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# Effective alternative curing/clearance of biofilm associated and antibiotic resistant bacterial infections: A relevant probiotics model

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## ABSTRACT

Diverse bio-inhibitory agents have been applied as control model for microbial proliferation with specific relevance/capability in effectively balancing various nexus microbiome. However, with recent reports on growing resistance mechanism and health concerns associated with bacterial (*Pseudomonas aeruginosa*) infections, an alternative model for treatment becomes imminent. One suggestive and alternative bio-inhibitory agent of relevance is probiotics which was prepared from the Genus *Bacillus*. This study determine the effective curing/clearing capacity of probiotics; a model in wound infection control and biofilm inhibition. Briefly, biofilm producing *P. aeruginosa* were used to challenge surfaces as infectious-strain, while surface challenge test was done to determine probiotics effectiveness as surface cleaner. *In vitro* antibacterial susceptibility testing using probiotics was done on 21 selected biofilm producing *P. aeruginosa* of clinical and environmental origin. Time kill assays was done at different time interval (0–24 h) and different dilutions (1:10 and 1:20) on biofilm producing strains with probiotics. In addition, probiotics was used as treatment for infected wound dressing to determine its effectiveness. Following statistical evaluations, the surface challenge test showed promising reports with notable probiotics inhibitory properties on tested strains as well as susceptibility testing. Also a reduction/biofilm clearance was observed especially for commercially obtained probiotics used for wound dressing with significant indications. From the foregoing, it is suggested that probiotics are model alternative therapeutic strategic agents for controlling/removing resistant potential pathogens. It may also be a model alternative surface cleaner, surface decontaminant, diverse microbiome control agent, removal of biofilm and treatment of recalcitrant wound infection pathogens with little or no identified side effect on both humans and environment.

## Introduction

Probiotics, a Greek origin word meaning "for life" has been defined by diverse global systems as "live microorganism which on administration to a clinical-based case in adequate amounts would confers a health benefit to any host" [1]. There may occur and be applied as cleaners or antimicrobials/disinfectants. As cleaners, Probiotics are unique blend of fermented bacteria and essential oils that eventually produce compounds called bio-detergents with capacity to hydrolyse contaminants and breaks down impurities. However, it is noteworthy to put in mind that probiotics do not kill all bacteria, which is an important/substantial aspect of its usage [2] and/or application. According to Perkins, the use of probiotics implies "Using 'good' bacteria to eliminate 'bad' bacteria which are/is similar to how kombucha balances

bacteria in the digestive system". "This means that one will not kill 99 % of the gut microbiota (which require active toxins in all cases), but we are preventing other bacteria from getting into our face, as nature has done for billions of years." Suffice it to say that disinfecting a home and killing every good bacterial may result several health problems, including autoimmune diseases and weight gain [3]. Furthermore, strict sanitary rules have also contributed to the medical community's main priority of limiting illness distribution and spread as well as multiple antibiotics resistance [4,5]. As part of a larger effort to reduce microbial resistance and/or transmission in a variety of settings, the "Association for Professionals in Infection Control and Epidemiology (APIC)" has recently implemented stringent infection control guidelines that have been integrated into a system of norms, particularly in hospitals. Hence related investigators emphasized the application of APIC standards for

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clarity while discussing "important components in infection control". No living germs or viruses may exist in a sterile environment except some emerging strains with superbug potentials [6]. In a medical institution/public-health system, this is often performed using machines or chemical agents. In hospital, sterilization methods include high-pressure steam, dry heat, ethylene oxide gas, or liquid chemicals etc which are typically employed [7]. However, variety of circumstances may reduce or even remove the disinfecting effects of treatment processes, including the initial state of cleanliness, the presence of organic matter, the type and number of microorganisms, the germicide concentration and exposure time, the item's physical configuration, the disinfection temperature and disinfection pH, have been discovered to influence disinfection success. These impacts and encourage superbug development and multiple antibiotic resistant organisms in addition to reduce impact on spores [8]. Other factors that may affect the disinfectant's effectiveness against pathogenic organisms include temperature and pH of the disinfection process, the surface material, the chemical properties of the disinfectant or ground, the antibacterial tolerance of the microbes, the fabrication of microbes in biofilm communities, the dosages of disinfection, and the period of exposure to disinfection [9]. Be that as it may, the use of such compounds to control surface pollution has been considered to have significant environmental effects and limitations. Increasing demand for chemical cleaning and disinfection products and evidence of the effects of chemical products on human health are mainly related to disease cases [10–13]. Such potential problems may be resolved by choosing a suitable and alternative bio-agent that works well even in cold weather [9]. Other methods with promising relevance are very expensive, and may not suit all surfaces or not available in low-income countries [13–15].

Furthermore one known disease-causing strain with superbug and surface tolerance capacity is a Gram-negative bacterium called "*Pseudomonas aeruginosa*". It is frequently found in water, soil, healthy individuals and other standard medical instruments, including mechanical ventilators, surgical tools, or catheters for the urine and intravenous systems, were it is implicated in minor to major clinical cases etc [16,17]. Nevertheless, those that are immune-compromised, like those with cancer or AIDS, as well as burn sufferers, are more in danger since they are more prone to diseases like dermatitis, pneumonia, urinary tract infections, and blood infections, which may occasionally be fatal [17,18].

