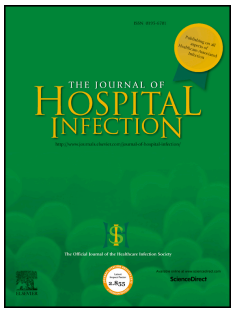
Accepted Manuscript



Tolerance of *Pseudomonas aeruginosa* in in vitro biofilms to high level peracetic acid disinfection

Ayorinde Akinbobola, Leighann Sherry, William G. Mckay, Gordon Ramage, Craig Williams

PII: S0195-6701(17)30346-8

DOI: 10.1016/j.jhin.2017.06.024

Reference: YJHIN 5147

To appear in: *Journal of Hospital Infection*

Received Date: 3 March 2017

Accepted Date: 17 June 2017

Please cite this article as: Akinbobola A, Sherry L, Mckay WG, Ramage G, Williams C, Tolerance of *Pseudomonas aeruginosa* in in vitro biofilms to high level peracetic acid disinfection, *Journal of Hospital Infection* (2017), doi: 10.1016/j.jhin.2017.06.024.

Tolerance of *Pseudomonas aeruginosa* in in vitro biofilms to high level peracetic acid disinfection

Ayorinde Akinbobola[1], Leighann Sherry[1,2], William G Mckay[1], Gordon Ramage[2], Craig Williams[1]

[1] Institute of Healthcare Policy and Practice, School of Health, Nursing, and Midwifery, University of the West of Scotland, Paisley, UK. [2]Oral Sciences Research Group, Glasgow Dental School, School of Medicine, Dentistry and Nursing, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK.

Key words: Peracetic acid, disinfectant, biofilm, resistance

Running title: Can *P. aeruginosa* biofilms survive high level disinfection?

Corresponding Author: Craig Williams, Institute of Healthcare Policy and Practice, School of Health, Nursing and Midwifery, University of the West of Scotland, Paisley, UK. Tel: (+44) 01418483000, email: craig.williams@uws.ac.uk

Summary

Background: Biofilm has been suggested as a cause of disinfection failures in flexible endoscopes where no lapses in the decontamination procedure can be identified. To test this theory, the activity of peracetic acid (PAA), one of the commonly used disinfectants in the reprocessing of flexible endoscopes, was evaluated against both planktonic and sessile communities of *Pseudomonas aeruginosa*.

Aim: To investigate the ability of *P. aeruginosa* biofilm to survive high level PAA disinfection.

Method: The susceptibility of planktonic cells of *P. aeruginosa* and biofilms 24, 48, 96 and 192 h old to PAA was evaluated by estimating their viability using resazurin viability and plate count methods. The biomass of the *P. aeruginosa* biofilms was also quantified using crystal violet assay. Planktonic cells of *P. aeruginosa* were treated with 5 – 30 ppm concentration of PAA in the presence of 3.0 g/L of Bovine serum albumin (BSA) for 5 min. Biofilms of *P. aeruginosa* were also treated with various PAA concentrations (100 - 3000 ppm) for 5 min.

Results: Planktonic cells of *P. aeruginosa* were eradicated by 20 ppm of PAA, whereas biofilms showed an age dependent tolerance to PAA, and 96 h old biofilm was only eradicated at PAA concentration of 2500 ppm.

Conclusion: 96 h old *P. aeruginosa* biofilm survives 5 min treatment with 2000 ppm of PAA, which is the working concentration used in some endoscope washer disinfectors. This implies that disinfection failure of flexible endoscopes could occur when biofilms are allowed to build up in the lumens of endoscopes.

Introduction

High level disinfection (HLD) is used in the decontamination of heat sensitive medical equipment such as flexible endoscopes.[1] It entails the eradication of most microorganisms except for small numbers of bacterial spores and achieves a 6-log reduction of mycobacterium species. [2] This level of decontamination is acceptable for medical devices designated as semi-critical equipment, which according to the Spaulding classification, are devices that only touch the mucous membrane during use.[3, 4] Commonly used disinfectants for HLD include peracetic acid (PAA), glutaraldehyde, ortho-phthalaldehyde, chlorine dioxide and hydrogen peroxide with peracetic acid.[2, 5] Despite the efficacy of these biocides, there are reports of endoscope disinfection failure, where no clear lapses in the decontamination procedures can be identified; most of these involve duodenoscopes used for endoscopic retrograde cholangiopancreatography (ERCP) owing to their complex design.[6-9] Most endoscope disinfection failures are however due to lapses in the decontamination procedure, which may allow biofilm formation in endoscope channels.[10, 11] Endoscope disinfection failure may also be due to the gradual accumulation of organic soils and wear on the surfaces of endoscope channels resulting in reduced exposure of bacteria to biocide and subsequent biofilm formation on endoscope channels.[10, 12, 13] There are evidences that bacterial species can form biofilm in endsocope lumens.[10, 14, 15] Biofilm formation can also occur within an endoscope reprocessor due to faults in it, or inadequate preventative maintenance for the unit. [14]

