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Reduced susceptibility to chlorhexidine in staphylococci: is it increasing and does it matter?

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Antiseptic agents are increasingly used for hand hygiene and skin decolonization as key tools for the prevention of healthcare-associated infections. Chlorhexidine, a divalent, cationic biguanide, has a broad spectrum of activity and is one of the most frequently used topical antiseptic agents. Notably, there are an increasing number of prevalence studies that report reduced levels of susceptibility to chlorhexidine. In contrast to bacterial resistance to antibiotics, using parameters such as the MIC to define resistance to antiseptics, including chlorhexidine, is not straightforward. A range of methods have been used for the detection of reduced susceptibility to chlorhexidine, but, importantly, there is no standardized method and no consensus on the definition of chlorhexidine 'resistance'. In this review we have assessed the methods available for the detection of reduced susceptibility to chlorhexidine and the prevalence of coresistance to other antimicrobial agents. We have focused on the development of reduced susceptibility to chlorhexidine and the presence of efflux-mediated resistance genes in staphylococci, and have reviewed the clinical significance of this phenomenon. Lastly, we have identified unanswered questions to further our understanding of this emergent threat. We anticipate that clinical use of chlorhexidine will continue to increase, and it will be important to be alert to the possibility that this may lead to the emergence of new clones with reduced susceptibility. Indiscriminate chlorhexidine use in the absence of efficacy data should be discouraged.

Keywords: antiseptic, biocide, resistance, *Staphylococcus aureus*

Introduction

Over the past decade, there has been considerable focus on the prevention of healthcare-associated infections (HCAIs), including those caused by methicillin-resistant *Staphylococcus aureus* (MRSA). One of the cornerstones of preventative measures has been the use of antiseptic agents for hand hygiene and skin decolonization prior to invasive procedures. Due to its broad spectrum of activity, acceptable tolerability and good safety record, chlorhexidine is one of the most frequently used antiseptic agents, or biocides.[1] Consequently, there is a plethora of information available about chlorhexidine and its application for the prevention of *S. aureus* infections, in particular those caused by MRSA. The decreased availability of triclosan products following concerns about safety[2] and selection of antimicrobial resistance[3] has exacerbated the increasing exposure to chlorhexidine. Furthermore, there is increasing attention on the control of methicillin-susceptible *S. aureus* (MSSA), which will likely include further use of chlorhexidine-containing products.[4,5]

Not surprisingly, therefore, there are an increasing number of prevalence studies that report reduced levels of susceptibility to chlorhexidine, with emphasis on the susceptibility of MRSA. Importantly, a range of methods have been used for the detection of reduced susceptibility to chlorhexidine, but there is no standardized method and no consensus on the definition of chlorhexidine 'resistance'. Notably, many investigations of the mechanisms of chlorhexidine resistance include only Gram-negative organisms[6,7] and chlorhexidine may not be included in the panel of antiseptics tested.[8–10] There are a number of thorough reviews on bacterial resistance to biocides; however, these provide an overview of all biocides and include Gram-positive and -negative bacteria.[11–14] In addition, the Scientific Committee on Emerging and Newly Identified Health Risks have produced a report that assesses the antibiotic resistance effects of biocides.[15]

In contrast, we have reviewed the evidence for reduced susceptibility to chlorhexidine in staphylococci. We begin with a brief description of chlorhexidine, and a summary of its uses, benefits and disadvantages. We then focus on the development of reduced susceptibility to chlorhexidine in staphylococci, along with the presence of efflux-mediated resistance genes and the prevalence of coresistance. We conclude by posing questions that remain to be answered.

Description of chlorhexidine

Chlorhexidine [1,6-*bis*(4′-chlorophenylbiguanide)hexane] is a topical antiseptic that was first described in 1954.[16] It is a

divalent, cationic biguanide agent that exists as gluconate, acetate and hydrochloride salts.[17] Chlorhexidine is most commonly used at various concentrations (0.5%–4%) of the water-soluble gluconate form.[18] Chlorhexidine acts by binding to the negatively charged bacterial cell wall and affecting the osmotic equilibrium of the cell.[19] Briefly, the biguanide groups of the chlorhexidine molecules bind strongly to exposed anionic sites on the cell membrane and cell wall. The formation of bridges between adjacent phospholipid head groups displaces the divalent cations (Mg^{2+} and Ca^{2+}) that naturally stabilize the cell membrane, and as a result the cell membrane becomes leaky to potassium ions and protons.[20] At higher, in-use, concentrations, binding of chlorhexidine causes the membrane to lose structural integrity, which results in cell death.[17] Further details about the mechanism of action of chlorhexidine, and other antiseptics, can be found in an article by Gilbert and Moore.[20]

Chlorhexidine is most active against Gram-positive bacteria, but also has activity against Gram-negative bacteria, anaerobes, fungi and some enveloped viruses.[19,21,22] Evidence of chlorhexidine activity against mycobacteria is inconclusive and it has limited activity against non-enveloped viruses.[1] The agent is not active against bacterial spores.[1] Chlorhexidine is known to be less effective in the presence of organic material, such as serum.[23]

Uses and benefits of chlorhexidine

Hand hygiene is acknowledged to be one of the key elements of effective infection prevention and control in healthcare facilities, as multiresistant organisms, such as MRSA, are known to be transmitted between patients via hands.[24] The WHO recommends the use of alcohol-based hand rubs as 'the gold standard for hand hygiene in health care'.[25] Alcohol-based hand rubs may contain additional active ingredients, such as chlorhexidine, but the inclusion of such agents is not essential.

Chlorhexidine effectively reduces numbers of bacterial skin flora[26] and is available for use in aqueous form, combined with 70% isopropyl alcohol or as a dusting powder, depending on the application.[27] Chlorhexidine has been used in preparations for hand cleansing, both general and pre-surgical, for >50 years.[18,26] Equally important is its use in skin disinfection prior to surgical procedures and the insertion of peripheral and central vascular catheters. Guidelines recommend the use of 2% chlorhexidine gluconate in 70% isopropyl alcohol solution for skin preparation before catheter insertion.[28–30] Screening for carriage of MRSA followed by decolonization of colonized individuals has been a widespread strategy for the prevention and control of MRSA in hospitals. Decolonization usually consists of the application of a topical antibiotic, such as mupirocin, to the anterior nares and bathing with an antiseptic-containing body wash,[26,31] with chlorhexidine being the most frequently used antiseptic-containing body wash. In recent years, chlorhexidine has been used in more novel ways in order to prevent HCAIs, including vascular catheter-related infections and ventilator-acquired pneumonia. Table 1 summarizes the breadth of chlorhexidine use in healthcare settings.

As well as a broad range of activity, a key potential advantage of chlorhexidine, especially when used as a skin disinfectant, is its 'residual activity'.[24,26] When compared with povidone iodine, chlorhexidine not only produces a greater reduction in the skin flora,[32] it also has longer residual activity.[33] Measurements of the efficacy and potential residual activity of chlorhexidine are dependent on the removal or neutralization of chlorhexidine after the defined point of exposure.[15] If a validated neutralization step is not included in the test procedure, the efficacy of chlorhexidine may be overestimated, as the biocide may continue to cause damage to cells after exposure.[34,35] The inclusion of a valid neutralizing step is missing from many published studies, leading some authors to challenge the data demonstrating the residual potency of chlorhexidine, specifically relating to hygienic hand disinfection.[34] A potential source of false elevation of MICs is if bacteria are not adequately dispersed *in vitro*, in effect partially simulating a biofilm mode of growth.

