www.czasopisma.pan.pl PAN www.journals.pan.pl

Polish Journal of Veterinary Sciences Vol. 15, No. 2 (2012), 329-335

DOI 10.2478/v10181-012-0051-1

Original article

Limited genetic diversity of *Aerococcus viridans* strains isolated from clinical and subclinical cases of bovine mastitis in Slovakia

T. Špaková[1], J. Elečko[2], M. Vasil[2], J. Legáth[3], P. Pristaš[4], P. Javorský[4]

[1] Faculty of Medicine, Pavol Jozef Šafárik University, Trieda SNP 1, 040 11 Košice, Slovakia
[2] Institute of Animal Husbandry, University of Veterinary Medicine, Komenského 73 041 81 Košice, Slovakia
[3] Department of Pharmacy, Pharmacology and Toxicology, University of Veterinary Medicine, Komenského 73, 041 81 Košice, Slovakia
[4] Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, 040 01 Košice, Slovakia

Abstract

The *Aerococcus viridans* isolates from bovine mastitis in Slovakia were isolated and characterized by classical microbiological and biochemical, and molecular techniques including IGS-PCR and rep-PCR, ARDRA and 16S rDNA gene sequencing. The substantial variability of antibiotic resistance patterns was observed. The majority of strains were resistant to beta-lactam antibiotics, the resistance to tetracycline was observed in 3 tested strains, resistance to lincomycin was found in 4 strains and practically all tested strains were sensitive to neomycin and ciprofloxacin. While variable at a phenotypic level, no significant genetic variability among *A. viridans* isolates was detected by molecular DNA based methods. The data obtained suggest that a few *A. viridans* strains spread among cow's population in Slovak farms.

Key words: *Aerococcus viridans*, genetic diversity, mastitis, raw milk

Introduction

Bovine mastitis is a widespread disease that affects dairy cows. It causes changes in glandular tissues as well as it affects the quality and quantity of milk. Bacteria involved in bovine mastitis are broadly classified as either contagious or environmental pathogens based on their epidemiological association with the disease (Sandholm et al. 1990). Contagious pathogens are those organisms transmitted from cow to cow where the primary reservoir harbouring the pathogens is the cow. The predominant contagious pathogens involved in bovine mastitis are *Staphylococcus aureus*, *Streptococcus uberis*, *Strep. dysgalactiae*, and *Strep. agalactiae*. Environmental pathogens are transmitted during milking from the environment serving as the primary source of these organisms. The main pathogens in this group are *Strep. equinus*, *Strep. mitis*, *Strep. salivarius*, *Strep. saccharolyticus*, *Enterococcus faecalis*, *E. faecium*, *E. avium*, and *Aerococcus viridans* (Forsman et al. 1997). However, these organisms have a profound importance in both human and veterinary medicine.

Aerococci exhibit many biochemical and physiological similarities with pediococci, enterococci, lactococci and leuconostocs, and are often confused with

Correspondence to: P. Pristas, e-mail: pristas@saske.sk, tel.: +421 55 6783120

streptococci (Facklam et al. 1989). Isolates assigned to this genus are facultatively anaerobic, show a weak reaction in the catalase test but do not contain cytochrome; they have a G+C content of 35 to 40 mol%. The genus *Aerococcus* was initially described including a single species, *Aerococcus viridans* (Williams et al. 1953). Five new species of *Aerococcus* have been further identified: *A. urinae*, *A. sanguinicola*, *A. christensenii*, *A. urinaeequi*, and *A. urinaehominis* (Euzeby 1997). *A. viridans* are catalase-negative Gram-positive cocci that resemble staphylococci by Gram stain, but have biochemical and growth characteristics of streptococci and enterococci (Facklam and Elliott 1995, Ruoff et al. 1995, Skov et al. 1995). *Aerococcus viridans* has been associated with different human infections such as endocarditis, urinary tract infections, arthritis, or meningitis (Gopalachar et al. 2004, Popescu et al. 2005). Moreover, this species is a pathogen for crustaceans, causing gaffkemia in marine lobsters (Battison et al. 2004, Stewart et al. 2004), and it has been associated with septicemia in sea turtles (Torrent et al. 2002). *Aerococcus viridans* has also been isolated from the milk of cows with subclinical mastitis (Devriese et al. 1999) and has been associated with septicemia in immunodeficient mice (Dagnaes-Hansen et al. 2004). The role of *A. viridans* in the etiology of bovine mastitis, however, is still unclear (Devriese et al. 1999).