In addition, *P. aeruginosa* has reportedly evolved resistance to widely administered antibiotics and disinfectants, such as "ciprofloxacin", "piperacillin", "gentamicin", and "carbenicillin" [19] and other reports of some cross-resistance between such agents exist. It has also shown aggressive rejection to antibiotic/drugs treatment with frequent therapeutic challenges multi-resistance plasmids [18,20,21].

Diverse related investigators are currently proposing a change in the paradigm using probiotic cleansers with capacity to effectively fight harmful bacteria on the surface and replace them with good bacteria, keep surfaces healthy [22], maintain a healthy immune system and good resistance using bacteria in various nexus [23]. It is to this end the study determine the effectiveness/curing/clearing capacity of probiotics; a model in wound infection control and biofilm inhibition in a bid to understand the interaction of probiotics on *P. aeruginosa* as a microbial agent, disinfectant (surface cleaner) and its effect on biofilms producing organisms.

## Materials and methods

The Study and experiments were carried out in the microbiology laboratory of Manchester metropolitan university United Kingdom. Probiotics used were provided by the WE ARE PROBIOTICS disinfectant company. Experiment was performed on surfaces that people come into daily contact with, i.e., metal surfaces. These steel surface mostly used as door handles or knob were employed. The surface coupons used were contaminated with different strains of *P. aeruginosa* (surface test).

Antibiotic susceptibility testing was done with M26 susceptibility disc and probiotics filter paper against 21 different strains of *P. aeruginosa*.

## Bacterial strains and media

Different strains of *P. aeruginosa* (PAO1, PA111074, PA115531, PA106167, PA115480, PA111070, PA111055, PA111124, PA111050, PA111139, PA115508, PA115524, PA106705, PA115530, PA106176, PA115489, PA115482, PA111053, PA106188, PA106177, and PA111091) used during the study was provided by the laboratory.

## Microbial mortality and viability test

The determination of microbial/organism mortality, Time kill kinetic assay and biofilm kill assay was performed at different times, from 0, 2, 4, 6, and 24 h. In addition, surface challenge test was done and lastly using probiotics as wound dressing was explored.

## Preparation of agar and broth

The preparation of Mueller-Hinton Agar (OXOID) in 1 L of distilled water was done according to the instructions of the manufacturers. *Pseudomonas*-specific agar (OXOID code: cmo559) was also prepared according to the instructions of the manufacturers. To the agar, 7.5 g of agar medium, and 5 mL of glycerol was added in 500 mL of sterile distilled water [24]. After incubation C-N selective supplement was added. Tryptic Soy Agar (OXOID- TSA) was prepared using the instructions from the manufactures (OXOID). Mueller-Hinton Broth (OXOID) was prepared in 1 L of sterile distilled water according to the instructions of the manufacturers. Tryptone Soy Broth (OXOID) was done according to the manufacturer's directives in 500 mL of sterile distilled water.

## Antibiotics and probiotics susceptibility assay

For this study, twenty-one (21) distinct strains of *P. aeruginosa* were used. New cultures of various strains were inoculated into 1 mL of sterile saline, and the turbidity was compared with 0.5 McFarland standards to evaluate if a good flash was required to get the same turbidity as the McFarland standard. 100 µL of the diluent was poured onto a Mueller-Hinton agar plate and distributed using a sterile spatula on the plate. To the plate, an antimicrobial disc containing eight different antibiotics (Ampicillin, Chloramphenicol, Colistin, Kanamycin, Nalidixic Acid, Nitrofurantoin, Streptomycin, and Tetracycline) MAS-TRING-S M26/NCE, was added and incubated at 37 °C for 24 h. Same procedure was done for another set of plates using 20 µL of Probiotics in triplicate on a sterile filter paper and placed on the centre of an agar plate, then incubated at 37 °C for 24 h. A negative control was also conducted which consisted the use of sterile water. After incubation the zones of inhibition was measured both on the plates containing antibiotics disc, negative control disc and the plates containing the probiotics [21].

## Surface challenge test

This study was carried out using contact plates containing *Pseudomonas* selective agar and steel coupons as the surface to contaminate. The steel coupons were contaminated with *P. aeruginosa* strain (PAO1). A pure overnight broth culture of PA 01 was regulated to an OD<sub>600</sub> of 0.1. Approximately 30 µL of *P. aeruginosa* strain PAO1 was pipette on the steel coupon and left on a protected and/or secured glass petri dish to dry for 24 h. 100 µL of probiotics was added to the steel and allowed to dry. The contact plate was pressed on the steel coupon for 5 s and incubated for at 37 °C for 24 h to check for growth on the plate. This same process was repeated using a filtered disinfectant. This experiment was repeated in triplicates alongside with the positive and

negative controls. All plates were labeled appropriately before incubation. To one plate steel coupon containing the probiotics and *P. aeruginosa* strain PAO1, another steel coupon was containing only the *P. aeruginosa* strain PAO1 used, to the other steel coupon was containing only disinfectant and *P. aeruginosa* PAO1 and lastly the steel coupon contained neither *P. aeruginosa* PAO1, the disinfectant or the probiotics.

