



## INFLUENCE OF NANO BEE PROPOLIS ON METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN YOGHURT

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### Summary

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Methicillin-resistant *Staphylococcus aureus* (MRSA) presents a serious public health concern due to its resistance to conventional antibiotics. This study evaluated the antibacterial activity of ethanolic propolis extract and its nanoemulsion (PNE) against MRSA and their application in yogurt as a functional food. *S. aureus* was isolated from 33.3% (20/60) of yogurt samples and identified using microbiological and biochemical methods. PCR detection of the *mecA* gene confirmed MRSA in 18 out of 20 isolates (90%), highlighting the reliability of molecular diagnostics. Antibacterial activities of propolis and PNE were assessed by agar well diffusion and minimum inhibitory concentration (MIC) assays. Both showed significant inhibitory effects, with PNE exhibiting superior activity at lower concentrations. Characterisation via dynamic light scattering revealed that PNE had an average droplet size of 123.47 nm and a low polydispersity index (PDI=0.226). MIC for both forms was 0.7%, with complete inhibition observed below this level. Yogurt was fortified with propolis and PNE (0.7%, 1.5%, 3%) and inoculated with MRSA. During 11 days of cold storage, PNE at 3% showed the most effective bacterial reduction. Sensory evaluation by 30 panelists revealed that yogurts enriched with 0.7% propolis and 1.5% PNE maintained high acceptability (scores 4.5 and 4.7, respectively), similar to control sample (4.9). However, higher concentrations of raw propolis reduced acceptability due to bitterness. In conclusion, propolis, particularly in nanoemulsion form, demonstrated strong anti-MRSA activity and potential as a natural preservative in dairy products without compromising the sensory quality.

**Key words:** methicillin resistant *Staphylococcus aureus*, nano bee propolis, yoghurt

### INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant global health concern due to its resistance to beta-lactam antibiotics, making infections challenging to treat (Vestergaard *et al.*, 2019). MRSA

is commonly found in dairy products, including yogurt, posing a serious risk to food safety and public health (Titouche *et al.*, 2022). The presence of *S. aureus* in dairy products often results from contami-

nation during milk handling, processing, or storage, and its ability to produce enterotoxins further exacerbates the potential for foodborne illnesses (Kadariya *et al.*, 2014).

Recent advancements in nanotechnology have cleared the path for innovative antimicrobial solutions, including nanoformulations of natural compounds (Elsherif *et al.*, 2025). Propolis is a natural bee-derived product composed of various bioactive substances, including vegetable balsam, wax, resins rich in phenolic acids and flavonoids, as well as aromatic and essential oils (Salomão *et al.*, 2021). Its chemical composition varies depending on the geographical location of the hive and the botanical sources available to bees. Propolis has attracted considerable scientific interest due to its wide range of biological activities, including antiviral, antioxidant, antibacterial, anti-inflammatory, antifungal, and anticancer effects. Its use in human medicine has increased significantly owing to its natural origin, low cost, minimal side effects, and broad therapeutic potential (Silva-Carvalho *et al.*, 2015).

Beyond its medicinal value, propolis is also employed in food preservation due to its strong antimicrobial and antioxidant properties. However, its intense flavour and aroma can alter the sensory qualities of food, limiting its consumer acceptance. To address this challenge, encapsulation of propolis in nanoemulsion form – typically within a particle size range of 20 to 200 nanometers – has been developed. This approach not only reduces the impact of propolis on food taste but also enhances its antimicrobial effectiveness by improving solubility, stability, and interaction with microbial cells (El-Sharkawy *et al.*, 2014).

Nanoemulsions of propolis (PNE) represent an advanced delivery system designed to overcome the limitations of crude propolis extracts, particularly poor water solubility, strong flavour, and limited bioavailability. PNE typically consist of tiny oil droplets containing dissolved propolis, dispersed in an aqueous phase with the aid of surfactants and mechanical processes such as ultrasonication or high-pressure homogenisation. Nano-encapsulation of propolis enhances its bioavailability, stability, and antimicrobial potential by increasing its surface area and facilitating better interaction with bacterial cells (Amin *et al.*, 2023). Moreover, nano-encapsulation protects sensitive phenolic constituents from environmental degradation, such as oxidation or light exposure, thereby prolonging the shelf life and efficacy of propolis in food or pharmaceutical applications. The small droplet size also facilitates deeper penetration into microbial biofilms and tissues, increasing the therapeutic potential of the formulation. Additionally, PNE enable controlled release of bioactive components, ensuring a sustained antimicrobial effect over time. From a sensory perspective, encapsulating propolis in nanoemulsion form reduces the impact of its strong taste and aroma, making it more acceptable for incorporation into food products such as yogurt and beverages (Hassanien *et al.*, 2021). These advantages make PNE a promising platform for natural preservative systems and functional food development, particularly in combating antibiotic-resistant pathogens like MRSA. Nano bee propolis has demonstrated superior antibacterial effects compared to conventional propolis extracts due to its enhanced penetration into bacterial membranes and improved dispersion in aqueous environments (Javed *et al.*, 2022). These characteristics make

nano-propolis a promising candidate for combating MRSA contamination in dairy products such as yogurt.