Aerococcus viridans strains, like the other members of the *Streptococcaceae*, are naturally susceptible to penicillins, macrolides and related drugs, tetracyclines, and chloramphenicol and are intrinsically resistant at a low level to aminoglycosides (Horaud and Delbos 1987).

The aim of the present study was to monitor the diversity of *A. viridans* isolates from subclinical cases of bovine mastitis in Slovakia.

Materials and Methods

Collection of milk samples and isolation of bacteria

The samples were taken during 2009 and 2010 years from 6 different farms in Slovakia (Roštár, Kriváň, Liptovská Kokava, Trhovište, Budča, Zuberec) with standard zoohygienic conditions using free stabling. The samples from twelve cows of Holstein breed (4) and Slovak spotted breed (8) on second and thrird lactation were used, respectively. Cows had a latent (5), subclinical (4) or subacute (3) forms of mastitis. A complex examination of the health status of the animals included clinical examination of the mammary gland, bacteriological examination of collected milk samples (quarter samples), cytological examination the first portion of milk, NK-test reaction with subsequent collecting of individual milk samples (mixed quarters' samples) for bacteriological examination, and subsequent cultivation and identification of pathogenic bacteria. The positive bacteriological results were commonly detected in all animals.

Quarter foremilk samples were collected aseptically from 12 cows. Before sampling, the first streams of milk were discarded, and teat ends were disinfected. The milk samples were transported on ice to the laboratory. From each sample, 0.01 mL of milk was cultured on Columbia Blood Agar Bass (Oxoid, England) with 5% of defibrinated ram blood and incubated for 48 h at 37°C; the plates were examined after 24 and 48 h of incubation. Selected isolates were further characterized. All isolates were recovered in pure culture from individual animal samples.

Conventional identification of bacterial isolates

Isolates were obtained from milk samples or gland skin and tested using conventional tests as previously described. Twelve selected isolates were typed using commercially available STREPTO test 24 (PlivaLachema, Czech Republic) identification system followed by 16S rRNA sequencing (Table 1).

Antimicrobial susceptibility testing

A commercially available HiComb MIC test strips (Hi Media Laboratories, Pvt. Ltd., India) were used to determine the minimum inhibitory concentration (MIC) for 9 antimicrobial agents (amoxicillin, amoxyclav, ciprofloxacin, erythromycin, lincomycin, methicillin, neomycin, streptomycin and tetracycline) according to the protocols supplied by the manufacturer.

DNA analysis, PCR primers and amplifications from A. viridans isolates

Genomic DNA from selected isolates was extracted from 1.5 ml overnight cultures, grown in BHI medium, using the E.Z.N.A.® Bacterial DNA Kit (Omega).

About 10 ng of extracted DNA was used as the template in PCR reactions. The 16S rRNA genes were amplified from purified DNA using the primers fD1 and rP2 to generate an amplicon of about 1500 bp (Weisburg et al. 1991). The rep-PCR (random

Table 1. Characteristics of Aerococcus viridans isolates tested in this study.

Isolate	Origin		Identification			Mastitis
	Farm	Source	STREPTO test 24	16S rRNA Accession number	Blastn best hit (%)	
R 089	Roštár	milk	*A. viridans*	HQ917007	*A. viridans* 100%	subclinic
K 123	Kriváň	milk	*A. viridans*	HQ917004	*A. viridans* 100%	subacute
LK 13	Liptovská Kokava	milk	*A. viridans*	HQ917005	*A. viridans* 99.7%	latent
T 13	Trhovište	milk	*E. faecium*	HQ917006	*A. viridans* 99%	latent
T 120	Trhovište	milk	*A. viridans*	HQ917008	*A. viridans* 100%	latent
S 23	Budča	milk	*Leuconostoc spp.*	HQ917009	*A. viridans* 99.8%	subclinic
Str 13	Budča	milk	*A. viridans*	HQ917010	*A. viridans* 99.9%	subacute
Str 54	Budča	milk	*A. viridans*	HQ917011	*A. viridans* 99.9%	subacute
208	Zuberec	udder skin	*A. viridans*	HQ917013	*A. viridans* 99.5%	na
219	Zuberec	milk	*A. viridans*	HQ917012	*A. viridans* 99.6%	latent
293	Zuberec	udder skin	*A. viridans*	HQ917015	*A. viridians* 99.6%	na
301	Zuberec	milk	*A. viridans*	HQ917014	*A. viridans* 99.6%	subclinic

