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Resistance to Widely Used Disinfectants

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Hypervirulent Clostridium difficile PCR-Ribotypes Exhibit Resistance to Widely Used Disinfectants

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Abstract

The increased prevalence of Clostridium difficile infection (CDI) has coincided with enhanced transmissibility and severity of disease, which is often linked to two distinct clonal lineages designated PCR-ribotype 027 and 017 responsible for CDI outbreaks in the USA, Europe and Asia. We assessed sporation and susceptibility of three PCR-ribotypes; 012, 017 and 027 to four classes of disinfectants; chlorine releasing agents (CRAs), peroxygens, quaternary ammonium compounds (QAC) and biguanides. The 017 PCR-ribotype, showed the highest sporation frequency under these test conditions. The oxidizing biocides and CRAs were the most efficacious in decontamination of C. difficile vegetative cells and spores, the efficacy of the CRAs were concentration dependent irrespective of PCR-ribotype. However, there were differences observed in the susceptibility of the PCR-ribotypes, independent of the concentrations tested for Virkon®, Newgenn®, Proceine 40® and Hibiscrub®. Whereas, for Steri7® and Biocleanse® the difference observed between the disinfectants were dependent on both PCR-ribotype and concentration. The oxidizing agent Perasafe® was consistently efficacious across all three PCR ribotypes at varying concentrations; with a consistent five Log10 reduction in spore titre. The PCR-ribotype and concentration dependent differences in the efficacy of the disinfectants in this study indicate that disinfectant choice is a factor for limiting the survival and transmission of C. difficile spores in healthcare settings.

Introduction

Clostridium difficile-infection (CDI) is an antibiotic associated diarrhoea, caused by C. difficile, a Gram-positive, spore-forming anaerobic bacillus. CDI clinical symptoms can range from mild diarrhoea to life threatening pseudomembranous colitis. Antibiotic therapy is proposed to elicit CDI by disruption of the intestinal microbiota, which enables colonization of the gastrointestinal tract by indigenous or ingested C. difficile. C. difficile was first recognized as a pathogen over 30 years ago, and primarily CDI was associated with immune suppressed and elderly patients, receiving antibiotic treatment [1]. However, in the last 10 years C. difficile has emerged as a global pathogen, with epidemics across Europe, Asia and the USA, culminating in the transcontinental spread of ‘hypervirulent’ PCR-ribotypes [2,3,4]. Evolutionary and genetic analysis of C. difficile have revealed five distinct clonal lineages, Clades 1–5 inclusive, which are conserved across analysis methods such as microarray [5], MLST sequence type (ST) [6] and whole genome sequencing [7]. The most notable being the PCR-ribotype 027/Clade 1/ST-1 and 017/Clade 4/ST-37, which have brought a concomitant increase in disease severity, mortality, recurrence rate, enhanced relative transmissibility and decreased mean age of infection [4,8,9]. Consequently, C. difficile is the most frequent cause of nosocomial diarrhoea worldwide [10,11]. C. difficile has a unique advantage over other healthcare associated communicable infections such as methicillin resistant Staphylococcus aureus (MRSA), due to its ability to form spores, which are central to transmission of C. difficile. Patients with C. difficile are estimated to excrete between 1 × 10^6 and 1 × 10^7 spores per gram of faeces [12,13]. Spores are highly infectious and readily transmissible [13], hence they are particularly problematic in healthcare settings [14], as they are able to persist on a variety of surfaces [15,16,17,18] and are resistant to many disinfectants [19,20,21]. The use of disinfectants in combating the spread of CDI in hospitals and the community is central to infection control strategies, particularly as studies indicate a correlation between overlapping resistance mechanisms to disinfectants, antiseptics and antibiotics [22,23]. Adaptation to altered antibiotic treatment regimes has been met with modified antimicrobial resistance patterns within C. difficile isolates [24,25,26], which is particularly apparent within the 027 lineage, whereby some 027 isolates have acquired fluoroquinolone resistance [7].

Resistance to antibiotics and disinfectants is a potential problem in managing infection control. There is a broad selection of disinfectants available, with differing active compounds. Presently, the UK Department of Health and Health Protection Agency guidelines advocate the use of chlorine-based disinfectants at a concentration of 1000 ppm for disinfection of C. difficile.

