Comparison of bacterial growth on antimicrobial coated and non-coated disposable curtains supplied to the NHS

Introduction

The aim of this study was to provide Opal contracts with a better understanding of the interactions of bacteria in relation to disposable privacy curtains used in the health care environment. A review of the literature identified published testing methodology including ISO and Japanese standards and from this we adopted a methodology rationale based on these standards. The project focussed on two disposable curtains types provide by Opal; antimicrobial vs non antimicrobial coated polyethylene (non-fabric). These disposable curtains have now mainly replaced traditional laundered woven fabric privacy curtains. Along with the economic benefits of using disposable curtains Opal Contracts also aim to raise both customer and health care workers (HCW's) awareness to antimicrobial resistance. Although, this pilot study did not investigate the psychological aspects of HCWs as to whether anti-microbial coatings lead staff into a false sense of security with regards to hand washing or cleaning, this may be an area of future research.

Review of the literature

Studies have been undertaken within hospital environments as discussed by Cheng and co-workers (2015), where the aim of the study was to enumerate the number of hand-touch contacts sites by patients, health care workers (HCW's) and visitors with hospital environmental items. This study demonstrated that many areas of the patients' surroundings received high contact levels with bed curtains being one of the frequently mutually-touched surfaces by a range of people. This presents a risk for transmission of nosocomial infection.

With this in mind and the renewed attention to the seriousness of antibiotic resistance, it raises the question if anti-microbial coatings are necessary for privacy curtains. Recent reviews by Muller *et al.* (2016) discussed health care-associated infection (HCAI) and aimed to determine whether antimicrobial surfaces prevented HCAI. Reductions in HCAIs were reported in this study when antimicrobials were incorporated; copper silver 1), metal-alloy or organosilane-treated surfaces however, not all types of antimicrobial coatings were evaluated in this study.

A comparative evaluation of antimicrobials for textile applications was made by Winder and co-workers (2013). This review considered market dominance, application rates, durability of effectiveness and subsequent environmental effects and highlighted that each antimicrobial technology has specific risks and benefits that should be taken into account in evaluating the suitability of different antimicrobial products. Nanoscale silver and silver salts were indicated as having clear potential benefits for textile use.

Various studies have been undertaken within health care environments including those of hospital curtains. A study by Schweizer *et al.* (2012) was undertaken regarding privacy curtains and a comparison of coated vs non-coated was evaluated. One of their findings

showed curtains containing "complex element compound" or antimicrobial properties were significantly less contaminated compared to a standard curtain after swabbing at time points corresponding to 2-10 days. However, after these sampling times (up to 25 days) no relevant differences were observed between the two types. This study focussed on textiles containing PurThread's highly effective antimicrobial technology vs standard curtains. Again, this study highlighted that consideration must be given to the type of antimicrobial coating, as well as the nature of the surface or fabric being tested, when comparing various studies.

Along with renewed attention to the seriousness of antibiotic resistance awareness is the increasing awareness regarding biocide resistance and this has been discussed by Fraise (2002) in an article *Biocide abuse and antimicrobial resistance - a cause for concern*? *In-vitro* evidence has shown that biocides can also play a role in the development, or selection and dissemination, of bacterial pathogens showing resistance phenotypes to both biocides and antibiotics (SCENIHR 2009). Meyer and Cookson (2010) report that increased numbers of healthcare-acquired infections, including those caused by antibiotic resistant microbes, have created a need for improved infection control use of disinfection regimes and concluded that the current risks to healthcare delivery caused by resistance related biocides are low, provided that biocides are used under appropriate conditions and further research is required.

Many factors must be considered in considering the use of antimicrobial coatings in a hospital context and procurement departments within the Health Care Sector are often unaware of antimicrobial or biocide resistance with purchase of items being mainly price-driven. Health care workers and clinicians including infection control departments may also not be aware of current research which should influence considerations over the appropriateness of use of antimicrobials including surface coatings.

Study undertaken on Opal and a competitors Curtains

Our study evaluated only one coating of antibacterial technology.

Our study was undertaken on a non-porous disposable curtain. In developing the methodology we looked at the International Standard BS ISO 22196:2011 which describes the criteria for *Measurement of antimicrobial activity on plastics and other non-porous surfaces*. However the methodology described within this standard does not take into account environmental aspects such as the effect of bio burden on the efficacy of antimicrobial coatings. This method also maintains a high humidity on the surface during incubation because it is designed to look specifically at measuring the antimicrobial activity levels of the surfaces against bacteria. It therefore does not reflect the concern that contamination occurring on curtains on a hospital ward may be allowed to dry on surfaces by HCWs.