The designation, and origin of isolates is shown together with identification results from phenotypic (STREPTO test 24) and molecular (16S rRNA Accession number and blastn best hit) typing, results of NK test and final classification of animal health status. (na = not applicable).

Table 2. Primer sequences and PCR conditions used in the study.

Primers	Primer sequence (5' – 3')	Target gene	PCR conditions[a]
fD1	AGAGTTTGATCCTGGCTCAG	16S rRNA	1
rP2	ACGGCTACCTTGTTACGACTT		
IGSRev	TACTTAGATGTTTCAGTTCC	intergenic spacer	
IGSFor	TGGGGTGAAGTCGTAACAAGGTA	region of rDNA	2
ERIC 1R	ATGTAAGCTCCTGGGGATTCAC	fingerprint of whole genome	3

a – 1 = 35x(93°C 60s, 52°C 60s, 72°C 120s); 2 = 35x(94°C 30s, 40°C 45s, 72°C 45s); 3 = 44x(94°C 30s, 35°C 60s, 72°C 90s).

amplified polymorphic DNA) technique with the primer ERIC 1 was used to characterize interspecific diversity of *Aerococcus* isolates. The IGS region (16S-23S spacer-region) from each isolate was amplified via PCR using primer pair IGSrev and IGSfor (Gurtler and Stanisich 1996). The oligonucleotide primers and PCR conditions used in this study are summarized in Table 2.

The PCR mixtures (50 μl) contained each deoxynucleoside triphosphate at a concentration of 200 μM, 2 mM MgCl2, each primer at a concentration of 1 μM, 1.25 U of *Taq* DNA polymerase (Invitrogen, Paisley-UK), and the PCR buffer supplied with the enzyme. All reactions were carried out in a Personal Thermal Cycler MJ Mini (Bio-Rad Laboratories, Richmond, USA). An aliquot of the PCR product was electrophoresed in a 0.8% agarose gel, stained with ethidium bromide, and quantified by using a standard (1 kb DNA Ladder; Invitrogen, USA).

Endonuclease digestion of amplified 16S rDNA

Amplified 16S rDNAs were digested using restriction endonuclease: RsaI (Fermentas, USA). Approximately 2 mg of DNA was digested with 10 U of enzyme and appropriate Buffer (Fermentas, USA) for at least 1 h at 37°C. Digests were electrophoresed in a 1% agarose gel stained with ethidium bromide. Restriction fragment sizes were determined by comparison to 100 bp DNA ladder (Invitrogen, USA).

Sequencing of 16S rRNA genes and phylogenetic analysis

16S rDNA PCR products were used to construct a clone library with InsTAclone™ PCR Cloning Kit (Fermentas, Germany). Recombinant colonies were

Table 3. Antimicrobial susceptibility of Aerococcus viridans isolates.

Strain	Amoxicillin	Amoxyclav	Ciprofloxacin	Erythromycin	Lincomycin	Methicillin	Neomycin	Streptomycin	Tetracycline
str T 13	5	10	5	0.1	0.1	0.1	0.1	5	0.1
LK 13	10	10	5	0.1	5	60	5	0.1	5
str 54	10	30	0.1	0.01	5	240	5	5	0.1
str 13	30	10	0.1	0.1	0.01	240	5	5	0.1
K 123	10	10	0.1	0.1	0.1	120	0.1	5	0.1
S 23	30	30	0.25	0.1	3	240	7.5	0.1	30
T 120	0.5	2	0.01	5	10	120	0.1	5	5
R 089	0,5	4	4	0.1	10	240	5	0.1	0.25
293	0.5	1	5	0.01	5	240	10	60	0.25
208	1	2	0.25	0.01	15	240	3	1	0.01
219	1	2	0.25	0.1	1	240	3	3	0.01
301	1	2	0.01	5	10	240	10	5	0.5

Minimal inhibitory concentration are shown in µg per mililiter.

selected and plasmid DNA extracted using a plasmid miniprep kit (Qiagen). Sequencing was performed on both strands. Phylogenetic affiliation of each sequence was attributed using the Blastn search algorithm (http://blast.ncbi.nlm.nih.gov/).

