



RESEARCH ARTICLE

Legionella contamination of a cold-water supplying system in a German university hospital – assessment of the superheat and flush method for disinfection

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Keywords

Legionella • Hospital-acquired Legionnaires' disease • Superheat and flush • Water-supplying system

Summary

Introduction. In case of a contamination of water-supplying systems in hospitals with legionella, usually chemical disinfection measures are used for remediation. Unfortunately, it is reported, that these methods may not be sustainable, have an impact on water quality, and can even fail. As an alternative, the superheat and flush method does not need any special equipment, can be initiated in a short lead of time and does not affect the water quality. However, evidence on this disinfection measurement against legionella is lacking. We therefore investigated and report on the effectiveness and long-term results of the superheat and flush disinfection method.

Methods. During routine periodical examinations, a rising count of legionella was detected in the cold-water supplying system at a German university hospital. Adapted to an analysis of risks, effort

and benefit, the superheat and flush procedure was applied twice within 6 months.

Results. While 33 out of 70 samples had a higher legionella count than the legal threshold of 100 CFU/100 mL (CFU - Colony Forming Units) before the first disinfection was carried out, this number could be reduced to 1 out of 202 samples after the first intervention. Additionally, in contrast to previously published studies, the effect was long-lasting, as no relevant limit exceedance occurred during the following observation period of more than two years.

Conclusion. The superheat and flush disinfection can provide an economic and highly effective measure in case of legionella contamination and should be shortlisted for an eradication attempt of affected water-supplying systems in hospitals.

Introduction

The contamination of water-supplying systems in hospitals with *Legionella spp.* can be a major challenge due to outbreaks of nosocomial legionnaires' disease and economic issues, as well. Over the last decades, authors have regularly reported on this topic, especially for facilities with old building substance [1-4]. In the majority of cases, different chemical disinfection measures, especially hyperchlorination, are used to control the presence of legionella [2-5]. These measures often do not have a sustainable effect and need to be applied regularly, or even continuously [6], which may have also an impact on the water quality [4]. Other options for controlling a legionella infestation include *inter alia* the installation of sterile water filters, irradiation with ultraviolet (UV) light, and copper/silver-ionization. Sterile water filters need to be mounted at each point-of-use water fixture, have to be changed regularly, and are therefore very complex in installation and maintenance [7]. UV irradiation can

either be applied at the main water supply, or near/at the water tapping points [8]. Again, special equipment is required for the application of this method. It has been evaluated in only few clinical studies and the results were varying, in some cases not long-lasting, and depending on the structural conditions of the building [9-11]. Thus, the current evidence describes UV irradiation as one possibility among many, with no clear debate for or against [12]. Silver and/or copper ionization can provide an effective measure to reduce legionella contamination and the risk of legionnaires' disease [12]. However, strict limits must be met, and the use of copper as a biocide in Europe is strictly regulated by the European Commission [13]. Despite all possibilities of disinfection measures, in some cases it is still not possible to achieve a sufficient reduction in the load of legionella [3, 14, 15]. As a last resort, only an entire reconstruction of the water-supplying system can eliminate the problem [16]. Beneath the effects of negative publicity due to reports in the local and/or national press [17, 18], these measures can essentially harm the economic base of a hospital [19-21].

In addition to the measures described above, other methods are also the subject of current discussions [6]. A physical disinfection approach is the superheat and flush method, which is based on the principle that water-carrying pipes are flushed for a short time with hot water at a temperature of approximately 70°C [22]. There is only few information in the current literature regarding the effectiveness and sustainability of this disinfection method, with even contradictory results. While early studies found this method to be as effective as hyperchlorination [23], other studies were found to be of only incomplete [24] or short-lasting success (60% efficacy and reoccurrence after 4-5 months) [25]. Therefore, this procedure deemed to be of lower interest during the last twenty years and was mainly used in a combination to other procedures [25]. However, this method has the advantage that it can be initiated without any special equipment in a short lead of time and does not negatively affect the water quality. Due to a currently only poor evidence base, further investigations are urgently needed to evaluate this disinfection control measure, since it may help to avoid the chemical methods and the difficulties associated with them.

