Effect of UV pulse light on the growth and resistance of *Pseudomonas* spp.

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I. INTRODUCTION

Pseudomonas spp. are aerobic, gram-negative bacteria that can be isolated from diverse environments, such as water, soil, plants, and animals. *Pseudomonas* spp. besides being a spoilage indicator in meats, can also be opportunistic pathogens, namely *P. aeruginosa* that causes nosocomial and community-acquired lung infections [1,2,3]. Additionally, due to overuse and misuse of antibiotics, *Pseudomonas* spp. have become increasingly more resistant, especially to the carbapenem class of antibiotics and to colistin. Colistin is an antibiotic frequently used as a last resort to treat infections by multidrug-resistant and extensively drug-resistant *Pseudomonas* spp., thus the increase in resistance is very concerning [1,2,4]. This increase in resistance coupled with the extensive presence of these bacteria in the food chain increases the possibility of cross-contamination[1,2]. As such it is vital to find new methodologies to control and eliminate these bacteria from the food processing environment. The UV pulse light technology is an emergent and non-thermal process, generally considered as a surface decontamination technology, but its effectiveness is dependent on several factors [5,6,7]. The aim of this work was to evaluate the influence of a UV pulse light treatment on the growth of carbapenem and colistin-resistant *Pseudomonas* spp. versus the effect of the treatment on the growth of susceptible *Pseudomonas* spp.. In addition, the effect of the treatment on the resistance profile of the strains was also evaluated.

II. MATERIALS AND METHODS

Two *Pseudomonas* spp. isolated from the pork meat chain were utilized, one resistant to meropenem and colistin, the OS1S E9 (*Pseudomonas lactis*), and one susceptible to both antibiotics, TSB5 (*Pseudomonas fragi*). Both species belong to the Laboratory of Food Technology collection of the Faculty of Veterinary Medicine (University of Lisbon). To test the UV light treatment on simulated early biofilm formation on food-grade surfaces, both strains were inoculated, to a concentration of 10^8 bacteria/cm^2, in one square centimeter of food-grade stainless steel surface finish category 2B and left to dry overnight at room temperature. This was done to simulate a *Pseudomonas* biofilm on a food-processing surface. After the incubation, the inoculated discs underwent the UV pulse light treatment. For each strain four different treatments were tested, the control (no treatment), treatment A (2.582 J/cm^2), treatment B (4.303 J/cm^2), and treatment C (7.746 J/cm^2). After treatment, the bacteria were removed from the discs through sonication and vortex agitation with glass beads. The bacteria were then inoculated in *Pseudomonas* CFC medium (Scharlau, Spain) according to ISO 13720:2010. After enumeration, five colonies of each isolate and treatment were recovered to study their antimicrobial profile. The antibiotics tested were meropenem and colistin, utilizing the e-test methodology (Biomerieux, France). The control utilized was *E. coli* ATCC 25922.

III. RESULTS AND DISCUSSION

The effect of the UV pulse light treatments on the two isolates can be seen in Table 1. Overall, for both *Pseudomonas* spp. the treatments were shown to be significantly more effective when in comparison with the control ($\alpha \leq 0.05$). For the strain OS1S E9 the most significantly effective treatment was treatment C (7.746 J/cm^2) which had the highest fluence. The TSB5 isolate was more affected by treatment B,

however it was not significantly different from treatment C (α>0.05). Additionally, it can be seen in treatment B and treatment A, that the resistant strain OS1S E9 was significantly less susceptible to the treatments than the antibiotic susceptible strain TSB5. The results demonstrate that while all treatments had a high bactericidal effect (>5 Log), treatment C was the most effective overall *Pseudomonas* spp..

Table 1. Average values for the enumeration in Log cfu/cm^2 for each treatment and the two *Pseudomonas* spp. OS1S E9 and TSB5.

Strains	Control	Treatment A	Treatment B	Treatment C
OS1S E9	5.77^{a}	2.31^{b}	1.55^{b,c}	0.00^{d}
TSB5	5.09^{a}	1.31^{c}	0.34^{d}	0.69^{c,d}

a,b,c,d – average values followed by different letters represent significant differences (α<0.05). To facilitate the statistics the limit of detection <1 log cfu/cm^2 was assumed as zero.

In this study besides the bactericidal effect of the treatment on the bacteria, it was also evaluated the effect of the treatment on the antibiotic profile of the strains. As mentioned above, the OS1S E9 before treatment was found to be resistant to meropenem and colistin while the TSB5 was found to be susceptible to these antibiotics. After the treatments the recovered colonies were tested for their antibiotic resistance profile against the two antibiotics. It was found that TSB5 isolates recovered after the highest treatment, treatment C, were found to have gained resistance to meropenem. In contrast, the OS1S E9 isolates recovered after treatment A and B were found to be susceptible to colistin. These preliminary results indicate a potential influence of the UV pulse light treatment on the resistance of the bacteria.

IV. CONCLUSION

The results present in this work demonstrated the capacity of this new technology, the UV pulse light, to control *Pseudomonas* spp. in conditions like the food processing environment. The highest treatment with a fluence of 7,746 J/cm^2 demonstrated to be effective independently of the resistance profile of the strains. However, this same treatment led to the appearance of resistance to meropenem in the recovered strains, which can be concerning since it can lead to transmission of resistance in the environment. On the contrary, these preliminary results also demonstrated that the treatment with UV pulse light can also lead to loss of resistance to colistin in resistant isolates. In conclusion, the effect of this emergent technology on the antibiotic profile should be further investigated.

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