

Antimicrobial resistance in *Staphylococcus* spp., *Escherichia coli* and *Enterococcus* spp. in dogs given antibiotics for chronic dermatological disorders, compared with non-treated control dogs

By M. Rantala¹, E. Lahti², J. Kuhalampi¹, S. Pesonen¹, A-K. Järvinen¹, L. Saijonmaa-Koulumies¹, T. Honkanen-Buzalski²

¹Department of Clinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Helsinki, and ²National Veterinary and Food Research Institute, Helsinki, Finland.

Rantala M, Lahti E, Kuhalampi J, Pesonen S, Järvinen A-K, Saijonmaa-Koulumies L, Honkanen-Buzalski T: Antimicrobial resistance in *Staphylococcus* spp., *Escherichia coli* and *Enterococcus* spp. in dogs given antibiotics for chronic dermatological disorders, compared with non-treated control dogs. Acta vet. scand. 2004, 45, 37-45. - The aim of this study was to evaluate antimicrobial resistance in canine staphylococci, *Escherichia coli* and enterococci, which were isolated from 22 dogs with pyoderma and a history of previous antibiotic treatment, compared to bacterial isolates from 56 non-treated control dogs. Two isolates of each bacterial species per dog were investigated, if detected. Staphylococcal isolates from dogs with pyoderma (35 isolates) were more resistant to sulphatrimethoprim than the isolates from controls (56 isolates) (57% vs. 25%, $p < 0.004$). Multiresistance in staphylococci was also more common in dogs with pyoderma (29% vs. 9%, $p = 0.02$). A similar trend among isolates of *E. coli* was detected (24 and 74 isolates from treated and control dogs, respectively), but the differences were not significant. Resistance for macrolide-lincosamides was approximately 20% among staphylococci in both groups. Resistance to ampicillin among enterococci was 4%-7%. The age of the dogs might have an impact on resistance: multiresistance among staphylococcal isolates from younger dogs (≤ 5 years) was more common than in older dogs (≥ 6 years) (24%, vs. 0%, 63 and 27 isolates, respectively, $p = 0.02$). Staphylococci in younger dogs were more resistant to tetracycline (48% vs. 11%, $p < 0.001$) and sulphatrimethoprim (48% vs. 15%, $p < 0.01$) than those in older dogs. In contrast, the isolates of *E. coli* from older dogs tended to be more resistant, although a significant difference was detected only in resistance to tetracycline (13% vs. 2% of 40 and 50 isolates respectively, $p = 0.04$). The results of this small study indicate that resistance in canine staphylococci in the capital area of Finland is comparable with many other countries in Europe. Resistance in indicator bacteria, *E. coli* and enterococci, was low.

antimicrobial resistance; normal flora; *Staphylococcus* spp.; *Enterococcus faecalis*; *Enterococcus faecium*; *Escherichia coli*; pyoderma; dog.

Introduction

Staphylococcus intermedius, as well as other staphylococci, is habitant of the normal bacterial flora of the dog and can be isolated from healthy dogs, especially from the anal region

(Devriese & DePelsmaecker 1987). *S. intermedius* is also an important skin pathogen in dogs (Medleau et al. 1986). Antimicrobial resistance among canine staphylococci is com-

mon (Noble & Kent 1992, Lloyd et al. 1996, Werckenthin et al. 2001, Holm et al. 2002). In addition, bacteria of the normal flora of the gut, such as *Escherichia coli* and *Enterococcus* spp., can easily acquire and transfer resistance genes. These bacteria can thus be used as indicators of changes in antimicrobial resistance (Caprioli et al. 2000). Regular monitoring of the level of resistance in pathogens and bacteria of the normal flora has been recommended (Martel et al. 2001). Monitoring programmes for antimicrobial resistance have been established in veterinary medicine, but only for food producing animals (Martel et al. 2001). Not many reports of the level of antimicrobial resistance in canine normal flora have been published (Hirsh et al. 1980, Monaghan et al. 1981, Devriese et al. 1996, van Belkum et al. 1996). The aim of this study was to investigate whether the bacteria of the normal flora are more resistant in dogs, which have received antimicrobials for the treatment of chronic dermatological disorders, when compared to the bacteria of non-treated control dogs. We chose to study staphylococci, *E. coli*, *Enterococcus faecalis* and *Enterococcus faecium*.

