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The potential for developing new antimicrobial resistance from the use of medical devices containing chlorhexidine, minocycline, rifampicin and their combinations: a systematic review

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Background: Catheter infections remain one of the most persistent adverse events causing significant morbidity, economic impact and mortality. Several strategies have been proposed to reduce these infections including the use of catheters embedded with antibiotics and/or antiseptics. One reoccurring challenge is the fear that antimicrobial medical devices will induce resistance. The aim of this systematic review is to evaluate the evidence for induced antimicrobial resistance caused by exposure to antimicrobial medical devices.

Methods: Four electronic databases [MEDLINE, Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL) and Scopus] were screened for studies published between 1983 and 2019 regarding assessment of microbial resistance with use of medical devices containing chlorhexidine, minocycline, rifampicin or combinations thereof. Development of new resistance, selection for tolerant organisms and 'no change in resistance' were assessed.

Results: Forty-four publications, grouped by study type and stratified by drug assessed, were included for analyses. The majority of studies found no change in resistance after exposure to antimicrobial medical devices (13 *in vitro*, 2 *in vivo*, 20 clinical). Development of new resistance was commonly reported with the use of rifampicin as a single agent and only reported in one study assessing the minocycline/rifampicin combination (M/R); however, the increase in MIC was well below clinical relevance.

Conclusions: Emergence of new resistance to combinations of M/R, minocycline/rifampicin/chlorhexidine (M/R/CH) and chlorhexidine/silver sulfadiazine (CHXSS) was rare. No clinical trials confirmed its occurrence and some refuted it. The risk of development of new resistance to these antimicrobial combinations appears more fear-based than substantiated by clinical and experimental evidence but warrants continued surveillance.

Introduction

Central line-associated bloodstream infections (CLABSIs) remain one of the most persistent post-insertion adverse events causing significant morbidity as well as substantial economic impact and mortality.[1] Central lines coated with antimicrobial agents were introduced to reduce the risk of CLABSIs.[2] While using antimicrobial catheters can potentially reduce the risk of CLABSI, their use introduces other risks to patients.[3,4] These include irritation and inflammatory responses to the antimicrobial agents, allergic reactions to the antimicrobial agents, breakthrough infections by virulent organisms against which the antimicrobial agents have limited effectiveness and the induction of antimicrobial resistance through prolonged exposure to the antimicrobial agents on the catheters. Allergic reactions by patients to the agents in the antimicrobial central lines (most commonly chlorhexidine) have been rare and doses of antimicrobial agents on catheters have been titrated to levels generally producing acceptably biocompatible responses following contact with the antimicrobial agents on the devices.[5] Antimicrobial resistance is the result of organisms defensively adapting to exposure to subinhibitory or sublethal doses of antimicrobial agents and developing defensive mechanisms whereby the microbes are able to thwart and render ineffective the mechanisms of action of the antimicrobial agents.[6] The consequence of antimicrobial resistance is that microbes may become more virulent and, when they cause infections, there may be fewer

and potentially more toxic antimicrobial drugs that are available to treat the infections.

Two antimicrobial catheter treatments that are combinations of different agents have been widely studied and have been recommended by the CDC at the level of category 1A. One is a triple combination of two antiseptic agents and one antibiotic, specifically chlorhexidine/silver sulfadiazine (CHXSS), and the other is a combination of two antibiotics, specifically minocycline and rifampicin (M/R).[7] Studies on the first-generation CHXSS catheter, performed at a time before rigorous hygienic insertion practices were widely adopted, demonstrated significant reduction in CLABSIs. Subsequent large prospective randomized clinical trials (RCTs) conducted following the adoption of modern insertion practices with a second-generation CHXSS catheter, having significantly higher chlorhexidine content, have repeatedly failed to significantly reduce CLABSIs.[8–10] A peripherally inserted central catheter (PICC) containing chlorhexidine as a sole antimicrobial agent similarly failed to reduce CLABSIs in a randomized prospective trial when compared with a non-antimicrobial PICC.[11] Several meta-analyses have reinforced the ineffectiveness of CHXSS catheters in preventing CLABSI.[12] Nevertheless, chlorhexidine-based catheters remain widely used. In contrast, the M/R catheter has significantly reduced CLABSIs in multiple randomized prospective clinical trials and several meta-analyses have further reinforced the effectiveness of the M/R combination in reducing CLABSIs.[12] The enhanced antimicrobial activity from combining chlorhexidine with M/R on catheters has been reported and the combination proposed for future use.[13] We therefore focus in this systematic review on the evidence for induced antimicrobial resistance caused by exposure to catheters and other medical devices containing chlorhexidine, minocycline, rifampicin and combinations thereof because of the prevalence of their use and extensive history of published *in vitro*, *in vivo* and clinical studies.

Methods

Search strategy

Four electronic databases [MEDLINE, Embase, Cumulative Index to Nursing and Allied Health Literature (CINAHL) and Scopus] were searched for studies assessing resistance to rifampicin, minocycline or chlorhexidine that were published between 1983 and February 2019. Search terms were mapped to the MeSH headings (MEDLINE) and Emtree terms (Embase). The following search string was used: (rifampin OR rifampicin OR minocycline OR chlorhexidine) AND (central venous catheter OR catheters OR catheters, indwelling OR vascular access devices OR catheterization, central venous OR CVC OR catheterization, peripheral OR PICC OR bandages OR bandages, hydrocolloid OR biological dressings OR occlusive dressings OR prosthesis and implants OR dental implants OR penile prosthesis OR pacemaker, artificial OR pacemaker OR catheter dressing OR dressing OR penile implant OR disinfection OR wipes) AND (drug resistance OR drug resistance, microbial OR drug resistance, bacterial OR drug resistance, fungal OR resistance OR antibiotic resistance OR emerging antibiotic resistance).

Search results were first screened by title and abstract by two independent reviewers. Any disagreements were discussed by authors to an agreed-upon consensus. Resulting articles were read for full-text review and data abstraction. Relevant references cited in primary literature were also screened to be included in analyses. Manuscripts were excluded if they were in a language other than English, were conference abstracts, did not assess the drug combination of interest (minocycline, rifampicin, chlorhexidine or combination thereof), did not assess a medical device (i.e. systemic use of drug agents), did not directly assess development of resistance or were a descriptive case series. Literature reviews were also excluded from the primary assessment and will be summarized independently.

