Adaptive bacterial response to low level chlorhexidine exposure and its implications for hand hygiene

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ABSTRACT Chlorhexidine digluconate (CHG) is commonly used in healthcare, e.g. in skin antiseptics, antimicrobial soaps, alcohol-based hand rubs and oral or wound antiseptics. Aim of the literature review was to evaluate the potential of bacteria to adapt to low level CHG exposure. A maximum 4fold MIC increase to CHG was found after low level exposure in most of the 71 evaluated bacterial species. A strong adaptive mostly stable MIC change was described in strains or isolates of the healthcare-associated species E. coli, S. marcescens and P. aeruginosa (up to 500fold, 128fold or 32fold, respectively). The highest MIC values after adaptation were 2,048 mg/l (S. marcescens) and 1,024 mg/l (P. aeruginosa). A new resistance to tetracycline, gentamicin, meropeneme or triclosan was found in some adapted isolates. In E. coli horizontal gene transfer was induced (sulfonamide resistance by conjugation), pointing out an additional risk of sublethal CHG. The use of CHG in patient care - but also all other settings such as consumer products and households - should therefore be critically assessed and restricted to indications with a proven health benefit or justifiable public health benefits. Additional CHG has no health benefit when used in alcohol-based hand rubs and is not recommended by the WHO. For routine hand washing of soiled hands the use of plain soap is sufficient, CHG in soaps has no health benefit. In surgical hand antisepsis alcohol-based hand rubs should be preferred to CHG soaps. Implementation of these principles will help to reduce avoidable selection pressure.

INTRODUCTION Chlorhexidine digluconate (CHG) is a commonly used antiseptic agent in human healthcare and veterinary medicine, mainly used for hand hygiene (e.g. at 2% - 4% as the only active agent in antiseptic soaps or at 0.5% or 1% as an additional active agent in alcohol-based hand rubs), in alcohol-based skin antiseptics at 2% and in mouth rinse solutions at 0.12% - 0.2% [1]. The widespread CHG use in various types of applications has probably lead to an increase of acquired bacterial resistances, mainly in Gram-negative species such as Pseudomonas aeruginosa (minimal inhibitory concentration (MIC) of up to 800 mg/l), Serratia marcescens (MIC of up to 400 mg/l) or Klebsiella pneumoniae (MIC of up to 256 mg/l) [1]. In some types of applications such as skin antiseptics CHG has been shown to reduce healthcare associated infections, e.g. catheter-associated bloodstream infections [2]. Recent evidence also suggests a contribution to the prevention of surgical site infections [3] although the single effect of CHG for this application is still under controversial debate [4-6].

Its widespread use in hand hygiene by healthcare workers in many countries suggests to look specifically at all possible applications in this area. The WHO has published a recommendation on hand hygiene for healthcare in 2009 with the aim to reduce healthcare-associated infections [7]. Three types of applications can be distinguished. The use of alcohol-based hand rubs is recommended on clean hands in five specific clinical situations: before touching a patient, before clean or aseptic procedures, after body fluid exposure, after touching a patient and after touching patient surroundings [7, 8]. Hand washing with either plain soap or antiseptic soap and water is

Abbreviations:

BAC – benzzalkonium chloride,
CHG – chlorhexidine digluconate,
MIC – minimal inhibitory concentration,
PHMB – polyhexanide.

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Bacterial response to sublethal chlorhexidine
digluconate can cause strong and stable bacterial tolerance in isolates or strains of many mainly Gram-negative species.

Cross resistance to tetracycline, gentamicin, meropenem or triclosan was found in some isolates.

Horizontal gene transfer (sulfonamide resistance by conjugation) was induced in E. coli.

The use of CHG in patient care and other settings such as consumer products and households should be restricted to indications with a proven health benefit.

Adaptive bacterial response in Gram-negative species

Some antiseptic agents are more likely than others to cause a bacterial tolerance or even resistance [11]. The magnitude of any adaptive response to CHG is expressed as an MIC change and assigned to one of the following three categories: No adaptive response (no MIC increase), weak adaptive response (MIC increase ≤ 4fold) and strong adaptive response (MIC increase > 4fold). For some bacterial species two or more studies were found resulting in data from various isolates or strains. That is why some bacterial species can be found in two or three categories depending on the results obtained with the various isolates or strains of the same species.

Adaptive bacterial response in Gram-positive species

No adaptive response was found in isolates or strains of 15 species (Acinetobacter baumannii, Aeromonas hydrophila, Campylobacter coli, Campylobacter jejuni, Chryseobacterium indologenes, Citrobacter spp., Cronobacter sakazakii, E. coli, K. pneumoniae, Moraxella osloensis, P. aeruginosa, Pseudomonas nitroreductans, Pseudomonas putida, Pseudoxanthomonas spp. and Sphingobacterium multivorum). Some isolates or strains of 12 species were able to express a weak adaptive response (MIC increase ≤ 4fold) such as A. xylosidans, A. jandaei, Chrysochromobacterium spp., E. cloacae, Enterobacter spp., E. coli, H. gallinarum, K. pneumoniae, P. aeruginosa, S. Typhimurium, Serratia spp. and S. maltophilia (Table 1).

A strong but unstable MIC change (> 4fold) was found in isolates or strains of four species (Burkholderia cepacia, E. coli, Salmonella enteritidis, Salmonella Typhimurium). A strong and stable MIC change (> 4fold) was described for isolates or strains of seven species (E. coli, K. pneumoniae, P. aeruginosa, Salmonella Virchow, Salmonella spp., S. marcescens, Stenotrophomonas maltophilia) in isolates or strains of six species (Acinetobacter baylyi, Acinetobacter proteolyticus, E. coli, Pseudomonas spp., Raistonia spp., S. marcescens) the adaptive response was strong but its stability was not described.

