Is the use of chlorhexidine contributing to increased resistance to chlorhexidine and/or antibiotics?

Technical Report

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1. Review Team and Background

Review Team:
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Background

The National Health and Medical Research Council (NHMRC) commissioned this independent literature review to provide assurance that the revision of the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (2010 Guidelines) is grounded in the most up-to-date and relevant scientific evidence.

Methods

Literature review

The clinical questions were:

1. Does exposure (different dosages, duration of use, and stratification of exposure) to any form of chlorhexidine result in 'chlorhexidine resistance' within different healthcare settings?
2. Does exposure (different dosages, duration of use, and stratification of exposure) to any form of chlorhexidine increase the incidence and/or prevalence of antibiotic-resistant strains of bacteria in any person within different healthcare settings?

Table 1: PICOS overview Question 1

	Population and setting	Intervention	Outcome	Types of studies
Qu 1	All patients (isolates) / participants (isolates) including children and adults in different health care settings including acute care, residential aged care, paediatric, neonatal and primary care and rehabilitation as well as the laboratory setting.	All forms of use of chlorhexidine in humans and all different exposures (dosage form, duration, stratification of exposure) across different settings.	1. 'Chlorhexidine Resistance' (with definition / measures used) to chlorhexidine established. 2. A specific intervention identified as contributing to resistance to Chlorhexidine in a specific population and / or setting. 3. A specific exposure of a specific intervention identified as contributing to resistance to Chlorhexidine in a specific population and / or setting.	Follow stepped approach. Systematic reviews if possible. Primary research studies may include: • Characterization studies • Comparative (nonrandomised and observational) studies • Concurrent control or cohort studies • Case-control • Historical control • Interrupted time series • Case series

Table 2: PICOS overview Question 2

	Population and setting	**Intervention**	**Outcome**	**Types of studies**
Qu. 2	All patients (isolates) / participants (isolates) including children and adults in different health care settings including acute care, residential aged care, paediatric, neonatal and primary care and rehabilitation as well as the laboratory setting	All forms of use of chlorhexidine in humans and all different exposures (dosage form, duration, stratification of exposure) across different settings.	1. 'Resistance against antibiotics' defined by using the clinical breakpoints for resistance as specified by the European Committee on Antimicrobial Susceptibility testing (EUCAST) or the Clinical and Laboratory Standards Institute (CSLI). 2. Increase in the incidence (rate) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use of chlorhexidine to be recorded. 3. Increases in the prevalence (frequency) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying specific dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use of chlorhexidine to be recorded.	Follow stepped approach. Systematic reviews if possible. Primary research studies may include: • Characterization studies • Prevalence studies • Cohort studies • Cross-sectional studies

Search Strategy

Types of participants and settings
All patients (isolates) / participants (isolates) including children and adults in different health care settings including acute care, residential aged care, paediatric, neonatal and primary care and rehabilitation as well as the laboratory setting were included.

Types of interventions
All forms of use of chlorhexidine in humans and all different exposures (dosage form, duration, stratification of exposure) across different settings were included.

Type of Comparison
This review investigated all uses of chlorhexidine in health care in relation to 'chlorhexidine resistance' and, the incidence and/or prevalence of antibiotic-resistant strains of bacteria. Other than non-use of chlorhexidine, there was no comparison.

Types of outcome measures
In broad terms the outcomes were chlorhexidine resistance however defined or measured in relation to chlorhexidine use and chlorhexidine use leading to antibiotic-resistant strains of bacteria.

To address the question 'Does exposure (different dosages, duration of use, and stratification of exposure) to any form of chlorhexidine results in 'chlorhexidine resistance' within different healthcare settings?' the outcomes included:

- 'Chlorhexidine Resistance' (with definition / measures used) to chlorhexidine established.
- A specific intervention identified as contributing to resistance to Chlorhexidine in a specific population and / or setting.
- A specific exposure of a specific intervention identified as contributing to resistance to chlorhexidine in a specific population and / or setting.

To address the question 'Does exposure (different dosages, duration of use, and stratification of exposure) to any form of chlorhexidine increases the incidence and/or prevalence of antibiotic-resistant strains of bacteria in any person within different healthcare settings? ' the outcomes included:

- 'Resistance against antibiotics' defined by using the clinical breakpoints for resistance as specified by the European Committee on Antimicrobial Susceptibility testing (EUCAST) or the Clinical and Laboratory Standards Institute (CSLI).
- Increase in the incidence (rate) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use of chlorhexidine to be recorded.
- Increases in the prevalence (frequency) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying specific dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use of chlorhexidine to be recorded.

Publication Date and limits

The reviewer considered all relevant studies regardless of publication status (published, unpublished, in press, and ongoing) in the last ten years - from 2006 to October 2016 following the stepped approach described below. There was no search time limit for randomized controlled trials

(RCTs) and none were identified that addressed chlorhexidine use resulting in chlorhexidine/antibiotic-resistance strains of bacteria. The search was limited to English language publications.

Electronic searches

The following information sources were searched:

- CENTRAL (Cochrane Central Register of Controlled Trials, The Cochrane Library)
- CINAHL (Cumulative Index to Nursing & Allied Health Literature)
- Cochrane Database of Systematic Reviews
- DARE (Database of Abstracts of Reviews of Effects)
- Joanna Briggs Institute EBP Database
- EMBASE-OvidSP
- MEDLINE-OvidSP
- Science Citation Index Expanded (Web of Science)

The two core biomedical databases MEDLINE/EMBASE were searched; The Cochrane Library and also relevant allied health databases e.g. CINAHL and Joanna Briggs. In addition, two multidisciplinary databases that index high quality journals and have good health coverage e.g. Scopus and Web of Science were also included. The NCCHTA and WHO Library Information System databases were not searched.

The databases searched form the base set used for most health sciences literature searches at this University and are generally supplemented with other databases depending on the subject area, including multidisciplinary databases. The point of searching additional databases and in particular, multidisciplinary databases, is to capture papers that are not indexed by the two core biomedical databases. This is especially relevant when the types of studies may not be higher level evidence e.g. RCTs.

Grey literature

A grey literature search was conducted by the Lead Reviewer to identify studies not indexed in the databases listed above.

- AHRQ (Agency for Healthcare Research and Quality)- www.ahrq.gov
- Grey Literature Report (New York Academy of Medicine) http://greylit.org/
- NICE (National Institute for Health and Clinical Excellence) www.nice.org.uk/
- Open Grey http://www.opengrey.eu/)

Key international infection control and health care organisations were searched for relevant reports related to one of the review objectives. These international organisations included:

- USA - Department of Health & Human Services (http://www.hhs.gov/)
- USA - Agency for Healthcare Research and Quality (http://www.ahrq.gov/)
- USA - Infectious Disease Society of America (www.idsociety.org).
- Australia - Department of Health (http://www.health.gov.au/)
- Australia - National Health and Medical Research Council (http://www.nhmrc.gov.au/)
- Australian Institute for Health and Welfare (https://www.aihw.gov.au/)

- Australian Commission on Safety and Quality in Health Care (http://www.safetyandquality.gov.au/)
- NZ – Department of Health (http://www.health.govt.nz/)
- World Health Organization (http://www.who.int/en/)
- Centres for Disease Control and Prevention (http://www.cdc.gov/)
- European Centre for Disease Prevention and Control (http://ecdc.europa.eu/en/Pages/home.aspx)
- European Society for Clinical Microbiology and Infectious Diseases (www.escmid.org)
- British Society for Antimicrobial Chemotherapy (www.bsac.org.uk)
- Infectious Diseases Research Network (www.idrn.org).
- Canada - IPAC (http://www.ipac-canada.org/)
- UK Healthcare Infection Society (https://www.his.org.uk/)
- Therapeutic Goods Administration (https://www.tga.gov.au/)

Trial Registries

The following registries were searched for ongoing and completed trials:

- Australian New Zealand Clinical Trials registry http://www.anzctr.org.au/BasicSearch.aspx
- ClinicalTrials.gov, US National Institutes of Health (NIH) http://clinicaltrials.gov/
- ICTRP (International Clinical Trials Registry Platform, Word Health Organization (WHO) http://www.who.int/ictrp/en/
- metaRegister of Controlled trials- www.controlled-trials.com

Keywords

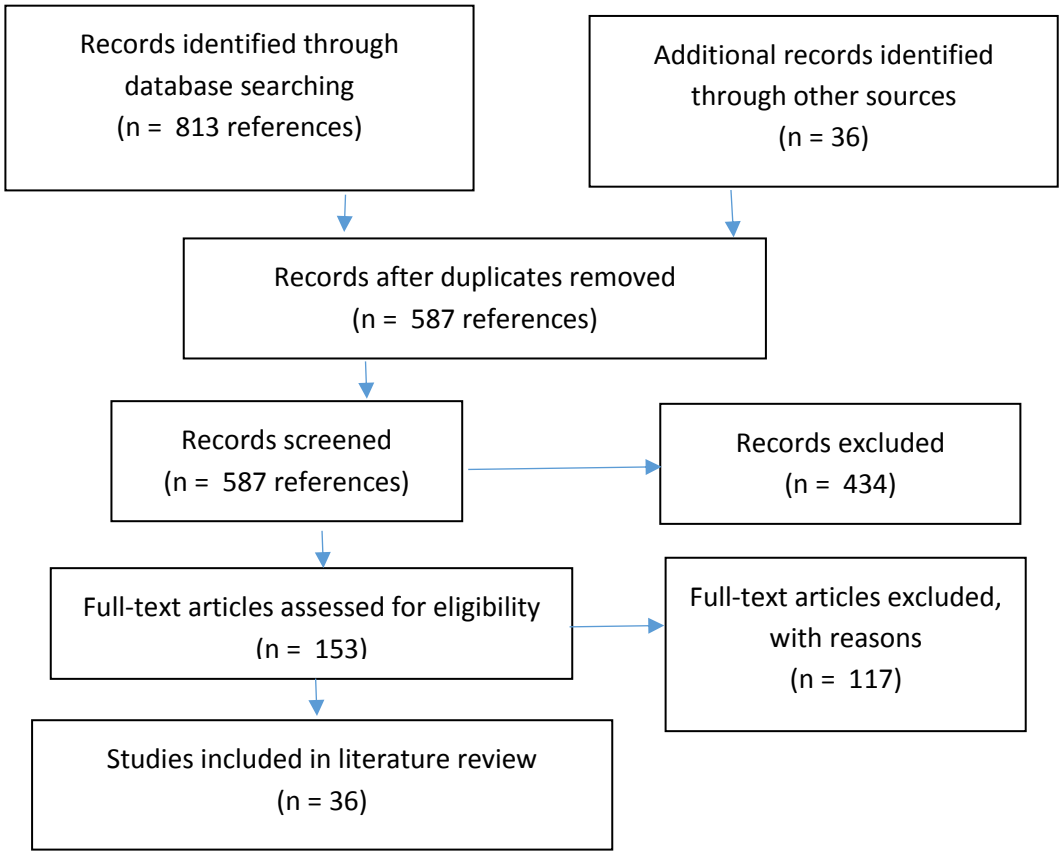
A combination of the search terms from concepts 1-4 (see Table 3) were used to identify potentially relevant peer reviewed publications. Synonymous terms, related MeSH headings, truncation symbols and wildcards were used to expand the search as appropriate. This formative phase of the search strategy was an integral part of a three-phase search process. The second phase of the search process involved the analysis of text words contained in the title and abstract of retrieved citations and of the index terms used to describe identified publications. The third step involved an integrated validation search using all identified key words and index terms, through the same databases.

Table 3. Key words and MeSH terms used in the search strategy.

Concept	Key words	MeSH
1.	Chlorhexidine, CHG, mk412a or mk-412a, Novalsan, Sebidin, Tubulicid, Gluconate, Biocide*, Eludril, Corsodyl, Chlorhexamed forte, Chlorohex, Cholorohexadine, Consepsis, Dentosan, Denzin, Eburos, Fimeil, Hexadol, Periogard, Promax, Soretol	Chlorhexidine/
2.	Bacteriocid*, Microbicid*, Skin decolonization, Root canal implant*, Dressing, Gel, Jelly, Lotion, Solution, Liquid, Pad, Sponge, Cream, Vaginal, Bactericid*, Bacteriostatic, Antiseptic, Disinfectant, Anti-infective agents, Anti-microbial* agents, Anti-mycobacterial agents	Anti-infective agents/, Anti-bacterial agents/, Anti-infective agents, local/, Hand disinfection/, Hand sanitizers Disinfectants/, Dental disinfectants/, "root canal irrigants"/, Anti-infective agents, urinary/
3.	Efflux system*, Efflux pump*, Time Kill, Time to Kill, Kill time, MIC, MBC, Kirby Bauer, Minimum inhibitory concentration, Minimum bacterial concentration	
4.	Susceptibility, Resistance, Tolerance	

Figure 1 details the overall results in a PRISMA (Moher et al. 2009) Flow Diagram.

Figure 1 PRISMA Flow Diagram



Inclusion and exclusion criteria for selecting studies

A stepped approach to the inclusion of studies was as follows.

Step 1: Systematic reviews (SRs) were searched – none were identified.

Step 2: Primary research studies (published and unpublished) including all types of observational and interventional studies were sourced. Please see Appendix 2 for the inclusion criteria checklist used.

Primary research studies included (n=29):

- Susceptibility testing /Controlled laboratory studies (n=24)
- Case-control / Interrupted time series / cross sectional / comparative (n= 5)

To identify missed papers, the bibliographies of the relevant papers were checked for articles missed by the initial search.

Studies included:

- Made clear the population of study
- Used isolates from humans
- Made clear the intervention – dosage form and exposure
- Made clear the health care setting or laboratory setting
- Defined or measured 'chlorhexidine resistance' / reduced susceptibility to chlorhexidine / non – susceptibility to chlorhexidine
- Defined antibiotic-resistant strain of bacteria

No use of chlorhexidine was excluded from the literature review. Studies were excluded if:

- Focus only on the use and effectiveness of chlorhexidine and not resistance
- Chlorhexidine resistance however stated not systematically assessed
- Isolates not from humans
- Focus was antibiotic resistance not related to chlorhexidine use
- Setting was schools or domestic home

Step 3: To complement what was identified in step 2, step 3 searched to see of any experimental and theoretical investigations could be included – none were identified.

Step 4: To ensure a broader understanding to address the literature review questions and to complement what was identified in all previous steps, case reports (n = 2) and evidence based / expert reviews (n= 5) were collated but only provided supported / background information.

In summary the number of primary research studies focused on review Question1 totalled 24/29. Studies showing a correlation between chlorhexidine use and increase in tolerance/reduced susceptibility totalled n=20/24. Studies showing no correlation between chlorhexidine use and an increase in tolerance/reduced susceptibility totalled n= 4/24 and of those four studies one (n=1) was on *Staphylococcus epidermidis*. The number of primary research studies that focused on review Question 2 totalled 9/29. Studies showing a link to chlorhexidine use and antibiotic resistance totalled n=8/9. Expert / Literature reviews and case reports were not included in these numbers.

Methodological Quality

The majority of the N=36 publications included in the review were controlled laboratory / susceptibility studies [n=24 (66%)], n=5 (14%) were case control/ cross sectional/ retrospective cohort studies and n=2 (6%) were case reports. The remaining publications n=5 (14%) were literature/ expert reviews.

Critical appraisal of the case control/cross sectional/ retrospective cohort studies and the case reports was undertaken using the Critical Review Form – Quantitative Studies McMasters University by two reviewers. No biases were noted by any researchers in these studies. Findings from these low level evidence studies need to be interpreted with caution. None of the five literature/expert reviews included search strategies to check the publications included.

The quality of the laboratory/susceptibility studies were difficult to determine. Given the specialised expertise and potential for controlled laboratory / susceptibility testing to be prone to numerous biases, an Expert in Microbiology worked with the Lead Reviewer in screening publications to be included in this review and to ensure publications included were suitable.

The McMasters Quantitative Study critical appraisal tool was used by two reviewers to appraise the case-control, cross sectional, comparative and interrupted time series included.

Table 4 is a summary of the critical appraisals.

Table 4 – Summary of critical appraisals												
Authors	**Type of study and level of evidence**	**Was the purpose stated clearly?**	**Was relevant back-ground literature reviewed?**	**Any biases stated that may have been operating and the direction of their influence on the results?**	**Sample described in detail?**	**Were the outcome measures listed and reliable / valid?**	**Intervention was described in detail?**	**Results were reported in terms of statistical significance?**	**Were the analysis method(s) appropriate?**	**Clinical importance was reported?**	**Drop outs reported ?**	**Conclusions were appropriate given study methods and results?**
Batra, R., Cooper, B.S., Whiteley, C., Patel, A.K., Wyncoll, D. and Edgeworth, J.D., 2010.	**Retrospective interrupted time series laboratory study**	**Y**	**Y**	**N**	**Y**	**N**	**Y**	**Y**	**Y**	**N**	**N**	**Y**
Ho, C.M., Li, C.Y., Ho, M.W., Lin, C.Y., Liu, S.H. and Lu, J.J., 2012.	**Case Control study**	**Y**	**Y**	**N**	**Y**	**N**	**Y**	**Y**	**Y**	**Y**	**N**	**Y**
Johnson, R.C., Schlett, C.D., Crawford, K., Lanier, J.B., Merrell, D.S. and Ellis, M.W., 2015.	**Case Report**	**Y**	**Y**	**N**	**Y**	**N**	**Y**	**Y**	**N**	**N**	**N**	**Y**
Lee, A.S., Macedo-Vinas, M., François, P., Renzi, G., Schrenzel, J., Vernaz, N., Pittet, D. and Harbarth, S., 2011.	**Nested case control study**	**Y**	**Y**	**N**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	**Y**

Vali, L., Dashti, A.A., El-Shazly, S. and Jadaon, M.M., 2015.	**Survey / case report**	**Y**	**Y**	**N**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	**Y**
Warren DK., Prager M., Munigala S., Wallace MA., Kennedy CR., Bommarito KM., Mazuski JE.and Burnham CD 2016	**Retrospective cohort over 8 years 2002 – 2012**	**Y**	**Y**	**N**	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	**Y**
Zhang, M., O'Donoghue, M.M., Ito, T., Hiramatsu, K. and Boost, M.V., 2011.	**Comparative cross-sectional**	**Y**	**Y**	**N**	**Y**	**Y**	**Y**	**Y**	**Y**	**N**	**N**	**Y**

Data Extraction

Data was extracted using the form included as Appendix 3. A summary table was used to present extracted data from all included studies (Appendix 4).

Data analysis and synthesis

In keeping with the literature review approach, data was summarised using tables and narrative discussion and presented in the literature review report. Despite the different terms used to describe data synthesis approaches all involve four distinct phases according to Evans (2002):

I. Gather the sample of studies,

2. Identify the key findings of each study,

3. Determine how these findings relate to those of other studies, and

4. Bring common findings together to generate a description of the phenomenon.

Declared interest(s) of the author(s) of each paper

Table 5 states the declared interests of authors of papers included in the literature review.

Table 5: Declared interests

Authors	Declared Interests / Transparency declarations
Abuzaid, A., Hamouda, A. and Amyes, S.G.B., 2012.	None declared. The study was funded by the General department of medical Services, Ministry of Interior, Saudi Arabia, which supported the grant to A. Abuzaid.
Aka, S.T. and Haji, S.H., 2015.	None declared.
Batra, R., Cooper, B.S., Whiteley, C., Patel, A.K., Wyncoll, D. and Edgeworth, J.D., 2010.	None declared. Funding through Guy's and St Thomas' Charity (to R.B. and J.D.E), Department of Health, via the National Institute for Health Research comprehensive Biomedical Research Centre award to Guy's and St Thomas' National Health Service Foundation Trust in partnership with King's College London (to J.D.E.). R.B. receives 50% salary support in the form of an unrestricted educational grant from Novartis UK.