Data abstraction

All primary literature to be abstracted were first classified into *in vitro*, *in vivo* or clinical study types. Manuscripts with more than one study type had data extracted from each type. All data abstracted from each manuscript were recorded electronically in the data abstraction form (DAF). Data from the DAF were then organized by spreadsheet for assessment. Quality control was assessed periodically to ensure accurate transfer of abstracted data. Data, including (i) study objective; (ii) device assessed; (iii) drug assessed; (iv) method for drug attachment to device; (v) method for assessing resistance; (vi) results; and (vii) conclusions were collected from each study type. Data from *in vitro* studies also included organisms assessed (challenge organisms) and their antimicrobial resistance profiles. Data abstraction pertaining to *in vivo* animal models included species and number of animals tested as well as organisms assessed (challenge organisms) and their antimicrobial resistance profiles. Clinical studies included data abstraction for study design (retrospective, prospective, case–control or RCT) and objective, number of patients assessed, causative organisms being treated with the antimicrobial device and their resistance profiles.

Definitions

Based on authors' conclusions regarding the potential for developing resistance after exposure to antimicrobial devices, each manuscript was categorized as follows:

1. No change in (antimicrobial) resistance
2. Selection for (antimicrobial agent-) tolerant strains (clinical studies only)
3. Development of new (antimicrobial) resistance
4. (Antimicrobial) resistance not assessed

'No change in antimicrobial resistance' was defined as no or inconsequential shift in MIC after use of antimicrobial devices. 'Selection for antimicrobial agent-tolerant strains' was defined as an increasing shift in MIC for the single agent or combination of agents; however, MIC remained below the threshold for clinical susceptibility (i.e. below CLSI cut-offs for resistance).[14] 'Development of new antimicrobial resistance' was defined as a clinically consequential shift in MIC to concentrations above the CLSI resistance concentration cut-off for clinical susceptibility.

Quality assessment

To assess quality and bias in each manuscript, any critique to study design, methodology or conclusions based on presented data was also recorded in the DAF. Clinical studies were assessed for bias using the National Heart, Lung, and Blood Institute Study Quality Assessment Tools (2018).[15] Clinical studies were stratified by study type (observational, case–control and controlled intervention trial) and then scored based on the questions examining various reporting measures. Observational studies were scored out of 12 points and had questions focused on study objective, population, exposures of interest and validated measurements of outcome. Case–control studies were scored out of 13 points and had questions focused on study objective, population, selection of cases and controls, exposures of interest and validated measurements of outcome. Clinical intervention trials were scored out of 14 points and had questions focused on study objective, randomization, blinding, interventions and adherence to intervention protocols, sample size and validated measurements of outcome.

For *in vitro* and *in vivo* studies, articles were assessed for bias using the Animal Research: Reporting *in vivo* experiments (ARRIVE) guidelines.[16] While the ARRIVE guidelines were originally developed for *in vivo* studies,

they have been adapted and assessed for quality criteria in *in vitro* studies.[17] *In vitro* studies were scored out of 19 and had questions pertaining to reporting of study objective and design, experimental procedure, use of appropriate controls, validated measurements of outcome and analyses. *In vivo* studies were scored out of 23 and had questions pertaining to reporting of study objective and design, experimental procedure, animal husbandry, exposures and controls, blinding, validated measurements of outcome and analyses.

Finally, all studies were graded with a score of A–D on methodology used for assessment of examining development of resistance. Articles were scored as follows:

1. Grade A if the article described AND referenced a validated model for assessing resistance such as CLSI or EUCAST methods for MIC or MBC.
2. Grade B if the article either described OR referenced a validated model for assessing resistance.
3. Grade C if the article assessed resistance with a method other than a microbiologically validated model (i.e. presence of resistance genes) or resistance was assessed only from the hospital record.
4. Grade D if the article did not describe or reference any method for resistance (i.e. only stated that resistance was assessed).

Results

Search strategy

Searches in MEDLINE, Embase, CINAHL and Scopus identified 526, 1040, 85 and 421 studies, respectively, for a total 2072 citations. Title and abstract screening identified 183 studies, 66 of which were duplicates, resulting in a total of 127 studies for full-text review. During full-text review, 19 studies were excluded because they did not assess one of the target drugs or drug combination, 13 studies were excluded because no antimicrobial device was assessed, 4 were excluded because they were conference abstracts/proceedings that did not contain complete data for analyses, 4 studies were excluded because they were descriptive case series and 23 were excluded because they didn't directly assess development of resistance. These studies typically reported whether organisms broke through with the use of an antimicrobial device, indicating that the antimicrobial device was not efficacious, not whether organisms developed resistance. Though CHXSS is a drug combination of chlorhexidine and silver sulfadiazine, which is not specifically our drug combination of interest (chlorhexidine, minocycline and rifampicin), it was included for assessing the potential for development of resistance against chlorhexidine combinations. Additionally, 26 manuscripts were identified as literature reviews and will be summarized independently. An additional six studies were identified by screening bibliographies of primary literature and were included in analyses. A total of 44 publications were included for qualitative assessment of the potential for developing new resistance after being exposed to antimicrobial medical devices (Figure 1).

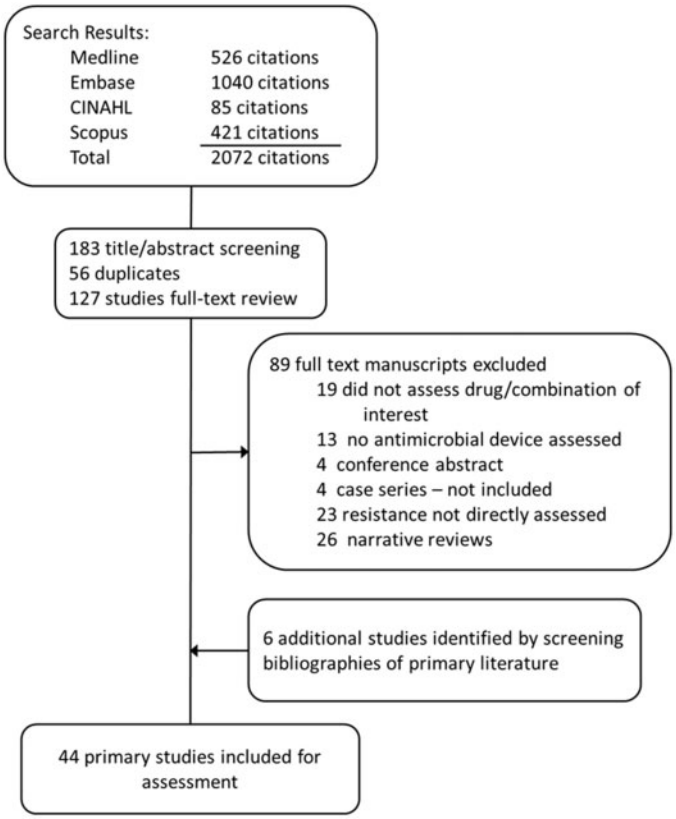

Figure 1. PRISMA flow chart for identification of primary literature included in review.