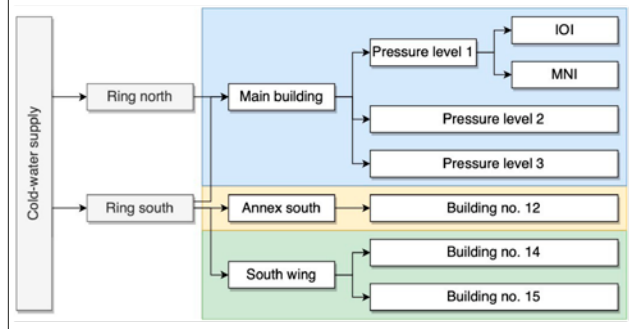
Within the scope of this study, we therefore investigated and report on the effectiveness and long-term results of the superheat and flush disinfection method on the contamination of the cold-water system with legionella in a German university hospital.

Methods

Local legislations (Ordinance on the Quality of Water intended for Human Consumption – “Trinkwasserverordnung, TrinkwV”) prescribe a periodic testing of all water-supplying systems of hospitals regarding the appearance of *Legionella spp.* [26]. In 2014/2015, during a routine periodical examination, we detected rising counts of colony forming units (CFU) of legionella in the cold-water supplying system at the University Hospital Knappschaftskrankenhaus Bochum (UKB) in Bochum, Germany. In the hot water supply, there was no increased number of *Legionella spp.* detected. Fortunately, cases of nosocomial Legionnaires’ disease were not observed.

According to recommendations of the Federal Environment Agency of Germany (“Umweltbundesamt”, UBA) a detailed risk assessment was performed [26, 27]. According to these results in March 2014, a structural renovation and renewal of parts of the water pipe system was performed until May 2015, accompanied by health protection measures, e.g. assembly of sterile water filters at distal sites. Pipe sections with a low frequency of use of the tapping points were considered as a risk factor regarding the appearance and growth of *Legionella spp.* These pipe sections were identified by architectural drawings and local inspection and according to this detection all water tap installations were included in a flushing schedule. This measure required a sufficient water flow of at least five minutes every 72 hours.

Fig. 1. Schematic representation of the cold-water supplying system at the study hospital (IOI: Interdisciplinary, operative ICU; MNI: Medical, neurological ICU meta-analysis).



The flushing schedule was continued until the present day and was set as a standard procedure. In addition, dismantling of unused pipes was performed, when possible. Re-evaluating these measures, we still found increased numbers of *Legionella spp.* CFUs exceeding the technical threshold of 100 CFU/100 mL. A total dismantling of the affected pipe systems would have meant a very high effort including a disruption of the daily business of the hospital. Thus, different measures of disinfection of the cold-water supplying system were evaluated under consideration of the applicability within the branched pipe-system as well as the feasibility during the regular daily business.

HOSPITAL BUILDINGS’ WATER SUPPLY

Two independent pipe strands build the basic structure of the water supplying system (ring north and ring south). The main hospital building, a fifteen-floor high tower built in 1972, is supplied by both of these rings and the water distribution is divided into three pressure levels to maintain a sufficient water pressure up to the highest floor. The first pressure level *inter alia* provides water for the two intensive care units (ICU), on the second floor of the building. The second pressure level feeds the patient-wards up to the seventh floor, and the third pressure level serves the floors eight to fifteen. The southern water distribution ring serves to the annex south (two patient wards and an outpatient department, four floors, built in 2011) and the south wing of the main building (four patient wards, four floors, built in 1963). A schematic representation of the hospitals cold-water supplying system is provided in Figure 1.

SUPERHEAT-AND-FLUSH MEASURE

In a first superheat and flush procedure in March 2017, the entire cold-water supplying system was flushed with hot water section by section, including all distal sites. The entire measure covered a period of 14 days. In a first preparatory step, it had to be determined, which devices were sensitive to heat and might take damage from this process. These devices (like coffee machines and drinking fountains) were temporarily disconnected from the water pipes. To maintain an effective elimination of *Legionella spp.*, a water

temperature of at least 70°C had to be maintained for at least three minutes at all distal sites. A big challenge during this measure was to avoid scalding of patients and hospital staff. Therefore, each cold-water tap had to be blocked and observed by hospital staff during the disinfection measure. Moreover, the observers proofed the achievement and maintenance of the target temperature, as well as the regression to a value below 20°C afterwards for protection against accidental scalding. The water temperature before and after flushing, as well as the duration of flushing was documented. After the disinfection measure, periodical follow-up examinations were taken out. As a result of an expected short impact of success according to literature, in November 2017 a second superheat and flush disinfection following the same scheme was carried out. Furthermore, periodical testing was continued until the present day without another disinfection measure.