Selected strains or isolates revealed substantial MIC changes: E. coli (up to 500fold), Salmonella spp. (up to 200fold), S. marcescens (up to 128fold), P. aeruginosa (up to 32fold), or A. proteolyticus, K. pneumoniae, and Pseudomonas spp. (all up to 16fold). The highest MIC values after adaptation were found in S. marcescens (2,048 mg/l), P. aeruginosa (1,024 mg/l), Salmonella spp. (> 1,000 mg/l), B. cepacia complex (700 mg/l), K. pneumoniae (> 512 mg/l) and E. coli (500 mg/l). Most maximum MIC values are above the proposed epidemiological cut-off value of 16–64 mg/l to determine CHG resistance in Gram-negative bacterial species [12].

Cross resistance to various antibiotics such as tetracycline, gentamicin or meropenem was found in some isolates of Bacteroides fragilis, B. cepacia complex and Salmonella spp.. In addition, a lower susceptibility to other biocidal agents was described for E. coli and S. Virchow to triclosan, for A. baylyi to hydrogen peroxide and for S. Typhimurium to benzalkonium chloride (BAC).

Other adaptive changes include a significant up-regulation of efflux pump genes in B. fragilis and B. cepacia complex. Horizontal gene transfer (sulfonamide resistance by conjugation) was induced in E. coli. VanA-type vancomycin resistance gene expression was increased vanA Enterococcus faecium (≥ 10fold increase of vanHAX encoding). Enhanced biofilm formation was described for K. pneumoniae and S. marcescens, adherence to polyethylene was increased in S. marcescens. Biofilm formation was decreased in B. cepacia.

Adaptive bacterial response in Gram-positive species

No adaptive response was found in isolates or strains from 18 species (Bacillus cereus, Corynebacterium xerosis, Enterococcus saccharolyticus, Eubacterium spp., Methylobacterium phyllosphaerae, Micrococcus luteus, Staphylococcus aureus, Staphylococcus capitis, Staphylococcus caprae, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus kloosii, Staphylococcus lugdunensis, Staphylococcus saprophyticus, Staphylococcus warneri and Streptococcus mutans).
| Species             | Strain/isolate                      | Type of exposure                  | Increase in MIC | MICmax (mg/l) | Stability | Associated changes                                                                 | Ref       |
|---------------------|-------------------------------------|-----------------------------------|-----------------|--------------|----------|-----------------------------------------------------------------------------------|-----------|
| *A. xylosoxidans*   | Domestic drain biofilm isolate MBRG 4.31 | 14 d at various concentrations   | 2fold           | 31.2         | No data  | None reported                                                                     | [36]      |
| *A. baumannii*      | Strain MBRG 15.1 from a domestic kitchen drain biofilm | 14 passages at various concentrations | None            | 7.8          | Not applicable | None reported                                                                     | [37]      |
| *A. baylyi*         | Strain ADP1                          | 30 min at 0.000001%               | Protection from lethal CHG concentration (0.00007%) | No data      | No data  | More resistance to a lethal hydrogen peroxide concentration (300 mM) | [38]      |
| *A. hydrophila*     | Domestic drain biofilm isolate MBRG 4.3 | 14 d at various concentrations   | None            | 15.6         | Not applicable | None reported                                                                     | [36]      |
| *A. jandaei*        | Domestic drain biofilm isolate MBRG 9.11 | 14 d at various concentrations   | 2fold           | 15.6         | No data  | None reported                                                                     | [36]      |
| *A. proteolyticus*  | Domestic drain biofilm isolate MBRG 9.12 | 14 d at various concentrations   | 16fold          | 125          | No data  | None reported                                                                     | [36]      |
| *B. fragilis*       | ATCC 25285                           | 12 h at 0.06%                     | No data         | No data      | Not applicable | Induction of multiple antibiotic resistance*; 2.7fold – 6fold increase of 6 efflux pumps | [39]      |
| *B. cenocepacia*    | 6 strains from clinical and environmental habitats | Up to 28 d at 15 mg/l            | Survival        | 100          | No data  | No degradation of CHG                                                             | [40]      |
| *B. cepacia*        | ATCC BAA-245                         | 40 d at various concentrations   | 8fold           | 29           | Unstable for 14 d | Decrease biofilm formation                                                       | [41]      |
| *B. cepacia complex*| *B. lato* strain 383                 | 5 min at 50 mg/l                 | No data         | 700          | Not applicable | Reduced susceptibility** to ceftazidime (30 – 33 mm), ciprofloxacin (11 – 20 mm) and imipenem (15 – 21 mm); 2 of 4 experiments) and to meropenem (33 mm; 1 of 4 experiments); up-regulation of transporter and efflux pump genes | [42]      |
| *C. coli*           | ATCC 33559 and a poultry isolate     | Up to 15 passages with gradually higher concentrations | None            | 0.031        | Not applicable | None described                                                                     | [15]      |
| *C. jejuni*         | NCTC 11168, ATCC 33560 and a poultry isolate | Up to 15 passages with gradually higher concentrations | None            | 1            | Not applicable | None described                                                                     | [15]      |
| *C. indolgenes*     | MBRG 4.29 (kitchen drain biofilm isolate) | 40 d at various concentrations   | None            | 7.3          | Not applicable | None described                                                                     | [41]      |
| *C. indolgenes*     | Domestic drain biofilm isolate MBRG 9.15 | 14 d at various concentrations   | None            | 31.2         | Not applicable | None reported                                                                     | [36]      |
| *Chrysobacterium spp.* | Domestic drain biofilm isolate MBRG 9.17 | 14 d at various concentrations   | 2fold           | 7.8          | No data  | None reported                                                                     | [36]      |
| *Chrysobacterium spp.* | 2 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 5fold – 6fold | 30           | Unstable | Cross-adaptation* to BAC (2fold - 100fold; 2 strains), triclosan (4fold; 1 strain) and didecyldimethyl-ammonium bromide (16fold; 1 strain); cross-resistance* to ceftazidime and ceftazidime (2 strains each), sulfamethoxazole, ampicillin and tetracycline (1 strain each) | [43]      |
TABLE 1 (continued) : Adaptive response of Gram-negative bacterial species to sublethal CHG exposure, adapted from [35].