Study characteristics

Characteristics and results of all *in vitro* studies included are summarized in Table 1, *in vivo* studies in Table 2 and clinical studies in Table 3.

In vitro

A total of 18 *in vitro* studies were included in the analyses (Table 1), including 4 studies assessing rifampicin alone,[18–21] 9 studies assessing chlorhexidine (CH or CHXSS),[22–30] 2 studies assessing M/R[31,32] and 1 study assessing the triple combination of M/R/CH.[33] Two studies assessed multiple drugs: Sampath *et al*.[34] assessed the CHXSS and M/R combinations and Tambe *et al*.[35] assessed minocycline, rifampicin, M/R and CHXSS. Three different devices (wipes/scrub,[23–30] vascular grafts[18,19,21] and catheters[20,22,31–35]) were assessed. Figure 2a depicts the device and drug combinations.

Authors reported no change in resistance in eight studies assessing exposure to devices containing chlorhexidine,[22–27,29,30,34] two studies assessing M/R,[31,32] one study that assessed minocycline alone[35] and the one study that assessed M/R/CH.[33] Development of new resistance was reported in five studies, the majority of which assessed rifampicin alone.[18–21,35] Two studies concluded that resistance developed after exposure to M/R[34,35] and one with exposure to chlorhexidine.[28]

In vivo

Three *in vivo* studies were included in the analyses (Table 2), all of which assessed rifampicin alone[18,36,37] in vascular graft models. Two studies assessed rifampicin-soaked vascular grafts in a sheep model[36,37] and one study assessed colonized vascular grafts with aqueous rifampicin in a subcutaneous mouse model.[18] Figure 2a depicts the device and drug combinations studied.

Most *in vivo* studies with full-text review assessed efficacy of the antimicrobial device (breakthrough) and did not assess

Table 1. *In vitro* assessment of potential resistance to rifampicin, minocycline and/or chlorohexidine

Citation	Drug	Device	Concentration(s)	Organisms assessed	Methodology; resistance measurement	Results	After contact with the device, which of the following does the study show: (i) no change in resistance; or (ii) development of new resistance
Rifampicin ($n = 4$)							
Bayston *et al.*, 2009[20]	R	peritoneal catheter	0.2%	SE	Growth within ZOI during serial plate transfer; MIC Etest	MIC of SE increased from 0.008 to >32 mg/L after 9 days exposure to 0.2% R	development of new resistance
Berard *et al.*, 2019[21]	R	vascular graft	5000 mg/L	SE, MRSA, EC, CA	EUCAST; agar dilution, confirmed by sequencing *rpoB* gene	MIC of SE and SA increased to >512 mg/L after 7 days exposure to R; mutations of *rpoB* gene confirmed by sequencing	development of new resistance
Bergamini *et al.*, 1996[19]	R	vascular graft	4×, 64×, 100×, 1000× MIC	SE colonized on vascular graft	Biofilm eradication; R MIC by disc diffusion	R decreased biofilm concentration at 4 h but was ineffective at 18 and 42 h due to MIC increasing from 0.1 to >30 mg/L R	development of new resistance
Garrison *et al.*, 1997[18]	R	uncoated vascular graft	aqueous R at 4×, 1000× MIC	SE	Biofilm eradication; MIC broth dilution	MIC of all recovered SE increased from baseline (0.1 mg/L) to >30 mg/L	development of new resistance
Chlorhexidine ($n = 9$)							
Aarestrup *et al.*, 2004[23]	CH	environmental (used for disinfection in food animals)	—	*Salmonella* (156), EC (202), SA (43), *Staphylococcus hyicus* (38), *Enterococcus faecalis* (52), *Enterococcus faecium* (78)	MIC agar dilution; development of resistance, defined as bimodal population	*E. faecium* showed high frequency of isolates at differing concentrations (0.5–1 versus 4–8 mg/L); all isolates formed one large population	no change in resistance
Apisarnthanarak *et al.*, 2014[30]	CH	CH wipes	—	AN (100): pre-CH (50), post-CH (50)	MIC microbroth dilution	Increase in CH MIC after initiation CH baths; however, did not achieve threshold for new CH resistance	no change in resistance
Ekizoglu *et al.*, 2016[24]	CH	—	MIC 0.125–512 mg/L; eradication 0.02%–4%	PS (22); AN (19); SM (13); KB (14); EB (15); MSSA (6); MRSA (15); EN (17)	MIC agar dilution, planktonic eradication; not conducted	Some organisms tested showed resistant MIC; however, CH at 4% was effective against all strains within 5 min of contact	no change in resistance (per Discussion section)
Johnson *et al.*, 2013[25]	CH	—	—	MRSA: *qacA/B*+ (5), *smr* + (5), no plasmid (5)	MBC	All isolates tested had MBC <16 mg/L regardless of presence of resistance genes	no change in resistance

Martro *et al.*, 2003[26]	CH	Hibiscrub	4%	AN (9) from prior to, during and after outbreak	Planktonic eradication	4% CH (Hibiscrub) eradicated all AN strains tested	no change in resistance
Modak *et al.*, 1992[22]	CH, CHXSS	planktonic, associated with CHXSS CVC	—	SA, EC	MIC after 25 serial subinhibitory passages	2-Fold increase in MIC for SA and EC tested against CH, SS and CHXSS	no change in resistance
Skovgaard *et al.*, 2013[27]	CH	CH hand scrub	—	SE from hospital, community or historic (prior to hand scrub)	MIC/MBC by disc diffusion	No difference in CH susceptibility in hospital versus community versus historic; no selection for *qacA/B* genes in hospital versus community versus historic (no genes present)	no change in resistance
Suwantarat *et al.*, 2014[28]	CH	CH cloths for skin antisepsis	2%	EN (30), SA (20), CoNS (29), other G+ (2), AN (3), CB (1), EB (3), EC (5), KB (12), PS (11), other G− (10)	MIC/MBC by microbroth dilution	Patients with daily CH bathing were more likely to have an organism with reduced CH susceptibility (86% versus 64% $P = 0.028$)	development of new resistance
Wesgate *et al.*, 2016[29]	CH	—	CH (0.00005%)	SA, EC	MIC measures of short and long exposure	No change in the CH susceptibility profile after short and long exposures to the CH	no change in resistance
M/R ($n = 2$)							
Munson *et al.*, 2004[32]	M/R	Spectrum CVC (Cook Critical Care)	—	SA, SE, EN, EC, PS	Organisms cultured from border of ZOI from M/R catheter; MIC disc diffusion	Colonies sampled from growth around ZOI failed to demonstrate emergence of resistance to M or R by disc diffusion	no change in resistance
Norton *et al.*, 2001[31]	M/R	umbilical catheters	11 mg M; 10.5 mg R	MRSA, MSSA, CoNS, CA, EN, AN, SM, PS, VRE	ZOI, biofilm colonization; disc diffusion	ZOI showed efficacy to all organisms tested except PS and CA; CoNS and SM in the study remained sensitive to M and R	no change in resistance
M/R/CH ($n = 1$)							
Rosenblatt *et al.*, 2019[33]	M/R/CH	CVC	—	SA, SE, VRE, KB, EC, EB, PS, AN, CA, CP	20 serial passages through subinhibitory concentration; MIC microbroth dilution	All except one organism remained within 2-fold change in MIC after 20 passages; one strain of EB showed a 4-fold increase in MIC after 20 passages but after passages in broth (no M/R/CH) the MIC returned within the 2-fold limit	no change in resistance
Multiple drug combinations ($n = 2$)							