The safety and tolerability of chlorhexidine is good.[36] It is less likely to cause dry skin than non-medicated soap[22] and in one study it caused less dermatitis of the hands of nurses than soaps containing other antiseptic agents.[37] Irritation of the skin and allergic reactions, such as dermatitis, are more likely at higher concentrations, but are relatively uncommon. There

Table 1. Uses of chlorhexidine in clinical practice

Application	Commonly used dilution of chlorhexidine (formulation[27])	Reference
Hand disinfection		
general	0.5% (hand rub), 4% (liquid)	88
pre-operative		33,89
Pre-procedure skin disinfection		
pre-surgical	2% in 70% isopropyl alcohol (liquid)	90
insertion of vascular catheters		91
Care of vascular catheters while *in situ*	2% in 70% isopropyl alcohol (gel)	29,30
Bathing patients on ICU	4% (liquid)	92
MRSA decolonization	1% (dusting powder), 4% (liquid)	83,87,93
Prevention of vascular catheter infections		
impregnation of catheter site dressing	2% in 70% isopropyl alcohol (gel)	94
impregnation of catheter	425 μg/cm	95–98
oropharyngeal decolonization to prevent ventilator-acquired pneumonia	0.12% (rinse), 0.2% (rinse), 2% (gel)	99

Table 2. Possible outcomes of an interaction of bacteria with chlorhexidine[11,12,43]

Term	Definition
Intrinsic resistance/insusceptible	bacteria that are intrinsically resistant to chlorhexidine, such as mycobacteria or bacterial spores
Phenotypic tolerance	survival in the presence of chlorhexidine due to low metabolism or due to a transient condition, such as the presence of a biofilm
Chlorhexidine tolerance	a bacterial strain that is inhibited but not killed by chlorhexidine, such as exposure to bacteriostatic concentrations of chlorhexidine used *in vitro* (4 mg/L)
Chlorhexidine resistance	a bacterial strain that can survive exposure to chlorhexidine at a concentration that kills the rest of the bacterial population, such as the in-use bactericidal concentration of chlorhexidine (40 000 mg/L)

are rare cases of severe side effects, such as anaphylaxis[38] and ototoxicity if applied to the inner ear.[39] The use of chlorhexidine in babies <2 months old is not advised, in part because of concerns about the systemic absorption of the agent and the potential for neurotoxicity.[40]

Reduced susceptibility to chlorhexidine

Bacteria may be described as insusceptible, phenotypically tolerant, tolerant or resistant to antiseptics (Table 2). As with antibiotic agents, resistance to antiseptics can be either intrinsic or acquired. Intrinsic resistance, or insusceptibility, to chlorhexidine is demonstrated by bacterial spores and mycobacteria. In both cases the outer layers of the cell form an impermeable barrier to the ingress of molecules.[1] In Gram-negative bacteria, such as *Proteus* and *Providencia* species, intrinsic properties of the outer membrane also confer resistance to chlorhexidine at in-use concentrations.[41] Phenotypic tolerance refers to the survival of a microorganism in the presence of a biocide during specific growth conditions, such as the 'protective' setting of a biofilm.[11] Once removed from the biofilm, the organism generally returns to a susceptible phenotype.[11] Efflux pumps are common mechanisms of resistance to antiseptics, such as chlorhexidine.[12,42,43] Efflux pumps are energy dependent, powered by ATP or the proton-motive force (PMF), and have the capacity to remove both antiseptics and antibiotics according to their substrate range. Efflux is the primary mechanism of reduced susceptibility to chlorhexidine in *S. aureus*.[14] Both Gram-negative and -positive bacteria can acquire genes encoding efflux pumps, which are commonly present on mobile genetic elements. While intrinsic resistance/insusceptibility and phenotypic tolerance are reasonably easy to define, the same cannot be said for chlorhexidine 'resistance'.

Bacterial resistance to antibiotics is generally clear and readily defined, using parameters such as the MIC; however, resistance measured in these terms is of less relevance to antiseptics, including chlorhexidine, for several reasons. Firstly, lethal rather than inhibitory effects are more important.[41] *In vitro*, bacteria are tested against much lower concentrations of biocide (i.e. in the case of chlorhexidine, 4 mg/L) and bacterial survival at an MIC concentration, such as 4–32 mg/L, does not necessarily guarantee bacterial survival at the much higher concentrations that are achieved in practice (i.e. 40 000 mg/L in 4% aqueous chlorhexidine solution).[44] For this reason, the use of time–kill tests and measurement of MBC may be more appropriate.[6] Also, both the MIC and MBC relate to specific concentrations attainable in body fluids (e.g. serum and urine), which are not relevant to antiseptics.[43]

In the purest sense, MIC values indicate tolerance to chlorhexidine rather than resistance; however, chlorhexidine 'resistance' has been used extensively in the literature to date. When bacteria are able to survive at in-use concentrations of a biocide, noting that these vary considerably according to the application/product (Table 1), the bacteria may be defined as resistant; however, when tested at lower concentrations *in vitro*, the bacteria should be considered tolerant to the biocide.[12] Taking these issues into consideration, our preferred term for phenotypic 'resistance' is 'reduced susceptibility' to chlorhexidine, whereas genotypic resistance could be described as the presence of efflux-mediated resistance genes.

Phenotypic detection of reduced susceptibility to chlorhexidine

Susceptibility to chlorhexidine is commonly tested using phenotypic and MIC- or MBC-based, methods. MIC methods measure the lowest possible concentration of a biocide that will inhibit growth of the organism, whereas an MBC method measures the lowest possible concentration required to kill the organism.[45] In staphylococci, chlorhexidine resistance is often defined as an MIC ≥4 mg/L,[46–48] although, as discussed, this may be more accurately described as reduced susceptibility to chlorhexidine. Crucially, a standardized method for chlorhexidine MIC determination is not available[47] and some authors do not consider MIC-based methods to be suitable for measuring the susceptibility of bacteria to chlorhexidine.[44,49] Alternative methods of chlorhexidine assessment have been used, such as surface disinfection tests and biocide residue tests.[49] Chlorhexidine has a low diffusion rate through solid agar and, thus, is not suited to susceptibility testing methods based on disc diffusion.[50] Table 3 summarizes the methods that have been used to test the phenotypic susceptibility of staphylococci to chlorhexidine.

It has been suggested that the method used for phenotypic susceptibility testing should mimic the in-use conditions as closely as possible.[6] Bacteria are not likely to achieve optimal growth *in vivo*, due to environmental stresses, such as lack of available nutrients, whereas *in vitro* bacteria are encouraged to grow and inocula are generated using nutrient-rich media. The results of phenotypic biocide susceptibility testing may be affected by the experimental conditions used, such as the culture media, inoculum size and age of the culture, as reviewed

Table 3. Common methods used to determine reduced susceptibility to chlorhexidine

Method	Details and comments
MIC by agar dilution[45,47,48,61]	incorporation of different concentrations of chlorhexidine into nutrient agar followed by the application of a controlled number of bacterial cells to the surface of the agar
	five or more doubling dilution concentrations are used and an MIC value can be determined
	chlorhexidine may be tested at specific concentrations necessary for differentiation of susceptible, intermediate or resistance isolates (breakpoint agar method)
	a multipoint or spiral plate method can be used
	microbial contamination and heterogeneity are easily detected
	chlorhexidine has a low diffusion rate through solid agar
MIC by broth dilution[48,61,62]	serial dilution of chlorhexidine into broth and addition of a controlled number of bacterial cells
	macrodilution method (i.e. large volumes of broth in tubes) or microdilution method (i.e. small volumes in the wells of a microtitre plate)
	can be readily converted to the MBC test; an appropriate neutralization step must be included
	other substances can be added to the broth if necessary (i.e. neutralizer)
MBC by broth dilution[74]	as MIC by broth dilution, including neutralization, followed by inoculation of solid biocide-free agar with an aliquot from the broth
Time–kill study[80]	the measurement of growth of bacteria exposed to a biocide in broth for different lengths of time (h)
	the study of inactivation kinetics can provide useful information about clonal populations and aggregation depending on the shape of the resultant inactivation kinetic

by Maillard.[12] For instance, when staphylococci were grown in suspension, lower amounts of the biocides tested (benzalkonium chloride/chlorhexidine digluconate) were taken up by cells compared with when staphylococci were grown on agar; as such, staphylococci grown in broth may appear susceptible, while agar-cultured strains appear resistant.[51]

The presence of organic matter, biofilms and biocide residues at sublethal concentrations are additional factors that need to be taken into account when comparing the *in vitro* and *in vivo* susceptibility of biocides. These factors may contribute to the survival of a subpopulation of bacterial cells in the presence of a particular biocide, compared with the majority of cells that will remain susceptible. The concept of heterogeneous resistance to chlorhexidine, defined as the presence of subpopulations of staphylococci that can survive at in-use concentrations of the agent, is a plausible one, given that heteroresistance to vancomycin is observed in some populations of *S. aureus*;[52] however, information about heterogeneous susceptibility to chlorhexidine in staphylococci is non-existent.

In summary, the results of phenotypic susceptibility testing are highly dependent on the method used and there are no nationally/internationally agreed breakpoint values for biocide susceptibility testing, which makes it difficult to standardize research in the field, and indeed to interpret the significance of results from published studies.

Chlorhexidine resistance genes

Phenotypic methods are a valuable way to screen isolates directly for chlorhexidine susceptibility; however, confirmation of the presence of DNA sequences known to encode efflux-mediated chlorhexidine resistance genes is an alternative method to identify specific mechanisms.