| Species          | Strain/isolate                        | Type of exposure                        | Increase in MIC | MIC<sub>max</sub> (mg/l) | Stability       | Associated changes                                                                 | Ref  |
|------------------|---------------------------------------|-----------------------------------------|-----------------|--------------------------|----------------|-------------------------------------------------------------------------------------|------|
| Citrobacter spp. | Domestic drain biofilm isolate MBRG 9.18 | 14 d at various concentrations          | None            | 1.9                      | Not applicable | None reported                                                                        | [36] |
| C. sakazakii     | Strain MBRG 15.5 from a domestic kitchen drain biofilm | 14 passages at various concentrations | None            | 7.8                      | Not applicable | None reported                                                                        | [37] |
| E. cloacae       | 2 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 10fold – 16fold  | 80                       | Stable for 20 subcultures (1 strain) | Cross-adaptation* to BAC (6fold; 2 strains), triclosan (6fold - 15fold; 2 strains) and didecyldimethylammonium bromide (6fold; 1 strain); cross-resistance* to imipenem, ceftazidime and sulfamethoxazole (2 strains each), cefotaxime and tetracycline (1 strain each) | [43] |
| E. ludwigii      | 2 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 6fold – 8fold   | 40                       | Unstable       | Cross-adaptation* to BAC (6fold – 8fold; 2 strains), triclosan (8fold – 10fold; 2 strains) and didecyldimethylammonium bromide (4fold – 6fold; 2 strains); cross-resistance* to imipenem, ceftazidime and sulfamethoxazole (2 strains each) | [43] |
| Enterobacter spp. | 6 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 4fold – 10fold | 80                       | Stable for 20 subcultures (1 strain) | Cross-adaptation* to BAC (3fold – 20fold; 6 strains), triclosan (4fold – 100fold; 6 strains) and didecyldimethylammonium bromide (4fold – 6fold; 3 strains); cross-resistance* to ceftazidime and imipenem (3 strains each), cefotaxime and sulfamethoxazole (2 strains each) | [43] |
| E. coli          | ATCC 25922                             | 40 d at various concentrations          | None            | 7.3                      | Not applicable | None described                                                                        | [41] |
| E. coli          | NCTC 8196                              | 6 x 48 h at variable concentrations     | None            | 0.7                      | Not applicable | None reported                                                                        | [44] |
| E. coli          | ATCC 25922 and strain MBRG 15.4 from a domestic kitchen drain biofilm | 14 passages at various concentrations | 1.5fold - 5fold | 11.7                     | Stable for 14 d | No reported                                                                            | [37] |
| E. coli          | NCIMB 8545                             | 0.00005% for 30 s, 5 min and 24 h       | ≤ 6fold         | 39                       | Unstable for 10 d | No increase of MBC; unstable resistance* to tobramycin                               | [45] |
| E. coli          | NCTC 8196                              | 12 w at various concentrations          | 32fold          | No data                  | No data        | None described                                                                        | [46] |
| E. coli          | NCTC 12900 strain O157                 | 6 passages at variable concentrations   | Approx. 500fold | Approx. 500              | Stable for 30 d | Increased tolerance** to triclosan (15 mm)                                            | [47] |
| E. coli          | CV601                                  | 24.4 µg/l for 3 h                       | No data         | 4.9                      | Not applicable | Induction of horizontal gene transfer (sulfonamide resistance by conjugation)         | [48] |
| H. gallinarum    | Domestic drain biofilm isolate MBRG 4.27 | 14 d at various concentrations          | 2fold           | 31.2                     | No data        | None reported                                                                        | [36] |
| K. oxytoca       | 2 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 2fold – 8fold   | 40                       | Unstable       | Cross-adaptation* to BAC (60fold; 1 strain), triclosan (3fold – 8fold; 2 strains) and didecyldimethyl-ammonium bromide (6fold; 1 strain) | [43] |
| K. pneumoniae    | 7 “Murray isolates” from the pre-CHG era | Up to 5 w at various concentrations   | None (5 isolates) | 256                      | Stable for 10 d | None reported                                                                        | [49] |
| K. pneumoniae    | 7 modern isolates / strains            | Up to 5 w at various concentrations   | 4fold - 16fold  | > 512                    | Stable for 10 d | None reported                                                                        | [49] |
TABLE 1 (continued): Adaptive response of Gram-negative bacterial species to sublethal CHG exposure, adapted from [35].

