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A Survey of Antibiotic Resistance in *Micrococcaceae* Isolated from Italian Dry Fermented Sausages

FAUSTO GARDINI,[1]* ROSANNA TOFALO,[2] AND GIOVANNA SUZZI[3]

[1]*Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Via Fanin 46, 40127 Bologna, Italy;* [2]*Dipartimento di Biologia, Difesa e Biotecnologie Agroforestali, Università degli Studi della Basilicata, Contrada Macchia Romana, 85100 Potenza, Italy; and* [3]*Dipartimento di Scienze degli Alimenti, Università degli Studi di Teramo, Mosciano Sant'Angelo, Teramo, Italy*

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ABSTRACT

The transfer of bacteria that are resistant to antimicrobial agents or resistance genes from animals to humans via the food chain is increasingly a problem. Therefore, it is important to determine the species and the numbers of bacteria involved in this phenomenon. For this purpose, 148 strains of microstaphylococci were isolated from three types of Italian dry fermented sausages. Eight of 148 strains belonged to the genera *Kocuria* and *Micrococcus*. The remaining 140 strains belonged to 11 different species of the genus *Staphylococcus*. The species most frequently isolated was *Staphylococcus xylosus,* followed by *Staphylococcus saprophyticus* and *Staphylococcus aureus*. Antibiotic resistance levels differed among the species and depended on the strain origin. Microstaphylococci were generally susceptible to β-lactams, but 12 strains were resistant to methicillin, 8 were resistant to oxacillin, and 9 were resistant to penicillin G. No resistance was observed for aminoglicosides and cephalosporines. Many strains were resistant to sulfonamide, colistin suphate, tetracyclin, and bacitracin. Two strains of *S. aureus,* four strains of *S. xylosus,* and one strain of *Staphylococcus sciuri* were able to grow in the presence of 8 μg of vancomycin per g, but all strains were susceptible to teicoplanin. Twenty-two microstaphylococci were resistant to at least five of the tested antibiotics. The multiresistant strain *S. aureus* 899 was unaffected by eight antibiotics, including vancomycin and methicillin, indicating that a more prudent use of antibiotics in animal husbandry and better hygienic conditions during production should be encouraged because they can play a major role in reducing the incidence of such multiresistant microorganisms and the possible spread of the genetic elements of their resistance.

Microstaphylococci are a major component of the secondary microflora of dry fermented sausages. They are involved in desirable reactions occurring during the ripening of dry fermented sausages that contribute to the formation of flavor and aroma *(5, 27, 28, 39),* as well as color stabilization, the decomposition of peroxides *(3),* nitrate reductase activity *(9),* and proteolytic and lipolytic activity *(26).* Moreover, some microstaphylococci can reduce the presence of biogenic amines, hazardous compounds that can be accumulated in fermented sausages by other microorganisms *(8, 21, 24).* All of these processes can take place in sausages because of some intrinsic characteristics of microstaphylococci, with many species being halotolerant and psychrotrophic. These processes have often been attributed mainly to strains belonging to the coagulase-negative species (particularly *Staphylococcus xylosus* and *Staphylococcus carnosus*), which are often used as starter cultures *(9, 33).* Some members of the genus *Staphylococcus* are important human pathogens (*S. aureus*), and some coagulase-negative species (*S. epidermidis, S. saprophyticus,* and *S. haemolyticus*) are opportunistic pathogens *(22).* There is growing interest in some staphylococci, particularly *S. aureus* and *S. epidermidis,* because they are the most important pathogens involved in nosocomial infections owing to their antibiotic resistance. This important characteristic is obtained through the production of an extracellular slime matrix that decreases the efficiency of antibiotics or through the acquisition of resistance to multiple antibiotics *(13).* More than 50% of *S. aureus* strains isolated from nosocomial infections in the United States are resistant to all commonly used antibiotics, leaving only vancomycin as the antibiotic of last resort *(14).* However, methicillin-resistant *S. aureus* strains with intermediate vancomycin resistance have already been isolated *(12, 13).*

Antimicrobial agents are used in animal husbandry both for therapeutic reasons and as growth promoters. Nevertheless, the administration of antibiotics to animals with these different purposes can select antibiotic-resistant species, depending on the spectrum of activity of the antimicrobial agents *(29, 40).* As a consequence, an emerging reservoir of antibiotic-resistant microbes can occupy the niches of antibiotic-sensitive species or spread resistance genes to other microorganisms via the horizontally mobile genetic elements, such as viruses, plasmids, and transposons *(11).* Because most genes for antibiotic resistance can be exchanged or transferred among different staphylococcal species *(2),* increasing attention has been paid to the antibiotic resistance of microstaphylococci found in fermented sausages *(25, 29, 34).*

In this paper, the antibiotic resistance of microstaphylococci isolated from Italian dry fermented sausages is described. The effects of antibiotics were analyzed with re-

* Author for correspondence. Tel: +390512096575; Fax: +390512096574; E-mail: fgardini@kaiser.alma.unibo.it.

