Vitenskapskomiteen for mattrygghet
Norwegian Scientific Committee for Food Safety

Quaternary ammonium compounds in cosmetic products

Risk assessment of antimicrobial and antibiotic resistance development in microorganisms

Opinion of the Panel on Biological Hazards of the Norwegian Scientific Committee for Food Safety:

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Terminology and definitions

Acquired resistance: Describes decreased susceptibility or insusceptibility that is the result of genetic changes in a microorganism due to mutation or the acquisition of genetic material.

Antibiotics: The term has traditionally referred to natural organic compounds synthesised by microorganisms that kill or inhibit growth of other microorganisms. Many antibacterial agents in clinical use are derived from natural products, but most are then chemically modified (i. e. semi-synthetic) to improve their properties. Some agents are totally synthetic (e. g. sulfonamides, quinolones). Therefore, the terms "antibacterial agent" or "antimicrobial agent" are preferred to "antibiotic" to include both natural and synthetic compounds. However, in the literature the term antibiotic is often used also on semi-synthetic and synthetic compounds.

Antimicrobial agents: A general term for the drugs (antibiotics), chemicals, or other substances that either kill or stop the growth of microbes. The concept of antimicrobial agents applies to disinfectants, preservatives, sanitising agents and biocidal products in general.

Antimicrobial resistance: The characteristic of a strain of a microorganism enabling it to avoid being killed or inhibited by a defined concentration of an antimicrobial agent. While the terminology regarding antimicrobial action and resistance is well understood, that relating to biocidal resistance is still the subject of debate. A culture is considered resistant to a biocide when it is not inactivated by a commonly found in-use concentration of a biocide, or a biocide concentration that inactivates other strains of that organism.

Antimicrobial susceptibility: Describes the degree to which a target microorganism is affected by an antimicrobial agent. There are no clear "cut-off" concentrations that are widely accepted to denote sensitivity or resistance of the various bacterial species to disinfecting agents.

Antiseptic agent: A substance applied topically to living tissue that prevents or inhibits the growth of microorganisms.

Biocide/ Biocidal products: According to the Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market, biocidal products are defined as "Active substances and preparations containing one or more substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert to controlling effect on any harmful organism by chemical or biological means". The word "biocide" is in common use, and means a "biocidal product".

Biofilm: Microbial biofilms are populations of microorganisms that are concentrated at an interface (usually solid/liquid), and typically surrounded by an extracellular polymeric slime matrix. Flocs are suspended aggregates of microorganisms surrounded by an extracellular polymeric slime matrix that is formed in liquid suspension.

Co-resistance: The process in which selection for one type of antimicrobial also selects for resistance to another antimicrobial agent due to linkage of the resistance genes on the same genetic unit.

Cross-resistance: The process in which resistance to one antimicrobial agent confer resistance to another since the same mechanism of resistance applies to both drugs.

Disinfectant: A substance that is used in the inanimate environment to destroy or eliminate a specific species or groups of microorganism.

Intrinsic resistance: A natural property of an organism resulting in decreased susceptibility to a particular antimicrobial agent.

Minimum Inhibitory Concentration (MIC): The lowest concentration of a given agent that inhibits growth of a microorganism under standard laboratory conditions.

Normal flora: Indigenous microbial flora of human external, and some internal, surfaces like the skin, mouth and gastrointestinal tract and the upper respiratory tract.
The normal flora contains numerous bacterial species, and numerous strains within each species. Although it may contain pathogens, the vast majority are commensals that contribute to general health as well as to resistance to colonization by pathogens. However, some of the low-virulent bacteria of the normal flora may, under certain circumstances, become opportunistic pathogens.

Selection: A process by which some bacterial species or strains of bacteria, in a population are selected for by having a specific advantage over other microorganisms. Antibacterial substances may provide a more resistant sub-population with such an advantage, enabling them to increase their relative prevalence.

Strain: A subset within a bacterial species differing by some minor, but identifiable differences.

Summary

The fields of application of quaternary ammonium compounds (QACs) are wide ranging, and comprise numerous industrial purposes, water treatment, antifungal treatment in horticulture, as well as inclusion in pharmaceutical and every day consumer products. In cosmetic products, QACs are added as preserving agents, due to their ability to condition hair and/or their claimed effects on skin, nails or lips.

The most important QACs in cosmetic formulations are benzalkonium chloride, stearalkonium chloride, cetrimonium chloride, cetrimonium bromide and cetylpyridin chloride.

It is evident that resistance towards QACs is widespread among a diverse range of microorganisms, and that microbial resistance to QACs are facilitated by several mechanisms. Currently, available literature on development of resistance due to QACs in cosmetics is lacking. However, it is likely that QACs in such products will add to the selection pressure towards more QAC resistant microorganisms among the skin and mouth flora. Furthermore, there are increasing evidence of co-resistance and cross-resistance between QACs and a range of other unrelated antibacterial agents as antibiotics and disinfectants. Thus, the contribution to increased occurrence of resistance to clinically important antimicrobial agents by QACs in cosmetic products cannot be excluded.

Samandrag

Kvartære ammoniumssambindingar (QAC) vert brukte på ei rekkje område som i industrien, til handsaming av vatn, mot soppinfeksjonar i landbruket og som tilsetjing til farmasøytiske preparat og i vanlege konsumentprodukt. I kosmetiske produkt blir QAC nytta som konserveringsmiddel eller grunna evna desse stoffa har til å gi fylde til hår, eller påstått ynskte effektar på hud, negler eller lepper.

Dei viktigaste QAC i kosmetiske produkt er benzalkonium klorid, stearalkonium klorid, cetrimonium klorid, cetrimonium bromid og cetylpyridin klorid.

Det er vel kjent at resistens mot QAC er utbreidd hos ei rekkje ulike mikroorganismar, og at denne resistensen er knytt til mange mekanismar. Det er ikkje tilgjengeleg vitskapeleg litteratur om utvikling av resistens som fylje av bruk QAC i kosmetikk. På den andre sida er det sannsynleg at tilsetjing av QAC til slike produkt vil bidra til seleksjon av QAC resistente mikroorganismar i hud og munnholefloraen. Vidare er det aukande grunnlagsinformasjon som peikar på at det kan oppstå ko- og kryss-resistens mellom QAC og fleir andre ulike antibakterielle stoff, som antibiotika og desinfeksjonsmiddel. Ein kan på denne måten ikkje sjå bort frå at bruken av QAC i kosmetiske produkt kan bidra til ein auke i førekomsten av resistens mot klinisk viktige antimikrobielle stoff.

1. Background

In 2009, the Norwegian Scientific Committee for Food Safety (VKM), Panel on Biological Hazards, received a request from the Norwegian Food Safety Authority to develop a risk assessment, regarding development of resistance in microorganisms resulting from the use of QACs in cosmetic products. In response, an *ad hoc* Working Group of experts was appointed with the mandate to draft an assessment regarding this issue.

2. Definition of cosmetic products

According to the EU Cosmetics Directive (76/768/EEC), "a *cosmetic product* shall mean any substance or preparation intended to be placed in contact with the various external parts of the human body (epidermis, hair system, nails, lips and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odours and/or protecting them or keeping them in good condition." Furthermore, Norwegian regulations define *cosmetic products* as products that come into contact with the human body surface (skin, hair, nail, lip, and external genitals), teeth or mucous membranes of the oral cavity (Kosmetikklova, 2005*).*

3. Terms of reference

The Norwegian Food Safety Authority commissioned the Norwegian Scientific Committee for Food Safety to undertake a risk assessment on the application of Quaternary ammonium compounds (QACs) in cosmetics with special emphasis on the following topics[1]:

1- May use of cosmetic products containing QACs facilitate the development of resistance/reduced susceptibility among microorganisms?
2- May QACs in cosmetic products affect the efficacy of clinically important antimicrobials due to induction of microbial cross- or co-resistance? If so, which classes of agents?
3- May use of QAC containing cosmetic products alter the microflora of the skin and the oral cavity, or influence the virulence of these microorganisms?

4. Hazard identification

Hazard identification is implicit in the title of this risk assessment and in the terms of reference.

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[1] **Oppdrag**

Mattilsynet ba VKM om en risikovurdering i forhold til bruk av kvartære ammoniumsforbindelser i kosmetiske produkter. Følgende spørsmål ble spesielt ønsket besvart:

1- Kan bruk av QAC i kosmetiske produkter føre til resistens/nedsatt QAC følsomhet hos mikroorganismer?
2- Kan man få redusert effekt av klinisk viktige antimikrobiell midler som følge av mulig resistensutvikling ved bruk av QAC i kosmetiske produkter? I tilfelle hvilke klasser av antimikrobielle midler kan bli berørt?
3- Kan bruk av QAC i kosmetiske produkter gi endringer i huds og munnhulens mikrobiota og dens virulensegenskaper?
4-

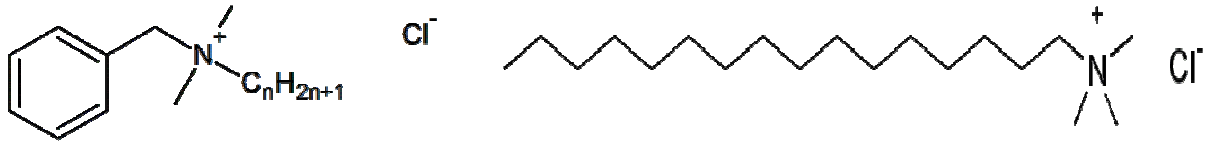
5. Hazard characterization

5.1. Characteristics

5.1.1. Chemical structure and properties

Quaternary ammonium compounds (QACs), also known as "quats", are of the general structure $NR_1R_2R_3R_4^+ X^-$, with R being hydrogen atoms, plain alkyl groups or alkyl groups substituted with other functional groups, and X being an anion. Figure 1. shows benzalkonium chloride and cetrimonium chloride, as examples of the chemical structure of QACs. In solution, the QACs dissociate into a quaternary ammonium cation and its anionic counterpart. Unlike the ammonium ion (NH_4^+) itself and the primary, secondary, or tertiary ammonium cations, the quaternary ammonium cations are permanently charged, independent of the pH of the solvent in which they are found. QACs are characterised by their ability to reduce the surface tension of water. This property places the QACs in the broad chemical group of surface active compounds, commonly referred to as either wetting agents, detergents, emulsifiers or dispersing agents. Surface active compounds are often divided into anionic detergents of acidic nature, and cationic detergents of which QACs are the most important.

Commonly applied QACs are benzalkonium chloride (BC), stearalkonium chloride (SC), cetrimonium chloride/bromide (CC/CB) and cetylpyridin chloride (CPC). Common trade quality of BC is a mixture of alkyl-benzyl-dimethyl-ammonium chlorides with various even-numbered straight alkyl chain lengths, usually from C_8 to C_{18} (Wikepedia 2008), with C_{12} to C_{14} predominating in pharmaceutical products (Xue et al., 2004). In its dry form BC is a yellowish to white powder. It is readily soluble in water, ethanol and acetone making solutions with a colour ranging from clear to a pale yellow (O´Neil 2006). Aqueous solutions tend to foam strongly when shaken. Standard concentrates of BC in solutions are commonly manufactured as 50% to 80% w/w products. Stearalkonium chloride is alkyl-benzyl-dimethyl-ammonium chloride with alkyl carbon chain-length of 18. The bromine and chlorine salts of cetrimonium (cetyl trimethyl ammonium) are common QACs with an alkyl chain length of 16. Of these, cetrimonium chloride is the most frequently used in household products, shampoos and cosmetics (Wikepedia 2009; Merianos, 2001).



n = 8, 10, 12, 14, 16, 18

Benzalkonium chloride

Cetrimonium chloride

Figure 1. Basic structure of benzalkonium chloride, where n is possible numbers of carbons in the alkyl chain of the molecule and cetrimonium chloride (Wikepedia 2008).