Authors	Declared Interests / Transparency declarations
Bock, L.J., Wand, M.E. and Sutton, J.M., 2016.	None declared. This study was supported by Public Health England Development Fund 108716 and GIA Grant Project 109506.
Edgeworth, J.D., 2011.	J. D. E. is supported by the Department of Health via the NIHR comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London. Transparency declarations. The article forms part of a Supplement sponsored by the BSAC. The author received an honorarium for writing this article.
EKİZOĞLU, M., SAĞIROĞLU, M., Kilic, E. and HASÇELİK, A.G., 2016.	The study was funded by the Hacettepe University Scientific Research Projects Coordination Unit (Project No. 01.02.30.10.008).
Harbarth S., Tuan Soh S., Horner C., & Wilcox, MH. 2014	SH has received consultant and speaker honoraria from bioMerieux (Marcy l'Etoile, France), Da Volterra (Paris, France) and Destiny Pharma (Brighton, UK). SH has received research funds from Pfizer Europe (grant WS1980748), B. Braun Germany (grant OPM-CIC-G-H-1001), the Centre de Recherche Clinique at the Geneva University Hospitals (grant 08-059) and the European Commission (SATURN contract 241796, AIDA contract 278348, R-Gnosis contract 282512, Rapp-ID contract 115153 and COMBACTE network contract 115523). MHW has received honoraria for consultancy work, financial support to attend meetings and research funding from Actelion, Alere, Astellas, Astra-Zeneca, bioMe´rieux, Cerexa, Durata, Cubist, Nabriva, Novacta, Pfizer, Roche, Sanofi-Pasteur, Summit, The Medicines Company and VH Squared. Funding source- STS was supported by a training grant from the Ministry of Health of Malaysia.
Ho, C.M., Li, C.Y., Ho, M.W., Lin, C.Y., Liu, S.H. and Lu, J.J., 2012.	The work was supported by grants from China Medical University Hospital (DMR-101-092), China Medical University (CMU99-NTU-03), Chang Gung Memorial Hospital (CMRPG3B0641), and the National Science Council (NSC-101-2320-B-182A-002-MY3), Taiwan.
Horner, C., Mawer, D., and Wilcox M. 2012	None declared

Authors	Declared Interests / Transparency declarations
Johnson, R.C., Schlett, C.D., Crawford, K., Lanier, J.B., Merrell, D.S. and Ellis, M.W., 2015.	This work (IDCRP-055) was supported by the Infectious Disease Clinical Research Program, a Department of Defense (DoD) program executed through the Uniformed Services University (USU) of the Health Sciences. This project has been funded in whole, or in part, with federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), under interagency agreement Y1-AI-5072. Additional funding was provided by Centers for Disease Control and Prevention, National Center for Emerging and Zoonotic Infectious Diseases, Division of Healthcare Quality Promotion interagency agreement 09FED914272 (M.W.E.); DoD Global Emerging Infections Surveillance program C0366-11-HS (M.W.E.); and USU DoD program project HT9404-12-1-0019 (D.S.M.). R.C.J. is supported by a fellowship from the Henry M. Jackson Foundation. The views expressed in this paper are those of the authors and do not necessarily represent the views of the USU of the Health Sciences, the DoD, or other federal agencies. The authors thank Kimberly Bishop-Lilly for her expertise and valuable discussions.
Kampf G, Acquired resistance to chlorhexidine – is it time to establish an “antiseptic stewardship” initiative? 2016,	None declared
Kawamura-Sato, K., Wachino, J.I., Kondo, T., Ito, H. and Arakawa, Y., 2010.	None declared. The work was supported by grants (H15-Shinkou-9 and H18-Shinkou-11) from the Ministry of Health, Labor and welfare, Japan, and a H17-Gakushin grant from the Nagoya University Graduate School of Medicine.
Kawamura-Sato, K., Wachino, J.I., Kondo, T., Ito, H. and Arakawa, Y., 2008.	None declared. The work was supported by a H18-Shinkou-11 grant from the Ministry of Health, Labor and welfare, Japan, and in part of a H17-Gakushin grant from the Nagoya University Graduate School of Medicine.

Authors	Declared Interests / Transparency declarations
Lee, A.S., Macedo-Vinas, M., François, P., Renzi, G., Schrenzel, J., Vernaz, N., Pittet, D. and Harbarth, S., 2011.	S. H. has received consulting fees from Roche, is a member of the speakers' bureau for bioMe´rieux, and is a member of the advisory board of Destiny Pharma. All other authors: No reported conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed in the Acknowledgements section. This work received financial support for MRSA research activities from the European Commission under the Life Science Health Priority of the 6th Framework Program (MOSAR network contract LSHP-CT-2007-037941 to A. L. and S. H.) and the Centre de Recherche Clinique of the University of Geneva Hospitals and Faculty of Medicine (to M. M. -V.).
Liu, Q., Zhao, H., Han, L., Shu, W., Wu, Q. and Ni, Y., 2015.	None declared. The work was supported by grants from Natural Science Foundation, Science and Technology Commission of Shanghai (no. 12ZR1425000) and the National Natural Science Foundation of China (no. 81371872).
Longtin J, Seah C, Siebert K et al. 2011	The authors acknowledge N. Noguchi and the Network on Antimicrobial Resistance in Staphylococcus aureus (NARSA) for supplying strains.
Lu, Z., Chen, Y., Chen, W., Liu, H., Song, Q., Hu, X., Zou, Z., Liu, Z., Duo, L., Yang, J. and Gong, Y., 2014.	None declared. The work was supported by a grant from the Natural Scientific Foundation of China (No. 811021681) and the National Key Program for Infectious Diseases of China (No. 2013ZX10004217002001) from the Ministry of Science and Technology, China.

Authors	Declared Interests / Transparency declarations
McDanel, J.S., Murphy, C.R., Diekema, D.J., Quan, V., Kim, D.S., Peterson, E.M., Evans, K.D., Tan, G.L., Hayden, M.K. and Huang, S.S., 2013.	This project was funded under contract number HHSA290-2005-0033I from the Agency for Healthcare Research and Quality, U.S. Department of Health and Human Services, as part of the Developing Evidence to Inform Decisions about Effectiveness (DEcIDE) program. The authors of this report are responsible for its content. Statements in the report should not be construed as endorsement by the Agency for Healthcare Research and Quality or the U.S. Department of Health and Human Services. J.S.M., C.R.M., V.Q., D.S.K., E.M.P., K.D.E., G.L.T., and S.S.H. report no conflicts of interest relevant to this article. D.J.D. has received research funding from bioMérieux, Innovative Biosensors, PurThread Technologies, Cerexa, Pfizer, and Merck. M.K.H. has received products for research from Sage, Inc.
McNeil, J.C., Kok, E.Y., Vallejo, J.G., Campbell, J.R., Hulten, K.G., Mason, E.O. and Kaplan, S.L., 2016.	This study was funded by NIAID grant K23AI099159-01A1 (to J.C.M.). The S. aureus surveillance study was supported by Pfizer Pharmaceuticals (to S.L.K.). HHS|NIH| National Institute of Allergy and Infectious Diseases (NIAID) provided funding to J. Chase McNeil under grant number K23AI099159-01A1.
Mendoza-Olazarán, S., Camacho-Ortiz, A., Martínez-Reséndez, M.F., Llaca-Díaz, J.M., Pérez-Rodríguez, E. and Garza-González, E., 2014.	None declared
Morrissey, I., Oggioni, M.R., Knight, D., Curiao, T., Coque, T., Kalkanci, A., Martinez, J.L. and BIOHYPO Consortium, 2014.	Morrisey and Knight were employees of Quotient Bioresearch at the time of the study
Naparstek, L., Carmeli, Y., Chmelnitsky, I., Banin, E. and Navon-Venezia, S., 2012.	None declared. The work was supported in part by the European Commission research grant FP7: SATURN – Impact of Specific Antibiotic Therapies on the Prevalence of Human Host Tolerant Bacteria.
Noto, MJ & Wheeler, AP. 2015	None declared
Oggioni, R., Rosado Coelho, M., Furi, J., R Knight, D., Viti, C., Orefici, G., Martinez, J.L., Teresa Freitas, A., M Coque, T. and Morrissey, I., 2015.	None declared. The work was supported by national funds through FCT – Fundacao para a Ciencia e a Techologia, under projects Pest-OE/EEI/LA0021?2013 the EC FP7 project BIOHYPO KBBE-227258

Authors	Declared Interests / Transparency declarations
Otter, J.A., Patel, A., Cliff, P.R., Halligan, E.P., Tosas, O. and Edgeworth, J.D., 2013.	J. A. O. is employed part-time by Bioquell UK Ltd. All other authors have no conflicts of interest to declare. The research was supported by a grant from the Guy's and St Thomas' Charity. J. D. E. is supported by the Department of Health via the NIHR comprehensive Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London.
Prag, G., Falk-Brynhildsen, K., Jacobsson, S., Hellmark, B., Unemo, M. and Söderquist, B., 2014.	B Söderquist has been a consultant for Pfitzer and Janseen-Cilag. The study was supported by a grant from the Orebro County Research Committee.
Shamsudin, M.N., Alreshidi, M.A., Hamat, R.A., Alshrari, A.S., Atshan, S.S. and Neela, V., 2012.	None declared. The work was supported by a grant 91857 from Research University Grant Scheme (RUGS, UPM).
Sheng, W.H., Wang, J.T., Lauderdale, T.L., Weng, C.M., Chen, D. and Chang, S.C., 2009.	None declared
Skovgaard, S., Larsen, M.H., Nielsen, L.N., Skov, R.L., Wong, C., Westh, H. and Ingmer, H., 2013.	None declared. The work was supported by the Danish Council for Strategic Research 2101-08-0030.
Smith, K., Gemmell, C.G. and Hunter, I.S., 2008.	None declared. K Smith received a 3 year scholarship from the Carnegie Trust of the University of Scotland.
Vali, L., Dashti, A.A., El-Shazly, S. and Jadaon, M.M., 2015.	None declared. The work was funded by Kuwait University Research Administration Grant number NM02/10 and the Kuwait Foundation for Advancement of Science (KFAS), Grant no. 2011130204. Authors declare that the sponsors had no involvement in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.
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Wang, J.T., Sheng, W.H., Wang, J.L., Chen, D., Chen, M.L., Chen, Y.C. and Chang, S.C., 2008.	None declared. The study was funded by the National Science Council, Taiwan (NSc-94-231-B-002-163).

Authors	Declared Interests / Transparency declarations
Warren DK., Prager M., Munigala S., Wallace MA., Kennedy CR., Bommarito KM., Mazuski JE. and Burnham CD 2016	The work was supported by the Prevention Epicentre Program from the Centre for Disease Control and Prevention (IU54CK000 162-01). DK Warren has served as a consultant to Centene Corporation, Sagentia, and Novaerus Corporation. CD Burnham has served as a consultant to ThermoFisher. JE Mazuski has served as a consultant to Astra-Zeneca, Merck and Bayer. All other authors declared no interests.
Wu, D., Lu, R., Chen, Y., Qiu, J., Deng, C. and Tan, Q., 2016.	None declared. The study was supported by the Natural Science Foundation of Guangxi Autonomous Region [Nos. 2014GXNSFAA118176 and 2012GXNSFAA276037] and Nanning City Science and Technology Plan [20131062].
Zhang, M., O'Donoghue, M.M., Ito, T., Hiramatsu, K. and Boost, M.V., 2011.	None declared. The study was supported by a research grant of the Hong Kong Polytechnic University.

Description of how comments from independent methodological review of the draft research protocol were addressed

The following table outlines the response of the review team to the independent methodological review of Protocol 3: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Table 6: Response to independent methodological review

Reviewer comment	Response
QUESTION, REVIEW TYPE AND PICO FORMAT The protocol states the research questions are: 1. Does the use of chlorhexidine contribute to resistance to chlorhexidine? 2. Does the use of chlorhexidine contribute to resistance to antibiotics? The Review Team is advised to use the PICOS (population, intervention, comparator, outcome, study type) format for the research question. The protocol implies that the research question could be: "Does the use of chlorhexidine increase the prevalence of chlorhexidine/antibiotic-resistant strains of bacteria in hospital settings?" This research question may not need the C component of the PICOS format, however the use of the PICOS format would substantially contribute to a more transparent and replicable review.	These comments have been addressed and the PICO format has been used so as to contribute to a more transparent and replicable review. A table outlining PICO is included in the protocol. In relation to the review questions they are now stated as: 1. Does exposure (different dosages, duration of use, and stratification of exposure) to any form of chlorhexidine results in 'chlorhexidine resistance' in any person within different healthcare settings? 2. Does exposure (different dosages, duration of use, and stratification of exposure) to any form of chlorhexidine increases the incidence and/or prevalence of antibiotic-resistant strains of bacteria in any person within different healthcare settings?
POPULATION The protocol states that the population is "all types of patients/participants including children and adults." The settings include "acute care, residential aged care, paediatric, neonatal and rehabilitation." as well as "the laboratory setting." This is an acceptable approach and would be improved by stating how these different populations and/or settings will be incorporated and presented in the present review.	These comments have been addressed and now made clear – please see review questions, the stepped approach to inclusion of studies and section titled 'Data analysis and synthesis'.

Reviewer comment	Response
INTERVENTION/COMPARATOR The protocol does not adequately state what the types of intervention are and includes outcomes in their statement. The protocol might include a more detailed description of the intervention e.g. 'chlorhexidine coated urethral catheter', 'chlorhexidine impregnated central venous catheter' or 'topical chlorhexidine'. Another approach would be to state what uses of chlorhexidine would be excluded from inclusion in the review. The rationale for the 2006 search date for all studies except for RCTs is not stated. It is suggested that these search dates are reconsidered and reasons for any limits be provided. Limiting inclusion to studies published in the English language and human studies is acceptable, although the language restriction may introduce publication bias.	These comments have been addressed and now made clear that 'No use of chlorhexidine will be excluded from the literature review'. It now stated for Publication Date and limits: As directed, the reviewer will consider all relevant studies regardless of publication status (published, unpublished, in press, and ongoing) in the last ten years - from 2006 to 2016 following the stepped approach described. There is no search time limit for randomized controlled trials (RCTs) should any be identified addressing chlorhexidine use resulting in chlorhexidine/antibiotic-resistance strains of bacteria. The search is limited to English language publications. The following section has also been updated. Keywords A combination of the following search terms will be used to identify potentially relevant peer reviewed publications. Synonymous terms and related MeSH headings will be used to expand the search as appropriate. Chlorhexidine/ eludril / corsodyl/ Tubulicid/Novalsan/Sebidin/CHX/ MK-412A/MK 412A/MK412/ Biocides/ skin decolonization / anti-infective agent / anti-bacterial agent / anti-infective agents local / hand disinfection / hand sanitisers/ disinfectants/ dental disinfectants / root canal implants / anti-infective agents urinary / Chlorhexidine Dressing / Chlorhexidine Gel/Jelly/ Chlorhexidine Lotion/ Chlorhexidine Solution / Chlorhexidine Liquid / Chlorhexidine Pad / Chlorhexidine Sponge / Chlorhexidine Cream / Vaginal chlorhexidine/ Resistance/ Chlorohexidine Tolerance / Chlorhexidine Susceptibility /Anti-microbial resistance / Antibiotic-resistance bacteria These terms will form the basis of the initial search. The search parameters may be subsequently expanded to incorporate additional search terms. This formative phase of the search strategy will be an integral part of the three-step search process. The second phase of the search process will involve the analysis of text words contained in the title and abstract of retrieved citations and of the index terms used to describe identified publications. The third step will involve an integrated validation search using all identified key words and index terms, through the same databases.

Reviewer comment	Response
OUTCOMES The outcomes should be more clearly stated e.g. 'the incidence/prevalence of antibiotic resistance', and the definitions of each term should be provided.	These comments have been addressed and stated as: Question 1: Outcomes 1. 'Chlorhexidine Resistance' (with definition / measures used) to chlorhexidine established. 2. A specific intervention identified as contributing to resistance to Chlorhexidine in a specific population and / or setting. 3. A specific exposure of a specific intervention identified as contributing to resistance to Chlorhexidine in a specific population and / or setting. Question 2: Outcomes 1. 'Resistance against antibiotics' defined by using the clinical breakpoints for resistance as specified by the European Committee on Antimicrobial Susceptibility testing (EUCAST) or the Clinical and Laboratory Standards Institute (CSLI). 2. Increase in the incidence (rate) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use chlorhexidine to be recorded. 3. Increases in the prevalence (frequency) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying specific dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use chlorhexidine to be recorded.

Reviewer comment	Response
METHODS TO IDENTIFY AND SELECT RELEVANT STUDIES The protocol states that this review will be an 'integrative review' and supplies a reference to substantiate this. The reference supplied describes a review where quantitative and qualitative study designs are synthesised however the protocol states that qualitative studies are excluded from the present review. It is implied in the protocol that the present review will be a synthesis of different quantitative study designs – this should be clarified in the protocol and more detail should be provided regarding which study designs will be included and how the inclusion and synthesis of these different study designs will be managed. The protocol would be improved by stating how particular study designs might be incorporated and presented in the review. For example, it might be anticipated that the review would locate systematic and/or narrative reviews. The protocol should be explicit how it defines these study designs and would critically appraise them. It should also articulate how other primary studies would be included or excluded.	These comments have been addressed and a stepped approach to the inclusion of studies and how studies will be critically appraised has been included. To ensure the best available evidence is included, a stepped approach to the inclusion of studies will be followed. Step 1: Systematic reviews (SRs) will be searched and critically appraised as the first step. Preliminary reviews have identified limited evidence being derived from step 1 to address the literature review questions. Step 2: To complement what is identified in Step 1, we will search and compare primary research studies (published and unpublished) including all types of observational and interventional studies and critically appraise those collated. Given the review questions, characterization studies, for example colonising isolates from scrub nurses and comparing to nonusers of chlorhexidine or colonising isolates from nares of carriers in residential aged care and to the isolation of caries pathogens from carious dentine specimens, will also be included. Primary research studies may include: • Characterization studies • Comparative (nonrandomised and observational) studies • Concurrent control or cohort studies • Case-control • Historical control • Interrupted time series • Case series • Prevalence studies To identify missed papers, the bibliographies of the relevant papers will be checked for articles missed by the initial search; and a citation search, will be conducted to identify papers that have cited the identified relevant studies, some of which may be subsequent primary research (How to review the evidence: systematic identification and review of the scientific literature"(NHMRC 1999).