This study aims to investigate the influence of bee propolis and its nano form on MRSA in yogurt, evaluating its efficacy in reducing bacterial contamination and ensuring food safety. The findings will contribute to the development of novel, natural antimicrobial agents suitable for application in the dairy industry.

## MATERIALS AND METHODS

### *Sample collection*

A total of 60 yogurt samples were collected from various dairy shops in Assiut Governorate, Egypt in sterile separate containers, labelled, and transported in an ice tank to ensure they reached the laboratory with minimal delay for bacteriological examination.

### *Isolation and identification of *S. aureus**

The procedures followed the guidelines of the FDA 2001. The isolated samples were inoculated onto Baird Parker agar and incubated aerobically at 37 °C for 48 h. *Staphylococcus aureus* was identified based on colony morphology, Gram staining, DNase activity, catalase production, and the fermentation of mannitol using conventional methods (Oxoid, England).

*Staphylococcus aureus* isolates were inoculated onto MRSA Agar Base (7420 Acumedia) for the identification of MRSA strains according to the National Committee for Clinical Laboratory Standards (NCCLS, 1997; Elsherif *et al.*, 2024a).

### *PCR molecular identification of MRSA*

The molecular identification of MRSA isolates was conducted at the Reference Laboratory for Veterinary Quality Con-

trol, Biotechnology Unit, Animal Health Research Institute, Dokki, Giza, Egypt. Genomic DNA was extracted using the QIAamp DNA Mini Kit following the manufacturer's protocol. The presence of the *mecA* gene, a key determinant of methicillin resistance in *Staphylococcus aureus*, was confirmed by polymerase chain reaction (PCR). PCR amplification was carried out in a T3 Thermal Cycler (Biometra, Germany) using a specific primer set targeting the *mecA* gene: forward primer (*mecA*-F): 5'-AAA ATC GAT GGT AAA GGT TGG C-3', and reverse primer (*mecA*-R): 5'-AGT TCT GCA GTA CCG GAT TTG C-3'

The reaction mixture (25 µL) contained 1× PCR buffer, 2.5 mM MgCl<sub>2</sub>, 200 µM dNTPs, 0.5 µM of each primer, 1 U Taq DNA polymerase (Thermo Fisher, USA), and 5 µL of extracted DNA. The PCR cycling conditions were set as followed: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 45 s, with a final extension step at 72 °C for 5 min. The amplified PCR products (310 bp) were resolved by electrophoresis on a 1.5% agarose gel, stained with ethidium bromide, and visualised under a UV transilluminator using a gel documentation system (Alpha Innotech, USA). A 100 bp DNA ladder (Thermo Fisher, USA) was used as a molecular size marker. The detection of a 310 bp amplicon confirmed the presence of the *mecA* gene, indicating methicillin resistance in the tested *S. aureus* isolates (Kumari *et al.*, 1997)

### *Source and composition of propolis*

Propolis powder (PP) was obtained from the Faculty of Agriculture Farm, Al-Azhar University, Assiut Branch, Assiut, Egypt. The chemical composition of Assiut pro-

polis was characterised with a diverse range of bioactive compounds, primarily organic acids, esters, flavonoids, and other minor constituents. Among the identified acids were lactic acid, hydroxyacetic acid, palmitic acid, 4-methoxy-cinnamic acid, 3,4-di-methoxy-cinnamic acid, isoferulic acid, and caffeic acid. Additionally, various esters such as 3-methyl 2-butenyl-cis-4-coumarate, 3-methyl 3-butenyl-trans-4-coumarate, 2-methyl 2-butenyl-trans-4-coumarate, 3-methyl 2-butenyl-trans-4-coumarate, isopentenyl caffeate, 2-methyl-2-butenyl caffeate, and 3-methyl-2-butenyl caffeate were identified. The flavonoid compounds included pinostrobin chalcone, pinocembrin, pinobanksin, and chrysin, which contribute significantly to its biological activities. Other detected components were glycerol, phosphoric acid, benzyl-2-methyl propyl, 3-hydroxypyridine, and 1,2,3-trihydroxybutanal (Hegazi *et al.*, 2001).

#### *Preparation of propolis nanoemulsion*

A novel methodology was employed for the preparation of propolis nanoemulsion (PNE) using an organic surfactant. Propolis powder (200 mg) was dispersed in 100 mL of double-deionised water containing 3% Tween 80 under constant stirring at 40 °C for 7 h. The mixture was then subjected to sonication for 10 min to ensure homogeneity, followed by filtration through a 200 nm membrane filter to remove any residual large particles. This method aimed to enhance the solubility and bio-availability of propolis by reducing its particle size to the nanoscale, thereby improving its potential biological applications (Hassanien *et al.*, 2021).