The first observations of an antimicrobial activity among the QACs were published in 1916, but the full potential for this class of agents were not realised until the 1930s. At this time it was reported that long chain QACs of which at least one of the four radicals was a plain or substituted aliphatic group, with a number of carbons between 8 and 18, possessed germicidal activity (Rahn & van Eseltine 1947).

5.1.2. Stability

QACs are generally referred to as "hard antibacterial agents" since they are poorly metabolized and mainly excreted in a non-metabolized form (Thorsteinsson *et al.,* 2003).

Surfactants used in the industry or for disinfection of surface, instrument and skin will inevitably reach the environment via the waste water (Kümmerer 2004). As expressed by the water/octanol distribution coefficient, QACs are not likely to bioaccumulate in food webs (Kümmerer 2004). However, due to their positive charge the QAC cations tend to adsorb strongly to the negative surfaces of sludge, soil and sediments.

The fate of QACs in the environment has been a subject for study by several authors. The biodegradability of QACs is generally considered to be poor, even though considerable variations are observed depending on the substance (Kümmerer 2004; Sullivan 1983). In a recent study (Clara *et al.*, 2007) the occurrence of surfactants in sewage, before and after treatment in full scale municipal plants, were reported. The authors found significant differences in the concentration of BCs in the sewage depending on the alkyl chain length. In this study, the C_{12} and C_{14} BC homologues were found to be the most prevalent, with a maximum concentration in untreated sewage of 170 and 110$\mu g l^{-1}$, respectively. The observed removal of the BCs in the sewage treatment plants under study, varied from 91 to >99 %, as measured in terms of differences of influx and efflux concentrations. The authors conclude that the main mechanism of BC reduction was biotransformation (80-94%), and adsorption to particles that were subsequently removed as excess sludge material (6-20%). In general the biodegradability seems to be increasing under anaerobic conditions (Clara *et al.,* 2007), with decreasing concentration of the substance in question (Dean-Raymond & Alexander 1977; Sutterlin *et al.,* 2008a). Sutterlin *et al.* (2008a) conducted a closed bottle biodegradation test of dissolved QACs held under dark conditions. In this test BC, was found to have a biodegradability of 36 % within the experimental period of 28 days.

5.1.3. Antimicrobial activity

QACs have antimicrobial effect against a broad range of microorganisms including vegetative bacteria, yeast, moulds, algae and viruses (Fredell 1994; Merianos 2001). QACs can inhibit germination of bacterial spores and the growth of vegetative bacteria, yeast, moulds and algae. The growth inhibitory activity of QACs is higher for Gram-positive bacteria and algae compared to Gram-negative bacteria and moulds. At higher concentrations, QACs are lethal to vegetative bacteria, yeasts, mould, algae, and lipophilic viruses, but not to bacterial spores, mycobacteria or hydrophilic viruses (Fredell 1994; Merianos 2001). The lethal effect of QACs is in some studies over-estimated since residual disinfectants may inhibit growth of surviving microorganisms.

The lethal and inhibitory effects of QACs are highly dependent on environmental factors (Fredell 1994). The efficacy is reduced at high microbial density and in the presence of organic material. This may be the case in some practical situations, for example for microorganisms growing in biofilms or attached to surfaces. Reduced efficacy on surfaces has been reported (Gronholm *et al.,* 1999; Ntsama-Essomba *et al.,* 1997). The antimicrobial effect of QACs increases with increasing temperature and exposure time and in the presence of chelators, such as EDTA. QACs are less active in "hard water", thus high levels of mineral salts as calcium and magnesium ions inactivates QACs.

The antimicrobial activity of QACs is a function of the compounds *n*-alkyl chain length, and thus the lipophilicity. For Gram-positive bacteria and yeast the optimal activity is achieved with chain lengths of n = 12-14, while for Gram-negative bacteria the activity maximises with chain lengths of n = 14-16. Compounds with *n*-alkyl chain lengths of $n < 4$ or $n > 18$ are virtually inactive (Gilbert & Moore 2005).

5.1.4. Mode of action and pharmacokinetics

The antimicrobial activity of QACs are primarily related to their cationic surfactant properties (Fredell 1994). QACs affect a number of different molecules, such as proteins and lipids. The lethal activity of QACs on virus is linked to detachment of the envelope of enveloped viruses causing release of nucleic acid (Shirai *et al.,* 2000). Exposure of non-enveloped viruses to QACs leads to formation of micelles but is not lethal. QACs may also act through denaturation of viral proteins (Maillard 2001). The main mode of action of QACs against microbial cells is interaction with cell membranes causing disruption and leakage of the cellular content (Gilbert & Moore 2005; Ioannou *et al.,* 2007). QACs predominantly act at the level of the cytoplasmic membrane. The action involves association of the positively charged quaternary nitrogen with the head group of acidic-phospholipids before integration of the hydrophobic tail into the hydrophobic membrane core. At high concentrations, QACs solubilise hydrophobic cell membrane components by forming mixed micellar aggregates (Gilbert & Moore 2005). Disruption and denaturation of structural proteins and enzymes has been suggested as other mechanisms behind antimicrobial activity (Fredell 1994).

In a study using rats as model animals (Xue *et al.,* 2004), it was found that after a single parenteral administration of 15 mg/kg of BC, the plasma elimination half lives ($t_{1/2}$) were found to be between 1.3 to 1.5 hours depending on the site of injection. Similar investigations have not been conducted in humans.

As additives to cosmetics, the QACs will generally be used as a “leave-on agent” on skin or mucous membranes. There is, however, no information about possible absorption through the skin.

Data from the seventies indicate that the initial oral retention of CPC after a single mouthrinse of a 10 ml, 2.2 mM solution will be around 65 % of the administered dose. The oral retention after a similar mouthrinse with chlorhexidine is lower (around 32 %) (Bonesvoll & Gjermo 1978). The binding of CPC seems to be weaker than that of chlorhexidine (Rolla & Melsen 1975), resulting in a faster oral clearance of CPC. However, CPC may be measured in saliva samples more than 24 hours after the mouthrinse. Chlorhexidine on the other hand, may be detected in the mouth even days after application.

5.2. Resistance mechanisms

Bacterial resistance to QACs can be considered as being either intrinsic or acquired.

5.2.1. Intrinsic resistance

Intrinsic resistance to QACs is demonstrated by Gram-negative bacteria (especially *Pseudomonas aeruginosa*), bacterial spores, mycobacteria, and under certain conditions staphylococci. Spore coats and cortex seem to be responsible for the high resistance of bacterial spores to QACs, and the most likely mechanism for high resistance of mycobacteria is their complex lipid-containing cell walls that provide an effective barrier of entry. In Gram-negative bacteria intrinsic resistance was initially attributed to lower permeability due to the outer membrane preventing the QAC reaching the cytoplasmic membrane. In *P. aeruginosa*

the outer membrane is responsible for its high resistance due to the high cation content aiding in formation of strong LPS-LPS links, and the presence of small sized porins not permitting general diffusion. A less acidic outer membrane LPS probably contributes to some *Proteus* strains high intrinsic resistance to QACs, whereas the slime layer of mucoide *Staphylococcus aureus* seem to protect these Gram-positive cells against the QAC cetrimide (McDonnel & Russel 1999).

In addition, intrinsic resistance is often associated with the activity of basal levels of efflux by pumps actively removing QAC from the membrane core. These efflux pumps are either acting alone, or in concert with decreased expression of porins. Furthermore, chromosomal efflux pumps can be induced so that an apparently susceptible strain can overproduce a pump to become resistant. The multidrug efflux pump SdeY in *Serratia marcescens* (Chen *et al.,* 2003) and AcrAB-TolC multidrug efflux pumps in *Salmonella enterica* serovar Typhimurium and *Escherichia coli* contribute to intrinsic resistance to QACs. These AcrAB-TolC multidrug efflux pumps can further be up-regulated in response to external stimuli (Levy 2002; Randall *et al.,* 2007). BC is both an inducer and a substrate for the MexCD-OprJ multidrug efflux pump in *P. aeruginosa* (Morita *et al.,* 2003) and the MdrL efflux pump in *Listeria monocytogenes* (Romanova *et al.,* 2006).

The intrinsic resistance of hydrophilic viruses towards QACs is probably due to the hydrophobic tails of QACs being unable to insert in and thus disrupt the hydrophilic capsid of these viruses.

5.2.2. Acquired resistance

Acquired antimicrobial resistance can result from the mutation of normal cellular genes, the acquisition of foreign resistance genes, or a combination of these two mechanisms. Although mutations play an important role in the evolution of antimicrobial resistance, the predominant factor for the escalation of antimicrobial resistance is the acquisition of antimicrobial resistance genes.

Reported changes in susceptibility to QACs have been associated with hyper-expression or acquisition of efflux pumps that actively remove QAC from the membrane. Also, reduced permeability or stablisation of the membrane through modifications in LPS, phospholipids or membrane proteins have been linked to increased resistance (Braoudaki & Hilton 2005; Gilbert & Moore 2005).

Finally, changes in susceptibility may also include other hitherto unknown mechanisms. In *E. coli* gradually adapted to BC concentrations of seven to eight times the initial MIC, changes in expression levels were shown among others for efflux pumps, porins and components related to the multiple antibiotic resistance regulon and to protection against oxidative stress (Bore *et al.,* 2007).

Mutations can contribute to increased expression of the efflux pump protein or to an amino acid substitution in the efflux protein that makes the protein more efficient at export. MICs of antimicrobial agents for efflux mutants over-expressing the efflux pump protein typically are two to eight fold higher that the MICs of these agents for the susceptible strain. Increased QAC efflux can be achieved by over-expression of chromosomal efflux protein PmpM in *P. aeruginosa* (He *et al.,* 2004) and multidrug efflux proteins MdeA (Huang *et al.,* 2004) and MepA (Huet *et al.,* 2008; Kaatz *et al.,* 2005) in *S. aureus* transporting BC or NorA in *S.*

aureus and ArcAB-TolC in *Salmonella enterica* serovar Typhimurium transporting cetrimide (Piddock 2006).