Reviewer comment	Response
METHODS TO IDENTIFY AND SELECT RELEVANT STUDIES (continued)	Studies included must: • Make clear the population of study • Isolates must be from humans • Make clear the intervention – dosage form and exposure • Make clear what health care setting or laboratory setting • Define or measure 'chlorhexidine resistance' / reduced susceptibility to chlorhexidine / non – susceptibility to chlorhexidine • Define antibiotic-resistant strain of bacteria No use of chlorhexidine will be excluded from the literature review. Studies will be excluded if: • Focus is only on the use and effectiveness of chlorhexidine and not resistance • Chlorhexidine resistance however stated not systematically assessed • Isolates not from humans • Focus is antibiotic resistance not related to chlorhexidine use • Setting is schools or domestic home All included and critically appraised studies, where possible, will be categorised according to the NHMRC Level of Evidence (NHMRC 2009). Consideration will be given to: the quality of the studies and the likelihood that the results have been affected by bias during its conduct; the consistency of its findings to those from other studies; the clinical impact of its results; the generalisability of the results to the population for whom the guideline is intended; and the applicability of the results to the Australian (and/or local) health care setting NHMRC additional levels of evidence and grades for recommendations for developers of guidelines" (NHMRC 2009). Step 3: If after step 2, the evidence does not adequately address the literature review questions, to complement what is identified, step 3 we will include experimental and theoretical investigations that use for example mathematical modelling (e.g. Shen *et al* (2016) Experimental and Theoretical Investigation of Multispecies Oral Biofilm Resistance to Chlorhexidine Treatment, *Scientific Reports).*

Reviewer comment	Response
METHODS TO IDENTIFY AND SELECT RELEVANT STUDIES (continued)	Step 4: To complement what is identified in all previous steps, scientific letters, case reports and evidence based / expert reviews and grey literature will be collated and appraised using an appropriate critical appraisal tool for the relevant publication or by key criteria for bias. Qualitative studies will be excluded. The aim is to ensure a broader understanding to address the literature review questions can be provided to the NHMRC.
ARE THE SEARCH STRATEGIES APPROPRIATE TO IDENTIFY THE IMPORTANT AND RELEVANT STUDIES? The databases that have been proposed for searching and other search strategies are very comprehensive and are likely to find most of the important and relevant studies.	
WILL STUDIES THAT ARE IMPORTANT, RELEVANT AND OF AN APPROPRIATE DESIGN BE INCLUDED? The approach to searching and including studies as proposed in the protocol is likely to identify a large number of studies, most of which will not be relevant to the research question. It is strongly suggested that the inclusion/ exclusion criteria (especially what study designs be included and how different study designs be incorporated into the review) be reconsidered and revised.	These comments have been addressed within the stepped approach described previously.
ARE THE INCLUSION AND EXCLUSION CRITERIA DESCRIBED AND APPROPRIATE? The inclusion/exclusion criteria need to be more explicit. At present there is only one exclusion criterion. More details regarding the relevant study design(s) and how different study designs are to be synthesised should be provided. It is suggested that a stepped approach to inclusion of study designs is utilised.	These comments have been addressed and inclusion and exclusion criteria now made clear

Reviewer comment	Response
METHODS TO EXTRACT, APPRAISE AND SYNTHESISE DATA FROM INCLUDED STUDIES It is stated that the review authors will apply the pre-defined inclusion and exclusion criteria. As it is not clear what these are, the approach to the selection of studies is not adequate. The protocol does not provide adequate information about the critical appraisal for the included studies. In addition, there is no information about how any extracted data will be synthesised.	These comments have been addressed and the protocol rewritten to make clear all the areas requested. The McMasters Quantitative Study critical appraisal tool will be used to appraise characterization studies. The set of JBI Critical Appraisal Tools (JBI 2014) will be used for the relevant study. Critical appraisal tools include prevalence studies, observational studies including prospective and retrospective cohort studies, case-control studies, cross-sectional studies, and case series (JBI 2014). The JBI critical appraisal tool for Systematic Reviews will be used. Where there is no appropriate critical appraisal tool, the quality assessment will be by key criteria for bias. In keeping with the literature review approach, data will be summarised using tables and narrative discussion. Following data extraction (Appendix 1) the body of evidence will be synthesised. A systematic description of the definitions and measurements of 'chlorhexidine resistance' and 'resistance to antibiotics' in comparison of studies will be provided. It is anticipated that there will be variation. Causes of variation, such as different terminology, measurements, dosage forms, exposure or setting will be searched and where there are 'true' differences in the studies and populations then this will be reported. Whether different groups differed because of measurement method, intervention exposure or other factors then this will be recorded. Incidence and prevalence of antibiotic-resistant strain of bacteria through the use chlorhexidine will be reported. If possible, a response to the question as to whether bacteria that are non-susceptible to chlorhexidine that this also by the same mechanism confers resistance to other antibiotics or disinfectants will be recorded.

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6. Appendices

- Appendix 1 Search Strings……p.34
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- Appendix 3 Data Extraction Table……..……p.43
- Appendix 4 Summary Table……p.46
- Appendix 5 Excluded Studies……..p.118

Appendix 1 - Search Strings

Medline

Search #	Search terms	Results
1.	Chlorhexidine/	6993
2.	Chlorhexidine OR CHG OR mk412a OR mk-412a OR Novalsan OR Sebidin OR Tubulicid OR Gluconate OR Biocide* OR Eludril OR Corsodyl (.mp)	19074
3.	Chlorhexamed forte OR Chlorohex OR Cholorohexadine OR Consepsis OR Dentosan OR Denzin OR Eburos OR Fimeil OR Hexadol OR Periogard OR Promax OR Soretol (.mp)	443
4.	OR/ 1-3	19421
5.	Anti-infective agents/	45063
6.	Anti-bacterial agents/	280043
7.	Anti-infective agents, local/	15403
8.	Hand disinfection/	4948
9.	Hand sanitizers/	68
10.	Disinfectants/	11107
11.	Dental disinfectants/	600
12.	“root canal irrigants”/	2804
13.	Anti-infective agents, urinary/	2568
14.	Bacteriocid* OR Microbicid* OR Skin decolonization OR Root canal implant* OR Dressing OR Gel OR Jelly OR Lotion OR Solution OR Liquid OR Pad OR Sponge OR Cream OR Vaginal OR Bactericid* OR Bacteriostatic OR Antiseptic OR Disinfectant (.mp)	1366279
15.	(agents AND (Anti-infective OR Anti-microbial* OR Anti-mycobacterial)) (.mp)	120250
16.	OR/ 5-15	1672113
17.	Efflux system* OR Efflux pump* (.mp)	6944
18.	Time Kill OR time-kill OR Time to Kill OR Kill time OR Kill-time OR MIC OR MBC OR Kirby bauer (.mp)	35632
19.	MIC OR MBC OR Minimum inhibitory concentration OR Minimum bacterial concentration (.mp)	36730
20.	OR/ 17-19	45047
21.	Susceptibility OR Resistance OR Tolerance (ti,ab.)	897046
22.	AND/ 4, 16, 20-21	271
23.	Limit 22 to English language	255
24.	Limit 23 to humans	96
25.	Limit 24 to yr=”2006-Current”	74

Search String Revised Cochrane Search

#1	MeSH descriptor: [Chlorhexidine] this term only	m	1499
Edit #2	chlorhexidine or CHG or "mk 412a" or "mk-412a" or mk412a or novalsan or sebidin or tubulicid or gluconate or biocide* or eludril or corsodyl or "Chlorhexamed forte" or Chlorohex or Chlorohexidine or Consepsis or Dentosan or Dezin or Eburos or Fimeil or Hexadol or Hexident or Periogard or Promax or Soretol		3629
Edit #3	#1 or #2		3629
#4	MeSH descriptor: [Anti-Infective Agents] this term only	m	2421
#5	MeSH descriptor: [Anti-Bacterial Agents] this term only	m	9250
#6	MeSH descriptor: [Hand Disinfection] this term only	m	321
#7	MeSH descriptor: [Hand Sanitizers] this term only	m	7
#8	MeSH descriptor: [Disinfectants] this term only	m	233
#9	MeSH descriptor: [Dental Disinfectants] this term only	m	46
#10	MeSH descriptor: [Root Canal Irrigants] this term only	m	359
#11	MeSH descriptor: [Anti-Infective Agents, Urinary] this term only	m	247
Edit #12	(Agents and (anti-infective or anti infective or antiinfective or "anti microbial*" or anti-microbial* or antimicrobial* or antimycobacterial))		9108
Edit #13	bacteriocid* or microbicid* or "skin decolonization" or "skin decolonisation" or "root canal implant*" or Dressing or Gel or Jelly or Lotion or Solution or Liquid or Pad or Sponge or Cream or Vaginal or bactericid* or bacteriostatic or antiseptic or disinfectant		58990
Edit #14	#4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13		72117
Edit #15	"Efflux System*" or "efflux pump*" or "Time Kill" or time-kill or "time to kill" or kill-time or "kill time" or MIC or MBC or "kirby bauer" or MBC or MIC or "minimum inhibitory concentration" or "minimum bacterial concentration"		2267

Search String CINAHL

Search History/Alerts

Print Search History | Retrieve Searches | Retrieve Alerts | Save Searches / Alerts

Select / deselect all | **Search with AND** | **Search with OR** | **Delete Searches**

	Search ID#	Search Terms	Search Options	Actions
☐	S1	(MH "Chlorhexidine")	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (1,785)
☐	S2	chlorhexidine or CHG or "mk 412a" or "mk-412a" or mk412a or novalsan or "sebidin a" or tubulicid	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (2,211)
☐	S3	"Chlorhexamed forte" or Chlorohex or Chlorohexidine or Consepsis or Dentosan or Dezin or Eburos or Fimeil or Hexadol or Hexident or Periogard or Promax or Soretol	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (87)
☐	S4	(chlorhexidine and (glutonate or biocide or bactericidal or bacteriostatic or antiseptic or disinfectant or bactericidal))	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (419)
☐	S5	S1 OR S2 OR S3 OR S4	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (2,294)
☐	S6	(MH "Antiinfective Agents")	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (3,612)
☐	S7	(MH "Antiinfective Agents, Local")	**Expanders** - Apply related words **Search modes** - Boolean/Phrase	View Results (2,284)
☐	S8	(MH "Handwashing")	**Expanders** - Apply related words	View Results (5,416)

Search String Embase

Ovid® Wolters Kluwer

JBI Admin Support & Training Logged in as Carole Gibbs at University of South Australia Close

Database(s): **Embase Classic+Embase** 1947 to 2016 October 26

Search Strategy:

#	Searches	Results	Annotations
1	Chlorhexidine/	14740	
2	(chlorhexidine or CHG or "mk 412a" or "mk-412a" or mk412a or novalsan or sebidin or tubulicid or gluconate or biocide* or eludril or corsodyl).mp.	38679	
3	(Chlorhexamed forte or Chlorohex or Chlorohexidine or Consepsis or Dentosan or Dezin or Eburos or Fimeil or Hexadol or Hexident or Periogard or Promax or Soretol).mp.	656	
4	or/1-3	39151	
5	anti-infective agents/	170278	
6	Anti-Bacterial Agents/	169735	
7	Anti-Infective Agents, Local/	5890	
8	Hand Disinfection/	8522	
9	Hand Sanitizers/	292	
10	disinfectants/	13167	
11	dental disinfectants/	13626	
12	"root canal irrigants"/	18001	
13	anti-infective agents, urinary/	1052	
14	(bacteriocid* or microbicid* or skin decolonization or skin decolonisation or root canal implant* or Dressing or Gel or Jelly or Lotion or Solution or Liquid or Pad or Sponge or Cream or Vaginal or bactericid* or bacteriostatic or antiseptic or disinfectant).mp.	1906830	
15	(Agents and (anti-infective or anti infective or antiinfective or "anti microbial*" or anti-microbial* or antimicrobial* or antimycobacterial)).mp.	62175	
16	or/5-14	2096473	
17	(Efflux System* or efflux pump*).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading]	8376	
18	(Time Kill or time-kill or "time to kill" or kill-time or kill time or MIC or MBC or kirby bauer).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading]	50400	
19	(MBC or MIC or minimum inhibitory concentration or minimum bacterial concentration).mp. [mp=title, abstract, heading word, drug trade name, original title, device manufacturer, drug manufacturer, device trade name, keyword, floating subheading]	105211	
20	or/17-19	114081	
21	(susceptibility or resistance or tolerance).ti,ab.	1163626	
22	and/4,16,20-21	413	
23	limit 22 to english language	388	
24	limit 23 to humans	149	
25	limit 24 to yr="2006 -Current"	128	

Search String Scopus

((((TITLE-ABS-KEY (chlorhexidine OR chg OR "mk 412a" OR "mk-412a" OR mk412a OR novalsan OR "sebidin a" OR tubulicid) OR TITLE-ABS-KEY ("Chlorhexamed forte" OR chlorohex OR chlorohexidine OR consepsis OR dentosan OR dezin OR eburos OR fimeil OR hexadol OR hexident OR periogard OR promax OR soretol) OR TITLE-ABS-KEY (chlorhexidine AND (glutonate OR biocide OR bactericidal OR bacteriostatic OR antiseptic OR disinfectant OR bactericidal)))) AND (((TITLE-ABS-KEY (agents) AND TITLE-ABS-KEY (anti-infective OR anti infective OR antiinfective OR "anti microbial*" OR anti-microbial* OR antimicrobial* OR antimycobacterial OR bacteriocid*))) OR (TITLE-ABS-KEY (bacteriocide* OR microbicide*))) AND ((TITLE-ABS-KEY ("Time Kill" OR time-kill OR "time to kill" OR kill-time OR "kill time" OR mic OR mbc OR "kirby bauer") OR TITLE-ABS-KEY (mbc OR mic OR "minimum inhibitory concentration" OR "minimum bacterial concentration") OR TITLE-ABS-KEY ("Efflux Systems" OR "efflux pump*"))) AND (TITLE-ABS-KEY (susceptibility OR resistance))) AND (TITLE-ABS-KEY (human OR humans))) OR (((((TITLE-ABS-KEY (chlorhexidine OR chg OR "mk 412a" OR "mk-412a" OR mk412a OR novalsan OR sebidin OR tubulicid OR gluconate OR biocide* OR eludril OR corsodyl)) OR (TITLE-ABS-KEY ("Chlorhexamed forte" OR chlorohex OR chlorohexidine OR consepsis OR dentosan OR dezin OR eburos OR fimeil OR hexadol OR hexident OR periogard OR promax OR soretol))) AND ((TITLE-ABS-KEY (bacteriocid* OR microbicid* OR "skin decolonization" OR "skin decolonisation" OR "root canal implant*" OR dressing OR gel OR jelly OR lotion OR solution OR liquid OR pad OR sponge OR cream OR vaginal OR bactericid* OR bacteriostatic OR antiseptic OR disinf)) OR (TITLE-ABS-KEY ((agents AND (anti-infective OR anti infective OR antiinfective OR "anti microbial*" OR anti-microbial* OR antimicrobial* OR antimycobacterial))))) AND ((TITLE-ABS-KEY ("Efflux System*" OR "efflux pump*")) OR (TITLE-ABS-KEY ("Time Kill" OR time-kill OR "time to kill" OR kill-time OR "kill time" OR mic OR mbc OR "kirby bauer")) OR (TITLE-ABS-KEY (mbc OR mic OR "minimum inhibitory concentration" OR "minimum bacterial concentration"))) AND (TITLE-ABS-KEY (susceptibility OR resistance))) AND (human OR humans)) AND NOT ((((TITLE-ABS-KEY (chlorhexidine OR chg OR "mk 412a" OR "mk-412a" OR mk412a OR novalsan OR "sebidin a" OR tubulicid) OR TITLE-ABS-KEY ("Chlorhexamed forte" OR chlorohex OR chlorohexidine OR consepsis OR dentosan OR dezin OR eburos OR fimeil OR hexadol OR hexident OR periogard OR promax OR soretol) OR TITLE-ABS-KEY (chlorhexidine AND (glutonate OR biocide OR bactericidal OR bacteriostatic OR antiseptic OR disinfectant OR bactericidal)))) AND (((TITLE-ABS-KEY (agents) AND TITLE-ABS-KEY (anti-infective OR anti infective OR antiinfective OR "anti microbial*" OR anti-microbial* OR antimicrobial* OR antimycobacterial OR bacteriocid*))) OR (TITLE-ABS-KEY (bacteriocide* OR microbicide*))) AND ((TITLE-ABS-KEY ("Time Kill" OR time-kill OR "time to kill" OR kill-time OR "kill time" OR mic OR mbc OR "kirby bauer") OR TITLE-ABS-KEY (mbc OR mic OR "minimum inhibitory concentration" OR "minimum bacterial concentration") OR TITLE-ABS-KEY ("Efflux Systems" OR "efflux pump*"))) AND (TITLE-ABS-KEY (susceptibility OR resistance OR tolerance))) AND (TITLE-ABS-KEY (human OR humans)))) AND (LIMIT-TO (PUBYEAR , 2016) OR LIMIT-TO (PUBYEAR , 2015) OR LIMIT-TO (PUBYEAR , 2014) OR LIMIT-TO (PUBYEAR , 2013) OR LIMIT-TO (PUBYEAR , 2012) OR LIMIT-TO (PUBYEAR , 2011) OR LIMIT-TO (PUBYEAR , 2010) OR LIMIT-TO (PUBYEAR , 2009) OR LIMIT-TO (PUBYEAR , 2008) OR LIMIT-TO (PUBYEAR , 2007) OR LIMIT-TO (PUBYEAR , 2006)) AND (LIMIT-TO (LANGUAGE , "English"))

Search String Web of Science

# 18	110	#17 AND #16 *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2006-2016*
# 17	1,575,733	**TOPIC:** (human or humans) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2006-2016*
# 16	804	(#15 AND #14 AND #11 AND #10) *AND* **LANGUAGE:** (English) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=2006-2016*
# 15	3,735,654	#12 OR #11 *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years*
# 14	1,721,287	**TOPIC:** (Tolerance or susceptibility or resistance) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years*
# 13	51,959	**TOPIC:** ("Efflux System*" or "efflux pump*" or "Time Kill" or time-kill or "time to kill" or kill-time or "kill time" or MIC or MBC or "kirby bauer" or MBC or MIC or "minimum inhibitory concentration" or "minimum bacterial concentration") *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years*
# 12	45,159	**TOPIC:** ((Agents and (anti-infective or anti infective or antiinfective or "anti microbial*" or anti-microbial* or antimicrobial* or antimycobacterial))) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years*
# 11	3,699,627	**TOPIC:** (bacteriocid* or microbicid* or "skin decolonization" or "skin decolonisation" or "root canal implant*" or Dressing or Gel or Jelly or Lotion or Solution or Liquid or Pad or Sponge or Cream or Vaginal or bactericid* or bacteriostatic or antiseptic or disinfectant) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years*
# 10	20,504	**TOPIC:** (chlorhexidine or CHG or "mk 412a" or "mk-412a" or mk412a or novalsan or sebidin or tubulicid or gluconate or biocide* or eludril or corsodyl or "Chlorhexamed forte" or Chlorohex or Chlorohexidine or Consepsis or Dentosan or Dezin or Eburos or Fimeil or Hexadol or Hexident or Periogard or Promax or Soretol) *Indexes=SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, ESCI, CCR-EXPANDED, IC Timespan=All years*

Appendix 2: Inclusion criteria checklist

Chlorhexidine and Resistance
Inclusion Criteria

Endnote Number	
Author	
Year	

Types of studies		
	• Systematic review	
	• Primary research – observational and interventional studies	
	Type of research	
	Characterization studies	
	Comparative (nonrandomised and observational) studies	
	Concurrent control or cohort studies	
	Case-control	
	Historical control	
	Interrupted time series	
	Case series	
	Susceptibility study	
	Other – state	
	Did the research:	
	1. Make clear the population of study	
	2. Isolates were from humans	
	3. Make clear the intervention – dosage form and exposure	
	4. Make clear what health care setting or laboratory setting	
	5. Defined or measured 'chlorhexidine resistance' / reduced susceptibility to chlorhexidine / non – susceptibility to chlorhexidine – stated clearly	
	6. Defined antibiotic-resistant strain of bacteria – stated clearly	
	• Experimental and theoretical investigations	
	• Scientific letters, case reports and evidence based / expert reviews	
	• Grey literature	

Types of participants and settings		
	• Acute care	
	• Residential aged care	
	• Paediatric	
	• Neonatal	
	• Rehabilitation	
	• Human isolates	
	State where:	

Types of CHX intervention		
	• Form	
	• Dose	
	• Duration	
	• Exposure	

Types of outcome measures		
	• Chlorhexidine Resistance' (with definition / measures used) to chlorhexidine established.	
	• A specific intervention identified as contributing to resistance to chlorhexidine in a specific population and / or setting.	
	• A specific exposure of a specific intervention identified as contributing to resistance to chlorhexidine in a specific population and / or setting.	
	• Resistance against antibiotics' defined by using the clinical breakpoints for resistance as specified by the European Committee on Antimicrobial Susceptibility testing (EUCAST) or the Clinical and Laboratory Standards Institute (CSLI).	
	• Increase in the incidence (rate) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use of chlorhexidine to be recorded.	
	• Increases in the prevalence (frequency) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying specific dosage form, exposure and specific population and / or setting. Antibiotic-resistant strain of bacteria through the use of chlorhexidine to be recorded.	

