Research article

An investigation into the relative resistances of common bacterial pathogens to quaternary ammonium cation disinfectants

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Benzalkonium chloride is a common quaternary ammonium cation-based disinfectant used as an industrial-grade biocide, but little independent work has been undertaken quantifying the concentrations required for sterilization. This study investigated relative differences in resistance between common Gram-negative and Gram-positive bacterial pathogens and determined the complete sterilization concentrations for each. A membrane filtration methodology was used to quantify an enriched isolate of deionized water, which was subjected to various concentrations of disinfectant incubated on MacConkey agar. The colony forming units at each concentration were compared to an untreated control. Three main trends, defined as ‘phases of inhibition’, were observed across all isolates studied. Phase I occurred from 0 to 1 mL disinfectant/L water and displayed a moderate, consistent rate of inhibition. Phase II occurred from 0.1% to 0.4% biocide in solution and was characterized by a dramatic increase in inhibition and a divergence of inhibition rates for each organism. Phase III occurred from 0.4% biocide in solution onward and was characterized by the gradual decline in rate of inhibition until each organism reached total inhibition. It was found that the Gram-negative group, comprising *Escherichia coli* and *Pseudomonas aeruginosa*, was generally more resistant than the Gram-positive group, comprising *Enterococcus faecalis* and *Staphylococcus aureus*, *p* < 0.001, with the individual Gram-negative organisms, having the highest complete sterilization concentrations. It was also observed that a variation in resistance existed between organisms of the same Gram stain group. This resulted in some organisms exhibiting resistances comparable to that of organisms of the opposite group, namely between the *E. faecalis* and *P. aeruginosa*, which exhibited no significance difference, *p* = 0.080. Therefore, a model is proposed in which the Gram stain groups can be generalized as being distinct in terms of intrinsic resistance, but also that the range of resistance exists as a spectrum within each group which can cause a similarity between individual organisms of different groups.

Key words: *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, membrane filtration, benzalkonium chloride

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Introduction

Quaternary ammonium cations (QACs) are an extremely potent, versatile family of disinfectants commonly used as industrial grade biocides. Biocleanse® is one such established brand of QAC-based disinfectants and utilizes benzalkonium chloride (BAC) as its active antimicrobial agent. BAC possesses strong biocidal activity against most major forms of pathogenic microbes including fungi, encapsulated viruses and bacteria. QACs exhibit antimicrobial properties due to a membrane-active interaction which initiates autolysis and results in the leakage of intracellular constituents (Ioannou, Hanlon, and Denyer, 2007). The first materials to evacuate the cell are usually low molecular weight cytoplasmic bodies and later, degradation of heavier components occurs, such as proteins and nucleic acids. This activates autolytic enzymes that disintegrate the cytoplasmic membrane causing lysis (McDonnell and Russel, 1999).

Traditionally, there was a long-held belief that development of bacterial resistance to QACs was extremely unlikely due to its non-specific target of action (Gerba, 2014). The rationale for this being that any attempts by bacteria to evolve a structural or biochemical component to subvert the disinfection mechanism would often be responded to by a different but equally as effective membrane bound reaction on account of the amphiphilic properties of the QAC molecule (Ahlström and Edebo, 1998). Contrary to this, clinical reports of increasingly QAC resistant strains of methicillin-resistant Staphylococcus aureus have been on the rise in recent years, particularly in nosocomial environments (Minbile et al., 2016).

Bacterial resistance to disinfectants involves not just inactivating antimicrobials, or inhibiting their penetration, but also removing non-endogenous molecules from the cell as quickly as possible (Heinzel, 1998). In regards to this, the contribution of plasmid and chromosome encoded efflux pumps has been increasingly stated in recent years (Blair et al., 2015). Complex efflux systems are particularly widespread in Gram-negative bacteria. Most have no known QAC detoxifying properties, but isoforms of the resistance-nodulation-cell division (RND) superfamily, outer membrane factor lipoprotein family and membrane fusion protein family are transporters known to be involved in the detoxification process of a number of compounds, including ionically charged disinfectants (Piddock and Webber, 2003). These effluxes are particularly abundant in Escherichia coli, Pseudomonas aeruginosa and Salmonella enterica serovar typhi, of which there remain a number of homologues whose specificities are yet to be established (Poole, 2001).