Acquisition of plasmid-encoded efflux pumps are particularly important mechanisms for resistance to QACs. In staphylococci loss of susceptibility to QAC can be mediated by QacA, QacB, Smr, the semi-functional derivative QacEΔ1, QacG, QacH, and QacJ efflux (Bjorland *et al.,* 2003; Heir *et al.,* 1998; Kazama *et al.,* 1998; Paulsen *et al.,* 1996; Poole 2002). The Qac determinants are usually plasmid borne and they confer less susceptibility to cationic antiseptic agents including dyes (acriflavine, ethidium bromide), biguanides (chlorhexidine digluconate, *qacA* only), and QACs (BC). A number of studies carried out during the past 10-15 years demonstrate the presence of Qac determinants in different staphylococcal species isolated from various sources like humans, health care settings, livestock animals, pets, the environment of veterinary clinics and from the food industry (Anthonisen *et al.,* 2002; Bjorland *et al.,* 2005; Bjorland *et al.,* 2003; Correa *et al.,* 2008; Heir *et al.,* 1995; Noguchi *et al.,* 1999; Noguchi *et al.,* 2005; Sidhu *et al.,* 2007; Sidhu *et al.,* 2002b). This indicates widespread distribution of these determinants among the various staphylococcal species colonizing different hosts and occurring in diverse environments. The QacA, QacB, and Smr determinants are common in clinical strains of *S. aureus* and other staphylococci. The location of the Qac determinants on mobile genetic elements enables these to intermingle between the various staphylococcal species, including both the coagulase-negative and positive staphylococci. Smr and QacEΔ1 have also been identified in *Enterococcus faecalis* (Paulsen *et al.,* 1996; Poole 2002; Bjorland *et al.,* 2003; Kazama et al., 1998; Heir et al., 1998). The QacE determinant is the semi-functional derivative QacEΔ1. This determinant confers low level export of QACs, and may thus be masked by the intrinsic resistance to QACs in Gram-negative species. In such species, QacF, QacG, QacH, QacI, and OqxAB exporter proteins mediate reduced susceptibility to QACs as seen for *Klebsiella pneumoniae*, *P. aeruginosa*, *Enterobacter* spp., *Pseudomonas* spp., *Helicobacter pylori*, *S. enterica*, *S. marcescens*, *Vibrio* spp., *Campylobacter* spp., *Stenotrophomonas maltophilia*, *Citrobacter freundii*, *Aeromonas* spp., *Proteus* spp., and *Morganella morganii* (Ceccarelli *et al.,* 2006; Hansen *et al.,* 2007; L'abee-Lund & Sorum 2001; Naas *et al.,* 2001; Plante *et al.,* 2003; Ploy *et al.,* 1998; Poole 2008; Schluter *et al.,* 2005).

5.3. Resistance among microbes in the normal flora of humans, in foods and in the environment

The microflora of the skin and the nasal cavity

The skin is generally an unfavourable place for microbial growth. It is subjected to periodic drying and the pH-value ranges from 3 to 5, which is non-optimal for most bacterial species. However, certain species are able to establish themselves under such conditions, and then in turn constitute the normal flora of the skin. The skin may be considered as a single organ, but its flora varies from different location of skin surfaces. Bacterial populations in warm humid places like the axillas, umbilicus and interdigital spaces are rich and numerous in contrasts to the microflora on dryer parts of the skin. Hair follicles, sebaceous glands and sweat glands provide attractive habitats for microorganisms, where a variety of bacteria and fungi reside (Høiby 1993; Linton 1982; Madigan *et al.,* 1997; Tortora *et al.,* 1989; Tortora *et al.,* 1998). Many factors may influence the composition of the microflora of the skin, like the temperature, humidity, age, sex, race and occupation (Roth & James 1988).

The skin flora consists primarily of Gram-positive bacteria. These include, examined by cultivation-based studies, species of *Staphylococcus* spp., *Micrococcus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Propionibacterium* spp. and yeasts belonging to the genus *Pityrosporum* (Cogen *et al.,* 2008; Gao *et al.,* 2007; Høiby 1993; Madigan *et al.,* 1997; Tortora *et al.,* 1989). However, little is known about the presence of non-cultivable or rare species in the microbiota of the skin, suggesting that a more complex microflora may be present (Gao *et al.,* 2007).

The staphylococcal group can be divided into two subdivisions; the coagulase-positive and the coagulase-negative. The coagulase-positive staphylococci are regarded as the most virulent, and are more often associated with disease than the coagulase-negative staphylococci. *S. aureus* is the most common coagulase-positive *Staphylococcus* species in man. *S. aureus* can sometimes be found on the skin of individuals who are nasal carriers, as the skin can be contaminated with bacteria from the mucosal linings of the nose. Several species of coagulase-negative staphylococci can be found on human skin, *Staphylococcus epidermidis* being the most common (Cogen *et al.,* 2008). Gram-negative bacteria are almost always minor constituents of the normal skin flora, *Acinetobacter* spp. are exceptions (Cogen *et al.,* 2008; Gao *et al.,* 2007; Madigan *et al.,* 1997; Martro *et al.,* 2003).

The anterior nares (nostrils) are always heavily colonized by microorganisms. The most common bacterial species include *S. epidermidis*, other coagulase-negative staphylococci, *Corynebacterium* spp. and *Micrococcus* spp. (Lina *et al.,* 2003; Linton 1982). The nasal vestibule is the main carrier site for *S. aureus*, and it is estimated that 20-30 % of the general population is colonized with this pathogen (Lecomte *et al.,* 2001; Peacock *et al.,* 2001).

Since staphylococci are major inhabitants of the normal flora of skin, they can be exposed to QACs via various products like disinfectants, hygienic handwashes and cosmetic products. QAC exposure will constitute a selection pressure on the bacterial populations and it is believed that *qac* containing staphylococci have advantages, even if the user-concentrations of the various products (particularly in products for medical use) are many times higher than the MICs of resistant strains.

The selection pressure on staphylococci of the normal flora of the skin from QACs in cosmetic products is not easily predicted. The microorganisms may be exposed to highly variable QAC concentrations via the different cosmetic products. Of special concern are QAC concentrations enabling microbes with acquired resistance to survive better than other susceptible variants of the same species. This will lead to an emergence of strains with selective advantages and a reduction of susceptible variants. Sub-inhibitory selection pressure on *qac* containing staphylococci is particularly noteworthy as staphylococci are important pathogens involved in human infections. *S. epidermidis* and other coagulase-negative staphylococci are increasingly recognized as cause of nosocomial infections, they also play important roles in implant-related infections. *S. aureus* is of special concern since this is a leading human pathogen. Staphylococci harbouring *qac* genes can be resistant to other antimicrobial agents (described under section 5.5). Selection of *qac* containing staphylococci may therefore contribute to increased occurrence of strains resistant to antimicrobial agents.

There has been a dramatic increase in *S. aureus* strains being resistant to antimicrobial agents, including meticillin resistant *S. aureus* (MRSA). MRSA are considered resistant to all beta-lactam antibiotics and are considered a major threat in human medicine. Several studies have shown the occurrence of *qac* genes in MRSA isolates (Gillespie *et al.,* 1986; Mayer *et al.,*

2001; Noguchi *et al.,* 1999; Noguchi *et al.,* 2005). An early investigation showed that among MRSA strains isolated in Japan in 1992, 10.2% contained the *qacA/B* gene and 20.4% contained the *smr* gene (Noguchi *et al.,* 1999). In another study it is reported that among MRSA strains from Europe isolated during the period 1997 to 1999, 63 % contained the *qacA/B* gene and six % contained the *smr* gene (Mayer *et al.,* 2001). Numerous successful MRSA clones are described and characterized, many of them have a worldwide dissemination (Deurenberg *et al.,* 2007). The presence of *qac* genes among the many established MRSA clones is currently not known.

Acinetobacter spp. can be a part of the normal skin flora. *Acinetobacter baumannii* is an important nosocomial pathogen with increasing importance. Of special significance is the increasing frequency of resistance to a variety of antimicrobial agents. Unfortunately, little is known about the susceptibility of *Acinetobacter* spp. to disinfecting agents (Martro *et al.,* 2003; Wisplinghoff *et al.,* 2007).

There are currently no knowledge about reduced susceptibility to disinfectants among other microorganisms being part of the normal flora of the skin and nasal cavity.

The microbiota of the gastro-intestinal tract

The bacteria of the gastrointestinal (GI) tract include the commensal species that permanently colonise the tract and a variable set of living bacteria that transit temporarily through the tract. The flora is complex with 300-500 different species among which 30-40 species comprise 99%. Around 50% are non-cultivable. Microbiologically the GI tract can be divided into three parts, the stomach, the small intestines and the colon. The colon is by far the most heavily colonised part with a total population of 10^{11} to 10^{12} CFU/ml of content. The study of the gut flora has earlier relied on quantitative culture of faecal microorganisms indicating the dominant genera to be *Bacteriodes, Bifidobacterium, Eubacterium, Clostridium, Lactobacillus,* and *Fusobacterium* (Guarner 2006). Studies based on 16S rRNA analyses have confirmed that *Bacteroides* is the predominant group in adult feces (Kolida *et al.,* 2006).

There are currently no available data on possible effects of QACs on the GI tract flora, nor on the fate of QACs when ingested. By use of mouthrinses containing QACs, low doses might reach the GI tract.

Oral microbiota

The bacterial community of the oral cavity is diverse and abundant and one of the most complex mixtures of bacteria known. There are now more than 800 species identified and more species are continuing to be discovered. Common bacterial taxa include Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria, Proteobacteria and Spirochetes. More than 100 species are likely to be found at any oral site. Less than 50 % of the flora is cultivable. Commensal oral streptococci constitute a major part of the bacterial flora that gradually becomes more complex with time. The streptococci form the basis for adhesion of other colonizing bacteria as *Actinomyces* and *Veillonella* species. The composition varies at various sites in the oral cavity, depending on nutrient availability, salivary flow rate and oxygen tension as well as on the dental and gingival health conditions.

The oral bacteria exist mainly as biofilms on mucosal and tooth surfaces representing a wide variety of polymicrobial ecosystems. In health there is a balance between the bacterial flora and the host. The indigenous flora represents an important barrier preventing colonization by

potential pathogens. Thus disruption of the ecological balance by for instance antimicrobial agents may bear consequences for health.

The wide range of applications of QACs is related to the ability to adsorb onto a variety of surfaces and the broad spectrum of antimicrobial activity over a wide pH range. Being cationic, the molecules can bind to carboxyl-, phosphate- and hydroxyl-groups of negatively charged surfaces. This binding allows the agent to bind to oral mucosal surfaces and to remain in the oral cavity for prolonged periods of time. This so-called substantivity is considered important for their oral antibiofilm effects, although QACs lose some of their activity upon adsorption to surfaces (Baker *et al.,* 1978; Moran & Addy 1984).

Mouthwashes containing QACs are advertised mainly as anti-halitosis agents. The quaternary ammonium compounds BC, benzetonium chloride and CPC, have been studied also for their effect on oral biofilms. Being cationic surfactants, they have some similarities with chlorhexidine. The positive charge leads to attraction to negatively charged proteins on the bacterial surface resulting in physical disruption of the membrane, dissipation of the proton motive force, and inhibition of membrane associated enzymes. It is assumed that the interaction with bacteria occurs via cationic binding to phosphate groups of cell wall teichoic acid in Gram-positive bacteria (Albert 1942) and to phosphate groups in the cell wall and membrane of Gram-negative bacteria (Heptinstall *et al.,* 1970). Mouthrinses containing 0.07 or 0.1% CPC reduced oral biofilm formation by 15 % and 16 % respectively, up to 40 h after the rinse (Rawlinson *et al.,* 2008). Both dental biofilm and gingival inflammation was reduced by 16 % after six months twice daily rinses with 0.07% CPC mouthrinse (Mankodi *et al.,* 2005).