TABLE 1. *Numbers of strains of various species isolated from different types of dry fermented sausages*[a]

Species	No. of strains isolated from type of sausage: Salsiccia (30 samples)	Soppressata (18 samples)	Milan-type salami (5 samples)	Total no. of strains
Kocuria kristinae	2	1	—	3 (2.0)
Kocuria spp.	2	—	—	2 (1.3)
Micrococcus luteus	2	1	—	3 (2.0)
Staphylococcus aureus	7	5	2	14 (9.5)
S. carnosus	4	1	—	5 (3.4)
S. caseolyticus	7	3	—	10 (6.7)
S. epidermidis	—	2	1	3 (2.0)
S. hominis	1	1	—	2 (1.4)
S. intermedius	8	2	2	12 (8.1)
S. saprophyticus	7	12	6	25 (16.9)
S. sciuri	2	1	—	3 (2.0)
S. simulans	2	—	—	2 (1.3)
S. warneri	—	2	1	3 (2.0)
S. xylosus	36	23	2	61 (41.2)
Total	80 (54.0)	54 (36.5)	14 (9.5)	148

[a] —, no strains isolated. Percentages of the total number of strains are given in parentheses.

spect to species and isolation source. Moreover, multiresistant strains (i.e., strains resistant to more than five antibiotics) were further investigated.

MATERIALS AND METHODS

Isolation and identification of microstaphylococci. Microstaphylococci were isolated from samples of fermented sausages (Soppressata and Salsiccia) produced in the Basilicata region (southern Italy) and from Milan-type salami. Salsiccia is produced by casing a mix of pork meat (usually shoulder meat and a low proportion of bacon), NaCl (2 to 4%, wt/wt), and spices (dill seed or red pepper flakes) in 20- to 25-mm natural casings; this process is followed by drying (24°C at 80% relative humidity for 24 h, 18 to 22°C at 75 to 80% relative humidity for 5 days) and ripening (15 to 20°C at 80 to 85% relative humidity for 20 to 25 days). Soppressata is produced by casing a mix of lean pork meat (usually ham), lard (2 to 3%, wt/wt), NaCl (2 to 4%, wt/wt), and spices (black pepper grains) in 45- to 60-mm natural casings; this process is followed by drying and ripening (thermohygrometric conditions are similar to those used for Salsiccia ripening) for up to 40 days. In industrial plants, starter cultures, sugars (sucrose and/or lactose [0.2 to 0.6%, wt/vol]), potassium nitrate and nitrite (50 to 150 mg/kg), and ascorbic acid (0.08%, wt/wt) are used, and ripening is carried out in rooms in which temperature, air speed, and relative humidity are controlled. In artisanal plants, ripening is carried out in cellars in which the control of temperature and relative humidity is poor. Milan-type salami is made with 33% beef meat, 33% lean pork meat, and 33% pork lard. Usually, starter cultures, sugars, nitrates, and nitrites and salt (3.5%) are added. All of these sausages are self-stable.

TABLE 2. *Numbers of strains of various species isolated from Salsiccia and Soppressata at different ripening times*[a]

Species	No. of strains isolated at ripening time: 1–5 days	6–14 days	15–21 days	>21 days
Kocuria kristinae	3	—	—	—
Kocuria spp.	—	—	2	—
Micrococcus luteus	3	—	—	—
Staphylococcus aureus	5	3	1	3
S. carnosus	1	2	1	1
S. caseolyticus	4	3	2	1
S. epidermidis	2	—	—	—
S. hominis	—	2	—	—
S. intermedius	3	3	4	—
S. saprophyticus	18	1	—	—
S. sciuri	—	1	—	2
S. simulans	1	—	1	1
S. warneri	1	—	—	—
S. xylosus	16	19	17	7
Total	57 (38.5)	34 (23.0)	28 (19.0)	15 (10.1)

[a] —, no strains isolated. Percentages of the total number of strains are given in parentheses. Strains isolated from Milan-type salami are not reported in this table.