Continued

Table 1. *Continued*

Citation	Drug	Device	Concentration(s)	Organisms assessed	Methodology; resistance measurement	Results	After contact with the device, which of the following does the study show: (i) no change in resistance; or (ii) development of new resistance
Sampath *et al.*, 2001[34]	CHXSS, M/R	CVC, CHXSS (Arrowguard Plus), M/R (Spectrum)	—	SE, EC	20 serial passages through subinhibitory concentration; MIC tube dilution	CHXSS: 2-fold increase for SE, unchanged for EC; M/R: 8-fold increase for SE, 4-fold increase for EC	CHXSS: no change in resistance; M/R: development of new resistance
Tambe *et al.*, 2001[35]	M, R, M/R, CHXSS	antimicrobials associated with CVC	—	SE	20 serial passages through subinhibitory concentration; MIC tube dilution	M: NS increase in MIC; R: 25 000-fold increase in MIC; M/R: 10-fold increase for ATCC strain, 16-fold increase for clinical strain; CHXSS: NS increase in MIC	M, CHXSS: no change in resistance; R: development of new resistance; M/R: less development of resistance

M, minocycline; R, rifampicin; CH, chlorhexidine; SS, silver sulfadiazine; ZOI, zone of inhibition; NS, not significant; MRSE, methicillin-resistant SE; EN, *Enterococcus* species; AN, *Acinetobacter* species; KB, *Klebsiella pneumoniae*; PS, *Pseudomonas aeruginosa*; SM, *Stenotrophomonas maltophilia*; EB, *Enterobacter* species; CB, *Citrobacter* species; CA, *Candida albicans*; CP, *Candida parapsilosis* G+, Gram-positive organisms; G−, Gram-negative organisms; —, data not presented in the manuscript being abstracted.

development of resistance after exposure to the device and thus were not included in the final analysis. Only three studies assessed resistance after exposure to a rifampicin vascular graft, two of which reported no change in resistance.[36,37] Garrison *et al.*[18] reported development of new resistance after implanting biofilm formed on an uncoated vascular graft into a subcutaneous pocket then exposing it to high-dose rifampicin infused into the pocket. MICs increased from 0.1 to >30 mg/L.[18] This is consistent with other reports that *de novo* rifampicin resistance is more common with higher concentrations of organisms than those typically cultured from infected devices.

Clinical

In total, 24 clinical studies were included in the analyses (Table 3): 2 case–control studies,[38,39] 6 retrospective studies,[40–45] 8 prospective studies[46–53] and 8 RCTs.[54–61] Among the clinical studies, 15 assessed chlorhexidine in three different devices, 9 assessed wipes/scrub,[39,41,42,48–51,58,62] 4 assessed CHXSS central venous catheters (CVCs)[38,53,60,61] and 2 assessed chlorhexidine in dressings or sponges.[46,59] Nine studies assessed M/R-impregnated CVCs.[43–45,52,54–57,60] Darouiche *et al.*[60] assessed both CHXSS and M/R CVCs.[60] Only one study assessed rifampicin alone in vascular grafts.[40] Figure 2b depicts all device and drug combinations studied.

The majority of studies found no change in resistance after exposure to either chlorhexidine (13 studies)[39,42,46–51,53,58–61] or M/R CVCs (7 studies).[43–45,54,55,57,63] Three studies reported selection for more tolerant strains in chlorhexidine wipes,[41] CHXSS CVC[38] and rifampicin alone.[40] Wright *et al.*[52] reported development of new resistance of *Staphylococcus epidermidis* (SE) to rifampicin after exposure to M/R catheters, with an increase in rifampicin resistance from 3/12 SE isolates recovered from catheters prior to implementation of M/R catheters to 8/8 SE isolates recovered from catheters after implementation of M/R catheters. No other changes in susceptibility patterns were reported.

Quality assessment

Quality assessments of each included manuscript are presented in Table 4. Out of the *in vitro* articles assessed, scores ranged from 13 to 18 (out of 19), indicating that the majority of studies have a low risk of bias. Ten out of 18 studies (55%) were graded 'A' for methodology, 7 of 18 (38.9%) were graded 'B' and only 1 study (5.5%) was graded 'C' for methodology. For the three *in vivo* studies assessed, scores ranged from 18 to 21 (out of 23). Two of the three studies (66.7%) were graded 'A' for methodology while one was graded 'C'. In the assessment of clinical studies, observational studies scores ranged from 6 to 11 (out of 12), case–control studies scores from 11 to 13 (out of 13) and controlled intervention trials scores from 9 to 14 (out of 14). Six of the 24 clinical studies (25%) were graded 'A' for assessment of resistance methodology, 12 of 24 (50%) were graded 'B', 3 of 24 (12.5%) were graded 'C' and 3 of 24 (12.5%) were graded 'D'.

Reviews

Of the literature reviews assessed, the majority discussed literature pertaining to other primary endpoints such as breakthrough after antimicrobial exposure, efficacy of antibiotics/biocides in infection

Table 2. *In vivo* assessment of potential resistance to rifampicin, minocycline and/or chlorohexidine

Citation	Drug	Device: how is drug attached; concentration	Animal model species (*n*); implant site; duration	Organism(s) assessed	Methodology; resistance measurement	Results	After contact with the device, which of the following does the study show: (i) no change in resistance; or (ii) development of new resistance
Rifampicin ($n = 3$)							
Avramovic *et al.*, 1991[36]	R	vascular graft; soaked in 100 mg aqueous R	sheep (20); carotid artery; 3 weeks	SA	Breakthrough ZOI; R antibiotic disc	All SA isolated from R-treated grafts remained sensitive	no change in resistance
Garrison *et al.*, 1997[18]	R	uncoated vascular graft; aqueous 4× and 1000× MIC R infused in subcutaneous pocket	mouse (42); subcutaneous pocket; 4, 18 and 42 h	SE	Biofilm eradication; MIC broth dilution	MIC of recovered SE from high-dose R increased from BL (0.1 to >30 mg/L)	development of new resistance
Sardelic *et al.*, 1995[37]	R	vascular graft; soaked in 1.2 mg/mL aqueous R	sheep (9); carotid artery; 3 weeks	MRSA	Breakthrough MIC; agar dilution	Breakthrough MRSA had same R MIC as starting inoculum	no change in resistance