Biofilm growth enhances the ability of bacteria to survive treatment with antimicrobial agents, due to a number of factors.[16-18] These include reduced penetration of the agent into biofilms due to the presence of extracellular matrix (ECM), biofilm heterogeneity and biofilm specific phenotypes such as expression of efflux pump and persister cells. [19]

Pseudomonas aeruginosa is one of the organism mostly associated with reported cases of endoscope contamination and endoscope associated infection.[20-22] Therefore *P. aeruginosa* was used as a model organism in this study. PAA is an oxidising agent now commonly employed in the disinfection of endoscopes, with highly potent bactericidal and fungicidal activity at low concentrations. [23, 24] In endoscope reprocessing, it is used at a concentration of 2000 to 3500 PPM depending on endoscope disinfection method and guidelines. PAA and other oxidising agents such as hydrogen peroxide have been increasingly adopted in endoscope disinfection due to hazards associated with glutaraldehyde based disinfectant

and emergence of glutaraldehyde resistant *Mycobacterium* species.[25] The aim of this study was to evaluate the efficacy of PAA against bacterial biofilms and examine the effect of biofilm maturity. We demonstrate that 96 hour *P. aeruginosa* biofilms can survive treatment with the concentration of PAA currently used for endoscope disinfection.

Material and Methods

Inoculum preparation

For each experiment, fresh overnight cultures of *P. aeruginosa* PA14 were prepared by inoculating a single colony of the isolates in Luria-bertani broth (Fluka, St. Louis USA) and incubated overnight at 37^{o}C. Cells were recovered from the overnight culture through the centrifugation of 1 mL of the overnight culture at 10,000 x g for 5 min. The resulting pellets were re-suspended in 1 mL phosphate buffered saline (PBS) and washed twice by centrifugation at 10,000 x g for 5 min. The CFU of the inoculum for each experiment was standardised using their OD_{570nm} absorbance.

Neutraliser validation

A neutraliser solution containing 1.0 % w/v sodium thiosulphate pentahydrate (ACROS Organics, Loughborough UK), 0.5 % w/v sodium dodecyl sulphate (BDH Laboratory Supplies, poole UK), 1.0 % w/v granular lecithin (ACROS Organics New Jersey US), 15 % w/v of Polysorbate 80 (ACROS Organics Loughborough UK) and 0.1 % w/v L-Histidine (ACROS Organics New Jersey US) was prepared and sterilised by membrane filtration. Efficacy and toxicity of the neutraliser against *P. aeruginosa* PA14 was evaluated using a suspension challenge method as specified in BS EN 13727 2012+A1 2013.[26]

Biofilm formation

Biofilms were prepared in 24-well plates (CellBIND Clear polystyrene Corning). Briefly, *P. aeruginosa* PA14 cells were recovered from overnight culture as highlighted above. Adjusted cultures were serially diluted in LB broth to obtain a final bacterial concentration of ≈1 x 10^6 CFU/ml. Wells of the 24-well plate ware inoculate with 1 mL of diluted inoculum for biofilm formation. Plates were incubated at 37^{o}C for 24, 48, 96 and 192 h, as appropriate, with spent media aseptically replaced every 24 h.

Peracetic acid treatment

The susceptibility of *P. aeruginosa* PA14 planktonic cells to PAA was evaluated according to the suspension challenge method with slight modifications.[26] The *P. aeruginosa* cell suspension was prepared by adding 0.1 ml of cell suspension in PBS prepared as described above to 0.9 mL of different concentrations of PAA (5 – 30 PPM), containing 3.0 g/L of BSA (mimicking dirty soiling condition) to obtain ≈1 x 10^8 CFU/mL of bacteria in the final suspension. After 5 min exposure time, PAA activity was quenched by adding neutraliser to treated cells for 10 min. Preliminary experiments (data not shown) showed that the neutraliser used in this study (1.0 % w/v sodium thiosulphate pentahydrate, 0.5 % w/v sodium dodecyl sulphate, 1.0 % w/v granular lecithin, 15 % w/v of Polysorbate 80 and 0.1 % w/v L-Histidine) is effective in neutralising the activity of PAA and not toxic to *P. aeruginosa*. For the biofilm assay, biofilms were treated with 1.5 mL of PAA (100 - 3000 PPM) diluted in sterile distilled water for 5 min, then subsequently neutralised for 10 min. The viability of the treated cells was evaluated either through the evaluation of their metabolic activity using the resazurin dye assay or plate count evaluation of cell viability.