Appendix 3: Data Extraction Table

Article Details						
First Author				Year		
Reference Number						
Publication Focus						
Type of Study Design						
Does the publication refer to any specific Health Service? Describe	1. Acute Care 2. Aged Care 3. Paediatrics 4. Neonatal 5. Rehabilitation			Other?		
Does the publication refer to any specific population / isolate? Describe						
Purpose of article						
Laboratory setting – describe						
Chlorhexidine related details						
Type						
Strength						
Application						
Duration of use - Stratification of exposure i.e. prolonged exposure versus one off						
Antimicrobial related details						
Bacteria/ bacterium named						
Isolates – describe						
How is 'chlorhexidine resistance' defined / measured						

/ discussed?	

Antibiotic-resistance strain of bacteria	
Describe	
Incidence	
Prevalence	
How has resistance against antibiotics been defined? As specified by the European Committee on Antimicrobial Susceptibility testing (EUCAST) or the Clinical and Laboratory Standards Institute (CSLI)? Describe	
'Chlorhexidine Resistance' / definition / measurement	
Definition / measurement	
MIC – if explained describe	
MBC – if explained describe	
Phenotypic – if explained describe	
Other – if explained describe	

Outcome specifically related to chlorhexidine	
Any of the following identified? Y/N Describe outcome	
• 'Chlorhexidine Resistance' (with definition / measures used) to chlorhexidine established.	
• A specific intervention identified as contributing to resistance to chlorhexidine in a specific population and / or setting.	
• A specific exposure of a specific intervention identified as contributing to resistance to Chlorhexidine in a specific population and / or setting.	
• Increase in the incidence (rate) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying dosage form, exposure and specific population and / or setting.	
• 'Resistance against antibiotics' defined by using the clinical breakpoints for resistance as specified by the European Committee on Antimicrobial Susceptibility testing (EUCAST) or the Clinical and Laboratory Standards Institute (CSLI).	
• Increases in the prevalence (frequency) of antibiotic-resistant strains of bacteria established through the use of chlorhexidine identifying specific dosage form, exposure and specific population and / or setting.	
• If bacteria that are non-susceptible to chlorhexidine are reported is it also reported whether this is also by the same mechanism confers resistance to other antibiotics or disinfectants will be recorded.	
Other – describe	

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Primary Research Studies – Controlled Laboratory /Susceptibility Studies (n=24/36)				
Aka, S.T. and Haji, S.H., 2015.	Controlled Laboratory Study	Twenty two clinical isolates of *Pseudomonas aeruginosa* were collected from the lab of Rizgary Teaching Hospital in Erbil, Iraq. The origin of isolates was from specimens of ear infections. Chlorhexidine 4% (w/v) was a laboratory standard solution.	Both bacterial isolates (CHX-culture) and (CHX-free culture) incubated for 72 h, could form biofilm following cultivation in antibiotic-free broth. In fact, the OD values showed greater biofilm, which enhanced by CHX-culture compared with CHX-free culture, although the difference was not statistically significant. These cells may started to show resistance mechanism to survive the attack due to changes in the phenotypic level, i.e. the ability to form biofilm, which is an adaptive form of resistance. N.B. Antibiotic resistance was not measured.	Phenotypic change of chlorhexidine and induction of gene expression due to antibiotics action might enhance bacterial resistance and further stronger biofilm formation. Incubating the isolates of *P. aeruginosa* to sub-MIC of antibiotics exhibited induction of biofilm in the presence of chlorhexidine. The study concluded that incubating the isolates of *P. aeruginosa* in sub-MIC of antibiotics exhibited induction of biofilm in the presence of chlorhexidine. Therefore, this study will help establish the medical application to guide antibiotic therapy and hospital disinfection that would suppress the biofilm induction.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Bock, L.J., Wand, M.E. and Sutton, J.M., 2016.	Controlled Laboratory study	This study aimed to determine the activity of in-use chlorhexidine formulations against pre-chlorhexidine era and modern *K. pneumoniae* clinical isolates, and strains that were adapted in the authors' laboratory to chlorhexidine through continuous exposure. Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) for a range of chlorhexidine formulations were determined after 5 min, 15 min, 30 min and 24 h of exposure, and compared with chlorhexidine and chlorhexidine digluconate alone. All tested chlorhexidine formulations were chosen from those available on the National Health Service supply chain (https://www.supplychain.nhs.uk/) in May 2013, and ranged in chlorhexidine concentration from 0.02% to 4% (see Table I). These	Chlorhexidine formulations can be effective at controlling clinical isolates of *K. pneumoniae* when used at the correct concentration and exposure time. However, not all commercially available formulations reach the minimum required concentration to achieve a satisfactory level of bacterial kill. Additional ingredients can increase and, in some cases, decrease the activity of chlorhexidine, especially when used to kill strains that have adapted to chlorhexidine exposure. It is therefore of paramount importance to develop and test chlorhexidine formulations for their application in controlling Gram-negative organisms. Current standard methods for testing biocide efficacy and their varied formulations should include strains that are known to have reduced biocide susceptibility as indicator organisms in order to address the issues surrounding reduced susceptibility. N.B. Included strains that are resistant due to chlorohexidine exposure.	Not all chlorhexidine formulations kill MDR *K. pneumoniae* after the recommended exposure time. Activity, especially against chlorhexidine-adapted strains, depends on additional ingredients. Careful formulation of chlorhexidine products is therefore important to maintain and enhance the activity of chlorhexidine products, and avoid potential breakdown in infection control.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
EKİZOĞLU, M., SAĞIROĞLU, M., Kilic, E. and HASÇELİK, A.G., 2016.	Susceptibility Study	The susceptibility of 120 hospital isolated strains of 7 bacterial genera against chlorhexidine digluconate was determined by agar dilution test, using minimum inhibitory concentration (MIC) values and the EN 1040 Basic Bactericidal Activity Test to determine the bactericidal activity.	"…all hospital isolates that were studied were found to be susceptible to 4% chlorhexidine digluconate after 5 min of contact time. There was no decrease in the bactericidal activity against the isolates, except for MRSA, in 2% chlorhexidine digluconate (no data available for P. aeruginosa). Acinetobacter sp., *Enterobacter sp., S. maltophilia, Klebsiella sp., and Enterococcus sp*. isolates were found to be susceptible in 0.5% chlorhexidine digluconate, whereas 11 P. aeruginosa, 14 MRSA, and 5 MSSA isolates were found to be resistant. All of the Enterococcus isolates and 9 isolates of S. maltophilia were susceptible in 0.02% chlorhexidine digluconate. Chlorhexidine digluconate at a concentration of 0.02% was active against only 2 S. aureus isolates (4.7%), whereas at the same concentration it was active against all Enterococcus isolates. This result showed that S. aureus isolates (MRSA and MSSA) had a lower level of susceptibility than Enterococcus in low concentrations of chlorhexidine digluconate.' 52 N.B. Can accept that these hospital isolated strains were exposed to Chlorhexidine	Biocide resistance, similar to antibiotic resistance, is described as microbial growth when bacteria are tested with in-use concentrations. Furthermore, resistance or insusceptibility to biocides can be either intrinsic, as a result of natural characteristics of microorganisms, or it can be acquired. Acquired resistance to biocides may arise from mutation and horizontal transfer of genetic material such as plasmids or transposons. Efflux pumps are common mechanisms of acquired resistance to chlorhexidine digluconate. By means of this mechanism, not only chlorhexidine but also other chemical substances are excluded from the cell, which can therefore also lead to resistance to antibiotics. Antimicrobial effectiveness of chlorhexidine may differ within pathogenic bacteria. "It is crucial to use biocides at appropriate concentrations and to perform surveillance studies to trace resistance or low susceptibility patterns of *S. aureus, P. aeruginosa*, and other hospital isolates."

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Kawamura-Sato, K., Wachino, J.I., Kondo, T., Ito, H. and Arakawa, Y., 2010.	Susceptibility Study	The aim of this study was to investigate the susceptibility profiles to disinfectants and antimicrobial agents of 283 non-repetitive Acinetobacter clinical isolates obtained in 97 Japanese hospitals in March 2002.	No evident resistance to disinfectants was seen among the 283 strains of *Acinetobacter spp*. isolated in 2002, but the MIC90s of chlorhexidine gluconate, benzalkonium chloride and alkyldiaminoethyl glycine hydrochloride were 50, 50 and 400 mg/L, respectively. Our results showed no apparent correlations between specific disinfectants and antimicrobial agents, but our observations imply a trend towards overall cross resistance between multiple antimicrobials and disinfectants among clinically isolated Acinetobacter spp. A hospital outbreak caused by a strain of Proteus mirabilis demonstrating resistance to several antimicrobial agents, including gentamicin as well as chlorhexidine gluconate, was reported.(1987) Thus, the increased isolation of *Acinetobacter spp*. that had acquired multiple resistance to antimicrobials would be a good indicator for early recognition of the emergence of *Acinetobacter* DRS isolates in both acute and long-term healthcare settings.	In conclusion, no apparent acquisition of resistance to disinfectants was observed in this time-dependent survey using the 283 strains of *Acinetobacter spp.* clinically isolated in Japan in 2002. About 10% of the isolates (28 strains) were found to demonstrate reduced susceptibility to disinfectants and these DRS isolates also tended to show resistances to various antimicrobial agents. Compared with the disinfectant-susceptible isolates using in vitro stepwise exposure including MBC measurements and time–kill assays, the DRS isolates tend to survive much longer in sub-MIC concentrations of several disinfectants. Thus, susceptibility to disinfectants must be carefully checked on a case-by-case basis if several multidrug-resistant *A. baumannii* are recurrently isolated from clinical specimens despite proper precautionary measures.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Kawamura-Sato, K., Wachino, J.I., Kondo, T., Ito, H. and Arakawa, Y., 2008.	Susceptibility Study	The bactericidal activities of the four disinfectants against 283 strains of *Acinetobacter* species recovered from 97 Japanese hospitals in March 2002 were investigated by four different tests: MIC measurements, MBC measurements, time-killing assays and adaptation assays. Moreover, disinfectant efficacy was examined in the presence of BSA in two tests: MBC measurements and time killing assays.	Acinetobacter species usually cause hospital-acquired infections, including urinary- and respiratory-tract infections, and particularly ventilator-associated pneumonia, especially in debilitated individuals.1,2,21 Indeed, no apparent resistance properties of these DRS isolates against disinfectants were observed from the viewpoints of MIC and MBC measurements in the absence of organic materials, but the results obtained by the suspension test in the presence of BSA suggested that these DRS isolates may well survive in conditions of contamination by organic materials such as blood and exudation. Thus, care should be taken in monitoring the susceptibility profile of *Acinetobacter* species against disinfectants, especially when this microbe is frequently or continuously isolated from clinical samples.	In conclusion, no resistance to CHX, BZX, BZT and ADH was detected among clinically isolated *Acinetobacter* species by MIC measurements. However, the bactericidal effects of BZK, BZT and ADH, especially on the DRS isolates, were remarkably reduced in the presence of an organic material (3% BSA). Furthermore, the DRS isolates tended to adapt a higher concentration of CHX after repetitive passages in 1/2 MIC concentrations of CHX. To prevent hospital-acquired infections caused by this kind of microbe, the profile of susceptibility to disinfectants, as well as to antimicrobial agents, must be carefully monitored and checked among *Acinetobacter* species isolated from both clinical specimens and environments. Disinfectants are indispensable to perform appropriate infection control. Hence, this study highlights the need to ensure that these agents are being used appropriately in practice at the correct concentrations and for adequate contact times.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Liu, Q., Zhao, H., Han, L., Shu, W., Wu, Q. and Ni, Y., 2015.	Controlled Laboratory Study	Fifty three MuH MRSA isolates gathered in August 2005 to May 2008 from 6 university hospitals in China were analyzed for plasmid-borne genes (qacA/B, smr, qacG, qacH, andqacJ) by polymerase chain reaction (PCR); for chromosome-mediated genes (norA, norB, norC, mepA, mdeA, sepA, andsdrM) by PCR and quantitative reverse transcription-PCR (qRT-PCR); and for susceptibility to chlorhexidine by MIC and minimum bactericidal concentration (MBC).	The plasmid-borne genes qacA/B (83.0%) and smr (77.4%) and overexpressions of chromosome-mediated genes norA (49.0%) and norB (28.8%) were predominantly found in isolates studied, and 90.6% of the isolates revealed tolerance to chlorhexidine. In the presence of BSA, the average MBC of chlorhexidine for these isolates rose to 256 μg/mL. Altogether, our results suggest that surveillance of sensitivity to biocides among MuH MRSA isolates is essential for hospital infection control.	In conclusion, the results of the present study showed that the plasmid-borne biocide resistance genes existed extensively in our MuH MRSA isolates, and some isolates with overexpression of chromosome-encoded biocide resistance genes were also found. The high rate of high-level chlorhexidine tolerance isolates should cause concern even if this reduced sensitivity may not be enough to abolish the efficacy of this agent at in-use concentration because biocide tolerance may contribute to persistence of MRSA in hospital and make the elimination of MRSA a more difficult task in hospital infection control. Therefore, there is a need to establish the biocide surveillance system for continued monitoring of such isolates in China. Meanwhile, this study also implies that biocides should be used appropriately in practice at the correct concentrations.

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Longtin J, Seah C, Siebert K et al. 2011	Susceptibility Study	MRSA strains were provided by Mount Sinai Hospital (MSH) and Sunnybrook Health Sciences Centre (SHSC), Toronto, Ontario, Canada. We collected the initial strain from each patient colonized or infected with MRSA within their ICU stay during 2005 to 2009 (SHSC) and in 2008 and 2009 (MSH). A total of 334 MRSA isolates were collected from two Canadian intensive care units between 2005 and 2009.	We found that the qacA, qacB, and smr genes are relatively infrequent in MRSA isolated from patients in two Toronto ICUs. spa typing revealed that our clones are consistent with Canadian MRSA epidemiology, so we do not expect a selection bias. It is known that the global distribution of the qac and smr genes is highly variable. The local utilization of chlorhexidine and other antiseptics could affect the distribution of resistance genes, but a relationship is difficult to infer. Interestingly, we did not witness a clinically significant increase in CHDN MBC to be associated with the presence of the qacA or qacB gene. The QacA pump confers a reduced susceptibility to a broad range of hydrophobic compounds, including CHDN. QacB has a similar action but has a limited impact on CHDN because of an amino acid substitution at position 323, and sequencing is needed to differentiate qacA from qacB. The fact that we did not observe a significant increase in MBCs associated with the qac and smr genes is in line with the relatively small increase witnessed by other studies, usually within a 2 2-fold-dilution increase.	In conclusion, we infrequently found the qacA, qacB, and smr genes in MRSA from two intensive care units in Canada. However, the increase in CHDN usage in routine patient care warrants periodic monitoring of susceptibility in order to detect any raise in either gene associated with resistance, as well as phenotypic testing to identify any other mechanisms of resistance.

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Lu, Z., Chen, Y., Chen, W., Liu, H., Song, Q., Hu, X., Zou, Z., Liu, Z., Duo, L., Yang, J. and Gong, Y., 2014.	Comparative Susceptibility study	One hundred and forty-five MRSA and 178 MSSA from clinical specimens from seven hospitals in different regions of China, 70 MRSA from superficial sites of patients and 106 MRSA from environmental samples from an ICU were collected and screened for the presence of the qacA/B gene.	Currently, whether the presence of qacA/B is the main reason for chlorhexidine resistance has not been definitely determined. Some reports have shown that the presence of qacA/B did not cause a significant increase in chlorhexidine MIC or MBC in vitro.15 In this study, we witnessed a significant correlation between qacA/B carriage and reduced susceptibility to chlorhexidine. As the other antiseptic genes were rarely found, this suggested that the qacA/B gene was the main reason for the reduced chlorhexidine susceptibility in our isolates.	In conclusion, we observed a reduced susceptibility of *S. aureus* isolates to chlorhexidine and presented detailed molecular and phenotypic characteristics of qacA/B-positive *S. aureus* isolates in China. Further work is required to study how to reduce the spread of qacA/B-positive *S. aureus*, especially in ICU patients.

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McDanel, J.S., Murphy, C.R., Diekema, D.J., Quan, V., Kim, D.S., Peterson, E.M., Evans, K.D., Tan, G.L., Hayden, M.K. and Huang, S.S., 2013.	Susceptibility Study	MRSA isolates from colonized residents in nursing homes located in a large metropolitan county (Orange County, CA, with a population of 3.1 million). 829 MRSA isolates collected from the nares of residents in 25 of the 26 nursing homes; 1 nursing home had no MRSA carriers. Each isolate was from a unique patient. The number of MRSA isolates collected from residents at a single nursing home ranged from 1 to 81, with a median of 34 isolates. All isolates had a chlorhexidine MIC of<4 _g/ml. There is no CLSI method for testing of chlorhexidine, but this was done using the standard broth dilution approach described by CLSI, using a complete inhibition endpoint at 18 to 24 h of incubation Chlorhexidine digluconate 20% aqueous solution (Sigma-Aldrich, St. Louis, MO) was used as the starting material for broth dilution testing.	We found that fewer than 1% of the MRSA isolates carried the putative chlorhexidine resistance genes qacA and/or qacB, and none had chlorhexidine MICs that were 4 g/ml. Other health care facilities have reported a higher prevalence of qacA and/or qacB in MRSA isolates. Lee et al. identified qacA and/or qacB in 91% of the MRSA isolates from patients who had failed decolonization. The rarity of the qacA and/or qacB gene loci in our large collection of nursing home MRSA isolates is of interest, given the common use of chlorhexidine for preoperative bathing, as well as body surface antisepsis prior to placement of central lines or surgical incisions. At least one affiliated hospital was using it for daily bathing in the intensive care unit setting.	In summary, chlorhexidine resistance was not commonly found in MRSA isolates from nursing homes, but mupirocin resistance rates were higher in nursing homes than previously found in the community and from acute care facilities and varied substantially across facilities. Importantly, in contrast to other studies which have found a predominance of LLMR, we found that nearly all mupirocin-resistant isolates exhibited HLMR. These elevated HLMR rates in nursing homes are concerning and suggest that emerging resistance will be a barrier to prevention programs that include widespread use of mupirocin.