ZOI, zone of inhibition; BL, baseline.

prevention or mechanisms of potential resistance. Four reviews had varied conclusions on potential development of resistance with repeated or sublethal use of chlorhexidine.[64–67] In two of the reviews, authors had no definitive conclusions, stating some studies assessed had found potential for chlorhexidine resistance while others did not and needed additional study.[64,67] One of the reviews concluded that exposure to sublethal chlorhexidine increased the risk of resistance in Gram-negative organisms[65] while another review reported no evidence of resistance with repeated chlorhexidine exposure.[66] Three other reviews assessed the potential for antimicrobial resistance with the use of M/R and overwhelmingly concluded that the use of M/R devices is unlikely to cause resistance.[68–70]

Discussion

The focus of this review was evidence in the literature for the development of antimicrobial resistance as a consequence of the use of antimicrobial devices containing minocycline, rifampicin, chlorhexidine or combinations thereof. No trends in findings were seen based on which device was studied. There were trends based on the agents or combinations of agents in the devices so the discussion is structured accordingly. First, however, our analysis framework is described.

Development of new antimicrobial resistance versus development of antimicrobial tolerance versus selection of tolerant strains

For this review, development of new antimicrobial resistance to minocycline, rifampicin and chlorhexidine is taken as a complete loss of inhibitory or bactericidal effect at therapeutically achievable concentrations due to adaptive changes by organisms following exposure to minocycline, rifampicin and/or chlorhexidine. Organisms that were not susceptible to minocycline, rifampicin and chlorhexidine prior to *de novo* exposure could be classified as having pre-existing resistance; however, they would not have presented as having developed new antimicrobial resistance as a result of exposure to these agents (but rather as possessing an innate pre-existing absence of susceptibility). In contrast to newly developed antimicrobial resistance, we include development of antimicrobial tolerance as a milder form of reduced antimicrobial susceptibility following exposure to minocycline, rifampicin and/or chlorhexidine. Development of antimicrobial tolerance can be a result of organisms responding to the presence of antimicrobial agents by expressing similar adaptive genes that phenotypically alter the concentrations of antimicrobial agents required to be effective. In some cases there is a limit to the concentration of interfering molecules an organism can express and in others less efficient alternative pathway responses result in impairment but incomplete inactivation of the effects of the antimicrobial agents. Developed antimicrobial tolerance responses typically are seen as shifts in MICs of antimicrobial agents to higher MICs in order to be inhibitory or cidal but would still be within potentially therapeutically attainable concentrations for antimicrobial agents. In the extreme, if organisms lose susceptibility to minocycline, rifampicin and/or chlorhexidine at therapeutically achievable concentrations they would have crossed the threshold to have developed new antimicrobial resistance. Selection of tolerant strains from

Table 3. Clinical assessment of potential resistance to rifampicin, minocycline and/or chlorohexidine

Citation	Study type (*n* patients)	Device; drug	Design	Measure of resistance	Results	After contact with the device, which of the following does the study show: (i) no change in resistance; (ii) selection for tolerant strains; or (iii) development of new resistance
Rifampicin ($n = 1$)						
Bandyk *et al.*, 2001[40]	retrospective (27)	vascular graft soaked in aqueous R (45–60 mg/mL)	Patient with graft infections had graft replaced with R-soaked graft	not stated	Failure from MRSA infection and recurrent R-resistant SE infection in 2 patients; 18 patients remained infection free and survived	selection for tolerant strains; discussion: 'need for continued evaluation to determine whether R grafts select for resistant G+'
Chlorhexidine ($n = 14$)						
Batra *et al.*, 2010[41]	retrospective (4570: 2480 pre, 2090 post)	CH bathing; Hibitane, 1% CH dusting powder, Hibiscrub	Assessed for MRSA infection pre- and post-antiseptic protocol	MBC (CLSI)	Decrease in MRSA infections by endemic strain but increase in MRSA infection by outbreak strain after initiation of CH baths; all outbreak strains had *qacA/B* genes and 3-fold higher MBCs than endemic strain	selection for tolerant strains
Choudhury *et al.*, 2017[46]	prospective (77: 43, CH dressing, 34 no CH dressing)	CH dressings at catheter insertion sites	Prevalence of *smr* or *qacA/B* from DNA from skin with CH dressing versus skin with no (or non-CH) dressing	assessment of *smr* or *qacA/B* genes by PCR	No significant difference in frequency of *qacA/B* and *smr* recovered from CH dressing versus no CH dressing; no evidence that CH increases frequency of CH-tolerance genes at catheter sites	no change in resistance
Chung *et al.*, 2015[48]	prospective (3054: 1514 pre, 1540 post)	CH wipes	Assessment of AN infections pre- and post-CH bathing	MIC microbroth dilution	Prevalence of AN decreased from 25.8% to 18.2%; no difference in CH MIC between 42 AN in pre-CH bathing and 56 AN in post-CH bathing	no change in resistance
Ho *et al.*, 2012[38]	case–control (156: 96 MRSA; 60 MSSA)	CHXSS CVC (Arrowguard blue)	Assessment of MIC and *qacA/B* or *smr* genes from MRSA and MSSA catheter-related infection	MIC agar dilution; prevalence of genes by PCR	Significantly more MRSA isolates containing *qacA/B* genes caused CHXSS-impregnated CRBSI; MIC stratified for CHXSS catheter not assessed	selection for more tolerant strains
Lee *et al.*, 2011[39]	case–control (150: 75 case; 75 control)	CH wipes for decolonization	Assessed risk factors for persistence of MRSA carriage after decolonization	presence of resistance genes by PCR	Genotypic CH resistance alone did not predict persistent MRSA carriage	no change in resistance