The physicochemical characteristics of PNE were analysed using multiple analytical techniques. The polydispersity index (PDI) and zeta potential were deter-

mined using a Zetasizer (Malvern Analytical, Spectris Company, UK) at the Nanotechnology Unit, Faculty of Pharmacy, Al-Azhar University, to assess the stability and size distribution of the formulation. Furthermore, transmission electron microscopy (TEM) (Jeol, Japan) was performed at the Electron Microscopy Unit, Assiut University, to examine the morphological characteristics and size of the nanoemulsion particles (Elsharkawy & Elsherif, 2022a).

#### *Agar well diffusion assay*

Agar well diffusion assay was used for determining the minimum inhibitory concentration (MIC) of propolis and its nanoemulsion against MRSA. A bacterial suspension of MRSA was prepared by inoculating a loopful of the stock culture into Brain Heart Infusion (BHI) broth, followed by incubation at 37 °C for 24 h. The bacterial density of the freshly prepared suspensions was standardised to 0.5 McFarland turbidity using a McFarland standard apparatus (Scientific Device Laboratory, Inc., USA).

A 100 µL aliquot of the standardised MRSA suspension was evenly spread onto Mueller-Hinton agar (MHA) plates to ensure uniform bacterial distribution. Wells with a diameter of 4 mm were aseptically created using a sterile cork borer. Each well was filled with 100 µL of either pure propolis extract, its nanoemulsion, or their respective double-fold serial dilutions, ranging down to 0.39% (Jain *et al.*, 2020). A control well, filled with 100 µL of sterile deionised water, was included in each plate to ensure experimental validity. The plates were left at room temperature for 45 min to allow for proper diffusion of the test compounds into the agar medium. Subsequently, the plates were incubated at 37 °C for 24 h. Antibacterial efficacy was

assessed by measuring the diameters of the inhibition zones surrounding each well (Chuensombat *et al.*, 2013). All experiments were performed in triplicate to ensure reproducibility and statistical accuracy (Richter *et al.*, 2010).

#### *Antimicrobial properties of propolis and its nano-emulsion*

To evaluate the antibacterial efficacy of propolis and its nanoemulsion against MRSA, isolated MRSA strains were inoculated into Mueller-Hinton broth and incubated at 37 °C until reaching the exponential growth phase. The bacterial density was standardised to 0.5 McFarland turbidity, equivalent to approximately 10<sup>5</sup> CFU/mL, using a McFarland standard apparatus (Dégi *et al.*, 2022).

Yogurt was prepared performed following a standardised scientific method. Pasteurised full-fat cow's milk was heated at 90 °C for 10 min, then rapidly cooled to 43–45°C. The milk was inoculated with 2–3% commercial yogurt starter culture, consisting of *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*. A 1 mL aliquot of the prepared MRSA suspension was mixed with 100 mL of inoculated milk and distributed into sterile jars. Propolis extract and its nanoemulsion were incorporated at final concentrations of 0.7% and 1.5% propolis and 1.5% and 3% nano-propolis, while a positive control contained MRSA without propolis treatment, and a negative control was prepared without bacterial inoculation or propolis addition. The samples were incubated at 42–45 °C until pH 4.6 was reached (approximately 4–6 hours), then rapidly cooled to 4 °C to halt fermentation. The yogurt was stored at 4 ± 2°C for subsequent analysis.

Microbiological analysis was conducted to assess the inhibitory effects of

propolis and its nanoemulsion on MRSA. Samples were examined using Baird-Parker agar for *S. aureus* enumeration, and bacterial counts were recorded at the time of curd formation (time zero) and every two days until the end of the experiment (Bezerra *et al.*, 2023).

#### *Sensory evaluation*

A sensory evaluation was performed to assess the acceptability of yogurt fortified with propolis and its nanoemulsion. Control yogurt jars (free from MRSA but containing propolis and its nanoemulsion at concentrations of 0.7%, 1.5% propolis and 1.5, 3% nano-propolis) were prepared using the same methodology. A total of 30 panelists (15 males and 15 females of various ages and educational backgrounds) participated in the evaluation. Sensory attributes, including flavour and palatability, were assessed using a four-point hedonic scale: strongly agree (SA)=5, agree (A)=4–3, disagree (D)=2, and strongly disagree (SD)=1 (Santos *et al.*, 2020). Sensory scores were statistically analyzed to determine consumer preferences for propolis-enhanced yogurt formulations (Javed *et al.*, 2022).