Therefore the methodology used within our study was based upon both ISO22196:2011 and The Japanese Industrial Standard JIS Z 2801: 2000. However, test samples inoculated with microorganisms were allowed to dry as opposed to incubating under

humid conditions. Additional samples were tested to mimic "dirty conditions" as reported by Ojeil and co-workers (2013) as these conditions affect the efficacy of antimicrobial coatings.

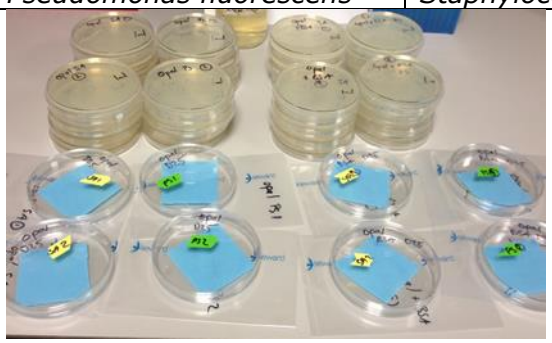
1. Schedule of work (as per quotation)

1. Chromosomally tagged (bioluminescent markers) *Pseudomonas fluorescens* and *Staphylococcus aureus* cultures were grown overnight at 37°C ±2°C.

2. Test pieces from Opal's non anti-microbial coated curtains and a competitors anti-microbial coated curtains measuring 50 mm X 50 mm were transferred to sterile petri dishes. Sufficient test pieces were produced to allow for each curtain type to be inoculated with both test organisms Pseudomonas fluorescens and Staphylococcus aureus and tested in duplicate, and for samples to be pre-treated with Bovine serum albumin (BSA) to mimic bioburden (Table 1.)

3. 1 ml of BSA (3 g/L) was spread across one set of samples to mimic "dirty conditions" or bioburden. Samples were allowed to dry overnight within a class II cabinet. Laboratory samples were labelled:

Table 1: Samples tested

Sample ID	Test inoculum	Test inoculum
Opal 1	*Pseudomonas fluorescens*	*Staphylococcus aureus*
Opal 2		
Opal 1 +BSA		
Opal 2 +BSA		
Competitor 1		
Competitor 2		
Competitor 1+BSA		
Competitor 2 +BSA		

Opal curtains test samples within Petri dishes

4. Overnight cultures of *Pseudomonas fluorescens* and *Staphylococcus aureus* were diluted into sterile nutrient broth at 1/500th strength so as to obtain ~ 2.5 X 10^5 / 10 X 10^5 cells ml^{-1}. Initial inoculum level were determined by plating serial dilutions onto Nutrient agar plates.

5. An aliquot (1000 µL per species) of each diluted culture was then placed onto 2 replicate sub-samples of curtain (25 $cm^{2)}$ of the treated (BSA) and non-treated surfaces under test.

6. Bacterial cultures were allowed to dry onto the samples within a class II cabinet. Once dried, levels of bioluminescence and bacterial counts were determined as described below (Day 0). Uninoculated curtain samples were also tested as a control for any back ground levels of contamination.

7. All test samples were incubated at 25°C ±2°C and again levels of bacteria and bioluminescence determined for Day 1, Day 3, Day 7, Day 25.

Bacterial enumeration

1. Inoculated test samples (25cm^2) were placed within a stomacher bag containing 9 ml sterile MRD and 1 ml neutralising buffer (Ojeil *et al*. 2013) and stomached for 30s.

2. Further serial dilutions were performed in sterile MRD and 100 µl spread onto duplicate Nutrient agar plates. Plates were incubated aerobically at 30°C ±2 °C for 24/48 h or until growth of colonies present were countable. As bacterial levels decreased 1 ml of initial 10^{-1} dilution was plated onto Nutrient agar plates and incubated as described above to improve detection levels.

Bioluminescence readings

1. Initially inoculated test piece(s) within petri-dishes were imaged directly using a BioSpac lab Photon imager. However, due to the low levels of bioluminescence observed, samples were evaluated using a tube luminometer.

For the tube luminometer method, 1 ml of the bacterial enumeration samples in MRD + neutralising buffer were tested for levels of bioluminescence using a tube luminometer.

Also, after stomaching the test material samples were placed onto Nutrient agar plates and incubated aerobically at 30°C ±2 °C for 24/48. This further incubation step allowed growth of residual bacteria not recovered by stomaching and enabled us to image test samples as can be seen in Figure 1.

