001). In Europe, prevalence of MRSA is increasing: from 0.1–1.5% for Denmark, Sweden and The Netherlands, to 30.3–34.4% for Spain, France and Italy (Voss, Milatovic, Wallrauch-Schwarz, Rosdahl, & Braveny, 1994). In the United States, the prevalence of methicillin resistance among nosocomial *S. aureus* extracts increased from 2.1% in 1975 to 35% in 1991 (Grosso et al., 2000) and it is greater than in community *S. aureus* isolates (Panlilio, Culver, & Gaynes, 1992).

Vancomycin is the antimicrobial commonly used in case of MRSA infection. In 1997, Hiramatsu et al. (1997) described the first clinical *S. aureus* isolate with intermediate resistance to vancomycin. Since then, other strains of *S. aureus* with reduced sensitivity to vancomycin were identified in Europe and in USA (CDCP, 1997). In June 2002, both *S. aureus* completely resistant to vancomycin and a vancomycin resistant *Enterococcus faecalis* were isolated from a patient in the United States (CDCP, 2002). The presence of *vanA* both in VRSA and in VRE suggested exchange of genetic material from enterococcus to staphylococcus. Importance of VRE is mainly due to the capacity of transferring vancomycin resistance genes to MRSA.

The prevalence of nosocomial VRE isolates has increased from 15% in the early 1990s to 24% in 1998 (Massachussetts Dep. Public Health, Div. of Epid. Immuniz., 2001): the use of antimicrobial avoparcin in pork and poultry meat manufacturing factories as a feed additive is correlated to the increase of VRE prevalence in food of animal origin, and consequently in community and in nosocomial isolates (Børgen, Sorum, Wasteson, & Kruse, 2001; Grosso et al., 2000; Müller, Ulrich, Ott, & Müller, 2001). The possibility of transmission of MRSA through food was unknown until 1994 (Kluytmans et al., 1995); however, today we know that when a few cells of *S. aureus* enter an immunocompetent organism, they are destroyed by the gastric juices, but when an immunocompromised patient's food contains cells of *S. aureus*, these can reach the circulatory system and cause infections that may evolve to septicaemia. Recent cases of MRSA infections have been reported in patients hospitalised in the Haematology Unit of the University Hospital in Rotterdam (The Netherlands), where the index case was recognized in a woman who had eaten a banana fruit infected from a nasal carriage of MRSA, and who had prepared the food for haematology patients (Kluytmans et al., 1995).

Another community acquired MRSA infection has been reported in 2001, when a family was involved in an outbreak from ingestion of MRSA with baked pork meat, contaminated from the food handler (Jones, Kellum, Porter, Bell, & Schaffner, 2002).

Food can be then considered an excellent way for introducing pathogenic microorganisms in general population and in immunocompromised people, and therefore it may transfer antibiotic-resistant bacteria to the intestinal tract of consumers very efficiently. It is exactly in the intestine that can occur the transfer of resistance genes between non-pathogenic bacteria and pathogenic or opportunist bacteria (Sorum & L'Abèe-Lund, 2002).

The present study was performed in order to evaluate the rates of food-isolated *S. aureus*, that was resistant to some of the antibiotics normally used in clinical therapy and particularly to methicillin and vancomycin (Monnet, 2000).

2. Methods

Strains of *S. aureus* were obtained from a one-year microbiological survey of raw meat of poultry, pork and beef, purchased from some butchers' shops and supermarkets in the Florence area (Italy). Meat items bought by butchers' shops usually come from local (Tuscany) or national breedings, while meat items sold by supermarkets usually derive from European countries, particularly from Italy, Germany, The Netherlands, and France.

Of the 176 meat samples that were analysed microbiologically: 42 came from poultry, 68 from beef and 66 from pork meat.

S. aureus isolated from Baird Parker agar (Oxoid, Milan, Italy), was confirmed by agglutination test (Staphylase, test, Oxoid, Milan, Italy), catalase test and biochemical tests (Api Staph, bioMérieux, Rome, Italy).

All isolates were tested against 12 antimicrobial agents: cefalotine, oxacillin, ampicillin, tetracycline, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, gentamicin (Polibiodisc, Biolife, Italy), methicillin, teicoplanin, penicillin G, vancomycin (Oxoid, Milan, Italy).

Antimicrobial sensitivity was monitored with the disk diffusion assay (Kirby–Bauer) recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2000) on Muller Hinton agar (Oxoid, Milan, Italy); the zone of inhibition was interpreted according to NCCLS guidelines (Bauer, Kirby, Sherris, & Turck, 1966; NCCLS, 2000).

Reference strain used for quality control was *S. aureus* ATCC 25923 (Culti-Loops, Oxoid, Milan, Italy).

3. Statistics

Statistical analysis were done by StataCorp. 2003. Stata Statistical Software: Release 8.0. College Station, TX: Stata Corporation.

4. Results

During the one-year survey, we analysed 176 samples of raw meat purchased by some retailers in the Florence area.