Representative isolates of the 012, 017 and 027 PCR-ribotypes were chosen for analysis; strain 630 is an 012 PCR ribotype is a virulent multidrug resistant strain isolated from an outbreak in a Zurich hospital in 1982 [27], and was therefore isolates before


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may of the disinfectants in this study were manufactured. Strain 630 was the first _C. difficile_ genome to be fully sequenced [28]. Strain R20291 is a representative 027 PCR-ribotype that was isolated from an outbreak in Stoke Mandeville hospital in 2006 and strain M68 is a representative 017 PCR-ribotype that was isolated from a CDI outbreak in Ireland in 2006, both of which have been fully sequenced [7]. We tested the susceptibility of spores and vegetative cells from the 012, 017 and 027 PCR-ribotypes to a panel of nine commercially available biocides from four categories of disinfectant. These include chlorine releasing agents (CRAs), peroxygen releasing agents, quaternary ammonium compounds (QACs), and a chlorhexidine based hand wash.

With the exception of Perasafe®, the disinfectants fell into three categories, i) those whose efficacy were concentration dependent, independent of the PCR-ribotype, ii) those whose efficacy were PCR-ribotype dependent and iii) those whose efficacies were dependent on both PCR-ribotype and concentration. Perasafe® was the only disinfectant consistently efficacious across all three PCR ribotypes at varying concentrations, where survival was below the limit of detection.

### Results

#### Sporulation of _C. difficile_ PCR-ribotypes

Spore production is a unique feature of _C. difficile_ among other important healthcare pathogens, therefore vegetative cell production and sporulation of three representative _C. difficile_ PCR-ribotypes 012, 017 and 027 (Figure 1a) was analysed. The 012 and 027 strains exhibited similar levels of sporulation in minimal media, $5.7 \times 10^4$ CFU/ml and $5.1 \times 10^4$ CFU/ml respectively, whereas the 017 strain spore titre was significantly higher, $1.8 \times 10^5$ ($p<0.0000$ Partial F-test) (Figure 1b). This observation was consistent with heat resistant spores and microscopy counted spores (spores were counted using a Neubauer-ruled Bright Line counting chambers; Hauser Scientific data not shown).

#### Susceptibility to disinfectants

The susceptibility of 012, 017 and 027 PCR ribotypes to a panel of disinfectants was assessed *in vitro* using pure _C. difficile_ cultures at $2.9 \times 10^5$ (±0.5). Preliminary investigations were performed with contact times of 2 minutes, 30 minutes and 4 hours. There were no significant differences between the data obtained at these time points, therefore a 30 minute contact was used throughout for experimental ease. The disinfectants used in the study are listed in Table 1. The data is expressed on a Log plot as normalized CFU/ml to take into account the differences in spore production between the three ribotypes. Statistical analysis (see methods) was performed to address three questions i) is there a strain dependent sensitivity to the disinfectants? ii) if so, what is the most appropriate concentration to use? and iii) which disinfectant has the greatest efficacy across all three PCR-ribotypes and concentrations?

#### Chlorine releasing agents

CRAs are halogenic compounds widely used in disinfection regimes. The active ingredients in Actichlor® and HazTab® are sodium dichloroisocyanurate (NaDCC), Adipic acid and NaDCC respectively. The manufacturers’ recommended working concentrations vary slightly for outbreaks and blood spills, but are conserved for general use (Table 1). The susceptibility of the 012, 017 and 027 ribotypes to chlorine releasing disinfectants revealed that at 5000 ppm survival was below the limit of detection of the assay in all ribotypes (Figure 2A and 2B), whereas at 1000 and 500 ppm, spores survived for all three ribotypes.) (Figure 2A and 2B). A Chi² interaction test and a partial F-test revealed that although concentration and PCR-ribotype were linked, the efficacy of the disinfectants were concentration dependent, irrespective of PCR-ribotype for both Actichlor® and HazTab® (Table 2 and 3). Overall, there were no significant differences between the efficacy of Actichlor® and HazTab® (Table 3).