The viability was determined through dilution series using the Miles and Misra technique on to a Mueller Hinton agar and incubated at 37 °C for 24 h. Colony count was carried out after incubation.

#### Time kill kinetic assay

Time-based elimination tests on probiotics using a well-established method of measuring microbial activity. At 0 and 24 h, the microbial viability was assessed at two probiotic dilutions (1:10 and 1:20, respectively). Using a pure overnight broth culture, 1 mL of *P. aeruginosa* strain PAO1 was regulated to an OD<sub>600</sub> of 0.025. To prepare 10 mL of neat, 9 mL of Mueller-Hinton broth was pipetted into a sterile container, into this container *P. aeruginosa* strain PAO1 with OD<sub>600</sub> of 0.025 was added. Then, to make a total of 10 mL, 1 mL of probiotics was added to the mixture. Two 96-well plates were prepared, one with 200 µL of neat and one with 180 µL of Mueller-Hinton broth. The neat was immediately placed in an incubator set at 37 °C, and subsequent tests were performed at different time intervals (2–24 h, respectively). A spot test was done with the neat and all the diluents using 20 µL for each spot and was repeated in triplicate on Mueller-Hinton agar plates and incubated at 37 °C for 24 h to check for cell viability. The mean of three counts were multiplied by the dilution factor to get the CFU/mL concentration. Colony count was done after incubation. Same process was repeated for the 1:20 dilution.

#### Biofilm kill kinetic assay

*P. aeruginosa* strain PAO1 was first grown overnight in Mueller-Hinton broth. To each of the 96 wells in the plates 100 µL of a pure overnight broth culture was pipette. Approximately 100 µL of probiotics or disinfectant was added to make total diluents of 200 µL in the well. All plates from 2 h to 24 h were placed in the incubator. The cells were decanted from the plate at 0 h and rinsed with water three times to eliminate excess cells. The plate was allowed to dry for 10 mins. Then 125 µL of 0.1 % of crystal violet stain in water was added to the wells and left to incubate for 10–15 mins at room temperature. After incubation plates were rinsed with water three times and allowed to dry before adding 125 µL of a 30 % acetic acid in water solution, the plate was incubated at room temperature for 10–15 mins. To test the absorbance of acetic acid at 550 nm, 125 µL of 30 % acetic acid was transferred to a new 96-well plate and placed in a spectrometric plate reader to get the readings.

#### Probiotics effective test in wound dressing

The CDC reactor used in this test was sterilized at 121 °C for 15 mins with eight (8) polypropylene rods containing 3 polycarbonate samples (a total of 24 samples, each 12.7 mm in diameter). *P. aeruginosa* strain PAO1 was inoculated into 50 mL of sterile TSB to an OD<sub>600</sub> of 1.5, which was then transferred to 450 mL of TSB in the CDC reactor to produce a final concentration of  $1 \times 10^7$  CFU/mL. Serial dilutions were made to control bacterial density and dilutions were spread using the spread plate method. Approximately 400 mL of the bacterial suspension was aseptically transferred to the CDC reactor and incubated for 24 h at 50 rpm at 37 °C.

A treatment sample of 2 × 2 cm tissue culture plate was prepared with 30 µL of probiotics. A standard was prepared in the same manner using 30 µL of filter disinfectant and set aside. The coupon was removed aseptically from the polypropylene rods and transferred to a sterile petri dish containing 20 mL of PBS to wash off loose cells. This process was

repeated 3 times to remove unattached cells. A 2 × 2 cm probiotic-treated dressing with 30 µL (100 % probiotics) was placed in a tissue culture dish above and below with the coupon in the middle (sandwich method) and activated with 1 % of PBS + TSB. Same method was done for disinfectant and control. The plate was covered with parafilm and incubated at 37 °C for 24 h.

After incubation, samples were neutralized for 5 mins at room temperature using 2 mL neutralizing agent, then sonicated for 5 mins to remove bacteria from treated samples. All samples were taken in triplicate. Using the suspension as neat, a dilution series was preformed, and bacterial viability was determined using Miles and Misra technique on to TSA agar. Recovery plates were incubated at 37 °C for 24 h.

#### Statistical analysis

The statistical analysis was conducted using GraphPad. The analysis of variance (ANOVA) was performed along with *t*-test to determine statistical relevance/relationship between antimicrobial and probiotics susceptibility testing using the ANOVA turkey multiple comparison test, the time kill kinetic assay and biofilm time kill assay using *t*-test method and lastly the probiotics as wound dressing using ANOVA turkey multiple comparison test.

## Results

#### Antimicrobial susceptibility test

The susceptibility testing analysis and reports using twenty-one distinct (21) strains of *P. aeruginosa*, and commercially obtained antimicrobial disc containing eight different antibiotics MASTRING-S M26/NCE. (Colistin, Streptomycin, Tetracycline, Probiotics etc) was demonstrated in Fig. 1. The effectively responding agents were described by the keywords as shown below.