Other potential explanations for the development of QAC resistance in bacteria have been proposed, including surface charge alterations and hydrophobicity changes in the local cellular environment, which have been examined in S. enterica serovars enteritidis, typhimurium and virchow (Hilton and Braoudaki, 2005). Despite this, efflux pump overexpression studies have been predominant in more thoroughly explaining bacterial resistance to QACs and appear to be remaining prevalent in current studies in the field.

The primary mechanism by which resistance in bacteria develops to QACs is prolonged exposure to sub-inhibitory concentrations due to intensified selective pressure, which is especially significant throughout exposure during the cellular exponential phase (Ibusquiza et al., 2012). This has also been shown to contribute to biofilm formation (Ortiz, López, and Martínez-Suárez, 2014), which is noteworthy as bacteria grown in a biofilm can be up to 1000 times more resistant to biocides than cultured bacteria (Bastian, Alabouvette, and Siz-Jimenez, 2009).

Gram-negative bacteria have been shown to possess higher complete sterilization concentrations than Gram-positive bacteria when exposed to BAC, therefore there is a greater probability of the organism being exposed to a sub-lethal concentration. Considering this, it is not surprising that Pseudomonas spp. have been shown to quickly develop resistance to QAC-based compounds in hand sanitizers (Mousavi et al., 2016). Furthermore, the rate at which this resistance develops in P. aeruginosa has been observed to slow considerably with inhibition of multidrug resistance efflux pumps (Lomovskaya et al., 2001).

The extent of the differences in resistance to BAC between Gram-positive and Gram-negative bacteria remains unquantified and there is no information regarding the complete sterilization concentrations stated in the product testing investigations of Biocleanse®. Consequently, this knowledge would better guide the decision of determining the most appropriate dilution factor to counteract the development of resistance due to the use of sub-inhibitory concentrations. Simultaneously, it may also reduce toxicity risk to individuals and the environment due to the use of excessively high concentrations.

The objective of this study was to determine the minimum concentrations of BAC required for complete sterilization of a number of common bacterial pathogens in vitro, and investigate the extent and variation in resistance between organisms of different Gram stain categories. Escherichia coli and P. aeruginosa constituted the Gram-negative members, and Enterococcus faecalis and S. aureus were used as their Gram-positive counterparts. The role of these organisms as opportunistic human pathogens is becoming increasingly relevant as they emerge as members of the next generation of ‘superbugs’ (Skalweit, 2008). Therefore, establishing the intrinsic resistance patterns and subsequently, quantifying how to ensure sterilization of them, is an important prerequisite to limiting development of resistance. This is particularly the case in nosocomial environments, where BAC-based agents are most frequently employed, and also where a significant reservoir of infection for many of these pathogens exists.

The alternative hypothesis developed for this study is that Gram-negative bacteria will exhibit greater resistance to disinfection attempts than Gram-positive bacteria and will demonstrate a greater complete sterilization concentration.
Methods

Bacterial strains

The organisms, E. coli (ATCC 11775), P. aeruginosa (ATCC 9027), E. faecalis (ATCC 19433) and S. aureus (NCTC 6571), were propagated within a maximum recovery diluent (MRD) broth at 22°C (±1°C). Where available, all strains were known disease-causing human pathogens of a biosafety level of two, signifying a potential mild disease causing effect, but difficult to contract via aerosolization, thus minimizing risk to the operator (Emmert, 2013). In regards to the E. coli strain, a pathogenic strain was not obtainable. As a consequence of this, the type strain ATCC 11775 was chosen instead.

The organisms were supplied as Vitroid® discs from the Sigma-Aldrich Corporation (Gillingham, UK). Vitroids® are certified reference material in the form of a water soluble matrix, inoculated with a defined quantity of viable microorganisms. Once the discs were fully dissolved within the MRD, they were agitated at 1500 revolutions per minute using a vortex mixer for approximately 15 s to prevent bacterial adhesion to tube surfaces. Four standard solutions were prepared from the 100 colony forming unit (CFU) Vitroids®, each containing an individual isolated species. This was performed by decanting the enriched MRD into 1 L of sterile deionized water. Isolates were homogenized vigorously prior to any inoculation, ensuring an even distribution of organisms throughout the vessel.

Membrane filtration

A prerequisite of using Vitroid® discs required inoculum volumes greater than 10 mL. Using volumes below this threshold was found to produce inconsistent and unreliable results. Directly pipetting this amount on an agar plate would saturate absorption of the liquid into the agar, and using a volume tolerated for absorption into the agar would not be sufficient to fulfill the aforementioned volumetric requirement. A membrane filtration technique was adopted as it allowed a large inoculum to be used through fixing the organisms onto a filter which was then placed onto the agar for incubation.