Samples of Salsiccia and Soppressata were collected at different times during the ripening of six different batches obtained by both industrial and artisanal procedures. The Milan-type salami was purchased at the market. Thirty Salsiccia samples, 18 Soppressata samples, and 5 Milan-type salami samples were analyzed. For microbiological analysis, the casing was aseptically removed and 10-g samples were homogenized in 90 ml of sterile diluent (0.1% bacteriological peptone, 0.85% NaCl) with a Stomacher 400 Laboratory blender (Seward Medical, London, UK). Tenfold serial dilutions were prepared with the same diluent. Microstaphylococci were enumerated on Baird Parker medium supplemented with egg yolk tellurite (Oxoid, Basingstoke, UK) incubated at 36°C for 36 h. All colonies were enumerated, and colonies with the typical *S. aureus* morphology were tested on Baird Parker medium with rabbit plasma and bovine fibrinogen supplement (Oxoid) and were then incubated at 37°C for 24 h to test the production of coagulase.

TABLE 3. *Number of strains of each species of microstaphylococci that were resistant to the antibiotic tested*[a]

Species	*n*	No. of strains resistant to antibiotic: AMP	MET	OXA	PEN	GEN	VAN	BAC	SUL	TRI	ACP	COL	TET	CLI	ERY	Mean[b]
Kocuria kristinae	3	—	1	1	—	—	—	—	3	—	3	3	1	—	—	4.00
Kocuria spp.	2	—	—	—	—	—	—	—	2	—	2	2	—	—	—	3.00
Micrococcus luteus	3	—	1	1	—	1	—	—	3	—	3	3	1	1	—	4.67
Staphylococcus aureus	14	—	1	—	5	—	2	1	10	2	9	7	4	—	—	2.93
S. carnosus	5	—	—	—	—	—	—	1	5	—	5	5	2	—	—	3.60
S. caseolyticus	10	—	—	—	—	—	—	1	7	1	6	4	1	–	—	2.00
S. epidermidis	3	1	—	—	—	—	—	1	3	–	3	3	3	—	—	4.67
S. hominis	2	—	—	—	—	—	—	—	2	—	2	2	—	—	—	3.00
S. intermedius	12	—	—	—	2	—	—	1	11	2	9	9	5	—	1	3.33
S. saprophyticus	25	—	2	4	—	1	—	4	24	—	24	18	8	1	—	3.44
S. sciuri	3	—	1	—	—	—	1	—	3	—	3	1	1	—	—	3.33
S. simulans	2	—	—	—	—	—	—	—	1	—	1	1	—	—	—	1.50
S. warneri	3	—	—	—	—	—	—	1	3	—	3	2	3	—	—	4.00
S. xylosus	61	—	6	2	2	—	4	5	46	3	47	30	11	2	2	2.62
Total	148	1 (0.7)	12 (8.11)	8 (5.41)	9 (6.1)	2 (1.4)	7 (4.7)	15 (10.1)	123 (83.1)	8 (5.4)	120 (81.1)	90 (60.8)	40 (27.0)	4 (2.7)	3 (2.0)	2.99

[a] No resistance was observed for amoxicillin, netilmicin, tobramycin, teicoplanin, piperidimic acid, cefoxitin, cephalothin, cefoperazone, cefuroxin, or nitrofurantoin. *n*, number of strains; AMP, ampicillin; MET, methicillin; OXA, oxacillin; PEN, penicillin G; GEN, gentamicin; VAN, vancomycin; BAC, bacitracin; SUL, sulfonamide; TRI, co-trimoxazole; ACP, piperidimic acid; COL, colistin sulphate; TET, tetracycline; CLI, clyndamycin; ERY, erythromycin. —, no strains were resistant. Percentages of the total number of strains are given in parentheses.

[b] Mean number of antibiotics to which strains were resistant.