Due to the superior effect of chlorhexidine, the interest in the QACs as oral hygiene adjuncts is waning. Higher concentrations and increased application frequency of products containing QACs is needed to obtain the same efficacy as with chlorhexidine. The bitterness of for instance CPC would normally dictate a tolerable concentration of no more than 0.1 %. The concentration generally found in marketed mouthwashes is 0.05-0.07 %.

There are no published reports on resistance to QACs among oral bacteria

Environment

As QACs enter the environment they may be expected to act on microbes found here. (Kümmerer *et al.,* 1997) found BC in a concentration of 4 to 5 mg/l^{-1} in hospital effluent water, and QACs are reported to be effective against aquatic microorganisms at these concentrations (Tubbing & Admiraal 1991). The toxicity of QACs to *Vibrio fisheri* and *Pseudomonas putida* have been examined (Sutterlin *et al.,* 2008b). The authors found BC to have an EC_{10} (Effect Concentration), defined as the concentration giving 10% reduction in cell proliferation or bioluminescence, of 3.9 mg/l for *P. putida* and 0.2 mg/l for *V. fisheri*. The study also shows that ion pair formation with other non biocide anionic compounds had a minor effect on the EC_{10} under the test conditions applied. In the study of (McBain *et al.,* 2004) the effect of QACs on bacterial community dynamics and antimicrobial susceptibility in a drain microcosm with mixed cultures were examined. In this study QAC exposure caused both increased susceptibility among some strains (*Pseudomonas* spp. and *Enterococcus saccharolyticus*), and decreased susceptibility to QACs among other strains (*Psesudomonas* spp*., Eubacterium* spp., *Chryseobacterium* spp., *Ralstonia* spp., and *Aranicola* spp.).

The effect of QACs on the microbes in biological waste water treatment plants is well known. Nitrifying bacteria used in biological filters in sewage treatment plants, seems to be particularly affected, as QACs interfere with their normal uptake of ammonium (Sutterlin *et al.,* 2008b).

Food-related microorganisms
QACs are often used for disinfection in food production environments. The targets in such environments are pathogenic and spoilage bacteria, moulds and yeasts.

In suspension, user-concentrations of QAC are lethal to most vegetative food pathogens such as *S. aureus, L. monocytogenes, S. enterica* serovar Enteritidis*, S. enterica* serovar Typhimurium and *E. coli* O157:H7 (Gonzales-Fandos *et al.,* 2005; Gronholm *et al.,* 1999; Jacquet & Reynaud 1994; Van de *et al.,* 1993).

Reduced susceptibility of the *Staphylococcus* spp., *L. monocytogenes* and lactic acid bacteria isolated from food or from the food production environment has been reported (Aarestrup *et al.,* 2007; Aase *et al.,* 2000; Heir *et al.,* 1995; Heir *et al.,* 1999; Sidhu *et al.,* 2001b). The level of resistance in these species in suspension is however far below the user concentration of QAC. Survival after exposure to user-concentrations of QACs has been reported for *L. monocytogenes* grown as biofilm (Aarnisalo *et al.,* 2003; Best *et al.,* 1990).

High intrinsic resistance and survival after exposure to in-use concentrations of QACs have been reported for some Gram-negative spoilage bacteria such as *Pseudomonas* spp. and *S. marcescens* (Langsrud *et al.,* 2003a; Langsrud & Sundheim 1997; Sidhu *et al.,* 2002b; Willinghan *et al.,* 1996). In general, bacteria isolated after disinfection are more resistant to QAC than laboratory strains (Langsrud *et al.,* 2003b). As Gram-negative bacteria may adapt to growth at higher concentrations of QAC when repeatedly exposed to sub-lethal concentrations (Ishikawa *et al.,* 2002; Langsrud & Sundheim 1997; Langsrud *et al.,* 2004; Mangalappalli-Illathu & Korber 2006; Nicoletti *et al.,* 1993) it has been speculated that inefficient disinfection may contribute to development of resistant strains in food production environments.

Bacteria in biofilm survive higher concentrations of QAC than their planktonic counterparts. Sub-MIC concentrations of QACs have been shown to induce biofilm formation in *S. epidermidis* but inhibit biofilm formation in *E. coli* (Houari & Di Martino 2007). This implies that exposure of some bacteria to low concentrations of QACs may result in increased biofilm formation and subsequent higher survival after disinfection.

The resistance of food-associated moulds and yeast varies among species and strains within species, with some isolates being resistant to the in-use concentration of QACs (Bundgaard-Nielsen & Nielsen 1996; Korukuoglu *et al.,* 2006). Ascospores from moulds are more resistant than vegetative yeast or mould cells (Jones *et al.,* 1991).

5.4. Susceptibility testing

The wide range of areas of applications and target organisms make it difficult to establish relevant and standardised tests for microbial susceptibility. For QACs, both the ability to inhibit growth and to kill microorganisms may be of importance, depending on the area of use. The test conditions highly influence the results and make it difficult to compare between

investigations and to extrapolate from laboratory conditions to practical conditions (Bloomfield *et al.,* 1995; Gilbert *et al.,* 1987; Johnston *et al.,* 2000; Langsrud & Sundheim 1998; Nicoletti *et al.,* 1993). Test conditions of special importance is temperature, time, level of organic material, growth conditions and growth phase of the microorganisms to be tested, and the method for detection of surviving microorganisms. Although laboratory tests give some information about antimicrobial activity of QACs, they have to be tested in practical situations to get relevant information.

Screening for antimicrobial activity
In tests for the general inhibitory activity, the microorganisms are exposed to QACs in nutrient suspension or nutrient agar, and growth is determined after incubation for a specific time. The main advantages of the MIC-method are that it is easy to perform and many strains or QACs can be tested in the same experiment. The applicability of the MIC method is limited for strains/species with low susceptibility because of precipitation of QAC in the nutrient broth/agar at sub-inhibitory concentrations. There are no standard methods for determining the MIC of QACs, and various approaches are therefore described in the literature.
In biocidal tests, microorganisms in suspension or on a surface are exposed to QAC for a specific period of time, followed by neutralisation of QAC and determination of the number of viable microbes. The main advantages of these methods are that all types of disinfectants can be tested and the effects of temperature and interfering substances may be included. Biocidal tests are easy to perform, but more time-consuming and less reproducible than the MIC test (Nicoletti *et al.,* 1993) and for that reason they are mostly used when investigating a few strains and a small number of antibacterial agents.

Testing susceptibility under practical-use conditions
Standardised challenge tests have been developed to test efficacy of preservatives in cosmetic products (British Pharmacopeia 2000; United States Pharmacopeia 2002). These methods could potentially be adapted to test susceptibility of microorganisms under practical-use conditions. The challenge tests are based on inoculation of the pharmaceutical/cosmetic product with bacteria, incubation and sampling for survivors during the storage period (Russell 2003).

5.5. Resistance link between QAC and other antimicrobial agents

A number of efflux pumps have been shown to mediate export of both QACs and other antimicrobial agents by the same pump.

Among such chromosomally encoded efflux pumps are:

- The MdrL pump in *L. monocytogenes* extruding macrolides and cefotaxime in addition to QAC (Romanova *et al.,* 2006).
- Efflux protein MdeA in *S. aureus* transporting BC and the antibiotics fusidic acid, mupirocin, virginiamycin, and novobiocin (Huang *et al.,* 2004).
- MepA in *S. aureus* transporting a range of structurally different cations such as BC, ethidium bromide, chlorhexidine, and pentamidin and to a lesser extent fluoroquinolones (Huet *et al.,* 2008; Kaatz *et al.,* 2005).
- NorA in *S. aureus* transporting cetrimide, chloramphenicol, and fluoroquinolones,
- *E. coli* AcrAB-TolC pump exporting biocides such as QACs, triclosan and chlorhexidine as well as multiple antibiotics (Levy 2000).

- AcrAB-TolC in *S. enterica* serovar Typhimurium transporting cetrimide, the antibiotics chloramphenicol, quinolones, and tetracycline, as well as triclosan (Piddock 2006).
- Pump SdeY in *S. marcescens* (Chen *et al.,* 2003) conferring reduced susceptibility to several antimicrobial agents including erythromycin, tetracycline, norfloxacin in addition to BC.
- The MexCD-OprJ pump in *P. aeruginosa* induced by BC to enhance resistance also to clinically relevant antibiotics such as quinolones, macrolides, tetracyclines, lincomycin, chloramphenicol, novobiocin, meropenem, most penicillins, and most cephems (Masuda *et al.,* 2000; Morita *et al.,* 2003).
- Efflux protein PmpM in *P. aeruginosa* transporting BC and fluoroquinolones (He *et al.,* 2004).

Among plasmid encoded efflux pumps are the QacA efflux protein and OqxAB. QacA efflux protein is common in clinical strains of *S. aureus* and other staphylococci specifying resistance to a range of structurally dissimilar cations such as BC, cetrimide, and chlorhexidine (Paulsen *et al.,* 1996; Poole 2002). The OqxAB has been found in *E. coli* strain of porcine origin, and was found on a plasmid conferring reduced susceptibility to chloramphenicol, quinolones, trimethoprim, quinoxalines, BC, and triclosan (Hansen *et al.,* 2007).

In addition there are a number of examples of co-resistance of QAC determinants and other antimicrobial agents by linkage of different resistance mechanisms on the same genetic unit such as on the same plasmid (A), transposon or integron (B) or rather on a combination of these (C-E).

A. QAC determinants are typically found on multi-resistance plasmids from clinical strains of *S. aureus* and other staphylococci (Archer *et al.,* 1986; Paulsen *et al.,* 1996). *qacA/B* and a gene conferring resistance to β-lactams have proved to co-reside on large plasmids in various staphylococcal species both of clinical and food-processing origin (Anthonisen *et al.,* 2002; Bjorland *et al.,* 2005; Sidhu *et al.,* 2002a; Sidhu *et al.,* 2001a) and such plasmids can be taken up by plasmid-free *S. aureus* indicating that the resistance genes has a potential to be transferred to pathogens under selective stress (Sidhu *et al.,* 2001a).
B. *qacH* has been found in clinical isolates of *S. maltophilia* on an integron also encoding resistance to aminoglycosides and chloramphenicol (Chang *et al.,* 2007).
C. The OqxAB determinant is located on a transposon adjacent to a gene conferring resistance to β-lactams on a plasmid showing broad host range among *Enterobacteriaceae*. This plasmid also has predicted regions encoding self-transfer of the plasmid as well as plasmid addiction systems that maintain the plasmid in the bacteria after entry (Norman *et al.,* 2008).
   A transposon encoding QacF and a predicted plasmid addiction system has also been found on a self-transmissible plasmid encoding resistance to amoxicillin, spectinomycin, streptomycin and sulfonamides in *E. coli* (Schluter *et al.,* 2007).
D. Self-transmissible broad host range plasmids from wastewater treatment plant bacteria have been shown to encode QAC efflux pumps in combination with resistance to other antimicrobials on the same integron such as the MexCD-OprJ multidrug efflux together with an aminoglycocide resistance determinant or combination of QacF, aminoglycoside, β-lactam, and sulphonamide resistance (Schluter *et al.,* 2007).

