Gazi University Journal of Science
GU J Sci
28(3):359-364 (2015)

Antibiotic Resistance and Slime Production in Coagulase-Negative *Staphylococcus* Strains from Retail Meat Samples

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Received: 27/02/2015 Revised: 09/04/2015 Accepted: 28/04/2015

ABSTRACT

In this study, a total of 100 retail meat samples were analysed for the presence of coagulase negative *Staphylococcus* (CoNS). A total of 208 CoNS strains were isolated from these samples. *Staphylococcus saprophyticus* was the most prevalent species. Resistance to methicillin was detected in 63.4% of CoNS isolates. Methicillin-resistant CoNS (MRCoNS) strains were determined to be more resistant to antibiotics than methicillin-susceptible CoNS strains. Resistance of methicillin was found in 76.1% of slime positive and in 57.4% of slime negative strains. Most of isolated slime producing CoNS were methicillin resistant, which makes the detection of these microorganisms necessary to prevent their dissemination in the environment, veterinary medicine and public health.

Key words: Antibiotic resistance, slime, CoNS, retail meat

1. INTRODUCTION

Coagulase-negative *Staphylococcus* (CoNS) are ubiquitous microorganisms and predominate in normal skin flora. They are commonly isolated in clinical specimens and several species are recognized as important agents of nosocomial infections, especially in neonates, immunocompromised individuals and patients with internal prosthetic devices where their pathogenic role is well established [1]. The origin of these infections can be endogenous or exogenous, coming from hospital environment or from personel hands. CoNS are also involved in animal diseases and many studies have shown that they are the bacteria most frequently recovered from infected bovine, caprine mammary glands and from a wide range of food stuffs such as meat, cheese and milk, and environmental sources such as soil, sand, air and water [2, 3]. *S. saprophyticus* was isolated as common contaminant of various food samples, especially of raw beef and pork [4]. Other CoNS species, *S. xylosus*, *S. sciuri and S. haemolyticus* were also reported in cow's beddings, nares, teat skin, chicken and environment. *S. warneri* and *S. epidermidis* have also been isolated from cow's skin.

CoNS infections are often diffucult to treat since CoNS strains of clinical origin are freuquently resistant to a wide variety of antibiotics [5]. Many CoNS isolates are resistant to penicillin and methicillin by beta-lactamase and PBP 2a production, respectively. These microorganisms can be reservoirs of antimicrobial resistance genes that could be transferred to pathogenic species such as *S. aureus*. It is suggested that the extensive use of antimicrobial agents in animal husbandry could have contribued to creating animal reservoirs of antibiotic-resistant microorganisms. From this reservoir, resistant strains or resistance genes may spread to humans through the food chain [6]. On the other hand, CoNS are characterized by their ability to adhere to and grow on solid surfaces and subsequently to produce polysaccharide slime. The extracellular

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slime may protect the bacteria against immunological host defense mechanisms and antimicrobial therapy. Thus, slime is considered an important virulence factor [7].

The purposes of the present study were 1) to determine the antibiotic resistance patterns 2) to evaluate the association between methicillin resistance and slime production of CoNS isolated from retail meat samples.

2. EXPERIMENTAL

2.1. Sample collection

A total of 100 retail meat samples (20 minced meat, 20 calf chunks, 20 drumsticks, 20 wings and 20 chicken breasts) were purchased from randomly selected different supermarkets in Ankara between May 2008 and January 2009. The retail meat samples (about 25g each) were collected in sterile polyethylene bags, transported on ice to the laboratory, and analyzed within 2 h.

2.2. Isolation and identification of CoNS species

Samples (25 g) were weighed into sterile stomacher bags, diluted with 225 ml of buffered sterile peptone water (BPW) (Oxoid, Basingstoke, Hampshire; Uk), and homogenized in a stomacher (Lab-Blender 400, PBI, Milan, Italy) for about 2 min, seeded onto Baird-Parker (BP) agar (Oxoid CM 275) supplemented with egg yolk-tellurite emulsion (Oxoid SR 54), and incubated at 37°C for 24h. From each plate, suspected colonies of CoNS with similar morphologies, were isolated and cultured separetely on slants of Brain-Heart Infusion (BHI, Oxoid CM 225). Preliminary identification of CoNS isolates was performed on the basis of colonial morphology, cultural characteristics on agar media, gram reaction or (gram staining), catalase and coagulase activity. The species of CoNS were identified using the BBL Crystal ™ GP Identification System, Gram-Positive ID kit (Beckton Dickinson), by following the manufacturer instructions. Pure cultures of the isolates were taken and cultured individually on slants of skimmed milk at -20°C.

