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Glutaraldehyde-resistant *Mycobacterium chelonae* from endoscope washer disinfectors

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P.A. GRIFFITHS, J.R. BABB, C.R. BRADLEY AND A.P. FRAISE. 1997. Glutaraldehyde is used to disinfect flexible and other heat-sensitive endoscopes often with the aid of automated systems. *Mycobacterium chelonae* is being isolated with increasing frequency from these washer disinfectors and processed endoscopes. This has, on occasions, led to misdiagnosis and iatrogenic infections. Recent reports suggest that disinfecting machines, on a sessional or regular basis, with 2% glutaraldehyde may have selected and therefore encouraged the growth of strains of *Myco. chelonae,* possibly in biofilm, with decreasing susceptibility to glutaraldehyde. In view of this, the resistance of three strains of *Myco. chelonae* var. *chelonae* (the type strain NCTC 946 and two machine isolates) was tested against 2% glutaraldehyde and a wide range of alternative disinfectants. Disinfectants tested were a chlorine releasing agent, sodium dichloroisocyanurate at 1000 ppm and 10 000 ppm av Cl, 0·35% peracetic acid (NuCidex, Johnson & Johnson), 70% industrial methylated spirit (IMS), 1% peroxygen compound ('Virkon', Antec International) and 10% succine dialdehyde ('Gigasept', Sanofi Winthrop). Suspension and carrier tests were carried out in the presence and absence of an organic load. Results showed the type strain, which had not been exposed to the selective pressure of disinfectant usage, to be very sensitive to most disinfectants with the exception of 1% Virkon. The washer disinfector isolates, on the other hand, were extremely resistant to 2% glutaraldehyde and showed greater resistance to 1% Virkon and 1000 ppm NaDCC. Purchasing machines in which the entire fluid pathways, including those for delivering rinse water, are disinfected with an appropriate agent during each cycle are preferred. If this is not possible then sessional cleaning and disinfection at the start of each day and regular maintenance should prevent biofilm formation and contamination with disinfectant-resistant strains of mycobacteria. In addition to machine disinfection, the use of sterile or bacteria-free (filtered $< 0{\cdot}45 \mu$m) water is essential for bronchoscopes and all invasive endoscopes. If there is doubt over the effectiveness of the machine disinfection procedure or water quality, the channels and surfaces of endoscopes may be rinsed with 70% IMS after automated processing.

INTRODUCTION

Mycobacterium chelonae, the turtle bacillus of Friedman 1903, has long been recognized as an environmentally associated mycobacterium being found in soil, dust and water. It has been isolated from, and found to multiply in, both natural and treated waters including tap water (Goslee and Wolinsky 1976; Collins *et al.* 1984). In the hospital environment problems ascribed to the presence of *Myco. chelonae* have been reported in haemodialysis fluids, pharmaceutical preparations and some disinfectant solutions (Carson *et al.* 1978). It is also being isolated with increasing frequency from decontaminated (i.e. cleaned and disinfected) flexible fibreoptic

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endoscopes and endoscope washer disinfectors (Nye *et al.* 1990; Fraser *et al.* 1992; Spach *et al.* 1993).

Automated systems are now used for decontaminating endoscopes in most hospitals as they are more convenient than manual processing and protect staff from skin and eye contact and, in some instances, from respiratory exposure to glutaraldehyde vapour (Bradley and Babb 1995). In a recent study of gastrointestinal endoscopy units in the UK, 98·6% were reported to be using 2% glutaraldehyde (Wicks 1994). Two per cent glutaraldehyde is recommended by the Department of Health and professional societies as the most suitable disinfectant for endoscope disinfection.

Reports of contaminated endoscopes have highlighted the need to destroy or remove atypical mycobacteria, including *Myco. chelonae*, present in instrument rinse water and automated systems. If these organisms are not removed or destroyed, they may be deposited in, or on endoscopes, during processing. In bronchoscopy, this has, on occasions, led to misdiagnosis of tuberculosis as acid-fast bacilli are deposited in the channel of the bronchoscope and these are transferred to bronchial lavage samples for ZN staining (Uttley *et al.* 1990).