Results

Isolation of bacteria from mastitis milk samples

During two years numerous Gram-positive isolates were obtained from mastitis cows in Slovakia. Most frequently (more than 21.9%) bacteria of *Streptococcus* genus, mainly *S. uberis*, were detected followed by coagulase negative staphylococci, *Arcanobacterium* sp., and *Bacillus* sp. *Aerococcus viridans* isolates represented of about 1% of isolates. As knowledge on *A. viridans* is fairly limited, selected isolates from 6 farms and 12 animals were further analysed.

Antimicrobial susceptibility of A. viridans isolates

Limited data are available on the antimicrobial susceptibility of *A. viridans* isolates. Thus, the antimicrobial susceptibility to different commonly used antimicrobials was determined (Table 3). Since no specific minimal inhibition concentrations (MIC) values for aerococci are available, the MIC values from Hogeveen (2005) and Watson et al. (1991) were used. The high level resistance to beta lactam antibiotics was observed when most strains were resistant to methicillin (MIC more than 60 µg/ml). In about half of these strains methicillin resistance was accompanied by amoxicillin (MIC more than 5 µg/ml) and amoxyclav (amoxicillin and clavulanic acid) resistance indicating lacks of beta-lactamase dependent mechanism of resistance. Variable resistance patterns were observed for other antibiotics. Practically all tested strains were sensitive to neomycin and ciprofloxacin, sporadically resistance to streptomycin and erythromycin was detected. Resistance to tetracycline was observed in 3 tested strains, two of them originated from Budča farm. Similarly, resistance to lincomycin was found in 4 strains, mainly from Zuberec farm.

Molecular identification of A. viridans isolates

While extremely variable at resistance patterns, *A. viridans* isolates showed very low variability at molecular level. Partial 16S ribosomal RNA gene nucleotide sequence of all isolates showed high similarity (at least 99%) to known isolates of *A. viridans* (Table 1) Surprisingly, in three cases complete identity was observed to DSD-PW4-OH13 strain of *A. viridans* isolated from sea water (GenBank accession number HQ425688).

Genotypic variability of A. viridans isolates

Limited variability detected on 16S rRNA sequence level was confirmed by ARDRA analysis (Amplified Ribosomal DNA Restriction Analysis). All isolates produced constantly identical profiles when tested by restriction endonucleases (data documented for RsaI restriction endonuclease, Fig. 1A). Similarly, DNA fingerprinting methods targeted to more variable part of ribosomal RNA operon (16S-23S intergenic spacer region) confirmed limited variability of *A. viridans* isolates. The PCR amplification resulted in production of single type of RISA