An enriched isolate (10 mL) was filtered through a nitrocellulose membrane with a 0.45 nm pore diameter at a pressure of 60 kPa. It was ensured that only one valve was opened at a time to maintain constant pressure, and closed immediately after the water was fully filtered.

The membranes were aseptically removed from the filtration bases and applied to MacConkey agar plates using a gentle rolling technique. A number of different media able to culture all target organisms at identical incubation parameters were trialled, and MacConkey agar was the most selective media trialled that exhibited a complete sterilization profile within 95% confidence intervals to that of a non-selective nutrient agar control. An uninoculated plate filtered only with 100 mL of sterile water was produced at the beginning and end of each filtration period as negative controls.

Disinfectant treatment

The Biocleanse® concentrate was obtained from Metlab Supplies (Deeside, UK). The disinfectant concentrations investigated in this study were produced through sequential serial dilution of the Biocleanse® concentrate, altering the disinfectant to sterile water ratio in order to obtain the following concentrations; 1.2%, 1.1%, 1%, 0.75%, 0.6%, 0.5%, 0.4%, 0.23% and 0.1% biocide in solution of concentrate.

The dilutions were prepared in sterile 15 mL culture tubes using a calibrated 1000 μL autopipette and disposable 10 mL pipettes. Once completed, the culture tubes were sealed with sterile microbiological lids and stored at 4°C (±3°C) in a calibrated, thermally monitored refrigerator when not in use.

The various disinfectant dilutions (1 mL) were pipetted onto the membrane using a calibrated 1000 μL autopipette. One millilitre was chosen due to it being the minimum volume tested that fully covered the membrane without oversaturating the plate. This adequately simulated a chemical persistence of the disinfectant that would be experienced by organisms in an uncontrolled environmental setting. Drying was encouraged by rotating and inclining the plate about a central axis in order to ensure an even distribution of disinfectant throughout the membrane, normalizing disinfectant volume per unit area. Once the plates were dry, they were then incubated at 37°C (±1°C) in a cyclic incubator for 24 h (±1 h).

Observation of colonial growth

Upon completion of the incubation cycle, the plates were observed for colonial growth. E. coli exhibited easily visible, red, non-mucoid colonies. P. aeruginosa demonstrated very minute brown coloured colonies that were easily identified under ultraviolet light due to their fluorescent properties. E. faecalis appeared similar to E. coli, sharing the same red colouring, however were observed to be smaller in size. S. aureus appeared as opaque pale pink colonies. Seven repeats were performed and recorded as CFU/10 mL. In order to be able to make comparisons between the responses of the different organisms, a percentage CFU was calculated, with 100% being the CFU observed with 0% biocide in solution for each organism.

Statistical analysis

A Two-Way Analysis of Variance (ANOVA) was used to determine the extent of significance difference between the Gram stain groups and between individual organisms across the full cohort of concentrations. Significance was defined with a 95% confidence interval (p < 0.05).

Results

Gram-positive and Gram-negative cohort

The Gram-negative bacteria were found to be more resistant to disinfection compared with the Gram-positive bacteria.
Fig. 1 shows that 0.7% biocide in solution was the lowest concentration investigated that exhibited total inhibition of Gram-positive bacteria. In comparison, the Gram-negative bacteria exhibited total inhibition was observed at 1.1% biocide in solution due to singular colonies being observed on a minority of plates at 1% biocide in solution.

Between 0% biocide in solution and 0.4% biocide in solution the most significant concentrations for increasing the observed bacterial inhibition were established, as over 90% of the total bacterial inhibition was observed for the Gram-positive bacteria and over 60% of the Gram-negative. After 0.4% biocide in solution, the level of inhibition slowed progressively until total inhibition was observed for both sets of organisms.

Three main phases of inhibition were identified and appeared ubiquitously across all isolates studied. The first phase, appearing between 0% biocide in solution and 0.1% biocide in solution, appeared as a gradual increasing inhibition, with all organism cohorts displaying extremely similar levels of inhibition. The second region, observed from the concentrations 0.1% biocide in solution to 0.4% biocide in solution, exhibited a trend in which all four organisms exhibited a phase of a pronounced increasing rate of inhibition, with the four organism trendlines appearing generally to diverge from one another as concentration progressively increased. The third and final region, established at the concentrations greater than 0.4% biocide in solution, displayed a net increase reduction with increasing disinfectant concentrations, until all organisms reached maximum inhibition.