Materials and methods

Sampling

Dogs with chronic dermatological disorders (n=22) treated with antimicrobials during the 6 months preceding the study were sampled in the treated group. The last treatment was to have ended at least 2 weeks before the samples were taken. Dogs in the control group (n=56) were presented to a veterinarian for other reasons and had not received antimicrobials during the previous 6 months. The following data were collected from the patients: breed, age, sex, antimicrobials given during the previous 6 months and the duration of the treatment.

Samples were collected at the Small Animal Veterinary Teaching Hospital of the Helsinki

University and at 2 veterinary clinics in the same area between December 1997 and July 1998. For the isolation of staphylococci, a sample was taken by swabbing the perianal mucosal area with a sterile cotton swab. For the isolation of *E. coli* and enterococci, a faecal sample was taken from the rectum with a sterile glove. Samples were directly transported to the National Veterinary and Food Research Institute, Helsinki, Finland, where the bacteriological analysis was carried out immediately after sample arrival. If samples were taken during a weekend, they were first stored at +4°C and then transported to be analysed on Monday.

Isolation and identification of bacteria

For the isolation of staphylococci, samples were streaked onto 5% bovine blood agar and Staphylococcus medium 110 -agar (Difco Laboratories, Detroit, Michigan, USA) and incubated at 37°C for 18-24 h. Identification to the species level was performed by conventional methods (Quinn et al. 1994).

For the isolation of *E. coli*, faecal samples were cultured on Rapid *E. coli* -agar (Sanofi Diagnostics, Marnes La Coquette, France) and incubated at +44°C for 24 h. Five blue colonies were picked, streaked onto blood agar and incubated further at +37°C for 24 h. *E. coli* was identified by colony morphology, oxidase and indole tests. The identity was further confirmed with API20E (bioMérieux, Marcy l'Etoile, France). For the isolation of enterococci, faecal samples were inoculated onto Slanetz-Bartley agar (Merck, Darmstadt, Germany) and incubated at +44°C for 48 h. Five red colonies were subcultured on Tryptone soya agar (BBL, Cockeysville, Maryland, USA) and incubated at 37°C for 24 h. Isolates were identified as enterococci on the basis of colony morphology on Slanetz-Bartley agar, a negative catalase test and the ability to grow in Brain Heart Infusion (BHI) (Difco Laboratories) containing 6.5%

Table 1. Susceptibility breakpoint zone diameters (mm) used in the study.

	Staphylococci	<i>E.coli</i>	Enterococci	Respective MIC -value for susceptible strains *
Betalactams				
Penicillin G 10 IU	≥29			≤0.12 µg /ml
Ampicillin 10 µg		≥17	≥17	≤8 µg /ml
Amoxillin-clavulanate (2:1) 30 µg	≥17			-
Cephalotin 30 µg	≥18			≤8 µg /ml
Cefotaxime 30 µg		≥18		-
Oxacillin 1 µg	≥13			≤2 µg /ml
Macrolides and lincosamides				
Erythromycin 15 µg	≥16			-
Clindamycin 2 µg	≥21			≤0.5 µg /ml
Aminoglycosides				
Streptomycin 10 µg		≥15		-
Gentamicin 10 µg		≥19		-
Others				
Chloramphenicol 30 µg		≥20		-
Enrofloxacin 5 µg		≥21		≤0.25 µg /ml
Trimethoprim/sulfamethoxazole 1.25/23.75 mg (SXT)	≥16	≥16		≤2/38 µg /ml
Tetracycline 30 µg	≥21	≥19		for <i>E.coli</i> : ≤4 µg /ml
Vancomycin 30 µg			≥18	-

*The respective MIC -value is, if found, from the NCCLS standards. Susceptibility breakpoints are those used by The National Veterinary and Food Research Institute at the time this study was made, and they partly differed from the NCCLS (1997) standards.

NaCl and at pH 9.6. *E. faecium* and *E. faecalis* were identified on the basis of tests for motility, fermenting reactions with L-arabinose, mannitol, melibiose, raffinose and sorbitol. The identification of isolates was further confirmed with APIStrept (bioMérieux, Marcy l'Etoile, France). If *Staphylococcus* spp., *E. coli*, *E. faecium* and/or *E. faecalis* were identified, 2 colonies per sample were stored in BHI supplemented with glycerol at -70°C.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of the isolates was determined by the agar diffusion test on Iso-Sensitest agar (CM471, Oxoid, Basingstoke, UK) according to NCCLS standards (1997).