Lowe *et al.*, 2017[47]	prospective (4037: 2039 controls, 1998 CH)	CH wipes	MRSA and VRE infections in 7 month intervention of CH bathing versus non-medicated soap/water bathing	MIC by agar dilution; presence of resistance genes by PCR	In intervention group, one MRSA and one MSSA contained resistance genes though MIC was susceptible; one MRSA had resistant MIC (8 mg/L) but was not PCR positive	no change in resistance
Mendoza-Olazaran *et al.*, 2014[49]	prospective (149: 80 prior, 69 CH bath)	CH wipes	AN infections in 6 months prior and 6 month intervention of CH bathing	MIC agar dilution	AN isolates in the CH bathing period showed a significant decrease in CH MIC likely due to change in clonality of infecting isolate	no change in resistance
Maki *et al.*, 1997[61]	RCT (403: 195 controls, 208 CHXSS)	CHXSS CVC	Susceptibility to CHXSS CVC in organisms cultured from CHXSS versus control catheters	ZOI	None of isolates from infected catheters showed resistance to fresh CHXSS catheters by ZOI	no change in resistance
McNeil *et al.*, 2016[42]	retrospective (247)	CH wipes	Assessed CH MIC, *smr* and *qacA/B* over time; increased CH bathing over time	MIC microbroth dilution; prevalence of genes by PCR	Increased prevalence of *smr* and *qacA/B* in SA infections over time; however, non-significant difference of CH MIC for isolates positive and negative for genes	no change in resistance
Schlett *et al.*, 2014[58]	cluster randomized trial (30 209: 10 030 CH bathing)	CH wipes, Hibiclens	MIC, *qacA/B* from MRSA SSTI among standard bathing versus CH bathing	MIC microbroth dilution; prevalence of genes by PCR	No difference in MIC or prevalence *qacA/B* in CH bathing versus control	no change in resistance
Schuerer *et al.*, 2007[53]	prospective (4630: 2079 pre, 2551 post)	CHXSS CVC	Assessment of CRBSI in control versus CHXSS	not conducted	No significant difference in rate of CRBSI or microbiological profile organisms cultured in control versus CHXSS	no change in resistance
Soma *et al.*, 2012[50]	prospective (29 swabs: 4 no CH, 15 moderate CH, 10 heavy CH)	CH wipes	MIC, *qacA/B* among CoNS sampled from patients with no, moderate or heavy CH baths	MIC microbroth dilution; prevalence of genes by PCR	No significant difference in CH MIC in no versus moderate versus heavy CH samples. 11/17 CoNS had *qacA/B*	no change in resistance
Timsit *et al.*, 2009[59]	RCT (1636: 819 controls, 817 CH sponge)	CH-impregnated sponges	Assessment of CRI, and MBC in CH sponge versus control	MBC	Reduction in CRI with use of CH sponge; no difference in MBC for control versus CH sponge	no change in resistance
Velazquez-Meza *et al.*, 2017[51]	prospective (156: 61 pre, 52 during, 45 post)	CH wipes (2%)	MIC of SA isolates from pre-, during and post-CH bathing	MIC agar dilution	No isolates showed reduced susceptibility to CH in pre- versus during versus post-intervention	no change in resistance

Continued

Table 3. *Continued*

Citation	Study type (*n* patients)	Device; drug	Design	Measure of resistance	Results	After contact with the device, which of the following does the study show: (i) no change in resistance; (ii) selection for tolerant strains; or (iii) development of new resistance
M/R ($n = 8$)						
Chatzinikolaou *et al.*, 2003[54]	RCT (130: 66 M/R, 64 controls)	M/R CVC, haemodialysis	CRBSI; MIC of CoNS cultured from colonized catheters	MIC microbroth dilution	MIC of CoNS isolates from colonized catheters were similar in M/R versus control	no change in resistance
Chatzinikolaou *et al.*, 2003[45]	retrospective (672: 212 M/R, 460 controls)	M/R CVC	CRBSI in BMT (M/R) versus leukaemia (no M/R)	MIC (CLSI)	All 67 SA and SE isolates were susceptible to M; one isolate (M/R) and 9 isolates (control) were resistant to R	no change in resistance
Gilbert *et al.*, 2016[55]	RCT (1485: 502 controls, 486 M/R, 497 heparin)	M/R CVC	CRBSI in control versus M/R versus hep CVC	varied by centre (MIC)	8/12 patients with MIC tested on BSI cultures were resistant to M, R or both: 3/5 control; 2/2 M/R; 3/5 heparin	no change in resistance
Hanna *et al.*, 2004[56]	RCT (356: 182 M/R, 174 controls)	M/R CVC	MIC for organisms cultured from catheter and/or skin at insertion site	MIC microbroth dilution	Mean M and R MIC of SE from M/R catheter was lower than control catheters; no difference in MIC from skin cultures before insertion versus removal	no change in resistance
Raad *et al.*, 1997[57]	RCT (298: 151 controls, 147 M/R)	M/R CVC	Colonization or CRBSI control versus M/R; ZOI with new M/R CVC from all organisms from colonized CVC; MIC (M, R) for SA, SE from M/R CVC versus insertion site	ZOI of new M/R CVC; MIC microbroth dilution	Significant reduction in colonization and CRBSI with M/R CVC; M/R CVC had activity by ZOI for all organisms isolated from patient CVC; SA or SE from M/R CVC and insertion site remained at $\leq$2 mg/L	no change in resistance
Ramos *et al.*, 2011[43]	retrospective (4732 isolates: 2451 pre, 2281 post)	M/R CVC	Susceptibility of SA, SE to tetracycline and R prior to M/R (1999) and 7 years after use of M/R CVC (2006)	MIC (CLSI)	Decrease in percentage of SA and SE resistant to tetracycline or R after 7 years of M/R CVC use; all decreases were significant except R resistance in SA	no change in resistance
Turnbull *et al.*, 2018[44]	retrospective (9703 isolates: 2818 pre, 6885 post)	M/R CVC	Susceptibilities of SA to tetracycline and R in ICU prior to and after use of M/R	not stated (reported at hospital)	Rate of R and tetracycline resistance unchanged prior to and after use of M/R CVC	no change in resistance

Wright *et al.*, 2001[52]	prospective (40: 23 pre, 17 post)	M/R CVC	Susceptibilities of M and R in organisms cultured from CVC	MIC microbroth dilution	Pre: 3/12 SE isolates resistant to R; post: 8/8 SE isolates resistant to R; no other changes in susceptibility patterns	development of new resistance
Comparison of multiple drug combinations ($n = 1$)						
Darouiche *et al.*, 1999[60]	RCT (738: 356 M/R, 382 CHXSS)	M/R CVC, CHXSS CVC	Susceptibilities of M and R in organisms cultured from CVC or skin prior to insertion	MIC microbroth dilution	No differences in M or R MIC ranges for SE and EN cultured from M/R CVC, CHXSS CVC or skin	no change in resistance

M, minocycline; R, rifampicin; CH, chlorhexidine; SS, silver sulfadiazine; MRSE, methicillin-resistant SE; EN, *Enterococcus* species; AN, *Acinetobacter* species; PS, *Pseudomonas aeruginosa*; SM, *Stenotrophomonas maltophilia*; EB, *Enterobacter* species; CB, *Citrobacter* species; CA, *Candida albicans*; G+, Gram-positive organisms; G-, Gram-negative organisms; CRBSI, catheter-related bloodstream infection; CRI, catheter-related infection; SSTI, skin and soft tissue infection; BMT, bone marrow transplant patients.

