Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supporting Information: **Novel Long-acting Antimicrobial Nanomicelle Spray**

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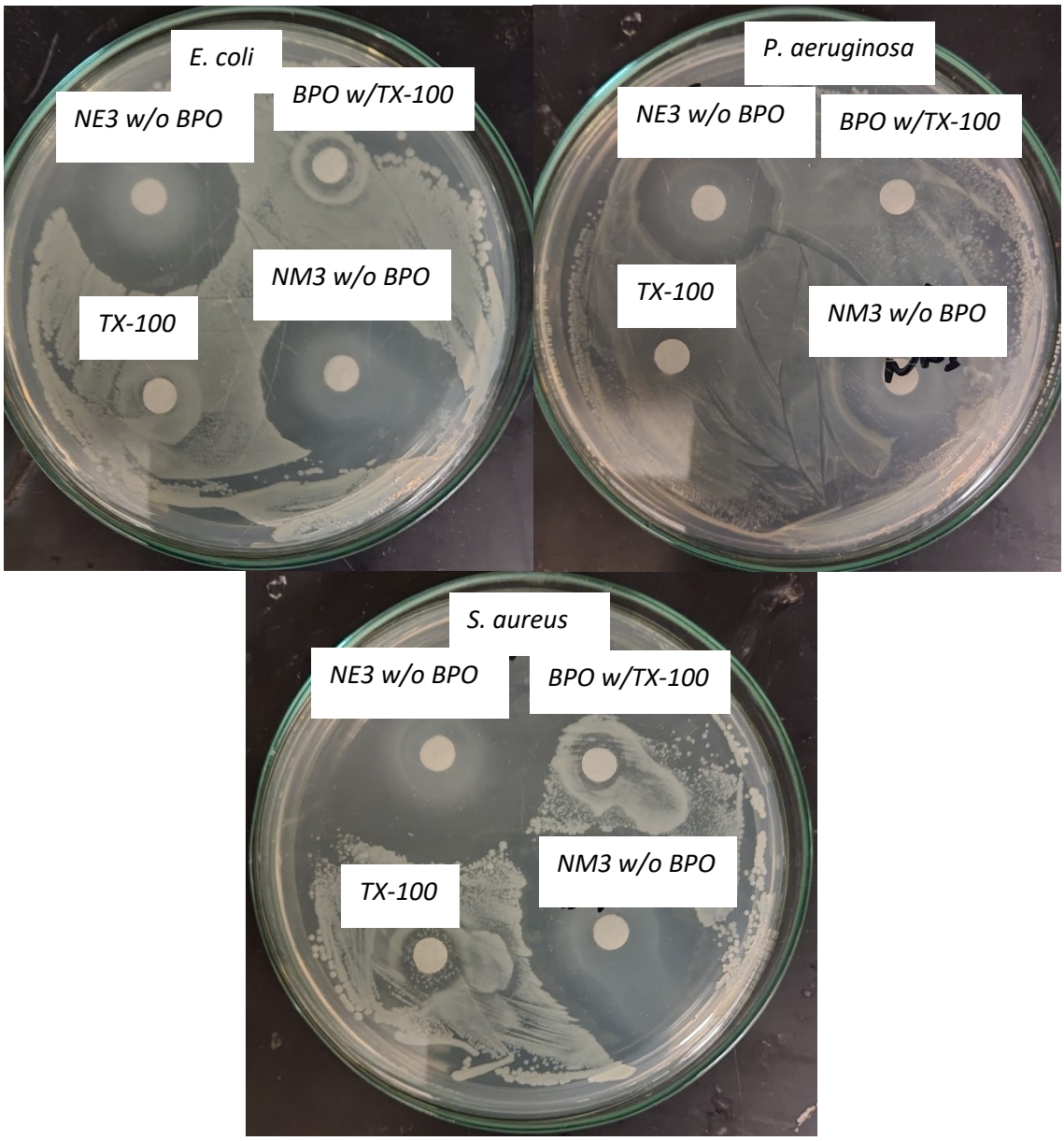
Abstract