Additional testing

Two strips of both Opal and the competitors curtains were affixed to the laboratory hand washing areas. Students and staff were asked to rub their hands on two marked areas (100cm^2) on both test samples before hand washing.

These areas were swabbed over a total period of 31 days and each swab was swabbed over a nutrient agar plate surface to recover bacterial contamination. Plates were incubated at 25°C ±2 °C for 48-72h, until colonies were visible to count. Results can be seen in Figure 3

Results

Enumeration of Inoculated Microorganisms on Samples with and without Soiling

Results are given in Table 2 and are expressed as viable bacteria (cfu) per 25 cm^2 test sample.
The data presented are the average plate counts from duplicate plates and duplicate samples.

Table 2. Enumeration of microorganisms.

Sample ID and test inoculum	Day 0	Day 1	Day 3	Day 7	Day 25
Opal Sa*	8.6×10^5	5.0×10^3	22.5	0	0
Opal Ps	5.1×10^6	$< 10^3$	212	2.5	0
Opal Sa+BSA	4.0×10^5	4.0×10^4	4.4×10^3	1.2×10^3	1.38×10^2
Opal Ps+BSA	5.0×10^4	7.5×10^3	132	2.5	0
Competitor Sa	8.5×10^5	$< 10^3$	20	0	0
Competitor Ps	1.5×10^6	5.0×10^3	65	50	0.75
Competitor Sa+BSA	2.0×10^6	1.2×10^5	8.2×10^3	2.0×10^3	0.75
Competitor Ps+BSA	6.5×10^5	1.8×10^4	7.7×10^3	250	0.25
Opal control	0				
Competitor control	0				

*Abbreviations **Ps**:*Pseudomonas fluorescens* **Sa** :*Staphylococcus aureus*

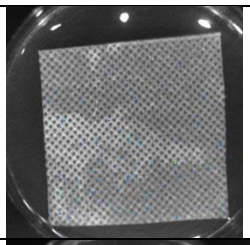
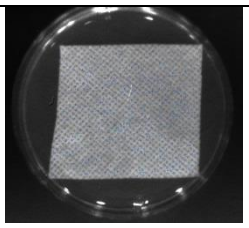
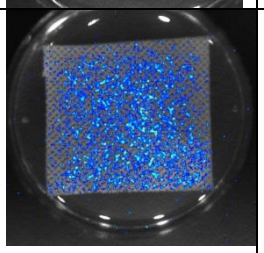
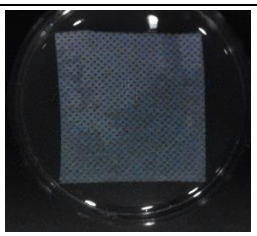
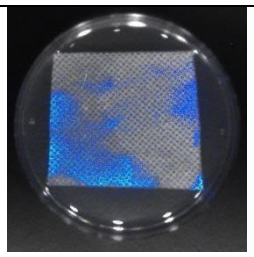
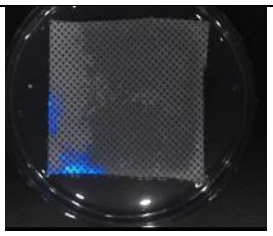
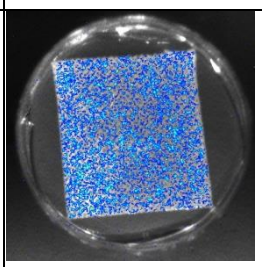
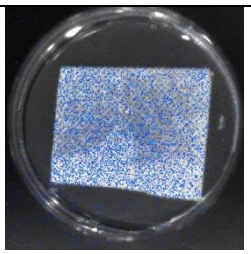
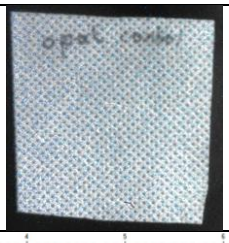
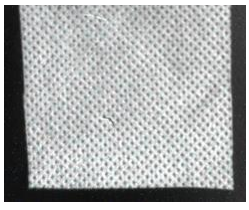
Initial inoculum added to each test sample:
Pseudomonas fluorescens 2.1×10^7 cfu ml^{-1}
Staphylococcus aureus 5.0×10^6 cfu ml^{-1}
Counts are given as cfu per 25 cm^2

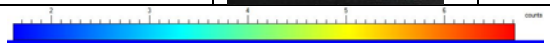
Comments
In general on Day 0 there was a decrease in cell viable counts recovered from the curtain samples compared to the initial amount of viable cells added (Table 1). This is an effect of drying cells onto the curtains, which can cause some cells to die off, and also reflects the ability to recover cells by the method used.