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McNeil, J.C., Kok, E.Y., Vallejo, J.G., Campbell, J.R., Hulten, K.G., Mason, E.O. and Kaplan, S.L., 2016.	Survey: Susceptibility testing	Nosocomial *Staphylococcus aureus* isolates from 2007 to 2013. Two hundred eighty infections were initially identified from the database, with 247 cases ultimately meeting the inclusion criteria. The median age of patients included in the study was 2.4 months Isolates and patients were identified from an *S. aureus* surveillance study at Texas Children's Hospital.	Overall, 111 isolates had one or both antiseptic tolerance genes (44.9%). Eighty-two isolates (33.1%) were positive for smr, 56 isolates (22.7%) were positive for qacA/B, and 27 isolates had both genes (10.9%). Among MRSA isolates, the proportions of isolates positive for smr and qacA/B were 44/98 (44.9%) and 26/98 (26.5%), respectively. The proportions of isolates with antiseptic tolerance genes varied over the time period, with the largest proportions seen in 2009 and 2013. There was no statistically significant difference in the proportions of isolates positive for qacA/B or smr by hospital unit. Genotypic antiseptic tolerance is common among nosocomial *S. aureus* at TCH, accounting for 44.9% of the isolates. smr-positive *S. aureus* strains are strongly associated with methicillin and ciprofloxacin resistance. In contrast, qacA/B-positive *S. aureus* strains are associated with the presence of CVLs, a diagnosis of CLA-BSI, and elevated vancomycin MICs. In addition, the presence of these genes seems to have a synergistic impact on the MIC/MBC to chlorhexidine. In contrast to the high	While the changes in chlorhexidine MICs are modest between staphylococci that are positive for these genes and those that are negative, there were statistically significant changes in the MBC90s. Of particular note is that the MBC90s for isolates that were positive for both smr and qacA/B were significantly higher than the MBC90s in isolates bearing either of these genes in isolation, suggesting that together, they may have a synergistic effect on antiseptic efflux. Despite the fact that the in vitro chlorhexidine MICs for these organisms are well below the concentrations in commercially available preparations, the associated co-resistance to systemic antimicrobials is of clinical importance. smr-positive *S. aureus* strains were more often associated with methicillin resistance, fluoroquinolone resistance, and a trend toward higher rates of clindamycin resistance. qacA/B-positive *S. aureus* isolates were more often associated with a vancomycin MIC of 2 g/ml than qacA/B-negative strains were.

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Mendoza-Olazarán, S., Camacho-Ortiz, A., Martínez-Reséndez, M.F., Llaca-Díaz, J.M., Pérez-Rodríguez, E. and Garza-González, E., 2014.	Cohort Susceptibility Study	The study was conducted at the Hospital Universitario Dr. José Eleuterio González, a 460-bed tertiary care hospital in Monterrey, Mexico. *A baumannii* is endemic in this hospital and 69% of isolates are meropenem-resistant. Our hospital is equipped with 4 ICUs (neonatal, pediatric, medical, and surgical ICUs, respectively). This study was performed in the adult medical and surgical ICUs with a combined 20-bed area. The hospital ICU has an infection control program that is based on proper handwashing practices that are supervised by the hospital's epidemiology unit based on the recommendations of the World Health Organization. All patients with potential or proven colonization-infection by multidrug resistant A baumannii are placed on contact precautions.	One of the most relevant results of our study was the observation that CHG bathing affected clonal displacement; that is, clone A predominated baseline cultures but was displaced by clone B, which predominated during the intervention period. The main difference between clones was biofilm production. Clone B showed higher biofilm production (OD595 ¼ 0.758) than clone A (OD595 ¼ 0.511). Both clones were positive for OXA51-like and OXA24-like and were resistant to the antibiotics tested. Contrary to what was expected it seemed that bathing patients with CHG facilitated the establishment of a "more virulent" *A baumannii* clone. To explain the observed decreasing MIC values following CHG administration during the intervention period and the replacement of baseline clones with intervention period clones, we hypothesized that microorganisms infecting/colonizing our patients during the intervention period were not colonizing the skin of patients (where only CHG-resistant *A baumannii* strains would be expected), but were transmitted to patients via fomites that facilitated bacterial survival due to strong	Overall, *A baumannii* isolates recovered from patients who received body washing with 2% CHG presented with a significant decrease in CHG MICs associated with a change in clonality associated with increased biofilm production.

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Morrissey, I., Oggioni, M.R., Knight, D., Curiao, T., Coque, T., Kalkanci, A., Martinez, J.L. and BIOHYPO Consortium, 2014.	Controlled laboratory	The aim of the present work is to establish appropriate breakpoints for defining biocide resistance for those biocides as triclosan (TRI), benzalkonium chloride (BZC), chlorhexidine (CHX) and hypochloride for which more concerns on the potential coselection of antibiotic resistance have been raised. These breakpoints will be the hallmarks for future studies to define mechanisms of biocide resistance as well as for analyzing the potential selection of antibiotic resistance by biocides in natural isolates. For this purpose, we have made use of the concept of epidemiological cut-off values (ECOFFs, http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_Presentations/2011/EW1_Brown_Definitionsf2.pdf). These breakpoints are not based, as clinical breakpoints are, on the	In order to discuss biocide resistance, we require a more suited definition, one which is based on the ‘‘natural’’ susceptibility to antimicrobials of a given species and not just on the clinical success of the treatment. This ecological concept of resistance states that ‘‘a microorganism is defined as wild type for a species by the absence of acquired and mutational mechanisms of resistance to the agent’’ (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_Presentations/2011/EW1_Brown_Definitionsf2.pdf). The definition of the wild-type MIC phenotype is obtained by the study of several unrelated isolates, which allow establishing the epidemiological cut-off value (ECOFF), which is the upper limit of the normal MICs distribution for a given antimicrobial and a given species. Any isolate presenting a MIC above this value is considered as resistant irrespective of whether or not the achieved level of resistance compromises therapy. As a starting point for distinguishing between wild-type and resistant organisms, we set out to determine the distributions of the MICs and the MBCs of TRI, BZC, CHX and NaOCl for natural isolates of different relevant	To the best of our knowledge, this is the largest analysis on biocide MICs or MBCs and the only one to determine ECOFFs for biocides. These data provide a baseline to measure biocide susceptibility to assist with future surveillance studies. The finding that in most cases, we did not find bimodal distributions indicates the lack of a relevant percentage of biocide resistant isolates at natural populations. If biocide resistant mutants are rare, this would imply that co-selection or cross-selection of antibiotic resistance should also be a rare event in natural populations. Nevertheless, two other issues must be taken into consideration. Firstly, most biocides have been widely used for decades; the fact that we did not find bimodal MIC/MBC distributions in current populations may reflect the lack of resistance but also a full replacement of susceptible microorganisms by more resistant ones. This situation that has been named as MIC-creep, which can be defined as ‘‘the constant rise over time in the basal intrinsic resistance of an average isolate of a given bacterial species]’’ has been described for different antibiotics. Secondly, our analysis reflects the current steady state of the overall susceptibility to biocides of the studied microbial populations. These observed distributions are the consequence of the emergence of resistance, but also of its spread and stability, the latter being mainly dependent on the fitness costs associated to the acquisition of resistance. As stated above, several recent studies (all before 2006) have shown that microorganisms can evolve to acquire biocide resistance, which in several cases, may be associated to resistance to antibiotics. Although careful studies on this issue are still scarce, it is possible that the

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Naparstek, L., Carmeli, Y., Chmelnitsky, I., Banin, E. and Navon-Venezia, S., 2012.	Comparative Laboratory Controlled Study	One hundred and twenty-six XDR K. pneumoniae strains isolated from unique patients and various clinical sources by the Clinical Microbiology Laboratory of Tel-Aviv Sourasky Medical Centre were included in the study.	The MICs of chlorhexidine ranged from 8 to >256 mg/mL (mean 140 mg/mL), which were generally higher than those observed for *K. pneumoniae* ATCC13883 and E. coli ATCC25922 control strains (16 mg/mL and 2 mg/mL, respectively). The 70 ST258 isolates tested (Group I) showed a narrow distribution of higher MICs of chlorhexidine (32e256 mg/mL) compared with much wider distribution of generally lower MICs of chlorhexidine among the 56 non-ST258 isolates (Group II) (8e256 mg/mL). This difference in distribution was statistically significant ($P < 0.0001$). Ninety-nine percent of Group I strains had MICs of chlorhexidine of >32 mg/mL, compared with 52% of Group II strains ($P < 0.0001$).	The findings demonstrate the existence of tolerant subpopulations. Hetero-resistance towards antibiotics has been described previously for other opportunistic pathogens such as *Acinetobacter baumannii*; however, to the authors' knowledge, this is the first study to demonstrate population heterogeneity towards a disinfectant. The presumably transient nature of these subpopulations raises questions about the underlying mechanism; further investigation is required. Finally, the clinical relevance of higher MICs of chlorhexidine for *K. pneumoniae* ST258 should be considered in the context of the global threat of these extremely drug-tolerant strains. It is possible that the resistance of this strain to chlorhexidine contributes to its ability to persist in the hospital environment.

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Oggioni, R., Rosado Coelho, M., Furi, J., R Knight, D., Viti, C., Orefici, G., Martinez, J.L., Teresa Freitas, A., M Coque, T. and Morrissey, I., 2015.	Susceptibility study and survey	To investigate the relationship between susceptibility profiles of biocides and antibiotics, we determined the susceptibility profiles of the most commonly used antibiotics in 1632 clinical *S. aureus* isolates with known susceptibility profiles of the biocides chlorhexidine, benzalkonium chloride, sodium hypochlorite and triclosan.	Using the non-linear correlation approach, no strong relationship between any biocide and antibiotic phenotypes was evidenced. Indeed, the data analysed showed weak to moderate bivariate correlations. The result of this study matches with that of a previous study of a smaller group of antibiotics where only the profiles of both benzalkonium chloride and chlorhexidine were associated with multi-drug resistance. With respect to the biocides, a series of observations have to be made which include (i) that whether the MICs to chlorhexidine and benzalkonium chloride have a statistically significant coefficient of 0.5 in accordance with the fact that both compounds are effluxed by the NorA and QacABCGHJ efflux pumps; on the contrary, absence of any correlation between MICs and MBCs for both chlorhexidine and benzalkonium chloride is in accordance with the absence of correlation of any known death-preventing and MBC-increasing resistance mechanisms, and (iii) a correlation coefficient of 0.6 between the MICs and MBCs for triclosan which are in accordance with the molecular characterisation of phenotypes conferred by fabI-	The data here show that in *S. aureus* there is no correlation of susceptibility profiles to triclosan or sodium hypochlorite and any clinically relevant antibiotic. The data further show that there is in contrast a significant relationship with a moderate correlation between susceptibility profiles to the bis-biguanide chlorhexidine and the quaternary ammonium compound benzalkonium chloride and some classes of antibiotics. In the light of the recently published observations that most clinically relevant bacterial species do not show the presence of subpopulations with decreased biocide susceptibility, our data suggest that the global use of biocide to date appears not to have resulted in a clinically relevant impact on antibiotic resistance. While our data do not allow for inference as to the direction of selective pressure in the case of the association between susceptibility profiles to some biocides and antimicrobial resistance, they clearly rule out the possibility that such evidence exists at present for other compounds. While not addressing toxicity of the biocides, this report should answer some of the other questions relating to risk for human health raised by the recent FDA report on the Safety and Effectiveness of Consumer Antiseptics.

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Otter, J.A., Patel, A., Cliff, P.R., Halligan, E.P., Tosas, O. and Edgeworth, J.D., 2013.	Susceptibility study: Controlled Laboratory Study	A chlorhexidine-based antiseptic protocol for all admissions to the ICU and the linked high-dependency units was introduced in April 2004. They evaluated the carriage of qacA, qacB and smr and in vitro chlorhexidine susceptibility in MRSA bloodstream infection (BSI) isolates between 2001 and 2009.	There were 602 single patient MRSA BSI isolates identified between 2001 and 2009, comprising CC22 (n¼224), CC30 (n¼197), ST239-TW (n¼58) and a group of sporadic clones (n¼123). The population chlorhexidine MIC profiles of CC22, CC30 and ST239-TW were comparable to 135/137 (98.5%) isolates having an MIC of either 2 mg/L (73.7%) or 1 mg/L (24.8%). Univariate analysis showed that the carriage of qacA in CC22 isolates was associated with a chlorhexidine MIC ≥2 mg/L, whereas carriage of qacA in CC30 isolates was associated with a chlorhexidine MIC ,2 mg/L. In multiple logistic regression analysis, CC22 isolates carrying qacA were more likely to have a chlorhexidine MIC ≥2 mg/L than CC30 isolates carrying qacA (OR, 21.67; CI, 2.54–185.20).	The limitations of this study include the lack of a validated method for detecting clinically significant reduced chlorhexidine susceptibility to link with qacA genotype or a clinical response, for which there is clearly an urgent need. We also did not have detailed clinical data and matched isolates from a cohort of MRSA-colonized patients to assess whether bloodstream or other infections following chlorhexidine decolonization were more likely in patients colonized with CC22 rather than CC30. This would add additional important clinical evidence for a differential effect of chlorhexidine on these two clones. Finally, unlike the ICU, there was no specific date for a step-change increase in chlorhexidine use or detailed data on compliance with the policy for MRSA decolonization on the general wards; instead, there was a progressive focus on education and guideline adherence from 2004 that coincided with the changing relative prevalence of the two clones. This study did, however, have important strengths. It analysed consecutive BSI isolates over an extended time period and linked clone, qacA carriage and an in vitro susceptibility phenotype with changing MRSA clonal epidemiology in the face of an effective infection control programme. In summary, this study provides the first evidence that qacA might confer a selective advantage in response to chlorhexidine based decolonization in some, but not other, MRSA clones. These data, combined with previously published evidence, support a hypothesis that infection control practice may drive changing MRSA epidemiology, perhaps helping to explain the increasing global dominance of CC22 and ST239 clones. This is a particular concern given that these clones have been linked with increased virulence.

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Prag, G., Falk-Brynhildsen, K., Jacobsson, S., Hellmark, B., Unemo, M. and Söderquist, B., 2014.	Susceptibility study: Controlled Laboratory Study	The study included a total of 143 *Staphylococcus epidermidis* isolates. The origin of the isolates was as follows: Sixty-one isolates were obtained from multiple tissue biopsies taken peri-operatively from 61 different patients during revision surgery for prosthetic joint infections (PJIs) with extraction or exchange (hip (n = 46); knee (n = 13); elbow (n = 1); shoulder (n = 1)). The revisions were conducted from 1993 to 2008. From the LOGIP (15) and the LOGIX (16) trials, performed from 2000 to 2002 and from 2007 to 2009, respectively, 31 S. epidermidis isolates that caused deep surgical site infections (mediastinitis and/or sternitis) were examined. These trials investigated the effect of prophylactic use of locally administered gentamicin containing sponges (collatamp-G; Schering Plough, Stockholm,	In the present study, we found a strong correlation between presence of MDR and genes encoding qacA/B. These MDR strains were also associated with decreased susceptibility to chlorhexidine. MDR *S. epidermidis* was predominantly isolated from clinical infections, i.e., PJIs and SSIs following cardiac surgery, probably representing nosocomial strains that successively accumulate resistance genes including genes encoding resistance against QAC. In the present study, *S. epidermidis* isolated from the skin, following pre-operative preparation with showers three times with chlorhexidine soap and subsequent disinfection with chlorhexidine in alcohol immediately before incision, did not display a higher prevalence of genes encoding resistance against QAC than commensals. In addition, they did not display multi-drug resistance. Thus, preoperative strategies to reduce post-operative infections by using chlorhexidine did not seem to select for isolates with decreased susceptibility against chlorhexidine, and the isolates present could be members of the commensal flora not completely eradicated by the disinfection procedure. However, this question	When the bacteria are exposed to efficient concentrations of chlorhexidine, the bacteria may be killed by membrane damage. However, if the bacteria do have mechanism for counteracting chlorhexidine, e.g., efflux pumps, the concentration of chlorhexidine that the microbe is exposed to and the duration of exposure might be important. A limitation of the present study is the fact that the isolates used were collected from various previous studies representing various time periods and that the number of isolates from the specific studies is limited. In conclusion, in the present study, *S. epidermidis* isolated from clinical infections displayed higher prevalence of genes encoding resistance against QAC as well as decreased susceptibility against chlorhexidine compared with commensal strains.

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Shamsudin, M.N., Alreshidi, M.A., Hamat, R.A., Alshrari, A.S., Atshan, S.S. and Neela, V., 2012.	Susceptibility study: Controlled Laboratory Study	60 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from Malaysia to three antiseptic agents benzalkonium chloride (BZT), benzethonium chloride (BAC) and chlorhexidine digluconate (CHG) were determined.	Our findings are in agreement with a previous study showing that CHG and QACs have comparable efficacy against MRSA. Hence, the antiseptics commonly used in the hospital environment should be effective against clinical isolates of MRSA if used at recommended in-use concentrations. However, a significant association was identified between the presence of qacA/B genes and degree of susceptibility to CHG and BAC ($P < 0.001$) (Table I). This means that isolates carrying qacA/B may be able to persist on the skin where concentrations of disinfectants may be lower than in-use concentrations.	In conclusion, this is the first time that the carriage rate of qacA/B and smr gene has been reported for Malaysian MRSA isolates. The presence of these antiseptic resistance genes is potentially a serious concern. The findings of the present study emphasize that the carriage of qacA/B is associated with reduced susceptibility, albeit in the susceptible range. Continuous monitoring to ensure proper usage of antiseptics in the hospital is recommended together with continued surveillance of resistance gene carriage.

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Sheng, W.H., Wang, J.T., Lauderdale, T.L., Weng, C.M., Chen, D. and Chang, S.C., 2009.	Susceptibility: Controlled Laboratory Study	206 MRSA clinical isolates from the Taiwan Surveillance of Antimicrobial Resistance program III and IV (years 2002 and 2004) from 26 hospitals.	The MIC50 and MIC90 of chlorhexidine for all 206 isolates were 2 and 8 μg/mL, respectively. Seventy-three (35.4%) isolates carried qacA/B gene, but none carried smr. For the 72 (35.0%) MRSA isolates with chlorhexidine MIC ≥4 μg/mL, 53 were ST239 (49 of them carried qacA gene), 12 were ST5 (all carried qacB gene), 5 were ST241 (4 carried qacA gene), 1 was ST338 (and carried qacA gene), and 1 was ST573 (and carried qacA gene). Compared with other sequence-type MRSA isolates, ST239 MRSA isolates were the most resistant to both chlorhexidine and other antimicrobial agents. Methicillin-resistant S. aureus strains with disinfectant resistance qacA/B genes are common in Taiwan. High frequency of qacA/B genes among specific sequence types (ST239, ST5, and ST241) resulted in low susceptibility to chlorhexidine. Periodic surveillance of antiseptic susceptibility among MRSA isolates is important for the control of nosocomial hospital-acquired infections. The qacA/B genes can confer resistance to cationic antiseptic agents (such as quaternary ammonium compounds, chlorhexidine digluconate, and	In conclusion, surveillance of MRSA isolates with high chlorhexidine MICs is necessary for the acquisition of knowledge that might lead to a reconsideration of chlorhexidine use as the recommended hand hygiene agent in hospitals. Presence of qacA/B genes in certain MRSA clones, such as ST239-III in Taiwan, is usually associated with high resistance to chlorhexidine and various antiseptic agents, might limit the choice of drugs for treating MRSA infections, and presents a difficult problem in MRSA infection control.