Antimicrobial disks were from Oxoid. The breakpoint zone diameters used in the study, and respective minimum inhibitory concentrations (MIC), if available, are presented in Table 1. In addition, for staphylococci, the ability to produce β-lactamase was tested (Nitrocefin, AB Biodisk, Sweden). Both resistant and intermediate isolates are reported as resistant when interpreting resistance percentages. An isolate was defined multiresistant if it showed resistance to 3 or more different classes of antimicrobials. *E. coli* (ATCC 25922), *E. faecalis* (ATCC 29212) and *Staphylococcus aureus* (ATCC 25923) were used as controls. Five staphylococcal isolates, which first gave an unusual macrolide resistance pattern (isolates

resistant or intermediate to clindamycin but sensitive to erythromycin) were re-tested both with E-test (AB-Biodisk, Solna, Sweden) and disk diffusion test. The susceptibility breakpoint in E-test both to erythromycin and clindamycin was MIC ≤ 0.5 $\mu\text{g/ml}$. The phenotypes of these isolates were tested with double-disk method (Leclercq & Courvalin 1991). Enterococcal isolates, which had reduced susceptibility to vancomycin according to the disk diffusion test, were tested on the presence of *van*-genes with a multiplex PCR (polymerase chain reaction) detection at a National Public Health Institute with the method described by Patel et al. (1997).

Statistical analysis

The results are presented as the number of bacterial isolates. Fisher's 2-tailed exact test was used to compare resistance in bacterial isolates between the 2 groups. The statistical analysis was made with the SAS software. Difference between the groups was considered significant if $p \leq 0.05$.

Results

A total of 56 dogs, with a median age of 5 years, were included in the control group, and 22 dogs, with a median age of 3 years in the treated group. Of the treated dogs, 9 had received antimicrobials during at least 2 treatment periods lasting more than 3 weeks, and the rest of the dogs were given antibiotics for less than 3 weeks. The number of treatment periods with antimicrobials in dermatological patients was: cephalexin 14, amoxicillin-clavulanate 4, amoxicillin 3, penicillin V 2, sulphatrimethoprim (SXT) 1 and clindamycin 1. Information on the antimicrobial agent used was missing for 4 dogs with pyoderma. The number of investigated bacterial isolates in the control and treated group, respectively, was as follows: *Staphylococcus* spp. 56/35, *E. coli* 74/24, *E.*

faecalis 28/21 and *E. faecium* 45/16. The respective mean number of bacterial isolates investigated per dog was: *Staphylococcus* spp. 1.8/1.8, *E. coli* 1.9/1.8, *E. faecalis* 1.6/1.9 and *E. faecium* 1.9/1.8. The number in parentheses (n) hereafter is the number of bacterial isolates, if not stated otherwise.

The staphylococcal isolates of the treated dogs were more resistant to SXT compared to those of the control group (57%, $n=35$ and 25%, $n=56$, respectively, $p < 0.004$, Fig. 1). Multiresistant staphylococci were also more common in the treated group (29% vs. 9%, $p=0.02$). The most common multiresistant phenotypes were penicillin-SXT-tetracycline-macrolide/lincosamides ($n=4$) and penicillin-SXT-tetracycline ($n=4$). β -lactamase production was detected in 71% of the staphylococcal isolates in the treated group and in 75% of the isolates in the control group. No differences were detected in macrolide-lincosamide resistance between the groups: among control group staphylococci, the level of resistance both to erythromycin and clindamycin was 21%. 20% of staphylococcal isolates from treated dogs were resistant to erythromycin and 16% were resistant to clindamycin. Phenotypes of 5 staphylococcal isolates, which first gave unusual macrolide-lincosamide resistant pattern, were as follows: 3/5 showed inducible resistance to the macrolide-lincosamides, and 2/5 were susceptible to macrolide-lincosamides. No resistance to oxacillin, amoxicillin-clavulanate and first generation cephalosporins was detected. Isolates of *E. coli* from dogs in the treated group tended to be more resistant to sulphatrimethoprim, streptomycin and ampicillin, but the differences were not statistically significant (Fig. 1). No resistance to gentamycin or cefotaxime was detected.