Van Klingeren and Pullen (1993) showed that machine-associated isolates of *Myco. chelonae* var. *abscessus* were far more tolerant to 2% glutaraldehyde than a laboratory strain of *Myco. chelonae* and *Myco. terrae* ATCC 15755, the official test organism for mycobactericidal testing in Germany and the Netherlands. In view of these findings we have investigated the resistance of machine isolates of atypical mycobacteria and a type strain to a wide range of instrument disinfectants. The strains tested were two strains of *Myco. chelonae* var. *chelonae* which were being consistently isolated from two separate endoscope washer disinfectors and processed endoscopes, and the type strain of *Myco. chelonae* var. *chelonae* (NCTC 946). In the absence of any UK or harmonized European standard tests to establish mycobactericidal activity, suspension and carrier tests based on the methods of Best *et al.* (1988) were carried out in the presence and absence of 10% serum as an organic load.

Clinical isolates of *Myco. chelonae* could have been used for disinfectant testing, the original source of infecting or colonizing micro-organisms and the disinfectant exposure record is unlikely to be known. It was for this reason that we chose the type strain with a known history for comparison with machine isolates.

METHODS AND MATERIALS

Test organisms

The type strain NCTC 946 and two endoscope washer disinfector isolates of *Myco. chelonae* var. *chelonae* from two different UK hospitals were used in this study. Unlike the two machine isolates, the type strain of *Myco. chelonae* var. *chelonae* had not been exposed to the selective pressure of disinfectant usage and, as such, was included for comparative purposes.

The washer disinfector isolates were received from the respective hospitals on Lowenstein Jensen slopes. They were immediately transferred to Middlebrook 7H11 (Becton Dickinson) agar plates to check for purity and from there, one colony was inoculated into 100 ml of Middlebrook 7H9 broth (Difco) and incubated at 30°C for 14 d. The suspensions were sonicated daily (50–60 Hz) for 10 min and inverted several times to minimize clumping.

The homogeneous suspensions were then mixed with 10% glycerol as a preservative and 1 ml aliquots were decanted into 1·5 ml microcentrifuge tubes and frozen at –70°C until required. The washer disinfector isolates were typed to subspecies level at the Mycobacteria Reference Laboratory, Cardiff, and were shown, using gas chromatography, to be *Myco. chelonae* var. *chelonae*. The NCTC strain was obtained freeze dried from the Central Public Health Laboratory, Colindale, London. This was reconstituted in Middlebrook 7H9 broth and cultured on 7H11 agar plates. Broth cultures of the NCTC strain were prepared in the same way as that described for the washer disinfector isolates and stored in 1 ml aliquots at –70°C until required.

Disinfectants

Several of the most widely used instrument and equipment disinfectants were tested. These included 'Asep', a 2% activated alkaline glutaraldehyde (Galen, Craigavon, N. Ireland), 'Sanichlor', sodium-dichloroisocyanurate, a chlorine-releasing agent, at concentrations of 1000 ppm and 10 000 ppm av Cl (G.H. Wood, Swindon), 70% v/v Industrial methylated spirit IMS 74 OP (Genta Medical, York), 'NuCidex' containing 0·35% v/v peracetic acid (Johnson and Johnson Medical, Ascot), 1% w/v 'Virkon', a peroxygen compound (Antec International, Sudbury) and 10% v/v 'Gigasept' a succine dialdehyde and formaldehyde mixture (Sanofi Winthrop Medicare, Guildford). All disinfectants were freshly prepared prior to testing. Sterile distilled water was used as a diluent and as a disinfectant control.

Neutralization/recovery system

Prior to testing the efficacy of each disinfectant, neutralization tests were carried out to determine the most suitable recovery system. To mimic test conditions, 100 μl of sterile distilled water was added to 900 μl of the disinfectant at the highest use concentration, mixed and left for 1 min. Ten μl of this mixture were then added to 990 μl of the neutralization/recovery medium, i.e. Ringer's solution containing 0·5% Tween 80. Ten μl of the undiluted test suspension of

Myco. chelonae were added to this mixture (neat), vortex mixed for 20 s and serially diluted to 10^{-5} in Ringer's solution only. One hundred μl of the neat and subsequent dilutions were spread onto 7H11 agar in duplicate, using sterile spreaders. The plates were incubated at 30°C for up to 5 d (in plastic bags to prevent drying out during prolonged incubation) and colony-forming units (cfu) were enumerated. The undiluted test suspension was used as the initial count. The test was repeated using water instead of the disinfectant as the control. The neutralizer was deemed suitable as there was no difference in colony size, growth rate or the number of cfu retrieved from tests and controls. This shows the neutralization/recovery system was effective and not inhibitory.

