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LACTIC ACID BACTERIA AND FERMENTED MAIZE SUPERNATANT (*Omidun*) HAVE ANTI-BIOFILM PROPERTIES AGAINST STAPHYLOCOCCI AND ENTEROAGGREGATIVE *Escherichia coli* STRAINS

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ABSTRACT

Bacterial infections caused by biofilm forming organisms are of public health concern due to their propensity to contribute to persistent chronic diseases, chiefly because of their ability to resist antibiotics and host immune functions. Probiotics are considered useful therapeutic option in combating pathogenic biofilms. This study evaluates the anti-biofilm properties of potential probiotic Lactic Acid Bacteria (LAB) and fermented maize supernatant (*Omidun)* against selected biofilm-forming pathogens. Crystal violet biofilm assay was used to determine LAB and *Omidun* biofilm inhibition and dispersion in selected pathogens (*Pseudomonas aeruginosa,* Coagulase-negative staphylococci (CoNS), *S. aureu* and Enteroaggregative *Escherichia coli*) at different concentration (1%, 10%, 50%, 100%) of neutralized and non-neutralized cell free supernatant (CFS). Percentage biofilm inhibitions and dispersions were evaluated, and data were analysed with ANOVA. *Omidun* and LAB showed promising biofilm inhibitory and dispersive effect against the selected pathogens. *L. plantarum* showed the greatest biofilm inhibitory effect (*P. aeruginosa*: 7.85%, CoNS: 27.75%, *S. aureus*: 66.90%, EAEC: 39.73%) and dispersive effect (*P. aeruginosa*: 15.94%, CoNS: 23.27%, *S. aureus*: 24.90%, EAEC: 32.09%) against the selected pathogens while *Omidun* showed the least biofilm inhibitory and dispersive effect against the selected pathogens. There was no significance difference in the percentage of biofilm inhibition and dispersion produced under different concentrations, neutralized and non-neutralized state. *Pseudomonas aeruginosa* was the most resistant pathogen while *Enteroaggregative Escherichia coli* (EAEC) was the most susceptible. Inhibition and dispersion of biofilm can be mediated by LAB and *Omidun*, these effects appear to be independent of the produced organic acids.

Keywords: biofilm, lactic acid bacteria, pathogens, *omidun*, probiotics

INTRODUCTION

Biofilms are aggregates of microorganisms that are embedded in a self-produced extracellular polymeric substance (EPS) in a sessile state (Bjarnsholt *et al.,* 2018). Bacterial biofilms are of important medical concern owing to their ability to contribute to antibiotic resistance and persistent chronic infections (Sharma *et al.,* 2019). The biofilm extracellular matrix serves as a protective barrier against unfavourable environmental conditions, antibiotics and host's immune cells (Percival *et al.,* 2015: Sharma *et al.,* 2019). There is genetic basis for biofilm formation with some bacteria possessing genes that can activate biofilm formation in response to stress associated with various environmental conditions such as alteration in cell density, pH, osmolarity, nutrition or temperature (Gjermansen *et al.,* 2010).

Biofilms are difficult to detect with routine diagnostic tests, this makes diagnosis of bacteria infections caused by biofilm forming organisms challenging (Sharahi *et al.,* 2019). Biofilm associated diseases are great threat to public health due to the resistance it poses towards many available antibiotics (Khan *et al.,* 2014). The exopolymer in biofilms limit and disrupt the penetration of leucocytes and their inherent ability to produce reactive oxygen species, hence preventing the phagocytosis of pathogens (Thurlow *et al.,* 2011). Biofilm forming bacteria are about tenfold more resistant to antibiotics than their planktonic variants, largely due to their improved survival mechanisms (Beser *et al.,* 2019). Colonization of implanted medical devices such as prosthetic heart valves, urinary catheters, joint prostheses, pacemakers have also been identified as one of mode of transmission of biofilm infections (Barzegari *et al.,* 2020). Organisms such as Methicillin resistant *Staphylococcus aureus* (MRSA) *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus mutans*, *Gardnerella vaginalis* etc are some of the most common biofilms producing bacteria causing nosocomial infections (Marhur *et al.,* 2018). *Escherichia coli* appears to be the most prevalent bacteria biofilm forming pathogen associated with medical devices as well as a representative model for the study of bacterial biofilm (Sharma *et al.,* 2019).

Considering the challenges with the different approaches aimed at inhibiting biofilm formation and dispersion such as quorum sensing inhibitor and development of new classes of antibiotics, there is a need to explore more roboust alternative strategies (Igarashi, 2019). The possible use of probiotics in inhibiting biofilm formation in pathogenic bacteria is a subject of many recent research. Probiotics are live microorganisms which when administered in adequate amount confer health benefits on the host (FAO, 2006). They have been reported to be effective in the treatment of viral gastroenteritis, inflammatory bowel diseases, antibiotic-associated diarrhea, cystic fibrosis, uropathogens, dental caries and periodontal diseases (Guandalini *et al.,* 2000; Chapman *et al.,* 2006; Alexandre *et al.,* 2014; Ayeni *et al.,* 2009; Saha *et al.,* 2012). Probiotic bacteria are generally 'regarded as safe' (GRAS) and have been documented to also inhibit or delay the incidence of pathogenic biofilm formation on medical devices (Barzegari *et al*., 2020; Fabio *et al.,* 2021). Lactic acid bacteria are prominent members of beneficial bacteria found in the gut of animals and numerous natural environments including fermented food such as Kunu, *Ogi* (fermented maize slurry) and its supernatant (*Omidun*). (Afolayan *et al.* 2017, Sowemimo *et al.* 2021). We have previously reported the antiviral (Sunmola *et al.* 2019) , antidiarrheageni *E. coli* (Kwasi *et al.* 2019), anti plasmodium (Omeiza *et al.* 2020), and anticolitis (Audu *et al.* 2019) properties of *Omidun*. However, there is little information

about possible anti-biofilm properties of *Omidun*. This study therefore evaluates the ability of lactic acid bacteria and '*Omidun*' to inhibit and disperse biofilms formed by selected bacterial pathogens.

MATERIALS AND METHODS

Bacterial strains and growth condition

Lactic acid bacterial strains (*Lactobacillus plantarum* OBISE A9, *Enterococcus lactis* and *Leuconostoc pseudomesenteroides* OBISE A10) from our research group were used in this study. Pathogenic strains of Enteroaggregative *Escherichia coli* (EAEC DO28J), *Pseudomonas aeruginosa* (EO102), Coagulase-negative staphylococci (OAU AAA 061) and *S. aureus (*OAU AAA 059A) were obtained from the Molecular Laboratory of Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan. The strains have been demonstrated to be excellent biofilm formers. LAB strains were cultivated in Man de Rogosa Sharpe (MRS) broth. *Pseudomonas aeruginosa* EO102, EAEC DO28J, *Staphylococcus aureus* OAU AAA 059A and *Staphylococcus* OAU AAA 061 (CoNS) were cultured on Cetrimide, MacConkey and Mannitol Salt Agar (MSA) respectively and incubated at 37 °C for 24 h. All strains were maintained at −80◦C in the appropriate cultivation broth containing 20% (v/v) glycerol.