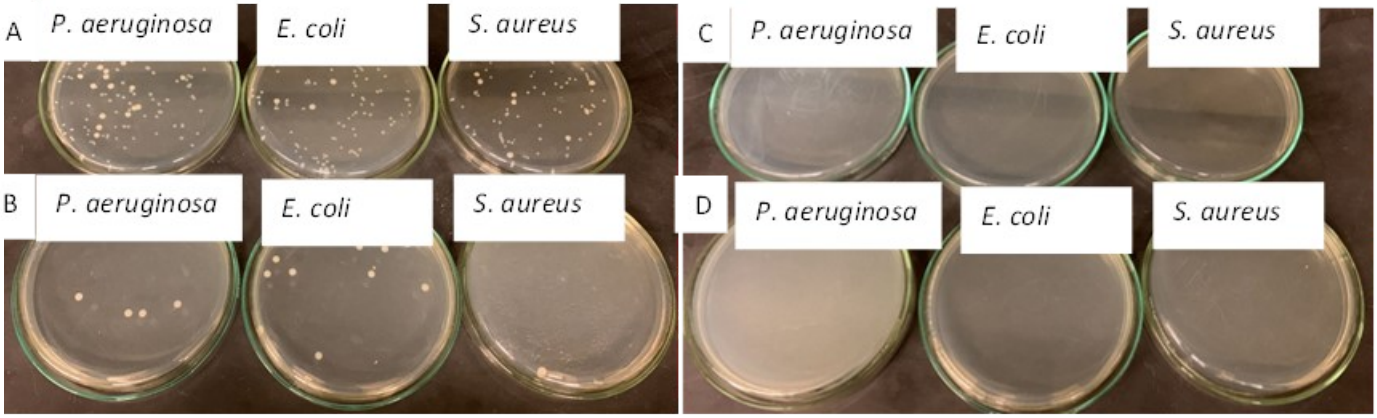
Contaminated surfaces play a major role in disease transmission to humans. The vast majority of commercial disinfectants provide short-term protection of surfaces against microbial contamination. Covid-19 pandemic has attracted attention to the importance of long-term disinfectants as they would reduce the need for staff and save time. In this study, nanoemulsion and nanomicelles containing a combination of benzalkonium chloride (BKC; a potent disinfectant and a surfactant) and benzoyl peroxide (BPO; a stable form of peroxide that is activated upon contact with lipid/membranous material) were formulated. The prepared nanoemulsion and nanomicelle formulae were of small sizes <80 nm and high positive charge >45 mV. They showed enhanced stability and prolonged antimicrobial efficacy. The antibacterial potency was evaluated in terms of long-term disinfection on surfaces as verified by repeated bacterial inoculums. Additionally, the efficacy of killing bacteria upon contact was also investigated. A nanomicelle formula (NM-3) consisting of 0.8% BPO in acetone and 2% BKC plus 1% TX-100 in distilled water (1:5 volume ratio) demonstrated overall surface protection over a period of 7 weeks upon a single spray application. Furthermore, its antiviral activity was tested by the embryo chick development assay. The prepared NM-3 nanoformula spray showed strong antibacterial activities against *Pseudomonas aeruginosa, Escherichia coli, and Staphylococcus aureus* as well as antiviral activities against infectious bronchitis virus due to the dual effects of BKC and BPO. The prepared NM-3 spray holds a strong potential as an effective solution for prolonged surface protection against multiple pathogens.



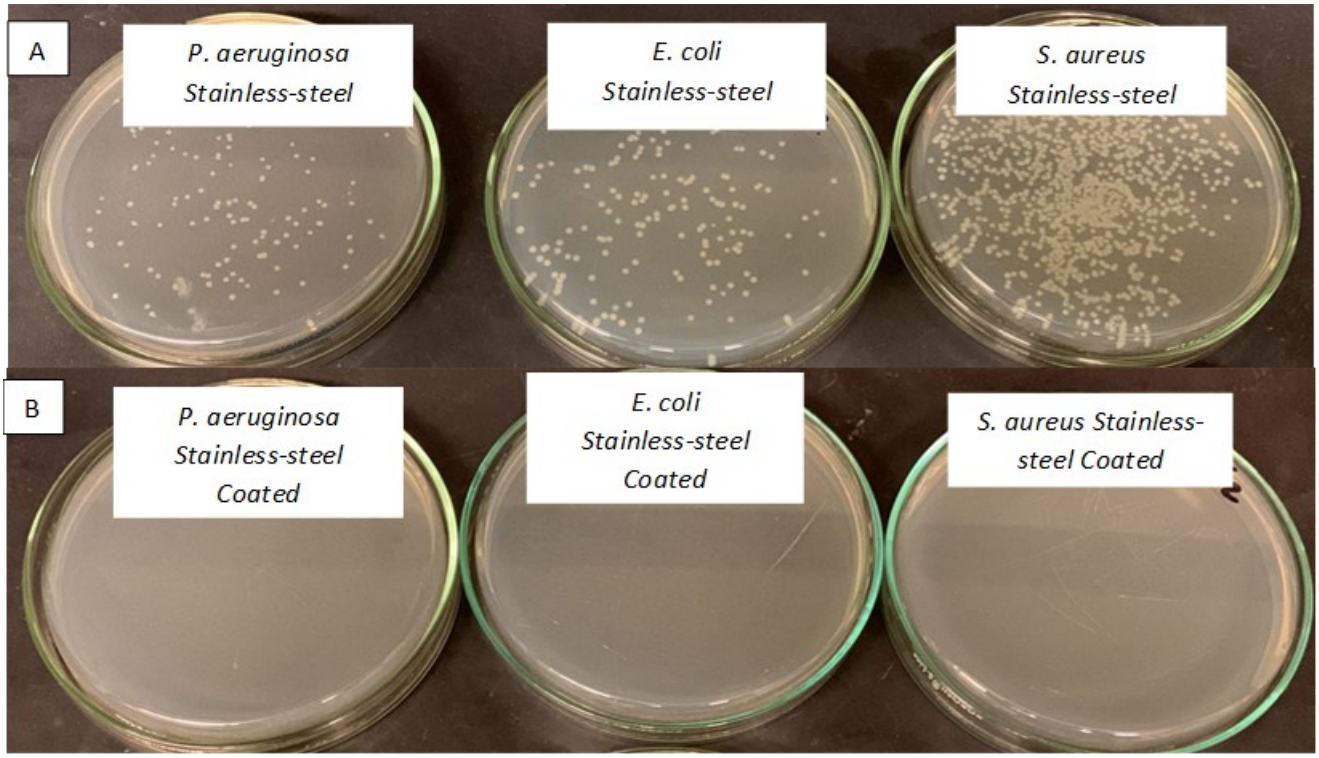
Supplementary Figure 1 Disk diffusion method of empty NM3 (w/o BPO) and NE3 (w/o BPO) and BPO with Tx-100 and without TX-100