It was hoped to be able to use this combination of dilution in Ringer's solution and 0·5% Tween 80 for all disinfectants in an attempt to standardize the neutralization procedure. However, although this combination was shown to neutralize most disinfectant residues without inhibiting the growth of small numbers of surviving test organisms, it was ineffective in neutralizing NuCidex, and for this disinfectant alone, a combination of 50 g l^{-1} sodium thiosulphate, 0·25 g l^{-1} catalase and dilution was used on the recommendation of the manufacturers, Johnson & Johnson. This was assessed in the same way and found to be suitable.

Preparation of test organism

In the absence of an internationally recognized test method, much work has been carried out (Griffiths 1997) in an attempt to obtain a standard, reproducible test suspension of a high titre with minimal clumping. Ideally the initial inoculum should have a sufficiently high titre to enable the assessor to demonstrate at least a 5 $\log_{10}$ reduction (99·999%) in carrier and suspension tests (Anon. 1987). The preparation methods investigated were a broth method, a plate method and the method currently proposed by the European Standards disinfectant testing committee, i.e. CEN/TC216/WG1, subgroup on mycobactericidal testing. As a result of these preliminary studies, a broth method only was used for preparing suspensions for storage prior to efficacy testing and a similar method to that proposed by CEN/TC216/WG1 was used to obtain the test suspension.

Immediately prior to testing, one of the suspensions stored at –70°C was removed from the freezer, thawed at room temperature, centrifuged, washed twice in sterile distilled water and a loopful spread onto a Middlebrook 7H11 agar plate. After 5 d incubation, 7–10 loopfuls of growth were harvested, added to glass beads, moistened with sterile water and shaken for 5 min. Ten ml of sterile distilled water were added, agitated and the suspension left to settle for 30 min. The supernatant fluid was removed to a second sterile bottle and left to settle for a further 2 h. The supernatant fluid from this suspension was sonicated for 10 min to disperse the organisms and this was used as the challenge in disinfectant tests. Films were prepared of these challenge test suspensions and stained by Ziehl Neelsen (ZN) to check for homogeneity. Although this method of preparation did not completely eliminate clumping, it vastly reduced it.

Organic material

To simulate an organic load, horse serum (Tissue Culture Services Ltd, Buckingham) was added to the initial test suspension to give a final concentration of 10%.

Suspension test

One hundred μl of the test suspension were added to 900 μl of freshly prepared disinfectant in microcentrifuge tubes and vortex mixed for 20 s. The disinfectant/test suspension mixture was held at room temperature and sampled at 1, 4, 10, 20 and 60 min intervals. After the required contact time, 10 μl were removed and added to 990 μl of Ringer's/Tween 80 or Ringer's/sodium thiosulphate/catalase neutralizer recovery medium, vortex mixed for 20 s and then serially diluted to 10^{-3}. One hundred μl of the neat and subsequent dilutions were spread onto Middlebrook 7H11 agar, in duplicate, using sterile hockey stick spreaders. Plates were incubated at 30°C for up to 1 week and cfu were enumerated.

Carrier test

Ten μl of the test suspension were placed on the base of a sterile glass cup (capacity 600 μl), supported in a 24 well cell culture plate, and left to dry at 25°C for 90 min. Care was taken to ensure the culture was only placed on the base of each cup. This inoculum was overlaid with 60 μl of freshly prepared disinfectant and left at room temperature for contact times of 1, 4, 10, 20 and 60 min. After the required contact time the glass cup was removed, using sterile forceps, and placed aseptically into 2940 μl of neutralization/recovery medium, vortex mixed for 20 s and serially diluted to 10^{-3}. One hundred μl of the neat and subsequent dilutions were spread onto Middlebrook 7H11 agar, in duplicate, incubated at 30°C for up to 1 week and cfu were enumerated.

Controls

Controls were carried out in duplicate at 1 and 60 min intervals using 900 μl (suspension test) and 60 μl (carrier test) of sterile distilled water instead of the disinfectant. The mean of the two counts obtained in the controls was taken to be the initial challenge for calculation of disinfectant efficacy.