Preparation and viability test for *Omidun*

Omidun was prepared according to the method described by Afolayan *et al.,* (2017) with a slight modification. The white variety of maize grains was washed appropriately and soaked in moderate quantity of sterile for 72 h at room temperature. The water was then decanted and the grain wet-milled in a clean grinding machine. The resulting pastes was sieved using sterile muslin cloths, with the filtrate being collected into a sterile container and allowed to settle for 3 days during which fermentation takes place by the natural flora of the grains. After three days, the starch granules became settled leaving a clear supernatant at the top called O*midun* which was collected in a sterile container, the settled starch granules (*Ogi*) were lightly scraped at the surface and then mixed with collected *Omidun* to fully obtain LAB that might have settled on its surface (Kwasi *et al.,* 2019). The collected supernatant and the slightly scraped sediments were pooled together to obtain a uniform mixture. The *Omidun* was used within 3 days of milling after which a fresh batch was prepared. The LAB in *Omidun* with lightly scrapped *Ogi* surface was quantified by viable count technique.

Preparation of cell free supernatant

Distinct colonies of LAB were inoculated into 10 ml MRS broth and incubated for 24 h at 37°C with 160 rpm. The overnight cultures of the LAB strains and fully fermented *Omidun* (at the third day of fermentation) in 10 ml tubes were centrifugation at 5,000 × *g* for 30 minutes at 4 °C, then the cell free supernatants (CFS) were collected and filtered through a nitrocellulose membrane. The following concentration of various CFS were obtained (1%, 10%, 50%, 100%) and a portion of each CFS were neutralized to pH 6.5.

Antibiofilm assay

Enteroaggregative Escherichia coli and Pseudomonas aeruginosa

Initiation of biofilm formation was done by picking single colonies from MacConkey and Cetrimide agar plates for EAEC and *Pseudomonas aeruginosa* respectively. They were inoculated in 5 ml of sterilized Luria Bertani Broth (LB) at 37 °C with agitation (200 rpm) for 18 h. Five uL of each LAB CFS (neutralized and non-neutralized) were first put into the different wells, then 190 uL mixture of Dulbecco Modified Eagle Medium and 5 uL of overnight inoculum of EAEC and *Pseudomonas aeruginosa* were then introduced into the wells already containing the CFS respectively and then incubated at 37 °C for 24 h. Thereafter, the medium was pipetted out and washed with water and dried by inversion. Fixing was done by adding 200 uL of 75% ethanol and allowed to dry. Then 0.5% crystal violet was used for staining for 5 minutes, after which the plates were washed thoroughly with water and then allowed to dry completely. Then, 200 uL of 95% ethanol was added to each well and allowed to stand for 20 minutes at room temperature. The absorbance was determined by plate reader at 570 nm. The control followed the same process but without the CFS and the assay was done in triplicate.

The percentage biofilm inhibition was calculated by the formula below:

% Biofilm Inhibition= [Σ(Control)-Σ(Pathogen+CFS)]/Σ(Control) x 100%

Adapted from Melo et al., (2016).

Coagulase Negative Staphylococci and *S. aureus*

To initiate biofilm formation, single colonies were picked from Tryptic Soy Agar (TSA) culture plates and inoculated in 10 ml Tryptone Soya Broth (TSB) supplemented with 1 % (w/v) glucose at 37°C with agitation (250 rpm) for 18 h. The overnight culture of CoNS was diluted with fresh TSB at ratio 1:100. Each well of the 96 well microtiter plate was filled aseptically with 180 µl aliquots of the diluted culture and then, 20 µL of CFS (neutralized and non-neutralized) were added to each well on a separate well plate respectively and 200 µ L of bacteria was put into other wells to be used as a control and incubated at 37 ºC for 24 h for optimum biofilm formation. The remaining medium in the plates was removed by careful pipetting and the plates were washed twice with phosphate-buffered saline (PBS), dried for 1 h at 50 °C, 1 % crystal violet was added, and the plates were incubated for a further 30 minutes at 25 °C. Each well was washed twice with PBS and allowed to dry well by inversion. Then, 200 uL of 95% ethanol was added to each well and allowed to stand for 20 minutes at room temperature. The absorbance was determined by plate reader at 570 nm. Control was done with same process above but without CFS and all studies were done in triplicates using a well mapped out 96 well plate.

The percentage biofilm inhibition was calculated by the formula below:

% Biofilm Inhibition = [Σ(Control)-Σ(Pathogen+CFS)]/Σ(Control) x 100%

Adapted from Melo et al., (2016).

Biofilm dispersive assay

Pseudomonas aeruginosa and enteroaggregative *Escherichia coli*

Biofilm formation was initiated by picking single colonies of *Pseudomonas aeruginosa* and EAEC from Cetrimide and MacConkey culture plates respectively and inoculated in 5 ml of Luria Bertani Broth (LB) at 37 °C with agitation (200 rpm) for 18 h. 200 uL of overnight inoculum of *Pseudomonas aeruginosa* and EAEC were first put into the wells, then incubated at 37 °C for 24 h to allow maximum biofilm formation. Thereafter, the medium was pipetted out carefully and rinsed gently with PBS. Then, 5 uL of CFS (neutralized and non-neutralized respectively) and 195 uL of Dulbecco Modified Eagle Medium was added accordingly to the rinsed wells and incubated at 37 °C for 24 h. The medium was pipetted out and washed well with water and dried by

inversion, then, fixing was done by 200 uL of 75 % ethanol and allowed to dry. After the plate has dried, 0.5% crystal violet was used for staining for 5 minutes, after which the plates were washed thoroughly with water and then allowed to dry completely. Then, 200 uL of 95 % ethanol was added to each well and allowed to stand for 20 minutes at room temperature. Then, absorbance was determined by plate reader at 570 nm. Control was done with the same process above but without CFS and all studies were done in triplicates using a well mapped out 96 well plate.

The percentage biofilm Dispersion was calculated by the formula below:

% Biofilm Dispersion = [Σ(Control)-Σ(Pathogen+CFS)]/Σ(Control) x 100%

Adapted from Melo et al., (2016).

Coagulase Negative Staphylococci and S. aureus

To initiate biofilm formation, single colonies was picked from TSA culture plates and inoculated in 10 ml TSB supplemented with 1 % (w/v) glucose at 37 °C with agitation (250 rpm) for 18 h. The overnight culture of CoNS and *S. aureus* were diluted with fresh TSB at ratio 1:100 respectively. Each well of the 96 well microtiter plates were filled aseptically with 200 µL aliquots of the diluted culture and simultaneously with 200 µL of bacteria being put into other wells to be used as a control and incubated at 37 ºC for 24 h. After 24 h, the remaining medium in the plates was removed by careful pipetting and the plates were washed once with PBS, then 100 uL of Tryptone Soya Broth and 50 uL of CFS (neutralized and non-neutralized) was added respectively to all the rinsed well except the control which only took in 200 uL Tryptic Soy Broth. These set ups were incubated for another 24 h at 37°C.

The remaining medium in the plates was removed by careful pipetting and the plates were washed twice with PBS, dried for 1 h at 50°C, 1 % crystal violet was added, and the plates were incubated for a further 30 min at 25°C. Each well was washed twice with PBS and allowed to dry well by inversion. Then, 200 uL of 95% ethanol was added to each well and allowed to stand for 20 min at room temperature. The absorbance was determined by plate reader at 570 nm. The control was done with same procedure above but without CFS and all studies were done in triplicates using a well mapped out 96 well microtitre plate.