SAMPLE COLLECTION, PROCESS AND ANALYSIS

Sampling sites were selected randomly. All samples were taken in accordance with DIN EN ISO 19458 and DIN EN ISO 11731 (DIN EN ISO – German standard which has also been adopted as a European standard). The sampling technique is visualized in Figure 2. After an initial disinfection of the water tapping point by flaming, one liter of water was drained and discarded. Afterwards, without further closing and opening of the water tap, a sample container was filled, and the temperature of the sample was documented [26]. For determination of legionella count 100 mL of sampled water was filtered with a sterile mixed cellulose ester (MCE) membrane filter (Millipore EZ-Pak, Merck

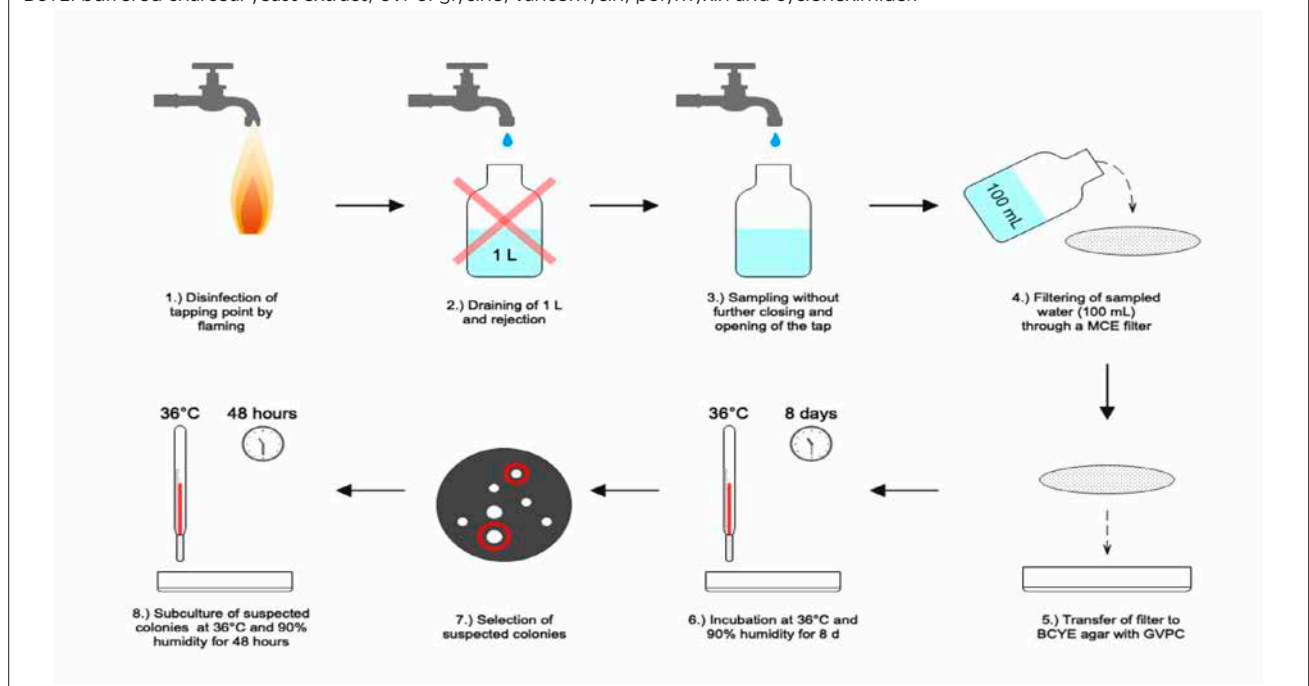
KGaA, Darmstadt, Germany), 47 mm in diameter with a pore size of 0.45 µm. Each membrane filter was transferred to a Buffered Charcoal Yeast Extract (BCYE) agar with glycine, vancomycin, polymyxin and cycloheximide (GVPC), incubated at $36 \pm 1^\circ\text{C}$ in a humid atmosphere (90% humidity), and examined after 8 days of incubation. Afterwards, suspected colonies were subcultured on BCYE agar with GVPC and Columbia blood agar. After another incubation period of 48 hours at $36 \pm 1^\circ\text{C}$, only colonies grown on GVPC agar were included in the results. These were reported in colony forming units (CFU) per 100 mL.