#### *Statistical analysis*

All experiments were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD). Data analysis was performed using SPSS software (version X.0, IBM, USA). For microbiological data, bacterial counts (log CFU/mL) were analysed using one-way analysis of variance (ANOVA) to compare different treatment groups: MRSA control, propolis, and propolis nanoemulsion. Tukey's *post hoc* test was applied for multiple comparisons to determine significant differences between groups. For sensory evaluation, the responses

from 30 panelists were analysed using a chi-square test to assess differences in consumer preferences for yogurt formulations containing different concentrations of propolis and its nanoemulsion. A P-value <0.05 was considered statistically significant in all analyses.

## RESULTS

The incidence of *Staphylococcus aureus* in yogurt samples is presented in Table 1. Out of a total of 60 yogurt samples examined, *S. aureus* was detected in 20 samples, representing an incidence rate of 33.3%. This finding indicates a considerable level of contamination, underscoring the potential public health risk associated with the consumption of improperly handled or stored dairy products.

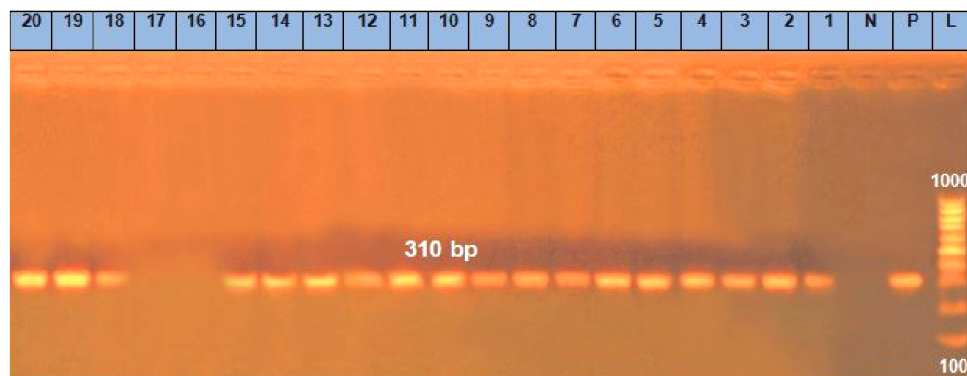
Polymerase chain reaction (PCR) was performed to detect the presence of the *mecA* gene, which is responsible for methicillin resistance in *Staphylococcus aureus* isolates. The target amplicon was observed at 310 base pairs (bp). The results confirmed the presence of the *mecA* gene in 18 out of 20 MRSA isolates. Spe-

**Table 1.** Incidence of *Staphylococcus aureus* in yoghurt

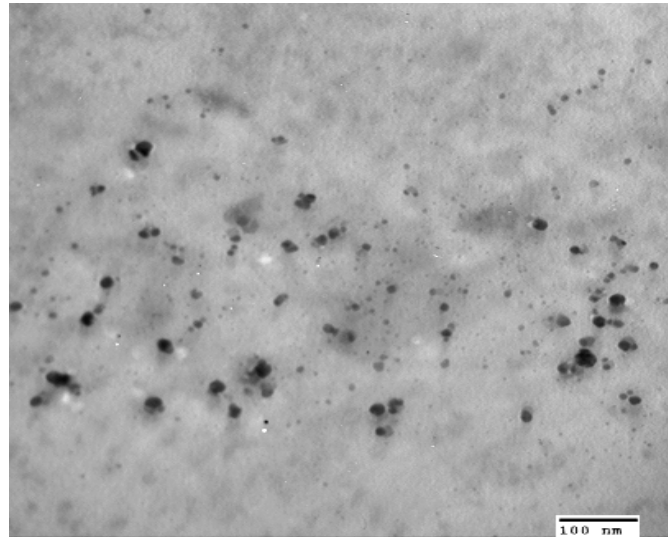
Sample type	Yoghurt
Number of examined samples	60
Positive samples	20
Percentage (%)	33.3

cifically, lanes 1 to 15 and lanes 18 to 20 showed clear amplification bands at 310 bp, indicating *mecA*-positive isolates. In contrast, lanes 16 and 17 showed no amplification, confirming the absence of the *mecA* gene in those isolates (Fig. 1). These findings reinforce the reliability of PCR as a rapid and specific method for confirming methicillin resistance in *S. aureus* isolates.

Transmission electron microscopy (Fig. 2) revealed that the prepared propolis nanoemulsion (PNE) exhibited a spherical and well-separated morphology, with an average particle size of approximately 76.34 nm. The uniform shape and dispersion confirm the successful formulation of the nanoemulsion at the nanoscale. Further physical characterisation of PNE was done using dynamic light scattering (DLS) (Table 2). The average droplet size



**Fig. 1.** PCR results for detection of *mecA* gene of MRSA isolates at 310 bp. Lanes 1–15, 18–20: positive isolates; lanes 16, 17: negative isolates; lane N: negative control; lane P: positive control; lane L: marker ladder.