Figure 2. *In vitro*, *in vivo* and clinical studies included in assessment by device and drug. Devices and drug/drug combinations for *in vitro* and *in vivo* studies (a) and clinical studies (b).

exposure to a device containing minocycline, rifampicin or chlorhexidine results when, as a consequence of prior exposure, organisms have different innate susceptibilities to particular antimicrobial agents or combinations. This is not an adaptive response but rather a separation of low-susceptibility strains from high-susceptibility ones based on their ability to tolerate the presence of threshold concentrations of minocycline, rifampicin, chlorhexidine or combinations thereof. Based on finite doses of antimicrobial agents on devices, tolerant organisms (higher MICs) will be able to survive and colonize antimicrobial devices before highly susceptible organisms (lower MICs) can. Differences in tolerance can explain why some organisms are able to break through and colonize antimicrobial devices before others are able to, as well as why organisms can preferentially colonize devices with one combination of antimicrobial agents versus a different combination. The selection of tolerant strains following exposure to an antimicrobial device is not indicative of development of antimicrobial tolerance nor indicative of development of new antimicrobial resistance unless, prior to exposure to the antimicrobial agents on the device, the organisms were susceptible to those agents.

Single antimicrobial agent device studies

Rifampicin alone

Four *in vitro* studies[19,20,34,35] reported that devices containing rifampicin alone exhibited newly developed resistance following exposure. The culprit organism was SE in these studies and

Table 4. Quality assessment

In vitro studies			*In vivo* studies			Clinical studies		
citation	quality score	method score	citation	quality score	method score	citation	quality score	method score
Modak, 1992	14/19	A	Avramovic, 1991	19/23	C	Maki, 1997	11/14	C
Bergamini, 1996	16/19	B	Sardelic, 1995	18/23	A	Raad, 1997	14/14	B
Garrison, 1997	14/19	A	Garrison, 1997	21/23	A	Darouiche, 1999	11/14	B
Sampath, 2001	14/19	B				Bandyk, 2001	10/12	D
Norton, 2001	13/19	C				Wright, 2001	8/12	B
Tambe, 2001	15/19	B				Chatzinikolaou, 2003	11/12	A
Martro, 2003	16/19	B				Chatzinikolaou, 2003	13/14	B
Aarestrup, 2004	18/19	A				Hanna, 2004	12/14	B
Munson, 2004	16/19	A				Schuerer, 2007	9/12	D
Bayston, 2009	17/19	A				Timsit, 2009	9/14	B
Skovgaard, 2013	16/19	A				Batra, 2010	8/12	A
Johnson, 2013	14/19	B				Lee, 2011	11/13	B
Apisarntharak, 2014	11/19	B				Ramos, 2011	10/12	A
Suwantarat, 2014	18/19	A				Ho, 2012	12/13	B
Wesgate, 2016	15/19	A				Soma, 2012	8/12	A
Ekizoglu, 2016	16/19	B				Schlett, 2014	10/14	A
Berard, 2019	16/19	A				Mendoza-Olazaran, 2014	10/12	A
Rosenblatt, 2019	13/19	A				Chung, 2015	8/12	B
						McNeil, 2016	8/12	B
						Gilbert, 2016	12/14	C
						Lowe,2017	9/12	B
						Turnbull, 2018	6/12	D
						Velazquez-Meza, 2017	8/12	B
						Choudhury, 2017	8/12	C

resistance was assessed through increases in MICs following exposure. Several *in vivo* studies assessed rifampicin devices; however, most did not assess development of new resistance. Two studies on soaked vascular grafts[36,37] reported no changes in resistance for *Staphylococcus aureus* (SA) or MRSA but one study[18] reported development of new resistance for SE. One human study[40] evaluating vascular grafts soaked in rifampicin reported recurrent breakthrough infections in two patients with two rifampicin-resistant SE and with MRSA. These results were consistent with selection for rifampicin-resistant strains but it is not possible to determine whether exposure to the rifampicin grafts induced newly developed resistance or whether the rifampicin resistance was pre-existing.

Resistance to rifampicin has been reported by occurrence of single point mutations in the β-subchain of bacterial RNA polymerase.[71] These point mutations so impair rifampicin binding and subsequent inactivation of bacterial RNA polymerase that the MIC increases to greater than the limit of testing (i.e. resistant). Point mutations have the greatest likelihood of spontaneously arising and being selected for when a bacterial population is in the presence of rifampicin alone since they involve changes to only a single amino acid. Consequently, development of new resistance has been repeatedly observed and use of rifampicin alone appears unwise. If other antimicrobial agents are present, they can continue to inhibit or kill bacteria even with favourable RNA polymerase mutations, so rifampicin-resistant bacterial populations might not be able to survive or propagate in the presence of rifampicin in combination with other antimicrobial agents.

Minocycline alone

One *in vitro* study[35] reported no development of new resistance following repeated exposure to devices containing minocycline alone. No *in vivo* or human studies were conducted on devices containing minocycline alone. Minocycline resistance has been reported to occur through expression of efflux pumps and less commonly through expression of ribosomal protection proteins.[72,73] In contrast to rifampicin, these types of resistance require acquisition of new genes and expression of new proteins, which are less probable than the occurrence of point mutations. Similarly, bacteria that do acquire minocycline efflux pump or ribosomal protection protein genes when in the presence of minocycline with other antimicrobial agents might not be able to survive and propagate in the presence of the combination because of the antimicrobial activity of the other agent.

Chlorhexidine alone

Eight *in vitro* studies[22–25,27,29,30] assessing devices with chlorhexidine alone reported no change in resistance following exposure. One *in vitro* study[28] reported development of new resistance. The

experimental design assessed MICs of colonizing organisms following exposure to chlorhexidine wipes. The results of a significant increase in prevalence of reduced chlorhexidine susceptibility following chlorhexidine exposure are consistent with selection for more tolerant strains. Insufficient information was provided to determine whether the exposure induced new chlorhexidine resistance or whether the reduced susceptibility was pre-existing prior to the exposure. No *in vivo* studies with devices containing chlorhexidine alone assessed development of new resistance. Two human studies[38,41] reported selection for chlorhexidine-tolerant strains following chlorhexidine exposure and nine studies[39,42,46,48–51,58] reported no changes in resistance. Chlorhexidine resistance has been reported to be due to the presence of efflux pumps,[74] requiring acquisition of new genes and expression of new proteins. Development of new chlorhexidine resistance appears to be rare but continued surveillance is warranted.