Other such examples are *qacE* in *Klebsiella aerogenes* on a broad-host range plasmid on an integron also encoding trimethoprim resistance (Paulsen *et al.,* 1993),
QacF in clinical strains of *Enterobacter aerogenes* and *Enterobacter cloacae* encoded on a plasmid on an integron also encoding resistance to aminoglycosides, chloramphenicol, and β-lactams (Plante *et al.,* 2003; Ploy *et al.,* 1998),
qacG in a clinical *P. aeruginosa* isolate on a plasmid on an integron also encoding resistance to an extended spectrum of β-lactams, chloramphenicol, and aminoglycosides (Laraki *et al.,* 1999), and
QacH in clinical isolates of *Vibrio cholerae* O1 encoded on an integron on a large conjugative plasmid also encoding resistance to aminoglycosides, chloramphenicol, tetracycline, trimethoprim, β-lactams, and sulphonamide (Ceccarelli *et al.,* 2006).

E. The QacI determinant has been found on a plasmid- and transposon-located integron also encoding resistance to an extended spectrum of β-lactams, chloramphenicol, aminoglycosides, rifampicin, and sulphonamide in a clinical isolate of *E. coli* (Naas *et al.,* 2001).

Given the ability of efflux systems to accommodate both QACs and antimicrobials used to treat infectious diseases and co-localisation of QAC determinants and other resistance determinants on the same genetic unit, the possibility exists of QACs selecting for organisms expressing/over-expressing these efflux systems and, so, resistance to antibiotics. This is supported by the recent study of bacterial isolates from the hand of primary caregivers in households using antibacterial consumer products containing QACs for a year, compared to hand floras in households not using QACs, finding that bacterial isolates with BC MICs higher than the median BC MIC distribution were more likely to be resistant to one or more antibiotics after assigned product usage (Carson *et al.,* 2008).

Biocides have been used for a longer period in clinical practice than antibiotics, and a *qac* resistance gene, or remnant thereof, is a normal feature of class 1 integrons widespread in clinical isolates. Diverse *qac* gene cassettes are a dominant feature of cassette arrays from environmental class 1 integrons, and they also occur in the absence of any antibiotic resistance gene cassettes. Evidence suggests that the clinical class 1 integron originated when one of these chromosomal class 1 integrons mobilized into a Tn*402*-like transposon and the initial selective advantage was conferred by resistance to biocides, mediated by *qac*. The abundance of *qac* gene cassettes makes them a likely source of a readily selectable phenotype. Thus, the increasing use of QACs seems likely to promote the fixation of further novel genetic elements (Gillings *et al.,* 2009).

Further, co-localisation of QAC determinants and other resistance determinants on mobile elements such as plasmids and transposons may contribute to the transfer of resistance into other bacteria. In addition, the finding of QAC determinants together with other resistance determinants on integrons which are collectors of resistance cassettes that readily pick up new additional resistance determinants, on broad-host range self-transmissible plasmids is particularly disturbing as the resistance collectors have found an efficient vehicle in order to travel among different species.

6. Link between QAC resistance and pathogenicity; genotype and phenotype

Efflux pumps that confer antimicrobial resistance in microorganisms may probably have greater clinical relevance than previously assumed. Certain classes of efflux pumps not only harbour resistance to antimicrobial agents used in therapy, but also have a role in bacterial pathogenicity. Efflux pumps that export antimicrobial agents may also export virulence determinants, such as adhesins, toxins, and other proteins which are important for colonization and survival of bacteria in host (Piddock 2006). Piddock (2006) reviews several studies that have demonstrated that the lack of efflux pump expression by Gram-negative bacteria like *S. enterica* serovar Typhimurium, *E. coli*, *Erwinia amylovora*, *P. aeruginosa*, *Campylobacter jejuni*, and *Neisseria gonorrhoeae* has a deleterious effect on the ability of the bacterium to be pathogenic in animal models.
Such efflux pumps, which can mediate cross-resistance due to export of both QACs and other antimicrobial agents have been mentioned under section 5.5.

7. Exposure assessment

The Nordic countries have established an open web based system for registration of substances in consumer products (SPIN, Substances in Products in the Nordic Countries, http://www.spin2000.net**)**. The SPIN register does not include components used in cosmetic products. Table 1. shows the total registered consumption of QACs excluding cosmetic products in Norway during 2007, divided into different application categories.

Category	**Amount (metric tons)**
Production and maintenance of equipment, machinery or vehicles as cars, boats and airplanes	64.3
Oil, gas and petro chemistry related activity	49.4
Building and construction related activity	42.4
Chemical industry	17.0
Private consumption	15.4
Timber and paper production	13.6
Food, feed and beverage production	12.4
Cleaning	7.7
Paint, varnish and ink production	6.3
Hotel and restaurants	4.4
Transport (sea and land based)	4.3
Soaps, detergents, perfumes and toilet supplies	3.6
Textile and furniture production	3.5
Metallurgy	2.0
Agri-and aquaculture, fishing and catch related activity	0.8
Sewage and renovation related activity	0.2
Pharmaceutical raw material production	0.1
Miscellaneous	10.4
Sum	**257.8**

Table 1. shows the total registered consumption of all SPIN registered QACs in Norway, excluding cosmetics, during 2007. Information extracted from the SPIN data base (Substances in Products in the Nordic Countries, http://www.spin.net). This database contains information on substances in consumer products, excluding cosmetics, in the Nordic countries.

According to the SPIN register on non cosmetic application of QACs, the total consumption of BC in Norway during 2006 was 17.4 tons, and this QAC were found in 19 products

including formulations for “cleaning”, “sewage and sanitation related activity” and for “private application”. SC does not seem to be applied in the Nordic countries after 2003, with the exception of Denmark where 16.8 tons were applied in a total of 649 formulations. This is also the case for CB, and this substance was applied in Denmark in 2005 and 2006. In 2006, 0.1 tons were applied in eight formulations in Denmark. CC is found in four products in Norway, but the consumption of this compound is too small to be declared in the SPIN data base. Furthermore data for CPC is not available at SPIN.

The most important QAC compounds in cosmetic products are benzalkonium chloride (BC), stearalkonium chloride (SC), cetrimonium chloride (CC), cetrimonium bromide (CB) and cetylpyridin chloride (CPC).

In cosmetic products, QACs are added as preserving agents, foam boosters and detergents due to their ability to condition hair and/or their claimed positive effects on skin, nails or lips. QACs are also added to mouthwashes with the intention of halitosis reduction, to prevent dental biofilm formation and gingivitis development. As an example, BC is reported used in the following product types: eye drops/artificial tears, decongestion nose drops, facial moisturizers/treatment formulations, sunscreens and other products with a sun protection factor, facial cleansers, acne treatment formulations, baby lotions, moisturizers, pain relief and hand sanitizers.

In the following the amount consumed in Norway, and common concentrations are summarised, with the Norwegian Food Safety Authority (2009) as the source. The total consumption of QACs included in cosmetic products has been estimated to 30 metric tons. This figure does not include QACs found in cosmetics purchased by Norwegians during travel abroad. The approximate consumption of BC accounts for 0.13 tons annually, SC 3 to 5 tons, CC and CB 16 tons, and CPC 0.02 tons.

According to current EU legislation **(**Council Directive 76/768/EEC), the permitted concentration of BC and SC used as preserving agents is 0.1 %, whereas the permitted concentrations when used for other purposes are from 0.1 % to 3.0 %. The highest concentration (3.0 %) is only allowed in products intended for immediate removal, as is the case for hair conditioners.

As for BC and SC, the permitted concentration of the cetrimonium salts (CC and CB) used as preserving agents is 0.1 %. For CC and CB, the cosmetic industry use concentration up to 2.5 % in products intended for rapid removal, such as hair conditioners. Information presented in journals from the cosmetic branch itself, indicates the normally found concentrations of these cetrimide salts to be between 1 and 2 %.

Currently there are no regulations regarding the permitted concentration of CPC in cosmetic products. Reported concentrations of CPC in cosmetic products vary from 0.1 to 2.0 %.

8. Data gaps

Published information on the pharmacokinetics of QACs is scarce. Thus, the stability of these agents on the skin and mucous membranes are not well described and the absorption, distribution, metabolism and excretion in animals and humans is not thoroughly described in the available literature.

There seems to be a lack of reliable statistics on the consumption of QACs in the food industry, in dental practice, as well as for veterinary- and human medical purposes.

Conclusive information on the development of resistance due to QACs in cosmetic products is currently lacking.

Knowledge about changes in the virulence of microbes of the skin microbiota due to QAC exposure is currently lacking.

9. Risk characterization: Answer to the questions

9.1. May use of cosmetic products containing QACs facilitate the development of resistance/reduced susceptibility among microorganisms?

It is evident that resistance towards QACs is widespread among a diverse range of microorganisms. Microbial resistance to QACs is facilitated by several mechanisms, as modifications in the membrane composition, expression of stress response and repair systems or increased expression of efflux pump genes excluding these agents from the cell. Plasmid mediated, and thus transferable efflux pumps are particularly important mechanisms for resistance to QACs. Published literature on resistance development from QACs in cosmetic products is lacking.

Development of resistance both in pathogenic and non-pathogenic bacteria, related to practical application, in human medicine and food industry has been well documented. QACs in cosmetic products will inevitably come into very intimate contact with skin or the mucosal linings in the mouth, and it is likely that in such products they will add to the selection pressure towards more QAC resistant microorganisms among the skin and mouth flora.

9.2. May QACs in cosmetic products affect the efficacy of clinically important antimicrobials due to induction of microbial cross- or co-resistance? If so, which classes of agents?

Many reports have shown that there are resistance links between QAC and other antimicrobial agents, and a recent study supports the possibility of QACs selecting for organisms resistant to antibiotics (see section 5.5.).

Cross-resistance can be mediated by a number of efflux pumps both in Gram-negative and Gram-positive bacteria that have been shown to export both QACs and clinically important antimicrobial agents. Such plasmid encoded efflux pumps may confer resistance to clinically important antibacterial agent classes such as quinolones, amphenicols, trimethoprim, and quinoxalines, or to the biocides triclosan and chlorhexidine. Further, such chromosomally encoded efflux pumps may also transport clinically important antibacterial agents in classes as macrolides, β-lactams, quinolones, amphenicols, fusidic acid, mupirocin, tetracyclines, and lincomycin, the anti-protozoan pentamidin or other biocides than QAC, such as chlorhexidine and triclosan. However, chromosomally encoded pumps often must be induced to confer resistance and after over-expression the levels of antibiotic resistance are sometimes relatively low and unlikely to compromise therapeutic effectiveness.

Reports of multi-resistant plasmids showing co-resistance of QAC determinants and other antimicrobial agents in clinical strains is increasing and dissemination of such plasmids is clearly evidenced. Such plasmids have been reported to encode resistance to clinically important antibacterial agent classes such as β-lactams, aminoglycosides, amphenicols, sulfonamides, trimethoprim, tetracycline, and rifampicin. Thus, there is a potential for both cross- and co-resistance of QAC to other antimicrobial agents.