STATISTICAL ANALYSES

Baseline characteristic and outcomes were analyzed as follows: Continuous, not normally distributed variables are presented as median and interquartile range (IQR), categorical variables are expressed as frequency and percentage. The normality of distribution of continuous variables was tested by one-sample Kolmogorov-Smirnov test. Comparison of CFUs before and after disinfection measures was performed using the Mann-Whitney U test. For this purpose, all sampling points of each water supply line were pooled according to time point of sampling (before and after superheat and flush disinfection). To a certain extent, samples were taken before and after superheat and flush disinfection at the same sampling point. These related samples were additionally compared with a one-sided Wilcoxon signed-rank test.

Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software Inc., San Diego, CA, USA) and The R Project for Statistical Computing 4.0 (The R Foundation for Statistical Computing, Vienna,

Fig. 2. Visualization of the sampling technique for determination of legionella colony forming units per 100 mL (MCE: mixed cellulose ester; BCYE: buffered charcoal yeast extract; GVPC: glycine, vancomycin, polymyxin and cycloheximide).



Austria). A p-value of less than 0.05 was considered to be statistically significant.

Results

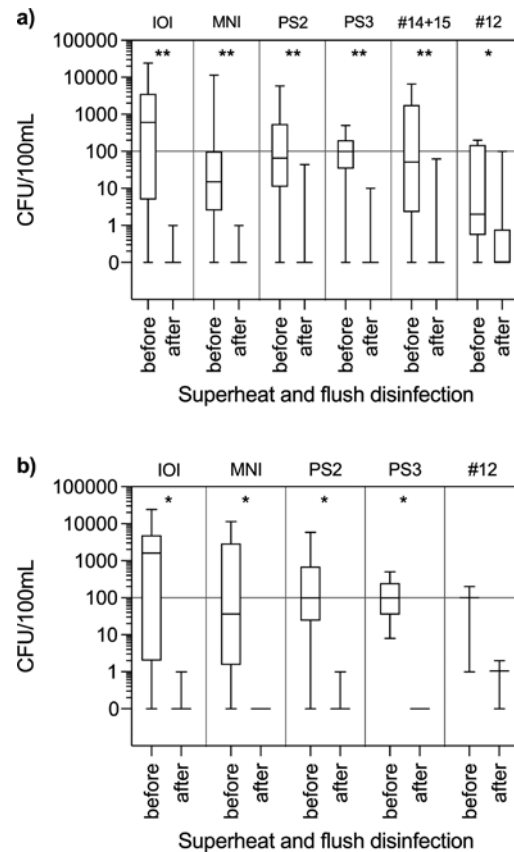
The count of CFU's before and after superheat and flush procedure are shown in Table I and Figure 3. The frequency of exceeding the limit value of 100 CFU/100 mL pre- and post-disinfection is also presented in Table I.

Independently of localization of testing, before thermal disinfection the bacterial load of legionella exceeded by far the legal limits of 100 CFU/100 mL. Especially at the interdisciplinary, operative ICU (IOI), 73% of all samples surpassed this cutoff value up to 30 times. Also, at other sampling points (medical, neurological ICU (MNI), pressure stage 2 and 3, building 12, 14 and 15) 29 to 54% of all samples presented an exuberant load of *Legionella* spp. None of the mentioned sampling sites showed acceptable CFU counts at that time.

After superheat and flush disinfection, CFUs of legionella at nearly all sampling sites, except for one, were found below the cutoff level of 100 CFU/100 mL, while even no proof of *Legionella* spp. could be found at some sites. Statistical analysis of all sampling sites showed a highly significant reduction of legionella load in the cold-water supplying system after the measure, shown in Figure 3a. This was also observed in matched samples of identical locations before and after disinfection, although not all differences were statistically significant due to only a small size of matched samples as it is shown in Figure 3b.

The one sampling site still showing elevated legionella levels after the first intervention was attributed to a

Fig. 3. Distribution of Colony Forming Units (CFU) per 100 mL of *Legionella* spp. within the cold-water supplying system for a) all sampling points or b) only matched, identical sampling points, before/after superheat and flush disinfection. Horizontal line indicating the limit value of 100 CFU / 100 mL (IOI: interdisciplinary, operative ICU; MNI: medical, neurological ICU; PS2: pressure stage 2; PS3: pressure stage 3; #14+15: building no. 14 and 15; #12: building no. 12; * p-value < 0.05; ** p-value < 0.001).