2.3. Determination of slime production

Congo Red agar was prepared by adding to initially autoclaved (both from Sigma, Missouri, USA) 1 L of Brain Heart Infusion agar (Oxoid, Basingstoke, Hampshire, England). Plates were incubated for 24 h at 37°C. Slime-producing CoNS species grew as red colonies. The original test was optimized by using a colorimetric scale with six tonalities: very black, black and almost black were considered as positive results, while bordeaux, red and very red were considered as negative results.

2.4. Antimicrobial susceptibility test

CoNS species were analysed for antimicrobial resistance using the disc diffusion assay on Mueller Hinton agar (Oxoid CM337) plates according to the Clinical and Laboratory Standarts Institue Guidelines (CLSI, 2006). The antimicrobial susceptibility test disc were purchased from (Oxoid, Basingstoke, UK). The antimicrobials and the respective quantities (μg) per disk were as follows: ampicillin (10 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), penicilin (10 μg), cephalothin (30 μg), tetracyline (30 μg), trimethoprim-sulphamethoxazole (1.25/23.75 μg) and vancomycin (30 μg). The CLSI criteria were applied for the interpretation of the results (CLSI, 2006). The disks containing 1 μg oxacillin (Oxoid) (zone diameter $\geq$ 13 mm sensitive, $\leq$ 10 mm resistant) were used for resistance to methicillin. *Staphylococcus epidermidis* ATCC 35984 and *Staphylococcus aureus* ATCC 25923 were used as quality control organisms.

2.5. Statistical Analysis

SPSS 11.0 for Windows was used to compare the data, and chi-square or fisher's exact test was used.

3. RESULTS AND DISCUSSION

Meat is an important source of nutrient compounds for human health, and is consumed worldwide. Therefore, raw meat and ready-to-eat meat products are sold in the different market and consumed. A total of 100 retail meat samples were found highly contaminated with some CoNS species (Table 1). The predominant species identified were *S. saprophyticus* (n=76, 36.5%), *S. vitulinus* (n=63, 30.2%), *S. sciuri* (n=17, 8.2%), *S. xylosus* (n=16, 7.7%) and *S. lentus* (n= 13, 6.2%). This distribution of these species are in parallel with the literature information [8]. Likewise, *S. saprophyticus* (34%) was reported being the most common isolate in raw beef and pork samples by Raz *et al.*, [4]. *S. simulans*, *S. warneri*, *S. auricularis*, *S.cohnii ssp. cohnii*, *S. capitis*, *S. haemolyticus*, *S. epidermidis*, *S. hominis*, and *S. lugdunensis* were less frequent, although these strains have also been isolated from dairy and meat samples [9]. The reason for CoNS contamination of retail meat products could be poor personel hygiene of the food-workers especially hands, asymptomatic carriers suffering from infected skin lesions, the surfaces of equipments, cutting, storing, packing or unhygienic ways of selling in retail outlets.

Table 1. The distribution of CoNS species and their slime production in meat samples.

CoNS	n (%)	Slime positive n (%)
S. saprophyticus	76 (36.5)	39 (51.3)
S. vitulinus	63 (30.2)	11 (17.5)
S. sciuri	17 (8.2)	4 (23.5)
S. xylosus	16 (7.7)	1 (6.3)
S.lentus	13 (6.2)	4 (30.8)
S.simulans	5 (2.4)	4 (30.8)
S. warneri	5 (2.4)	2
S. auricularis	3 (1.4)	-
S. cohnii ssp. Cohnii	3 (1.4)	1
S. capitis	2 (0.9)	-
S. haemolyticus	2 (0.9)	-
S.epidermidis	1 (0.5)	1
S. hominis	1 (0.5)	-
S.ludgunensis	1 (0.5)	-
Total	208	