Colistin sulphate presents statistically ( $p < 0.0001$ ) the highest zone of inhibition compared to streptomycin, tetracycline, and probiotic against all the *Pseudomonas* PAO1 strains. Inhibition effect of streptomycin was observed only against PA11074, PA 11070 and PA111091 strains. Interestingly, no inhibition effect of streptomycin, tetracycline and probiotic were seen against PA106705 and PA106177 strains. Inhibition zone on PA106176, PA111124, PA 115508 was detected upon treatment only with colistin sulphate and tetracycline. Furthermore, PA1110754 and PA111091 strains showed sensitivity against all the antibiotics used, however, no inhibition zone was observed against the probiotic. It is Noteworthy to state that only probiotics and colistin sulphate treatments inhibited the growth of PA115482 and PA106188. PA11070 strain was the most sensitive against the three antibiotics and the probiotics used. The highest level of resistance against the treatments was seen against PA106705 and PA106177, with only inhibition zone shown by colistin sulphate (20 mm and 22 mm respectively). Overall, colistin sulphate was effective against all ( $n = 21$ ) *P. aeruginosa* strains tested, whereas, tetracycline, probiotic and streptomycin were effective against only 15, 14 and 3 respective strains.

#### Surface challenge test

The Fig. 2 below represents the report of the growth of the steel surface challenged test which was grouped into sections (A, B, C, D). Section A shows the contact plate photo of probiotics and *P. aeruginosa*, section B of the figures below shows the negative control plate, section C shows the contact plate with disinfectant and *P. aeruginosa*, while section D shows a positive control which is used as a killing standard check.

Probiotics was effective against the test strain as there was no observable colonies on the surface of the contact plate (Fig. 2A), as predicted there was no growth on the negative control plate (Fig. 2B).

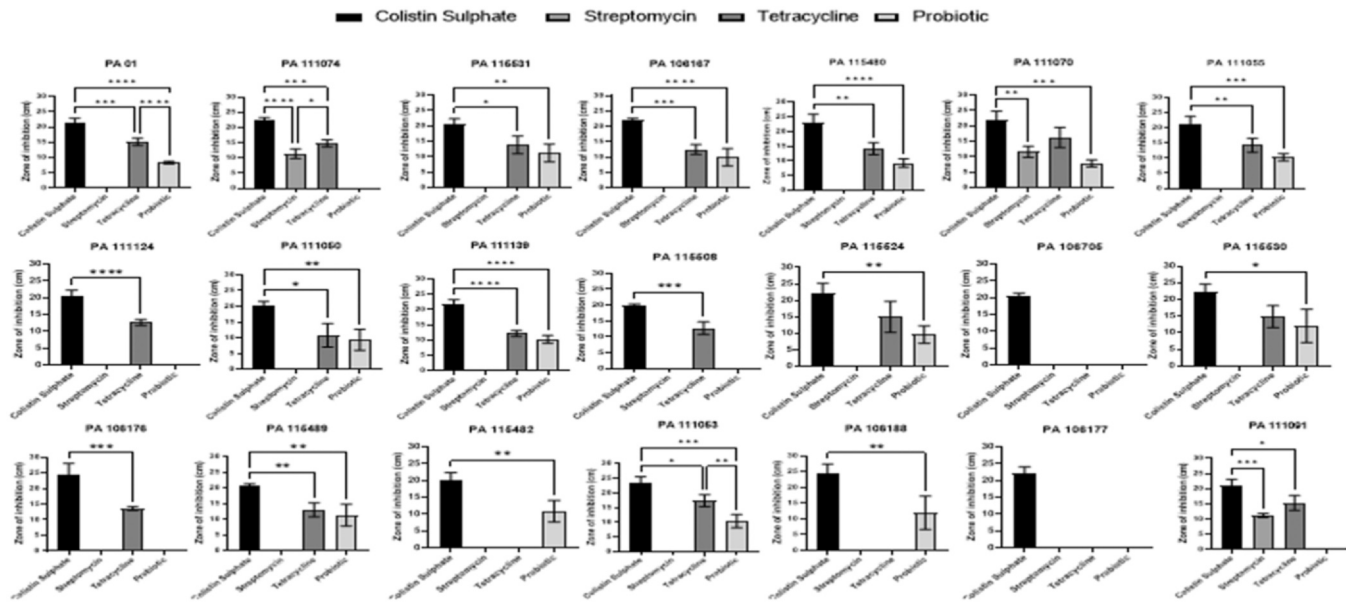


Fig. 1. The comparison of conventionally used antibiotics and probiotics on 21 different strains of *Pseudomonas aeruginosa*.