Alamar blue resazurin evaluation of metabolic activity

For the resazurin assay, 1 mL of 10% Alamar blue (Invitrogen, Frederick, MD) diluted in LB broth was added to treated cells and incubated in the dark at 37^{o}C with a negative control of 10% alamar blue in LB broth. Based on preliminary optimisation experiments (data not shown), after 6 h of incubation, 100 µL of the 10% alamar blue dye was transferred to 96 well flat bottom plates. The absorbance of the dye was taken at 570 and 595nm using 96 well plate reader (Tecan Infinite F200 PRO). Percentage cell viability was calculated using a viability formula.[27]

Plate count evaluation of colony forming unit (CFU)

The drop plate technique was also used to evaluate the viability of the treated cells on LB agar.[28] 20 µL of serially diluted treated cells re-suspended in 1 mL of PBS were transferred to LB agar plates in triplicates. Plates were allowed to dry at room temperature and subsequently incubated at 37^{o}C. After 24 h of incubation, colonies on plates were counted

and CFU/well of the surviving cells were calculated. For the biofilm experiment, biofilms were scraped into 1 mL of PBS in micro-centrifuge tubes and disrupted in a water bath sonicator (Fisherbrand FB11201) at 37 KHz for 5 min.[29] Sonicated biofilm were vortexed for 30 s, prior to serial dilution and viability evaluated using the drop plate technique.

Quantification of bacterial biofilm biomass

The total biomass of 24, 48, 96 and 192 h old *P. aeruginosa* PA14 biofilms was quantified using a crystal violet assay with slight modifications.[30] Biofilms were grown for 24, 48, 96 or 192 h in 24-well plates as previously described. Biofilms were washed with PBS after the removal of growth media. 1 ml of 0.1% w/v crystal violet was added and plates incubated for 30 min at room temperature. Crystal violet was subsequently removed and biofilm washed with sterile distilled water. Crystal violet remnant binding to the biofilm biomass was dissolved in 1 mL 95% ethanol for 30 min. The absorbance (570nm) of the dissolved crystal violet was measured in a 96 well plate reader (Tecan Infinite F200 PRO) using 100 µl of dissolved crystal violet.

Statistical analysis

All data are presented as mean of three independent replicates ±SD. One way ANOVA was used for statistical analysis using the Graphpad prism 6 with $P < 0.05$ considered as statistically significant**.**

Results

PAA was very active against planktonic cells of *P. aeruginosa* at the soiling condition used for this study. Planktonic cells were killed at a concentration of 20 ppm after 5 min of exposure to PAA (Figure 1). For the planktonic study both cell viability resazurin evaluation of cell viability and the plate count study showed similar results. Initial experiments to evaluate the susceptibility of biofilm of various ages to PAA concentration ranging from 100 - 800 PPM showed that biofilm mediate more than a 100-fold increase in minimum eradication concentration of PAA against *P. aeruginosa* which increase with biofilm age. The minimum concentrations of PAA which killed 24 h and 48 h biofilm were 400 and 800 PPM respectively (Figure 2). However, 800 PPM of PAA which is the highest concentration used

for the initial biofilm experiment only achieved a 1 $\log_{10}$ reduction in the CFU of 96 and 192 h biofilms as shown in Figure 2B. For the aged biofilm experiment, there was no good correlation between cell viability resazurin and the plate count assay. The cell viability resazurin assay for instance showed about 95% and 60% loss in viability of 96 and 192 h biofilms, respectively, treated with 800 PPM of PAA. By contrast,, only a 1 $\log_{10}$ reduction in CFU of the treated biofilm was estimated by the plate count assay, as summarised in Figure 2. Further experiments with concentrations of PAA up to 3000 PPM showed that 96 h biofilms retained viability as assessed by plate count assay at 2000 PPM, but were killed at 2500 ppm (Figure 3).

To evaluate the contribution of biofilm biomass to age associated biofilm tolerance to PAA, the biomass of 24, 48, 96 and 192 h old biofilm was evaluated using the crystal violet quantification assay. Biofilm biomass increased significantly with age from 24 to 192 h ($P < 0.001$). Despite the wide difference in concentration of PAA needed to kill 48 and 96 h old biofilms, there was no significant difference in their biomass quantified using the crystal violet assay (Figure 4).

Discussion and Conclusion

Biofilm associated reduced bacterial susceptibility to disinfectants has been reported[29, 31] but exact mechanisms of this resistance remain to be elucidated. Unlike antibiotics, which often target specific bacterial pathways, disinfectants have non-specific activity and target multiple cellular components or metabolic pathways simultaneously. [32] This has led to a relaxed attitude to potential disinfectant resistance as it is assumed that the recommended concentration of disinfectants is always well above the level required to kill the target organism. However, this thinking ignores the potential effects of the biofilm phenotype. High level disinfectants such as PAA used in the reprocessing of semi-critical medical equipment are active against vegetative bacteria, mycobacteria, bacterial spores, fungi and viruses.[2] However, the presence of organic debris and biofilm extracellular matrix (ECM) can significantly reduce the activity of disinfectant underlining the importance of the physical cleaning step prior to disinfection.[33] Here we have shown that the biofilm phenotype mediates a 100-fold increase in the minimum eradication concentration of PAA against *P. aeruginosa* biofilms bringing the concentration needed to kill the organism to the

working concentration of the disinfectant (which ranges between 2000 and 3500 PPM depending on the reprocessing procedure). Reduced biofilm susceptibility to PAA in other bacterial species have also been shown [17, 34, 35], and previous studies have compared the susceptibility of the planktonic form of these bacteria and their biofilm phenotype reporting similar or even higher susceptibility of biofilm bacteria to PAA. [36, 37] However, these studies did not consider the reactivity of PAA with culture media used in the planktonic experiments.