#### Peroxygens

Peroxygens are oxidizing agents, two differently acting peroxygens were tested, Perasafe® and Virkon® (Table 1). The efficacy of

**Figure 1. Vegetative cells and Spore counts of _C. difficile_ PCR ribotypes 012, 017 and 027.** A) Total cell counts and spore counts were obtained by plating cultures and heat resistant samples of _C. difficile_ on blood plates containing 0.1% taurocholate. B) Percentage spore counts were obtained by calculating the number of heat resistant spores as a proportion of the total cell counts. Data consists of three biological and two technical replicates from separate cultures. Student T-tests were performed between total counts and spores for each strain and significant differences are marked with a bracket * ($p<0.05$). A comparison for percentage survival of spores was performed using linear regression and a partial F-test, where M68 was the reference strain, a significant difference ($p<0.01$) in spore production between the three strains is marked with a bracket **.

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Table 1. The disinfectants used in this study.

<table>
<thead>
<tr>
<th>Disinfectant name</th>
<th>Biocide type</th>
<th>Active ingredient(s)</th>
<th>Recommended concentration</th>
<th>Recommended uses</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actichlor®</td>
<td>CRA</td>
<td>sodium dichloroiodoacetate</td>
<td>1000 ppm (5000 ppm*)</td>
<td>blood and body fluid spills</td>
<td>Ecolab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Trichloroacetic acid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bioclense®</td>
<td>QAC</td>
<td>Benzalkonium chloride</td>
<td>5%</td>
<td>surfaces and general hygiene</td>
<td>Teknon</td>
</tr>
<tr>
<td>HazTab®</td>
<td>CRA</td>
<td>Sodium Dichloroisocyanurate</td>
<td>1000 ppm (10000 ppm*)</td>
<td>blood and body fluid spills</td>
<td>Guest Medical LTD</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Sodium dichloro-1,3,5 triazinetrione dihydroxy)</td>
<td></td>
<td>and for general hygiene</td>
<td></td>
</tr>
<tr>
<td>Hibiscrub®</td>
<td>Cationic bis-biguanide</td>
<td>chlorhexidine gluconate</td>
<td>100%</td>
<td>Handwash</td>
<td>Regent Medical</td>
</tr>
<tr>
<td>NewGenn®</td>
<td>QAC</td>
<td>Di-decyl dimethyl ammonium chloride</td>
<td>0.8%</td>
<td>surfaces, general hygiene and equipment</td>
<td>NewwGenn research</td>
</tr>
<tr>
<td>PeraSafe®</td>
<td>Peroxygen</td>
<td>peracetic acid</td>
<td>1.62%</td>
<td>medical devices, surfaces and general hygiene</td>
<td>Micro Medical</td>
</tr>
<tr>
<td>Proceine 40®</td>
<td>QAC</td>
<td>alkyl-amino-alkyl glycines</td>
<td>0.6%</td>
<td>small spills, surfaces and general hygiene</td>
<td>AGMA</td>
</tr>
<tr>
<td>Steri 7®</td>
<td>QAC</td>
<td>isothiazolium-benzalkonium chloride</td>
<td>100%</td>
<td>general hygiene and surfaces</td>
<td>Sentinal International LTD</td>
</tr>
<tr>
<td>Virkon®</td>
<td>Peroxygen</td>
<td>potassium peroxymonosulfate</td>
<td>1%</td>
<td>hazardous spills, surfaces and equipment</td>
<td>DuPont</td>
</tr>
</tbody>
</table>

The active ingredients, biocide type and recommended working concentrations and recommended uses are listed. Outbreak or blood spill concentrations are highlighted with * where they differ from the standard working concentrations. There were no minimum contact times provided for the disinfectants.

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Discussion

The efficacy of disinfectants against the nosocomial pathogen C. difficile is central to infection control strategies, especially as colonization rates near infected individuals are as high as 58% [16]. Recent publications have indicated aerosolization of spores as well as environmental contamination contribute to dissemination of C. difficile [13]. The transmissibility and virulence of C. difficile is continually evolving, through ecological and environmental influences. The spores produced by C. difficile enhance transmission due to their ability to survive in the environment [18,29,30] and resists biocides [19,20,21]. Cross resistance has been observed between biocides and antibiotics [31,32,33,34], which is enhanced by exposure to sub-inhibitory concentrations of biocide [35].