Among enterococci, the resistance to ampicillin was low in both groups (4%-7%) and there was no difference between the groups. Two *E. fae-*

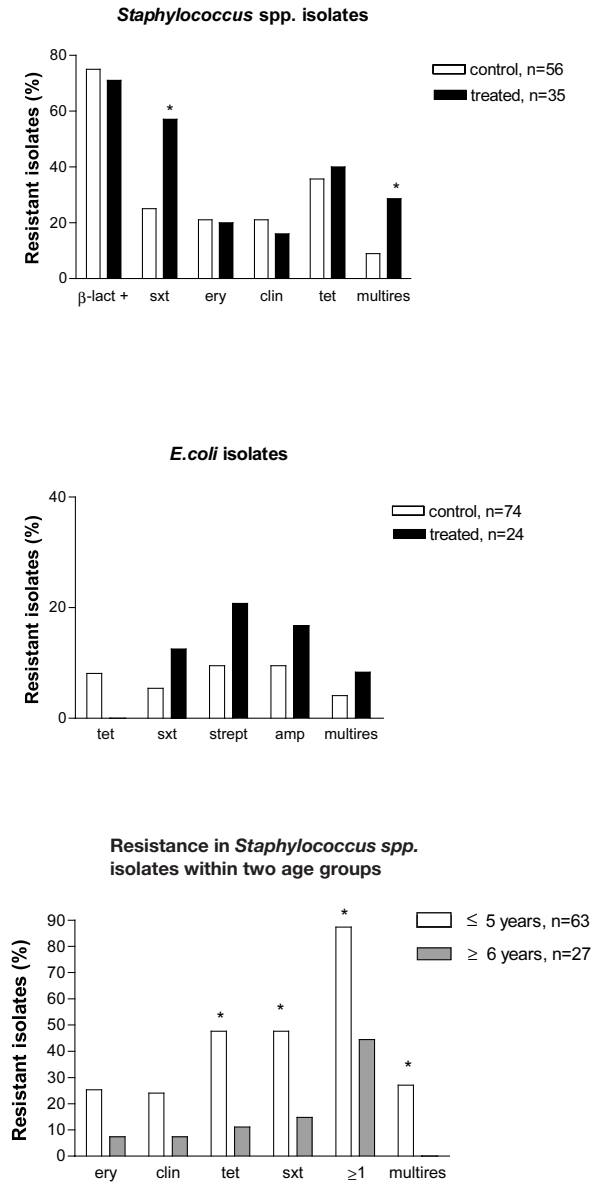


Figure 1. Upper panel: percentage of resistant staphylococcal isolates from control and treated dogs. Middle panel: respective results of *E.coli*. Lower panel: resistance in staphylococcal isolates from younger and older dogs regardless of treatment history. Significant differences marked by an asterisk. Abbreviations: β-lact+ = betalactamase positive, amp=ampicillin, clin=clindamycin, ery=erythromycin, strep=streptomycin, sxt=sulphatrimethoprim, tet=tetracyclin, >1=resistant to one or more antimicrobials, multires=multiresistant isolates (resistant to three or more different class of antimicrobials).

calis isolates from 2 control dogs were classified as resistant to vancomycin, but no *van* - genes were present in multiplex PCR testing. None of *E. faecium* isolates were resistant to vancomycin.

The impact of the age of the dog on the results was further studied by dividing the dogs into 2 age groups (≤ 0.5 years, and ≥ 6 years) regardless of the previous treatment history. Multiresistant staphylococcal isolates were more commonly found in younger than older dogs (24%, $n=63$ vs. 0%, $n=27$, $p=0.02$). Staphylococcal isolates from younger dogs were also more resistant to tetracycline (48% vs. 11%, $p<0.001$) and SXT (48% vs. 15%, $p<0.01$, Fig. 1). *E. coli* isolates showed the opposite pattern in resistance: isolates from older dogs tended to be more resistant compared with isolates from younger dogs, but a significant difference was detected only in the resistance to tetracycline (13%, $n=40$ vs. 2%, $n=58$, $p=0.04$).