Currently, there are ≥11 genes known to encode efflux-mediated resistance to biocides (*qacA*, *B*, *E*, *EΔ1*, *F*, *G*, *H*, *J*, *Z*, *smr* and *norA*) (Table 4); however, not all of the 11 resistance genes have been identified in staphylococci. The genes can be categorized into two families, the major facilitator superfamily (MFS) and the small multidrug resistance (SMR) family, according to DNA homology, protein structure, substrate specificity and plasmid association of the proteins they encode. Proteins of both families encode efflux-mediated resistance to a range of structurally unrelated cationic, lipophilic substrates across the cell membrane, powered by the PMF.[42,53] In general, the MFS family encodes resistance to biocides, including chlorhexidine, whereas the SMR family confers resistance to certain biocides but not chlorhexidine. Staphylococci have the capacity to efflux lipophilic cations, such as quaternary ammonium compounds, intercalating dyes, diamidines and biguanidine compounds, including chlorhexidine. In fact, the substrate range for QacA comprises 30 cationic lipophilic compounds distributed across 11 chemical classes.[54]

The *qacA* gene in *S. aureus* was the first gene encoding a PMF-dependent efflux system to be sequenced and described.[55–57] The QacA and QacB proteins, which belong to the MFS family 1, are large proteins known to have 14 membrane-spanning regions. Conserved sequences are found in the N-terminal region of the protein, likely to be involved in the generation of energy to transport molecules, whereas the C-terminal region contains variable sequences responsible for substrate specificity. Of note, there are seven nucleotide differences present in the sequence of *qacB* compared with *qacA*. These nucleotide differences result in a single amino-acid substitution from Asp to Ala at codon 323. Subsequently, *qacB* is not able to efflux divalent cations.

Of the 11 known biocide resistance genes, *qacA* is the gene that is commonly associated with reduced susceptibility to chlorhexidine in staphylococci. Although the majority of the 'wild-type' genes are not strictly able to efflux chlorhexidine in their native state (Table 4), there are examples of *qac* genes that occasionally encode phenotypic reduced susceptibility to

Table 4. Summary of described efflux-mediated biocide resistance genes

	Gene, family of proteins										
	qacA, MFS family 1	*qacB*[a], MFS family 1	*smr*[b], SMR	*qacE*, SMR	*qacEΔ1*, SMR	*qacF*, SMR	*qacG*, SMR	*qacH*, SMR	*qacJ*, SMR	*qacZ*, SMR	*norA*, MFS family 2
Genera											
staphylococci	✓	✓	✓		✓		✓	✓	✓		✓
enterococci					✓					✓	✓
Gram-negative bacteria				✓	✓	✓					✓
Substrate											
quaternary ammonium compounds	R	R	R	R	R	R	R	R	R	R	R
intercalating dyes	R	R	R	R	R	NT	R	R	R	X	R
diamidines	R	R	X	NT	NT	NT	X	NT	NT	NT	NT
biguanidines (including chlorhexidine)	R	X	X	NT	R	NT	X	NT	NT	X	R
Reference	53	53	53	100	101	102	103	104	105	106	42

R, isolates of the genera tested exhibited resistance to the class of agents; X, the compound is not a substrate for the efflux pump; NT, not tested.
[a]Seven nucleotide differences exist between *qacA* and *qacB*.
[b]Alternative names for *smr* are *qacC*, *qacD* and *ebr*.

chlorhexidine. For instance, isolates of coagulase-negative staphylococci found to carry only *qacC* (now known as *smr*), a gene that does not usually encode phenotypic reduced susceptibility to chlorhexidine, exhibited chlorhexidine MICs >4 mg/L.[58] These raised MICs may be due to the presence of base-pair mutations in the *qacC* gene that cause a change in substrate specificity or there may be another undetected mechanism of resistance, i.e. overexpression of *norA* can also produce a similar resistance phenotype.[42] These instances justify the importance of surveillance for other *qac* genes, not just *qacA*.

The presence of *qacA* does not necessarily mean that an isolate will express phenotypic resistance to chlorhexidine. Staphylococci may appear susceptible despite the presence of *qacA*; conversely, efflux-mediated resistance gene(s) may be absent but the bacteria may display reduced susceptibility to chlorhexidine (Table 5). One way to investigate this variability further would be to measure RNA expression of chlorhexidine resistance genes during exposure to the agent. Two studies that have used this approach include an investigation of the molecular mechanisms of chlorhexidine tolerance in *Listeria monocytogenes*[59] and in *Burkholderia cenocepacia* biofilms.[60]

Staphylococci may carry more than one biocide efflux-mediated resistance gene that confers resistance to a similar range of substrates (Table 5).[10,61,62] A possible selective advantage of this phenomenon may relate to the fact that *qacA* encodes resistance to the widest range of biocides, whereas other genes (e.g. *smr*), although not enabling the same level of resistance to biocides, may be associated with resistance to other antibiotic agents or additional mobile genetic elements, so increasing the chance of microbe survival.[62] Biocide resistance genes are part of tightly regulated virulence systems. In addition to the presence of single genes encoding chlorhexidine resistance, the wider effects of global regulatory systems may result in an efflux-based resistance phenotype.[63,64]

Relationship between chlorhexidine resistance genes and other antimicrobial resistance genes in staphylococci

In addition to reduced susceptibility to chlorhexidine and other biocides, the presence of biocide efflux-mediated resistance genes in staphylococci also has implications for other antimicrobial agents in terms of cross-resistance and coresistance. For instance, the multidrug efflux pump encoded by *norA* is capable of removing biocides and fluoroquinolones.[63] On the other hand, coresistance occurs because of the shared location of chlorhexidine resistance genes on the same mobile genetic elements as other antimicrobial resistance genes.[11]

The *qacA* gene was first described from a plasmid that encoded resistance to heavy metals and β-lactam agents (Tn*4002*).[65] Other transmissible plasmids that *qacA* has been found associated with include pSK1, pSK105, pSK107, pSK4032, pSK4769, pSK638 and pSK57.[58] Each family of plasmids is known to carry resistance to other antibiotics, such as aminoglycosides (Tn*4001*) and trimethoprim (Tn*4003*).[55] The *qacA/B* genes have been identified in MRSA strains carrying SCC*mec* types I–V, most commonly types II and III.[47,66] Various plasmids (e.g. PuB110 and pT181) and transposons (i.e. Tn*554*, Tn*4001* and Tn*5801*) can be found integrated into certain allotypes of the SCC*mec* element (types I, II, III, IVa and IVc), and staphylococci encoding these SCC*mec* types will carry additional antibiotic resistance genes, in particular coding for resistance to aminoglycosides, tetracycline and the macrolide–lincosamide–streptogramin group.[67]

Genetic linkage between the *qacA/B* genes and β-lactamase resistance mediated by *blaZ* has been reported;[47] an association that has been noted in both *S. aureus* and coagulase-negative staphylococci.[68] Associations between chlorhexidine resistance genes and resistance to other antibiotic classes have also been

Table 5. Studies showing the discrepancy between the presence of *qacA/B* and/or *smr* genes and reduced susceptibility to chlorhexidine in MRSA

Location of study	Isolates (*n*)	Genes		Isolates with reduced susceptibility to chlorhexidine by MIC/MBC method (*n*)	Isolates with reduced susceptibility in the presence of resistance genes (*n*)	Isolates with reduced susceptibility in the absence of resistance genes (*n*)	Susceptible isolates (MIC ≤2 mg/L) in the presence of resistance genes (*n*)	Reference
		qacA/B	*smr*					
Canada	334	7 (2%)	23 (7%)	88 isolates had MBC ≥5 mg/L	31	57	NT	74
USA	493	5 (1%)	NT	16 isolates had MIC ≥8 mg/L	5	11	none	69
Taiwan	206	73 (35%)	NT	72 isolates had MIC ≥4 mg/L	67	5	6	47
Taiwan	240	46 (19%)	NT	83 isolates had MIC ≥4 mg/L	46	37	11	48

NT, not tested.

identified. A study of 237 *S. aureus* isolates identified a significant association between isolates carrying the *qacA/B* genes and resistance to the following antibiotic agents: ciprofloxacin ($P=0.005$), trimethoprim/sulfamethoxazole ($P=0.001$), clindamycin ($P=0.023$) and tetracycline ($P=0.01$). No significant association between the *qacA/B* genes and resistance to gentamicin, fusidic acid or erythromycin was identified.[62] Such associations could simply reflect the copresence/absence of resistance genes that are subject to selection pressures, some of which may overlap with those for chlorhexidine resistance mechanisms.