The percentage biofilm dispersion was calculated by the formula below:

% Biofilm Dispersion = [Σ(Control)-Σ(Pathogen+CFS)]/Σ(Control) x 100%

Adapted from Melo et al., (2016).

Statistical analysis

The data obtained were analyzed by one-way Analysis of Variance (ANOVA), P value < 0.05 was statistically significant.

RESULTS AND DISCUSSION

The biofilm forming potential of *Staphylococcus aureus* and CoNS were evaluated. *S. aureus* OAUAAA 059A and *Staphylococcus* OAU AAA 061 are excellent biofilm formers with the mean absorbance readings of *S. aureus* OAU AAA 059A *and Staphylococcus* OAU AAA 061 being 0.488 ± 0.021 and 0.488 ± 0.015 respectively. *Leuconostoc pseudomesenteroides, L. plantarum*, *E. lactis* and *Omidun* had a cfu/ml of 1.4×10^8, 1.5×10^8 and 1.6×10^8 respectively while *Omidun* has higher value of 3.0×10^9 cfu/ml.

The anti -biofilm formation results are comparable among the three tested LAB strains and *Omidun.* The highest percentage inhibition by non-neutralized CFS of *L. plantarum* yielded 7.29% inhibition against *P. aeruginosa* at 10% concentration, 23.15% against CoNS at 50% strength, 66.90% against *S. aureus* at 1% strength and 36.95% against EAEC at 100% concentration. Also, highest percentage inhibition by neutralized CFS achieved 7.85% inhibition against *P. aeruginosa* at 10% concentration, 27.75% inhibition against CoNS at 100% concentration, 24.01% inhibition against *S. aureus* also at 1% strength and lastly, 39.73% against EAEC at stock concentration (Figure 1- 4). For *Leuconostoc pseudomesenteroides*, the highest percentage inhibition by non-neutralized CFS was 24.33% against *P. aeruginosa* at 100% concentration, 21.05% against CoNS at 1% strength, 25.35% against *S. aureus* also at 100% strength and 38.19% against EAEC also at 100% concentration. Also, the highest percentage inhibition by neutralized CFS yeilded 8.04% inhibition against *P. aeruginosa* at 100% concentration, 22.01% inhibition against CoNS at 1% concentration, 23.79% inhibition against *S. aureus* also at 1% strength and 39.96% against EAEC at stock concentration as seen in Figure 1- 4. For *E. lactis*, the highest percentage of inhibition by non-neutralized CFS was 10.56% inhibition against *P. aeruginosa* at 50% concentration, 24.98% against CoNS at 1% strength, 19.74% against *S. aureus* also at 100% strength and 33.66% against EAEC at 50% concentration. Also, the highest percentage inhibition by neutralized CFS was 11.67% inhibition against *P. aeruginosa* at 50% concentration, 20.08% inhibition against CoNS at 50% concentration, 24.16% inhibition against *S. aureus* also at 1% strength and 33.37% against EAEC at 100% concentration. (Figure 1- 4) For *Omidun*, the highest percentage inhibition by non-neutralized CFS was 5.62% inhibition against *P. aeruginosa* at 10% concentration, 22.59% against CoNS at 10% strength, 20.71% against *S. aureus* also at 1% strength and 35.72% against EAEC at 100% concentration. Also, the highest percentage inhibition by neutralized CFS resulted in 8.57% inhibition against *P. aeruginosa* at 10% concentration, 19.90% inhibition against CoNS at 1% concentration, 27.17% inhibition against *S. aureus* at 1% strength and 43.19% against EAEC at 100% concentration. However, the general analysis of variance (ANOVA) of the whole data involving four CFS against the pathogens in their respective categories revealed that there is no significance difference in the percentage biofilm inhibition produced under different concentrations and under neutralized and non-neutralized state.

The biofilm dispersal results are comparable among the three tested LAB strains and *Omidun.* For *L. plantarum*, the highest percentage dispersion by non-neutralized CFS was 13.14% inhibition against *P. aeruginosa* at 10% concentration, 16.41% against CoNS at 50% strength, 24.90% against *S. aureus* at 1% strength and 32.09% against EAEC also at 50% concentration. Also, the highest percentage dispersion by neutralized CFS was achieved as 15.94% against *P. aeruginosa* at 1% concentration, 23.27% dispersion against CoNS at 50% concentration, 24.13% dispersion against *S. aureus* also at 10% strength and 21.29% against EAEC at 100% concentration (Figure 5-8). *Leuconostoc pseudomesenteroides* had the highest percentage dispersion by non-neutralized CFS as 15.38% dispersion against *P. aeruginosa* at 10% concentration, 16.33% against CoNS at 50% strength, 24.57% against *S. aureus* also at 50% strength and 33.05% against EAEC also at 100% concentration. The highest percentage dispersion by neutralized CFS was recorded as 10.80% dispersion against *P. aeruginosa* at 1% concentration, 25.25% dispersion against CoNS at 50% concentration, 24.76% dispersion against *S. aureus* also at 1%

strength and 20.07% against EAEC at stock concentration (Figure 5-8). The highest percentage dispersion by non-neutralized CFS of *E. lactis* was 6.62% dispersion against *P. aeruginosa* at 100% concentration, 17.63% against CoNS at 50% strength, 23.39% against *S. aureus* also at 1% strength and 29.33% against EAEC also at 100% concentration. Also, the highest dispersion by neutralized CFS was 8.32% dispersion against *P. aeruginosa* at 10% concentration, 24.78% dispersion against CoNS at 1% concentration, 24.36% dispersion against *S. aureus* at 10% strength and lastly, 16.24% against EAEC at 100% concentration [Figure 5-8]. *Omidun* had the highest percentage dispersion by non-neutralized CFS of 9.79% against *P. aeruginosa* at 1% concentration, 17.63% against CoNS at 50% strength, 24.50% against *S. aureus* also at 50% strength and 26.35% against EAEC also at 100% concentration. Furthermore, the highest percentage dispersion by neutralized CFS yielded a 4.49% dispersion against *P. aeruginosa* at 10% concentration, 19.23% dispersion against CoNS at 1% concentration, 24.36% dispersion against *S. aureus* at 10% strength and 19.12% against EAEC at 100% concentration as seen in Figure 5- 8. However, the ANOVA result of the whole data involving the four tested CFS against the pathogens in their respective categories suggests that there is no significance difference in the percentage biofilm dispersion produced under different concentrations and under neutralized and non-neutralized state.