| Species                  | Strain/isolate                  | Type of exposure                        | Increase in MIC | MICmax (mg/l) | Stability          | Associated changes                                                                 | Ref |
|--------------------------|---------------------------------|-----------------------------------------|-----------------|---------------|--------------------|-------------------------------------------------------------------------------------|-----|
| *K. pneumoniae*          | ATCC 13883                       | 40 d at various concentrations          | 6.9fold         | 14.5          | Stable for 14 d    | Increase biofilm formation                                                            | [41]|
| *Klebsiella spp.*        | Bioicide-sensitive strain from organic foods | Several passages with gradually higher concentrations | 2fold           | 30            | Unstable           | Cross-adaptation* to BAC (12fold) and triclosan (12fold); cross-resistance* to imipenem and ceftazidime | [43]|
| *M. osloensis*           | Strain MBRG 15.3 from a domestic kitchen drain biofilm | 14 passages at various concentrations | None            | 2.0           | Not applicable     | None reported                                                                      | [37]|
| *P. agglomerans*         | 5 bioicide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 5fold – 10fold | 50            | Unstable           | Cross-adaptation* to BAC (30fold – 40fold; 5 strains); triclosan (8fold – 100fold; 5 strains) and didecyldimethylammonium bromide (4fold – 6fold; 2 strains); cross-resistance* to cefotaxime and ceftazidime (3 strains each), tetracycline and sulfamethoxazole (2 strains each) and imipenem (1 strain) | [43]|
| *P. ananatis*            | 2 bioicide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 10fold – 50fold | 50            | Unstable           | Cross-adaptation* to BAC (20fold – 30fold; 2 strains); triclosan (60fold – 100fold; 2 strains) and didecyldimethylammonium bromide (6fold; 2 strains); cross-resistance* to cefotaxime (2 strains), sulfamethoxazole, imipenem, ceftazidime and tetracycline (1 strain each) | [43]|
| Pantoea spp.             | 3 bioicide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 5fold – 16fold | 80            | Unstable           | Cross-adaptation* to BAC (6fold – 60fold; 2 strains); triclosan (8fold; 3 strains) and didecyldimethylammonium bromide (4fold – 6fold; 3 strains); cross-resistance* to tetracycline (2 strains), ampicillin, ceftazidime, cefotaxime, sulfamethoxazole and imipenem (1 strain each) | [43]|
| *P. aeruginosa*          | 178 CHG sensitive strains Exposure to CHG | None at various concentrations          | None            | 625           | Not applicable     | None reported                                                                      | [50]|
| *P. aeruginosa*          | ATCC 9027                        | 40 d at various concentrations          | 2fold           | 14.5          | Unstable for 14 d  | None reported                                                                      | [41]|
| *P. aeruginosa*          | ATCC 9027                        | 14 passages at various concentrations   | 4fold           | 31.3          | Stable for 14 d    | None reported                                                                      | [37]|
| *P. aeruginosa*          | NCIMB 10421                      | 6 x 48 h at variable concentrations     | 7fold           | 70            | Stable for 15 d    | High MICs to BAC did not change in a relevant extent                                | [44]|
| *P. aeruginosa*          | NCTC 6749                        | 12 w at various concentrations          | 8fold – 32fold  | 1,024         | Stable for 7 w     | None described                                                                      | [46]|
| *P. nitroreductans*      | Domestic drain biofilm isolate MBRG 4.6 | 14 d at various concentrations          | None            | 3.9           | Not applicable     | None reported                                                                      | [36]|
| *P. putida*              | Strain MBRG 15.2 from a domestic kitchen drain biofilm | 14 passages at various concentrations | None            | 7.8           | Not applicable     | None reported                                                                      | [37]|
| *Pseudomonas spp.*       | Domestic drain biofilm isolate MBRG 9.14 | 14 d at various concentrations          | 16fold          | 15.6          | No data            | None reported                                                                      | [36]|

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Some isolates or strains of 12 species were able to express a weak adaptive response (MIC increase ≤ 4fold) such as *B. cereus*, *Corynebacterium pseudogenitalum*, *Corynebacterium renale* group, *Enterococcus casseliflavus*, *Enterococcus faecalis*, *E. faecium*, *M. luteus*, *S. aureus*, *S. capitis*, *S. haemolyticus*, *S. lugdenensis* and *S. warneri*.

A strong but unstable MIC change (> 4fold) was also described for isolates or strains of *S. aureus* which could be stable or of unknown stability.

The largest MIC increase was noticed in *S. aureus* (up to 16fold) and *E. faecalis* (up to 6.7fold) leading to MIC values as high as 24.2 mg/l in *E. faecalis* and 20 mg/l in *S. aureus* (Table 2). Some maximum MIC values are above the proposed epidemiological cut-off value (8 mg/l for *S. aureus*) and some below (64 mg/l for *E. faecalis*) to determine CHG resistance in Gram-positive bacterial species [12].

### Table 1 (continued): Adaptive response of Gram-negative bacterial species to sublethal CHG exposure, adapted from [35].