Whether the presence of genes that encode efflux-mediated resistance to chlorhexidine selects for the presence of antibiotic resistance genes is a well-debated question.[11,43] When the prevalence of antiseptic resistance genes in staphylococci was compared between nurses and the general population, a higher incidence of *qac* genes was identified in staphylococci that were colonizing hospital nursing staff compared with staphylococci from the general population.[62] This result may indicate that the use of biocides in the hospital setting could select for strains that are able to survive in the presence of biocides.

Prevalence of chlorhexidine resistance genes in staphylococci

The reported prevalence of *qac* and *smr* genes in staphylococci varies according to geographical location, ranging from 1% in the eastern states of the USA[69] to 80% in Brazil.[70] Table 6 summarizes the findings of 18 studies that describe the geographical distribution of phenotypic and/or genotypic chlorhexidine resistance. Two studies include the prevalence of other genes that may be associated with chlorhexidine resistance genes, such as *norA/blaZ*.[49,71] Four studies compare the association between chlorhexidine resistance genes and methicillin resistance,[10,46,62,72] and four studies investigate the prevalence of chlorhexidine resistance genes in coagulase-negative staphylococci.[10,58,62,71] As mentioned above, there is only one study that compared the prevalence of chlorhexidine resistance genes between healthcare workers and the general population.[62]

While the number of studies investigating the prevalence of chlorhexidine resistance genes and reduced susceptibility to chlorhexidine in staphylococci has increased, a definitive conclusion about whether the prevalence of resistance genes and/or the proportion of staphylococci with reduced susceptibility are changing cannot be made. Also, geographical differences in the prevalence of chlorhexidine resistance genes are likely to be the result of numerous factors, such as clonal spread, the population/case mix under study, differences in infection control policies and, potentially, the pressure of use of chlorhexidine. Notably, a lack of consensus regarding chlorhexidine susceptibility testing methodology adds further confusion.

Clonal association of chlorhexidine resistance genes in staphylococci

The clonal predominance of particular strains of staphylococci likely influences the prevalence of chlorhexidine resistance genes.[9,46] Hence, choice of locale, period of study and isolate selection are key considerations when interpreting such data.

Table 6. Studies reporting the prevalence of chlorhexidine resistance in staphylococci

Location of study	Organism (no. of isolates)	Year of isolate collection	Isolate type	Single or multicentre	Genes		Susceptibility test
					qacA/B	*smr*	
Toronto, Canada[74]	MRSA (334)	2005–09	clinical	m	7 (2%)	23 (7%)	MBC
Eastern USA[69]	MRSA (493)	2003	clinical	m	5 (1%)	NT	MIC and MBC by broth dilution
Hong Kong[62a,b]	MRSA (12) MSSA (225) CoNS (602)	not stated	nurses and general population	s	MRSA: 6 (50%) MSSA: 36 (16%) CoNS: 256 (43%)	MRSA: 2 (17%) MSSA: 14 (6%) CoNS: 90 (15%)	MIC and MBC by broth dilution
Tunisia[10a,b]	MRSA (23) MSSA (16) CoNS (71)	not stated	clinical	s	MRSA: 6 (26%) MSSA: 4 (25%) CoNS: 35 (49%)	MRSA: 3 (13%) MSSA: 2 (13%) CoNS: 36 (51%)	MIC by broth microdilution (CHX not tested)
Taiwan[47]	MRSA (206)	2002/2004	clinical	m	73 (35%)	NT	MIC by agar dilution method
Edinburgh, Scotland[49c]	MRSA (120)	2006	clinical	s	10 (8%)	53 (44%)	surface disinfection and biocide residue tests
Glasgow, Scotland[75]	MRSA (94)	not stated	clinical	m	14 (15%)	4 (4%)	MBC and tolerance tests
Taiwan[48]	MRSA (240)	1990, 1995, 2000, 2005	clinical	s	57 (24%)	NT	MIC by agar dilution method
China[107]	MRSA (131)	2003–04	clinical	m	80 (61%)	NT	not measured
Rio de Janeiro, Brazil[70]	MRSA (74)	2002–03	clinical	m	59 (80%)	NT	not measured
Japan[66]	MRSA (283)	1999–2004	clinical	m	96 (34%)	4 (1%)	MIC by agar doubling dilution
Various, Asia[61]	MRSA (894)	1998–99	clinical	m	372 (42%)	28 (3%)	MIC by agar doubling dilution (CHX not tested)
Tokyo, Japan[9]	MRSA (65)	2003	clinical	s	34 (52%)	1 (2%)	MIC to AEG, AF, BTC and BKC, not CHX
Sapporo, Japan[46a]	MRSA (334) MSSA (188)	1993–95, 1997, 1999, 2001	clinical	s	MRSA: 109 (33%) MSSA: 14 (7%)	MRSA: 11 (3%) MSSA: 11 (6%)	not measured
Norway[71b,c]	MSSA (61) CoNS (177)	1991–92, 1995–96	clinical and skin colonization	m	MSSA: 17 (28%) CoNS: 48 (27%)	MSSA: 1 (2%) CoNS: 1 (0.5%)	MIC by broth microdilution
Various, Europe[72a]	MRSA (297) MSSA (200)	1997–99	clinical	m	MRSA: 186 (63%) MSSA: 24 (12%)	MRSA: 19 (6%) MSSA: 10 (5%)	not measured
Tokyo, Japan[8]	MRSA (98)	1992	clinical	m	10 (10%)	20 (20%)	MIC
Sydney, Australia[58b]	CoNS (164)	1979–84	clinical	m	20 (12%) *qacA* + *qacC* = 16/164 (10%)	4 (2%)	MIC

AEG, alkyldiaminoethylglycine hydrochloride; AF, acriflavin; BTC, benzethonium chloride; BKC, benzylalkonium chloride; CHX, chlorhexidine; CoNS, coagulase-negative staphylococci; s, single; m, multicentre.

[a]Studies that compare the association between genotypic chlorhexidine resistance between MRSA and MSSA.

[b]Studies that include the presence of chlorhexidine resistance genes in coagulase-negative staphylococci.

[c]Studies that include the prevalence of other genes (*norA*, *blaZ*).

Clonal expansion of a single MRSA clone was responsible for the spread of *qacA/B* resistance genes in a single centre in Japan (1993–2001). Of 522 clinical isolates of *S. aureus*, 109 MRSA isolates were positive for *qacA/B* genes, compared with 14 MSSA isolates. The MRSA isolates represented 11 *spa* types, with one *spa* type (S10) accounting for 38% of *qacA/B*-positive MRSA isolates.[46]

There is also circumstantial evidence of horizontal transfer of plasmids carrying the *qacA/B* genes among strains of *S. aureus* and other staphylococci,[66] and transfer of plasmids encoding *qacB* has been demonstrated *in vitro* between strains of *S. aureus*.[73] From a collection of 334 MRSA isolates from patients present on two intensive care unit (ICU) wards in a single institute in Canada between 2005 and 2009, 88 (26%) isolates were identified to harbour *qacA/B* or *smr* genes and these isolates were genotyped using *spa* typing. Seventeen *spa* types were identified, with two being prevalent (t002 and t008). The *qacA/B* genes were associated with two *spa* types from MRSA typically associated with healthcare (t002 and t037), whereas *smr* genes were associated with more *spa* types (t002, t007, t008 and t064).[74]

Surveillance of 240 isolates from a single hospital in Taiwan over four periods (1990, 1995, 2000 and 2005) showed that the number of lineages with reduced susceptibility to chlorhexidine increased over the study period (one in 1995 to six in 2005).[48] In 1990, no *qacA* genes were identified in the three dominant lineages [ST254, $n=27$; ST30, $n=22$; and ST239, $n=10$ (where ST stands for sequence type)], although a single isolate of ST239 displayed reduced susceptibility (≥4 mg/L) to chlorhexidine. In 1995, isolates of ST239 and ST254 were still circulating along with two additional lineages, ST59 and ST241. Reduced susceptibility was identified in 1/5 isolates of ST59 and 29/33 isolates of ST241; however, the *qacA* gene was only identified in 16 of the ST241 isolates. In 2000, a higher number of lineages ($n=6$) were circulating that had reduced susceptibility to chlorhexidine (ST1, ST5, ST59, ST239, ST241 and ST254). In 2005, there were eight different lineages circulating, with four having reduced susceptibility to chlorhexidine. Since 1990, five lineages (ST1, ST59, ST239, ST241 and ST594) were associated with *qacA* genes or reduced susceptibility to chlorhexidine. The increase in the prevalence of lineages may be due to increased chlorhexidine use in the area or some other factor.[48] In a separate study, 11 multilocus STs were identified among 206 MRSA isolates from multiple hospitals in Taiwan between 1998 and 2004.[47] Four lineages carried *qacA* genes (ST239, ST241, ST338 and ST573), whereas isolates of ST5 carried the *qacB* gene and exhibited reduced susceptibility to chlorhexidine (MIC ≥4 mg/L). MRSA lineages ST1, ST6, ST8, ST59, ST89 and ST900 were also circulating, all of which exhibited reduced susceptibility to chlorhexidine but did not carry *qac* genes. In conclusion, ST239 is a prevalent MRSA lineage that has been associated with reduced susceptibility to chlorhexidine and carriage of chlorhexidine resistance genes.