We have found that the concentration of PAA required to eradicate *P. aeruginosa* biofilm is dependent upon the age of the biofilm. A similar observation has been reported by Cabeca *et al*, who tested benzalkonium chloride *against Staphylococcus aureus* biofilm. Age related resistance to glutaraldehyde, ahigh level disinfectant also used in endoscope disinfection, has been reported in *P. aeruginosa* and *P. flourescens.* [38] Resistance to disinfection may be due to the components of the ECM, which react with the disinfectant before the agent reaches the bacterial cell surface. If this is the case then higher biomass should result in reduced effectiveness of disinfection. However, we have shown that the difference in biomass between the 48 h and 96 h old biofilms is not significant compared with the difference between 24 h and 48 h biofilms, yet there is a wider difference in the susceptibility of 48 h and 96 h biofilm to PAA, suggesting that other biofilm associated mechanisms cannot be ruled out.

In conclusion, our study shows that mature biofilms may not be killed by currently recommended concentrations of PAA. However, for this to occur bacterial contaminants must be left to mature into biofilms for prolonged periods, 96 h in our system. In practical terms this would be any organisms left in endoscope channels for prolonged periods, for example biofilm build-up in endoscope channels due to endoscope reprocessing failure lasting for days before detection and subsequent rectification. However, if disinfection occurs daily biofilms sufficiently mature to cause problems with currently used concentrations disinfectants should not occur. To this end, periodic microbiological monitoring of endoscope as prescribed by some guidelines should be encouraged as a control measure in endoscope reprocessing. [39, 40] However a sampling and detection method that can detect possible microbial biofilm build up in endoscope lumen should be employed. Likewise regular servicing of endoscopes to avoid biofilm build up should be encouraged. This study examined the susceptibility of biofilm grown in multiple well plates

using LB replaced daily. However, biofilm build-up in endoscopes occurs under more stringent conditions; this study therefore depicts a worst case scenario on biofilm in endoscopes.

Refrences

1. Petersen BT, Chennat J, Cohen J, Cotton PB, Greenwald DA, Kowalski TE, et al. Multisociety guideline on reprocessing flexible gastrointestinal endoscopes: 2011. Gastrointestinal endoscopy. 2011;73(6):1075-84.
2. Rutala WA, Weber DJ, Control CfD. Guideline for disinfection and sterilization in healthcare facilities, 2008: Centers for Disease Control (US); 2008.
3. Spaulding EH, Cundy K, Turner F. Chemical disinfection of medical and surgical materials. Disinfection, sterilization, and preservation: Lea & Febinger; 1977. p. 654-84.
4. Rutala WA, Weber DJ. New developments in reprocessing semicritical items. American journal of infection control. 2013;41(5):S60-S6.
5. Foliente RL, Kovacs BJ, Aprecio RM, Bains HJ, Kettering JD, Chen YK. Efficacy of high-level disinfectants for reprocessing GI endoscopes in simulated-use testing. Gastrointestinal endoscopy. 2001;53(4):456-62.
6. Epstein L, Hunter JC, Arwady MA, Tsai V, Stein L, Gribogiannis M, et al. New Delhi metallo-beta-lactamase-producing carbapenem-resistant Escherichia coli associated with exposure to duodenoscopes. Jama. 2014;312(14):1447-55.
7. Wendorf KA, Kay M, Baliga C, Weissman SJ, Gluck M, Verma P, et al. Endoscopic Retrograde Cholangiopancreatography-Associated AmpC Escherichia coli Outbreak. Infection Control and Hospital Epidemiology. 2015;36(6):634-42.
8. Qiu L, Zhou Z, Liu Q, Ni Y, Zhao F, Cheng H. Investigating the failure of repeated standard cleaning and disinfection of a Pseudomonas aeruginosa-infected pancreatic and biliary endoscope. American Journal of Infection Control. 2015;43(8):E43-E6.
9. Gastmeier P, Vonberg RP. Klebsiella spp. in endoscopy-associated infections: we may only be seeing the tip of the iceberg. Infection. 2014;42(1):15-21.
10. Pajkos A, Vickery K, Cossart Y. Is biofilm accumulation on endoscope tubing a contributor to the failure of cleaning and decontamination? Journal of Hospital Infection. 2004;58(3):224-9.
11. Humphries RM, McDonnell G. Superbugs on duodenoscopes: the challenge of cleaning and disinfection of reusable devices. Journal of clinical microbiology. 2015;53(10):3118-25.
12. Hervé RC, Keevil CW. Persistent residual contamination in endoscope channels; a fluorescence epimicroscopy study. Endoscopy. 2016.
13. Alfa MJ, Olson N, DeGagne P, Jackson M. A survey of reprocessing methods, residual viable bioburden, and soil levels in patient-ready endoscopic retrograde choliangiopancreatography duodenoscopes used in Canadian centers. Infection Control & Hospital Epidemiology. 2002;23(04):198-206.
14. Roberts CG. The role of biofilms in reprocessing medical devices. American Journal of Infection Control. 2013;41(5):S77-S80.
15. Kovaleva J, Meessen NEL, Peters FTM, Been MH, Arends JP, Borgers RP, et al. Is bacteriologic surveillance in endoscope reprocessing stringent enough? Endoscopy. 2009;41(10):913-6.
16. Stewart P, Rayner J, Roe F, Rees W. Biofilm penetration and disinfection efficacy of alkaline hypochlorite and chlorosulfamates. Journal of Applied Microbiology. 2001;91(3):525-32.
17. Vázquez-Sánchez D, Cabo ML, Ibusquiza PS, Rodríguez-Herrera JJ. Biofilm-forming ability and resistance to industrial disinfectants of Staphylococcus aureus isolated from fishery products. Food Control. 2014;39:8-16.
18. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. International journal of antimicrobial agents. 2010;35(4):322-32.
19. Stewart PS, Costerton JW. Antibiotic resistance of bacteria in biofilms. The lancet. 2001;358(9276):135-8.
20. Kovaleva J, Peters FT, van der Mei HC, Degener JE. Transmission of infection by flexible gastrointestinal endoscopy and bronchoscopy. Clinical microbiology reviews. 2013;26(2):231-54.