Calculation of disinfectant efficacy

The efficacy of the disinfectants in suspension and carrier tests was established by converting the pre- and post-disinfection counts to the $\log_{10}$ system and subtracting the mean $\log_{10}$ post-disinfection count from the mean $\log_{10}$ pre-disinfection count. The pre-disinfection count was that obtained from the mean of the controls, i.e. water substituted for disinfectant for contact with test organisms for 1 and 60 min. In summary:

$\log_{10}$ pre-disinfection count–$\log_{10}$ post-disinfection count =

$\log_{10}$ reduction (RF)

The neutralization/recovery system largely based on dilution has a limit of sensitivity of $\log_{10}$ reductions less than 3·48 in the carrier test (dilution of 1/3000) and less than 3·00 (dilution of 1/1000) in the suspension test. As a $> 5{\cdot}00$ $\log_{10}$ reduction is often used as an indication of acceptable efficacy (Ayliffe 1993; Anon. 1987), the initial $\log_{10}$ challenge must therefore be $> 8{\cdot}48$ in carrier tests and $> 8{\cdot}00$ in the suspension tests.

RESULTS

Table 1 shows the activity of six disinfectants against a type strain *Myco. chelonae* var. *chelonae* NCTC 946, in suspension and dried onto carriers, in the presence and absence of 10% serum. The type strain of *Myco. chelonae* was very susceptible to most disinfectants, i.e. a 5 $\log_{10}$ reduction was achieved following exposure for 1 min to 2% glutaraldehyde, 70% industrial methylated spirits, 1000 and 10 000 ppm av Cl sodium dichloroisocyanurate and 0·35% peracetic acid (NuCidex). However, 10% succine dialdehyde (Gigasept) required a contact time of 20 min and 1% peroxygen (Virkon) failed to achieve a > 5 $\log_{10}$ reduction in 60 min in the carrier tests.

The susceptibility of the two endoscope washer disinfector isolates of *Myco. chelonae* var. *chelonae* (WD A and WD B) to the same disinfectants under clean and dirty (10% serum) conditions is shown in Tables 2 and 3. Table 2 shows the suspension test results, while Table 3 shows the carrier test results. Both machine isolates were very resistant to 2% glutaraldehyde and 1% Virkon. Sixty minutes exposure was insufficient to achieve a > 5 $\log_{10}$ reduction. Ten per cent Gigasept was similarly ineffective with one of the two machine isolates. However, sensitivities to the chlorine releasing agents, alcohol and peracetic acid, were good and similar to those obtained with the type strain NCTC 946. Carrier tests are a little more stringent than the suspension tests and the lower concentration of NaDCC, i.e. 1000 ppm av Cl, failed to give a 5 $\log_{10}$ reduction under dirty conditions in 60 min.

DISCUSSION

The presence of atypical mycobacteria in both naturally occurring and treated waters, including tap water, is well recognized. Nye *et al.* (1990) were the first to indicate that tap water was the initial source of contamination of the bronchoalveolar lavage fluid specimens, although it had been the suspected source in a number of earlier incidences (Pappas *et al.* 1983). It is currently recommended that sterile or bacteria-free water is used to rinse bronchoscopes and all invasive or surgical instruments whether they are processed automatically or manually (Anon. 1989; Ayliffe *et al.* 1992; Cooke *et al.* 1993). This is usually achieved by the use of steam sterilized or filtered water ($< 0{\cdot}45\mu$m). Other systems currently being considered are u.v., chlorination, heat treatment, reverse osmosis and ozonization.

To further minimize the risk of instrument contamination during rinsing, current UK guidelines advise that if machines cannot be disinfected during each cycle they should at least be disinfected at the start of each day or session (Anon. 1993). Two per cent activated alkaline glutaraldehyde, at ambient temperature, is currently used in the UK and elsewhere to disinfect both washer disinfectors and endoscopes. This is due to its wide spectrum of microbicidal activity and its non-damaging effect on the components of the endoscopes, accessories and processors. However, it appears that constantly disinfecting washer disinfectors with this agent, has selected strains and thereby encouraged the proliferation of *Myco. chelonae* with a decreasing susceptibility to glutaraldehyde (Van Klingeren and Pullen 1993).

This study has shown similar results. Two endoscope washer disinfector isolates of *Myco. chelonae* var. *chelonae* proved very tolerant to 2% glutaraldehyde with little or no loss of viability (< 1 $\log_{10}$) during a 1-h period of exposure to the disinfectant. However, unlike Van Klingeren and Pullen, no cross resistance to peracetic acid was found and this along with higher concentrations of a chlorine releasing agent and 70% IMS, were very effective in destroying these organisms, even when dried onto carriers with 10% serum present.