X^2=14.508, p=0.013 n: number of isolates

Antibiotics are used in medicine and veterinary medicine for different purposes such as growth promotion, prophylaxis, or therapeutics. However, their excesive use human therapy as well as in animal production and treatment has resulted in increased bacterial resistance to many antibiotics [10]. As a result, chicken and red meat can harbor antimicrobial-resistant strains that can be transmitted to humans. Several studies have reported the presence of antibiotic resistance genes in CoNS isolated from various foods [11, 12]. Bacterial β- lactam resistance mechanisms include production of β- lactamases and low-affinity penicilin-binding protein 2a (PBP2a). In 1996, methicillin resistant, CoNS were isolated from young and healthy chickens' nares and skin [12] and it may easily spread all methicillin-resistant CoNS, probably through transposons [13]. At present, more than 70% of the CoNS isolates worldwide are resistant to methicillin or oxacillin. In this study, 132 of 208 (63.4%) CoNS isolates were found to be methicillin resistance (Table 2). Similarly, Rather et al., [14] reported a 66,7% resistance rate among bovine strains to methicillin. Methicillin-resistant staphylococci are resistant to all other penicillins, carbapenems, cephems and beta-lactam/ beta-lactamase inhibitor combinations. Consequently, these antibiotics should not be used for treating of methicillin-resistant staphylococci infections [15]. Recently, Kanuer et al., [16] reported that the MRCoNS strains acquired in hospitals have become resistant to many widely used antimicrobial agents (aminoglycosides, tetracyline, quinole, macrolide group antibiotics). The resistance to penicillins (67,4%), ampicillin (44,6%) and tetracycline (59,8%) in the retail meat isolates (Table 2) are similar to those found by Turutoglu et al., [17] in Turkey. Antibiotic resistant strains are also found in foods and genes for microbial resistance to tetracycline and β-lactams have been detected in CoNS isolated from starter cultures, probiotic bacteria, fermented food and meat [18]. These results are expected, since β-lactam antibiotics and tetracycline are widely used in veterinery medicine to treat infections in animals in Turkey [19]. This indicates that the resistance pattern might originate from one source (organisms) and spread to other animals through close contact. A small percentage of MRCoNs isolates demonstrated resistance to erythromycin (12.8%), clindamycin (10.6%), gentamicine (0.7%) (Table 2). Clindamycin is used widely in veterinary medicine to treat a variety of bacterial infections including skin, wound and bone infections, pneumonia, oral cavity infections and may be considered as the choice of antibiotic for treatment when methicillin-resistnt *S. aureus* is isolated [20]. The absence of vancomycin resistant CoNS from retail meat samples in this study is consistent with previous studies [21]. Vancomycin resistance has emerged first in *Enterococcus* and, more recently in *S.aureus* and CoNS [22]. Vancomycin resistance has been associated with the clinical use of this antibiotic and animal use of avoparcin. However, a recent study suggets that the predominant phenotype (Van A) in both clinical (human and animal) and dairy isolates does not occur by clonal dissemination of resistant strains, but rather through the transfer of vanA gene by transposable elements, like Tn1546 [23].

Table 2. The antibiotic resistance of methicillin resistant (MR) and methicillin susceptible (MS) CoNS strains isolated from meat samples.

Antibiotics	MS-CoNS No =76 (36.5%)		MR- CoNS No=132(63.4%)	
	n	%	n	%
Ampicillin(AM)	5	6,5	59	44,6
Cephalothin(KF)	-	-	1	0,7
ClindamycinDA)	3	3,9	14	10,6
Erythromycin (E)	6	7,8	17	12,8
Gentamicin (CN)	-	-	1	0,7
Penicillin (P)	11	14,4	89	67,4
Tetracyline(TE)	32	42,1	79	59,8
Trimethoprin/sulfamethoxazole(SXT)	-	-	1	0,7
Vancomycin	-	-	-	-

X^2=18.377, p=0.01

Slime production and multiple antibiotic resistance play an important role in the pathogenesis of staphylococci infections. Multi-resistant CoNS may adhere to medical devices and food surfaces through secreted slime which has a mucopolysaccaride structure, and in this way, they may easily colonize and spread within hospital and veterinary environment. Moreover, biofilm production has been recently reported to be a marker of pathogenic potential for the staphylococci and multidrug-resistant staphylococci [24]. Furthermore, the slime factor protects the CoNS from antibiotics, phagocytosis and chemotaxis. Regulation of slime production and methicillin resistance may use similar pathways, as insertion of a certain transposon influences both slime production and the expression of methicillin resistance [25]. Although, Koksal et al. [26] reported that slime production and antibiotic resistance have been detected in CoNS isolated from clinical samples, there have been a few published reports of CoNS isolates, methicillin resistance and presence of slime from food products [3, 27] in Turkey. In our study, the slime positivity was higher (51.3%) in *S.saprophyticus* than the other CoNS species (17.5% in *S. vitulinus,* 23.5 % in *S. sciuri,* 30,8% in *S. lentus* and 6.3% in *S. xylosus*) (Table 1). Slime positivity was 67 (32.2%) in CoNS strains. 38.6% of which was in MR-CoNS, and 21% in MSCoNS (Table 3). This study showed that methicillin resistance was significantly higher in slime positive CoNS strains (76.2%) than in slime negative CoNS strains (57.4%). Similary, methicilline resistance was found significantly higher in slime positive isolates (81%) than in slime negative isolates (57%) among blood cultures of septicemic patients [26]. It was reported that slime producing strains are more resistant to antibiotics than non-slime producing strains [28] and there is a coorelation between slime production and multiple antibiotic resistance [29].