Only one study specifically examined examples of healthcare-associated (HA) MRSA, community-associated (CA) MRSA and vancomycin-intermediate *S. aureus* (VISA) strains, and tested for an association with chlorhexidine resistance genes.[75] *qacA* genes were identified in 10/38 (26%) HA-MRSA strains and 4/6 (67%) VISA strains, i.e. strains that are likely to have been subjected to selection pressures in the hospital setting. None of the strains of CA-MRSA ($n=25$) or MSSA ($n=25$) tested were positive for *qacA* genes and these isolates had significantly lower chlorhexidine MBCs. The epidemic strain of MRSA in the UK remains EMRSA-15 (ST22-IV) and although it is possible for this strain to carry *qacA* genes,[49] these genes do not appear to be prevalent in this lineage.[76] Therefore, EMRSA-15 has been a successful lineage in the UK for many years, but without the widespread addition of chlorhexidine resistance genes. On the other hand, rates of MRSA bacteraemia associated with the other predominant MRSA lineage in the UK, EMRSA-16 (ST30-II), have been decreasing,[77] and yet chlorhexidine and mupirocin resistance was commonly found in isolates of this clone in one study.[49] Why chlorhexidine resistance genes may be prevalent in specific lineages is not known, but may be related to plasmid carriage, and the compatibility and transmissibility of mobile genetic elements.

Significance of reduced susceptibility to chlorhexidine in staphylococci

There have been a number of reports that MRSA is less susceptible to chlorhexidine than MSSA.[50,75,78,79] This observation is of particular concern given the widespread use of chlorhexidine for the purposes of MRSA decolonization. Although MRSA may be associated with higher chlorhexidine MICs/MBCs than MSSA, the clinical relevance of this finding has not been fully established. The concentrations of chlorhexidine achieved when used as recommended by the manufacturer are several orders of magnitude greater than the MIC and MBC tested *in vitro*.[75] It has been shown that chlorhexidine remains effective at killing *S. aureus* that have an elevated MIC under *in vivo* experimental conditions.[80]

In addition, several authors have shown that repeatedly exposing MRSA to subinhibitory concentrations of chlorhexidine *in vitro* leads to an increasing level of resistance, as measured by a rise in the MIC.[49,50,75] Block and Furman[81] found a significant inverse correlation between the intensity of chlorhexidine use and the overall susceptibility of a group of study bacteria, including *S. aureus*; however, the results were not significant when species were considered individually. Wang *et al.*[48] showed that in a Taiwanese hospital, where 4% chlorhexidine had been in use for hand hygiene for >20 years, the proportion of MRSA isolates with a chlorhexidine MIC ≥4 mg/L increased from 1.7% in 1990 to 46.7% in 2005.

If extrapolated to the healthcare environment, the residual activity of chlorhexidine after being applied to the skin or an inanimate surface may promote resistance in resident flora. For instance, the prevalence of the *qacA/B* genes in coagulase-negative staphylococci varies between 12% and 49%,[10,58,71] and was found to be higher in isolates from nurses compared with those from the general population (57% versus 14%, respectively; $P<0.001$).[62] Thus, exposure to chlorhexidine in the hospital environment may select for colonization by chlorhexidine-resistant staphylococci. Given the variety of chlorhexidine-containing products and the concentrations of chlorhexidine in clinical use (0.5%–4%), the consequence of the use of lower concentrations of chlorhexidine in terms of selection of staphylococci with reduced susceptibility requires further investigation.

Notably, the results of two recent studies suggest that reduced susceptibility to chlorhexidine in MRSA may have a clinical impact. Firstly, Batra *et al.*[82] showed that the use of a chlorhexidine-based surface antiseptic protocol in an ICU led to the spread of an MRSA strain with an elevated MBC to the agent. Secondly, the presence of genotypic chlorhexidine resistance (*qacA/B* genes) in combination with mupirocin resistance independently predicted failure of MRSA decolonization in a study by Lee *et al.*[76]

Batra *et al.*[82] aimed to assess the effects of three infection prevention interventions on the rates of MRSA transmission on two 15 bed ICU wards in St Thomas' Hospital, London, between January 2002 and April 2006. At the time of the study, EMRSA-15 (ST22-IV) and EMRSA-16 (ST30-III) were circulating in both units. In addition, both units had been affected by a 2 year outbreak with a non-endemic strain (ST239), which was also recorded during the study period. The interventions were as follows: Intervention A, introduced in July 2003, was based on staff education and covert audit of hand hygiene and barrier nursing practice; Intervention B, introduced in October 2003, involved nursing MRSA-colonized patients in cohorts or side rooms; and Intervention C, introduced in April 2004, was based on the use of a chlorhexidine-based antiseptic for the decolonization of patients colonized with MRSA. Importantly, the concurrent application of mupirocin for decolonization was not included in the third intervention. There was no reduction in MRSA acquisition of ST22 or ST30 strains on the units following intervention A or B; however, following intervention C, numbers of ST22 and ST30 (but not ST239) decreased. Twenty-one ST239 isolates were selected for further investigation; all 21 carried *qacA/B* genes and had chlorhexidine MBC values that were 3-fold higher than the chlorhexidine MBC values for the 21 non-ST239 isolates that were tested for comparison (78±4 mg/L versus 26±8 mg/L). Only one non-ST239 isolate carried *qacA/B* genes. Batra *et al.*[82] concluded that although the use of chlorhexidine antisepsis for MRSA decolonization can lead to an immediate and sustained reduction in the transmission of susceptible strains, caution must be taken in the use of such a protocol in areas where strains with reduced susceptibility to chlorhexidine are circulating. These observations are particularly useful, because mupirocin was not included in the decolonization regimen due to concerns about resistance.

Lee *et al.*[76] found that the presence of *qacA/B* genes in combination with mupirocin resistance independently predicted failure of MRSA decolonization. The study took place in a large single centre in Geneva that implemented the use of intranasal mupirocin and chlorhexidine bathing to decolonize MRSA carriers in 1994. Mupirocin resistance increased between 1999 (9%) and 2008 (81%), and by 2008 >99% MRSA isolates had low-level resistance to mupirocin (MIC 8–256 mg/L). The study identified low-level mupirocin resistance in 49/75 (65%) cases versus 26/75 (35%) controls prior to decolonization ($P<0.001$); carriage of *qacA/B* genes was associated with 68 (91%) cases and 51 (68%) controls ($P<0.001$). Forty-seven (63%) of the mupirocin-resistant isolates carried *qacA/B* genes. Low-level mupirocin resistance and genotypic chlorhexidine resistance were strongly associated with persistent MRSA colonization after decolonization therapy ($P=0.004$). All low-level mupirocin-resistant isolates were SCC*mec* type I and contained the V588F point mutation in the native tRNA synthetase gene that encodes resistance to mupirocin. Isolates collected before and after decolonization from the same patient were indistinguishable, suggestive of recolonization rather than acquisition of an MRSA strain from an exogenous source. All 150 MRSA isolates were typed using multilocus sequence typing and, although the *qacA/B*-positive genotypes were not recorded, there was a significant association of *qacA/B* genes with isolates of ST228 identified in cases compared with the controls (65 cases versus 49 controls; $P=0.002$). Lee *et al.*[76] acknowledged that it is difficult to separate the effects of the individual agents contributing to the failure of decolonization. The presence of *qacA/B* genes alone did not predict decolonization failure ($P=0.44$) nor did low-level mupirocin resistance alone ($P=0.32$). The combination of both low-level mupirocin resistance and genotypic chlorhexidine resistance was an independent risk factor for failure of decolonization ($P=0.004$). As phenotypic chlorhexidine susceptibility was not measured, it is not possible to determine how many of the isolates actually had reduced susceptibility to chlorhexidine. Studies commonly do not include follow-up with chlorhexidine susceptibility testing;[83–86] however, when investigated, an increase in reduced susceptibility to chlorhexidine following chlorhexidine use was not identified.[83,87]

Summary of current knowledge

The biguanide antiseptic chlorhexidine was introduced into clinical practice >50 years ago, and its use is likely to continue and, indeed, increase. In reviewing the information available about this antiseptic agent and its association with staphylococci, it is apparent that there are important gaps in the current knowledge.