| Species                     | Strain/isolate                          | Type of exposure | Increase in MIC | MIC<sub>0</sub> (mg/l) | Stability | Associated changes | Ref |
|-----------------------------|-----------------------------------------|------------------|-----------------|------------------------|-----------|--------------------|-----|
| *Pseudomonas*               | Domestic drain biofilm isolate MBRG 9.20| 14 d at various concentrations | None            | 0.97                   | Not applicable | None reported      | [36] |
| *Ralstonia*                 | Domestic drain biofilm isolate MBRG 4.13| 14 d at various concentrations | 21fold          | 167                    | No data    | None reported      | [36] |
| *S. Virchow*               | Food isolate                           | 6 passages at variable concentrations | Approx. 120fold | Approx. 120            | Stable for 30 d | Increased tolerance*** to triclosan (0 mm) | [47] |
| *Salmonella enterica*      | Strain SL1344                           | 5 min at 0.1, 0.5, 1 and 4 mg/l | 13fold – 27fold | 800                    | Unstable for 1 d | 3fold – 67fold increase of tolerance*** to BAC | [51] |
| *Salmonella*               | Strain 14028S                          | 5 min at 1 and 5 mg/l | 3fold – 33fold | 1,000                  | Stable for 1 d | 2.5fold – 20fold increase of tolerance*** to BAC | [51] |
| *S. enteritidis*           | ATCC 13076                              | 7 d of sublethal exposure | ≥ 10fold | > 50                   | Unstable | None reported      | [52] |
| *Salmonella*               | 3 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 5fold – 10fold | 50 | Unstable | Cross-adaptation* to BAC (8fold – 30fold; 2 strains) and triclosan (40fold - 80fold; 3 strains) cross-resistance* to cefotaxime, nalidixic acid and imipenem (2 strains each), tetracycline and sulfamethoxazole (1 strain each) | [43] |
| *Salmonella*               | 6 strains with higher MICs to biocidal products | 8 days at increasing concentrations | 50fold – 200fold (2 strains) | > 1,000 | “stable” | One strain with increased tolerance*** to tetracycline (> 16 mg/l), chloramphenicol (8 mg/l) and nalidixic acid (16 mg/l) | [53] |
| *S. marcescens*            | Strain GSU B6-828                      | 7 d exposure to CHG-containing contact lens solutions | 8fold | 50 | No data | Increased adherence to polyethylene | [54] |
| *S. marcescens*            | ATCC 13880                             | 40 d at various concentrations | 9.6fold | 116 | Stable for 14 d | Increase biofilm formation | [41] |
| *S. marcescens*            | Clinical isolate                       | 12 w at various concentrations | 32fold – 128fold | 2,048 | Stable for 7 w | None described | [46] |
| *Serratia*                 | Not described                          | 5 to 8 transfers | “resistance” to CHG | No data | “stable” | None described | [55] |
| *S. multivorum*            | Domestic drain biofilm isolate MBRG 9.19| 14 d at various concentrations | None | 15.6 | Not applicable | None reported | [36] |
| *S. maltophilia*           | Domestic drain biofilm isolate MBRG 9.13| 14 d at various concentrations | 4fold | 62.5 | No data | None reported | [36] |
| *S. maltophilia*           | MBRG 4.17 (kitchen drain biofilm isolate) | 40 d at various concentrations | 6fold | 29 | Stable for 14 d | None described | [41] |

*spiraling gradient endpoint method; **disc diffusion method; ***broth microdilution; ****macrodilution method
Cross tolerance to various antibiotics such as tetracycline, gentamicin or meropenem could be found in some isolates of *S. aureus*. In *E. faecium* (vancomycin-resistant enterococcus; VRE) a more than 10fold vanA up-regulation was detected as well as reduced daptomycin susceptibility. An increase in biofilm formation was described in *S. epidermidis*.

**DISCUSSION**

The strongest adaptation to low level CHG exposure was found in common nosocomial pathogens such as *E. coli* (up to 500fold MIC increase), *S. marcescens* (up to 128fold MIC increase), *P. aeruginosa* (up to 32fold MIC increase) and *K. pneumoniae* (up to 16fold MIC increase). After sublethal exposure the highest MIC values were also found in common nosocomial pathogens such as *S. marcescens* (2,048 mg/l), *P. aeruginosa* (1,024 mg/l), *K. pneumoniae* (> 512 mg/l) and *E. coli* (500 mg/l). It is probably no coincidence that these pathogens are among those species considered to have extreme or even pan resistance to antibiotics [13].

Low level CHG exposure also reduced the susceptibility to selected antibiotics in *Burkholderia* spp. or *Salmonella* spp. In *Burkholderia* spp. an up-regulation of transporter and efflux pump genes was found. Efflux pumps are often not agent-specific and may well result in resistance to other biocidal agents or antibiotics [1]. A quite alarming finding was that horizontal gene transfer was induced in *E. coli* by low level CHG exposure enabling the faster spread of resistance genes within the bacterial community.

Some mechanisms of the adaptive response have been described. Increased expression of efflux pumps is recognized as a mechanism of antibiotic and biocide resistance. The pumps may have limited or broad substrates, the so-called multiple drug resistance pumps [14]. The multiple antibiotic resistance (mar) locus and mar regulon in *E. coli* and other members of the enterobacteriaceae is a paradigm for a generalized response locus leading to increased expression of efflux pumps. One such pump, the AcrAB pump, extrudes biocides such as triclosan, chlorhexidine and quaternary ammonium compounds as well as multiple antibiotics [14]. In *P. aeruginosa*, a number of multidrug efflux pumps export a broad range of substrates [14]. In *C. jejuni* and *C. coli* active efflux was identified in adapted strains. In addition, the outer membrane protein profiles had changed, along with morphological changes [15]. In *K. pneumoniae* CHG adaptation was associated with mutations in the two-component regulator phoPQ and a putative Tet repressor gene (smvR) adjacent to the major facilitator superfamily (MFS) efflux pump gene, smvA [16]. And in *Salmonella* spp. a defense network was described that involved multiple cell targets including those associated with the synthesis and modification of the cell wall, the SOS response, virulence, and a shift in cellular metabolism toward anoxic pathways. In addition, results indicated that CHG tolerance was associated with more extensive modifications of the same cellular processes involved in this proposed network, as well as a divergent defense response involving the up-regulation of additional targets such as the flagellar apparatus and an altered cellular phosphate metabolism [17].

A major limitation of this review is that most of the data were obtained in laboratories under defined conditions. The findings are certainly suitable to describe the potential for adaptation to CHG. But it is less clear if or how the findings are transferred to the clinic. In 2002 Block et al. described that the MIC for CHG was higher among clinical isolates when more CHG was used for any type of application [18]. A similar correlation between CHG usage and MIC values was described in 2018 with *S. aureus* [19]. Lindford et al. described an outbreak by MDR *A. baumannii* in a burn unit. One of the measures to finally control the outbreak was to reduce moist low-concentration CHG dressings on burn wounds [20]. And yet the clinical impact of an elevated MIC value remains under controversial debate [21]. In hand hygiene it is known that a low bactericidal effect of CHG on the skin can only be achieved in the presence of small volumes of water, the water released by the skin as transepidermal water loss does not seem to be sufficient [22]. If the water realised by the skin is sufficient to allow adaptive changes of the bacterial species on the skin it is currently not known. And yet, the triclosan tale strongly suggested that “a chemical that constantly stresses bacteria to adapt, and behaviour that promotes antibiotic resistance needs to be stopped immediately when the benefits are null” [10]. CHG is obviously such a chemical that constantly stresses bacteria to adapt. Even if the clinical impact of isolates or strains with elevated MIC values cannot finally be evaluated at the moment it seems justified restricting the use of CHG to applications where health benefits are associated with its use.

