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RE: Laboratory report for contract services between Dr Andrew McDowell and C-TRIC (Maranna Sweeney) on behalf of Medi-Shower™

Assessment of the antibacterial properties of the Medi-Shower™ hose

- **Original research question:**
  Does the Medi-Shower™ hose (containing Biomaster™ antimicrobial technology) display anti-bacterial properties against a bacterial solution that has been lying in the system for 7 days (to mimic non-sterile stagnant water), versus other commercially available domestic shower hoses.

- **Null Hypothesis:**
  There is no difference in the anti-bacterial properties of Medi-Shower™ against non-sterile stagnant water versus other commercially available shower systems.

Brief Overview of Agreed Experimental Approach:

- ***Choice of bacterium***
  To address the null hypothesis, we utilised the Gram-negative bacterium *Pseudomonas aeruginosa* in our experiments. We chose this organism as it is an opportunistic pathogen that is frequently present in hospital water systems, where it has the potential to grow relatively quickly and achieve very high cell concentrations that remain stable for long periods of time (Favero *et al*., 1971). The bacterium has been responsible for a range of infections (e.g., burn wounds, urinary tract, neonates) within the hospital setting due to contamination of water systems (Kolomos *et al*., 1993; Ferroni *et al*., 1998; Walker *et al*., 2013).

- ***Artificial contamination of hoses***
  To mimic the situation where *P. aeruginosa* is present in an 'unflushed' shower hose, we artificially contaminated the Medi-Shower™ hose and a standard shower hose (the control) with an aqueous suspension (quarter strength Ringer's solution) of the bacterium [~10^4 colony forming units (CFU) per ml], which was left for a maximum of 4 days at room temperature. We then compared both hoses for levels of the bacterium at t=2 hrs and t=4 days to prove/disprove our null hypothesis.

Results:

Preparation of aqueous contaminating solution:

A fresh overnight culture of the *P. aeruginosa* clinical isolate strain PA0049 was prepared in nutrient broth. An inoculating suspension of the fresh culture in sterile quarter strength Ringer's solution was then prepared (final concentration of ~ 6 x 10^4 CFU/ ml; t=0). Average total bacterial counts up to 10^4 CFU/ ml have been observed in shower water within hospital settings (Perkins *et al*., 2009)

Artificial contamination of hoses:

The hoses were initially rinsed with sterile quarter-strength Ringer's solution to remove any potential debris/ extraneous material and then left to dry at room temperature. The hoses were then filled to capacity with this bacterial suspension (as requested during initial discussions). The Medi-Shower^{Tm} hose was able to accommodate 100 ml of the suspension, while the standard shower hose held 80 ml*. The hoses were then sealed at both ends with parafilm and left suspended between two laboratory retort stands at room temperature (both ends at the same height).

*(the standard shower hose was made of reinforced PVC)

Bacterial counts:

Samples were initially analysed for the levels of *P. aeruginosa* within the hoses at t=2 hrs (Table 1) by colony counting.

Table 1. *Bacterial counts at t=2 hrs for the Medi-Shower^{TM} and a standard shower hose*

Time	Medi-Shower^{TM}	Standard shower hose
0 hrs	6.3 x 10^4 ± 7.57 x 10^3 CFU/ ml	6.3 x 10^4 ± 7.57 x 10^3 CFU/ ml
2 hrs	4.0 x 10^4 ± 1.04 x 10^4 CFU/ ml	4.3 x 10^4 ± 6.11 x 10^3 CFU/ ml

At t=2 hrs we observed an initial small drop in the number of organisms cultured from the Medi-Shower^{TM} hose, but this was also observed in the standard shower hose and was not statistically significant (Student's unpaired t-test; $p>0.05$). The hoses were then left until t= 4d and re-analysed for *P. aeruginosa* levels (Table 2).

Table 2. *Bacterial counts at t=4 days for the Medi-Shower^{TM} and a standard shower hose*

Time	Medi-Shower^{TM}	Standard shower hose
0 hrs	6.3 x 10^4 ± 7.57 x 10^3 CFU/ ml	6.3 x 10^4 ± 7.57 x 10^3 CFU/ ml
4 days	2.3 x 10^5 ± 1.96 x 10^4 CFU/ ml	6.3 x 10^6 ± 3.06 x 10^5 CFU/ ml

At t=4 days we observed an increase in the levels of *P. aeruginosa* in both hoses compared to t=0, but levels in the standard shower hose were significantly higher (30-fold) compared to Medi-shower^{TM} (Student's unpaired t-test; $p<0.001$). When compared

with the initial levels of *P. aeruginosa* at t=0, an overall 100-fold increase in bacterial levels within the standard shower hose product was detected, compared to a much smaller 3-fold increase within the Medi-Shower™ hose (Figure 1.)