Firstly, the development of a standardized method for the detection of reduced susceptibility and/or resistance to in-use concentrations of chlorhexidine, along with a consensus definition of chlorhexidine 'resistance' are crucial for taking this area of research forward. Investigation of the impact of environmental factors on the development of reduced susceptibility to chlorhexidine and the frequency with which reduced susceptibility to chlorhexidine develops would then be possible.[11] The existence of subpopulations of staphylococci that are able to survive at in-use concentrations of chlorhexidine, or heterogeneous chlorhexidine resistance, is an important area of further investigation considering the effect of residual concentrations of biocides encountered in the healthcare environment.

Secondly, the relationship between the carriage of chlorhexidine resistance genes, such as *qacA*, and phenotypic reduced chlorhexidine susceptibility is not clear. Questions relating to the transmission of resistance associated with *qac* genes and mobile genetic elements leading to cross-resistance and/or coresistance with antibiotics remain unanswered. In order to answer these questions, the measurement of biocide resistance gene expression in relation to phenotypically reduced susceptibility to chlorhexidine may be of benefit.

Finally, although there is no shortage of information about the *in vitro* susceptibility to chlorhexidine of staphylococci, information about the clinical impact of *in vivo* reduced susceptibility to chlorhexidine is limited. Of relevance here, the role of selection pressure for coresistance and transmission of genes needs to be examined in a way that controls for confounding. The effect of coadministered antiseptics (e.g. alcohol) and residual

concentrations on the emergence of reduced susceptibility needs to be clarified. As reviewed here, only two studies have investigated staphylococci exhibiting reduced susceptibility to chlorhexidine and/or the presence of chlorhexidine efflux-mediated resistance genes, which may be associated with clinical failure of the decolonization/treatment of staphylococci. A correlation between the occurrence of reduced chlorhexidine susceptibility and decolonization failure does not indicate causality, as there are possible confounding factors. Investigation of a correlation and causality between increased chlorhexidine use, in particular the types of use, and an increased prevalence of reduced susceptibility to chlorhexidine in staphylococci is required. Ultimately, large, multicentre studies are needed.

We anticipate that clinical use of chlorhexidine will continue to increase and it will be important to be alert to the possibility that this may lead to the emergence of new clones with reduced susceptibility. Indiscriminate chlorhexidine use in the absence of efficacy data should be discouraged.

Transparency declarations

None to declare.

References

1 McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. *Clin Microbiol Rev* 1999; **12**: 147–79.

2 Scientific Committee on Consumer Products. *Opinion on Triclosan*. http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_166.pdf (13 May 2012, date last accessed).

3 Scientific Committee on Consumer Products. *Opinion on Triclosan (Antimicrobial Resistance)*. http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_023.pdf (13 May 2012, date last accessed).

4 HPA. *Quarterly Epidemiological Commentaries on MRSA, MSSA, Escherichia coli Bacteraemia and C. difficile Infection*. http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1259151891722 (26 April 2012, date last accessed).

5 Bode LG, Kluytmans JA, Wertheim HF *et al*. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N Engl J Med* 2010; **362**: 9–17.

6 Walker EM, Lowes JA. An investigation into in vitro methods for the detection of chlorhexidine resistance. *J Hosp Infect* 1985; **6**: 389–97.

7 Walsh SE, Maillard JY, Russell AD *et al*. Development of bacterial resistance to several biocides and effects on antibiotic susceptibility. *J Hosp Infect* 2003; **55**: 98–107.

8 Noguchi N, Hase M, Kitta M *et al*. Antiseptic susceptibility and distribution of antiseptic-resistance genes in methicillin-resistant *Staphylococcus aureus*. *FEMS Microbiol Lett* 1999; **172**: 247–53.

9 Sekiguchi J, Hama T, Fujino T *et al*. Detection of the antiseptic- and disinfectant-resistance genes *qacA*, *qacB*, and *qacC* in methicillin-resistant *Staphylococcus aureus* isolated in a Tokyo hospital. *Jpn J Infect Dis* 2004; **57**: 288–91.

10 Zmantar T, Kouidhi B, Miladi H *et al*. Detection of macrolide and disinfectant resistance genes in clinical *Staphylococcus aureus* and coagulase-negative staphylococci. *BMC Res Notes* 2011; **4**: 453.

11 Chapman JS. Disinfectant resistance mechanisms, cross-resistance, and co-resistance. *Int Biodeter Biodegr* 2003; **51**: 271–6.

12. Maillard JY. Bacterial resistance to biocides in the healthcare environment: should it be of genuine concern? *J Hosp Infect* 2007; **65**: 60–72.

13. Meyer B, Cookson B. Does microbial resistance or adaptation to biocides create a hazard in infection prevention and control? *J Hosp Infect* 2010; **76**: 200–5.

14 Russell AD. Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *Symp Ser Soc Appl Microbiol* 2002; **92**: 121S–35S.

15 Scientific Committee on Emerging and Newly Identified Health Risks. *Assessment of the Antibiotic Resistance Effects of Biocides*. 2009. http://ec.europa.eu/health/ph-risk/committees/04-scenihr/docs/scenihr_o_021.pdf (11 July 2012, date last accessed).

16. Davies GE, Francis J, Martin AR *et al*. 1:6-Di-4′-chlorophenyldiguanidohexane (hibitane); laboratory investigation of a new antibacterial agent of high potency. *Br J Pharmacol Chemother* 1954; **9**: 192–6.

17 Russell AD. Chlorhexidine: antibacterial action and bacterial resistance. *Infection* 1986; **14**: 212–5.

18 Milstone AM, Passaretti CL, Perl TM. Chlorhexidine: expanding the armamentarium for infection control and prevention. *Clin Infect Dis* 2008; **46**: 274–81.

19 Hugo WB, Longworth AR. Some aspects of the mode of action of chlorhexidine. *J Pharm Pharmacol* 1964; **16**: 655–62.

20 Gilbert P, Moore LE. Cationic antiseptics: diversity of action under a common epithet. *J Appl Microbiol* 2005; **99**: 703–15.

21 Denton GW. Chlorhexidine. In: Block SS, ed. *Disinfection, Sterilization, and Preservation*. Philadelphia: Lippincott Williams and Wilkins, 2001; 321–36.

22 Kampf G, Kramer A. Epidemiologic background of hand hygiene and evaluation of the most important agents for scrubs and rubs. *Clin Microbiol Rev* 2004; **17**: 863–93.

23 Russell AD, Day MJ. Antibacterial activity of chlorhexidine. *J Hosp Infect* 1993; **25**: 229–38.

24 Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HIPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *Am J Infect Control* 2002; **30**: S1–46.

25 WHO. *Guidelines on Hand Hygiene in Healthcare*. 2009. http://whqlibdoc.who.int/publications/2009/9789241597906_eng.pdf (11 July 2012, date last accessed).

26 Lowbury EJ, Lilly HA. Use of 4 per cent chlorhexidine detergent solution (Hibiscrub) and other methods of skin disinfection. *Br Med J* 1973; **1**: 510–5.

27 Joint Formulary Committee. *British National Formulary*. http://www.bnf.org (15 June 2012, date last accessed).

28 Department of Health. *High Impact Intervention: Care Bundle to Prevent Surgical Site Infection*. http://hcai.dh.gov.uk/files/2011/03/2011-03-14-HII-Prevent-Surgical-Site-infection-FINAL.pdf (27 March 2012, date last accessed).

29 Department of Health. *High Impact Intervention: Peripheral Venous Cannula Care Bundle*. http://hcai.dh.gov.uk/files/2011/03/2011-03-14-HII-Peripheral-intravenous-cannula-bundle-FIN%E2%80%A6.pdf (27 March 2012, date last accessed).

30 Department of Health. *High Impact Intervention: Central Venous Catheter Care Bundle*. http://hcai.dh.gov.uk/files/2011/03/2011-03-14-HII-Central-Venous-Catheter-Care-Bundle-FINAL.pdf (27 March 2012, date last accessed).

31 Coia JE, Duckworth GJ, Edwards DI *et al*. Guidelines for the control and prevention of meticillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities. *J Hosp Infect* 2006; **63** Suppl 1: S1–44.