Chronic exposure of the environment to QAC might expose microbial communities to sub-inhibitory concentrations causing emergence of more resistant clones with changes in their susceptibility to third-party antibiotics. Further, co-localisation of QAC determinants and other resistance determinants on mobile elements such as plasmids and transposons may contribute to transfer of resistance into other bacteria.

In addition, recent publications suggest that use of QACs may have driven the fixation and spread of certain integrons (resistance cassette collectors), now responsible for a major part of antibiotic resistance. Thus, broader and more indiscriminate use of QACs may drive the emergence of new genetic elements, with unpredictable consequences.

In summary, there are increasing evidence of co- and cross-resistance between QACs and a range of unrelated antibacterial agents such as antibiotics and disinfectants. Therefore, application of QACs in cosmetic products may contribute to increased occurrence of resistance to clinically important antimicrobial agents. The contribution of QACs in cosmetics to such resistance compared to the use in other applications or the use of other antibacterial agents is not clear.

9.3. May use of QAC containing cosmetic products alter the microflora of the skin and the oral cavity, or influence the virulence of these microorganisms?

The selection pressure on microorganisms of the skin microbiota from QACs in cosmetic products is difficult to predict, as knowledge about the concentrations and the stability of the QAC compounds under practical conditions is lacking. QACs in the many different cosmetic products may lead to exposure to highly variable concentrations. This may represent lethal concentrations for some microorganisms, whereas others may be exposed to sub-inhibitory concentrations. This may in turn influence the composition of the microflora leading to reduction of species being highly susceptible to QACs, and to an increase of microorganisms with selective advantages. However, cosmetic products are in many cases applied on relatively small and limited areas of the skin. The possibility of a re-colonisation with microbes from other areas of the skin surface may contribute to re-establishment of the common skin flora. The overall effect on the skin microbiota from QACs in cosmetic products is therefore uncertain. On the other hand, staphylcocci constitute a significant proportion of the normal flora of skin and sub-inhibitory selection pressure on *qac* containing staphylococci should be considered with concern. Staphylococci are important pathogens involved in human infections; *S. epidermidis* and other coagulase-negative staphylococci are frequently recognized as the cause of nosocomial infections and implant-related infections. *S. aureus* is a leading pathogen and the emergence of MRSA represents a major health threat in human medicine. Selection of *qac* containing staphylococci may therefore contribute to a microflora containing a higher level of bacterial species being potentially pathogenic to humans.

Knowledge about changes in virulence of microbes of the skin microbiota due to QAC exposure is currently lacking.

It is well known that horizontal gene transfer readily occurs among bacteria in oral biofilms. Thus resistance genes are likely to be shared and spread among oral bacteria. Although data are presently lacking, it cannot be ruled out that oral exposure to QACs might alter and influence virulence of the oral microbiota.

10. Conclusions

The fields of application of the quaternary ammonium compounds (QACs) are wide ranging, and include the addition of QACs as benzalkonium chloride, stearalkonium chloride, cetrimonium chloride, cetrimonium bromide and cetylpyridin chloride in cosmetic products.

Even though some information is available, there seems to be a lack of reliable statistics on the amounts of QACs used, including the application in cosmetic products.

The main conclusions on the questions raised by The Norwegian Food Safety Authority in the Terms of reference are:

- It is evident that resistance towards QACs is widespread among a diverse range of microorganisms, and that the observed QAC resistance is facilitated by several mechanisms.
- Conclusive information on the development of resistance due to QACs in cosmetic products is currently lacking.
- It is likely that QACs in cosmetic products will add to the selection pressure towards more QAC resistant microorganisms among the skin and mouth flora.
- There are increasing evidence of co- and cross-resistance between QACs and a range of unrelated antibacterial agents such as antibiotics and disinfectants. Thus, addition of QACs in cosmetic products may contribute to increased occurrence of resistance to clinically important antimicrobial agents.
- Even though, the overall effect on the skin microbiota from QACs in cosmetic products is uncertain, selection for QAC resistant staphylococci should be considered with concern as these microorganisms are important pathogens involved in human infections.
- Knowledge about changes in the virulence of microbes of the skin microbiota due to QAC exposure is currently lacking.
- Although data are presently lacking, it cannot be ruled out that exposure to QACs may alter and influence the virulence of the oral microbiota.

References

Aarestrup, F. M., Knochel, S., & Hasman, H. 2007, "Antimicrobial susceptibility of Listeria monocytogenes from food products", *Foodborne.Pathog.Dis.,* **4**, 216-221.

Aarnisalo, K., Autio, T., Sjoberg, A. M., Lunden, J., Korkeala, H., & Suihko, M. L. 2003, "Typing of Listeria monocytogenes isolates originating from the food processing industry with automated ribotyping and pulsed-field gel electrophoresis", *J Food Prot.,* **66**, 249-255.

Aase, B., Sundheim, G., Langsrud, S., & Rorvik, L. M. 2000, "Occurrence of and a possible mechanism for resistance to a quaternary ammonium compound in Listeria monocytogenes", *Int J Food Microbiol,* **62**, 57-63.

Albert, A. 1942, "Chemistry and physics of antiseptics in relation to mode of action", *Lancet,* **2**, 633-636.

Anthonisen, I. L., Sunde, M., Steinum, T. M., Sidhu, M. S., & Sorum, H. 2002, "Organization of the antiseptic resistance gene qacA and Tn552-related beta-lactamase genes in multidrug-resistant Staphylococcus haemolyticus strains of animal and human origins", *Antimicrobial Agents and Chemotherapy,* **46**, 3606-3612.

Archer, G. L., Coughter, J. P., & Johnston, J. L. 1986, "Plasmid-encoded trimethoprim resistance in staphylococci", *Antimicrobial Agents and Chemotherapy,* **29**, 733-740.

Baker, P. J., Coburn, R. A., Genco, R. J., & Evans, R. T. 1978, "The in vitro inhibition of microbial growth and plaque formation by surfactant drugs", *J Periodontal.Res.,* **13**, 474-485.

Best, M., Kennedy, M. E., & Coates, F. 1990, "Efficacy of a variety of disinfectants against Listeria spp", *Appl.Environ.Microbiol,* **56**, 377-380.

Bjorland, J., Steinum, T., Kvitle, B., Waage, S., Sunde, M., & Heir, E. 2005, "Widespread distribution of disinfectant resistance genes among staphylococci of bovine and caprine origin in Norway", *J Clin.Microbiol,* **43**, 4363-4368.

Bjorland, J., Steinum, T., Sunde, M., Waage, S., & Heir, E. 2003, "Novel plasmid-borne gene qacJ mediates resistance to quaternary ammonium compounds in equine Staphylococcus aureus, Staphylococcus simulans, and Staphylococcus intermedius", *Antimicrobial Agents and Chemotherapy,* **47**, 3046-3052.

Bloomfield, S. F., Arthur, M., Gibson, H., Morley, K., Gilbert, P., & Brown, m. R. W. 1995, "Development of reproducible test inocula for disinfectant testing: , v. 36, p. 311-331.", *International Biodeterioration and Biodegradation,* **36**, 311-331.

Bonesvoll, P. & Gjermo, P. 1978, "A comparision between chlorhexidine and some quaternary ammonium compounds with regard to retention, salivary concentration and plaque-inhibiting effect in the human mouth after mouth rinses", *Arch Oral Biol.,* **23**, 289-294.

Bore, E., Hebraud, M., Chafsey, I., Chambon, C., Skjaeret, C., Moen, B., Moretro, T., Langsrud, O., Rudi, K., & Langsrud, S. 2007, "Adapted tolerance to benzalkonium chloride in

Escherichia coli K-12 studied by transcriptome and proteome analyses", *Microbiology,* **153**, 935-946.

Braoudaki, M. & Hilton, A. C. 2005, "Mechanisms of resistance in Salmonella enterica adapted to erythromycin, benzalkonium chloride and triclosan", *Int.J Antimicrob.Agents,* **25**, 31-37.

British Pharmacopeia 2000, *Efficacy of Antimicrobial Preservation (Appendix XVIC),* HMSO, London.

Bundgaard-Nielsen, K. & Nielsen, P. V. 1996, "Fungicidal effect of 15 disinfectants against 25 fungal contaminants commonly found in bread and cheese manufacturing", *J Food Prot.,* **59**, 268-275.

Carson, R. T., Larson, E., Levy, S. B., Marshall, B. M., & Aiello, A. E. 2008, "Use of antibacterial consumer products containing quaternary ammonium compounds and drug resistance in the community", *J Antimicrob.Chemother.,* **62**, 1160-1162.

Ceccarelli, D., Salvia, A. M., Sami, J., Cappuccinelli, P., & Colombo, M. M. 2006, "New cluster of plasmid-located class 1 integrons in Vibrio cholerae O1 and a dfrA15 cassette-containing integron in Vibrio parahaemolyticus isolated in Angola", *Antimicrob Agents Chemother,* **50**, 2493-2499.

Chang, L.-L., Li, H.-H., Chan, C.-Y., & Lu, P. L. 2007, "Increased incidence of class 1 integrons in trimethoprim/sulfamethoxazole-resitant clinical isolates of *Stenotrophomonas maltophilia*", *J Antimicrob Chemother,* **59**, 1038-1045.

Chen, J., Kuroda, T., Huda, M. N., Mizushima, T., & Tsuchiya, T. 2003, "An RND-type multidrug efflux pump SdeXY from Serratia marcescens", *J Antimicrob Chemother,* **52**, 176-179.

Clara, M., Scharf, S., Scheffknecht, C., & Gans, O. 2007, "Occurrence of selected surfactants in untreated and treated sewage", *Water Research,* **41**, 4339-4348.

Cogen, A. L., Nizet, V., & Gallo, R. L. 2008, "Skin microbiota: a source of disease or defence?", *Br J Dermatol.,* **158**, 442-455.

Correa, J. E., De Paulis, A., Predari, S., Sordelli, D. O., & Jeric, P. E. 2008, "First report of qacG, qacH and qacJ genes in Staphylococcus haemolyticus human clinical isolates", *J Antimicrob.Chemother.,* **62**, 956-960.

Dean-Raymond, D. & Alexander, M. 1977, "Bacterial metabolism of quaternary ammonium compounds", *Appl.Environ.Microbiol,* **33**, 1037-1041.

Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A., & Stobberingh, E. E. 2007, "The molecular evolution of methicillin-resistant Staphylococcus aureus", *Clin Microbiol.Infect,* **13**, 222-235.

Fredell, D. L. 1994, "Biological properties and applications of cationic surfactants," in *Cationic surfactants*, J. Cross & E. J. Singer, eds., Marcel Dekker, New York.

Gao, Z., Tseng, C. H., Pei, Z., & Blaser, M. J. 2007, "Molecular analysis of human forearm superficial skin bacterial biota", *Proc.Natl.Acad.Sci.U S A,* **104**, 2927-2932.

Gilbert, P., Brown, M. R., & Costerton, J. W. 1987, "Inocula for antimicrobial sensitivity testing: a critical review", *J Antimicrob.Chemother.,* **20**, 147-154.