Table 3. Prevalance of slime production in MRCoNS and MSCoNS strains isolated from meat samples.

	MR		MS	
	n	%	n	%
Slime positive n=67 (32.2%)	51	76.1	16	23.9
Slime negative n=141 (67.8%)	81	57.4	60	42.5
Total n=208	132	63.5	76	36.5

Fisher's exact test, p=0.009

In conclusion, the results showed the presence of methicillin resistance and multiresistant CoNS species in retail meat samples, with risks of infections and possibility of transmission of resistances to other bacteria. Production of slime factor has important implications for the safety of retail meat for the consumers. Indeed, slime producer MRCoNS can reach to immunocompromised people through food or a human carrier, cause infections and lead to failure of antibiotic therapies. Personal hygiene during marketing is also very important since these bacteria could easily be transmitted to meat via the food handlers, salespersons and consumers. Therefore, hygienic measures for retail meat samples should be strongly enforced in retail supermarkets. Besides, proper cleaning and disinfection programs are needed in order to eliminate slime producing MRCoNS.

ACKNOWLEDGEMENTS

Financial support was provided by the Gazi University Research Fund Project No: 05/2008-51.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] Kogan, G., Sadovskaya, I., Chaignon, P., Chokr, A. and Jabbouri, S., "Clinical strains of *Staphylococcus* that do not contain polysaccharide intercellular adhesin", ***FEMS Microbiol Lett.***, 255: 11–16, (2006).

[2] Kloos, W.E. and Scheifer, K.H., Genus IV. *Staphylococcus*, Rosenbach 1884.In: Sneath, PHA, Mair NS, Sharpe ME, Holt JG.(Eds). Bergey's Manual of Systematic Bacteriology. Vol 2. Williams &Wilkins, Baltimore, 1013-1035, (1986).

[3] Citak, S. and Duman, T. "*Staphylococcus aureus* and coagulase-negative *Staphylococcus* from raw chicken samples in Turkey: Prevalence and antimicrobial resistance", ***J. Food Agriculture Environ.***, 9: 156-158, (2011).

[4] Raz, R., Colodner, R. and Kunin, C., "Who Are You—*Staphylococcus saprophyticus*?", ***Clin. Infect. Dis.***, 40: 896-898, (2005).

[5] Agvald-Ohman, C., Lund, B. and Edlund, C., "Multiresistant coagulase-negative Staphylococci disseminate frequently between incubated patients in a multidisciplinary intensive care unit", ***Crit. Care***, 8: 42-47, (2004).

[6] Swartz, M.N., "Human diseases caused by foodborne pathogens of animal origin", ***Clin. Infect. Dis.***, 34: 111-122, (2002).

[7] Gotz, F., "*Staphylococcus* and biofilms", ***Mol. Microbiol.***, 43:1367-1378, (2002).

[8] Moodley, A. and Guardabassi, L., "Clonal spread of methicillin-resistant coagulase-negative *Staphylococci* among horses, personel and environmental sites at eqine facilities", ***Vet. Microbiol.***, 137: 397- 401, (2009).

[9] Al-Tarazi, Y.H., Albetar, M.A. and Alaboudi, A.R., "Biotyping and enterotoxigenicity of Staphylococci isolated from fresh and frozen meat marketed in Jordan", ***Food Research Int.***, 42: 374-379, (2009).

[10] Mathew, A.G., Cissell, R. and Liamthong, S., "Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production", ***Foodborne Pathog. Dis.***, 4: 115–133, (2007).

[11] Perreten, V., Schwarz , F., Cresta, L., Boeglin, M., Dasen, G. and Teuber, M., "Antibiotic resistance spread in food", ***Nature***, 389: 801-802, (1997).

[12] Kawano, J., Shimizu, A., Saitoh, Y., Yagi, M., Saito, T. and Okamoto, R., "Isolation of methicillin-resistant coagulase-negative staphylococci from chickens", ***J. Clin. Microbiol.***, 34**:** 2072–2077, (1996).