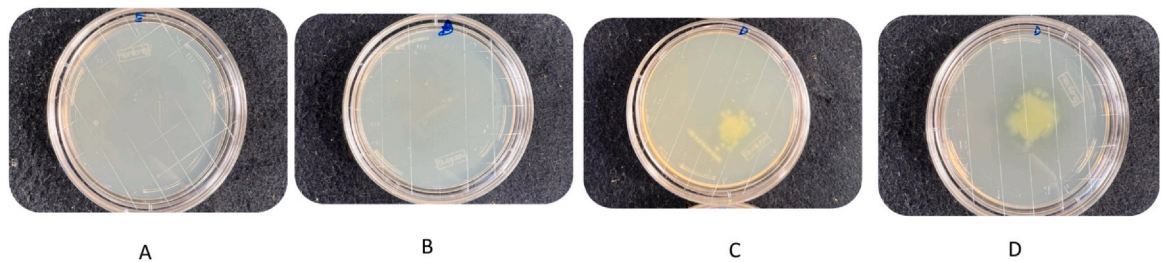


Fig. 2. *P. aeruginosa* strain PAO1 used as surface contaminant and treated with probiotics and disinfectants with the control contact plates.

Fig. 2C showed partial inhibition when compared to Fig. 2D. Obvious colony bacterial growth was observed on Fig. 2D. It is important to note that the bacterial count obtained from this experiment was  $2.6 \times 10^9$  cfu/mL. Plate A showed complete inhibition of bacterial growth.

Time kill kinetic assay

The invitro desmonstration of time kill kinetic assay using probiotics in two (2) dilutions 1:10 and 1:20 to ascertain the time kill kinetic in a period of time.

The time kill assay shown in Fig. 3 illustrates the probiotics effect when tested in different dilutions, 1:10 and 1:20 (line orange and line red respectively). Statistically ( $p = 0.0084$ ) significant reduction in colony counts was observed when compared probiotics 1:20 to the control (line black). However, no significant difference was observed

between the CFU/mL across the different times points tested (2, 4, 6 and 24 h). On the other hand, treatment with probiotics 1:10 presented statistically ( $p < 0.0001$ ) higher reduction in colony counts with sharp decrease in CFU/mL observed between 0 – 6 h. Interestingly, data for longer than 6 h recorded no colony formation. Overall, probiotics 1:10 used demonstrated higher bactericidal effect against *P. aeruginosa* strain PAO1 compared to probiotics 1:20.

Biofilm kill assay

The biofilm killing and anti-biofilm potential of the probiotics used was observed during this experiment. *P. aeruginosa* strain PAO1 was used for this experiment.

Statistically significant reduction ( $p = 0.0009$ ) in bacterial absorbance was observed over 24 h in the presence of the probiotic. Exposure

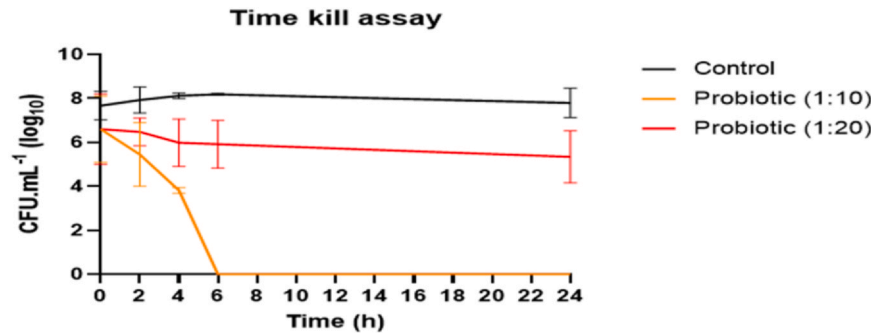


Fig. 3. The time kill assay for *Pseudomonas aeruginosa* using probiotics in two (2) different dilutions to determine cell viability at different time intervals.

to probiotic caused a sudden reduction in absorbance in the first 2 h of treatment, followed by further decrease in the remaining 22 h of experiment (line blue). The control had continuous growth throughout the experiment (line orange). A reverse proportional relation was seen between the time and absorbance. As time of exposure increases, there was an observable reduction on the bacterial absorbance.

#### Probiotics as wound dressing

The use of probiotics and disinfectant as wound dressing treatment.

Higher reduction in bacterial viability (CFU/mL) was observed on bacteria exposed to probiotic compared to disinfectant and the control. Particularly, approximately 1.5 log CFU/mL difference was detected between the probiotic and disinfectant use. Similar trend was seen in Fig. 5B as the lowest levels of crystal violet were recorded under probiotic treatment. Statistically significant differences were seen between the probiotic ( $p < 0.0001$ ), the disinfectant and the control samples. Overall, the data from both Figures showed the probiotic use as the treatment with the highest reduction in bacterial viability and also the treatment with the highest reduction in crystal violet levels.

#### Discussion

The growing reports on antimicrobial resistance mechanism, biofilm producing strains and their associated health concerns continues to arouse interest as seeking potential alternative strategy promises a way forward. Demonstrating the effectiveness of probiotics as a potential cleaning, wound dressing, biofilm inhibiting agent has been the focus of this study while revealing the antimicrobial chattels of probiotics against biofilm forming *Pseudomonas aeruginosa* strains. The study describes probiotics effect of agent on surfaces, time kill effect, biofilm inhibiting assay, antimicrobial activities of probiotics and wound dressing/healing potential.