21. Machado AP, Pimenta ATM, Contijo PP, Geocze S, Fischman O. Microbiologic profile of flexible endoscope disinfection in two Brazilian hospitals. Arquivos de gastroenterologia. 2006;43(4):255-8.
22. Kampf G, Fliss PM, Martiny H. Is peracetic acid suitable for the cleaning step of reprocessing flexible endoscopes? World journal of gastrointestinal endoscopy. 2014;6(9):390.
23. Park E, Lee C, Bisesi M, Lee J. Efficiency of peracetic acid in inactivating bacteria, viruses, and spores in water determined with ATP bioluminescence, quantitative PCR, and culture-based methods. Journal of water and health. 2014;12(1):13-23.
24. van der Veen S, Abee T. Mixed species biofilms of Listeria monocytogenes and Lactobacillus plantarum show enhanced resistance to benzalkonium chloride and peracetic acid. International Journal of Food Microbiology. 2011;144(3):421-31.
25. Psaltikidis EM, Leichsenring ML, Nakamura MHY, Bustorff-Silva JM, Passeri LA, Venâncio SI. High-level disinfectants alternative to glutaraldehyde for processing flexible endoscopes. Cogitare Enfermagem. 2014;19(3).
26. EN N. 1040 (2006)-Chemical disinfectants and antiseptics-Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics-Test method and requirements (phase 1). Irradiated Count-Tact 3PTM agar (CT3P) 13006E-en-2013/11 bioMérieux SA. 2013;69280.
27. Pettit RK, Weber CA, Kean MJ, Hoffmann H, Pettit GR, Tan R, et al. Microplate Alamar blue assay for Staphylococcus epidermidis biofilm susceptibility testing. Antimicrobial agents and chemotherapy. 2005;49(7):2612-7.
28. Herigstad B, Hamilton M, Heersink J. How to optimize the drop plate method for enumerating bacteria. Journal of Microbiological Methods. 2001;44(2):121-9.
29. Cochran W, McFeters G, Stewart P. Reduced susceptibility of thin Pseudomonas aeruginosa biofilms to hydrogen peroxide and monochloramine. Journal of applied microbiology. 2000;88(1):22-30.
30. O'Toole GA. Microtiter dish biofilm formation assay. JoVE (Journal of Visualized Experiments). 2011(47):e2437-e.
31. Wong H, Townsend K, Fenwick S, Trengove R, O'Handley R. Comparative susceptibility of planktonic and 3-day-old Salmonella Typhimurium biofilms to disinfectants. Journal of applied microbiology. 2010;108(6):2222-8.
32. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clinical microbiology reviews. 1999;12(1):147-79.
33. Jaglic Z, Červinková D, Vlková H, Michu E, Kunová G, Babák V. Bacterial Biofilms Resist Oxidising Agents Due to the Presence of Organic Matter. Czech Journal of Food Science. 2012;30(2).
34. Castelijn GA, Parabirsing J-A, Zwietering MH, Moezelaar R, Abee T. Surface behaviour of S. Typhimurium, S. Derby, S. Brandenburg and S. Infantis. Veterinary microbiology. 2013;161(3):305-14.
35. Cabeca TK, Pizzolitto AC, Pizzolitto EL. Activity of disinfectants against foodborne pathogens in suspension and adhered to stainless steel surfaces. Brazilian Journal of Microbiology. 2012;43(3):1112-9.
36. Ibusquiza PS, Herrera J, Cabo M. Resistance to benzalkonium chloride, peracetic acid and nisin during formation of mature biofilms by Listeria monocytogenes. Food microbiology. 2011;28(3):418-25.
37. Spoering AL, Lewis K. Biofilms and planktonic cells of Pseudomonas aeruginosa have similar resistance to killing by antimicrobials. Journal of Bacteriology. 2001;183(23):6746-51.
38. Vikram A, Bomberger JM, Bibby KJ. Efflux as a glutaraldehyde resistance mechanism in Pseudomonas fluorescens and Pseudomonas aeruginosa biofilms. Antimicrobial agents and chemotherapy. 2015;59(6):3433-40.