Gilbert, P. & Moore, L. E. 2005, "Cationic antiseptics: diversity of action under a common epithet", *J Appl.Microbiol,* **99**, 703-715.

Gillespie, M. T., May, J. W., & Skurray, R. A. 1986, "Plasmid-encoded resistance to acriflavine and quaternary ammonium compounds in methicillin-resistant *Staphylococcus aureus*", *FEMS Microbiol Lett.,* **34**, 47-51.

Gillings, M. R., Xuejun, D., Hardwick, S. A., Holley, M. P., & Stokes, H. W. 2009, "Gene cassettes encoding resistance to quaternary ammonium compounds: a role in the origin of clinical class 1 integrons?", *ISME.J,* **3**, 209-215.

Gonzales-Fandos, E., Sanz, J., Garcia-Fernandez, M. C., & Garcia-Arias, M. T. 2005, "Effectiveness of disinfectants used in the food industry on microorganisms of sanitary interest: , v. 34, p. 253-258.", *Acta Alimentaria,* **34**, 253-258.

Gronholm, L., Wirtanen, G., Ahlgren, K., Norstrom, K., & Sjoberg, A. M. 1999, "Screening of antimicrobial activities of disinfectants and cleaning agents against foodborne spoilage microbes: v. 208, p. 289-298.", *ZEITSCHRIFT FUR LEBENSMITTEL-UNTERSUCHUNG UND-FORSCHUNG A-FOOD RESEARCH AND,* **208**, 289-298.

Guarner, F. 2006, "Enteric flora in health and disease", *Digestion.,* **73 Suppl 1**, 5-12.

Hansen, L. H., Jensen, L. B., Sorensen, H. I., & Sorensen, S. J. 2007, "Substrate specificity of the OqxAB multidrug resistance pump in Escherichia coli and selected enteric bacteria", *J Antimicrob Chemother,* **60**, 145-147.

He, G. X., Kuroda, T., Mima, T., Morita, Y., Mizushima, T., & Tsuchiya, T. 2004, "An H(+)-coupled multidrug efflux pump, PmpM, a member of the MATE family of transporters, from Pseudomonas aeruginosa", *J Bacteriol.,* **186**, 262-265.

Heir, E., Sundheim, G., & Holck, A. 1998, "The *Staphylococcus qacH* gene product: a new member of the SMR family encoding multidrug resistance", *FEMS Microbiology Letters,* **163**, 49-56.

Heir, E., Sundheim, G., & Holck, A. L. 1995, "Resistance to quaternary ammonium compounds in Staphylococcus spp. isolated from the food industry and nucleotide sequence of the resistance plasmid pST827", *J Appl.Bacteriol.,* **79**, 149-156.

Heir, E., Sundheim, G., & Holck, A. L. 1999, "Identification and characterization of quaternary ammonium compound resistant staphylococci from the food industry", *Int J Food Microbiol,* **48**, 211-219.

Heptinstall, S., Archibald, A. R., & Baddiley, J. 1970, "Teichoic acids and membrane function in bacteria", *Nature,* **225**, 519-521.

Høiby, N. 1993, *Basal og klinisk mikrobiologi* FADL`s forlag., Copenhagn, Denmark.

Houari, A. & Di Martino, P. 2007, "Effect of chlorhexidine and benzalkonium chloride on bacterial biofilm formation", *Lett.Appl.Microbiol,* **45**, 652-656.

Huang, J., O'Toole, P. W., Shen, W., Amrine-Madsen, H., Jiang, X., Lobo, N., Palmer, L. M., Voelker, L., Fan, F., Gwynn, M. N., & McDevitt, D. 2004, "Novel chromosomally encoded multidrug efflux transporter MdeA in Staphylococcus aureus", *Antimicrob Agents Chemother,* **48**, 909-917.

Huet, A. A., Raygada, J. L., Mendiratta, K., Seo, S. M., & Kaatz, G. W. 2008, "Multidrug efflux pump overexpression in Staphylococcus aureus after single and multiple in vitro exposures to biocides and dyes", *Microbiology,* **154**, 3144-3153.

Ioannou, C. J., Hanlon, G. W., & Denyer, S. P. 2007, "Action of disinfectant quaternary ammonium compounds against Staphylococcus aureus", *Antimicrobial Agents and Chemotherapy,* **51**, 296-306.

Ishikawa, S., Matsumura, Y., Yoshizako, F., & Tsuchido, T. 2002, "Characterization of a cationic surfactant-resistant mutant isolated spontaneously from Escherichia coli", *J Appl.Microbiol,* **92**, 261-268.

Jacquet, C. & Reynaud, A. 1994, "Differences in the sensitivity to eight disinfectants of Listeria monocytogenes strains as related to their origin (short note)", *Int J Food Microbiol,* **22**, 79-83.

Johnston, M. D., Simons, E. A., & Lambert, R. J. 2000, "One explanation for the variability of the bacterial suspension test", *J Appl.Microbiol,* **88**, 237-242.

Jones, M., Johnson, M., & Herd, T. 1991, "Sensitivity of yeast vegetative cells and ascospores to biocides and environmental stress", *Letters in Applied Microbiology,* **12**, 254-257.

Kaatz, G. W., McAleese, F., & Seo, S. M. 2005, "Multidrug resistance in Staphylococcus aureus due to overexpression of a novel multidrug and toxin extrusion (MATE) transport protein", *Antimicrobial Agents and Chemotherapy,* **49**, 1857-1864.

Kazama, H., Hamashima, H., Sasatsu, M., & Arai, T. 1998, "Distribution of the antiseptic-resistance genes qacE and qacE delta 1 in gram-negative bacteria", *FEMS Microbiology Letters,* **159**, 173-178.

Kolida, S., Saulnier, D. M., & Gibson, G. R. 2006, "Gastrointestinal microflora: probiotics", *Adv.Appl.Microbiol,* **59**, 187-219.

Korukuoglu, M., Sahan, Y., & Yigit, A. 2006, "The fungicidal efficacy of various commercial disinfectants used in the food industry", *Annals of Microbiology,* **56**, 325-330.

Kümmerer, K. 2004, "Emission from medical care units," in *Pharmaceuticals in the environment - sources, fate, effects and risks*, K. Kümmerer, ed., Springer, Berlin, pp. 27-44.

Kümmerer, K., Eitel, A., Braun, U., Hubner, P., Daschner, F., Mascart, G., Milandri, M., Reinthaler, F., & Verhuef, J. 1997, "Analysis of benzalkonium chloride in the effluent from European hospitals by solid-phase extraction and HPLC with post-column ion-pairing for fluoresce detection", *J.Chromatogra.,* **774**, 281-286.

L'abee-Lund, T. M. & Sorum, H. 2001, "Class 1 integrons mediate antibiotic resistance in the fish pathogen Aeromonas salmonicida worldwide", *Microb.Drug Resist.,* **7**, 263-272.

Langsrud, S., Moretro, T., & Sundheim, G. 2003a, "Characterization of Serratia marcescens surviving in disinfecting footbaths", *J Appl.Microbiol,* **95**, 186-195.

Langsrud, S., Sidhu, M. S., Heir, E., & Holck, A. L. 2003b, "Bacterial disinfectant resistance - a challenge for the food industry", *International Biodeterioration & Biodegradation,* 283-290.

Langsrud, S. & Sundheim, G. 1997, "Factors contributing to the survival of poultry associated Pseudomonas spp. exposed to a quaternary ammonium compound", *J Appl.Microbiol,* **82**, 705-712.

Langsrud, S. & Sundheim, G. 1998, "Factors influencing a suspension test method for antimicrobial activity of disinfectants", *J Appl.Microbiol,* **85**, 1006-1012.

Langsrud, S., Sundheim, G., & Holck, A. L. 2004, "Cross-resistance to antibiotics of Escherichia coli adapted to benzalkonium chloride or exposed to stress-inducers", *J Appl.Microbiol,* **96**, 201-208.

Laraki, N., Galleni, M., Thamm, I., Riccio, M. L., Amicosante, G., Frere, J. M., & Rossolini, G. M. 1999, "Structure of In31, a blaIMP-containing Pseudomonas aeruginosa integron phyletically related to In5, which carries an unusual array of gene cassettes", *Antimicrob Agents Chemother,* **43**, 890-901.

Lecomte, F., Nouvellon, M., & Levesque, H. 2001, "Nasal carriage of Staphylococcus aureus", *N.Engl.J Med,* **344**, 1399-1400.

Levy, S. B. 2000, "Antibiotic and antiseptic resistance: impact on public health", *Pediatr.Infect Dis J,* **19**, S120-S122.

Levy, S. B. 2002, "Active efflux, a common mechanism forbiocide and antibiotic resistance", *J Appl Microbiol Suppl,* **92**, 65S-71S.

Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J., & Vandenesch, F. 2003, "Bacterial competition for human nasal cavity colonization: role of Staphylococcal agr alleles", *Appl.Environ.Microbiol,* **69**, 18-23.

Linton, A. H. 1982, *Microbes, Man and Animals. The Natural History of Microbial Interactions. , UK.* John Wiley and Sons, UK.

Madigan, M. T., Martinko, J. M., & Parker, J. 1997, *Brock Biology of Microorganisms* Prentice-Hall International Limited, London, UK.

Maillard, J.-Y. 2001, "Virus susceptibility to biocides: an understanding", *Reviews in Medical Microbiology,* **12**, 63-74.

Mangalappalli-Illathu, A. K. & Korber, D. R. 2006, "Adaptive resistance and differential protein expression of Salmonella enterica serovar Enteritidis biofilms exposed to benzalkonium chloride", *Antimicrobial Agents and Chemotherapy,* **50**, 3588-3596.

Mankodi, S., Bauroth, K., Witt, J. J., Bsoul, S., He, T., Gibb, R., Dunavent, J., & Hamilton, A. 2005, "A 6-month clinical trial to study the effects of a cetylpyridinium chloride mouthrinse on gingivitis and plaque", *Am J Dent.,* **18 Spec No**, 9A-14A.

Martro, E., Hernandez, A., Ariza, J., Dominguez, M. A., Matas, L., Argerich, M. J., Martin, R., & Ausina, V. 2003, "Assessment of Acinetobacter baumannii susceptibility to antiseptics and disinfectants", *J Hosp.Infect.,* **55**, 39-46.

Masuda, N., Sakagawa, E., Ohya, S., Gotoh, N., Tsujimoto, H., & Nishino, T. 2000, "Substrate specificities of MexAB-OprM, MexCD-OprJ, and MexXY-oprM efflux pumps in Pseudomonas aeruginosa", *Antimicrobial Agents and Chemotherapy,* **44**, 3322-3327.

Mayer, S., Boos, M., Beyer, A., Fluit, A. C., & Schmitz, F. J. 2001, "Distribution of the antiseptic resistance genes qacA, qacB and qacC in 497 methicillin-resistant and -susceptible European isolates of Staphylococcus aureus", *J Antimicrob.Chemother.,* **47**, 896-897.

McBain, A. J., Ledder, R. G., Moore, L. E., Catrenich, C. E., & Gilbert, P. 2004, "Effects of quaternary-ammonium-based formulations on bacterial community dynamics and antimicrobial susceptibility", *Appl.Environ.Microbiol,* **70**, 3449-3456.