#### Antimicrobial susceptibility test

Contrary to what was expected, the applied probiotics showed antibacterial inhibitory characteristics on 14 (66.7 %) strains out of the 21 stains of *P. aeruginosa* used during the study. Probiotics were observed to be more effective than some employed antibiotics (ampicillin, chloramphenicol, kanamycin, nalidixic acid, nitrofurantoin) which had zero bacterial inhibitory characteristics when their zone of inhibition are compared. When probiotics zone of inhibition was statistically compared with that of colistin, a highest significance (Fig. 1)  $p < 0.0001$ , was observed using one way ANOVA). When similar zone of inhibition was compared with streptomycin, it had a significance of (Fig. 1),  $p < 0.0001$  was observed, using one way ANOVA) and lastly when such inhibition was compared with tetracycline (Fig. 1)  $p < 0.0001$  was observed, using one way ANOVA. This comparison was done using the most virulent strain PA 01. Such significant inhibitory activity was previously reported in studies by various investigators [15,25–27] who reported a reduction and increased activity of probiotics over commercial antibiotics.

It is important to note that the use of probiotics in our study was not effective in all the strains tested, however the statistical significance in this study was encouraging which reveals probiotic better antibacterial them such employed antibiotic. It is also suggestive that antibiotics usage be replaced specific/identified probiotics as quickly as possible. Furthermore, multiple investigations have also proven that probiotics were more beneficial than antibiotics in pig husbandry. According to [28], the apparent total tract digestibility of gross energy was higher in pigs given a probiotic than in pigs fed with aureomycin. While some individuals thought the opposite was true, others concurred. However, some investigators who used antibiotics in feeding animals opined that the feeding is inadequately controlled hence the antibiotic activity was poor [8]. Heal et al. [29] discovered that antibiotics were more efficient

than probiotics in boosting growth in pigs. The recent study of Zambare, [30] further revealed that probiotics may be equally effective as antibiotics for weaning piglets in high-healthy farm, indicating that the effectiveness of probiotics depends on some factors. Other related investigators added that piglets can be exposed to a variety of stressors, including diseases and mild-contaminated feed [28], which can cause a significant inflammatory response and an imbalance in the antioxidant system. Thus, the potential for probiotics to replace antibiotics may be suggestive in such situation. This is not the case in our study as the *in vitro* test revealed a significantly high activity of probiotics revealing its antibacterial relevance.

#### Surface challenge test

The CFU/mL derived from this experiment was  $2.6 \times 10^9$ . Cleaning with disinfectant had partial inhibitory properties (Fig. 2C), as it inhibits the growth of some of the organism used to contaminate the steel surface. The contact plate containing the probiotics had complete inhibition with no visible growth of the organisms (Fig. 2A) suggesting the potency of probiotics usage. This experiment showed that at the same cfu/mL, probiotics was more effective against tested organisms than disinfectant used on a steel surface. This could be adjudged that disinfectants are not as effective as probiotics when used on a still surface. Although for this experiment, only steel surface was used therefore, we may not draw a conclusion that such observation would be reproducible when used on other surface types for example laminate surface, wood surface or tile surface. In a related study by Habeebuddin and his groups, [12], it was reported that probiotics was effective on skin surfaces than deep skin treatment revealing its potential cleansing activity. In another related study by Zambare, [30], it was reported that the rates of antibiotic resistance amongst bacteria isolated from surfaces cleaned with either conventional or probiotic cleaning solutions are not significantly different. However, it such probiotic cleaning solutions lower the prevalence of antibiotic-resistant bacteria, hence, to sustain this reduction in germs over time, a continuous usage is suggestive [30]. A similar discovery was also reported from an Italian laboratory study using a probiotic pill that contains *Bacillus* specie. Such bacteria recovered from surfaces cleaned with the probiotics product exhibited considerably lower levels of antibiotic resistance genes after such a long period of time than bacteria recovered from the identical surfaces washed with chemical disinfectants [31].

Also observed in our study was the poor effect of chemical disinfectants which were demonstrated to be effective against some surfaces in the near term, but ineffective in preventing recontamination and germ after only a few hours. The probiotics were effective all through the study hence probiotics cleansers are suggestive for usage due to its highly effect. The effect of probiotics on *P. aeruginosa* which are reported as multiple antibiotic resistant strain due to mutation and extended exposure to disinfectants including chlorhexidine [8], is also another affirmation on the need to employ the use of probiotics in the control of multiple antibiotic resistant organisms.