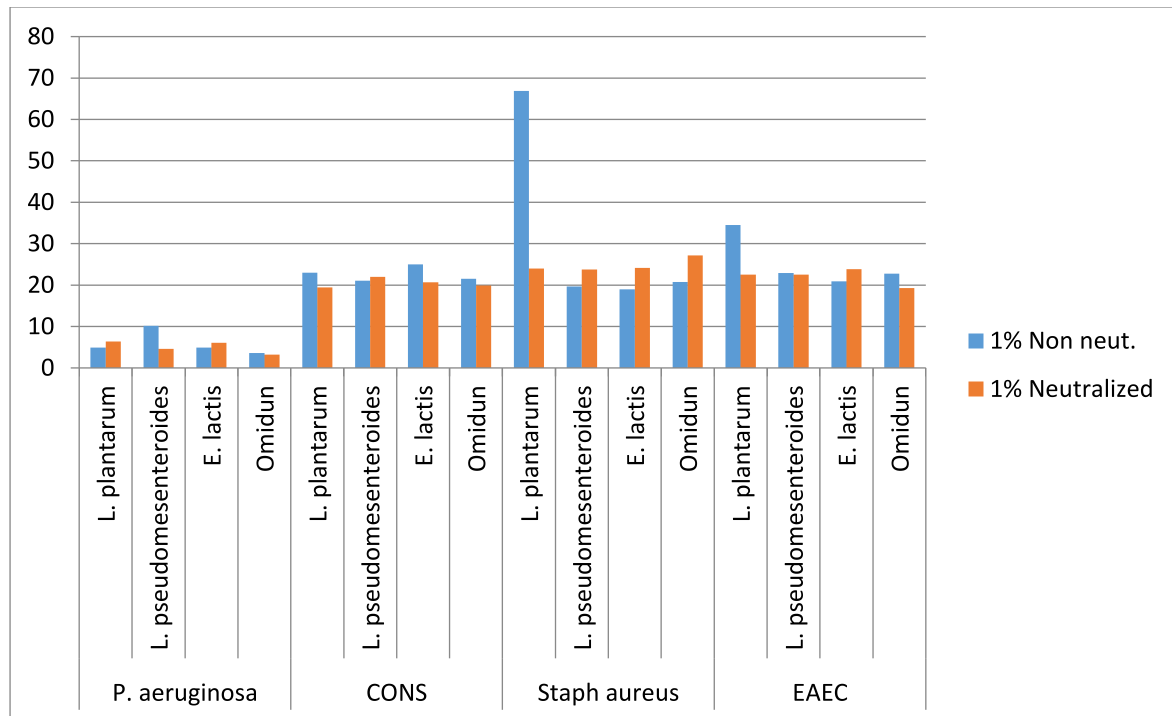

Figure 1: Inhibition of biofilm formation at 1% concentration (Neutralized and Non neutralized)

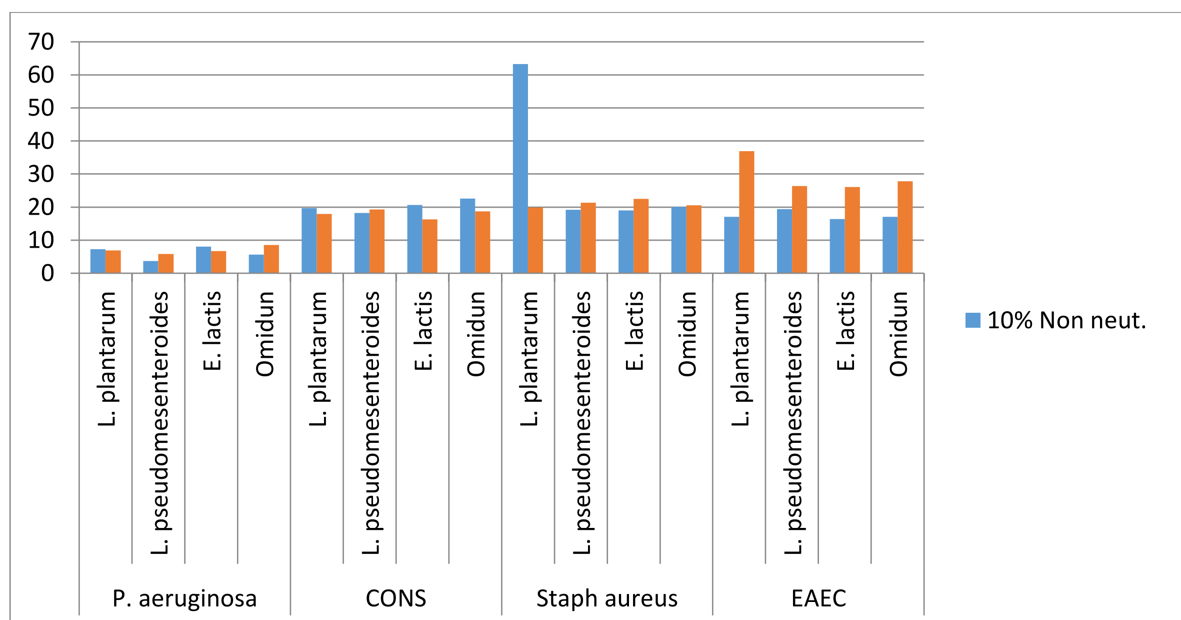

Figure 2: Inhibition of biofilm formation at 10% concentration (Neutralized and Non neutralized).

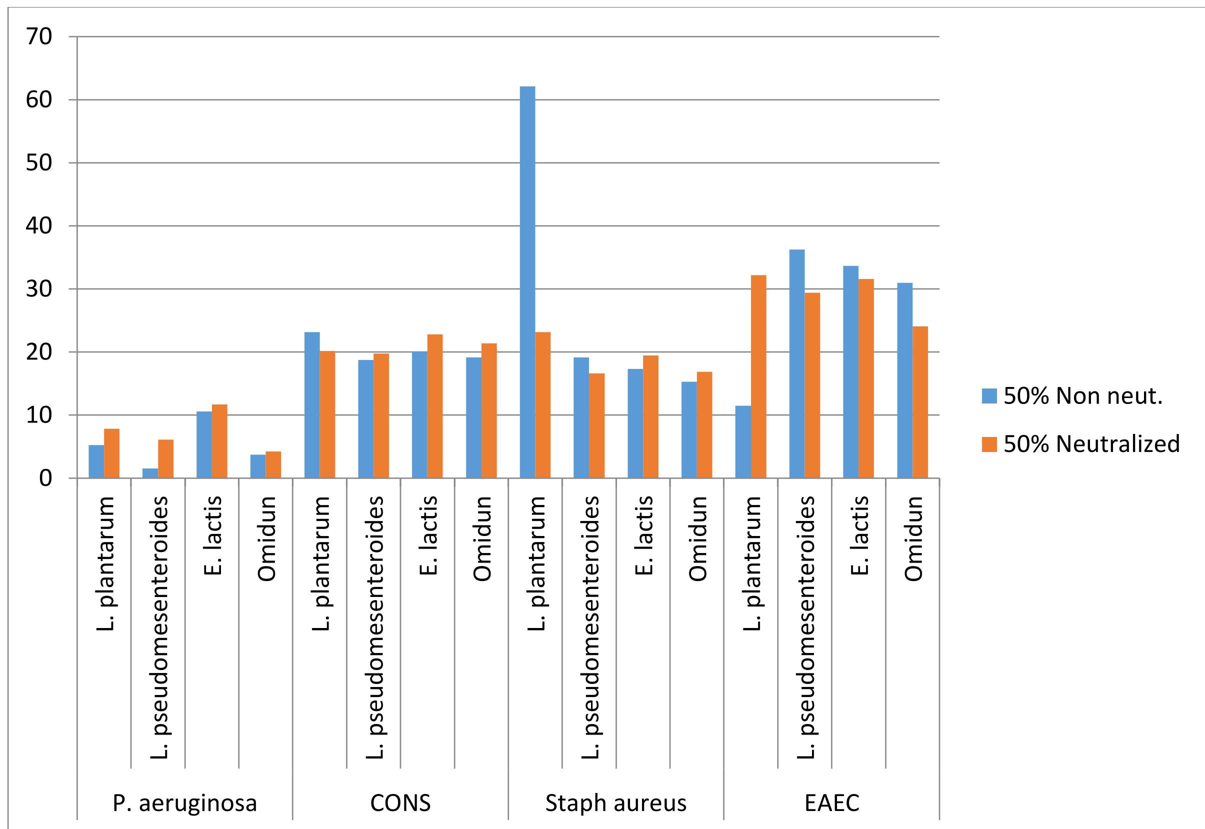

Figure 3: Inhibition of biofilm formation at 50% concentration (Neutralized and Non neutralized).