Supplementary Table 1: IC50 of Benzalkonium chloride for each bacterial strain.

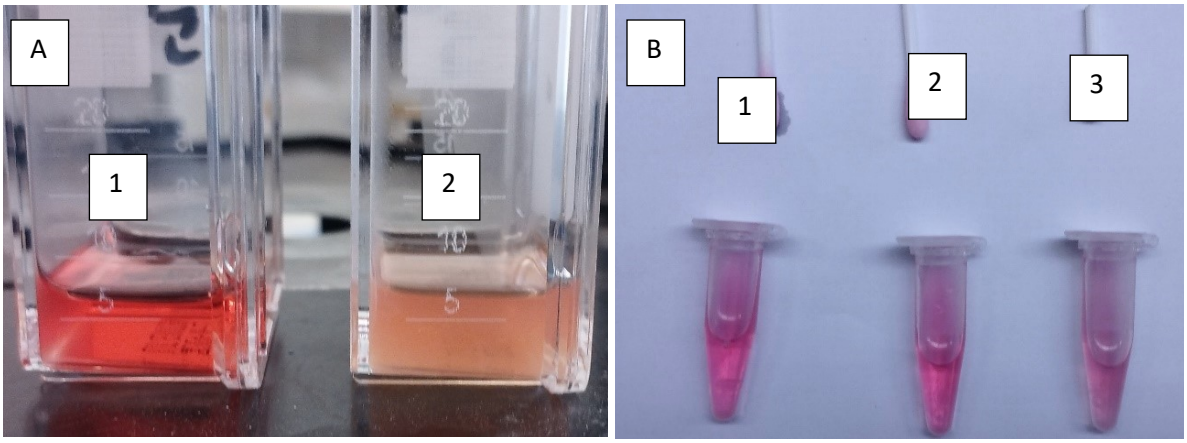
Bacteria	IC50 (W/V %)	Std. error (%)
Escherichia coli	0.01	0.006
Pseudomonas aeruginosa	0.1	0.03
Staphylococcus aureus	0.0123	0.003



Supplementary Figure 2 Week 3 of Antibacterial assay using ISO: 22196:2011 on NM-3 coated glass slides. A) log 3 dilution of inoculum from non-coated glass slides. B) log5 dilution of inoculum from non-coated glass slides. C) log0 dilution of inoculum from NM-3 Coated glass slides D) log 1 dilution of inoculum from NM-3 coated glass slides.



Supplementary figure 3 Antibacterial assay identical to ISO:22196:2011 but instead of NM-3 coated glass slides, NM-3 coated stainless-steel surface were used. (A) Log 3 dilution of inoculum from stainless steel surface of the 2nd week. (B) Log 0 dilution of inoculum from NM-3 coated stainless steel surface of the 2nd week



Supplementary figure 4 A) Growth medium with cells bleaching after addition of 600 µL of NM3 solution (2) in comparison to normal growth medium with cells (1). B) Cotton Swabs tested for bleaching media after soaking in PBS (1), NM3 (3), and rubbed on dry NM3 coat (2).