McDonnel, G. & Russel, A. D. 1999, "Antiseptics and disinfectants: activity, action, and resitance", *Clin Microbiol Rev,* **12**, 147-179.

Merianos, J. J. 2001, "Surface-active agents, *in* J. C. Block, ed., : ," in *Disinfection, sterilization and Preservation*, J. C. Block, ed., Lippincott Williams and Wilkins, Philidelphia.

Moran, J. & Addy, M. 1984, "The effect of surface adsorption and staining reactions on the antimicrobial properties of some cationic antiseptic mouthwashes", *J Periodontol.,* **55**, 278-282.

Morita, Y., Murata, T., Mima, T., Shiota, S., Kuroda, T., Mizushima, T., Gotoh, N., Nishino, T., & Tsuchiya, T. 2003, "Induction of mexCD-oprJ operon for a multidrug efflux pump by disinfectants in wild-type Pseudomonas aeruginosa PAO1", *J Antimicrob.Chemother.,* **51**, 991-994.

Naas, T., Mikami, Y., Imai, T., Poirel, L., & Nordmann, P. 2001, "Characterization of In53, a class 1 plasmid- and composite transposon-located integron of Escherichia coli which carries an unusual array of gene cassettes", *J Bacteriol.,* **183**, 235-249.

Nicoletti, G., Boghossian, V., Gurevitch, F., Borland, R., & Morgenroth, P. 1993, "The antimicrobial activity in vitro of chlorhexidine, a mixture of isothiazolinones ('Kathon' CG) and cetyl trimethyl ammonium bromide (CTAB)", *J Hosp.Infect.,* **23**, 87-111.

Noguchi, N., Hase, M., Kitta, M., Sasatsu, M., Deguchi, K., & Kono, M. 1999, "Antiseptic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant Staphylococcus aureus", *FEMS Microbiol Lett.,* **172**, 247-253.

Noguchi, N., Suwa, J., Narui, K., Sasatsu, M., Ito, T., Hiramatsu, K., & Song, J. H. 2005, "Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes qacA/B and smr of methicillin-resistant Staphylococcus aureus isolated in Asia during 1998 and 1999", *J Med Microbiol,* **54**, 557-565.

Norman, A., Hansen, L. H., She, Q., & Sorensen, S. J. 2008, "Nucleotide sequence of pOLA52: a conjugative IncX1 plasmid from Escherichia coli which enables biofilm formation and multidrug efflux", *Plasmid,* **60**, 59-74.

Ntsama-Essomba, C., Bouttier, S., Ramaldes, M., Dubois-Brissonnet, F., & Fourniat, J. 1997, "Resistance of Escherichia coli growing as biofilms to disinfectants", *Veterinary Research,* **28**, 353-363.

O´Neil, M. 2006, *The Merck Index, An encyclopedia of chemicals, drugs, and biologicals , .*, Fourtheenth edition edn, Merck & Co, NJ, USA.

Paulsen, I. T., Brown, M. H., Littlejohn, T. G., Mitchell, B. A., & Skurray, R. A. 1996, "Multidrug resistance proteins QacA and QacB from Staphylococcus aureus: membrane topology and identification of residues involved in substrate specificity", *Proc.Natl.Acad.Sci.U S A,* **93**, 3630-3635.

Paulsen, I. T., Littlejohn, T. G., Radstrom, P., Sundstrom, L., Skold, O., Swedberg, G., & Skurray, R. A. 1993, "The 3' conserved segment of integrons contains a gene associated with multidrug resistance to antiseptics and disinfectants", *Antimicrobial Agents and Chemotherapy,* **37**, 761-768.

Peacock, S. J., de, S., I, & Lowy, F. D. 2001, "What determines nasal carriage of Staphylococcus aureus?", *Trends Microbiol,* **9**, 605-610.

Piddock, L. J. V. 2006, "Multidrug-resistance efflux pumps - not just for resistance", *Nature Rev Microbiol,* **4**, 629-636.

Plante, I., Centron, D., & Roy, P. H. 2003, "Direct sequencing and PCR mapping of integrons reveals multiple class 1 integrons in the multiresistant strain Enterobacter cloacae SCH88040794", *FEMS Microbiol Lett.,* **221**, 59-62.

Ploy, M. C., Courvalin, P., & Lambert, T. 1998, "Characterization of In40 of Enterobacter aerogenes BM2688, a class 1 integron with two new gene cassettes, cmlA2 and qacF", *Antimicrobial Agents and Chemotherapy,* **42**, 2557-2563.

Poole, K. 2002, "Mechanisms of bacterial biocide and antibiotic resistance", *J Appl Microbiol Symp Suppl,* **92**, 55S-64S.

Poole, K. 2008, "Efflux pumps as antimicrobial resistance mechanisms", *Ann Med,* **39**, 162-176.

Rahn, O. & van Eseltine, W. P. 1947, "Quaternary ammonium compounds", *Annual Reviews of Microbiology,* **1**, 173-192.

Randall, L. P., Cooles, S. W., Coldham, N. G., Penuela, E. G., Mott, A. C., Woodward, M. J., Piddock, L. J., & Webber, M. A. 2007, "Commonly used farm disinfectants can select for mutant Salmonella enterica serovar Typhimurium with decreased susceptibility to biocides and antibiotics without compromising virulence", *J Antimicrob.Chemother.,* **60**, 1273-1280.

Rawlinson, A., Pollington, S., Walsh, T. F., Lamb, D. J., Marlow, I., Haywood, J., & Wright, P. 2008, "Efficacy of two alcohol-free cetylpyridinium chloride mouthwashes - a randomized double-blind crossover study", *J Clin.Periodontol.,* **35**, 230-235.

Rolla, G. & Melsen, B. 1975, "On the mechanism of the plaque inhibition by chlorhexidine", *J Dent.Res.,* **54 Spec No B**, B57-B62.

Romanova, N. A., Wolffs, P. F., Brovko, L. Y., & Griffiths, M. W. 2006, "Role of efflux pumps in adaptation and resistance of Listeria monocytogenes to benzalkonium chloride", *Appl.Environ.Microbiol,* **72**, 3498-3503.

Roth, R. R. & James, W. D. 1988, "Microbial ecology of the skin", *Annu.Rev Microbiol,* **42**, 441-464.

Russell, A. D. 2003, "Challenge testing: principle and practice", *International Journal of Cosmetic Science,* **25**, 147-153.

Schluter, A., Heuer, H., Szczepanowski, R., Poler, S. M., Schneiker, S., Puhler, A., & Top, E. M. 2005, "Plasmid pB8 is closely related to the prototype IncP-1beta plasmid R751 but transfers poorly to Escherichia coli and carries a new transposon encoding a small multidrug resistance efflux protein", *Plasmid,* **54**, 135-148.

Schluter, A., Szczepanowski, R., Puhler, A., & Top, E. M. 2007, "Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool", *FEMS Microbiol Rev,* **31**, 449-477.

Shirai, J., Kanno, T., Tsuchiya, Y., Mitsubayashi, S., & Seki, R. 2000, "Effects of chlorine, iodine, and quaternary ammonium compound disinfectants on several exotic disease viruses", *J Vet.Med Sci.,* **62**, 85-92.

Sidhu, M. S., Heir, E., Leegaard, T., Wiger, K., & Holck, A. 2002a, "Frequency of disinfectant resistance genes and genetic linkage with beta-lactamase transposon Tn552 among clinical staphylococci", *Antimicrobial Agents and Chemotherapy,* **46**, 2797-2803.

Sidhu, M. S., Heir, E., Sorum, H., & Holck, A. 2001a, "Genetic linkage between resistance to quaternary ammonium compounds and beta-lactam antibiotics in food-related Staphylococcus spp", *Microb.Drug Resist.,* **7**, 363-371.

Sidhu, M. S., Langsrud, S., & Holck, A. 2001b, "Disinfectant and antibiotic resistance of lactic acid bacteria isolated from the food industry", *Microb.Drug Resist.,* **7**, 73-83.

Sidhu, M. S., Oppegaard, H., Devor, T. P., & Sorum, H. 2007, "Persistence of multidrug-resistant Staphylococcus haemolyticus in an animal veterinary teaching hospital clinic", *Microb.Drug Resist.,* **13**, 271-280.

Sidhu, M. S., Sorum, H., & Holck, A. 2002b, "Resistance to quaternary ammonium compounds in food-related bacteria", *Microb.Drug Resist.,* **8**, 393-399.

Sullivan, D. E. 1983, "Biodegradation of a cationic surfactant in activated sludge", *Water Res,* **17**, 1145-1151.

Sutterlin, H., Alexy, R., Coker, A., & Kummerer, K. 2008a, "Mixtures of quaternary ammonium compounds and anionic organic compounds in the aquatic environment: Elimination and biodegradability in the closed bottle test monitored by LC-MS/MS", *Chemosphere,* **72**, 479-484.

Sutterlin, H., Alexy, R., & Kummerer, K. 2008b, "The toxicity of the quaternary ammonium compound benzalkonium chloride alone and in mixtures with other anionic compounds to bacteria in test systems with Vibrio fischeri and Pseudomonas putida", *Ecotoxicology and Environmental Safety,* **71**, 498-505.

Thorsteinsson, T., Loftsson, T., & Masson, M. 2003, "Soft antibacterial agents", *Curr Med Chem.,* **10**, 1129-1136.

Tortora, G. J., Funke, B. R., & case, C. L. 1989, *Microbiology: An introduction* The Benjamin/Cummings Publishing Company Inc., Redwood city, CA., USA.

Tortora, G. J., Funke, B. R., & case, C. L. 1998, *Microbiology: An introduction* The Benjamin/Cummings Publishing Company Inc., Redwood city, CA., USA.

Tubbing, D. M. J. & Admiraal, W. 1991, "Inhibition of bacterial and phytoplanktonic metabolic activity in the lower river Rhine by ditallowdimethylammonium chloride.57:3616-3622.", *Applied and Environmental Microbiology,* **57**, 3616-3622.

United States Pharmacopeia 2002, *US Pharmacopeial Convention (25. revision),* Rockville, MD.

Van de, W. A., Devleeschouwer, M. J., & Dony, J. 1993, "Bactericidal activity of disinfectants on Listeria", *J Appl.Bacteriol.,* **74**, 480-483.

Wikepedia. Benzalkonium chloride. 2008.
Ref Type: Internet Communication

Willinghan, E. M., Sander, J. E., Thayer, S. G., & Wilson, J. L. 1996, "Investigation of bacterial resistance to hatchery disinfectants", *Avian Diseases,* **40**, 510-515.

Wisplinghoff, H., Schmitt, R., Wohrmann, A., Stefanik, D., & Seifert, H. 2007, "Resistance to disinfectants in epidemiologically defined clinical isolates of Acinetobacter baumannii", *J Hosp.Infect.,* **66**, 174-181.

Xue, Y., Hieda, Y., Kimura, K., Takayama, K., Fujihara, J., & Tsujino, Y. 2004, "Kinetic characteristics and toxic effects of benzalkonium chloride following intravascular and oral administration in rats", *J Chromatogr.B Analyt.Technol.Biomed.Life Sci.,* **811**, 53-58