#### Time kill assay

The time kill assay was done using two (2) dilutions (Fig. 3). The 1:10 dilution showed drastic reduction from 2 h and continuously released at 4 h and had complete inhibition after the 6 h. The 1:20 dilution show reduced growth at 2 h but gradually continued growth after 4 h and continuously grew at a constant rate up on till 24 h. From the observations, probiotics is only effective in producing complete inhibitory effect at a higher concentration and is less effective at a lower concentration. This should be put into consideration when probiotics cleaners are manufactured and applied, in other to obtain an effective product. In some related studies, investigators have shown that probiotic cleaning solutions lower the prevalence of antibiotic-resistant bacteria; however, sustaining this reduction in germs over time is likely

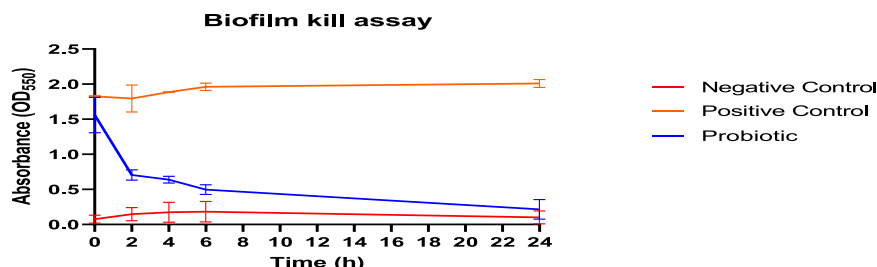


Fig. 4. The formation of biomass using biofilm forming *Pseudomonas aeruginosa* within a given period of time when treated with probiotics.

to need continuous usage [30]. Probiotic may help in the killing and removal of harmful germs from inaccessible locations by destroying various groups of pathogens.

#### Biofilm kill assay

Biofilm kill assay as seen in Fig. 4 also had a very interesting outcome. As seen in the figure, the formation of biofilm had a significant reduction from 2 h and continuously reduced the growth of biofilm formation till the 24th hour. Due to the antibiofilm potential of probiotics and its tendency to compete with pathogens for nutrients and space via several modes of action, probiotics are considered as a viable technique for treating biofilms forming pathogens. Probiotics used as antibiofilm may promote the colonisation and long-term stability of the human mucosa, preventing the formation of harmful microorganisms. In a related study, such antibiofilm potential of probiotics was also reported [16] which affirms the biofilm inhibitory properties of probiotics. This study has also shown the relevance of probiotics on biofilm producing organism. Biofilm has been a major source of concern since it helps *P. aeruginosa* to develop resistance against antimicrobial agents that were formerly effective. With the nature of our reports, probiotics may now be used since it can help in the killing and removal of harmful germs from inaccessible locations by destroying the biofilms that other pathogens rely on for protection. Our findings suggest that the probiotic

cleaning and antibiofilm action is most likely due to the ability of agent sources to produce diverse antimicrobial compounds [31].

#### Probiotics as wound dressing

The use of probiotics as wound dressing treatment did not show any significant reduction in the CFU/mL concentration (Fig. 5A). This might be associated with experimental procedures and growth on coupons (as model) which might have been in cluster. But Fig. 5B showed a surprising/impressive reduction of biofilm after treatment with probiotics ( $p < 0.0001$ ) when compared to the disinfectant used. This is contrary to what was expected as probiotics worked as a treatment for wound dressing according to the finding of related investigators and this study. Kabiri-Samani et al. [31] reported in a recent study that when probiotics were given to children, the requirement for grafting was much reduced, and when grafting was not performed, the time required for full wound healing was substantially reduced. Velazquez et al. [28] also reported that the mean grafted body surface area of adult patients receiving probiotic medicine was slightly bigger than that of the control group based on their findings. In the same study, average graft loss was considerably greater in the control group. Similarly, Rahimzadeh et al. [8] discovered no difference in the number of operating days for excision and grafting procedures or the time required for recovery in paediatric patients given probiotics vs those who were not.