Based on our findings, and providing it can be established that the washer disinfector components are not damaged, we recommend that if a glutaraldehyde-resistant strain of *Myco. chelonae* is present, the machine is disinfected by an initial purge with a chlorine releasing agent at 10 000 ppm av Cl followed by sessional disinfection with a 1000 ppm av Cl. If the quality of the water used for cleaning and rinsing cannot be assured we would recommend the last stage of the decontamination process is rinsing the instrument, including the channel(s) with 70% IMS. Apart from its value as a mycobactericidal agent, the alcohol evaporates leaving the surfaces of the instrument dry and free of irritant residues. Bacteria are then less likely to proliferate before the instrument is reused.

Table 1 Susceptibility of *Mycobacterium chelonae* NCTC 946 to disinfectants, in suspension and dried onto carriers, in the presence and absence of 10% serum

Disinfectant	Log initial count	Mean log reduction after 1 min	4 min	10 min	20 min	60 min
Suspension test						
2% glutaraldehyde	8·64	> 5·64	> 5·64	> 5·64	> 5·64	> 5·64
	8·82	**> 5·82**	**> 5·82**	**> 5·82**	**> 5·82**	**> 5·82**
1000 ppm av Cl NaDCC	8·27	> 5·27	> 5·27	> 5·27	> 5·27	> 5·27
	8·13	**> 5·13**	**> 5·13**	**> 5·13**	**> 5·13**	**> 5·13**
10 000 ppm av Cl NaDCC	8·45	> 5·45	> 5·45	> 5·45	> 5·45	> 5·45
	8·07	**> 5·07**	**> 5·07**	**> 5·07**	**> 5·07**	**> 5·07**
70% IMS	8·76	> 5·76	> 5·76	> 5·76	> 5·76	> 5·76
	8·64	**> 5·64**	**> 5·64**	**> 5·64**	**> 5·64**	**> 5·64**
1% Virkon	8·42	0·87	2·56	4·48	> 5·42	> 5·42
	9·07	**0·45**	**1·78**	**4·06**	**4·83**	**> 6·07**
10% Gigasept	8·67	0	0·51	> 5·67	> 5·67	> 5·67
	8·27	**0·18**	**0·19**	**> 5·27**	**> 5·27**	**> 5·27**
0·35% NuCidex	8·76	> 5·76	> 5·76	> 5·76	> 5·76	> 5·76
	8·47	**> 5·47**	**> 5·47**	**> 5·47**	**> 5·47**	**> 5·47**
Carrier test						
2% glutaraldehyde	8·80	> 5·32	> 5·32	> 5·32	> 5·32	> 5·32
	8·90	**> 5·42**	**> 5·42**	**> 5·42**	**> 5·42**	**> 5·42**
1000 ppm av Cl NaDCC	8·48	> 5·00	> 5·00	> 5·00	> 5·00	> 5·00
	8·55	**> 5·07**	**> 5·07**	**> 5·07**	**> 5·07**	**> 5·07**
10 000 ppm av Cl NaDCC	8·53	> 5·05	> 5·05	> 5·05	> 5·05	> 5·05
	9·16	**> 5·68**	**> 5·68**	**> 5·68**	**> 5·68**	**> 5·68**
70% IMS	8·51	> 5·03	> 5·03	> 5·03	> 5·03	> 5·03
	8·94	**> 5·46**	**> 5·46**	**> 5·46**	**> 5·46**	**> 5·46**
1% Virkon	9·18	0·55	1·86	2·92	4·46	4·47
	9·09	**0**	**0·18**	**1·89**	**3·62**	**3·41**
10% Gigasept	9·29	0·02	0·25	0·4	> 5·81	> 5·81
	9·35	**0**	**0·32**	**0·35**	**> 5·87**	**> 5·87**
0·35% NuCidex	8·93	> 5·45	> 5·45	> 5·45	> 5·45	> 5·45
	8·71	**3·48**	**> 5·23**	**> 5·23**	**> 5·23**	**> 5·23**

Log_{10} initial count = Control count, i.e. water used instead of disinfectant in the **presence** and absence of 10% serum. Mean of samples at 1 and 60 min.