**Individual organism cohort**

Fig. 2 shows that *E. coli* was more resistant than the *P. aeruginosa* up to 1% biocide in solution, although after this *P. aeruginosa* became more resistant, albeit at 1% colony formation, whereas *E. coli* was fully inhibited. *P. aeruginosa* was found to be the only organism to exhibit growth at the concentrations greater than 0.75% biocide in solution.

*Enterococcus faecalis* was found to be the more resistant out of the two gram-positive organisms, but still less resistant than the two Gram-negative organisms. *Staphylococcus aureus* was least resistant to disinfection out of the entire cohort.

Table 1 shows all statistical comparisons of disinfectant treatment between organisms.

Fig. 3 displays the minimum tested disinfectant concentration in which total inhibition of bacterial pathogens was
observed across all repeats. It can be inferred from the table that the range between the inhibitory concentrations of the two Gram-negative organisms was lower than that of the Gram-positive bacteria. In addition, the total range between the complete sterilization concentrations of the most resistant organism, *P. aeruginosa*, and least resistant organism, *S. aureus*, was determined to be 0.6% biocide in solution. It should be noted however, that trendline interpolation from Figs 1

**Table 1. Correlations between disinfectant concentration and organisms using a two-way ANOVA**

| Parameters tested | Subjects tested           | Mean  | Standard deviation | Significance (p) |
|-------------------|---------------------------|-------|--------------------|------------------|
| Concentration     | Gram stain                |       |                    |                  |
|                   | Gram-negative             | 28.88 | 39.34              | <0.001           |
|                   | Gram-positive             |       |                    |                  |
|                   | *Escherichia coli*        | 32.98 | 39.52              | 0.811            |
|                   | *Pseudomonas aeruginosa*  |       |                    |                  |
|                   | *Enterococcus faecalis*   | 30.60 | 39.59              | 0.005            |
|                   | *Escherichia coli*        | 28.77 | 39.36              | <0.001           |
|                   | *Staphylococcus aureus*   |       |                    |                  |
|                   | *Pseudomonas aeruginosa*  | 28.99 | 39.46              | 0.080            |
|                   | *Enterococcus faecalis*   |       |                    |                  |
|                   | *Pseudomonas aeruginosa*  | 27.15 | 39.16              | <0.001           |
|                   | *Staphylococcus aureus*   |       |                    |                  |
|                   | *Enterococcus faecalis*   | 24.78 | 38.87              | 0.037            |
|                   | *Staphylococcus aureus*   |       |                    |                  |

**Figure 2.** The mean percentage maximum colony formation for each individual organism observed at increasing concentrations of disinfectant. Obtained from data produced by subjecting 100 CFU of filtered isolate to 1 mL and calculating as a percentage of the mean colony formation of a 0% control, ± standard error, *n* = 7.
and 2 indicates that the true minimum complete sterilization concentration may be somewhat lower than stated in Fig. 3.

**Discussion**

**Phases of inhibition**

It is not possible from this study to offer a definitive explanation for the results obtained, but several well documented characteristics relating to the organisms studied may have some involvement in the developments of resistance observed. Therefore, the following discussion offers a speculative account of the cellular and molecular characteristics that could have a contribution to such observations.

During Phase I, it can be inferred that at extremely low sub-inhibitory concentrations, there are simply not enough BAC molecules present in solution to cause significant bacterial inhibition. At these concentrations, the LPS layer or any other membranous characteristics of the Gram-negative bacteria confers no additional resistance to the organism than protective measures exhibited by Gram-positive organisms. At lower concentrations, depolarization of the cell cannot yet be achieved, as a result any inhibitory affect may be accountable to a bacteriostatic effect. This could explain why the inhibition rates at these concentrations were very similar, regardless of organism or Gram group.

The magnitude of inhibition increases at Phase II was anticipated due to the exponential nature of reaction with increasing numbers of molecules per unit area, following Fick’s first law of diffusion (Nikaido, 2000). A wealth of evidence suggests that there are operational limits to the molecular functions of microbial detoxification, which may be the cause of the vastly increasing rate of inhibition between these concentrations. This has been demonstrated in the *E. coli* strain HN1157, where the AcrB pump demonstrated an efflux rate that plateaued at approximate 0.02 nmol mg⁻¹ s⁻¹, indicating that a saturation threshold was met (Nagano and Nikaido, 2008). As a consequence of this, efflux binding will become less effective as the active sites become less available to remove the non-endogenous molecules from the cell. At this point only minimal supplementation of an increase in concentration is required to cause catastrophic detoxification failure and lysis.