Tab. I. Colony forming units of *Legionella* spp. before and after first superheat and flush disinfection of the cold-water supplying system.

	Before disinfection			After disinfection			P-value
Sample location	CFUa [median (IQR)] / 100 mL	N	N above TLVb (%)	CFUa [median (IQR)] / 100 mL	N	N above TLVb (%)	
All sampling points							
Total	61 (3-100)	70	33 (47.1)	0 (0-0)	202	1 (0.5)	< 0.001
IOI ^c	600 (103-2,600)	11	8 (72.7)	0 (0-0)	36	0 (0.0)	< 0.001
MNI ^d	15 (3-100)	17	5 (29.4)	0 (0-0)	26	0 (0.0)	< 0.001
PS2 ^e	66 (11-450)	16	7 (43.8)	0 (0-0)	43	0 (0.0)	< 0.001
PS3 ^f	100 (35-200)	15	8 (53.3)	0 (0-0)	42	0 (0.0)	< 0.001
#14+15 ^g	52 (3-175)	6	3 (50.0)	0 (0-0)	27	0 (0.0)	< 0.001
#12 ^h	2 (1-100)	5	2 (40.0)	0 (0-0)	28	1 (3.6)	0.014
Matched sampling points ⁱ							
Total	100 (11-400)	33	18 (54.5)	0 (0-0)	33	0 (0.0)	< 0.001
IOI ^c	1,600 (4-4250)	7	4 (57.1)	0 (0-0)	7	0 (0.0)	0.018
MNI ^d	37 (10-85)	6	2 (33.3)	0 (0-0)	6	0 (0.0)	0.030
PS2 ^e	100 (37-400)	9	5 (55.6)	0 (0-0)	9	0 (0.0)	0.007
PS3 ^f	100 (36-200)	9	6 (66.7)	0 (0-0)	9	0 (0.0)	0.005
#14+15 ^g	No matched sampling points						
#12 ^h	101 (51-150)	2	1 (50.0)	1 (0-2)	2	0 (0.0)	0.500

^a CFU: colony forming units; ^b TLV: threshold limit value of 100 CFU/100 mL; ^c IOI: interdisciplinary, operative ICU; ^d MNI: medical, neurological ICU; ^e PS2: pressure stage 2; ^f PS3: pressure stage 3; ^g #14+15: building no. 14 and 15; ^h #12: building no. 12; ⁱ identical sampling points before and after measure.

local contamination, and therefore an exchange of the tapping point was performed. Afterwards no increased loads of *Legionella spp.* were observed at this site. Until the second episode of disinfection in November 2017, follow-up samples showed no increase of CFUs of legionella. Over the further course of time, until the present day, the periodical assessment of bacterial load of *Legionella spp.* detected no sampling site passing the intervention threshold of 100 CFU/100 mL. Only at one sampling site in December 2019 (toilet flush) the limit value was exceeded, which was again attributable to a local contamination from an external origin (feces).

Discussion

We evaluated the effects of the superheat and flush method on the bacterial load of legionella in the cold-water supplying system at the UKB and we could show that this measure was highly effective. While 33 out of 70 samples had a legionella load of more than 100 CFU/100 mL before the first disinfection was carried out, this number could be reduced to 1 out of 202 samples. Additionally, the effect was long-lasting, as no relevant limit exceedance occurred until the present day (June 2020).

Since the 1980s, multiple studies have been carried out to analyze methods controlling legionellae populations in water-conducting systems of hospitals. Often the focus was set on hot water-supplying systems, but it has also been shown that cold water supply systems can be contaminated with legionella [28]. The investigation of used methods differs widely though. In early studies, both chemical disinfection processes using chlorine, and thermal disinfection processes were deemed as effective [23]. Additionally, further chemical methods *e.g.* ozone, copper or silver came up. Nevertheless, beside reports of successful implementations of these chemical measures [29-31], there were also examples of failure [32]. All chemical methods have in common, that they need to be carried out continuously. Alternatively, an intermittent chemical disinfection of the pipes can be carried out. In this case, a ban on the use of all water intakes for the duration of the measure is required. It must also be ensured that a target concentration of the substance in the water is reached and subsequently rinsed out to a harmless level [33]. However, due to these measures, damage to piping systems was observed in the past. In addition, they involve a great deal of effort and are difficult to implement in medical supply buildings during the clinical workflow [34].