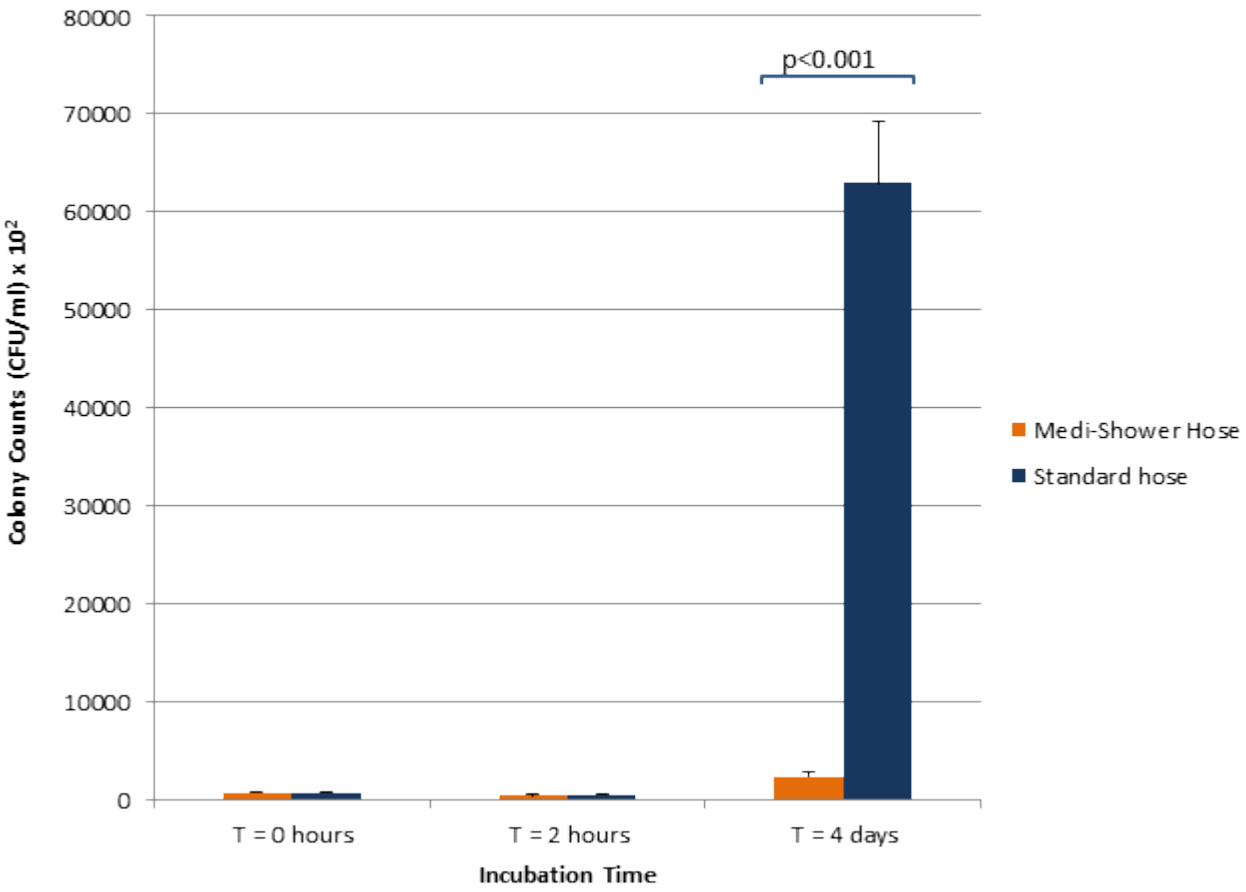

Figure 1. *Graphical representation of the differences in P. aeruginosa colony counts between the Medi-shower™ hose and a standard shower hose after 4 days at room temperature*

Conclusions:

Our study has disproved our original Null hypothesis and shown that the Medi-Shower™ hose has anti-bacterial properties against non-sterile stagnant water when compared with another commercially available shower hose. The Medi-Shower™ hose had significantly reduced numbers of *P. aeruginosa* growing within it over a 4 day period at room temperature when compared against the standard shower hose product. The results are therefore consistent with the antibacterial effects of the Biomaster™ technology previously described (http://www.biomastertechnology.com/). While we also observed an increase in *P. aeruginosa* levels in the Medi-Shower™ hose over the 4 day period (3-fold), it is possible this may not have occurred if lower loads of bacteria were initially present at t=0 (10-to-10^3 CFU/ml); the ratio of Biomaster™-to-bacteria would be higher in this instance. We can speculate the latter scenario may occur when

'fresh' water with lower bacterial levels enters and rests within the system after flushing. While we had originally planned to look at concentrations of the bacterium for up to 7 days, we decided not to progress beyond t= 4 days as the large differences observed between the hoses at this time point enabled us to successfully answer our original hypothesis.

REFERENCES

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