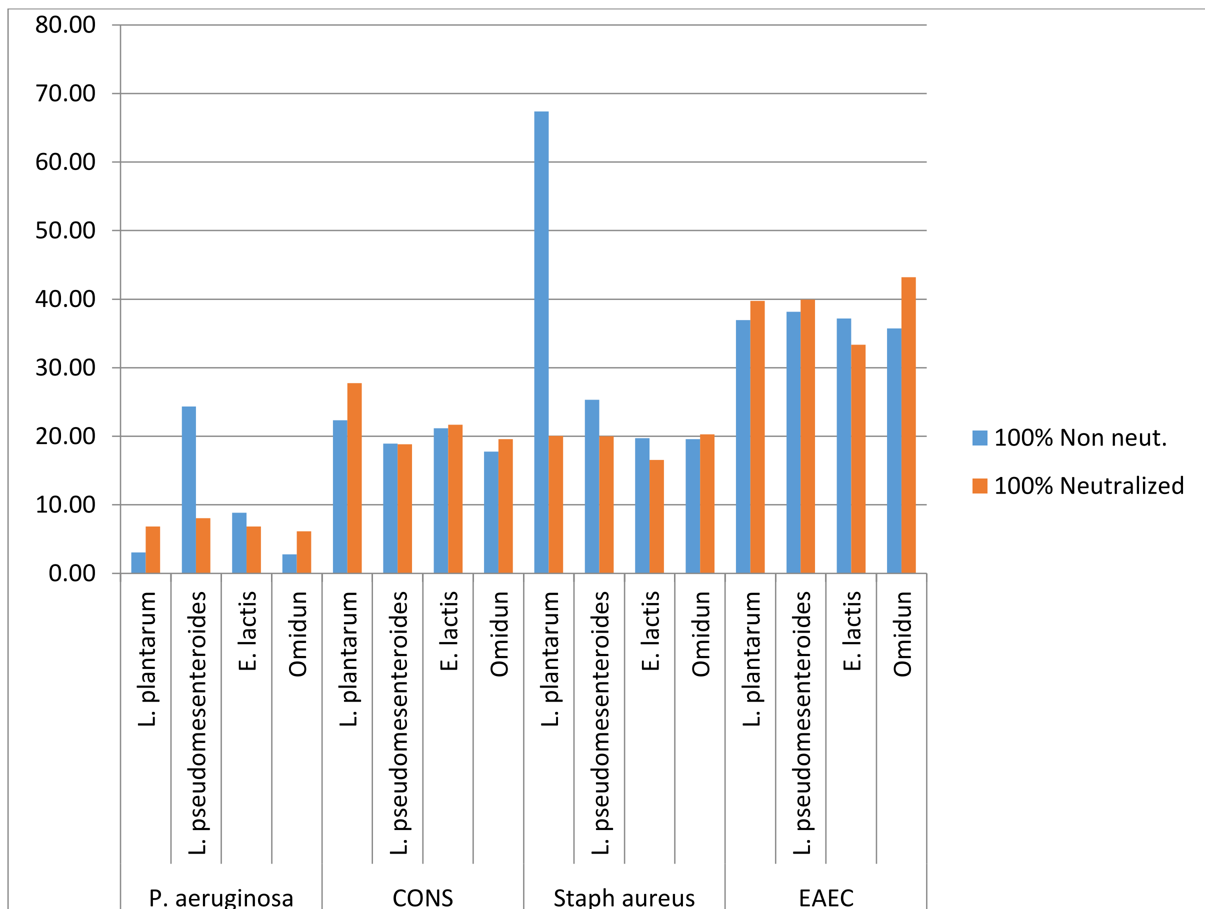

Figure 4: Inhibition of biofilm formation at 100% concentration (Neutralized and Non neutralized).

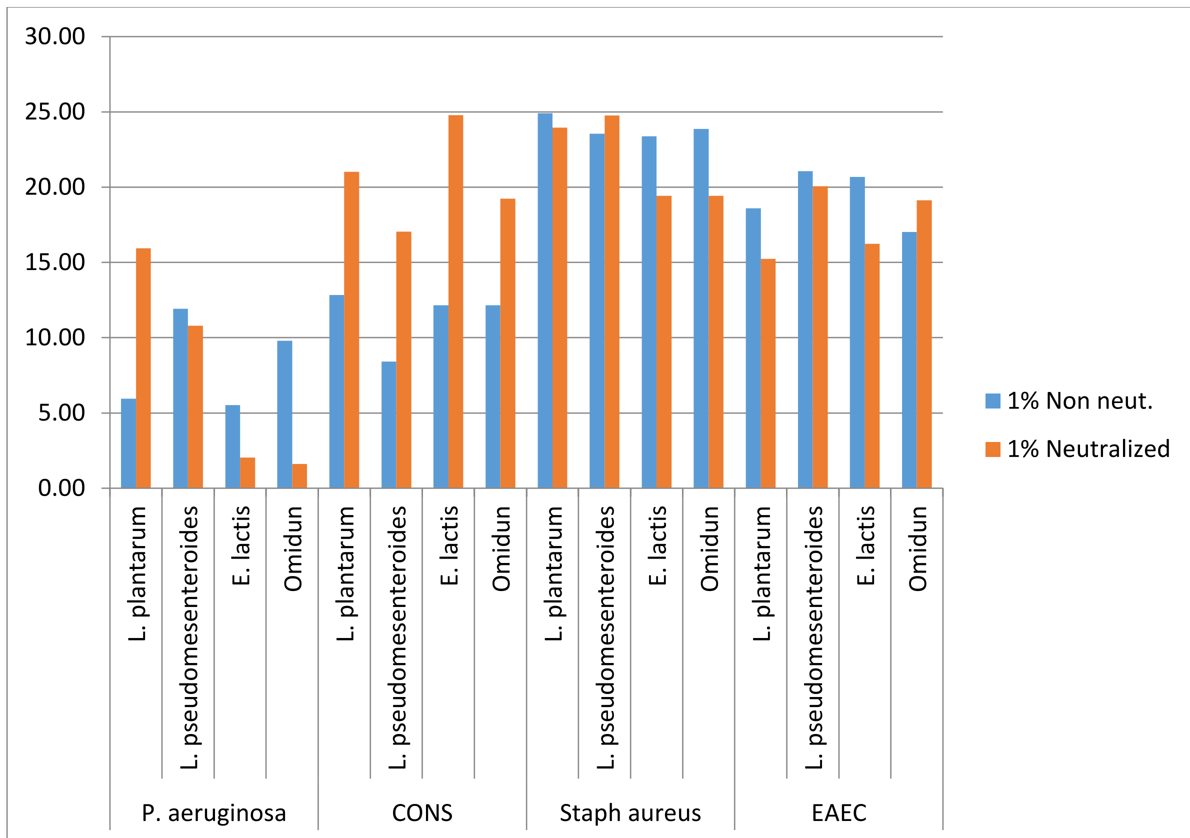

Figure 5: Pre-formed biofilm dispersive effect at 1% concentration (Neutralized and Non neutralized).