Antimicrobial combination device studies

M/R device studies

Two *in vitro* studies[34,35] on combination M/R devices reported development of new resistance to SE and *Escherichia coli* (EC). The increases in MIC for the combination were modest (4–16-fold) and were much lower than increases in rifampicin MICs (25 000-fold) following exposure to devices containing rifampicin alone. In practical terms, in one of the studies[35] the M/R MIC for one strain of SE was reported to increase from 0.02 to 0.25 mg/L following exhaustive sequential passaging at subinhibitory concentrations and for another strain it increased from 0.015 to 0.25 mg/L. These ultimate MICs remain below the CLSI thresholds for susceptibility for minocycline (less than or equal to 4 mg/L) and rifampicin (less than or equal to 1 mg/L);[14] thus, while drift of MICs against the combination plausibly occurred, its clinical relevance was more theoretical (as a tolerance shift) than practical because the organisms remained within the therapeutic susceptible range and thus the MIC drifts reported for the SE strains were not clinically relevant. Similarly, the other study[34] reported an MIC increase for SE from 0.02 to 0.31 mg/L and for EC from 0.25 to 1 mg/L. Again, the drift in MIC went from very susceptible to susceptible, reflecting increased antimicrobial tolerance; however, the clinical relevance was more theoretical than practical as the organisms remained susceptible and thus the tolerance shift was not clinically relevant, in that the organisms remained susceptible to the M/R combination. In contrast, two other *in vitro* studies[31,32] reported no development of new resistance following exposure to devices containing the combination of M/R. More substantively, eight human studies[43–45,54,55,57,60,63] reported no development of new M/R resistance following exposure to M/R devices. This includes two independent studies[43,44] over multi-year periods that reported no changes in rifampicin or tetracycline resistance for SA isolates when compared over prolonged periods prior to and following implementation of the routine use of M/R devices. In contrast, one small human study[52] reported the development of new SE resistance following implementation of the use of M/R catheters. In the period prior to use of the M/R devices, 3/12 infectious SE isolates were resistant to rifampicin, while following use of the M/R devices 8/8 SE isolates were rifampicin resistant. This study did not report development of new minocycline resistance or new resistance to the M/R combination and did not report results for MIC testing other than for rifampicin. In addition, since rifampicin-resistant isolates were present prior to use of the M/R device it is quite possible that the results reflect selection for more tolerant SE isolates rather than development of new resistance. The authors acknowledged that the clinical significance of their results was not clear.

It appears that development of new rifampicin resistance following exposure to devices containing the M/R combination might be theoretically possible for SE but was more a tolerance increase that was not clinically relevant because the culprit organisms remained susceptible to M/R combinations. Additionally, the development of new resistance against the M/R combination for SA was refuted by two independent studies. Since minocycline and rifampicin act with different mechanisms of action at entirely different places in microbial cells, the combination does appear to decrease the likelihood of development of newly resistant organisms, particularly over devices containing rifampicin alone, because two completely different resistance mechanisms would need to simultaneously emerge in the target cells.[75,76]

CHXSS device studies

Although silver sulfadiazine is not a component of the proposed M/R/CH catheter, studies on development of new resistance to CHXSS are included in this review to assess the potential for resistance to emerge against chlorhexidine combinations. No change in resistance was reported in three *in vitro* studies.[22,34,35] No *in vivo* studies assessed development of new resistance following exposure to CHXSS. Three human studies reported no change in resistance.[53,60,61] One human study[38] reported selection of more tolerant MRSA strains following exposure to CHXSS; however, this finding was entirely based on genetic analysis (i.e. more isolates contained *qacA/B* efflux pump genes) and more clinically significant measurement of increases in MICs were not performed. It appears there is a theoretical potential for chlorhexidine resistance to develop but it has been unlikely and has not been clinically widespread or confirmed, particularly when chlorhexidine antimicrobial combinations have been present.

M/R/CH device studies

One *in vitro* study assessed the potential for development of resistance with repeated exposure to subinhibitory concentrations of M/R/CH.[33] Organisms with high susceptibility to individual agents as well as low susceptibility to individual agents showed no development of resistance over 20 passages. Only one carbapenem-resistant *Enterobacter* showed a 4-fold increase in MIC during the 20 passages. While there was an increase in MIC, the increase was still well below clinical relevancy. Additionally, after the stressor (M/R/CH) was removed and the organism was passaged in broth alone, the MIC returned to baseline, indicating a phenotypic adaptation rather than development of new resistance. Further, for Gram-positive pathogens common to catheter infections, M/R/CH was highly effective. In the first few passages, subinhibitory concentrations were established for a few SA and SE; however, within two passages, the organisms died and passages could not continue. Based on this study and previous drug combination studies, with the addition of the third component

(chlorhexidine) to M/R there is no evidence of the potential for new resistance developing after exposure.

Conclusions

A systematic literature review was undertaken to assess evidence for the development of new antimicrobial resistance from exposure to medical devices containing rifampicin, minocycline, chlorhexidine or their combinations. Strengths of this study include: a search strategy that was conducted in multiple databases; use of systematic criteria to select studies and abstract data from these studies, thus reducing potential for selection bias; and use of standardized metrics for assessing the quality of studies. Limitations include: while multiple databases were searched, we did not include conference abstracts or search grey literature for additional studies; manuscripts included were limited to English language; and variability in the quality of studies. While the majority of our studies were scored as 'high quality' there were a few studies that did not present detailed methods or criteria and were scored as 'low quality' and should not be compared with equal weight.

Resistance was most likely to emerge in devices containing rifampicin as a single antimicrobial agent because only a single point mutation in RNA polymerase was required for rifampicin resistance to emerge. Development of rifampicin resistance was seen in multiple studies. In contrast, development of new resistance to minocycline or chlorhexidine single-agent devices was much less likely because acquisition of genes and expression of new proteins was required for new resistance to develop. Clinically meaningful development of new resistance against minocycline or chlorhexidine in devices was not confirmed in human studies although slight drifts in MICs were observed.

Emergence of new resistance to double combinations of M/R or CHXSS, although theoretically possible, was rarer and no clinical trials confirmed its occurrence and some refuted it. Studies did demonstrate that selection of more tolerant isolates capable of colonizing devices occurred when testing was performed on these combinations, but the lower innate susceptibilities of the tolerant strains likely existed prior to the exposure. The risk of development of new resistance to these antimicrobial combinations appears more fear-based rather than substantiated by clinical and experimental evidence but warrants continued surveillance.

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Transparency declarations

Drs I. I. Raad and J. Rosenblatt are co-inventors of the minocycline, rifampicin, chlorhexidine combination technology which is owned by the University of Texas MD Anderson Cancer Center and has been licensed by Cook Medical LLC. All other authors: none to declare.

Supplementary data

The Reviewer report is available as Supplementary data at *JAC-AMR* Online.

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