Overall a $\log_{10}$ (10 fold) decrease in viable counts was detected at each sampling point. In general there was no very significant difference between the Opal and the competitor curtains for survival of either *Staph. aureus* or *Ps. fluorescens* over the 25 day period. Mimicking "dirty conditions" (+BSA samples) enabled cells to be recovered at a higher level than with no bioburden and *Staph. aureus* in particular survived at much higher levels when a bioburden was present.

Bioluminescence was used as a potential indicator of bacterial viability which could be measured directly; if the bacteria are viable they produce light which can be measured quantitatively or by imaging. Initial luminometry readings or relative light units (RLU) on Day 0 (Table 3) were above control tests (empty tube blank and MRD+ neutralising buffer). A reduction in RLU could be observed during the sampling time however the levels of RLU were insufficiently above the control RLU after Day 0 to be of any scientific importance and so this approach was less useful in considering survival than the viable counts.
With some of the test samples plated for viable counts on day 25, no viable organisms were detected however, by further incubating these test samples on Nutrient agar plates and imaging for bioluminescence, low levels of bioluminescent bacteria were detected (as shown in Figure 1). This demonstrates that viable cells can remain on the curtains which are not recovered by the stomaching and plating method used. This was evident when BSA was present and could reflect that the protein acts as a keying layer for cells to attach and potentially form a biofilm which is more resistant to survival. Images of the plate counts from these are shown in Figure 2; recovery of bioluminescent bacteria shows that the isolates are the inoculated strain and not a contaminant.

Figure 1. Bioluminescent images of inoculated test samples after Day 25 and incubated on Nutrient agar plates

Sample ID and test inoculum		**Sample ID and test inoculum**	
Opal Sa 0 cfu ml^{-1}		Competitor Sa 0 cfu ml^{-1}	
Opal Ps 0 cfu ml^{-1}		Competitor Ps 0 cfu ml^{-1}	
Opal Sa+BSA 1.38 x 10^2 cfu ml^{-1}		Competitor Sa +BSA 0.75 cfu ml^{-1}	
Opal Ps+BSA 0 cfu ml^{-1}		Competitor Ps +BSA 0.75 cfu ml^{-1}	
Opal control 0 cfu ml^{-1}		Competitor control 0 cfu ml^{-1}	

 Low levels of bioluminescence blue, high red

Images obtained using Biospace photon counter, by overlaying bioluminescent images over inoculated test samples after Day 25 and further incubated on Nutrient agar plates. Blue areas indicate growth of either either *Pseudomonas fluorescens* Ps or *Staphylococcus aureus SA.*

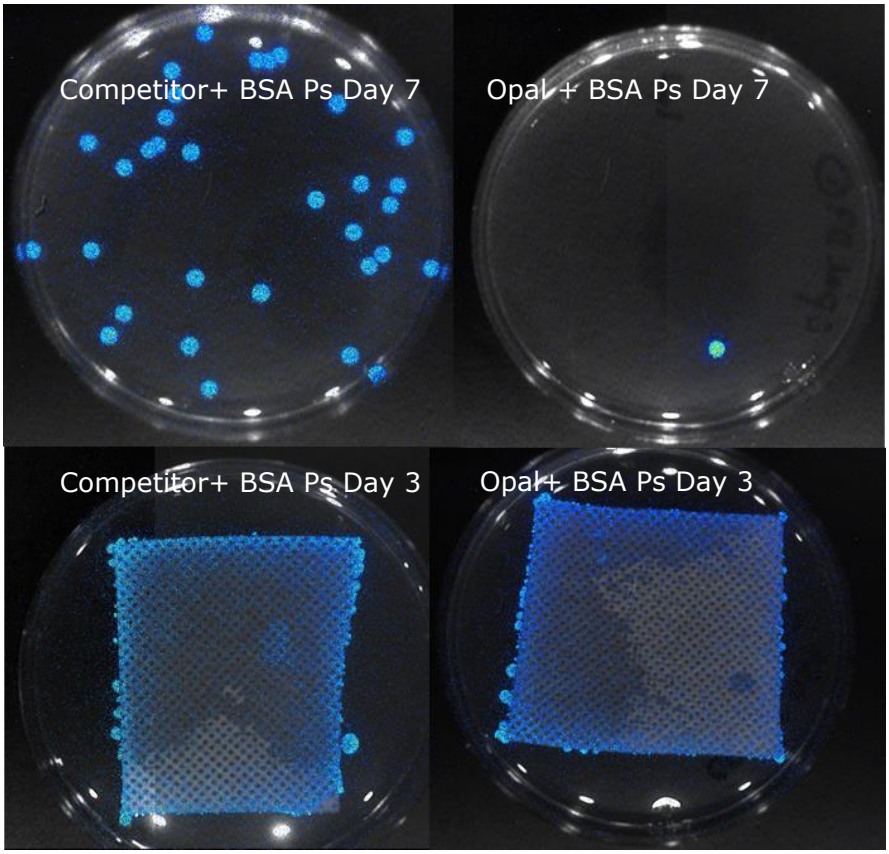
Results from Bioluminescence readings using tube luminometer