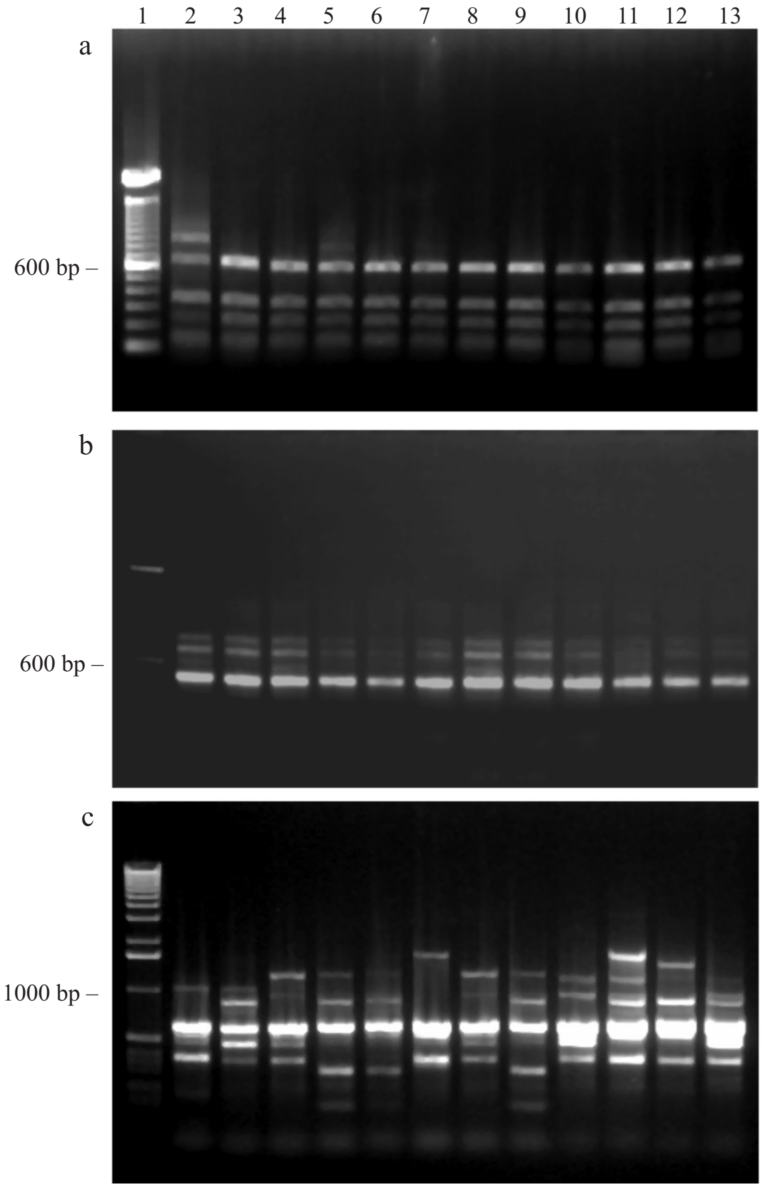

Fig. 1. Analysis of genetic variability in Aerococcus viridans isolates. Agarose gel electrophoresis of 16S rDNA amplificons digested by the restriction enzyme *Rsa*I (part A); RISA generated patterns (part B); ERIC1R generated patterns (part C). Lane 1 – standard of molecular weight; patterns for isolate R 089 – lane 2; K 123 – lane 3; LK 13 – lane 4; T 13 – lane 5; T 120 – lane 6; S 23 – lane 7; Str 13 – lane 8; Str 54 – lane 9; 219 – lane 10; 208 – lane 11; 301 – lane 12; 293 – lane 13. The size of selected standard band is shown in basepairs.

profile (Fig. 1B) across all isolates. Since 16S ribosomal RNA operons are usually highly conserved among the species, the whole genome fingerprinting technique based on repetitive element targeted PCR amplification was used to analyze genotypic variability in *A. viridans* isolates. Using ERIC1R primer similar profiles (Fig. 1C) for all isolates were obtained confirming limited variability among *A. viridans* isolates tested.

Discussion

Mastitis is one of the most economically important diseases of dairy animals. It causes great economic losses and affects the quality and quantity of milk. Mastitis generally results from interaction between a variety of microbial infections and host responses in the udder. Mastitis-causing bacteria classified as contagious are *Strep. agalactiae*, *Staph. aureus*, *Arcanobacter pyogenes*, *Mycoplasma*s; as environmental are *Strep. uberis* and *dysgalactiae*, *Escherichia coli* and other enterobacteria, yeasts and molds; and opportunist as coagulase negative *Staphylococcus* spp. *Aerococcus viridans* bacteria are regularly isolated from mastitis cows. However, it usually represents a minor part of bacterial population only. Devriese et al. (1999) reported that *A. viridans* species represented 15% of Gram-positive, catalase-negative, aesculin degrading cocci isolated from clinical and subclinical mastitis cases in dairy cows, but McDonald et al. (2005) found two *A. viridans* isolates among 100 *Streptococcus* spp. causing bovine mastitis only. However, *A. viridans* species was detected in 50% of 48 bulk tank milk samples from 48 dairy farms in USA (Zadoks et al. 2004).