339 39. Saviuc P, Picot-Gueraud R, Sing JSC, Batailler P, Pelloux I, Brenier-Pinchart M-P, et al.
340 Evaluation of the Quality of Reprocessing of Gastrointestinal Endoscopes. Infection Control and
341 Hospital Epidemiology. 2015;36(9):1017-23.
342 40. Force RGT, Petersen BT, Cohen J, Hambrick RD, Buttar N, Greenwald DA, et al. Multisociety
343 guideline on reprocessing flexible GI endoscopes: 2016 update. Gastrointestinal Endoscopy. 2017.

344

345

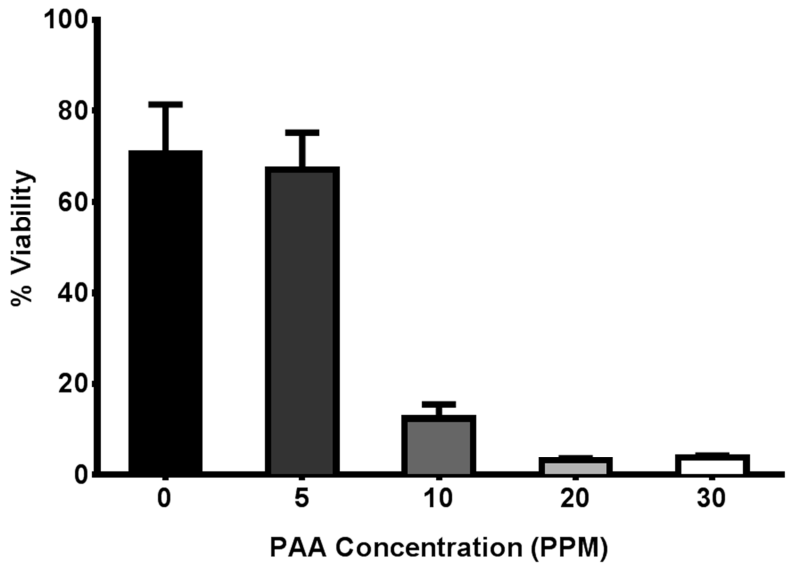
346

1

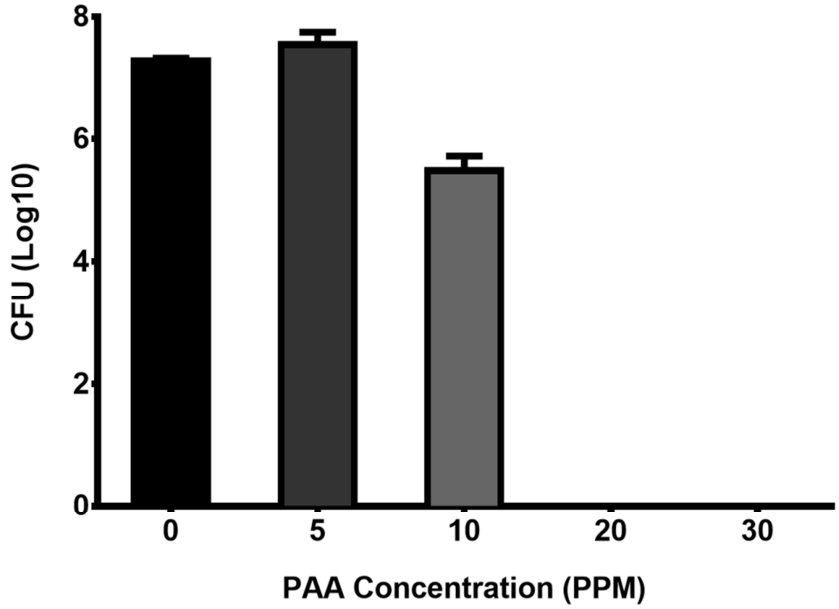
Experiments		*Log10(CFU)*
Efficacy test	Initial Inoculum	2.91 ± 0.14
	Test[a]	2.82 ± 0.06
	Control[b]	0.00
Toxicity test	Initial Inoculum	3.76 ± 0.13
	Test[c]	3.93 ± 0.19