TABLE 4. *Number of strains isolated from each type of sausage that were resistant to the antibiotic tested*[a]

Sausage	*n*	No. of strains resistant to antibiotic:													Mean[b]
		AMP	MET	OXA	PEN	GEN	VAN	BAC	SUL	TRI	ACP	COL	TET	CLI	ERY	
Salsiccia	80	—	5	4	3	1	4	6	64	4	61	44	11	3	2	2.65
Soppressata	54	1	7	4	5	1	3	7	45	4	45	33	19	1	1	3.26
Milan-type salami	14	—	—	—	1	—	—	2	14	—	14	13	10	—	—	3.86
Total	148	1 (0.7)	12 (8.1)	8 (5.4)	9 (6.1)	2 (1.4)	7 (4.7)	15 (10.1)	123 (83.1)	8 (5.4)	120 (81.1)	90 (60.8)	40 (27.0)	4 (2.7)	3 (2.0)	2.99

[a] No resistance was observed for amoxicillin, netilmicin, tobramycin, teicoplanin, piperidimic acid, cefoxitin, cephalothin, cefoperazone, cefuroxin, or nitrofurantoin. *n*, number of strains; AMP, ampicillin; MET, methicillin; OXA, oxacillin; PEN, penicillin G; GEN, gentamicin; VAN, vancomycin; BAC, bacitracin; SUL, sulfonamide; TRI, co-trimoxazole; ACP, piperidimic acid; COL, colistin sulphate; TET, tetracycline; CLI, clyndamycin; ERY, erythromycin. —, no strains were resistant. Percentages of the total number of strains are given in parentheses.

[b] Mean number of antibiotics to which strains were resistant.

For each sample, several colonies were randomly isolated with the use of a Harrison disk *(10).* The colonies were purified on tryptone soya broth (Oxoid), incubated at 37°C for 24 h, checked for morphology, Gram straining, and the presence of catalase, and identified by physiological tests *(18, 19).* Colonies were identified on the basis of physiological characteristics ascertained by the amplification of the 16S–23S rDNA intergenic region with universal primers *(37).*

Antibiotic resistance. Antibiotic resistance was tested by the agar diffusion method *(4)* according to the guidelines of the NCCLS *(30).* All experiments were carried out at least in duplicate. The strains were tested for their resistance to 24 antibiotics, namely, amoxicillin (10 ppm), ampicillin (10 ppm), methicillin (10 ppm), oxacillin (1 ppm), penicillin G (10 IU), netilmicin (30 ppm), tobramycin (10 ppm), gentamicin (10 ppm), co-trimoxazole (25 ppm), sulfonamide (300 ppm), cefoxitin (30 ppm), cefoperazone (30 ppm), cefuroxine (30 ppm), piperacillin (100 ppm), piperidimic acid (20 ppm), bacitracin (10 ppm), colistin sulphate (10 ppm), nitrofurantoin (300 ppm), tetracycline (30 ppm), clindamycin (2 ppm), erythromycin (15 ppm), teicoplanin (30 ppm), and vancomycin (8 ppm). Resistance was tested on plates of Muller-Hinton agar (Oxoid) supplemented with antibiotic disks (Susceptibility Discs, Oxoid). According to the procedure of the NCCLS, control strains (*Escherichia coli* ATCC 25922, *S. aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27893) were used to ensure the precision and accuracy of the test for the antibiotics. The resistance or susceptibility of other antibiotics was determined according to the directions of the manufacturer of Susceptibility Discs (Oxoid).

RESULTS

Isolation of microstaphylococci from dry fermented sausages. One hundred forty-eight strains of microstaphylococci were isolated from three types of dry fermented sausages. The first two products were sausages from the Basilicata region (southern Italy), namely, Salsiccia and Soppressata, and 80 and 54 strains, respectively, were isolated from these two products. Samples of these sausages were collected from various producers (including both artisanal and industrial producers) at various ripening times. Artisanal sausages differed from industrial ones owing to the smaller scale of production, the absence of starter cultures and sugar, and the less strictly controlled ripening conditions. The third type of product from which microstaphylococci (14 strains) were isolated was Milan-type salami obtained by industrial processes and purchased at the market. The initial number of microstaphylococci depended on the use of specific starter cultures. In any case, their numbers during ripening always reached values of 10^7 to 10^8 CFU/g.