The gradual decrease of level of inhibition during Phase III indicated that some members of the bacterial population were particularly resilient at resisting detoxification. Bacterial recovery within the MRD broth likely produced a population of organisms at different magnitudes of reactivation, and thus resilience to disinfection. This adequately simulates the variation in wellbeing of wild populations of bacteria.

**Differences in resistance by Gram stain group**

Gram-negative bacteria exhibited greater resistance to BAC disinfection attempts than Gram-positive organisms. The Gram-positive group experienced a greater, statistically significant, level of inhibition than the Gram-negative group indicating a difference in physicochemical interaction with the disinfectant.

The Gram-positive bacterial cell wall is composed of mainly peptidoglycan which is easily traversed by the BAC molecule, therefore the organism can mount little defence to the invasion of the disinfectant molecules, which have unparalleled access to the cell, resulting in disruption and cellular death (Russell et al., 1998). Gram-negative cell walls are comprised of two membranes reinforced by the expression of LPS on the cellular surface providing an additional protective property.

The twin-membrane structure of Gram-negative bacteria enable efflux pumps to effectively clear lipophilic agents migrating across the periplasm through the use of accessory proteins transecting the periplasmic space (Franklin and Snow, 2013). These protein complexes are known as tripartite pumps and are responsible for an increased efflux capacity in Gram-negatives. Conversely, in Gram-positive organisms, the pump terminals are located between the external environment and internal cell, therefore the migratory capture of exogenous molecules does not occur, and the pump mechanism can only

![Figure 3](https://academic.oup.com/biohorizons/article-abstract/doi/10.1093/biohorizons/hzx008/4055594)
be activated once the disinfectant molecule has penetrated the cell. This results in fewer molecules being excreted due to a lower surface area-to-volume ratio within the cell.

**Differences in resistance by organism**

The high complete sterilization concentration observed in *P. aeruginosa* may have been a result of less-permeable membranous characteristics possessed by the organism. It is understood that the threshold at which BAC becomes bactericidal over bacteriostatic is greater in *Pseudomonads*, which may account for the poorer sterilization response until higher concentrations were met. Such properties contributing to this includes membrane porins which only facilitate the entry of low molecular weight compounds, inhibiting entry of QACs which are typically relatively heavy due to a long alkyl chain (McDonnell and Russel, 1999). BAC has been shown to exclude divalent cations from the outer membrane, which *P. aeruginosa* has in abundance in the form of Mg$^{2+}$ ions (Tabata et al., 2003). This attenuates the protective effect of the Mg$^{2+}$ ions on forming strong lipopolysaccharide linkages (Russell, 2001). This may explain why throughout the second half of the dataset, up to 1% biocide in solution, that *P. aeruginosa* had consistently lower growth than the *E. coli*. It is at this point where mechanical factors are believed to have the greatest contribution and efflux saturation will have reached a maximum.

No significant difference was detected between *P. aeruginosa* and *E. coli* cohorts. Studies have shown that many gram-negative organisms contain homologues of the same efflux pumps and actively exchange them via horizontal gene transfer. In addition, the functionality of foreign effuxes across different genera have been confirmed, as the archetypical MDR *MexA-MexB-OprM* and *MexC-MexD-OprF* efflux systems in *P. aeruginosa* are functional and retain their substrate specificity in *E. coli* (Srikumar et al., 1998). This indicates that some homogeneity between the efflux mechanisms of *P. aeruginosa* and *E. coli*, and possibly similar magnitudes of operation.

The data indicate that there is resistance variation between organisms of the same Gram stain category, as displayed by the significant difference between the *S. aureus* and *E. faecalis*. *Enterococcus faecalis* is known to contain an additional component in its peptidoglycan structure in the form of lysine-alanine side chains (de Pedro, Blanot, and Vollmer, 2008). These repeated sequences of dipeptides have been observed to modify the surface charge density and structural organization of the peptidoglycan caused a decrease in sensitivity to cationic compounds (Grare et al., 2010). This may explain why *E. faecalis* was observed to have a greater resistance than *S. aureus*, despite both organisms being Gram-positive, as BAC is also cationic and therefore subject to the same charge-related interactivity predispositions.