32 Peterson AF, Rosenberg A, Alatary SD. Comparative evaluation of surgical scrub preparations. *Surg Gynecol Obstet* 1978; **146**: 63–5.

33 Aly R, Maibach HI. Comparative antibacterial efficacy of a 2-minute surgical scrub with chlorhexidine gluconate, povidone-iodine, and chloroxylenol sponge-brushes. *Am J Infect Control* 1988; **16**: 173–7.

34. Kampf G. What is left to justify the use of chlorhexidine in hand hygiene? *J Hosp Infect* 2008; **70** Suppl 1: 27–34.

35. Reichel M, Heisig P, Kampf G. Pitfalls in efficacy testing-how important is the validation of neutralization of chlorhexidine digluconate? *Ann Clin Microbiol Antimicrob* 2008; **7**: 20.

36 Rosenberg A, Alatary SD, Peterson AF. Safety and efficacy of the antiseptic chlorhexidine gluconate. *Surg Gynecol Obstet* 1976; **143**: 789–92.

37 Larson E, Friedman C, Cohran J *et al*. Prevalence and correlates of skin damage on the hands of nurses. *Heart Lung* 1997; **26**: 404–12.

38 Sijbesma T, Rockmann H, van der Weegen W. Severe anaphylactic reaction to chlorhexidine during total hip arthroplasty surgery. A case report. *Hip Int* 2011; **21**: 630–2.

39 Lai P, Coulson C, Pothier DD *et al*. Chlorhexidine ototoxicity in ear surgery, part 1: review of the literature. *J Otolaryngol Head Neck Surg* 2011; **40**: 437–40.

40 Mullany LC, Darmstadt GL, Tielsch JM. Safety and impact of chlorhexidine antisepsis interventions for improving neonatal health in developing countries. *Pediatr Infect Dis J* 2006; **25**: 665–75.

41 Russell AD. Bacterial resistance to disinfectants: present knowledge and future problems. *J Hosp Infect* 1999; **43** Suppl: S57–68.

42 Paulsen IT, Brown MH, Skurray RA. Proton-dependent multidrug efflux systems. *Microbiol Rev* 1996; **60**: 575–608.

43. Russell AD. Do biocides select for antibiotic resistance? *J Pharm Pharmacol* 2000; **52**: 227–33.

44 Platt JH, Bucknall RA. MIC tests are not suitable for assessing antiseptic handwashes. *J Hosp Infect* 1988; **11**: 396–7.

45 Wiegand I, Hilpert K, Hancock RE. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc* 2008; **3**: 163–75.

46 Alam MM, Kobayashi N, Uehara N *et al*. Analysis on distribution and genomic diversity of high-level antiseptic resistance genes *qacA* and *qacB* in human clinical isolates of *Staphylococcus aureus*. *Microb Drug Resist* 2003; **9**: 109–21.

47 Sheng WH, Wang JT, Lauderdale TL *et al*. Epidemiology and susceptibilities of methicillin-resistant *Staphylococcus aureus* in Taiwan: emphasis on chlorhexidine susceptibility. *Diagn Microbiol Infect Dis* 2009; **63**: 309–13.

48 Wang JT, Sheng WH, Wang JL *et al*. Longitudinal analysis of chlorhexidine susceptibilities of nosocomial methicillin-resistant *Staphylococcus aureus* isolates at a teaching hospital in Taiwan. *J Antimicrob Chemother* 2008; **62**: 514–7.

49 Vali L, Davies SE, Lai LL *et al*. Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant *Staphylococcus aureus* isolates. *J Antimicrob Chemother* 2008; **61**: 524–32.

50 Suller MT, Russell AD. Antibiotic and biocide resistance in methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*. *J Hosp Infect* 1999; **43**: 281–91.

51 Brill F, Goroncy-Bermes P, Sand W. Influence of growth media on the sensitivity of *Staphylococcus aureus* and *Pseudomonas aeruginosa* to cationic biocides. *Int J Hyg Environ Health* 2006; **209**: 89–95.

52 Tenover FC, Moellering RC Jr. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clin Infect Dis* 2007; **44**: 1208–15.

53 Littlejohn TG, Paulsen IT, Gillespie MT *et al*. Substrate specificity and energetics of antiseptic and disinfectant resistance in *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992; **74**: 259–65.

54 Mitchell BA, Brown MH, Skurray RA. *qacA* multidrug efflux pump from *Staphylococcus aureus*: comparative analysis of resistance to diamidines, biguanidines, and guanylhydrazones. *Antimicrob Agents Chemother* 1998; **42**: 475–7.

55 Littlejohn TG, DiBerardino D, Messerotti LJ *et al*. Structure and evolution of a family of genes encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *Gene* 1991; **101**: 59–66.

56 Rouch DA, Cram DS, DiBerardino D *et al*. Efflux-mediated antiseptic resistance gene *qacA* from *Staphylococcus aureus*: common ancestry with tetracycline- and sugar-transport proteins. *Mol Microbiol* 1990; **4**: 2051–62.

57 Tennent JM, Lyon BR, Midgley M *et al*. Physical and biochemical characterization of the *qacA* gene encoding antiseptic and disinfectant resistance in *Staphylococcus aureus*. *J Gen Microbiol* 1989; **135**: 1–10.

58 Leelaporn A, Paulsen IT, Tennent JM *et al*. Multidrug resistance to antiseptics and disinfectants in coagulase-negative staphylococci. *J Med Microbiol* 1994; **40**: 214–20.

59 Kastbjerg VG, Larsen MH, Gram L *et al*. Influence of sublethal concentrations of common disinfectants on expression of virulence genes in *Listeria monocytogenes*. *Appl Environ Microbiol* 2010; **76**: 303–9.

60 Coenye T, Van AH, Peeters E *et al*. Molecular mechanisms of chlorhexidine tolerance in *Burkholderia cenocepacia* biofilms. *Antimicrob Agents Chemother* 2011; **55**: 1912–9.

61 Noguchi N, Suwa J, Narui K *et al*. 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* 2005; **54**: 557–65.

62 Zhang M, O'Donoghue MM, Ito T *et al*. Prevalence of antiseptic-resistance genes in *Staphylococcus aureus* and coagulase-negative staphylococci colonising nurses and the general population in Hong Kong. *J Hosp Infect* 2011; **78**: 113–7.

63 DeMarco CE, Cushing LA, Frempong-Manso E *et al*. Efflux-related resistance to norfloxacin, dyes, and biocides in bloodstream isolates of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2007; **51**: 3235–9.

64 Paulsen IT, Brown MH, Littlejohn TG *et al*. Multidrug resistance proteins QacA and QacB from *Staphylococcus aureus*: membrane topology and identification of residues involved in substrate specificity. *Proc Natl Acad Sci USA* 1996; **93**: 3630–5.

65 Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus*: genetic basis. *Microbiol Rev* 1987; **51**: 88–134.

66 Noguchi N, Nakaminami H, Nishijima S *et al*. Antimicrobial agent of susceptibilities and antiseptic resistance gene distribution among methicillin-resistant *Staphylococcus aureus* isolates from patients with impetigo and staphylococcal scalded skin syndrome. *J Clin Microbiol* 2006; **44**: 2119–25.

67 International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob Agents Chemother* 2009; **53**: 4961–7.

68 Anthonisen IL, Sunde M, Steinum TM *et al*. Organization of the antiseptic resistance gene *qacA* and Tn*552*-related β-lactamase genes in multidrug-resistant *Staphylococcus haemolyticus* strains of animal and human origins. *Antimicrob Agents Chemother* 2002; **46**: 3606–12.

69 McGann P, Kwak YI, Summers A *et al*. Detection of *qacA/B* in clinical isolates of methicillin-resistant *Staphylococcus aureus* from a regional healthcare network in the eastern United States. *Infect Control Hosp Epidemiol* 2011; **32**: 1116–9.

70 Miyazaki NH, Abreu AO, Marin VA *et al*. The presence of *qacA/B* gene in Brazilian methicillin-resistant *Staphylococcus aureus*. *Mem Inst Oswaldo Cruz* 2007; **102**: 539–40.

71 Sidhu MS, Heir E, Leegaard T *et al*. Frequency of disinfectant resistance genes and genetic linkage with β-lactamase transposon Tn*552* among clinical staphylococci. *Antimicrob Agents Chemother* 2002; **46**: 2797–803.

72 Mayer S, Boos M, Beyer A *et al*. Distribution of the antiseptic resistance genes *qacA*, *qacB* and *qacC* in 497 methicillin-resistant and -susceptible European isolates of *Staphylococcus aureus*. *J Antimicrob Chemother* 2001; **47**: 896–7.