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Skovgaard, S., Larsen, M.H., Nielsen, L.N., Skov, R.L., Wong, C., Westh, H. and Ingmer, H., 2013.	Characterisation and Susceptibility Study	Authors address if the widespread use of chlorhexidine in the Danish hospital setting has selected for *S. epidermidis* strains with tolerance towards chlorhexidine and/or harbours the qacA/B genes and if those genes are associated with more antibiotic resistance. *S. epidermidis* were collected from nurses and patients recruited at the Copenhagen University Hospital, Hvidovre, Denmark.We recruited eight scrub nurses working within the sterile field, using the hand rub Iduscrub (85% denatured ethanol, 0.5% chlorhexidine/0.5% glycerol) (Brenntag Nordic A/S) as the last step in the disinfecting hand procedure performed before surgery. They were sampled on a Friday when disinfecting hand hygiene had been performed for a minimum of 3 of the last 4 days. Samples were	They investigated a large number of S*. epidermidis* isolates from healthy colonized people, scrub nurses heavily exposed to chlorhexidine, current isolates from blood and blood isolates from the pre-chlorhexidine era. They isolated S. epidermidis from eight scrub nurses with 2–4 different isolates obtained from each. From10 patients (non-users of chlorhexidine), *S. epidermidis* were isolated before hospitalization, representing 1–5 isolates from each. Also *S. epidermidis* were obtained from the same 10 patients after hospitalization, representing 1–6 isolates from each.	The use of chlorhexidine in the Danish hospital setting appears neither to have selected for measurable chlorhexidine tolerance in *S. epidermidis* nor qacA/B carriage when compared with community isolates. Importantly, the susceptibility of hospital isolates to chlorhexidine was similar to that of community isolates as well as to that of blood isolates obtained in the 1960s before the introduction of chlorhexidine. However, in contrast to current blood isolates, the qacA/B gene were absent in the isolates collected in the 1960s, suggesting that selection has occurred. This is the first study to indicate a recent introduction of qacA/B genes in *S. epidermidis* and we speculate it may be associated with the use of chlorhexidine or related compounds as has been suggested for *S. aureus*.

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Smith, K., Gemmell, C.G. and Hunter, I.S., 2008.	Controlled Laboratory study	Bacterial strains were provided by the Scottish MRSA Reference Laboratory (Stobhill Hospital, Glasgow, UK). Ninety-four clinical strains of S. aureus were selected from a large library of clones and subclones based on differences in their PFGE banding patterns. There were 38 HA-MRSA isolates, 25 CA-MRSA isolates, 25 methicillin-susceptible S. aureus isolates (MSSA) and 6 isolates with intermediate resistance to vancomycin (VISA). Two VISA strains were isolated in Scotland, two originated in the USA and two were isolated in Japan. Commonly used hospital biocides were obtained in commercial preparations. These were: Trigene, a product containing a mixture of the QACs (alkyl dimethyl benzyl ammonium chloride and dodecyl dimethyl ammonium chloride);	The continued exposure of bacteria to residual levels of biocides in the hospital environment is causing concern. This study has shown that clinical isolates of *S. aureus* including HA-MRSA, MSSA, CA-MRSA and VISA strains have MBCs of the commonly used hospital biocides Trigene, MediHex-4 and Mediscrub of 10–1000-fold less than the concentrations recommended for use by the manufacturer. However, HA-MRSA isolates had the ability to develop significantly increased tolerance to Trigene following repeated exposure to this agent. This may suggest that repeated exposure of *S. aureus* to subinhibitory concentrations of this biocide in the hospital environment could enhance tolerance. HA-MRSA and VISA isolates frequently carried qac efflux pump genes, which significantly increased (P, 0.0001) the MBC of Trigene and MediHex-4 for these isolates compared with isolates that did not carry qac genes. Trigene and MediHex-4 were found to induce the expression of the genes encoding the QacA/B efflux pumps, which confirms that these biocides are likely substrates. This suggests that in the presence of these biocides, efflux-mediated increased tolerance	All isolates had MBCs of Trigene, MediHex-4 and Mediscrub of 10–1000-fold lower than concentrations recommended for use by the manufacturers. This would suggest that, if these biocides are used in accordance with the manufacturers' instructions, 100% of bacteria should be killed. Problems may arise when biocides are used incorrectly, in dirty situations where surfaces are not cleaned of organic matter prior to using a biocide or 'topping up' biocides leading to the use of subinhibitory concentrations. In the hospital environment bacteria grow in biofilms on surfaces, which have been shown to afford the cells a 10–1000-fold higher tolerance of antimicrobials, and may be a contributing factor to failure of disinfection. If biocides are used at concentrations recommended for use by the manufacturer in the hospital environment, then *S. aureus* isolates should be killed, as even the increased tolerance displayed in isolates failed to develop into complete resistance. However, the presence of qac genes in the clinical *S. aureus* population and their ability to develop increased tolerance highlights the importance of effective and rigorous infection cleaning and infection control strategies and the use of biocides at concentrations recommended by the manufacturer.

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Wand ME, Bock LJ, Bonney LC, Sutton JM 2016	Controlled Laboratory study	The *K. pneumoniae* isolates used in this study are clinical strains with a variety of antibiotic 80 resistance markers e.g. blaNDM-1, blaSHV-18 and have been described previously. In this study we investigated whether adaptation of clinical *K. pneumoniae* isolates to chlorhexidine caused cross resistance to other biocides and antibiotics, and whether adapted strains maintained fitness and virulence. The underlying mechanisms of increased resistance to chlorhexidine in *K. pneumoniae* were also investigated, particularly in connection with the observed cross resistance to colistin.	This study has shown that adaptation of clinical *K. pneumoniae* isolates to chlorhexidine exposure can not only lead to stable resistance to chlorhexidine but also cross-resistance to colistin. This has important clinical implications for the treatment of MDR (particularly carbapenem-resistant) *K. pneumoniae* infections and outbreaks, given their increasingly prevalence in hospitals. Many carbapenem-resistant *K. pneumoniae* isolates are susceptible to very few antibiotics notably colistin; treatment often involves combination therapy including colistin. Therefore, any potential loss of colistin efficacy has implications for treatment of these infections. Whilst chlorhexidine has been successfully used as part of a multifaceted intervention to reduce the prevalence of carbapenem-resistant *K. pneumoniae* in hospitals the observation that exposure to chlorhexidine leads to colistin resistance means eradication of potentially colistin and carbapenem-resistant isolates is very problematic. Since the isolate has also acquired increased resistance to chlorhexidine this also makes prevention of colonisation with these isolates more	Overall this study has identified a novel resistance mechanism to chlorhexidine (smvA/R) that may potentially operate in a number of different species. Clearly increased smvA expression is important for chlorhexidine adaptation in *K. pneumoniae* but it is not the only mechanism and may operate in conjunction with other regulatory processes. Chlorhexidine-adaptation is also associated with the generation of mutations in PhoPQ, which affect a number of known regulatory targets (notably pmrD and pmrK). Upregulation of these genes also correlates with the presence of colistin resistance. That increased colistin and chlorhexidine resistance may occur in clinical isolates without significant loss of fitness/virulence highlights the potential challenges associated with critical infection control procedures and the use of chlorhexidine as an antiseptic to control healthcare–associated infections.

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Wang, J.T., Sheng, W.H., Wang, J.L., Chen, D., Chen, M.L., Chen, Y.C. and Chang, S.C., 2008.	Longitudinal susceptibility study	Six isolates in 1990 and 60 randomly selected isolates each in 1995, 2000 and 2005 from MRSA isolates causing nosocomial bloodstream infections at NTUH, a 2500 bed hospital in Taiwan, were enrolled first (only six nosocomial bloodstream infections in total in 1990). Because of the limited number of blood isolates in 1990, 54 nosocomial MRSA isolates from other clinical specimens in 1990 were also included (only 63 nosocomial MRSA isolates in total in 1990). The total number of nosocomial blood S. aureus isolates in 1990, 1995, 2000 and 2005 at NTUH was 596. The total number of nosocomial blood MRSA isolates in 1990, 1995, 2000 and 2005 at NTUH was 388.	Resistance of *S. aureus* to chlorhexidine is conferred by two gene families, qacA/B and smr.4 The qacA/B gene confers high-level resistance to antiseptics, whereas the smr gene confers low-level resistance. The current study aimed to understand the changes in susceptibility to chlorhexidine as well as the proportion of MRSA isolates carrying the qacA/B gene at NTUH, where a high prevalence of MRSA nosocomial infections and long-term chlorhexidine use were present. The chlorhexidine MIC ranges of MRSA isolates collected in 1990, 1995, 2000 and 2005 were 1–4, 0.5–8, 1–8 and 1–16 mg/L, respectively (for the six blood isolates in 1990, the MIC range was 0.5–2 mg/L) and the MIC90s were 2, 4, 8 and 8 mg/L, respectively. The proportion of tested MRSA isolates with high chlorhexidine MICs (4 mg/L) increased markedly from 1.7% in 1990 to 50% in 1995. After 1995, the proportion stabilized (40% in 2000 and 46.7% in 2005) (testing for heterogeneity of frequencies: with all four time points, P ¼ 0.003; with only 1995–2005, P ¼ 0.54). A total of 83 isolates (34.6%) expressed high chlorhexidine MICs.	In conclusion, the present study demonstrated that the proportion of MRSA isolates with high chlorhexidine MICs at NTUH increased from 1990 to 1995 and remained steady thereafter. More than half (55.4%) of the isolates with high chlorhexidine MICs harboured the qacA/B gene, and it is presumable that the presence of these genes may contribute to the spread of specific MRSA clones.

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Wu, D., Lu, R., Chen, Y., Qiu, J., Deng, C. and Tan, Q., 2016.	Susceptibility study	The *S. aureus* reference strain ATCC 25923 as well as 14 clinical isolates were exposed to antibiotics, CHX and RCE at sublethal doses for up to 14 days.	All isolates were cross-resistant to more than one other antibiotic following tetracycline exposure, and increased resistance (≥4-fold MIC increase) to RCE and CHX was observed in six and three isolates, respectively. Following selection by CHX, most of the treated strains showed no significant change in sensitivity to CHX. However, all strains developed cross-resistance to at least one antibiotic, and decreased susceptibility (≥4-fold MIC increase) to RCE appeared in seven strains. Following exposure to RCE, 11 isolates showed cross-resistance to at least one antibiotic. In addition, three RCE-exposed strains showed reduced susceptibility to CHX (4- or 8-fold MIC increase).	The results obtained in this study imply that antibiotics, biocides and antimicrobial Chinese herbs might employ some of the same mechanisms of action against bacteria, triggering mutual cross-resistance to further foster the development of bacterial resistance.

Primary research studies – retrospective time series / cross sectional / case control / cohort (n=5/36)

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Batra, R., Cooper, B.S., Whiteley, C., Patel, A.K., Wyncoll, D. and Edgeworth, J.D., 2010.	Retrospective interrupted time series laboratory study	MRSA acquisitions in two 15-bed intensive care units An evaluation of 3 interventions to prevent MRSA transmission (educational campaign, or cohorting or a chlorhexidine antiseptic protocol) in intensive care units using interrupted time series data to estimate the effects of the intervention. Emerging resistance is of concern with the use of antimicrobials and antiseptics as decolonisation agents.	All TW MRSA strains (21 of 21 isolates) and <5% (1 of 21 isolates) of non-TW MRSA strains tested carried the chlorhexidine resistance loci qacA/B. In vitro chlorhexidine minimum bactericidal concentrations of TW strains were 3-fold higher than those of non-TW MRSA strains, and in vivo, only patients with non-TW MRSA demonstrated a reduction in the number of colonization sites in response to chlorhexidine treatment. N.B. TW MRSA is a novel variant of ST-239 (sequence type) called TW (ST – sequence type and TW is Taiwanese)	A chlorhexidine-based surface antiseptic protocol can interrupt transmission of MRSA in the intensive care unit, but strains carrying qacA/B genes may be unaffected or potentially spread more rapidly. Raised the question “whether the carriage of qacA/B can account for some of the decolonization failures observed in randomized studies in which chlorhexidine is used as part of the protocol”

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Ho, C.M., Li, C.Y., Ho, M.W., Lin, C.Y., Liu, S.H. and Lu, J.J., 2012.	Case Control study	Sixty methicillin-sensitive Staphylococcus aureus (MSSA) and 96 MRSA isolates were collected from blood cultures of different patients from July 2008 to December 2009. Identification of these clinical isolates was achieved by the Bactec 9000 system (Becton, Dickinson, Sparks, MD), and the susceptibility of each isolate to oxacillin was determined by the BD Phoenix Automated Microbiology System (Becton, Dickinson). The basic and clinical information of each patient was obtained from medical records. Patients with community-acquired MRSA (CA-MRSA) infection were those without histories of surgery, long-term-care facility residence, dialysis, indwelling device or catheter usage within the recent 1 year, or hospitalization for less than 48 h before positive MRSA culture (1). Other	Because few MSSA isolates carried qacA/B (n = 2) and only one patient with CRBSI had chlorhexidine-impregnated catheter insertion, the 96 MRSA isolates were analyzed for their roles in CRBSI (Tables 4 and and5). The results showed no significant relationship between the existence of qacA/B and different clinical backgrounds (age, gender, frequency of chlorhexidine-impregnated catheter insertion, and hospital- or community-acquired infections), agr and spa genotypes, or chlorhexidine MIC, except that more SCCmec II and IV MRSA isolates (47.4% and 72.2%, respectively) were found to carry qacA/B. Multivariate logistic regression analyses with adjustments for gender and age revealed that the presence of qacA/B and chlorhexidine MIC of ≥2 μg/ml were the two risk factors for chlorhexidine-impregnated CRBSI caused by MRSA (OR, 6.097 and 4.373, respectively). This finding suggests that the transmission of qacA/B was not related to the clonal spreading of MRSA in our hospital but was related to the selective pressures in preventive procedures for nosocomial infections. The carrier rate of qacA/B in MRSA isolates determined in this study was 43.8%,	The clinical significance of the existence of these antiseptic-resistant genes remains to be investigated. Since there is no internationally standardized method for in vitro susceptibility tests of these antiseptics, the interpretation of susceptibility to these biocides may not be the same as that for systemic antibiotics. However, the possibility of increased CRBSI episodes as a result of more MRSA isolates containing qacA/B cannot be ignored. Thus, the threat of MRSA to infection control is not confined to glycopeptide resistance but also can affect resistance to the biocides commonly used in clinical procedures. Further investigations on the effects of qacA/B in chlorhexidine-integrated preventive procedures are warranted.

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Lee, A.S., Macedo-Vinas, M., François, P., Renzi, G., Schrenzel, J., Vernaz, N., Pittet, D. and Harbarth, S., 2011.	Nested case control study	The University of Geneva Hospitals is a tertiary care center with 1901 beds and 47,706 admissions in 2009. MRSA screening is performed for patients with a history of MRSA carriage or who are hospitalized in the intensive care unit, for contacts of newly identified carriers, and for patients who are about to be transferred to rehabilitation facilities. Universal screening at admission previously occurred hospital-wide from January through August 2003 and in surgical wards from July 2004 through May 2006. Screening swab samples are collected from the nares, groin, and other clinically indicated sites. MRSA carriers routinely receive decolonization therapy consisting of intranasal mupirocin twice daily for 5 days and chlorhexidine bathing (4% Lifo-Scrub; B. Braun) daily for 7 days.	Genotypic chlorhexidine resistance was more common than mupirocin resistance, with 68 case patients (91%) and 51 control patients (68%) carrying MRSA with the qacA/B genes ($P < .001$). In almost all instances, low-level mupirocin resistance coexisted with genotypic chlorhexidine resistance. Only 1 of the case patients had a baseline MRSA isolate that was resistant to mupirocin and not to chlorhexidine, and there were none among the control patients. Therefore, for further analyses, the combination of resistance to both agents was taken as the exposure of interest. Controlling MRSA transmission and infection is important in healthcare facilities, and decolonization is often recommended to achieve this goal (strength of evidence, IB–II). However, the results of this study emphasize the need to exercise caution when using this strategy. Our findings demonstrate that carriage of MRSA with both low-level mupirocin resistance and genotypic chlorhexidine resistance is strongly associated with persistent colonization after eradication therapy. Resistance to both these agents was closely linked in our	Rates of genotypic chlorhexidine resistance comparable to that seen in our institution have been described previously, in 63% of isolates in Europe and up to 80% of isolates elsewhere. This is of particular concern in view of increasing chlorhexidine use, not only for MRSA control but also for a variety of other indications, as well as reports of possible antibiotic cross-resistance with chlorhexidine. Our high resistance rates are likely due to selection of resistant strains. The V588F mutation, seen in all low-level mupirocin-resistant MRSA in this study, is not associated with substantial fitness costs. In addition, MRSA strains that carry the qacA/B genes have the potential for increased transmission when chlorhexidine-based surface antiseptic protocols are used. These factors may explain why resistant strains were able to predominate in our institution where targeted decolonization of MRSA carriers has been routine for more than 15 years. The association between resistance and decolonization failure may be underestimated in the current study. MRSA control is a priority in healthcare facilities, and eradication of carriage can be beneficial for the individual, as well as for patients at risk of MRSA acquisition. However, with any intervention using antimicrobial agents, the risk of emergence of resistance is invariably a potential threat. In this study of MRSA-colonized inpatients, carriage of strains with combined low-level mupirocin and genotypic chlorhexidine resistance significantly increased the risk of persistent MRSA carriage after decolonization therapy. Therefore, widespread use of decolonization therapies should be coupled with procedures to monitor for emergence of resistance. Alternative agents or practices are required in settings where resistance has rendered this

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Warren DK., Prager M., Munigala S., Wallace MA., Kennedy CR., Bommarito KM., Mazuski JE.and Burnham CD 2016	Retrospective cohort over 8 years 2002 – 2012	To determine the frequency of qacA/B chlorhexidine tolerance genes and high-level mupirocin resistance among MRSA isolates before and after the introduction of a chlorhexidine daily bathing intervention in a surgical intensive care unit (SICU) in a 1250 bed tertiary-care centre (Barnes-Jewish hospital). Patients admitted to SICU who had MRSA surveillance cultures of the anterior nares. A random sample of banked MRSA anterior nares isolates recovered during (2005) and after (2006–2012) implementation of a daily Chlorhexidine bathing protocol was examined for qacA/B genes and high-level mupirocin resistance. Staphylococcal cassette chromosome mec (SCCmec) typing was also performed.	Of the 504 randomly selected isolates (63 per year), 36 (7.1%) were qacA/B positive (+) and 35 (6.9%) were mupirocin resistant. Of these, 184 (36.5%) isolates were SCCmec type IV. There was a significant trend for increasing qacA/B (P=.02; highest prevalence, 16.9% in 2009 and 2010) and SCCmec type IV (P<.001; highest prevalence, 52.4% in 2012) during the study period. qacA/B(+) MRSA isolates were more likely to be mupirocin resistant (9 of 36 [25%] qacA/B(+) vs 26 of 468 [5.6%] qacA/B(−); P=.003).	A long-term, daily Chlorhexidine bathing protocol was associated with a change in the frequency of qacA/B genes in MRSA isolates recovered from the anterior nares over an 8-year period. This change in the frequency of qacA/B genes is most likely due to patients in those years being exposed in prior admissions. Future studies need to further evaluate the implications of universal Chlorhexidine daily bathing on MRSA qacA/B genes among hospitalized patients.