An unpublished study carried out by the authors on an endoscope washer disinfector artificially contaminated with a serum broth culture of one of these glutaraldehyde-resistant strains of *Myco. chelonae* showed that 1000 ppm av Cl was effective in eliminating the strain in 10 min. However, this does not mimic naturally occurring contamination where biofilm and limescale may have been present. In a further study by the authors a thorough machine clean and service, followed by disinfection with a chlorine releasing agent was also effective in eliminating a naturally occurring glutaraldehyde-resistant strain of *Myco. chelonae* from a contaminated machine but the benefit of the initial service, which included replacement of much of the pipework, was not independently assessed. No similar studies have so far been carried out with peracetic acid but on the basis of *in vitro* tests a similar response is anticipated.

At the time of this study NuCidex was relatively new and the compatibility of the disinfectant with the washer disinfector components was unknown. Alcohol, the other effective mycobactericidal agent, is a fixative and will only work on clean surfaces. It is also flammable and could not therefore be used in large quantities to disinfect the washer disinfectors.

Another important issue is whether or not these atypical

Table 2 Suspension tests—Susceptibility of two washer disinfector isolates of *Mycobacterium chelonae* to various disinfectants in suspension in the presence (dirty) and absence (clean) of 10% serum

Disinfectant	Log initial count	Mean log reduction after 1 min	4 min	10 min	20 min	60 min
Clean conditions						
2% glutaraldehyde	8·43	0·24	0·30	0·35	0·51	0·64
	9·1	**0**	**0·12**	**0·09**	**0·33**	**0·29**
1000 ppm av Cl NaDCC	8·54	2·90	> 5·54	> 5·54	> 5·54	> 5·54
	8·8	**1·79**	**> 5·8**	**> 5·8**	**> 5·8**	**> 5·8**
10 000 ppm av Cl NaDCC	9·00	> 6·00	> 6·00	> 6·00	> 6·00	> 6·00
	9·18	**> 6·18**	**> 6·18**	**> 6·18**	**> 6·18**	**> 6·18**
70% IMS	9·00	> 6·00	> 6·00	> 6·00	> 6·00	> 6·00
	9·04	**> 6·04**	**> 6·04**	**> 6·04**	**> 6·04**	**> 6·04**
1% Virkon	8·18	0	0	0	0·07	0·07
	8·3	**0·03**	**0·06**	**0·08**	**0·18**	**2·25**
10% Gigasept	8·44	0	0	2·69	> 5·44	> 5·44
	8·35	**0·09**	**0·16**	**0·06**	**0·17**	0·13
0·35% NuCidex	8·06	4·06	> 5·06	> 5·06	> 5·06	> 5·06
	9·12	**4·03**	**> 6·12**	**> 6·12**	**> 6·12**	**> 6·12**
Dirty conditions						
2% glutaraldehyde	8·83	0·81	0·98	0	0·94	1·08
	9·16	**0**	**0**	**0·01**	**0·02**	**0·04**
1000 ppm av Cl NaDCC	8·50	2·17	4·02	4·10	> 5·50	> 5·50
	9·47	**0·03**	**0·81**	**3·20**	**4·82**	**5·59**
10 000 ppm av Cl NaDCC	8·75	> 5·75	> 5·75	> 5·75	> 5·75	> 5·75
	8·19	**> 5·19**	**> 5·19**	**> 5·19**	**> 5·19**	**> 5·19**
70% IMS	8·69	> 5·69	> 5·69	> 5·69	> 5·69	> 5·69
	9·30	**> 6·30**	**> 6·30**	**> 6·30**	**> 6·30**	**> 6·30**
1% Virkon	8·00	0	0·14	0·14	0·10	0·34
	9·36	**0**	**0**	**0**	**0**	**0**
10% Gigasept	8·67	0	0	1·15	> 5·67	> 5·67
	9·28	**0**	**0**	**0·14**	**0·24**	**0·88**
0·35% NuCidex	8·07	4·07	> 5·07	> 5·07	> 5·07	> 5·07
	9·43	**4·15**	**> 6·43**	**> 6·43**	**> 6·43**	**> 6·43**

Mycobacterium chelonae washer disinfector isolates WD A and **WD B**.

mycobacteria are susceptible to heat as some of the recently introduced washer disinfectors utilize glutaraldehyde and rinse water at an elevated temperature of 45–60°C.