Eight of 148 strains belonged to the genera *Kocuria* and *Micrococcus,* while the remaining 140 belonged to 11 different species of the genus *Staphylococcus.* In Table 1, the total numbers and the relative percentages of strains of the different species are reported, along with numbers of strains isolated from the different sausages analyzed. The species most frequently isolated was *S. xylosus,* which accounted for 41.2% of the strains (61 strains); the incidence of *S. xylosus* was high in Salsiccia and Soppressata, while only two strains of this species (14.2%) were isolated from

TABLE 5. *Numbers of antibiotics to which strains of each species were resistant*

Species	No. of strains resistant to[a]: 0–2 antibiotics	3–4 antibiotics	>5 antibiotics
Kocuria kristinae	—	2	1
Kocuria spp.	—	2	—
Micrococcus luteus	—	2	1
Staphylococcus aureus	5	7	2
S. carnosus	—	5	—
S. caseolyticus	6	3	1
S. epidermidis	—	1	2
S. hominis	—	2	—
S. intermedius	3	6	3
S. saprophyticus	5	16	4
S. sciuri	—	3	—
S. simulans	1	1	—
S. warneri	—	2	1
S. xylosus	27	27	7
Total	47 (31.8)	79 (53.4)	22 (14.9)

[a] —, no strains were resistant. The percentage of the total number of strains is given in parentheses.

Milan-type salami. *S. saprophyticus* was the second most frequently isolated species (16.9%), with a consistent presence in Soppressata (accounting for 22.2% of the strains isolated) and Milan-type salami (accounting for 42.8% of the strains isolated). *S. aureus* strains accounted for 9.5% of the isolates and were found at similar levels in all of the types of sausages considered. In contrast, *Staphylococcus intermedius* was found predominantly in Salsiccia (8 of 12 strains).

The distribution of different species during ripening is shown in Table 2. The 14 isolates from Milan-type salami are not included in the table because for this commercial product it was impossible to define the ripening time. *S. saprophyticus* was found almost exclusively in the first days of ripening, and in this phase it was the most frequently isolated species (18 of 57 strains, 31.6%). In these first days of ripening, *S. xylosus* accounted for 28.0% of the isolates. *S. aureus* was isolated in relatively constant proportions throughout ripening.

Resistance to antibiotics in relation to species. The strains were tested for their resistance to 24 antibiotics, namely, amoxicillin, ampicillin, methicillin, oxacillin, penicillin G, netilmicin, tobramycin, gentamicin, co-trimoxazole, sulfonamide, cefoxitin, cefoperazone, cephalothin, cefuroxin, piperacillin, piperidimic acid, bacitracin, colistin sulphate, nitrofurantoin, tetracycline, clindamycin, erythromycin, teicoplanin, and vancomycin. Table 3 shows the numbers and the percentages of the isolates of each species that are resistant to these antibiotics.

All of the species were rather susceptible to the β-lactams tested in this experiment (amoxicillin, ampicillin, methicillin, oxacillin, and penicillin G). None of strains grew in the presence of 10 ppm of amoxicillin, and only a strain of *S. epidermidis* grew when 10 ppm of ampicillin was added to the medium. Eight strains were resistant to oxacillin (ppm), including four strains of *S. saprophyticus* and two strains of *S. xylosus.* Five of 14 strains of *S. aureus,* along with 2 strains of *S. intermedius* and 2 strains of *S. xylosus,* were resistant to penicillin G. In addition, 12 strains showed resistance to 10 ppm of methicillin (at a concentration of >10 ppm); 6 of these strains belonged to the species *S. xylosus,* but a strain of *S. aureus* was also resistant to this antibiotic.

The strains tested were also highly susceptible to aminoglicosides: only two strains of *Micrococcus luteus* and one strain of *S. saprophyticus* were resistant to 10 ppm of gentamicin, while no strain was able to grow in the presence of 30 ppm of netilmicin or 1 ppm of tobramycin. About 10% of the strains were resistant to 10 ppm of bacitracin (including four strains of *S. saprophyticus* and five strains of *S. xylosus*).

More than 80% of the strains were resistant to sulfonamide (300 ppm), whereas 5% of the strains were resistant to co-trimoxazole (25 ppm). An analogous situation was observed for quinolones: no strain was resistant to piperacillin (100 ppm), and 81% of the strains could grow in the presence of 20 ppm of piperidimic acid.

No resistance to any of the four cephalosporins tested or to nitrofurantoin was observed, while a few strains were resistant to erythromycin (two strains of *S. xylosus* and one strain of *S. intermedius*) and clindamycin (two strains of *S. xylosus,* one strain of *S. intermedius,* and one strain of *M. luteus*). The strains belonging to the genera *Micrococcus* and *Kocuria* and to the species *Staphylococcus hominis* and *S. epidermidis,* as well as 50% of the *S. aureus* and *S. xylosus* strains, grew in the presence of colistin sulphate (10.0 ppm).