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Zhang, M., O'Donoghue, M.M., Ito, T., Hiramatsu, K. and Boost, M.V., 2011.	Comparative cross-sectional	A minimum sample size of 202 was estimated based on an S. aureus carriage rate of 20% and an assumed 5% carriage rate of qac genes in S. aureus and CoNS with 3% error and 95% confidence intervals (CIs). Nurses were recruited from 15 local hospitals and designated as 'fresh' (<2 years of nursing experience in the hospital) or 'experienced' (2 years of work experience). qac gene positivity levels were compared with 186 S. aureus and a random selection of 200 CoNS isolated from 775 healthy adults with no healthcare association participating in a study of carriage of MRSA in the general population. They consisted of families of university students and their friends.	Samples were obtained from 249 nurses, of whom 157 (63.1%) were experienced and 92 (36.9%) fresh. There was no significant difference between S. aureus carriage rates of nurses (51/249; 20.5%) and the general population (186/775; 24%). Eight nurses (3.2%), seven experienced, were colonised with MRSA compared with only 4/775 (0.5%) of the general population (OR: 6.4; 95% CI: 1.9e21.4; P¼ 0.002). There was a significantly lower rate of meticillin resistance in CoNS isolated from the general public (11%) than from nurses (28.9%; 117/404) (OR: 3.3; 95% CI: 2.0e5.4; P< 0.001).Resistance to several antibiotics was significantly more frequent in qac gene-positive than -negative isolates (Table IV). Isolates with qac genes (N¼ 168) had significantly highermean MICs and MBCs to BC and CHG, with a wider range of MICs andMBCs (Table V).Whereas there were no differences in MICs for CHG in qac-positive isolates from nurses and the general public, the MBCs were significantly higher for nurses' isolates (MBC50 nurses 8 mg/L, general public 2mg/ L; MBC90 nurses 16 mg/L, general public 8 mg/L; P< 0.001). No such difference was	Use of antiseptics may be selecting for antibiotic-resistant strains and assisting their survival in the healthcare environment. The association between mecA and qacA/B/smr may contribute to survival of MRSA in the hospital environment. They may pose an infection control risk by persisting in areas with low level antiseptic residues.

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Expert / Literature Reviews (n=5)

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Edgeworth, J.D., 2011.	Literature review	There has been a notable rise in the proposed uses for chlorhexidine in ICUs. In addition to its role in MRSA decolonization discussed above, it is being: used for skin antisepsis prior to blood culture collection and the insertion of vascular catheters; applied to the catheter exit site in the form of impregnated sponges; impregnated into vascular catheters to prevent bloodstream infections; and for oropharyngeal antisepsis to prevent ventilator-associated pneumonias. Much of this broader use has been predicated on the notion that resistance is either restricted to certain non-fermenting Gram-negative bacteria or where potentially transferable resistance mechanisms are identified, they are not clinically significant. This increased use of chlorhexidine in ICUs does, however, raise concerns	Available evidence on the efficacy of decolonization, predominantly from ICU studies, combined with the introduction of national guidelines endorsing its implementation as part of a new performance management culture in the NHS, supports the proposal that the widespread uptake of decolonization has made the key additional contribution. Although there is little published evidence on decolonization efficacy or practice on UK general wards, it is now recommended for all MRSA-colonized patients and uptake is probably widespread. The recent observation that MRSA strains carrying the antiseptic resistance genes qacA/B can be clinically resistant to chlorhexidine raises a note of caution against its unfettered use. The dissemination of chlorhexidine-resistant MRSA would have implications for the decolonization of individual patients and for preventing transmission.	Chlorhexidine particularly is being recommended in the ICU for an increasing number of indications, including decolonization, universal patient bathing, oropharyngeal antisepsis in ventilated patients and vascular catheter insertion sites. Of concern for the future would be the emergence of resistance to decolonization agents. Mupirocin resistance is well known but chlorhexidine resistance in MRSA is an emerging threat and of additional concern. If qacA/B-positive MRSA strains are clinically resistant to chlorhexidine and selected for in response to its use in MRSA control programmes, this would have important implications for the many uses of chlorhexidine in preventing MRSA transmission and infection.

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Harbarth S., Tuan Soh S., Horner C., & Wilcox, MH. 2014	A point/ counterpoint review	Offers a differentiated perspective and possible answers to the question, 'Should we be worried about reduced susceptibility to disinfectants and antiseptics in healthcare settings?'	Consensus: reduced susceptibility to antiseptics could become an increasing problem, but its clinical impact needs further research. While examples of reduced susceptibility to in-use concentrations of antiseptics are rare, there are hints that emergence of strains with reduced susceptibility can have clinical consequences. This is particularly pertinent to the increasingly prevalent use of chlorhexidine.	In situations of widespread and increasing use of biocidal active ingredients, a better understanding of the significance of reduced susceptibility to such agents is required. To make progress in this area, international standards to determine reduced susceptibility to biocidal agents in vitro need to be established. Once a method has been agreed, the implications for use and related reduced susceptibility of antiseptics and disinfectants in health care can be investigated prospectively in a controlled and systematic manner. Once the implications of widespread antiseptic use have been investigated thoroughly, the appropriate and/or inappropriate use of biocidal active agents can be discussed. For example, does an alcohol-based hand rub require additional chlorhexidine when used for hygienic hand disinfection, when evidence suggests the contrary? It is important to raise awareness that biocidal agents should be used in a targeted manner, and should be restricted to indications with proven clinical benefit (e.g. central venous catheter care) rather than in an indiscriminate manner. Examples of cross-resistance between antiseptics and antibiotics have been very uncommon. However, recent examples of the emergence of co-resistance to the quaternary ammonium compound benzalkonium chloride and fluoroquinolones in several different bacterial species emphasize that this phenomenon is possible. A better understanding of the clinical risks of reduced susceptibility to antiseptics, including the underlying mechanisms, is required. Only the brave (or foolhardy) would dismiss the relevance of reduced susceptibility to antiseptics and disinfectants to clinical practice.

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Horner, C., Mawer, D., and Wilcox M. 2012	Expert Review	In this review we have assessed the methods available for the detection of reduced susceptibility to chlorhexidine and the prevalence of co-resistance 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	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. 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	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.

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Kampf G, Acquired resistance to chlorhexidine – is it time to establish an "antiseptic stewardship" initiative? 2016,	Literature review	Published data from clinical isolates with CHG minimum inhibitory concentrations (MICs) were reviewed and compared to epidemiological cut-off values to determine resistance.	CHG resistance is rarely found in Escherichia coli, Salmonella spp., Staphylococcus aureus or coagulase negative staphylococci. In Enterobacter spp., Pseudomonas spp., Proteus spp., Providencia spp. and Enterococcus spp., however, isolates are more often CHG resistant. CHG resistance may be detected in multi-resistant isolates such as extremely drug-resistant Klebsiella pneumoniae. Isolates with a higher MIC are often less susceptible to CHG for disinfection. Although cross-resistance to antibiotics remains controversial, some studies indicate that the overall exposure to CHG increases the risk for resistance to some antibiotic agents. Resistance to CHG has resulted in numerous outbreaks and healthcare associated infections. On an average intensive care unit, most of the CHG exposure would be explained by hand hygiene agents when liquid soaps or alcohol-based hand rubs contain CHG. Exposure to sub-lethal CHG concentration may enhance resistance in Acinetobacter spp., K. pneumoniae, and Pseudomonas spp., all species well known for emerging antibiotic resistance. In order to reduce additional selection pressure in	Kampf concluded: Based on the fairly high resistance rates in Enterobacter spp., Pseudomonas spp., Proteus spp., Providencia spp. and Enterococcus spp., the ability of Acinetobacter spp., K. pneumoniae and Pseudomonas spp. to adapt to Chlorhexidine and the potential for cross-resistance to some antibiotics, it seems prudent to restrict the use of Chlorhexidine to those applications with a clear patient benefit and to eliminate it from applications without any benefit or with a doubtful benefit.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Noto, MJ & Wheeler, AP. 2015	Expert review	Understanding chlorhexidine decolonization strategies. Authors discuss the use of chlorhexidine for the decolonization of the mouth and skin of critically ill patients.	The available evidence supporting chlorhexidine-based oropharyngeal decolonization to prevent lower respiratory tract infections suggests a small benefit but is inconclusive. Chlorhexidine bathing to decolonize patients' skin consistently reduces colonization by MDROs and may reduce the incidence of hospital-acquired bloodstream infections, particularly those caused by skin commensal organisms, some of which are likely the result of blood culture contamination. These findings, however, were not reproduced in a large trial of chlorhexidine bathing, suggesting that this practice is not universally beneficial to patients or effective in all settings. These strategies expose a large population of patients to chlorhexidine, the overwhelming majority of which will never experience an HAI. Although reductions in blood culture contamination may be beneficial, these could be attained through interventions targeting only the subset of patients that have blood cultured. Furthermore, adverse or allergic reactions to chlorhexidine are rare, but serious reactions have been reported. In addition, aspiration of chlorhexidine causes	In conclusion, although chlorhexidine-based decolonization may be of benefit in select situations and should remain in the armamentarium of strategies to prevent HAIs, universal implementation of these practices warrants caution and further consideration in light of the available evidence and potential for harm.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Case Reports (n=2/36)				
Johnson, R.C., Schlett, C.D., Crawford, K., Lanier, J.B., Merrell, D.S. and Ellis, M.W., 2015.	Case report	We describe the selection of reduced chlorhexidine susceptibility during chlorhexidine use in a patient with two episodes of cutaneous USA 300 methicillin-resistant Staphylococcus aureus abscess. The second clinical isolate harbors a novel plasmid that encodes the QacA efflux pump. Greater use of chlorhexidine for disease prevention warrants surveillance for resistance.	Despite its widespread use, the prevalence of chlorhexidine resistance in the United States is low (approximately 1%); this is in contrast to observations in other countries. When used in large trials in both community and hospital settings, chlorhexidine resistance has been only rarely reported. Nevertheless, with the widespread and increasing use of this agent, experience has shown that concern about the potential emergence of chlorhexidine resistance is appropriate. Additional studies that investigate the frequency of chlorhexidine use and selection of chlorhexidine-resistant strains must be conducted to ensure proper chlorhexidine stewardship.	In summary, to our knowledge, this is the first report of selection for increased chlorhexidine MICs while using chlorhexidine in a community-based patient with recurrent USA300 MRSA SSTIs. In light of recent clinical trials that show the benefit of chlorhexidine in the prevention of drug-resistant infections, the medical community should anticipate greater use of this agent and consequently increased resistance. Further study and surveillance for the emergence of chlorhexidine resistance should be considered in health care and community settings that use chlorhexidine for disease prevention.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations
Vali, L., Dashti, A.A., El-Shazly, S. and Jadaon, M.M., 2015.	Survey / case report	A survey of qnr-positive Klebsiella spp. was undertaken from January 2010 to December 2012. Three major hospitals that serve the six governorates of Kuwait, namely Al-Ahmadi hospital, Al-Amiri hospital and Adan hospital, were taking part in this study. All the three hospitals are tertiary health care providers with bed capacities of 300, 500 and 600, respectively. While the survey was ongoing, in 2012, K. oxytoca Y20 was isolated from the foot ulcer (right foot) of a 48 year old type II diabetic female patient. The patient was admitted to hospital on 12th February 2012 due to complications related to diabetes and the wound sample was sent to the microbiology laboratory on 22nd February 2014. The sample was processed by using conventional microbiological techniques.	Here we report for the first time the identification of a K. oxytoca isolate from a diabetic foot infection with reduced sensitivity to chlorhexidine. The pathogenic potential of K. oxytoca is not limited to causing intestinal infection and antibiotic-associated hemorrhagic colitis28 and its potential as an opportunistic pathogen in patients with diabetic foot ulcers should be further explored. In our study the severity of the diabetic foot infection increased with the presence of class 1 integrons producing ESBL enzymes and low sensitivity to chlorhexidine. The key finding in this study was the presence of the qacE gene in K. oxytoca located in the 30-CS of class 1 integrons. qacE gene belongs to the SMR family29 conferring efflux-mediated resistance to QACs. Several members of the SMR family have been shown to export a range of toxins, including ethidium bromide and QACs, through coupling with proton influx.30	In conclusion, this is the first report of K. oxytoca with reduced sensitivity to chlorhexidine that contains qacE gene in a diabetic ulcer. To avoid continuous low level exposure of K. oxytoca to biocides which may result in emerging strains with reduced sensitivity to these agents, dilution standards in hospitals specifically in developing countries and the hospital's adherence to infection control policies should be strictly monitored. Administering preventive measures by using the correct dose of biocides is essential.

Appendix 4 Summary Table: Is the use of Chlorhexidine contributing to increased resistance to Chlorhexidine and/or antibiotics?

Reference Authors	Type of study	Population /Study information / isolates Intervention- Chlorhexidine Use/Type and exposure	Results / Outcomes	Clinical importance/ conclusion/recommendations

Appendix 5 Excluded studies

	Reference	Reason for exclusion
1.	Abi-Rached, G. P. C., et al. (2014). "Efficacy of Ethylene-Diamine-Tetra-Acetic Acid Associated With Chlorhexidine on Intracanal Medication Removal: A Scanning Electron Microscopy Study." Microscopy Research and Technique 77(9): 735-739.	Does not address CHX resistance
2.	Andersson, D. I. and D. Hughes (2014). "Microbiological effects of sublethal levels of antibiotics." Nature Reviews Microbiology 12(7): 465-478	Not specific about CHX and resistance - does not address review questions
3.	Aykan, Ş. B., et al. (2013). "Investigation of the presence of disinfectant resistance genes qocA/B in nosocomial methicillin-resistant Staphylococcus aureus isolates and evaluation of their in vitro disinfectant susceptibilities." Mikrobiyoloji Bulteni 47(1): 1-10	Not specific about CHX and does not address review questions
4.	Azzimonti, B., et al. (2015). "Essential Oil from Berries of Lebanese Juniperus excelsa M. Bieb Displays Similar Antibacterial Activity to Chlorhexidine but Higher Cytocompatibility with Human Oral Primary Cells." Molecules 20(5): 9344-9357	Does not address review questions
5.	Bass, P., et al., 2013. “Impact of chlorhexidine-impregnated washcloths on reducing incidence of vancomycin-resistant enterococci colonization in hematology–oncology patients” . Am J Inf Control 41(4), pp.345-348.	About VRE colonisation rates not CHX
6.	Berkner, S., et al. (2014). "Antibiotic resistance and the environment - There and back again: Science & Society series on Science and Drugs." EMBO Reports 15(7): 740-744	Not specific about CHX and antibiotics resistance. Does not address review questions
7.	Bhatia, M., et al. (2016). "Reduced susceptibility of carbapenem-resistant Klebsiella pneumoniae to biocides: An emerging threat." Indian Journal of Medical Microbiology 34(3): 355-358	Not specific about CHX. Does not address review questions

	Reference	Reason for exclusion
8.	Bhardwaj., 2016. Chlorhexidine induces VanA-type vancomycin resistance genes in enterococci. *Antimicrobial agents and chemotherapy*, 60(4), pp.2209-2221	Not specifically about CHX :The goal of this study was to investigate the transcriptional responses of E. faecium 1,231,410, a vancomycin-resistant clinical isolate, to MIC levels of a CHG-containing consumer product
9.	Bi, D., et al. (2015). "Mapping the resistance-associated mobilome of a carbapenem-resistant Klebsiella pneumoniae strain reveals insights into factors shaping these regions and facilitates generation of a 'resistance-disarmed' model organism." Journal of Antimicrobial Chemotherapy 70(10): 2770-2774	Does not address review questions
10.	Bolla, J. M., et al. (2011). "Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria." FEBS Letters 585(11): 1682-1690	Does not address review questions
11.	Buffet-Bataillon, S., et al. (2012). "Molecular mechanisms of higher MICs of antibiotics and quaternary ammonium compounds for Escherichia coli isolated from bacteraemia." Journal of Antimicrobial Chemotherapy 67(12): 2837-2842	Does not address review questions
12.	Cabrera, C. E., et al. (2007). "Resistance to bacterial antibiotics, antiseptics and disinfectants a manifestation of the survival and adaptation mechanisms." Colombia Medica 38(2): 149-158	Does not address review questions
13.	Cavalcanti, A. L., et al. (2012). "In vitro susceptibility of streptococcus oralis to different mouthwashes." Acta Stomatologica Croatica 46(4): 291-296	CHX Effectiveness not resistance
14.	Cerf, O., et al. (2010). "Tests for determining in-use concentrations of antibiotics and disinfectants are based on entirely different concepts: "Resistance" has different meanings." International Journal of Food Microbiology 136(3): 247-254	Not about CHX and antibiotics resistance. Does not address review questions
15.	Chiang, W.C. ., et al. (2012). "The metabolically active subpopulation in Pseudomonas aeruginosa biofilms survives exposure to membrane-targeting antimicrobials via distinct molecular mechanism." FEMS Immunol Med Microbiol 65(3): 245-256	Not CHX specific. Does not address review questions

	Reference	Reason for exclusion
16.	Chung, Y. K., et al. (2015). “Effect of daily chlorhexidine bathing on acquisition of carbapenem-resistant Acinetobacter baumannii (CRAB) in the medical intensive care unit with CRAB endemicity” . American journal of infection control, 43(11), pp.1171-1177.	An effectiveness study not relevant
17.	Cimolai, N. (2010). "Methicillin-resistant Staphylococcus aureus in Canada: A historical perspective and lessons learned." Canadian Journal of Microbiology 56(2): 89-120	Does not address the review questions
18.	Cole, M. R., et al. (2013). "Minimizing human infection from Escherichia coli O157:H7 using GUMBOS." Journal of Antimicrobial Chemotherapy 68(6): 1312-1318	Escherichia coli was in food-producing animals – argued as a viable strategy to minimize human disease initiated by exposure to these microorganisms.
19.	Conceição, T., et al. (2016). "High prevalence of biocide resistance determinants in Staphylococcus aureus isolates from three African countries." Antimicrobial Agents and Chemotherapy 60(1): 678-681	Environment
20.	Correa, J. E., et al. (2008). "First report of qacG, qacH and qacJ genes in Staphylococcus haemolyticus human clinical isolates." Journal of Antimicrobial Chemotherapy 62(5): 956-960	Not specific to CHX
21.	Costa, S. S., et al. (2013). "Description of plasmid pSM52, harbouring the gene for the Smr efflux pump, and its involvement in resistance to biocides in a methicillin-resistant Staphylococcus aureus strain." International Journal of Antimicrobial Agents 41(5): 490-492	Not specific to CHX – difficult to determine population / setting
22.	Coulon, C., et al. (2010). "Resistance of Acanthamoeba Cysts to Disinfection Treatments Used in Health Care Settings." Journal of Clinical Microbiology 48(8): 2689-2697	Environment
23.	de Lucena, J., et al. (2013). "Antimicrobial effectiveness of intracanal medicaments on Enterococcus faecalis: chlorhexidine versus octenidine." International Endodontic Journal 46(1): 53-61.	Effectiveness – does not address review questions