73. Nakaminami H, Noguchi N, Nishijima S *et al*. Transduction of the plasmid encoding antiseptic resistance gene *qacB* in *Staphylococcus aureus*. *Biol Pharm Bull* 2007; **30**: 1412–5.

74 Longtin J, Seah C, Siebert K *et al*. Distribution of antiseptic resistance genes *qacA*, *qacB*, and *smr* in methicillin-resistant *Staphylococcus aureus* isolated in Toronto, Canada, from 2005 to 2009. *Antimicrob Agents Chemother* 2011; **55**: 2999–3001.

75 Smith K, Gemmell CG, Hunter IS. The association between biocide tolerance and the presence or absence of *qac* genes among hospital-acquired and community-acquired MRSA isolates. *J Antimicrob Chemother* 2008; **61**: 78–84.

76 Lee AS, Macedo-Vinas M, Francois P *et al*. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant *Staphylococcus aureus* carriage after decolonization therapy: a case–control study. *Clin Infect Dis* 2011; **52**: 1422–30.

77 Ellington MJ, Hope R, Livermore DM *et al*. Decline of EMRSA-16 amongst methicillin-resistant *Staphylococcus aureus* causing bacteraemias in the UK between 2001 and 2007. *J Antimicrob Chemother* 2010; **65**: 446–8.

78 Kampf G, Jarosch R, Ruden H. Limited effectiveness of chlorhexidine based hand disinfectants against methicillin-resistant *Staphylococcus aureus* (MRSA). *J Hosp Infect* 1998; **38**: 297–303.

79 Wootton M, Walsh TR, Davies EM *et al*. Evaluation of the effectiveness of common hospital hand disinfectants against methicillin-resistant *Staphylococcus aureus*, glycopeptide-intermediate *S. aureus*, and heterogeneous glycopeptide-intermediate *S. aureus*. *Infect Control Hosp Epidemiol* 2009; **30**: 226–32.

80 Cookson BD, Bolton MC, Platt JH. Chlorhexidine resistance in methicillin-resistant *Staphylococcus aureus* or just an elevated MIC? An in vitro and in vivo assessment. *Antimicrob Agents Chemother* 1991; **35**: 1997–2002.

81 Block C, Furman M. Association between intensity of chlorhexidine use and micro-organisms of reduced susceptibility in a hospital environment. *J Hosp Infect* 2002; **51**: 201–6.

82 Batra R, Cooper BS, Whiteley C *et al*. Efficacy and limitation of a chlorhexidine-based decolonization strategy in preventing transmission of methicillin-resistant *Staphylococcus aureus* in an intensive care unit. *Clin Infect Dis* 2010; **50**: 210–7.

83 Harbarth S, Dharan S, Liassine N *et al*. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1999; **43**: 1412–6.

84 Kassakian SZ, Mermel LA, Jefferson JA *et al*. Impact of chlorhexidine bathing on hospital-acquired infections among general medical patients. *Infect Control Hosp Epidemiol* 2011; **32**: 238–43.

85 Simor AE, Phillips E, McGeer A *et al*. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infect Dis* 2007; **44**: 178–85.

86 Wendt C, Schinke S, Wurttemberger M *et al*. Value of whole-body washing with chlorhexidine for the eradication of methicillin-resistant *Staphylococcus aureus*: a randomized, placebo-controlled, double-blind clinical trial. *Infect Control Hosp Epidemiol* 2007; **28**: 1036–43.

87 Ridenour G, Lampen R, Federspiel J *et al*. Selective use of intranasal mupirocin and chlorhexidine bathing and the incidence of methicillin-resistant *Staphylococcus aureus* colonization and infection among intensive care unit patients. *Infect Control Hosp Epidemiol* 2007; **28**: 1155–61.

88 Maki DG. The use of antiseptics for handwashing by medical personnel. *J Chemother* 1989; **1** Suppl 1: 3–11.

89 Smylie HG, Logie JR, Smith G. From Phisohex to Hibiscrub. *Br Med J* 1973; **4**: 586–9.

90 Mangram AJ, Horan TC, Pearson ML *et al*. Guideline for prevention of surgical site infection, 1999. Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* 1999; **20**: 250–78.

91 Chaiyakunapruk N, Veenstra DL, Lipsky BA *et al*. Chlorhexidine compared with povidone-iodine solution for vascular catheter-site care: a meta-analysis. *Ann Intern Med* 2002; **136**: 792–801.

92 Climo MW, Sepkowitz KA, Zuccotti G *et al*. The effect of daily bathing with chlorhexidine on the acquisition of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and healthcare-associated bloodstream infections: results of a quasi-experimental multicenter trial. *Crit Care Med* 2009; **37**: 1858–65.

93 Parras F, Guerrero MC, Bouza E *et al*. Comparative study of mupirocin and oral co-trimoxazole plus topical fusidic acid in eradication of nasal carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1995; **39**: 175–9.

94 Timsit JF, Schwebel C, Bouadma L *et al*. Chlorhexidine-impregnated sponges and less frequent dressing changes for prevention of catheter-related infections in critically ill adults: a randomized controlled trial. *JAMA* 2009; **301**: 1231–41.

95 Brun-Buisson C, Doyon F, Sollet JP *et al*. Prevention of intravascular catheter-related infection with newer chlorhexidine-silver sulfadiazine-coated catheters: a randomized controlled trial. *Intens Care Med* 2004; **30**: 837–43.

96 Ostendorf T, Meinhold A, Harter C *et al*. Chlorhexidine and silver-sulfadiazine coated central venous catheters in haematological patients—a double-blind, randomised, prospective, controlled trial. *Support Care Cancer* 2005; **13**: 993–1000.

97 Rupp ME, Lisco SJ, Lipsett PA *et al*. Effect of a second-generation venous catheter impregnated with chlorhexidine and silver sulfadiazine on central catheter-related infections: a randomized, controlled trial. *Ann Intern Med* 2005; **143**: 570–80.

98 Bassetti S, Hu J, D'Agostino RB Jr. *et al*. Prolonged antimicrobial activity of a catheter containing chlorhexidine-silver sulfadiazine extends protection against catheter infections in vivo. *Antimicrob Agents Chemother* 2001; **45**: 1535–8.

99 Chlebicki MP, Safdar N. Topical chlorhexidine for prevention of ventilator-associated pneumonia: a meta-analysis. *Crit Care Med* 2007; **35**: 595–602.

100 Kucken D, Feucht H, Kaulfers P. Association of *qacE* and *qacEΔ1* with multiple resistance to antibiotics and antiseptics in clinical isolates of Gram-negative bacteria. *FEMS Microbiol Lett* 2000; **183**: 95–8.

101 Kazama H, Hamashima H, Sasatsu M *et al*. Distribution of the antiseptic-resistance gene *qacEΔ1* in Gram-positive bacteria. *FEMS Microbiol Lett* 1998; **165**: 295–9.

102 Ploy MC, Courvalin P, Lambert T. Characterization of In40 of *Enterobacter aerogenes* BM2688, a class 1 integron with two new gene cassettes, *cmlA2* and *qacF*. *Antimicrob Agents Chemother* 1998; **42**: 2557–63.

103 Heir E, Sundheim G, Holck AL. The *qacG* gene on plasmid pST94 confers resistance to quaternary ammonium compounds in staphylococci isolated from the food industry. *J Appl Microbiol* 1999; **86**: 378–88.

104 Heir E, Sundheim G, Holck AL. The *Staphylococcus qacH* gene product: a new member of the SMR family encoding multidrug resistance. *FEMS Microbiol Lett* 1998; **163**: 49–56.

105 Bjorland J, Steinum T, Sunde M *et al*. Novel plasmid-borne gene *qacJ* mediates resistance to quaternary ammonium compounds in equine *Staphylococcus aureus*, *Staphylococcus simulans*, and *Staphylococcus intermedius*. *Antimicrob Agents Chemother* 2003; **47**: 3046–52.

106 Braga TM, Marujo PE, Pomba C *et al*. Involvement, and dissemination, of the enterococcal small multidrug resistance transporter QacZ in resistance to quaternary ammonium compounds. *J Antimicrob Chemother* 2011; **66**: 283–6.

107 Wang C, Cai P, Zhan Q *et al*. Distribution of antiseptic-resistance genes *qacA/B* in clinical isolates of meticillin-resistant *Staphylococcus aureus* in China. *J Hosp Infect* 2008; **69**: 393–4.