**Fig. 2.** Transmission electron microscopy for PNE with average size 76.34 nm and spherical shape.

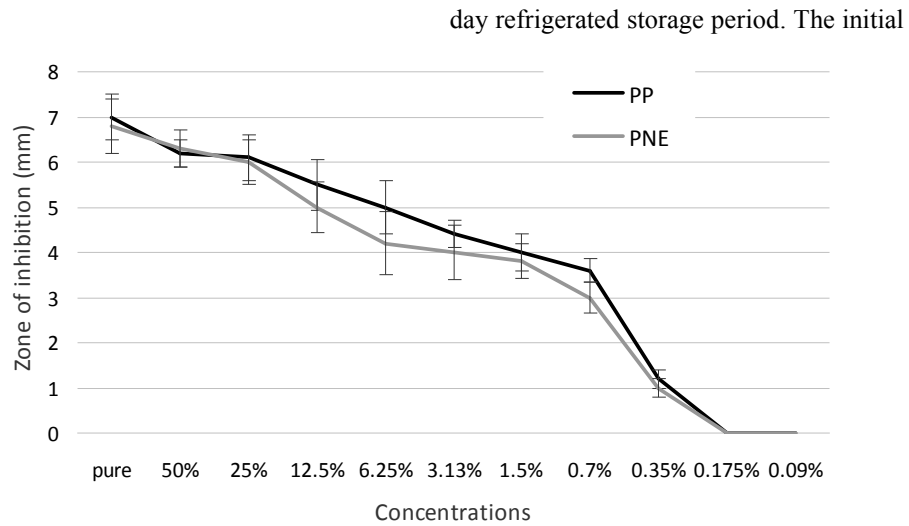
of the formulated nanoemulsion was 123.47 nm, with a polydispersity index (PDI) of 0.226, indicating a narrow size distribution and good stability of the nanoemulsion system. These findings support the nanoscale nature and homogeneity of the prepared PNE, which are essential characteristics for enhancing the antimicrobial efficacy and bioavailability of encapsulated compounds.

The antibacterial efficacy of ethanolic bee propolis extract (PP) and its nanoemulsion (PNE) against all isolated methicillin-resistant *Staphylococcus aureus* (MRSA) was evaluated using the agar well diffusion method at different concentrations. The results showed a concentration-dependent inhibition for both PP and PNE (Fig. 3). At the highest concentration (pure form), both PP and PNE exhibited the maximum zone of inhibition, with a larger inhibition zone (7 mm) for pure propolis compared to its undiluted nanoemulsion (6.8 mm). As the concentrations decreased from 50% to 0.09%, a gradual reduction in the inhibition zone

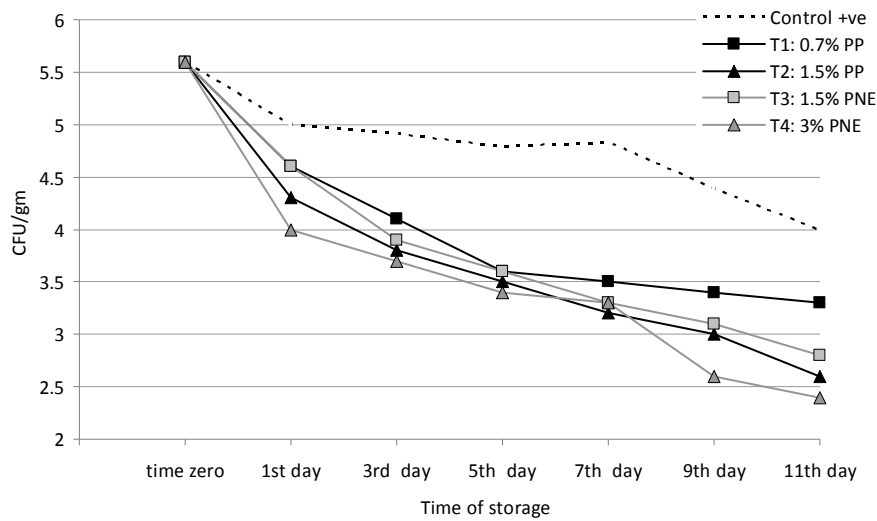
was observed. PP consistently demonstrated slightly higher inhibition zones compared to PNE at most concentrations, especially in the range between 12.5% and 0.7%. Although at lower dilutions, PNE maintained greater or comparable antimicrobial activity (50% and 25% dilutions showed 6.3 mm and 6 mm for PNE vs 6.2 mm and 6.1 mm for PP, respectively). However, the differences between the two treatments were relatively small and within the standard error range. The antimicrobial performance of PNE remained effective even at a 6.25% concentration (4.2 mm inhibition zone), while both forms lost activity at concentrations below 0.7%, indicating a concentration threshold necessary for bactericidal activity against MRSA.