We show variation in sporulation rates of C. difficile PCR-ribotypes 012, 017 and 027. The toxbin defective strain M68, an 017 PCR-ribotype, showed the highest sporulation frequency under these test conditions, with an average of 3.5 times the spore titre compared to 012 and 027 PCR-ribotypes. The high sporulation rate of the 017 PCR-ribotype, may have contributed to the transcontinental spread of the 017 PCR-ribotype, in spite of their lack of one of the major virulence factors, toxbin A from this lineage. Limiting the transmission of C. difficile spores in healthcare settings is an important factor in infection control; however, even sporidal disinfectants are relatively inactive against C. difficile spores, which are able to remain on various surfaces even after disinfection [36,37]. Contaminated surfaces have been implicated as reservoirs for airborne transmission of spores, which can be aerosolized by disturbance of these contaminated environments [13]. The transmission of environmental spores and efficacy of disinfectants to prevent patient-to-patient transmission has recently been addressed using a murine model, in which oxidizing
disinfectants had the most effective reduction in transmission efficiency of the 017 PCR-ribotype strain M68 [38]. This along with the data we present highlights the importance of disinfectant choice in limiting the spread of CDI.

In this study, with the exception of Perasafe®, the disinfectants fell into three categories, i) those whose efficacies were dependent on concentration, ii) those whose efficacy were dependent on PCR-ribotype and iii) those whose efficacies were dependent on both PCR-ribotype and concentration. The use of CRAs, peroxygen based compounds, QACs and biguanides is widespread in the hospital setting, with different biocides used for distinct applications, including; antiseptic, disinfectant or preservative treatments [39]. Biocide activity can be affected by several different factors, including; concentration, contact time, pH, temperature, organic matter, as well as the number and condition of the bacteria, such as vegetative cells, biofilms and spore [39]. Within our experimental system, the tests were performed on liquid cultures to enable direct comparisons to be made between different disinfectants. However, some of these disinfectants are surfactants, therefore the low level of activity of some of these compounds could be linked to the experimental methods used. The most effective biocides across all three PCR-ribotypes tested were the oxidizing agents, such as CRAs (Specifically Actichlor® and HazTab®) and peroxygens (specifically Perasafe®), which damage DNA, proteins and lipids [40]. It has been shown that oxidizing agents such as H₂O₂ interfere with the spore coat thus rendering the spore nonviable [38]. However, H₂O₂ has been shown to be less effective than other peroxygens [41]. There was a marked difference between the efficacies of two types of peroxygens tested. The peracetic acid containing peroxygen was active against all three PCR ribotypes, where the level of survival of C. difficile was below the limit of detection for the assay, indicating a 5–Log₁₀ reduction in spore titre, whereas the 012, 017 and 027 PCR-ribotypes were less susceptible to the potassium peroxymonosulphate containing peroxygen, with less than a 1-Log₁₀ decrease in spore titre at the recommended working concentration. However, there were PCR-ribotype dependent differences in the susceptibility to differing concentrations of Virkon®.

The CRAs Actichlor® and HazTab® showed a good efficacy at 5000 ppm and 1500 ppm, however, survival of spores was detected for all three PCR-ribotypes at lower concentrations,
which is consistent with published data indicating that CRAs are only sporidical at high concentrations [42].

Under the experimental methodology used in this study, the QACs were overall less effective against the three PCR-ribotypes than the CRAs and the peroxys, which may be linked to their use as primarily surfactants. However, interesting differences were observed between these disinfectants that were dependent on concentration and PCR-riboype. The PCR ribotype 027 was more susceptible to the majority of the QACs (except Biocleanse®) and the peroxys Virkon® than the 012 and 017 PCR-ribotypes. However, the 027 PCR-riboype was more resistant to the widely used hand wash Hibiscrub® than the 012 and 017 PCR-ribotypes. All disinfectants exhibited effective inactivation of vegetative cells at the majority of concentrations tested, with a few exceptions at low concentrations.

The comparative efficacy of the nine disinfectants was assessed using a Linear regression model controlling for strain (PCR-riboype), concentration and disinfectant, with Actichlor® as the reference. The coefficient of variance was used as an estimate for the relative efficacy of the disinfectants, with Perasafe® being the most effective disinfectant under the experimental conditions used.