Considerable differences between the species with respect to susceptibility to tetracycline were found. About 30% of the 148 strains tested showed resistance to 30 ppm of this antibiotic, but all of the strains belonging to the species *Staphylococcus warneri* and *S. epidermidis* grew in the presence of tetracycline. The resistance of the *S. aureus, S. carnosus,* and *S. saprophyticus* strains reflected the overall mean, while *S. xylosus* was more susceptible (18% resistance).

With regard to glycopeptides, no strain was found to be resistant to teicoplanin, but two strains of *S. aureus,* one strain of *S. sciuri,* and four strains of *S. xylosus* were not inhibited by 8 ppm of vancomycin.

Resistance to antibiotics in relation to the isolation source. Table 4 shows the antibiotic resistance of microstaphylococci in relation to their isolation sources. Resistance to a mean of 2.65 antibiotics characterized the strains isolated from Salsiccia; strains isolated from Soppressata and Milan-type salami were resistant to means of 3.26 and 3.86 antibiotics, respectively. No resistance to methicillin was observed for strains isolated from Milan-type salami, while 13.0% of the strains isolated from Soppressata and 6.3% of the strains isolated from Salsiccia grew in the presence of this antibiotic; an analogous situation was observed

TABLE 6. *Antibiograms of the multiresistant strains isolated*

Species	Sausage[a]	Time[b]	Resistance of strain to antibiotic[c]: AMO	AMP	MET	OXA	PEN	NET	TOB	GEN	TEI	VAN
Kocuria kristinae 61	A	1–5	—	—	R	R	—	—	—	—	—	—
Staphylococcus caseolitycus 455	A	1–5	—	—	—	—	—	—	—	—	—	—
S. intermedius 748	A	6–14	—	—	—	—	—	—	—	—	—	—
S. intermedius 760	A	15–21	—	—	—	—	—	—	—	—	—	—
S. xylosus 53	A	1–5	—	—	—	—	—	—	—	—	—	R
S. xylosus 811	A	15–21	—	—	—	—	—	—	—	—	—	—
S. epidermidis 36	B	1–5	—	R	—	—	—	—	—	—	—	—
S. epidermidis 38	B	1–5	—	—	—	—	—	—	—	—	—	—
S. saprophyticus 75	B	1–5	—	—	R	—	—	—	—	—	—	—
S. warneri 910	B	>21	—	—	—	—	—	—	—	—	—	—
S. xylosus 83	B	1–5	—	—	—	—	—	—	—	—	—	R
S. xylosus 347	B	6–14	—	—	—	R	—	—	—	—	—	—
S. aureus 30	C	ND	—	—	—	—	R	—	—	—	—	—
S. saprophyticus 16	C	ND	—	—	—	—	—	—	—	—	—	—
S. saprophyticus 19	C	ND	—	—	—	—	—	—	—	—	—	—
S. xylosus 799	A	1–5	—	—	—	R	—	—	—	—	—	—
S. intermedius 435	B	6–14	—	—	—	—	R	—	—	—	—	—
S. xylosus 741	A	15–21	—	—	—	—	—	—	—	—	—	—
S. xylosus 880	B	1–5	—	—	—	—	R	—	—	—	—	R
Micrococcus luteus 207	A	1–5	—	—	R	R	—	—	—	R	—	—
S. aureus 899	B	1–5	—	—	R	—	R	—	—	—	—	R
S. saprophyticus 352	B	1–5	—	—	R	R	—	—	—	R	—	—
Total no. resistant			0	1	5	5	4	0	0	2	0	4

[a] A, Salsiccia; B, Soppressata; C, Milan-type salami.

[b] Days of ripening. ND, not determined.

[c] AMO, amoxicillin; AMP, ampicillin; MET, methicillin; OXA, oxacillin; PEN, penicillin G; NET, netilmicin; TOB, tobramycin; GEN, gentamicin; TEI, teicoplanin; VAN, vancomycin; BAC, bacitracin; SUL, sulfonamide; TRI, co-trimoxazole; PIP, piperacillin; ACP, piperidimic acid; CEF, cefoxitin; CEP, cefoperazone; CET, cephalothin; CEX, cefuroxin; COL, colistin sulphate; NIT, nitrofurantoine; TET, tetracycline; CLI, clyndamycin; ERY, erythromycin; —, susceptible; R, resistant.