Therefore, other feasible and effective disinfection methods were evaluated in the past, including physical measures for eradication. Irradiation with ultra-violet (UV) light (wavelength 253.7 nm) reliably kills legionella [35]. Despite that, irradiation units must be operated permanently and maintained regularly in accordance with the water flow rate and the systems

have to be replaced annually. Moreover, disinfection by UV irradiation is locally limited and thus, if used centrally, contamination in the piping system is likely to remain [36]. There are no toxic by-products, but a decentralized use is very cost intensive.

A different approach is the superheat and flush procedure, which was the first disinfection measure used for eradication of legionella-colonized water distribution systems in hospitals [22]. This measure is based on a physical principle. Hot water is flushed through contaminated pipe sections for a sufficient period of time and kills legionella through a high temperature. However, there are older reports on only a short duration of effect with a recolonization of the water-supplying system within a short time after disinfection [25, 37-39]. But there is no need for special equipment and therefore the superheat and flush method can be initiated in a short period of time. These were the main reasons, why we chose this method for eradication in our hospital. Nevertheless, we were also aware to repeat this measure if a recolonization would have been occurred and thus, we scheduled two episodes of disinfection in advance, which represents a typical approach according to literature [40].

With regard to implementation of the superheat and flush method, there are also important things to consider. Some failures in the past were most likely attributable to non-systematic and simultaneous flushing of distal sites in a short period of time [40]. Moreover, it has to be ensured, that all pipe sections are included in the procedure. Therefore, we performed the superheat and flush disinfection section by section in only 14 days, where one structural section was disinfected at once within a few hours and repeated this intervention after 6 months. This meant a great deal of effort, as each tapping point had to be secured by personnel for prevention of accidental scalding. However, this ensured that no recontamination from areas that had not been disinfected yet could occur, which may be possibly one of the main reasons why the superheat and flush disinfection has shown such a great and long-lasting effect in our hospital. Legal thresholds were easily met and by far exceeded. The absolute legionella count could be reduced to almost 0 CFU/100 mL at all sampling points and no damage to pipelines or thermal damage to users occurred. Contrary to past studies, the effect was shown to be long-lasting after an observation period after more than two years. It remains speculative whether this result could have also been obtained with other disinfection methods. However, chemical disinfection might have required an interruption of the clinical workflow and environmental aspects can also not to be neglected. Drawback of superheat and flush method is an increased expenditure, which could be estimated at approximately 250 working hours for each implementation at the UKB. In contrast it has also to be taken into account, that other methods would have to be carried out by specialized companies.

The retrospective approach of the investigation and

the lack of comparison with other methods on the same object may limit our findings. However, several studies show that restrictions such as a development of tolerances and insufficient effectiveness may occur in chemical disinfection processes. Moreover, it remains speculative to what extent the removal of unused pipeline sections and the introduction of a flushing plan for less-used parts of the pipeline system influenced the effectiveness and long-term effect of the measure.

Despite the promising results of our study, a transferability into other hospitals or buildings is only limited. First of all, water supplying systems differ widely between hospitals as they represent grown structures which were extended and adjusted throughout the past during construction works and building expansions. Moreover, the location of contamination with legionella within the water system (*e.g.* pipes vs distal sites) may have an impact on effectiveness. Therefore, our measures cannot be transferred one-to-one to other hospitals with similar problems. Thus, it is essential to carry out an individual risk assessment and to choose and adapt disinfection measures according to local conditions. Nevertheless, our experience may encourage others to put the superheat and flush method on the shortlist of possible disinfection procedures.

Conclusions

The superheat and flush disinfection can provide an economic and highly effective measure in case of legionella contamination of water supplying systems, especially in hospitals with an older building structure. Nevertheless, according to local conditions, no general statement can be made for or against this disinfection measure. Affected hospitals have to carry out an individual risk assessment and selection of method for eradication. However, as there is no need for special equipment and it can be initiated in a short period of time, the superheat and flush procedure should be shortlisted for an eradication attempt.

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Conflict of interest statement

The authors declare no conflict of interest.

Authors' contributions

MU: writing the manuscript; Data analysis. TR: review the manuscript. TK: planning the work. CP: data collection. MB: data collection. MN: data collection. CS: data analysis. H-P J: planning the work. NP: review the manuscript; analyze literature. MA: planning the work; review the manuscript. HN: writing the manuscript; statistics.

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