Commercially available STREPTO test 24 rapid identification kit identified correctly the majority of *A. viridans* isolates in the study. Another two isolates included in the study were misidentified by the test but clearly identified to be *A. viridans* on the basis of 16S rRNA analysis. The *A. viridans* isolates were highly diverse in their antibiotic resistance patterns when a unique pattern was observed for every strain tested. There are very limited data on cow mastitis *A. viridans* antibiotic resistance in the recent literature. Owens et al. (1991) reported prevalent resistance to streptomycin (60%) followed by tetracycline (23%), erythromycin (17%), penicillin (16%) and ampicillin (13%). Completely different profiles were reported for *A. viridans* isolates from swine clinical specimens (Martin et al. 2007). These *A. viridans* isolates were commonly susceptible to beta-lactam antimicrobials, but resistant to streptomycin (90%), tetracycline (95%) and erythromycin (59%). The resistance patterns of our isolates are much more similar to the patterns of human clinical *A. viridans* isolates, for which the resistance to beta-lactams was found to be prevalent (Facklam et al. 2003).

Phylogenetic analysis confirmed very low intraspecies variability in *A. viridans*. All isolates showed the similarity higher than 99% to the known members of the *A. viridans* species. In three cases perfect match of 16S rRNA sequences was found with environmental isolate of *A. viridans* from sea water indicating widespread occurrence of this bacterium. Practically no variability was observed in ARDRA and RISA analysis indicating that the majority of phenotypic diversity observed is due to epigenetic factors and not due to evolutionary divergence. Very limited variability was observed by rep-PCR using ERIC1R primer. Despite restricted distribution of ERIC elements, DNA fingerprinting using repetitive element targeted probes is a useful tool for DNA based studies of clonal relationships among bacterial isolates (Versalovic et al. 1991). Our data suggest that only few *A. viridans* strains spread among cow population in Slovak farms. The identity of ERIC1R generated PCR profiles clearly suggests that strains LK 13 and Str 13 from Liptovska Kokava or Budča farms are clonaly related. This observation is in contrast to data obtained by Martin et al. (2007) who indicated an exposure of swine to multiple *A. viridans* strains, what is in accordance with the wide distribution of *A. viridans* (Woodward et al. 1998), and also suggests an opportunistic pathogenic character of this bacterium. Similarly, Kagkli et al. (2007) reported the presence of putative new *A. viridans* species in Gram-positive cocci from raw milk in a farm dairy environment. Despite being variable in antibiotic resistance profiles, *A. viridans* isolates from Slovakia represent homogenous group with limited genetic variability.

Acknowledgement

This study was supported by the Slovak Research and Development Agency (SRDA) grant No. 0586-07.

References

Battison AL, Cawthorn RJ, Horney B (**2004**) Response of American lobsters *Homarus americanus* to infection with a field isolate of *Aerococcus viridans* var. *homari* (Gaffkemia): survival and hematology. Dis Aquat Organ 61: 263-268.

Dagnaes-Hansen F, Kilian M, Fuursted K (**2004**) Septicaemia associated with an *Aerococcus viridans* infection in immunodeficient mice. Lab Anim 38: 321-325.

Devriese LA, Hommez J, Laevens H, Pot B, Vandamme P, Haesebrouck F (**1999**) Identification of aesculin-hydrolyzing streptococci, lactococci, aerococci, and enterococci from subclinical intramammary infections in dairy cows. Vet Microbiol 70: 87-94.

Euzeby JP (**1997**) List of bacterial names with standing in nomenclature: a folder available on the Internet. Int J Syst Bacteriol 47: 590-592.

Facklam R, Elliott JA (**1995**) Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. Clin Microbiol Rev 8: 479-95.

Facklam R, Hollis D, Collins MD (**1989**) Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. J Clin Microbiol 27: 724-730.

Facklam R, Lovgren M, Shewmaker PL, Tyrrell G (**2003**) Phenotypic description and antimicrobial susceptibilities of *Aerococcus sanguinicola* isolates from human clinical samples. J Clin Microbiol 41: 2587-2592.

Forsman P, Tilsala-Timisjarvi A, Alatossava T (**1997**) Identification of staphylococcal and streptococcal causes of bovine mastitis using 16S-23S rRNA spacer regions. Microbiology 143: 3491-3500.

Gopalachar A, Akins RL, Davis WR, Siddiqui AA (**2004**) Urinary tract infection caused by *Aerococcus viridans*, a case report. Med Sci Monit 10: CS73-CS75.