The global spread of CDI has seen the increase in other PCR ribotypes in the USA, Europe and Asia. The global spread of CDI has been genetically and phenotypically characterized and is good representatives of their distinct lineages. Strains were stored at −80°C and cultured on C.C.E.Y Agar (Oxoid), supplemented with 4% egg yolk emulsion (Bioconnections), 1% defibrinated horse blood (TCS Biosciences), and cycloserine/cefoxitin antiboitic supplement (Bioconnections) for 1 to 2 days under anaerobic conditions, in a Modular Atmosphere Control System 500 (Don Whitney Scientific) at 37°C. All cultures were performed in duplicate. Primary liquid cultures were inoculated with three single colonies into 10 ml of pre-reduced Yeast Peptone (YP) broth (16 g/L Peptone, 8 g/L Yeast, 5 g/L NaCl) with 0.2% (v/v) Tween 80 and incubated anaerobically for 24 hours on a shaking platform at 60 rpm. Secondary cultures were inoculated using 1/20 dilution of the primary cultures onto 40 ml of pre-reduced YP broth with 0.2% (v/v) Tween 80 and incubated anaerobically for 24 hours.

### Materials and Methods

**Bacterial culture and media**

*C. difficile* strains used are as follows: R20291 a PCR-riboype 027 from an outbreak at the Stoke Mandeville hospital, England; 006, strain M68 a PCR-riboype 017 from an outbreak in Dublin, Ireland 2006 and strain 630 a PCR-riboype 012 isolated from a patient in Zurich, Switzerland 1982. These strains have been geneticaly and phenotypically characterized and are good representatives of their distinct lineages. Strains were stored at −80°C and cultured on C.C.E.Y Agar (Oxoid), supplemented with 4% egg yolk emulsion (Bioconnections), 1% defibrinated horse blood (TCS Biosciences), and cycloserine/cefoxitin antibiotic supplement (Bioconnections) for 1 to 2 days under anaerobic conditions, in a Modular Atmosphere Control System 500 (Don Whitney Scientific) at 37°C. All cultures were performed in duplicate. Primary liquid cultures were inoculated with three single colonies into 10 ml of pre-reduced Yeast Peptone (YP) broth (16 g/L Peptone, 8 g/L Yeast, 5 g/L NaCl) with 0.2% (v/v) Tween 80 and incubated anaerobically for 24 hours on a shaking platform at 60 rpm. Secondary cultures were inoculated using 1/20 dilution of the primary cultures onto 40 ml of pre-reduced YP broth with 0.2% (v/v) Tween 80 and incubated anaerobically for 24 hours.

### Vegetative cells and spore counts

Vegetative cell counts were determined for all cultures, 1 ml of each culture was centrifuged at 8000 x g and washed with 1 ml of sterile phosphate buffered saline 1 x (PBS, Sigma), samples were centrifuged again and pellets were resuspended in 1 ml PBS, serially diluted in 1 x PBS and plated in duplicate onto blood agar base plates supplemented 7% (v/v) defibrinated horse blood (TCS) and 0.1% (v/v) taurocholate (Sigma). Bacterial counts were enumerated on plates after 24 hours and calculations were performed to give colony forming units per ml (CFU/ml).

Heat resistant spore counts: 1 ml of each culture was incubated at 56°C for 20 minutes to heat inactivate the vegetative cells. The heat resistant spores were then centrifuged and washed.

---

**Table 2. Chi² and partial F-test p-values.**

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Chi² p-value</th>
<th>Partial F-test p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actichlor®</td>
<td>0.004</td>
<td>0.0172*</td>
</tr>
<tr>
<td>Biocleanse®</td>
<td>0.0017</td>
<td>0.0000</td>
</tr>
<tr>
<td>Haztab®</td>
<td>0.0004</td>
<td>0.0164*</td>
</tr>
<tr>
<td>Hibiscrub®</td>
<td>n/a</td>
<td>0.0050;</td>
</tr>
<tr>
<td>Newgenn®</td>
<td>0.5131*</td>
<td>0.0000</td>
</tr>
<tr>
<td>Perasafe®</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Prociene 40®</td>
<td>0.0610*</td>
<td>0.0000</td>
</tr>
<tr>
<td>Steri7®</td>
<td>0.0000</td>
<td>0.0026</td>
</tr>
<tr>
<td>Virkon®</td>
<td>0.0306*</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

Chi² p-value is the probability that the differences observed for each disinfectant are independent of strain and concentration, p<0.01 indicates that strain and concentration are both a factor in the efficacy of the disinfectant, whereas *indicates there is no significant interaction between strain and concentration. A partial F-test was performed to determine whether there were significant differences between the three PCR-ribotypes (p<0.01). * indicates the exception to the partial F-test, where the strain difference for Hibiscrub was tested using a three variant Chi².