**IMPLICATIONS FOR HAND HYGIENE**

**Alcohol-based hand rubs**

In alcohol-based hand rubs with additional CHG used for hygienic hand disinfection there is no sound evidence for an additional effect of CHG *in vitro* [23]. There is also no evidence on the prevention of any type of healthcare-associated infection by the additional CHG in hand rubs. But there are obvious risks such as acquired bacterial resistance, anaphylactic reactions or skin irritation [24]. Its use in the immediate patient environment may therefore contribute to the selection pressure especially when the CHG concentration is sublethal [20]. Additional biocidal agents in alcohol-based hand rubs such as CHG are not recommended by the WHO [7].

The same applies to hand rubs used for surgical hand disinfection [24]. For surgical hand disinfection additional biocidal agents such as CHG are not recommended because they do not contribute to the prevention of surgical site infections [3, 25]. Replacing hand rubs with additional CHG by hand rubs without CHG will help to reduce avoidable CHG selection pressure. They should, however, have an equivalent efficacy, dermal tolerance and user acceptability [26].
| Species               | Strain/isolate                                      | Type of exposure                                      | Increase in MIC | MICmax (mg/l) | Stability | Associated changes                                                                 | Ref  |
|----------------------|-----------------------------------------------------|-------------------------------------------------------|-----------------|---------------|----------|-------------------------------------------------------------------------------------|------|
| B. cereus            | MRBG 4.21 (kitchen drain biofilm isolate)           | 40 d at various concentrations                        | None            | 14.5          | Not applicable                      | None described | [41]|
| B. cereus            | Domestic drain biofilm isolate MRBG 4.21            | 14 d at various concentrations                        | None            | 1.9           | Not applicable                      | None reported  | [36]|
| B. cereus            | 4 biocide-sensitive strains from organic foods      | Several passages with gradually higher concentrations  | 6fold – 16fold  | 80            | Stable for 20 subcultures (1 strain)      | Cross-adaptation* to BAC (≥ 100fold; 3 strains) and didecyl dimethylammonium bromide (6fold; 2 strains); cross-resistance* to imipenem (4 strains), sulfmethoxazole (2 strains), ampicillin and tetracycline (1 strain each) | [43]|
| B. licheniformis     | 2 biocide-sensitive strains from organic foods      | Several passages with gradually higher concentrations  | 4fold – 10fold  | 50            | Unstable                           | Cross-adaptation* to BAC (40fold – 75fold; 2 strains) and triclosan (8fold; 1 strain); cross-resistance* to imipenem (2 strains), cefotaxime and tetracycline (1 strain each) | [43]|
| B. subtilis          | 2 strains and 3 derivatives                         | 2 h at 0.00005%                                       | No data         | No data       | Not applicable                      | No increase of transfer of the mobile genetic element Tn916, a conjugative transposon | [56]|
| Bacillus spp.        | 4 biocide-sensitive strains from organic foods      | Several passages with gradually higher concentrations  | 4fold – 8fold   | 40            | Unstable                           | Cross-adaptation* to BAC (15fold – 100fold; 4 strains), triclosan (8fold; 4 strains) and didecyl dimethylammonium bromide (4fold - 6fold; 2 strains); cross-resistance* to imipenem and sulfmethoxazole (4 strains each), cefotaxime and tetracycline (1 strain each) | [43]|
| C. pseudogenitalum   | Human skin isolate MBRG 9.24                        | 14 d at various concentrations                        | 4fold           | 3.9           | No data                            | None reported  | [36]|
| C. renale group      | Human skin isolate MBRG 9.13                        | 14 d at various concentrations                        | 4fold           | 31.2          | No data                            | None reported  | [36]|
| C. xerosis           | WIBG 1.2 (wound isolate)                            | 40 d at various concentrations                        | None            | 3.6           | Not applicable                      | None described | [41]|
| E. casseliflavus     | 3 biocide-sensitive strains from organic foods      | Several passages with gradually higher concentrations  | 8fold – 20fold  | 100           | Stable for 20 subcultures (1 strain) | Cross-adaptation* to BAC (30fold - 100fold; 3 strains), triclosan (> 100fold; 1 strain) and didecyl dimethylammonium bromide (4fold - 6fold; 2 strains); cross-resistance* to imipenem (3 strains), cefotaxime and tetracycline (1 strain each) | [43]|
| E. durans            | Biocide-sensitive strain from organic foods         | Several passages with gradually higher concentrations  | 10fold          | 50            | Unstable                           | Cross-adaptation* to BAC (≥ 100fold), triclosan (10fold) and didecyl dimethylammonium bromide (16fold), cross-resistance to imipenem and ampicillin | [43]|
| E. faecalis          | 1 strain of unknown origin                          | 14 passages at various concentrations                 | 2fold           | 7.8           | Stable for 14 d                     | None reported  | [37]|
| E. faecalis          | Strain SS497                                        | 10 passages at various concentrations                 | 3.7fold         | 11            | No data                            | Significant increase of surface hydrophobicity | [57]|
| E. faecalis          | WIBG 1.1 (wound isolate)                            | 40 d at various concentrations                        | 6.7fold         | 24.2          | Unstable                           | None described | [41]|
### TABLE 2 (continued): Adaptive response of Gram-positive bacterial species to sublethal CHG exposure, adapted from [35].