Table 1
Antimicrobial sensitivity of *S. aureus* isolates ($n = 42$)

Agent	Resistance								Fisher's exact test
	Poultry (12)		Pork (10)		Beef (20)		Total (42)		
	n	(%)	n	(%)	n	(%)	n	(%)	
Cefalotine	0	0	1	10.00	0	0	1	2.38	0.238
Oxacillin	8	66.66	1	10.00	6	30.00	15	35.71	0.018
Ampicillin	7	58.33	2	20.00	9	45.00	18	42.86	0.204
Tetracycline	1	8.33	2	20.00	5	25.00	8	19.04	0.529
Trimethoprim-sulphamethoxazole	1	8.33	1	10.00	0	0	2	4.76	0.268
Eritromycin	1	8.33	2	20.00	5	25.00	8	19.04	0.529
Clindamycin	1	8.33	3	30.00	5	25.00	9	21.43	0.391
Gentamicin	2	16.66	1	10.00	1	5.00	4	9.52	0.674
Methicillin	0	0	0	0	0	0	0	0	1
Teicoplanin	0	0	0	0	0	0	0	0	1
Penicillin G	3	25.00	2	20.00	2	10.00	7	16.66	0.566
Vancomycin	0	0	0	0	0	0	0	0	1

S. aureus was present in 42 of the 176 samples (23.86%). Among them 12 (28.57%) were from poultry, 20 (29.41%) from beef, and 10 (15.15%) from pork meat.

All strains, tested for antibiotic sensitivity, were sensitive to methicillin, teicoplanin and vancomycin, and only one was resistant to cephalotine.

After the comparison of inhibition diameters and the relative frequencies, we could assess that the investigated strains had different patterns of antibiotic-resistance from those *S. aureus* isolated from nasal carriages or nosocomial infections. Table 1 shows the results of the antibiograms of all 42 *S. aureus* extracts; 13 strains (30.95%) were sensitive to all the tested antibiotics, 1 strain (2.38%) was sensible to all antibiotics except of oxacillin with an intermediate value, and 28 (66.67%) strains showed resistance to at least one antimicrobial substance.

We found similar resistance rates to those obtained in another study made in Tuscany (Italy) (Zanelli et al., 2002) from human nasal carriages only for a few antibiotics: the percentage of *S. aureus* resistant to gentamicin was 9.52% against a value of 13.2% coming from clinical samples, and no vancomycin-, teicoplanin-*o* methicillin-resistance was found. For the other tested antibiotics no similarity was found. Instead, we found a much stronger resistance against the other drugs, except for penicillin, where our results show lower levels. We found differences in antibiotic sensitivity also in another study made in Switzerland, where antibiotic sensitivity was studied in *S. aureus* isolated from food (Diekema et al., 2001; Valsangiacomo et al., 2000). Our strains present higher resistance levels for all the tested antimicrobial substances, except for ampicillin and penicillin G. Resistances were similar (Fisher's exact test > 0.05) in the three kind of meat except for oxacillin, where resistance was seen more frequently in isolates from poultry as in isolates from pork and beef (Fisher's exact test $= 0.018$).

Although MRSA strain has always shown multiresistance (Salmenlinna & Vuopio-Varkila, 2001; Joo Lee et al., 2001), we found multiresistance also in our strains

of *S. aureus* methicillin sensible (MSSA). Only 11 (26.19%) of all the tested strains showed single resistance: 6 of them (14.28%) resistant to oxacillin, 3 (7.14%) to ampicillin, 1 (2.38%) to clindamycin, and 1 to penicillin G. Double resistances were present in 4 (9.52%) of the total strains isolated; the other 13 strains (30.95%) show multiple ($\geqslant 3$ antibiotics) resistances, 6 of them (14.28%) presented triple resistances, 3 (7.14%) quadruple resistances, 1 quintuple resistances and 3 sextuple resistances.

5. Discussion

Meat is an important vector for the transfer of antibiotic resistances from animals to humans, and antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments. Such transfer can occur in three ways: by means of antibiotic residues in food, through the transfer of resistant food borne pathogens, or through the ingestion of resistant parts of the original food micro flora and resistance transfer to pathogenic microorganisms (Klein, 1999; Kruse & Sorum, 1994; Mayrhofer, Paulsen, Smulders, & Hilbert, 2004; Teuber, 1999).

The great use of avoparcin as a feed additive, at least in the last decade in Europe, in poultry and pork meat factories, has induced the occurrence of vancomycin-resistant enterococci and the potential transmission of this resistance to *S. aureus*. Today all European meat producers and breeders must comply with European directives that regulate the limits and kinds of additives and antimicrobial growth promoters admitted for breeding.

In our study, we analysed the major meat species (beef, poultry and pork) for the occurrence of *S. aureus* and antimicrobial resistance profile of isolates. The results showed a large presence of multiresistant *S. aureus* in food, with risks of infections and possibility of transmission of resistances to other bacteria. Indeed, these bacteria can reach immunocompromised people through food or a human carrier, cause infections and lead to failure of antibiotic therapies. Our study also showed that all *S. aureus* isolated

from food were sensitive to methicillin, vancomycin and teicoplanin. This fact demonstrates that these kinds of resistance are, actually, limited to bacteria in hospitals, that represent important reservoirs for multiresistent bacteria.

Resistance to ampicillin was high in all isolates (42.86%, Fisher's exact test = 0.204) in accordance with natural resistance for β lactams of staphylococcus induced by exposure to pennicillins (Fish, Piscitelli, & Danziger, 1995). Resistance to oxacillin was extremely high in poultry meat probably also because of the extended utilize of avoparcin used as an aid in the prevention of necrotic enteridis and as growth promoter in broiler chickens (Cormican, Erwin, & Jones, 1997).

It is to notice that 30.95% of all isolates were multiresistent (resistant at least to three antibiotics) and although we did not find any methicillin, or teicoplanin or vancomycin resistant we hypotize that it is necessary an accurate and active control against antibiotic resistances in food particularly on meat, community and in nosocomial infections, in order to detect any expansion and/or increment of new antibiotic resistance that can be transferred to other bacteria. Moreover, the microbiological safety of food has to be guaranteed in order to prevent the transmission of pathogen or opportunist microorganisms to the consumer, according to the hazard analysis and critical control point (HACCP) principles in food chain.

Acknowledgement

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