	Reference	Reason for exclusion
24.	De Silva, M., et al. (2015). "Evidence that a novel quaternary compound and its organic N-chloramine derivative do not select for resistant mutants of Pseudomonas aeruginosa." Journal of Hospital Infection 91(1): 53-58.	Not specific to CHX – difficult to determine population / setting
25.	de Souza, I. O. P., et al. (2016). "Bifunctional fluorescent benzimidazo[1,2-α]quinolines for Candida spp. biofilm detection and biocidal activity." Journal of Photochemistry and Photobiology B: Biology 163: 319-326.	Not specific to CHX
26.	Decker, E. M., et al. (2008). "Effect of xylitol/chlorhexidine versus xylitol or chlorhexidine as single rinses on initial biofilm formation of cariogenic streptococci." Quintessence International 39(1): 17-22.	Effectiveness – does not address review questions
27.	Delgado, R. J. R., et al. (2010). "Antimicrobial Effects of Calcium Hydroxide and Chlorhexidine on Enterococcus faecalis." Journal of Endodontics 36(8): 1389-1393.	Effectiveness – does not address review questions
28.	Desbois, A. P., et al. (2010). "Surface disinfection properties of the combination of an antimicrobial peptide, ranalexin, with an endopeptidase, lysostaphin, against methicillin-resistant Staphylococcus aureus (MRSA)." Journal of Applied Microbiology 108(2): 723-730.	Not specific to CHX – difficult to determine population / setting
29.	Dobson, A., et al. (2011). "Impact of the broad-spectrum antimicrobial peptide, lacticin 3147, on Streptococcus mutans growing in a biofilm and in human saliva." Journal of Applied Microbiology 111(6): 1515-1523.	Not specific to CHX – difficult to determine population / setting
30.	Faraj, J. A., et al. (2007). "Development of a peptide-containing chewing gum as a sustained release antiplaque antimicrobial delivery system." Aaps Pharmscitech 8(1): 9.	Not specific to CHX – difficult to determine population / setting
31.	Fernández-Cuenca, F., et al (2015). “Reduced susceptibility to biocides in Acinetobacter baumannii: association with resistance to antimicrobials, epidemiological behaviour, biological cost and effect on the expression of genes encoding porins and efflux pumps”. Journal of Antimicrobial Chemotherapy, 70(12), pp.3222-3229.	Not specifically about CHX

	Reference	Reason for exclusion
32.	Ferran, A. A., et al. (2016). "Comparison of the in vitro activity of five antimicrobial drugs against staphylococcus pseudintermedius and staphylococcus aureus biofilms." Frontiers in Microbiology 7 (AUG) (no pagination)(1187).	Not specifically about CHX
33.	Forbes, S., et al. (2013). "Comparative surface antimicrobial properties of synthetic biocides and novel human apolipoprotein E derived antimicrobial peptides." Biomaterials 34(22): 5453-5464.	Not relevant
34.	Forman, M. E., et al. (2016). "Structure-Resistance Relationships: Interrogating Antiseptic Resistance in Bacteria with Multicationic Quaternary Ammonium Dyes." ChemMedChem 11(9): 958-962.	Not specific to CHX and antibiotic resistance
35.	Frater, M., et al. (2013). "IN VITRO EFFICACY OF DIFFERENT IRRIGATING SOLUTIONS AGAINST POLYMICROBIAL HUMAN ROOT CANAL BACTERIAL BIOFILMS." Acta Microbiologica et Immunologica Hungarica 60(2): 187-199	Effectiveness and not specific to CHX
36.	Frese, F., et al. (2011). "Biological activity of Bacillus extracts against Legionella." International Journal of Medical Microbiology 301: 27.	Not relevant
37.	Furi, L., et al. (2013). "Evaluation of reduced susceptibility to quaternary ammonium compounds and bisbiguanides in clinical isolates and laboratory-generated mutants of staphylococcus aureus." Antimicrobial Agents and Chemotherapy 57(8): 3488-3497.	Community and hospital – not specific to CHX
38.	Furiga, A., et al. (2008). "In vitro anti-bacterial and anti-adherence effects of natural polyphenolic compounds on oral bacteria." Journal of Applied Microbiology 105(5): 1470-1476.	Not related to CHX

	Reference	Reason for exclusion
39.	Futoma-Koloch, B., et al. (2015). "Selection and electrophoretic characterization of Salmonella enterica subsp enterica biocide variants resistant to antibiotics." Polish Journal of Veterinary Sciences 18(4): 725-732.	Not specific to CHX and antibiotic resistance
40.	Gant, V. A., et al. (2007). "Three novel highly charged copper-based biocides: Safety and efficacy against healthcare-associated organisms." Journal of Antimicrobial Chemotherapy 60(2): 294-299.	Not specific to CHX
41.	Goldenberg, R. L., et al. (2006). "Use of vaginally administered chlorhexidine during Labor to improve pregnancy outcomes." Obstetrics and Gynecology 107(5): 1139-1146	Effectiveness not resistance
42.	Gullberg, E., et al. (2014). "Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals." mBio 5(5): e01918-01914	Not specific to CHX and antibiotic resistance
43.	Guo, W., et al. (2014). "Resistant mechanism study of benzalkonium chloride selected salmonella typhimurium mutants." Microbial Drug Resistance 20(1): 11-16	Not specific to CHX
44.	Guo, W., et al. (2015). "Determining the resistance of carbapenem resistant Klebsiella pneumoniae to common disinfectants and elucidating the underlying resistance mechanisms." Pathogens and Global Health 109(4): 184-192	Not specific to CHX
45.	Hall, T. J., et al. (2009). "A comparison of the antibacterial efficacy and cytotoxicity to cultured human skin cells of 7 commercial hand rubs and Xgel, a new copper-based biocidal hand rub." American Journal of Infection Control 37(4): 322-326	Not specific to CHX and resistance
46.	Hassan, K. A., et al. (2013). "Transcriptomic and biochemical analyses identify a family of chlorhexidine efflux proteins." Proceedings of the National Academy of Sciences of the United States of America 110(50): 20254-20259.	Not specific to CHX and resistance

	Reference	Reason for exclusion
47.	Hassan, K. A., et al. (2007). "Active export proteins mediating drug resistance in staphylococci." Journal of Molecular Microbiology and Biotechnology 12(3-4): 180-196	Not specific to CHX and resistance
48.	Hegstad, K., et al. (2010). "Does the wide use of quaternary ammonium compounds enhance the selection and spread of antimicrobial resistance and thus threaten our health?." Microb Drug Resist 16(2): 91-104	Not specific to CHX and resistance
49.	Heruzzo, I., (2015) "Is There A Correlation Between Antibiotic Resistance and Decreased Susceptibility to Biocides in Different Genus of Bacterial General"? J Antibiotic Res 1(1) 1-7	General article, nothing new or specific
50.	Hill, K. E., et al. (2010). "An in vitro model of chronic wound biofilms to test wound dressings and assess antimicrobial susceptibilities." Journal of Antimicrobial Chemotherapy 65(6): 1195-1206	Not specific to CHX and resistance
51.	Hurley, M. N., et al. (2013) Antibiotic adjuvant therapy for pulmonary infection in cystic fibrosis. Cochrane Database of Systematic Reviews DOI: 10.1002/14651858.CD008037.pub3	Not specific to CHX and antibiotic resistance
52.	Ivanov, I. B., et al. (2015). "The Effect of Brief Exposure to Sub-Therapeutic Concentrations of Chlorhexidine Digluconate on the Susceptibility of Staphylococci to Platelet Microbicidal Protein." Surgical Infections 16(3): 263-266.	Not specific to CHX and resistance
53.	Jayampath Seneviratne, C., et al. (2010). "Proteomics of drug resistance in candida glabrata biofilms www.proteomics-journal.com." Proteomics 10(7): 1444-1454.	Not specific to CHX and antibiotic resistance

	Reference	Reason for exclusion
54.	Kanaguchi, N., et al. (2012). "Effects of salivary protein flow and indigenous microorganisms on initial colonization of Candida albicans in an in vivo model." Bmc Oral Health 12: 8	Not specifically about CHX
55.	Karpinski, T. M. and A. K. Szkaradkiewicz (2015). "Chlorhexidine - pharmaco-biological activity and application." European Review for Medical and Pharmacological Sciences 19(7): 1321-1326.	Not specific to CHX and resistance
56.	Kawai, M., et al. (2009). "Cell-wall thickness: Possible mechanism of acriflavine resistance in meticillin-resistant Staphylococcus aureus." Journal of Medical Microbiology 58(3): 331-336.	Not specifically about CHX
57.	Kim, J. H., et al. (2016). "Biological Evaluation of Anodized Biodegradable Magnesium-Calcium Alloys." Acta Physica Polonica A 129(4): 728-735	Report seems to contradict itself.
58.	Lee, S. S., et al. (2015). "The effect of daily chlorhexidine bathing on the acquisition of methicillin-resistant Staphylococcus aureus in the medical intensive care unit." International Journal of Antimicrobial Agents 45: S94	Effectiveness not CHX resistance
59.	Leggett, M. J., et al. (2012). "Bacterial spore structures and their protective role in biocide resistance." Journal of Applied Microbiology 113(3): 485-498	Not specific to CHX and resistance
60.	Lepri, S., et al. (2016). "Indole Based Weapons to Fight Antibiotic Resistance: A Structure-Activity Relationship Study." Journal of Medicinal Chemistry 59(3): 867-891	Not specific to CHX and antibiotic resistance
61.	Liguori, G., et al. (2009). "Microbiological evaluation of the efficacy of two new biodetergents on multidrug-resistant nosocomial pathogens." Annals of Clinical Microbiology and Antimicrobials	Not specific to CHX and antibiotic resistance
	Reference	**Reason for exclusion**
62.	Lourenço, T.G.B., etal (2015). “Long-term evaluation of the antimicrobial susceptibility and	About composition and treatment not

	microbial profile of subgingival biofilms in individuals with aggressive periodontitis". Brazilian Journal of Microbiology, 46(2), pp.493-500.	specifically answering the question
63.	Luna, V. A., et al. (2010). "Susceptibility of 169 USA300 methicillin-resistant Staphylococcus aureus isolates to two copper-based biocides, CuAL42 and CuWB50." Journal of Antimicrobial Chemotherapy 65(5): 939-941	Not specific to CHX and antibiotic resistance
64.	Lynch, A. S. (2006). "Efflux systems in bacterial pathogens: an opportunity for therapeutic intervention? An industry view." Biochemical Pharmacology 71(7): 949-956	Not specific to CHX and resistance
65.	Machado, F. C., et al. (2011). "Use of Chlorhexidine Gel (0.2%) to Control Gingivitis and Candida Species Colonization in Human Immunodeficiency Virus-infected Children: A Pilot Study." Pediatric Dentistry 33(2): 153-157	Effectiveness and not specific to CHX resistance
66.	Madrid, I. M., et al. (2012). "Inhibitory effect of sodium hypochlorite and chlorhexidine digluconate in clinical isolates of Sporothrix schenckii." Mycoses 55(3): 281-285	Not specific to CHX and resistance
67.	Maillard, J. Y. (2007). "Bacterial resistance to biocides in the healthcare environment: should it be of genuine concern?" Journal of Hospital Infection 65(SUPPL. 2): 60-72	Too general
68.	Mavri, A. and S. S. Možina (2013). "Effects of efflux-pump inducers and genetic variation of the multidrug transporter cmeB in biocide resistance of Campylobacter jejuni and Campylobacter coli." Journal of Medical Microbiology 62(PART3): 400-411	Not specific to CHX and resistance

69.	Mavri, A. and S. Smole Možina (2013). "Development of antimicrobial resistance in Campylobacter jejuni and Campylobacter coli adapted to biocides." International Journal of Food Microbiology 160(3): 304-312	Not specific to CHX and resistance
70.	Mc Cay, P. H., et al. (2010). "Effect of subinhibitory concentrations of benzalkonium chloride on the competitiveness of Pseudomonas aeruginosa grown in continuous culture." Microbiology 156(1): 30-38	Not specific to CHX and resistance
71.	McNeil, et a;., 2014. "Decreased susceptibilities to Retapamulin, Mupirocin, and Chlorhexidine among Staphylococcus aureus isolates causing skin and soft tissue infections in otherwise healthy children" . Antimicrobial agents and chemotherapy, 58(5), pp.2878-2883	Mostly about Retapamulin, nothing other than on pg 2881 Not specifically relating to study questions
72.	McGann, P., et al. (2011). "Detection of qacA/B in clinical isolates of methicillin-resistant staphylococcus aureus from a regional healthcare network in the eastern United States." Infection Control and Hospital Epidemiology 32(11): 1116-1119	Not specific to CHX and antibiotic resistance
73.	Micek, S.T. (2010) "Current Concepts in the Prevention and Treatment of Ventilator-Associated Pneumonia". Jrn Ph Prac 23(1):25-32	It doesn't answer virtually any of the questions in the extraction tables
74.	Mima, E. G. D. O., et al. (2011). "Effectiveness of chlorhexidine on the disinfection of complete dentures colonised with fluconazole-resistant Candida albicans: In vitro study." Mycoses 54(5): e506-e512	Effectiveness - Not specific to CHX and resistance
75.	Moore, L. E., et al. (2008). "In vitro study of the effect of cationic biocides on bacterial population dynamics and susceptibility." Applied and Environmental Microbiology 74(15): 4825-4834	Not specific to CHX and resistance
76.	Mueller, G. and A. Kramer (2008). "Biocompatibility index of antiseptic agents by parallel assessment of antimicrobial activity and cellular cytotoxicity." Journal of Antimicrobial	Not specific to CHX and resistance

	Chemotherapy 61(6): 1281-1287	
	Reference	**Reason for exclusion**
77.	Munoz-Gallego, I., et al. (2016). "Chlorhexidine and mupirocin susceptibilities in methicillin-resistant Staphylococcus aureus isolates from bacteraemia and nasal colonisation." Journal of Global Antimicrobial Resistance 4: 65-69	Not able to understand CHX product
78.	Mutters, N. T., et al. (2015). "Is your antiseptic effective against clinical multidrug-resistant microorganisms? A chlorhexidine digluconate formulation demonstrates efficacy even in lower concentrations." Antimicrobial Resistance and Infection Control. Conference: 3rd International Conference on Prevention and Infection Control, ICPIC 4	Efficacy - Not specific to CHX and resistance
79.	Noszticzius, Z., et al. (2013). "Chlorine Dioxide Is a Size-Selective Antimicrobial Agent." PLoS ONE [Electronic Resource] 8(11): 10	Not specific to CHX and resistance
80.	O'Meara, S., et al. (2010). "Antibiotics and antiseptics for venous leg ulcers." Cochrane Database of Systematic Reviews(1): 99	Not specific to CHX and antibiotic resistance
81.	O'Meara, S., et al. (2013). "Antibiotics and antiseptics for venous leg ulcers." Cochrane Database of Systematic Reviews(12): 194	Not specific to CHX and antibiotic resistance
82.	O'Meara, S., et al. (2014). "Antibiotics and antiseptics for venous leg ulcers." Cochrane Database of Systematic Reviews(1): 194	Not specific to CHX and antibiotic resistance
83.	Oule, M. K., et al. (2012). "Akwaton, polyhexamethylene-guanidine hydrochloride-based sporicidal disinfectant: a novel tool to fight bacterial spores and nosocomial infections." Journal of Medical Microbiology 61(10): 1421-1427	Not specific to CHX and resistance

	Reference	Reason for exclusion
84.	Oztan, M. D., et al. (2006). "Antimicrobial effect, in vitro, of gutta-percha points containing root canal medications against yeasts and Enterococcus faecalis." Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics 102(3): 410-416	Not specific to CHX and resistance
85.	Pastrana-Carrasco, J., et al. (2012). "qacEΔ1 gene frequency and biocide resistance in extended-spectrum β-lactamase producing Enterobacteriaceae clinical isolates." Revista de Investigacion Clinica 64(6 PART 1): 535-540	Not specific to CHX
86.	Piddock, L. J. (2014). "Understanding the basis of antibiotic resistance: a platform for drug discovery." Microbiology 160(Pt 11): 2366-2373	Not specific to CHX and antibiotic resistance
87.	Pienaar, E. D., et al. (2010). "Interventions for the prevention and management of oropharyngeal candidiasis associated with HIV infection in adults and children." Cochrane Database of Systematic Reviews(11): 108	Not specific to CHX and resistance
88.	Polin, R. A., et al. (2012). "Strategies for prevention of health care-associated infections in the NICU." Pediatrics 129(4): e1085-e1093	Not specific to CHX and resistance
89.	Provenzano, J. C., et al. (2013). "Metaproteome Analysis of Endodontic Infections in Association with Different Clinical Conditions." PLoS ONE [Electronic Resource] 8(10): 9	Not specific to CHX and resistance
90.	Rondeau, C., et al. (2016). "Current molecular epidemiology of methicillin-resistant staphylococcus aureus in elderly French people: Troublesome clones on the horizon." Frontiers in Microbiology 7(JAN)	Not specific to CHX and resistance

	Reference	Reason for exclusion
91.	Sabatini, S., et al. (2013). "Re-evolution of the 2-phenylquinolines: Ligand-based design, synthesis, and biological evaluation of a potent new class of staphylococcus aureus NorA efflux pump inhibitors to combat antimicrobial resistance." Journal of Medicinal Chemistry 56(12): 4975-4989	Not specific to CHX and resistance
92.	Santos Costa, S., et al. (2015). "Impact of efflux in the development of multidrug resistance phenotypes in Staphylococcus aureus." BMC Microbiology 15: 232	Not specific to CHX and antibiotic resistance
93.	Sardana, K., et al. (2014). "The role of zinc in acne and prevention of resistance: Have we missed the "base" effect?" International Journal of Dermatology 53(1): 125-127	Not specific to CHX and resistance
94.	Sauerbrei, A., et al. (2007). "Hexon denaturation of human adenoviruses by different groups of biocides." Journal of Hospital Infection 65(3): 264-270	Not specific to CHX and resistance
95.	Sauerbrei, A. and P. Wutzler (2010). "Virucidal efficacy of povidone-iodine-containing disinfectants." Letters in Applied Microbiology 51(2): 158-163	Not specific to CHX and resistance
96.	Schlett, C. D., et al. (2014). "Prevalence of chlorhexidine-resistant methicillin-resistant Staphylococcus aureus following prolonged exposure." Antimicrobial Agents and Chemotherapy 58(8): 4404-4410	Community-based
97.	Schlusselhuber, M., et al. (2015). "Potent antimicrobial peptides against Legionella pneumophila and its environmental host, Acanthamoeba castellanii." Applied Microbiology and Biotechnology 99(11): 4879-4891	Not relevant
98.	Seaman, P. F., et al. (2007). "Small-colony variants: A novel mechanism for triclosan resistance in methicillin-resistant Staphylococcus aureus." Journal of Antimicrobial	Not specific to CHX and resistance

	Chemotherapy 59(1): 43-50	
	Reference	**Reason for exclusion**
99.	Shokraneh, A., et al. (2014). "Antibacterial effect of triantibiotic mixture versus calcium hydroxide in combination with active agents agents Enterococcus faecalis biofilm." Dental Materials Journal 33(6): 733-738	Not specific to CHX and resistance
100	Shtannikov, A. V., et al. (2007). "Evaluation of in vitro antibacterial activity of fosmidomycin and its derivatives." Antibiotiki i Khimioterapiya 52(7-8): 3-9	Not specific to CHX and resistance
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110	Uzunoglu, E., et al. (2016). "Final Irrigation Regimens Affect Fracture Resistance Values of Root-filled Teeth." Journal of Endodontics 42(3): 493-495	Not specific to CHX and resistance
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114	Vijaya Kumar, R., et al. (2016). "In vitro susceptibility of multidrug resistant pseudomonas aeruginosa clinical isolates to common biocides." International Journal of Research in Pharmaceutical Sciences 7(1): 110-116	Not specific to CHX and antibiotic resistance
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