| Species          | Strain/isolate                          | Type of exposure | Increase in MIC | MIC<sub>min</sub> (mg/l) | Stability | Associated changes                                                                 | Ref         |
|------------------|-----------------------------------------|------------------|-----------------|--------------------------|-----------|------------------------------------------------------------------------------------|-------------|
| *E. faecalis*    | Biocide-sensitive strain from organic foods | Several passages with gradually higher concentrations | 10-fold         | 50                       | Unstable  | Cross-adaptation* to BAC (80-fold) and didecyldimethy lammonium bromide (8-fold); cross-resistance* to imipenem and ceftazidime | [43]        |
| *E. faecium*     | 9 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 2-fold – 16-fold | 80                       | Stable for 20 subcultures (1 strain) | Cross-adaptation* to BAC (10-fold - 100fold; 9 strains), triclosan (4-fold - 100fold; 6 strains) and didecyldimethy lammonium bromide (4fold - 8fold; 7 strains); cross-resistance* to imipenem (9 strains), tetracycline (4 strains), ampicillin (2 strains) cefotaxime and ceftazidime (1 strain each) | [43]        |
| *E. faecium*     | VRE strain 410 (skin and soft tissue infection isolate) | 21 d at various concentrations | 4-fold          | 19.6                     | No data   | Subpopulation with reduced susceptibility* to daptomycin including significant alterations in membrane phospholipids | [58]        |
| *E. faecium*     | 3 vanA VRE strains | 15 min at MIC | No data | No data | Not applicable | ≥ 100-fold increase of vanHAX encoding VanA-type vancomycin resistance and of liaXYZ associated with reduced daptomycin susceptibility; vanA upregulation was not strain or species specific; VRE was more susceptible to vancomycin in the presence of subinhibitory chlorhexidine | [59]        |
| *E. saccharolyticus* | Domestic drain biofilm isolate MBRG 9.16 | 14 d at various concentrations | None            | 1.9                      | Not applicable | None reported | | [36]        |
| **Enterococcus spp.** | 6 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 2-fold – 10-fold | 50                       | Unstable  | Cross-adaptation* to BAC (30-fold - 100fold; 6 strains), triclosan (4fold - 15fold; 5 strains) and didecyldimethy lammonium bromide (4fold - 6fold; 4 strains); cross-resistance* to imipenem (9 strains), ceftazidime and sulfa-methoxazole (5 strains each), cefotaxime (4 strains) and tetracycline (3 strains) | [43]        |
| *Eubacterium spp.* | Domestic drain biofilm isolate MBRG 4.14 | 14 d at various concentrations | None            | 31.2                     | Not applicable | None reported | | [36]        |
| *M. phyllophthora* | Domestic drain biofilm isolate MBRG 4.30 | 14 d at various concentrations | None            | 15.6                     | Not applicable | None reported | | [36]        |
| *M. luteus*      | MBRG 9.25 (skin isolate) | 40 d at various concentrations | None            | 3.6                      | Not applicable | None described | | [41]        |
| *S. aureus*      | ATCC 6538 | 40 d at various concentrations | None            | 3.6                      | Not applicable | None described | | [41]        |
| *S. aureus*      | ATCC 6538 | 100 d at various concentrations | None            | 0.6                      | Not applicable | None described | | [60]        |
| *S. aureus*      | NCTC 6571 plus 2 MRSA strains | Several passages with gradually higher concentrations | 1.3-fold – 2-fold | 1                        | “unstable” | None described | | [61]        |
| *S. aureus*      | NCIMB 9518 | 0.00005% for 30 s, 5 min and 24 h | 2-fold – 5-fold | 20                       | Stable for 10 d | No increase of MBC | | [45]        |
| Species          | Strain/isolate         | Type of exposure | Increase in MIC | MIC<sub>max</sub> (mg/l) | Stability        | Associated changes                                                                 | Ref  |
|------------------|------------------------|-----------------|----------------|-------------------|------------------|------------------------------------------------------------------------------------|------|
| *S. aureus*      | ATCC 6538              | 7 d of sublethal exposure | 2.5fold     | 2.5               | Unstable for 10 d | None reported                                                                       | [52] |
| *S. aureus*      | 3 clinical MRSA strains| 10 passages at various concentrations | ≤ 4fold    | 8                 | No data          | No change of PHMB susceptibility**                                                  | [62] |
| *S. aureus*      | ATCC 6538              | 14 passages at various concentrations | 4fold       | 7.8               | Unstable for 14 d | None reported                                                                       | [37] |
| *S. aureus*      | ATCC 25923 and 14 clinical isolates | 14 d at various sublethal concentrations | 4fold - 6fold (6 isolates) | 6.3        | No data          | Increased tolerance* to ciprofloxacin (4fold - 64fold; 10 isolates), tetracycline (4fold - 512fold; all isolates), gentamicin (4fold - 512fold; 8 isolates), amikacin (16fold - 512fold; 11 isolates), cefepime (8fold - 64fold; 11 isolates) and meropenem (8fold - 64fold; 9 isolates) | [63] |
| *S. aureus*      | NCTC 4163              | 12 w at various concentrations | 16fold      | No data           | No data          | Stability                                                                         | [46] |
| *S. aureus*      | Strain SAU3 carrying plasmid pWG613 | 10 min at 0.00005% | No data       | No data           | Not applicable | No significant reduction of plasmid transfer frequency                                | [64] |
| *S. capitis*     | MRBG 9.34 (skin isolate) | 40 d at various concentrations | 1.7fold     | 6                 | Stable for 14 d  | None described                                                                     | [41] |
| *S. capitis*     | Human skin isolate MRGB 9.34 | 14 d at various concentrations | None       | 7.8               | Not applicable | None reported                                                                     | [36] |
| *S. caprae*      | MRBG 9.3 (skin isolate) | 40 d at various concentrations | None       | 3.6               | Not applicable | None reported                                                                     | [41] |
| *S. caprae*      | Human skin isolate MRGB 9.30 | 14 d at various concentrations | None       | 7.8               | No data          | None reported                                                                     | [36] |
| *S. cohnii*      | Human skin isolate MRGB 9.31 | 14 d at various concentrations | None       | 3.9               | Not applicable | None reported                                                                     | [36] |
| *S. epidermidis* | MRBG 9.33 (skin isolate) | 40 d at various concentrations | None       | 9.7               | Not applicable | None reported                                                                     | [41] |
| *S. epidermidis* | Human skin isolate M 9.33 | 14 d at various concentrations | None       | 7.8               | Not applicable | None reported                                                                     | [36] |
| *S. epidermidis* | CIP53124               | 1 d at various concentrations | No data    | No data           | Not applicable | Significant increase of biofilm formation at various sublethal concentrations       | [65] |
| *S. haemolyticus*| Human skin isolate MRGB 9.35 | 14 d at various concentrations | None       | 15.6              | Not applicable | None reported                                                                     | [36] |
| *S. haemolyticus*| MRBG9.35 (skin isolate) | 40 d at various concentrations | 2.1fold     | 3                 | Unstable for 14 d | None reported                                                                     | [41] |
| *S. hominis*     | Human skin isolate MRGB 9.37 | 14 d at various concentrations | None       | 7.8               | Not applicable | None reported                                                                     | [36] |
| *S. kloosii*     | Human skin isolate MRGB 9.37 | 14 d at various concentrations | None       | 7.8               | Not applicable | None reported                                                                     | [36] |
| *S. lugdunensis* | Human skin isolate MRGB 9.36 | 40 d at various concentrations | None       | 15.6              | Not applicable | None reported                                                                     | [36] |
| *S. lugdunensis* | MRBG 9.36 (skin isolate) | 40 d at various concentrations | 4fold       | 3.6               | Stable for 14 d  | None reported                                                                     | [41] |
| *S. saprophyticus* | Human skin isolate MRGB 9.29 | 14 d at various concentrations | None       | 3.9               | Not applicable | None reported                                                                     | [36] |
| *S. saprophyticus* | 4 biocide-sensitive strains from organic foods | Several passages with gradually higher concentrations | 2fold – 10fold | 50                | Unstable        | Cross-adaptation* to BAC (25fold - 100fold; 4 strains), triclosan (4fold - 8fold; 3 strains) and didecyldimethy lammonium bromide (6fold - 12fold; 2 strains); cross-resistance* to ceftazidime (4 strains), imipenem, sulfamethoxazole and cefotaxime (2 strains each) and tetracycline (1 strain) | [43] |
Antimicrobial soaps