[d] Total number of antibiotics to which strain is resistant.

for oxacillin. In contrast, the strains isolated from Milan-type salami were particularly resistant to colistin sulphate (92.8% of the strains were resistant), whereas <62% of the strains isolated from Salsiccia and Soppressata were resistant to this antibiotic. Moreover, 71.4% of microstaphylococci isolated from Milan-type salami were resistant to tetracycline, whereas 35.2% of the strains isolated from Soppressata and 13.8% of the strains isolated from Salsiccia were resistant to this antibiotic.

Resistance profiles of the microstaphylococcus strains. Table 5 shows the numbers of the antibiotics to which the strains of each species were resistant. Of the 148 strains tested, 47 (31.8%) were susceptible to less than two antibiotics (17 of these strains showed no resistance). More than half (53.38%) of the strains tested were resistant to three or four antibiotics, while 22 strains were resistant to five or more antibiotics. Seven of these multiresistant strains belonged to the species *S. xylosus,* four belonged to *S. saprophyticus,* three belonged to *S. intermedius,* two each belonged to *S. aureus* and *S. epidermidis,* and one each belonged to *S. warneri, Staphylococcus caseolyticus, Kocuria kristinae,* and *M. luteus*. The complete antibiograms of these multiresistant microorganisms are reported in Table 6. Nine of these 22 strains were isolated from Salsiccia, 9 were isolated from Soppressata, and 3 were isolated from Milan-type salami.

All of the multiresistant strains grew in the presence of 300 ppm of sulfonamide, 20 ppm of piperidimic acid, and 20 ppm of colistin sulphate. About 70% of the multiresistant strains were not inhibited by 30 ppm of tetracycline. Among these multiresistant strains, *S. aureus* 899, isolated from Soppressata in the first days of ripening, was resistant to both methicillin and vancomycin and was not inhibited by penicillin G, sulfonamide, piperidimic acid, colistin sulphate, or tetracycline, although it was susceptible to the other β-lactams tested (amoxicillin, oxacillin, and ampicillin). Combined resistance to vancomycin and methicillin was also observed for a strain isolated from Salsiccia and belonging to the species *S. sciuri*; this strain was also resistant to piperidimic acid and sulfonamide.

DISCUSSION

S. xylosus was the species most frequently isolated from the dry sausages examined. In other studies, the same species was also found to be dominant in Iberian ham *(36)* and in chorizo *(7). S. carnosus* was found in small numbers, confirming results obtained by Garcia-Varona et al. *(7).* Differences in the relative importances of some species de-

TABLE 6. *Extended*

Resistance of strain to antibiotics[c]:														
BAC	SUL	TRI	PIP	ACP	CEF	CEP	CET	CEX	COL	NIT	TET	CLI	ERY	Total[d]
—	R	—	—	R	—	—	—	—	R	—	—	—	—	5
R	R	R	—	R	—	—	—	—	R	—	—	—	—	5
—	R	R	—	R	—	—	—	—	R	—	R	—	—	5
R	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	R	—	—	5
R	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	R	—	—	5
R	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	R	—	—	5
R	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	R	—	—	5
R	R	—	—	R	—	—	—	—	R	—	R	—	—	5
R	R	—	—	R	—	—	—	—	R	—	R	—	—	5
—	R	—	—	R	—	—	—	—	R	—	—	R	R	6
—	R	R	—	R	—	—	—	—	R	—	—	—	R	6
R	R	R	—	R	—	—	—	—	R	—	R	—	R	7
—	R	R	—	R	—	—	—	—	R	—	R	—	—	7
—	R	—	—	R	—	—	—	—	R	—	R	R	—	8
—	R	R	—	R	—	—	—	—	R	—	R	—	—	8
R	R	—	—	R	—	—	—	—	R	—	R	R	—	9
9	22	6	0	22	0	0	0	0	22	0	18	3	3	

pending on the type of sausage and the ripening time have been observed. In particular, *S. saprophyticus* was isolated from Salsiccia and Soppressata, in which it was the most important species, only during the first days of ripening. This finding indicates that *S. saprophyticus* was the dominant species in the animals from which the meat was derived but was not competitive with other staphylococci during ripening.

Fermented sausages, representing complex natural microbial associations, can provide a suitable environment for the transfer of plasmids and transposons containing antibiotic resistance genes *(29).* Microstaphylococci can be a reservoir of resistance genes owing to the selection caused by antibiotic administration to animals whose skin is a natural habitat for these microorganisms. From there, microstaphylococci pass to the meat during slaughtering operations and are an important component of the wild microflora of the raw material used for sausage production *(31).*

The antibiotic resistance found in this work for *Kocuria* and *Micrococcus* confirms the observations of Martin and Maris *(23)* for gentamicin, oxacillin, ampicillin, and erythromycin. On the other hand, the same investigators found the resistance of these genera to tetracycline to be less pronounced.