[13] Maran ., "Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands in 2005", (2005). Available at: http://www.cidc-lelystad.wur.nl/UK/publications/

[14] Rather, P.N., Davis, A.P. and Wilkinson, B.J., "Slime production by bovine milk *Staphylococcus aureus* and identification of coagulase-negative staphylococcal isolates", ***J. Clin. Microbiol.***, 23: 858-862, (1986).

[15] Clinical and Laboratory Standart Institue (CLSI). Performance Standart for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document, M100-S16.vol.26-3; M7-A7, vol.26-2; M2-A9,vol. 26-1.Wayne, PA. USA, (2006).

[16] Knauer, A., Fladerer, P., Strempfl, C., Krause, R. and Wenisch, C., "Effect of hospitalization and antimicrobial therapy an antimicrobial resistance of colonizing *Staphylococcus epidermidis*", ***Wien Klin. Wochenschr***, 16: 489-494, (2004).

[17] Turutoglu, H., Ercelik, S. and Ozturk, D., "Antibiotic resistance of *Staphylococcus aureus* and coagulase- negative staphylococci isolated from bovine mastitis", ***Bull. Vet. Inst. Pulawy.***, 50: 41-45, (2006).

[18] Simeoni, D., Rizzotti, L., Cocconcelli, P., Gazzola, S., Dellaglio, F. and Torriani, S., "Antibiotic resistance genes and identification of staphylococci collected from the production chain of swine meat commodities", ***Food. Microbiol.***, 25: 196-201, (2008).

[19] Erginkaya, S. and Filazi, A., "Determination of antibiotic residues in milk samples", ***Kafkas University, The J. Vet. Faculty***, 16(Supp-A): 31-35, (2010).

[20] Rich, M., Deighton, L. and Roberts, L., "Clindamycin-resistance in methicillin-resistant *Staphylococcus aureus* isolated from animals", ***Vet. Microbiol.***, 111: 237-240, (2005).

[21] Virdis, S., Scarano, C., Cossu, F., Spanu, V., Spanu, C. and Luigi De Santis, E.P., "Antibiotic Resistance in *Staphylococcus aureus* and Coagulase Negative Staphylococci Isolated from Goats with Subclinical Mastitis", ***Vet. Med. Int.***, Article ID 517060, 1-6, (2010).

[22] Palazzo, I.C.V., Araujo, M.L.C. and Darini, A.L.C., "First report of vancomycin –resistant Staphylococci isolated from healty carriers in Brazil", ***J. Clin. Microbiol.***, 43: 179-185, (2005).

[23] Witte, W., Cuny, C., Klare, I., Nubel, U., Strommenger, B. and Werner, G., "Emergence and spread of antibiotic-resistant Gram positive bacterial pathogens", ***Int. Medical Microbiol.***, 298 (5-6): 365-377, (2008).

[24] Jain, A. and Agarwal, A., "Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci", ***J. Microbiology Met.***,76: *88-92*, (2009).

[25] Mack, K.D., Bartscht, S., Dobinsky, M.A., Horskotte, H., Kiel, K.M., Knobloch, A. and Schafer, P., Staphylococcal factors involved in adhesion and biofilm formation on biomaterials. In YH An, RJ Friedman (eds) Handbook of Bacterial Adhesion, Principles Methods and Application, Humana press, Totowa, NJ: 307-330, **(**2000).

[26] Koksal, F., Yasar, H., Samasti, M.,"Antibiotic resistance patterns of coagulase-negative *Staphylococcus* strains isolated from blood cultures of septicemic patients in Turkey", ***Microbiol. Research***, 16: 404-410, (2009).

[27] Citak, S., Varlik, O. and Gundogan, N., "Slime production and DNase activity of staphylococci isolated from raw milk", ***J. Food Safety***, 23: 281-288, (2003).

[28] Amorena, B., Gracia, E., Monzon, M., Leiva, J., Oteiza, C., Perez, M., Alabart, J.L. and Hernandez-Yago, J., "Antibiotic susceptibility assay for *Staphylococcus aureus* in biofilms developed in vitro", ***J. Antimicrob. Chemother***, 44: 43-55, (1999).

[29] Gunaydin, M., Leblebicioglu, H., Sanic, A. and Pirinciler, M., "Koagulaz negatif stafilokoklarda slime yapimi ve antibiyotik direnci ile ilişkisi", ***Microbiology Bulltein***, 105: 493-500, (1995).