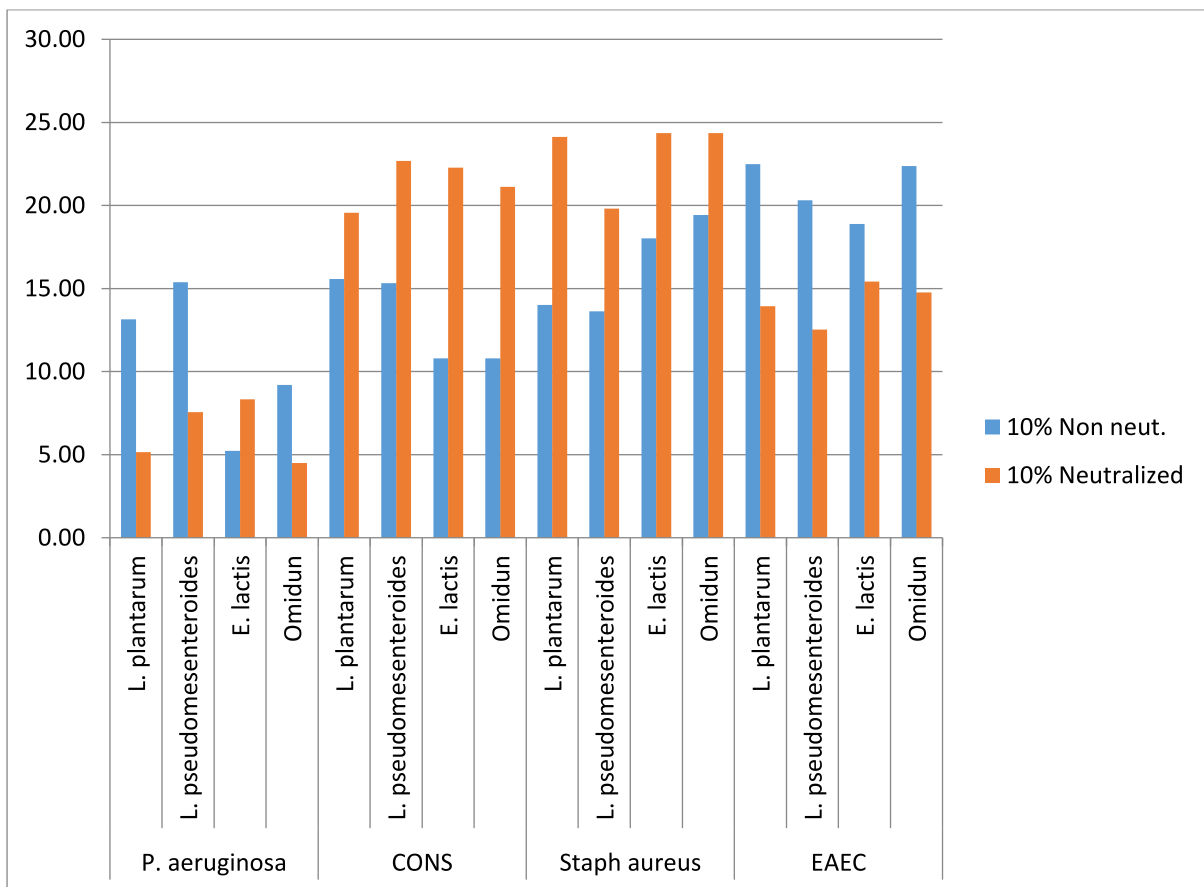

Figure 6: Pre-formed biofilm dispersive effect at 10% concentration (Neutralized and Non neutralized).

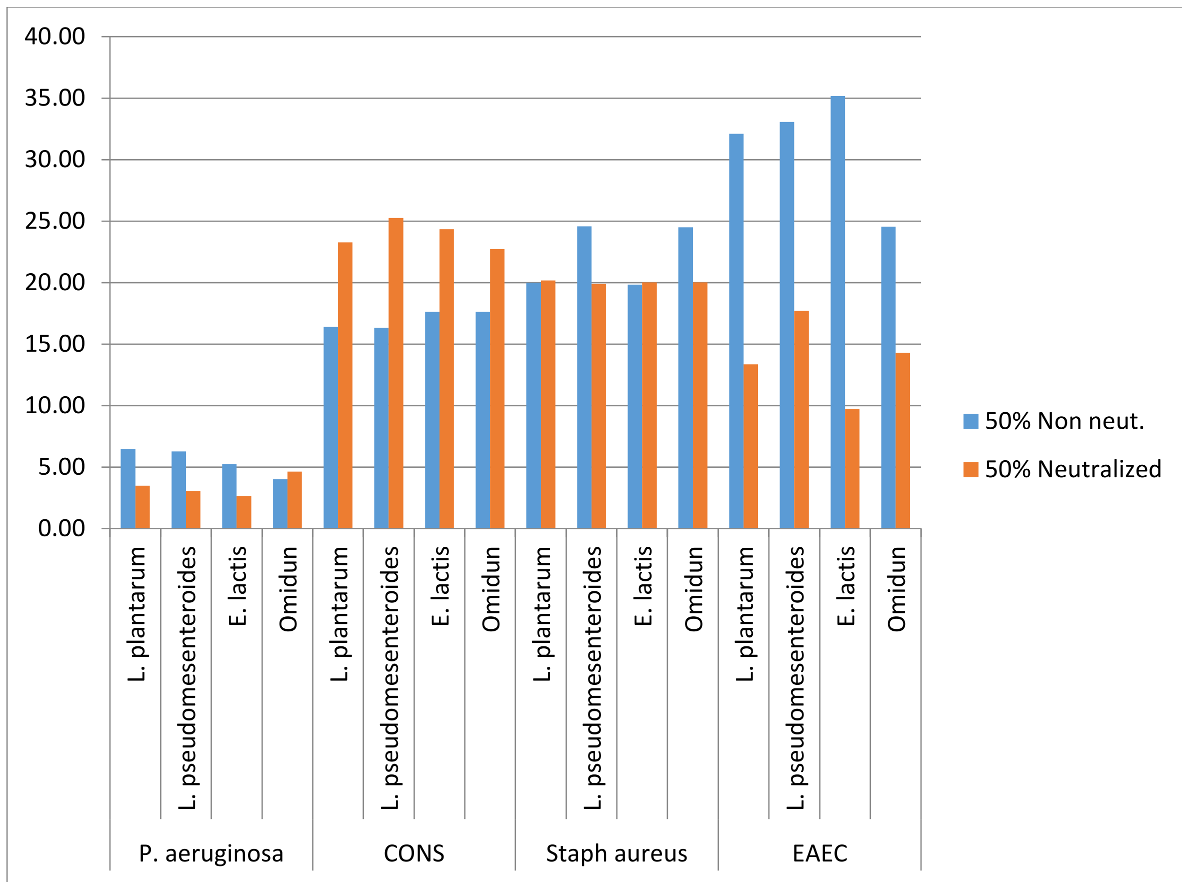

Figure 7: Pre-formed biofilm dispersive effect at 50% concentration (Neutralized and Non neutralized).