The water delivery system to the washer disinfector may also become contaminated with atypical mycobacteria and requires similar treatment. We would advise that if bacteria-retaining filters are used, these are steam sterilized or chemically disinfected together with any fluid pathways not accessed during the machine self-disinfect cycle. Water treatment with u.v. light in the absence of bacteria-retaining filters is unlikely to be effective as the presence of dead mycobacteria may also lead to a positive ZN and misdiagnosis of tuberculosis.

The presence of biofilm in endoscope washer disinfectors may harbour and protect micro-organisms, including *Myco. chelonae*, present within the matrix. These will be more difficult to access with the disinfectant than those in the planktonic state. Regular cleaning, disinfection and maintenance of the washer disinfector and the water delivery system will prevent the formation of biofilm and increase the effectiveness of the machine self-disinfect cycle. The use of softened water and the selection of biofilm antagonistic materials should further reduce this risk.

The regular use of a disinfectant may select and thereby encourage the proliferation of mycobacteria, or other micro-organisms, of increasing resistance to the agents used. We would therefore recommend sampling at periodic intervals or

Table 3 Carrier tests—Susceptibility of two washer disinfector isolates of *Mycobacterium chelonae* to various disinfectants dried onto carriers in the presence (dirty) and absence (clean) of 10% serum

Disinfectant	Log initial count	Mean log reduction after 1 min	4 min	10 min	20 min	60 min
Clean conditions						
2% glutaraldehyde	7·37	0·02	0·02	0	0·04	0·15
	8·59	**0·04**	**0·06**	**0·06**	**0·04**	**0·19**
1000 ppm av Cl NaDCC	9·21	0·83	**0·91**	4·18	5·73	> 5·73
	8·82	**1·88**	**3·44**	**> 5·34**	**> 5·34**	**> 5·34**
10 000 ppm av Cl NaDCC	8·52	> 5·04	> 5·04	> 5·04	> 5·04	> 5·04
	8·50	**> 5·02**	**> 5·02**	**> 5·02**	**> 5·02**	**> 5·02**
70% IMS	8·56	> 5·08	> 5·08	> 5·08	> 5·08	> 5·08
	8·80	**> 5·32**	**> 5·32**	**> 5·32**	**> 5·32**	**> 5·32**
1% Virkon	8·56	1·42	1·77	2·28	2·61	2·74
	8·80	**0·58**	**1·37**	**1·88**	**2·05**	**1·99**
10% Gigasept	8·90	0·03	0·15	2·71	3·63	> 5·42
	8·61	**0**	**0**	**0**	**0**	**0·23**
0·35% NuCidex	8·80	4·37	> 5·32	> 5·32	> 5·32	> 5·32
	8·50	**> 5·02**	**> 5·02**	**> 5·02**	**> 5·02**	**> 5·02**
Dirty conditions						
2% glutaraldehyde	7·80	0	0	0	0	0
	8·69	**0**	**0**	**0**	**0**	**0**
1000 ppm av Cl NaDCC	7·84	0·28	0·54	1·80	3·20	2·46
	8·94	**0·23**	**0·39**	**0·74**	**1·27**	**2·19**
10 000 ppm av Cl NaDCC	8·74	> 5·26	> 5·26	> 5·26	> 5·26	> 5·26
	8·62	**> 5·14**	**> 5·14**	**> 5·14**	**> 5·14**	**> 5·14**
70% IMS	8·80	> 5·32	> 5·32	> 5·32	> 5·32	> 5·32
	9·06	**> 5·58**	**> 5·58**	**> 5·58**	**> 5·58**	**> 5·58**
1% Virkon	9·12	0·05	0·32	0·69	1·11	1·47
	8·74	**0·35**	**0·84**	**1·03**	**0·90**	**1·18**
10% Gigasept	9·16	0·04	0·05	0·04	1·72	> 5·68
	8·80	**0**	**0·02**	**0**	**0·16**	**0·46**
0·35% NuCidex	8·54	2·73	> 5·06	> 5·06	> 5·06	> 5·06
	8·54	**4·47**	**> 5·06**	**> 5·06**	**> 5·06**	**> 5·06**

Mycobacterium chelonae washer disinfector isolates WD A and **WD B**.

should a problem occur (Bradley and Babb 1990). Samples should be taken from the lumens and external surfaces of the instrument after processing and from the washer disinfector and rinse water.

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