Discussion

The findings of this study indicate that antimicrobial resistance in canine staphylococci is at a similar level in the capital area of Finland compared with studies from Norway (Kruse et al. 1996), Sweden (Holm et al. 2002), France (Pellerin et al. 1998), Denmark (Pedersen & Wegener 1995), USA, UK and Germany (Werckenthin et al. 2001). Betalactamase production among canine *S. intermedius* has been reported to be common, 50%-90% of isolates produce betalactamase (Noble & Kent 1992, Pedersen & Wegener 1995, Kruse et al. 1996, Lloyd et al. 1996, Holm et al. 2002).

In spite of an extensive use of first generation cephalosporins and other β -lactamase stable antimicrobials in the canine practice, resistance to oxacillin is still rather rare (Pedersen & Wegener 1995, Kruse et al. 1996, Lloyd et al. 1996), although methicillin resistance in canine staphylococci has been reported to occur (Piritz

et al. 1996, Gortel et al. 1999, Pak et al. 1999). It should also be noted that the routine disk diffusion test is not optimal for detecting methicillin resistance (Gortel et al. 1999). In our study, resistance to first generation cephalosporins or oxacillin was not detected.

The level of SXT resistance among canine *S. intermedius* varies in different countries. In our study, SXT resistance was more common in staphylococci isolated from treated dogs (57%) compared to controls (25%). Pellerin et al. (1998) reported similar resistance figures to SXT among *S. intermedius* isolates from healthy dogs in France. In the same study, however, resistance among *S. intermedius* isolated from dogs with pyoderma increased from 6% to 36% during a 9-year follow-up period. In contrast, in the UK, resistance to SXT in staphylococci from canine pyoderma cases peaked at 15% in 1989, but fell to 8% by 1995 (Lloyd et al. 1996). Interestingly, in Norway and in Denmark, despite of extensive use of SXT products in canine practice, resistance to this agent was very low (1% and 0%) in 1996 (Pedersen & Wegener 1995, Kruse et al. 1996). Due to the molecular genetics of SXT resistance, removal of the selection pressure will not have an immediate impact on the level of resistance (Huovinen et al. 1995).

The relatively high macrolide-lincosamide resistance (on average 20%) in canine staphylococci in this study might be explained by an increased use of these antimicrobials in dogs during the last decades in our country. Similarly, Kruse et al. (1996) reported from 3% to 25% increase in resistance to macrolide-lincosamide antimicrobials during 1987-1994 in canine staphylococci in Norway, which was probably due to an increased use of these drugs. In France, macrolide-lincosamide resistance in canine staphylococci was as high as 40% (Pellerin et al. 1998). Usually, as a rule, bacteria which are resistant to clindamycin are also re-

sistant to macrolides. In susceptibility testing they thus show MLS_B phenotype (resistance to macrolide-lincosamide-streptogramin-B antimicrobials), which is mediated by *erm*-methylase genes (Leclercq & Courvalin 1991). In the study by Boerlin *et al.* (2001) it was reported that all erythromycin resistant canine *S. intermedius* strains carried *erm*(B)-gene. Interestingly, Malbruny *et al.* (2002) described one human strain of *Streptococcus pyogenes*, which showed resistance to clindamycin (\geq MIC 2 μ g/ml) but not to erythromycin in MIC-testing. Since the strain showed resistance to azithromycin, they still considered it to be of the MLS_B-phenotype. However, this strain did not carry any *erm*-genes, but instead a point mutation in 23S rRNA was found. Since the number of reports from macrolide resistance mechanisms in canine gram-positive bacteria is scarce, more studies will be needed.

Faecal gram-negative bacteria and enterococci are considered to be good indicators of the selection pressure caused by the use of antimicrobials (Caprioli 2000). In our study, the level of resistance was low in canine enterococci and *E. coli* isolates. Monaghan *et al.* (1981) investigated *E. coli* strains from healthy urban and rural dogs and observed more multiresistance in *E. coli* isolates from rural dogs living on dairy farms than in *E. coli* isolates from urban dogs. These dogs had not received antimicrobials, but the selection pressure was supposed to be derived from antimicrobials used for cows. Hirsh (1973) reported that 69% of *E. coli* strains isolated from canine urinary infections were resistant to at least 2 antimicrobials, and multiresistance was also common among patients who had not received antimicrobials before. The incidence of R-plasmids has been shown to be common in the faecal flora of healthy dogs (Hirsh *et al.* 1980)