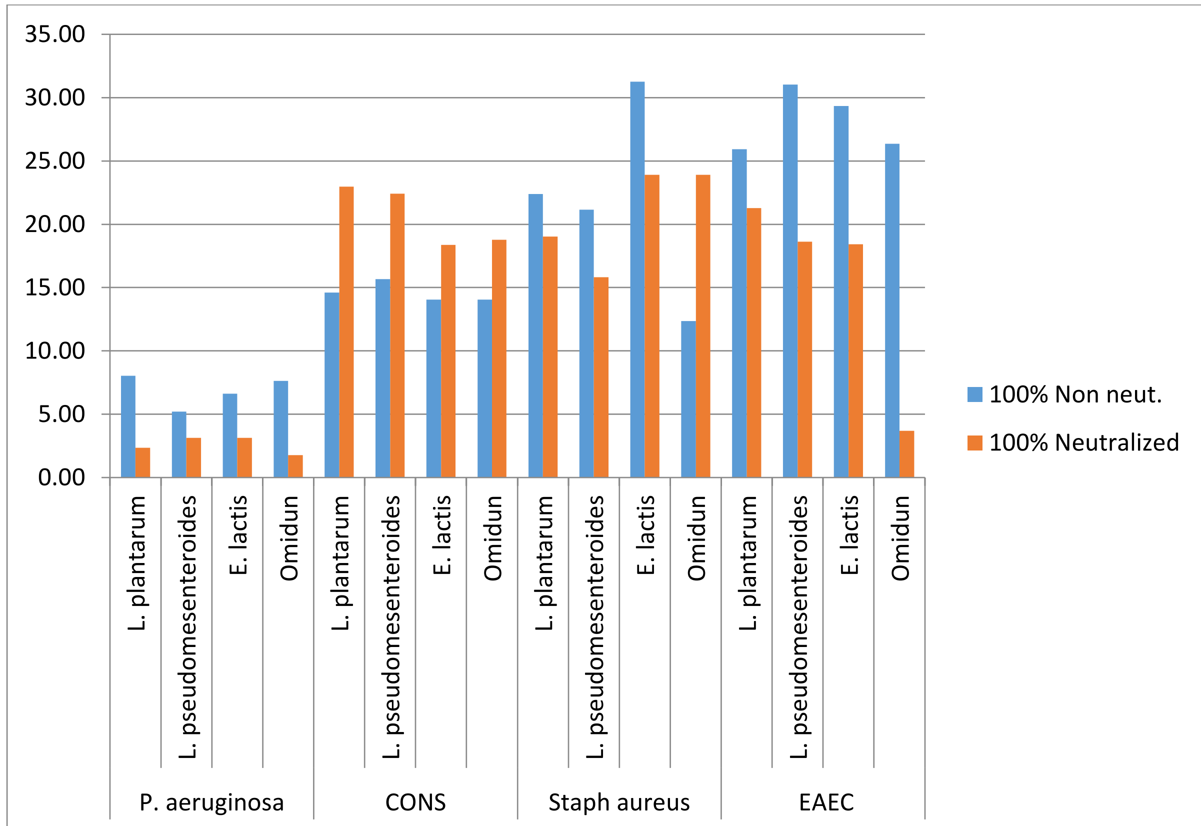

Figure 8: Pre-formed biofilm dispersive effect at 100% concentration (Neutralized and Non neutralized).

Discussion

Currently, pathogenic biofilm remains one of the most relevant virulence factors and the leading cause of antibiotic treatment failure, resulting in chronic infections (Sharma *et al.,* 2019). However, several recent evidence have shown that probiotics have opened new vistas for treatment of pathogenic biofilms. Probiotics have been documented to inhibit the activity of biofilm forming pathogenic bacteria through various mechanisms not limited to; prevention of quorum sensing, interfering with biofilm formation and biofilm eradication (Barzegari *et al.,* 2020). Their mechanism of antimicrobial activity involves the production of inhibitory sustances such as organic acids, bacteriocins and hydrogen peroxide, blockage of adhesion sites, competitive exclusion of pathogens etc. (Markowiak *et al.,* 2017).

This study revealed that non neutralized cell free supernatant of *L. plantarum* exhibited a greater biofilm inhibitory effect against *S. aureus,* EAEC CoNS and *P. aeruginosa* when compared with the neutralized CFS. This suggests that the antibiofilm activity of the studied *L. plantarum* could be directly related to the low pH produced in the medium. Organic acids and other antimicrobial metabolites produced by lactic acid bacteria have been reported to have antibiofilm potentials. This assertion is corroborated by the report of Soltani *et al., (*2022) where non neutralized CFS of *Lactobacillus* spp exhibited antibacterial and antibiofilm activitiy against uropathogenic *E. coli* as a result of the production of organic acid. Previous studies on biofilm inhibitory effect of *Lactobacillus spp* are in tandem with the outcome of this study. *Lactobacillus* strains have been associated with the inhibition of biofilms formed by enteropathogenic bacteria (Miquel *et al.,* 2016; Kaur *et al.,* 2018). Lee *et al.,* (2022) reported that non neutralized cell free supernatant of *L. plantarum* KU200656 demonstrated marked antibiofilm forming activity against *S. aureus and Escherichia coli,* the antibacterial activities in their study was thought to be due to co-aggregation, production of antimicrobial agents, and inhibition of pathogen adherence. Multi drug resistant staphylococci and CoNS have been equally reported to be inhibited by metabolites produced by *Lactobacillus* spp. (Bhola *et al.,* 2019). Specifically, the antimicrobial activities of *Lactobacillus* species are prominent against many pathogens (Varma *et al.,* 201; Adetoye *et al.,* 2018), these effects are strongly associated with the secretion of organic acids and other by-products that accumulate in the supernatant of *Lactobacillus spp*. (Cotar *et al.,* 2013). Organic acid and peroxides produced by lactic acid bacteria are known to cause a decline in cell adherence, preformed biofilm and inhibit biofilm formation (Wasfi *et al.,* 2018).