Table 3. Relative light units obtained from bioluminescent reporter strains (Reported as average of 1 ml samples from duplicate test pieces)

Sample ID and test inoculum	Day 0	Day 1	Day 3	Day 7	Day 25
Opal Sa*	420	98	106	101	147
Opal Ps	916	101	299	94	134
Opal Sa+BSA	424	117	314	134	99
Opal Ps+BSA	519	306	251	150	175
Competitor Sa	418	90	99	160	110
Competitor Ps	622	116	350	95	114
Competitor Sa+BSA	470	183	149	111	169
Competitor Ps+BSA	669	280	309	217	164
blank	44				
MRD+ neutralising buffer	94				

*Abbreviations: **Ps**: *Pseudomonas fluorescens* **Sa**: *Staphylococcus aureus*

Figure 2. Bioluminescent bacteria recovered from test samples



Top images: Total aerobic plate counts of *Pseudomonas fluorescens* imaged for bioluminescence after incubating aerobically at 30°C ±2 °C for 48 h.

Bottom images: Test samples incubated on Nutrient agar plates after sampling. Bioluminescence confirms all bacterial colonies are *Pseudomonas fluorescens* inoculum

Testing curtains contaminated by natural handling

Swabbing results

Figure 3 Swab results of the Competitor's and Opal's curtains

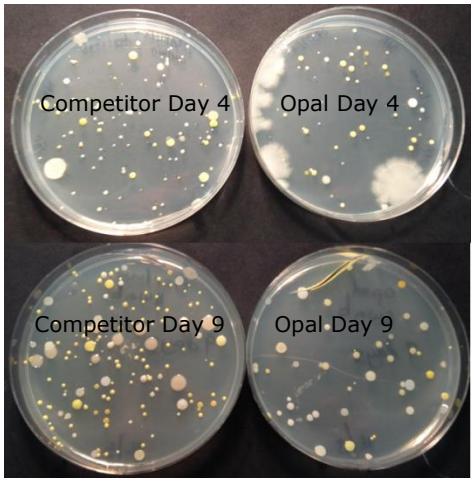


Table 4: Swabbing time post curtain hanging Opal Curtains reported as cfu/100 cm^2

Curtain	Day 1	Day 4	Day 9	Day 31
Competitor	105	102	134	16
Opal	54	75	44	31

Comments

The images of the counts from the curtains naturally contaminated by handling are shown in Figure 3 and the counts obtained by swabbing these samples are shown in Table 4. We anticipated no bacteria would be recovered one day post-hanging of the curtains, however from the results in Table 4 it is evident that bacteria could be detected even after one day and there was either repeated recontamination or survival over the following days which was detected on repeat swabbing of the same curtains. Although this is not a precise count measure, as samples were naturally contaminated and so may have received different levels of bacteria, it is clearly evident that no reduced level of contamination was observed by having an antimicrobial coating even after the first day.

Conclusions

In common with other research groups, we have identified the ability of pathogenic bacteria to survive on hospital privacy curtains. The presence of the particular antimicrobial coating used by the competitor did not appear to be effective in reducing counts when dried-on cultures were applied. The presence of protein applied to mimic biological soiling improved levels of survival. Natural contamination by handling also showed the presence of the antimicrobial coating gave no improvement in reducing bacterial contamination on curtains. This latter study, although less controlled because known counts were not applied, did mimic better the way curtains would be contaminated on a regular basis in hospitals through handling and showed that the antimicrobially coated curtains did not appear to prevent the presence of bacteria any better than the non-treated ones. This work was only done once because of the time constraints but it is an approach which could prove useful for further studies. Another area which would be interesting to investigate is the transferability of bacteria from curtains to other surfaces via handling.

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