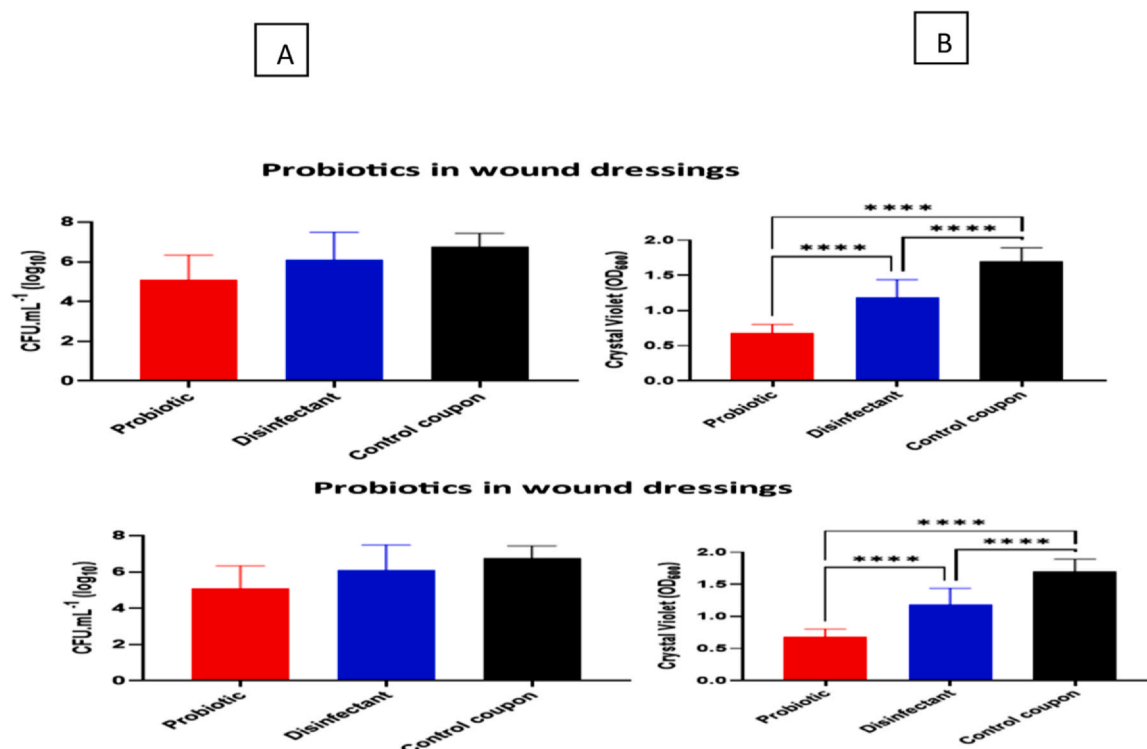


Fig. 5. A) The bacterial viability after treatment with probiotics and disinfectant B) showing the biomass reduction when treated with probiotics and disinfectant.

Furthermore, probiotics therapy for surgical wounds and diabetic foot ulcers also yielded positive outcomes following the studies of other investigators. According to Zambare, [30], dressing wounds with probiotic may result changes substantially less frequently (1.7 times/day) than by the antibiotics (3.3 times/day) or the antibiotics Plus placebo group (2.8 times/day). Similarly, in the groups that did not get probiotic medicine, there was a significant increase in the frequency of post-operative wound complications [31]. According to Kabiri-Samani et al. [31], adults treated with probiotics had a substantial improvement in the length, breadth, and depth of ulcer healing. Three trials on adults and children revealed evidence that probiotics therapy accelerated wound healing in some wound cases. It is possible that due to variances in the wound types evaluated, the people treated, and the criteria for evaluating the impact. Other related investigators report emphasize that they did not achieve a consensus or offer solid evidence for the efficacy of probiotic treatment for wound healing [7]. Hence it is suggested that while probiotic therapy has been demonstrated to speed wound healing, no studies have found any negative side effects. Graft survival, graft loss, ulcer size, number of dressing changes per day, and the incidence of postoperative wound complications were the primary efficacy markers in included trials. However, when the wounds were clinically graded, the administration of probiotic medication did appear to make some differences. Skin grafting is the treatment of choice for severe dermal burns because it allows for the removal of dead and inflammatory tissues while also facilitating quicker physiological wound healing. As observed in this study, probiotic-treated individuals had a decreased requirement for grafting materials and an increased healing effect which suggest further/additional research to confirm this benefit in other wound types.

#### Future works

In the future, it is suggested that study focus should be observing the potency of probiotics on other gram-negative organisms and some gram-positive organisms for examples multiple antibiotics *Klebsiella* spp (MAKS) and Meticillin resistant *Staphylococcus aureus* (MRSA). Also, studies should now focus on evaluating the inhibitory effects of probiotics on infecting pathogens: a strategy for controlling enterocyte infecting potential pathogens, Determination of the sublethal effect of probiotics on infected wound and superficial potential pathogens, evaluating the anti-plasmid potential of probiotics amongst multiple antibiotic resistance bacterial strains: a strategy for controlling Pan resistant strains and superbug and Investigating the mode/mechanism of action of probiotics against *P. aeruginosa*.

#### Conclusion

It is important to emphasize that the environment is a major source of different micro-organisms and may also aid spread of infection and diseases. *P. aeruginosa* which is usually harmless in its normal habitat may become a pathogen in infection cases when it becomes opportunistic. This experiment has shown the effectiveness of probiotics on *P. aeruginosa* strains. It has shown a significant evidence that utilizing a probiotic cleaning solution in *P. aeruginosa* associated case may reduced *P. aeruginosa* growth and biofilm formation. It also revealed that probiotics consist biocidal active agents which may aided in the prevention of pollution and the spread of pollution-resistant bacteria while also being environmentally friendly. It was observed that while chemical disinfectants are very effective at killing surface contaminants right away, they are not as effective at preventing recontamination and re-growth of bacteria or germs that can happen just a few hours later. The study results shows that probiotics were effective as a surface cleaner, as a biofilm formation inhibitor and effective when used as a wound dressing treatment against biofilm forming *P. aeruginosa*. It is therefore suggested that probiotics usage may be recommended in cases associated with *P. aeruginosa* and as a surface cleansing agent while

harnessing other potentials of probiotics both in the public health systems and other environments.

#### CRediT authorship contribution statement

**Bright E Igere:** Writing – review & editing, Visualization, Validation, Resources, Project administration, Formal analysis, Data curation. **Jennifer Imade Elijah:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Jonathan Butler:** Writing – review & editing, Writing – original draft, Supervision, Software, Funding acquisition, Data curation, Conceptualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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