Resistance to ampicillin in enterococci was low in our study, and no differences were detected

between the groups. Two (2/49) *E. faecalis* isolates had reduced susceptibility to vancomycin according to disk diffusion test, but these isolates did not carry any *van*-genes and thus were not real VRE isolates (=vancomycin resistant enterococcus). None of the 61 *E. faecium* isolates was resistant to vancomycin. Vancomycin resistant enterococci in dogs have been reported in other studies, probably due to avoparcin use in farm animals (Van Belkum *et al.* 1996). The VRE prevalence in enterococci from Dutch dogs was 48%, and half of these carried an identical *van*(A)-gene found also in human VRE-strains (Van Belkum *et al.* 1996). In Belgium, 8% of canine enterococci were resistant to vancomycin and also carried the *van*(A)-gene (Devriese *et al.* 1996). The use of avoparcin was banned in Finland in 1995, and the use of vancomycin for the treatment of animals is legally prohibited.

According to our results, the effect of age on resistance would need more studies, although material in this study was too small to do any further conclusions. In humans, Arstila *et al.* (1994) reported increased resistance among urinary *E. coli* isolates with increasing age, but they were not able to conclude if this was due to the ageing or the cumulative lifetime use of antimicrobials. We were not able to find reports where the effect of age on resistance had been studied in dogs.

In conclusion, our results support the fact that the use of antimicrobials, and the development of and prevailing antimicrobial resistance among bacteria are linked together. Although resistance among indicator bacteria was found to be low, resistance in staphylococci to commonly used antimicrobials is widespread: the efficacy of sulphatrimethoprim and macrolides is at risk to deteriorate. The rather high prevalence of multiresistant strains among canine staphylococci is also a phenomenon, which gives cause for concern. In order to detect early

changes in bacterial susceptibilities before a high prevalence of resistance is selected or developed, regular monitoring of antimicrobial resistance both among pathogenic bacteria and normal flora of companion animals will be needed. The genetic mechanisms, which mediate antimicrobial resistance in these bacteria, would also need further studies.

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Sammanfattning

Antibiotika resistens hos Staphylococcus spp., Escherichia coli och Enterococcus spp. isolerade från hundar med kroniska dermatologiska sjukdomar; i förhållande till friska hundar.

Målet med denna studie var att jämföra stafylokocker, *E. coli* och enterokockers antibiotikaresistens hos hundar med pyoderma (n=22), vilka tidigare behandlats med antibiotika, med antibiotikaresistensen hos icke-behandlade kontrollhundar (n=56). Resistens mot sulfatrimetoprim var mer allmän hos stafylokocker från hundar med pyoderma (35 isolat) än stafylokocker från kontrollhundar (56 isolat) (57% vs. 25%, p<0.004). Stafylokocker var mera multiresistenta hos hundar med pyoderma (29% vs. 9%, p=0.02). En liknande trend kunde konstateras även hos *E. coli* (24 isolat från pyoderma och 74 isolat från kontrollhundar), men skillnaden var inte signifikant. Ampicillin resistens var 4%-7% hos *Enterococcus* spp. isolat.

Hundens ålder kan ha betydelse för resistensen - multiresistenta stafylokocker var vanligare hos yngre hundar (≤ 5 år) än hos äldre (≥ 6 år) (24% vs. 0%, n=63 respektive 27, p=0.02). Stafylokocker isolerade från yngre hundar var oftare resistenta mot tetracyklin och sulfatrimetoprim. Däremot var *E. coli* från äldre hundar mera resistenta, men en signifikant skillnad kunde konstateras enbart mot tetracyklin (12.5% vs. 1.7%, n=40 respektive 58, p=0.04). Resultaten från denna lilla studie visar att resistensen hos stafylokocker hos hundar i Finland är jämförbar med resistensen i många andra länder i Europa. Resistensen hos *E. coli* och enterokocker verkar däremot vara låg i Finland.

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Reprints may be obtained from: M. Rantala, National Public Health Institute, Antimicrobial research laboratory, Kiinamyllynkatu 13, 20520 TURKU, FINLAND. E-mail: merja.rantala@ktl.fi, tel: +358-2-331 6629.