**Table 3. Disinfectant efficacy estimated using coefficient of variance.**

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>coefficient of variance</th>
<th>p-value</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perasafe®</td>
<td>−3.3024</td>
<td>0.000</td>
<td>0.296</td>
</tr>
<tr>
<td>Actichlor®</td>
<td>0</td>
<td>n/a</td>
<td>0.194</td>
</tr>
<tr>
<td>Haztab®</td>
<td>0.19</td>
<td>0.249</td>
<td>0.165</td>
</tr>
<tr>
<td>Biocleanse®</td>
<td>1.7004</td>
<td>0.000</td>
<td>0.358</td>
</tr>
<tr>
<td>Newgenn®</td>
<td>2.415</td>
<td>0.000</td>
<td>0.318</td>
</tr>
<tr>
<td>Steri7®</td>
<td>2.64</td>
<td>0.000</td>
<td>0.275</td>
</tr>
<tr>
<td>Virkon®</td>
<td>2.974</td>
<td>0.000</td>
<td>0.369</td>
</tr>
<tr>
<td>Prociene 40®</td>
<td>3.4749</td>
<td>0.000</td>
<td>0.267</td>
</tr>
<tr>
<td>Hibiscrub®</td>
<td>4.0972</td>
<td>0.000</td>
<td>0.292</td>
</tr>
</tbody>
</table>

A Linear regression was performed taking strain, concentration and disinfectant into consideration. Actichlor® was used as the reference and the output gave the coefficient of variance from the reference (p<0.01). This was then normalised to the reference to give the overall variance from Actichlor®. The larger the negative coefficient of variance the higher efficacy of the disinfectant. The standard error and p-values are listed, where *indicates no significant difference from the reference Actichlor® (p<0.01). doi:10.1371/journal.pone.0025754.t003
as outlined. Serial dilutions were performed in 1 x PBS and plated in duplicate onto blood agar base plates supplemented 7% (v/v) defibrinated horse blood (TCS Biosciences) and 0.1% (w/v) taurocholate (Sigma). Colony counts were enumerated on plates after 24 hours, and calculations were performed to give CFU/ml. Direct spore counts were also made from the liquid culture using a haemocytometer (Neubauer-ruled Bright Line counting chambers; Hauser Scientific) and a light microscope (Nikon) at 1000 x magnification.

Disinfectant assays

The disinfectants used in the study and concentrations are described in Table 1. Disinfectant survival assays were performed by mixing 1 ml of each duplicate culture with 1 ml of disinfectant at the appropriate concentration to give the desired final concentration. These were incubated for 30 minutes before 1 ml of the samples was centrifuged, washed, serially diluted and plated as outlined above. CFU counts were plotted in Graphpad Prism (v4) as percentage survival compared to heat resistant spore counts, error bars are standard error of the mean (SEM). The limit of detection for the assay is 25 CFU/ml.

Statistical analysis

For the comparison between total counts and spore counts, an unpaired two-tailed Students T-tests were performed in Graphpad Prism (v4), with a confidence interval of 95% (p<0.05). Analysis on the percentage spore production and the efficacy of the disinfectants was performed using Stata 12 statistical analysis program. Three questions were set to analyze the data i) is there a strain dependent sensitivity to the disinfectants? ii) if so, what is the most appropriate concentration to use? iii) which disinfectant has the greatest efficacy across all three PCR-ribotypes and concentrations?

Hypotheses i and ii were answered using an interaction test (Chi^2) performed on two Linear regression analyses (r^2) using log10 percentage survival data: The linear regression analyses performed were a) the regression accounting for concentration (independent of strain) and b) the regression accounting for both strain (PCR-ribotype) and concentration. The Chi^2 test to look for a relationship between strain and concentration was then performed on these two regression data sets (a and b), where a P<0.05 indicates that both strain (PCR-ribotype) and concentration are a factor in the efficacy of the disinfectant (Table 2).

References