**Table 2.** Physical properties of formulated propolis nano-emulsion (PNE)

Type of nano-emulsions	PNE
Average droplet size (nm)	123.47
Polydispersity index (PDI)	0.226



**Fig. 3.** The inhibitory effect (MIC) of different concentrations of propolis (PP) and propolis nano-emulsion (PNE) on MRSA. Data are presented as mean  $\pm$  SD (n=18).



**Fig. 4.** Changes in average MRSA counts (CFU/g) demonstrating the beneficial effect of propolis (PP) and its nano-emulsion (PNE) against MRSA inoculated in yoghurt. T1: 0.7% PP; T2: 1.5% PP; T3: 1.5% PNE; T4: 3% PNE; Control +ve: MRSA without propolis.

Fig. 4 illustrates the effect of incorporating different concentrations of propolis nanoemulsion (PNE) on the viability of methicillin-resistant *Staphylococcus aureus* (MRSA) in yoghurt samples over an 11-

bacterial count in all samples was approximately 5.5 log CFU/g. In the positive control group (without PNE), the MRSA count showed a slight reduction over time, reaching around 4.1 log CFU/g by the 11<sup>th</sup>

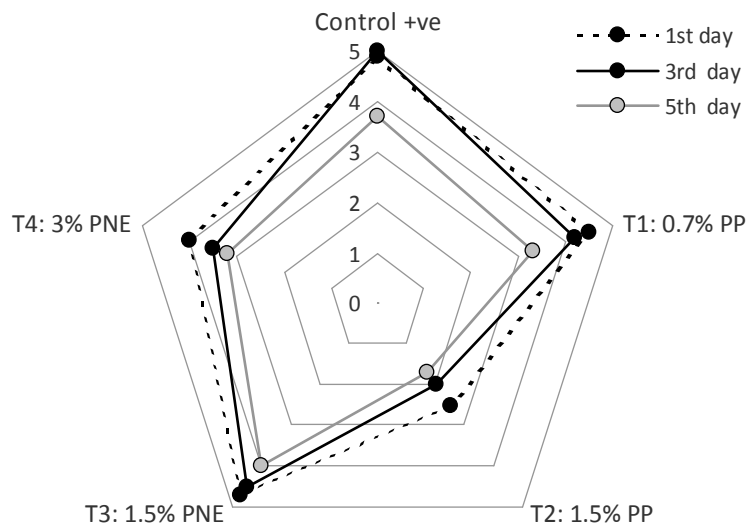
day, indicating limited natural inhibition under storage conditions. In contrast, yogurt samples treated with PNE exhibited a more pronounced reduction in bacterial counts. The degree of inhibition was concentration-dependent:

- T1 (0.7%) showed a moderate decrease, with MRSA counts gradually dropping to just below 4 log CFU/g by day 11;
- T2 and T3 (both 1.5%) showed enhanced antimicrobial activity compared to T1, with a greater and more consistent reduction, reaching approximately 3.3–3.5 log CFU/g by the end of the storage period;
- T4 (3%) demonstrated the strongest antibacterial effect. The MRSA count declined rapidly after the first day and continued to decrease steadily, reaching the lowest level (~3 log CFU/g) by day 11.

These findings confirm that higher

concentrations of PNE significantly enhance the antibacterial effect against MRSA in yogurt during refrigerated storage. Incorporating PNE at concentrations of 1.5% and above appears particularly effective in extending microbiological safety.

The radar chart (Fig. 5) illustrates the average sensory evaluation scores of yogurt samples fortified with different concentrations of propolis and propolis nanoemulsion (PNE) over a 5-day refrigerated storage period. The attributes were scored on a 5-point hedonic scale, where higher values indicate better sensory acceptability. On the 1<sup>st</sup> day, all treatments, including the control, received relatively high sensory scores ranging from 4.0 to 5.0, indicating good initial acceptability. By the 3<sup>rd</sup> day, a slight decrease in scores was observed in most samples, with more noticeable decline in T2 (1.5%) and T3 (1.5%), potentially due to stronger propolis flavour or colour changes. By the 5<sup>th</sup>



**Fig. 5.** Average scores from the sensory evaluation of propolis (PP) and its nano-emulsion (PNE) inoculated in yogurt during the storage period. T1: 0.7% PP; T2: 1.5% PP; T3: 1.5% PNE; T4: 3% PNE; Control +ve: MRSA without propolis.

day of storage, a significant decline in sensory scores was observed across all treatments, with the most pronounced reductions occurring in T2 and T3, where overall acceptability dropped to below 3.0. In contrast, both the control group and T1 (0.7% PNE) maintained comparatively higher scores, remaining close to 4. These findings highlight that yogurt supplemented with a lower concentration of PNE (T1) was able to retain superior sensory attributes during storage.