[a]inoculum exposed to neutralised 4000 PPM PAA, [b]inoculum exposed to unneutralised 4000 PPM PAA, [c]inoculum exposed to nuetralisation mixture

1A



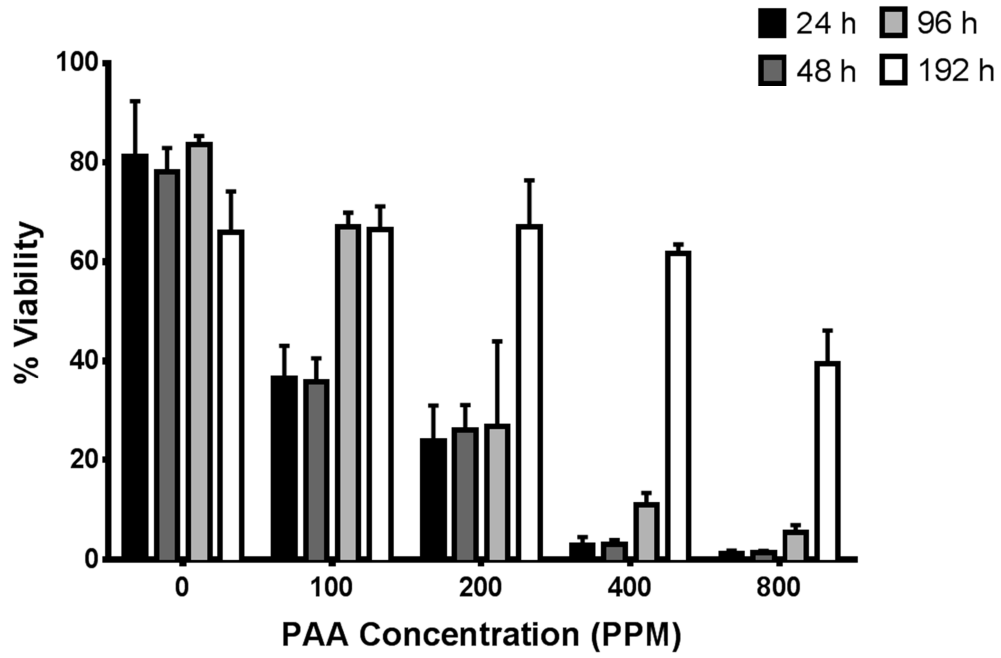
356 1B



357

358

359 2A



360

361

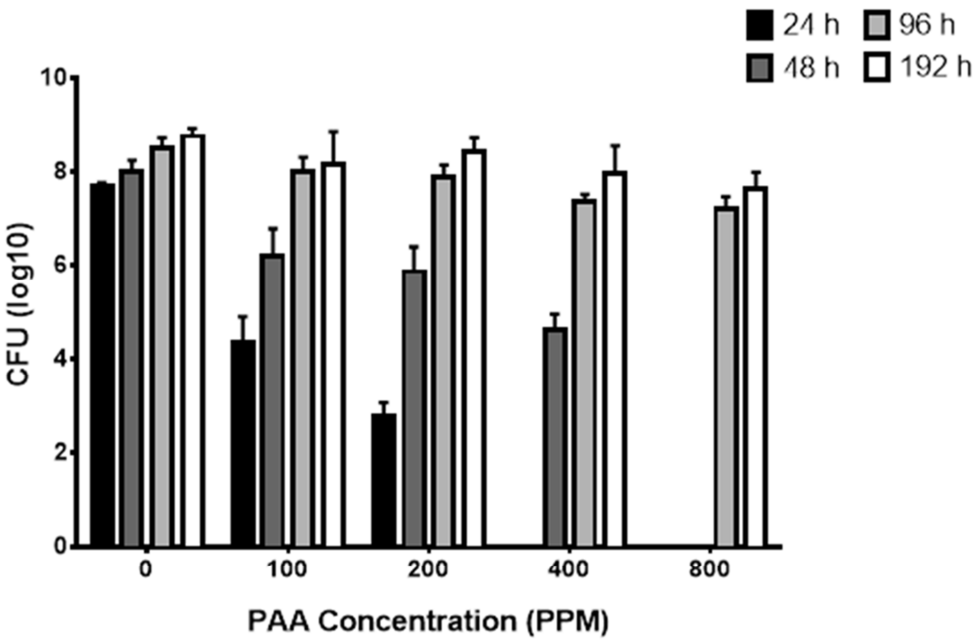
362

363

364

365

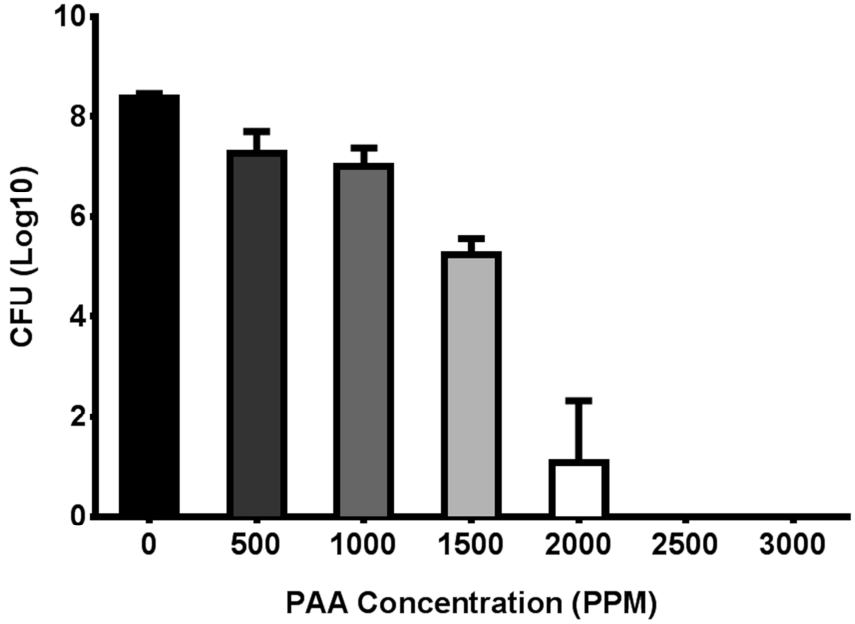
366 2B



367

368

369



370 **3**

371

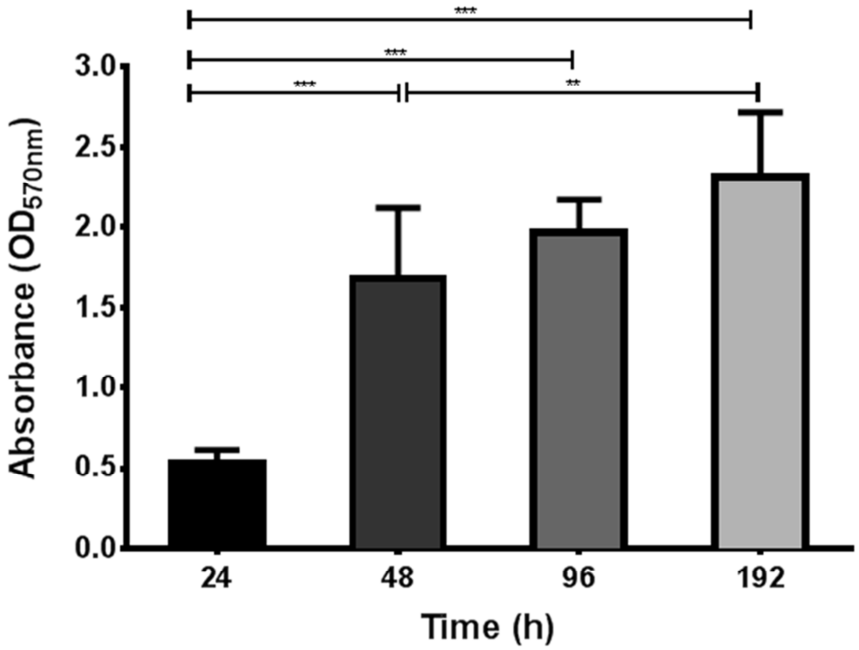
372

373

374

375

4



Legends

Table 1: Evaluation of the efficacy and toxicity of Peracetic acid neutraliser

Figure 1: PAA is very active in killing planktonic cells of *P. aeruginosa* in the presence of 3.0 g/L of BSA mimicking high soiling condition. Evaluation of PAA activity against planktonic cells measured by the resazurin viability assay (A) and the conventional plate count techniques (B) showed good correlation. Graph show the mean data from independent triplicate experiment ± SD.

Figure 2: *P. aeruginosa* biofilm demonstrated an age dependent tolerance to PAA. The evaluation of biofilm susceptibility to PAA using the resazurin viability assay (A) and the conventional plate count technique (B) showed better correlation for young biofilms (24 and 48 hs old) compared to the old biofilm (96 and 192 hs old). The highest concentration of PAA used in this study can only achieve a log reduction in the CFU of aged biofilms. Data is a mean of independent triplicate experiments ±SD.

Figure 3: 96 hs old biofilm is eradicated at 2500 ppm of PAA after 5 minutes' exposure. Biofilm viability after PAA treatment was evaluated by the plate count technique. Graph shown represents the mean of data from three independent replicates with error bar showing ±SD.

Figure 4: Biofilm biomass as evaluated by the crystal violet assay increase with biofilm age. There ws significant increase in biomass from 24 to 48, 96 and 192 hs (***P value <0.001) as well as between the 48 and 192 Hs old biofilm (**Pvalue <0.01). Graph is showing the average of data from indipendent triplicate experiment ±SD

Acknowledgements

We will like to appreciate Ms Tina Bradley (Queen Elizabeth Hospital Birmingham, UK) for her advice and support towards this research.