Another simple option to reduce CHG selection pressure is to ban CHG soaps in healthcare for regular handwashing. Based on the WHO recommendation for hand hygiene from 2009 hand washing is recommended to wash hands when they are visibly soiled. The use of plain soap, however, is adequate, there is no health benefit for antimicrobial soaps [7].

Another possible use of antimicrobial soaps is prior to surgery. Surgical scrubbing usually lasts for 6–10 min of scrubbing time and consumes between 5 and 20 l water per scrub [27-29]. Surgical scrub products may only be effective with additional post-scrub water-based CHG treatments of the hands which pose an additional contamination and selection pressure risk [30, 31]. Alcohol-based hand rubs with an appropriate concentration of alcohol(s) have a stronger effect on the resident hand flora, require typically 1.5 min for application, cause less skin irritation [32] and do not pose any selection pressure to bacterial species due to their volatility [33, 34].

CONCLUSION

Overall, the evidence on the adaptive potential of various pathogens to low level CHG exposure strongly suggests to critically review the use of CHG in patient care and to eliminate it in all applications where no health benefit has been shown or is realistically expectable.

METHODS

A systematic literature search was conducted via the National Library of Medicine (PubMed) and via ScienceDirect (only research articles) on 10th March 2018 and up-dated on 25th June 2018 using the term chlorhexidine in combination with low level exposure (17 hits PubMed, 5 hits ScienceDirect), adaptive response (6/24), sublethal (27/72), resistance and MIC (142/640), and resistant and MIC (116/648). In addition, studies deemed suitable for this review were also included. Publications were included and results were extracted from them when they provided original data on any type of adaptive response to the exposure of bacteria to sublethal concentrations of CHG, corresponding changes of MICs (CHG, antibiotics, and other biocidal agents), survival in CHG solutions, efflux pump activity, gene expression or biofilm formation. Articles were excluded when they described only data on fungi, outbreaks, pseudo-outbreaks or infections caused by contaminated CHG products or solutions, only biochemical changes, an adaptive effect with other chlorhexidine salts or when a CHG solution or product was used for disinfection during an outbreak but without being the suspected or proven source. Reviews were also excluded and screened for any original information within the scope of the review.

The susceptibility of isolates or strains to CHG is described as the minimum inhibitory concentration (MIC value). In most studies it was described as a single value and is presented as such unless stated otherwise. The magnitude of any adaptive response to CHG is expressed as an MIC change and assigned to one of the following three categories: no adaptive response (no MIC increase), weak adaptive response (MIC increase ≤ 4fold) and strong adaptive response (MIC increase > 4fold).

SUPPLEMENTAL MATERIAL

All supplemental data for this article are available online at www.microbialcell.com.
CONFLICT OF INTEREST
The author has worked until 2016 for Bode Chemie GmbH, Hamburg, Germany.

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