In contrast to the observations of this study, Mauriello et al. *(25)* found all coagulase-negative staphylococci isolated from Naples-type salami to be sensitive to methicillin and vancomycin but found >7% to be resistant to teicoplanin. In the same study, marked resistance to penicillin G and amoxicillin (with >64% of the strains being resistant) was also observed. Similarly, the susceptibility observed by Mauriello et al. *(25)* for erythromycin and clindamycin was considerably more extensive than that observed in the present study, whereas levels of resistance to tetracycline were comparable. Other studies have reported extensive resistance (>95%) to penicillin G for *S. aureus (31),* and the *S. carnosus* strains tested by Holley and Blaszyk *(16)* were sensitive to penicillin, tetracycline, and streptomycin.

In a study involving *S. xylosus,* no strain was found to be resistant to more than five antibiotics *(29).* On the other hand, two staphylococcal strains (identified as *Staphylococcus simulans* and *Staphylococcus* spp.) isolated from Italian salami were resistant to 12 antibiotics, and 75% of the strains showed resistance to at least 5 antibiotics, leading to hypothesis that the strains were derived from environments in which antibiotics are often used *(25).*

Several clones of methicillin-resistant *S. aureus* have arisen and been disseminated in the last decades. These clones have been particularly successful in adapting to the increasing antibiotic pressure for both mutation and the acquisition of exogenous genes *(15).* This trend can be of particular concern with regard to public health when resistance to vancomycin, the antibiotic representing the last resort against methicillin-resistant strains of *S. aureus,* is acquired.

All the methicillin-resistant *S. aureus* strains with reduced susceptibility to vancomycin that have been identified to date have had different patterns of antibiotic susceptibility, suggesting that these strains are developing antibiotic resistance independently after exposure to vancomycin *(12).* For staphylococci, combined resistance to

methicillin and vancomycin was more frequent for clinical isolates *(6).* Nevertheless, it is important to highlight this study's finding of the presence of a strain of *S. aureus* that was resistant to these two antibiotics. In addition, the same strain was resistant to six of the other antibiotics. The emergence of methicillin-resistant *S. aureus* strains with reduced vancomycin susceptibility is an undesirable phenomenon that calls for appropriate measures to prevent the spread of such strains *(12).* In addition, the methicillin resistance of coagulase-negative staphylococci is controlled by the same mechanism found in *S. aureus* and is based on the presence of the *mecA* gene *(35).*

The emergence of vancomycin resistance in methicillin-resistant *S. aureus* strains can be due to several mechanisms. It has been experimentally established that the vancomycin-resistant genes *vanA* and *vanB* can be transferred to *S. aureus* via plasmid-mediated gene transmission by microorganisms that easily acquire resistance to this antibiotic *(20, 32).* In addition, it has been demonstrated that the acquisition of resistance can be obtained by a step pressure procedure *(1, 38).*

The presence of an *S. aureus* strain resistant to both vancomycin and methicillin could be probably attributed to cross-contamination with a strain of human origin. Nevertheless, the presence of such a strain, reinforced by the isolation of an *S. sciuri* strain with the same resistance to these two antibiotics, poses a risk because of the possible transmission of these characteristics to other strains constituting a potential reservoir of resistance genes in food environments. This kind of transmission from human to food strains has been hypothesized for methicillin resistance in chicken *(17).* This possibility is also of particular interest in light of the possibility that once microorganisms acquire such resistance, its loss, also in the absence of the selective factor, may be not be immediate and complete. As stated by Heinemann et al. *(11),* resistance genes may acquire new functions, and the initial costs of resistances can evolve into advantages; decreasing drug use might not be enough to discourage the evolution of sensible microorganisms. Although the transfer of bacteria and resistance genes from humans to animals generally occurs on limited scale, it can assume particular significance because of the potential for the amplification of organisms within the animal population *(40).* Moreover, sausage-processing plants and the final products can also provide environments suitable for amplification and become reservoirs of antibiotic-resistant microorganisms. In conclusion, the more prudent use of antibiotics in animal husbandry and the implementation of better hygienic conditions that avoid cross-contamination during production should be encouraged because they can play a major role in reducing the incidence of multiresistant microorganisms and the consequent possible spread of the genetic elements of their resistance.

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