## DISCUSSION

The current study provides a critical insight into the microbiological safety of yogurt sold in local dairy shops within Assiut Governorate, Egypt. The collection of 60 yogurt samples in sterile, labelled containers and their prompt transport under chilled conditions ensured minimum external contamination and preserved the microbial integrity of each specimen. This strict adherence to standard sampling protocols is essential for accurate bacteriological examination, especially when targeting opportunistic pathogens such as *Staphylococcus aureus*.

The detection and identification of *S. aureus* were carried out following the Food and Drug Administration (FDA, 2001) guidelines, ensuring internationally accepted reliability and reproducibility of results. Baird Parker agar remains a gold-standard selective medium for *S. aureus*, as it supports the growth of characteristic black colonies with clear zones due to tellurite reduction and lecithinase activity. Complementary confirmatory tests – including Gram staining, catalase and DNase production, and mannitol fermentation – collectively provided a robust phenotypic profile for the accurate identification of *S. aureus*.

The results revealed a substantial prevalence of *S. aureus*: in 33.3% (20/60) of yogurt samples (Table 1). This rate is alarming, as it underscores lapses in hygienic practices during production, storage, or distribution. The presence of *S. aureus* in ready-to-eat dairy products such as yogurt represents a direct public health risk, particularly due to its potential to produce heat-stable enterotoxins, which are resistant to conventional pasteurisation. This prevalence aligns with findings from Alexandria, where *S. aureus* was identified in 5.7% of yogurt samples, indicating widespread contamination across different regions (Abd El Halem, 2019).

Further screening for MRSA using MRSA Agar Base followed the NCCLS 1997 guidelines and confirmed the presence of methicillin-resistant strains among the previously identified *S. aureus* isolates. This is a significant finding, given the clinical and epidemiological implications of MRSA as a multidrug-resistant pathogen. The emergence of MRSA in food products reflects not only the possible misuse of antibiotics in dairy farming but also the potential for zoonotic transmission of resistant strains through the food chain (Elsherif *et al.*, 2024b). The identification of MRSA strains among the isolates is particularly alarming. MRSA presence in dairy products has been documented in various Egyptian governorates. For instance, a study in Kafr El-Sheikh reported an 8.33% overall occurrence of MRSA in food samples, with raw milk showing the highest prevalence at 13.3% (Naeim *et al.*, 2023). Similarly, research in Mansoura revealed that 35% of yogurt samples were contaminated with MRSA strains, all harboring the *mecA* gene, which confers resistance to methicillin and other  $\beta$ -lactam antibiotics (Omran *et al.*, 2023).

The antimicrobial resistance profiles of these isolates are concerning. High resistance rates to commonly used antibiotics such as penicillin G, tetracycline, and amoxicillin have been observed, limiting treatment options (Omran *et al.*, 2023; Abd El Halem, 2019). The emergence of vancomycin-resistant *S. aureus* (VRSA) further complicates the scenario, as vancomycin is often considered a last-resort antibiotic. A recent study highlighted the presence of VRSA strains in Egyptian dairy herds, emphasising the potential for these resistant strains to enter the human food chain (Tartor *et al.*, 2024). These findings highlight the urgent need for stringent hygienic practices during milk production, processing, and distribution. Implementing regular screening protocols for *S. aureus* and MRSA in dairy products is essential. Moreover, educating dairy handlers about proper sanitation and the risks associated with antibiotic misuse can play a pivotal role in mitigating contamination and resistance spread.

The molecular identification of MRSA isolates through PCR amplification of the *mecA* gene remains a cornerstone in clinical microbiology, given its high sensitivity and specificity. In this study, the detection of the *mecA* gene in 18 out of 20 MRSA isolates (90%) underscores the reliability of PCR-based methods in confirming methicillin resistance.

The *mecA* gene encodes penicillin-binding protein 2a (PBP2a), which has a low affinity for  $\beta$ -lactam antibiotics, thereby conferring resistance to methicillin and related drugs. This mechanism is well-documented and continues to be a significant concern in both clinical and environmental settings. Recent studies have highlighted the prevalence of *mecA*-positive MRSA strains in various environments, emphasising the importance of

continuous surveillance. For instance, the study by Elhassan *et al.* 2015 reported the detection of the *mecA* gene in MRSA isolates from clinical and environmental samples, indicating the widespread nature of these resistant strains.

Furthermore, advancements in molecular diagnostics have led to the development of rapid and sensitive detection methods. A study by Lee *et al.* 2024 introduced a single-tube dual-gene detection system that simultaneously identifies the *mecA* and *nuc* genes, streamlining the diagnostic process for MRSA. Therefore, the detection of the *mecA* gene via PCR remains a gold standard for confirming methicillin resistance in *S. aureus* isolates. The high prevalence of *mecA*-positive MRSA strains in various environments underscores the need for ongoing surveillance and the development of rapid diagnostic tools to mitigate the spread of these resistant pathogens.