It was equally observed that *L. pseudomesenteroides* used in this study exhibited an overall greater biofilm inhibitory effect against the test pathogens in the non neutralized state. This further substantiatesthe veracity of the anti-biofilm capacity of organic acid produced by lactic acid bacteria against pathogenic bacteria exemplified by EAEC, *S. aureus,* CoNS and *P. aeruginosa* as demonstrated in this study in support of this result, there are several studies that have reported the antibiofilm properties of *Leuconostoc species*. Ahmad and Awad (2019) confirmed the antibiofilm potential of *Leuconostoc* species against biofilm forming pathogens isolated from food such as *Staphylococcus aureus, Samonella spp, Escherichia coli, Klebsiella spp, Pseudomonas aeruginosa, Bacillus subtilis and Bacillus cereus.*

In contrast to the foregoing, there appears to be no relationship between the pH and the biofilm inhibitory effect of *E. lactis* in this study against EAEC, CoNS, *S. aureus* and *P. aeruginosa* as there was no marked difference in the antibiofilm properties of both neutralized and non-neutralized CFS of *E. lactis*. In fact, it was observed that neutralized CFS produced greater antibiofilm activity against *P. aeruginosa.* It can be inferred that the antibiofilm potential of the tested *E. lactis* could be due to production of other compounds with the production of organic acids. Other mechanisms deployed by benficial bacteria against biofilm formation by pathogens not determined in this study such as quorum sensing, bacteriocin production, biosurfactants among others may be responsible for the antibiofilm property displayed by the tested *E. lactis.* Although, *Enterococcus* has not earned the *GRAS* status owing to its virulence and pathogenic potential; this accounts for the scanty evidence on its beneficial antibiofilm effects. Nevertheless, there are few cases where *Enterococcus* has been proven to have beneficial antibiofilm effect. Kanmani *et al*., (2013) reported the synthesis and functional characterization of an exopolysacharide (EPS) from a probiotic *Enterococcus faecium* MC13 isolated from the gut of fish which was found to exhibit inhibition against biofilm forming pathogens, prominently *Listeria monocytogenes*. A recent study on the evaluation of the antioxidant and potential antibiofilms effect of *Enterococcus lactis* Q1 and 4CP3 strains derived from raw shrimp against methicillin-resistant *Staphylococcus aureus* showed synergistic anti-adhesion, antibiofilm and anti-oxidant properties (Braiek *et al*., 2019)

Omidun equally exhibited a better biofilm inhibitory effect against most of the test pathogens in the neutralized state; this implies that the production of organic acids may not be the main antibiofilm property inherent in *Omidun* against EAEC. *S. aureus,* CoNS and *P. aeruginosa*. *Omidun* is derived from locally fermented food which is reported to be abundantly rich in LAB (Afolayan *et al.,* 2017; Kwasi *et al.,* 2019). Meanwhile, LAB are known to be active producers of organic acid, hydrogen peroxide, bacteriocins and several enzymes during fermentation (Imade *et al*., 2012). Bacteriocinogenic activity of LAB isolated from *Ogi* against foodborne pathogens including *S. aureus, E. coli, P. aeruginosa, Shigella spp and Salmonella* have been documented (Orji *et al*., 2020).

An important finding in this study is the observed susceptibility trend, which has a direct relationship with the magnitude of percentage inhibition. It was generally observed that Enteroaggregative *Escherichia coli* was the most susceptible, followed by *Staphylococcus aureus* and CoNS, while *Pseudomonas aeruginosa* exhibited the least resistance to the biofilm inhibition effect of CFS throughout the study. The percentage inhibition recorded by each LAB under neutralized and non-neutralized state against the pathogens was selected to represent antibiofilm effect against each pathogen. However, there was no significant difference in the percentage biofilm inhibition produced under different concentrations and under neutralized and non-neutralized state. Overall, the inhibition of biofilm by the tested LAB and *Omidun* in this study could be due to organic acids and the production of other antimicrobial metabolites such as bacteriocins, biosurfactants, hydrogen peroxide and inhibitory exopolysaccharides. Other possible mechanisms include competition with the pathogenic biofilm for nutrients and adhesion sites (Toushik *et al.,* 2021).

Biofilm dispersal is naturally one of the steps in biofilm formation where the organisms are released and dispersed into the environment for the purpose of colonizing new sites. For many pathogenic bacteria, biofilm dispersal plays an important role in the transmission of bacteria from environmental reservoirs to human hosts and in the exacerbation and spread of infection within a host (Kaplan,

2010). Biofilm dispersal is a promising area of research that may lead to the development of novel agents that inhibit biofilm formation or promote biofilm cell detachment. Such agents may be useful for the treatment of pathogenic biofilms infections. Although, dispersion results in dissemination of bacteria, it also leaves the former biofilm residents vulnerable- as they are now more susceptible to erstwhile resistant conditions including antimicrobial agents. Percentage dispersion in this study corresponds to magnitude of dispersive effect exerted by the LAB. A direct relationship exists between the percentage biofilm dispersion and biofilm dispersive effect of the LAB and also the susceptibility of the pathogens. There was no significance difference in the percentage biofilm dispersion produced under different concentrations and under neutralized and non-neutralized state. It was observed that *L. plantarum* exhibited the highest biofilm dispersive effect against EAEC followed by *S. aureus*, CoNS and *P. aeruginosa*. The biofilm dispersive properties of *L. plantarum, Omidun* and other LAB in this study are not directly associated with the pH as there appears to be no relationship between the percentage biofilm dispersion and the pH. The dispersive potential exhibited by LAB in this study may be due to its antagonistic activities in the surrounding medium resulting in environmentally induced dispersion. The egression of bacteria cells from the biofilm seems to be driven by competive exclusion leading to changes in chemical concentration gradients of essential nutrients, oxygen and waste products (Rumbaugh and Sauer, 2020).

The result of this current research is in tandem with the report of Rao *et al*., (2015) where the CFS of both *L. plantarum* and *L. pentosus* strains exhibited good biofilm disruptive activity against *P. aeruginosa, Klebsiella pneumoniae*, *Escherichia coli* and *B. subtilis*. Lactobacilli strains have been documented to have biofilm dispersal effect on biofilm forming pathogen, *Vibrio parahaemolyticus* at low pH (Kaur *et al.,* 2018). Varma *et al.,* (2011) also showed the ability of *Lactobacillus fermentum* supernatant to disrupt the surgical wounds and implant-associated *Staphylococcus aureus* and *Pseudomonas aeruginosa* pre-formed biofilm, *Lactobacillus acidophilus* was confirmed to have *in vitro* biofilm dispersive effects against cariogenic biofilm in dental caries (Mei *et al.,* 2013). Kim *et al*., (2022) reported on the biofilm dispersive effect of crude bacteriocin derived from *Lactobacillus brevis* on *Escherichia coli* and *Salmonella typhimurium.*

This study also revealed that *L. pseudomesenteroides* exhibited the highest biofilm dispersive effect against EAEC, followed by *S. aureus* and *P. aeruginosa*. Some previous studies support the biofilm dispersive potential of *Leuconostoc*. A biopolymer dextran produced by *L. pseudomesenteroides* was blended with Gentamycin and Polyvinylpyrrolidine (PVP) and was shown to have biofilm dispersive effect against *E. coli, P. aeruginosa* and *S. aureus* biofilm that are present on catheters (Salman and Salim, 2016). *E. lactis* exhibited the highest biofilm dispersive effect against EAEC, followed by *S. aureus* (non-neutralized: 23.39%, neutralized: 24.36%), CoNS and *P. aeruginosa*. *Omidun* recorded the highest biofilm dispersive effect against against *S. aureus*, followed by EAEC, CoNS and *P. aeruginosa*. There is also very scarce literature about biofilm dispersal effect of *Omidun.* However, the ability of *Omidun* to disperse biofilm can be assumed to be as a result of the antimicrobial substances secreted by its resident LAB flora.

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