Gütler V, Stanisich VA (**1996**) New approaches to typing and identification of bacteria using the 16S-23S rDNA spacer region. Microbiology 142: 3-16.

Hogeveen H (**2005**) Mastitis in Dairy Production: Current knowledge and future solutions. In: H Hogeveen (ed) Mastitis in Dairy Production. Wageningen Academic Publishers, The Netherlands, pp 131-200.

Horaud T, Delbos F (**1987**) *Streptococcaceae* and antibiotics. Lett Infectiol 19: 595-604.

Kagkli DM, Vancanneyt M, Hill C, Vandamme P, Cogan TM (**2007**) *Enterococcus* and *Lactobacillus* contamination of raw milk in a farm dairy environment. Int J Food Microbiol 114: 243-251.

Martin V, Vela AI, Gilbert M, Cebolla J, Goyache J, Dominguez L, Fernandez-Garayzabal JF (**2007**) Characterization of *Aerococcus viridans* Isolates from Swine Clinical Specimens. J Clin Microbiol 45: 3053-3057.

McDonald WL, Fry BN, Deighton MA (**2005**) Identification of *Streptococcus* spp. causing bovine mastitis by PCR-RFLP of 16S-23S ribosomal DNA. Vet Microbiol 111: 241-246.

Owens WE, Watts JL, Greene BB, Ray CH (**1990**) Minimum inhibitory concentrations and disk diffusion zone diameter for selected antibiotics against streptococci isolated from bovine intramammary infections. J Dairy Sci 73: 1225-1231.

Popescu GA, Benea E, Mitache E, Piper C, Horstkotte D (**2005**) An unusual bacterium, *Aerococcus viridans*, and four cases of infective endocarditis. J Heart Valve Dis 14: 317-319.

Ruoff KL (**1995**) *Leuconostoc*, *Pediococcus*, *Stomatococcus*, and miscellaneous gram-positive cocci that grow aerobically. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (eds) Manual of clinical microbiology, Washington DC: American Society for Microbiology, pp 315-323.

Sandholm M, Kaartinen L, Pyorala S (**1990**) Bovine mastitis why does antibiotic therapy not always work? An overview. J Vet Pharmacol Therap 13: 248-260.

Skov RL, Klarlund M, Thorsen S (**1995**) Fatal endocarditis due to *Aerococcus urinae*. Diagn Microbiol Infect Dis 21: 219-21.

Stewart JE, Cornick JW, Zwicker BM, Arie B (**2004**) Studies on the virulence of *Aerococcus viridans* (var.) *homari*, the causative agent of gaffkemia, a fatal disease of homarid lobsters. Dis Aquat Organ 60: 149-155.

Torrent A, Deniz S, Ruiz A, Calabuig P, Sicilia J, Oros J (**2002**) Esophageal diverticulum associated with *Aerococcus viridans* infection in a loggerhead sea turtle (Caretta caretta). J Wildl Dis 38: 221-223.

Versalovic J, Koeuth T, Lupski JR (**1991**) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. Nucleic Acids Res 19: 6823-6831.

Vulfson L, Pedersen K, Chriel M, Holmen Andersen T, Dietz HH (**2003**) Assessment of the aerobic faecal microflora in mink (*Mustela vison* Schreiber) with emphasis on *Escherichia coli* and *Staphylococcus intermedius*. Vet Microbiol 93: 235-245.

Watson CK, Cole JR, Pursel AR (**1991**) Comparison of a veterinary breakpoint minimal inhibitory concentration system and a standardized disk agar diffusion procedure for antimicrobic susceptibility testing. J Vet Diagn Invest 3: 66-71.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ (**1991**) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173: 697-703.

Williams REO, Hirch A, Cowan ST (**1953**) *Aerococcus*, a new bacterial genus. J Gen Microbiol 8: 475-480.

Woodward WD, Ward AC, Fox LK, Corbeil LB (**1988**) Teat skin normal flora and colonization with mastitis pathogen inhibitors. Vet Microbiol 17: 357-365.

Zadoks RN, Gonzalez RN, Boor KJ, Schukken YH (**2004**) Mastitis-causing streptococci are important contributors to bacterial counts in raw bulk tank milk. J Food Prot 67: 2644-2650.