Propolis, a resinous substance collected by honeybees from plant sources, is widely recognised for its broad-spectrum biological activities, largely attributed to its complex and regionally variable chemical composition. In this study, propolis powder was sourced from the Al-Azhar University farm in Assiut, Egypt, and characterised by a diverse matrix of organic acids, esters, flavonoids, and other minor constituents. Notably, the identified acids such as caffeic acid, isoferulic acid, and cinnamic acid derivatives – are well-known for their antimicrobial, antioxidant and anti-inflammatory properties (Hegazi *et al.*, 2001). The esters and flavonoids, including pinostrobin chalcone, chrysin, and pinocembrin, have been documented to enhance the bioactivity of propolis, particularly its antibacterial and anticancer potential (Pasupuleti *et al.*, 2017).

Despite its potent bioactive profile, the clinical and pharmaceutical applications of crude propolis are limited due to its poor water solubility and bioavailability. To overcome these challenges, a nanoemulsion formulation of propolis (PNE) was developed using Tween 80 as a surfactant. The formulation process consisting of prolonged stirring, sonication and membrane filtration, successfully reduced the particle size to the nanoscale, enhancing the physicochemical stability and dispersion of the active constituents (Hasanien *et al.*, 2021).

The dynamic light scattering (DLS) characterisation of PNE revealed an average droplet size of 123.47 nm with a low polydispersity index (PDI = 0.226), indicating a narrow and uniform size distribution. These parameters are within the optimal range for biological applications as smaller, more uniform particles improve cellular uptake and bioactivity (Elsharkawy & Elsherif, 2022b). Additionally, transmission electron microscopy (TEM) confirmed the spherical morphology of the nanoemulsion particles, with an average size of 76.34 nm. This slight discrepancy between DLS and TEM measurements is typical, as TEM reflects the dry particle size, while DLS includes the hydration shell in measurement.

The enhanced formulation of propolis into a nanoemulsion form holds promising potential for antimicrobial and therapeutic applications, particularly when addressing antibiotic-resistant pathogens such as MRSA.

The agar well diffusion assay performed in this study effectively demonstrated the antimicrobial potency of both crude propolis and its nanoemulsion (PNE) against MRSA, with clear zones of inhibition observed at decreasing concentrations. This assay is widely recognised

for its sensitivity and simplicity in evaluating the antimicrobial potential of both natural and synthetic agents (Jain *et al.*, 2020).

The MIC values for crude propolis and PNE were determined based on the lowest concentration that yielded visible zones of inhibition. This is consistent with previous studies indicating that nanoemulsification can enhance the antimicrobial efficiency of bioactive compounds due to improved dispersion and greater bioavailability (Yang *et al.*, 2022; Kazemi *et al.*, 2024).

Previous results support the hypothesis that nanoemulsified propolis can enhance the interaction between antimicrobial agents and bacterial cell membranes, possibly due to the increased surface area and improved solubility of the encapsulated compounds (Silva *et al.*, 2022). Compared to traditional antibiotics, which are increasingly challenged by rising MRSA resistance, natural products like propolis offer a promising alternative, especially when modified into nano-formulations (Hegazi *et al.*, 2001). The superior activity of PNE at submaximal concentrations is in agreement with findings by Kazemi *et al.* 2024, who reported similar trends with nanoencapsulated propolis against *Pseudomonas aeruginosa*.

Furthermore, the triplicate design and use of standardised McFarland turbidity ensured reproducibility and statistical validity of the results, as recommended in microbiological standard protocols (Richter *et al.*, 2010). The agar well diffusion method, despite some limitations in mimicking complex food matrices, remains an essential preliminary screening technique for antimicrobial candidate evaluation.

The study found that the higher raw propolis concentrations impart undesirable sensory characteristics, such as bitterness and resinous odour. This is in line

with prior findings by Viuda-Martos *et al.* (2010), who noted that unprocessed propolis can adversely influence the taste profile of dairy products at elevated levels. As storage progressed, a gradual decline in sensory acceptability was observed across all treatments, consistent with expected changes in yogurt properties such as acidity and microbial activity. Notably, the PNE-fortified samples (especially at 1.5% and 3%) maintained relatively high scores throughout storage, particularly when compared to their equivalent concentrations of crude propolis. These results confirm the findings from Silva *et al.* (2022), who demonstrated that nanoemulsion systems improve the organoleptic acceptability of functional foods by enhancing dispersibility and reducing the intensity of strong bioactive compounds. Furthermore, nanoencapsulation allows for more controlled release and interaction with food matrices, thus improving both the stability and sensory experience of enriched products (Pateiro *et al.*, 2021).

Overall, this study supports the use of propolis nano-emulsion as a superior delivery system for propolis in yogurt fortification, offering a balance between antimicrobial efficacy and consumer acceptability.

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