Another plausible reason for the high variation of resistance in the Gram-positive bacteria may be due to differences in the membrane structure imparted during peptidoglycan formation in different Gram-positive species, as it has been observed that the *lipid II* moiety, a precursor molecule involved in peptidoglycan synthesis, can undergo structural modifications conferring changes in the cell envelope charge (Münch and Sahl, 2015). This has been observed to result in alterations in the magnitude of interaction with antimicrobial peptides, which share identical mechanistic and charge characteristics with BAC (Zasloff, 2002). This may explain the fundamental difference in resistance observed between the two Gram-positive organisms, especially as peptidoglycan plays such a large role in the intrinsic protection of Gram-positive organisms to exogenous compounds. Additionally, it may also explain why this difference in resistance was not observed between the Gram-negative organisms, as the peptidoglycan expression in the twin-membrane system makes up a relatively low proportion of the total cell wall mass.

*E. faecalis* was unexpectedly found to have no statistical difference with the *P. aeruginosa*. More research should be undertaken into understanding similarities of interaction with biocides between the *E. faecalis* and *P. aeruginosa*, since as of yet, a plausible explanation for the similarities of interaction has not been widely adopted. Furthermore, this outcome resembles the findings of another study, in which resistance against several β-lactamase inhibitors were examined on *E. faecalis* and *P. aeruginosa*, which were both found to have similar magnitudes of susceptibility (Klepser et al., 1997).

**Limitations and directions for future study**

It is necessary to consider the feasibility of the 37°C incubation temperature causing interactions of a magnitude non-comparable with that when employed in clinical or domestic settings, which have temperatures typically considered to be around 21°C. Despite this, data obtained from a pilot study indicated that *E. faecalis* cultured on Slanetz and Bartley (S&B) agar displayed an identical complete sterilization concentration with those incubated on MacConkey. The incubation parameters of S&B were far lengthier and warmer than MacConkey, indicating that if temperatures around this range did influence biological activity of the disinfectant, it was negligible. The same was observed with *E. coli* on membrane lactose glucuronide agar, which has a shorter, cooler incubation period than MacConkey agar.

One must consider the possibility that the nutritional environment provided by MacConkey agar may select against growth of certain organisms investigated in this study. Regarding this, trial data indicated that the concentration of complete sterilization of MacConkey agar remained within 95% confidence intervals, therefore if there was any inhibition of cellular growth, it did not significantly affect colony formation.

It has been recognized in this study that there is a significant difference between the average Gram-negative and Gram-positive organism, and also that there is a degree of variation and overlap within and between these groups. However, only two organisms of each category were examined in this study, therefore the extent to which this variation is present has not yet been established.
In order to confirm if the double-membraned structure, and other properties unique to Gram-negative bacteria, confers a significantly greater intrinsic resistance, it is necessary to repeat and increase the scope of this study. Additional organisms that are similarly relevant in human bacterial infection should be investigated in order to better understand the magnitude of variation that is present between these organisms.

Furthermore, it is believed that BAC has a bacteriostatic effect at some lower concentrations. An additional useful metric for determining intrinsic resistance would be to establish the endpoints of the bacteriostatic effect at the point where it becomes bactericidal for each organism investigated.

In this study, efflux pumps were postulated to be a significant contributor to the intrinsic differences in resistance observed between organisms. In order to support these results, future studies should implement nucleic acid-probe techniques for the detection of the genes that encode these pumps. Furthermore, inactivation of these genes through site-directed mutagenesis and subsequent evaluation on its effect on sterilization concentration would provide additional support to the conjectures made.

Conclusions

In conclusion, the results demonstrate the well-established phenomenon of increased Gram-negative resistance to biocides, and consequently highlight the increased risk of development of resistance. However, the results also indicate the presence of a spectrum of resistances existing in which Gram-positive bacteria are believed to have an increased range of variants. It is feasible that the relative resistances between groups can be generalized as being distinct, but more importantly, that being categorized as Gram-positive or Gram-negative may not be the absolute determination of magnitude of resistance as previously thought. In order to solidify these findings and identify the extent to which this spectrum exists, study of additional organisms is necessary.

Author biography

Gregory Wickham is a first class honours biology graduate from the University of Chester. He has a particular interest in microbiology and molecular biotechnology and, since 2015, has worked as a microbiologist at ALS Environmental, the United Kingdom’s largest independent provider of environmental analysis. He is currently studying for an MSc in medical microbiology at the University of Manchester and intends to undertake a PhD in molecular microbiology upon the completion of his Master’s degree.

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