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Prevalence of resistant *Staphylococcus aureus* strains in frozen meat and their control using phytosynthesized selenium nanoparticles

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Abstract

Staphylococcus aureus continually threatens the safety of meat products, especially the multidrug-resistant (MDR) strains. The screening of MDR *S. aureus* prevalence in marketed meat products in Saudi Arabia was conducted via molecular identification of resistance genes (*cfr*, *gyrA,* and *gyrB*) that occurred in isolated bacteria. The green phytosynthesis of selenium nanoparticles (Se-NPs) was also conducted, using the fruits' extract of *Phyllanthus Emblica* (*Pe*E), for their evaluation as antibacterial nanocomposites against MDR *S. aureus* isolates. The prevalence percentage (PP) of *S. aureus* in meat samples was 14.2%, where the MDR *S. aureus* prevalence was 9.2%. The highest prevalence of *S. aureus* isolates was attained from minced meat samples, whereas the highest PP for multidrug-resistant (MDR) *S. aureus* was recorded from sausages samples. The *Pe*E-synthesized Se-NPs had negative charges, spherical shapes, and well-disperssion with mean diameters of 11.98 nm. The anti- *S. aureus* activities of *Pe*E, phytosynthesized Se-NPs, and their composite (*Pe*E/Se-NPs) were proved qualitatively and quantitatively against the different standard and MDR strains, the antibacterial action of *Pe*E/Se-NPs was the strongest. The treatment of MDR *S. aureus* with *Pe*E/Se-NPs led to severe cells' lyses/explosion after 8 h of exposure. The nanocomposites from *Pe*E/Se-NPs are recommended for controlling MDR *S. aureus* in meat.

Keywords: Green synthesis; multidrug-resistance; mode of action; *Staphylococcus* Prevalence.

1. Introduction

Bacterial pathogens' transmissions through meat products are major public health threats; the frequency of trade and exportation are continually increasing worldwide, which extends the potential risk factors from the global distribution of pathogenic bacteria through exported foods (Osman *et al*., 2017). The meat pathogens' risks seriously increased with their resistance to ordinary antibiotics, especially with the multidrug-resistant (MDR) strains (Chan *et al*., 2008; Osman *et al*., 2016). Amongst all identified bacteria, *Staphylococcus aureus* has extreme concerns associated with health caring because of its ability to cause a wide diversity of life-threatening infections (Paterson *et al*., 2014).

Various materials do exist with anti-staphylococcal activities, e.g. daptomycin, fluoroquinolones, linezolid, and tetracyclines, but they have rapidly become less effective due to bacterium ability to develop different mechanisms for neutralizing these compounds (Lowy, 2003).

The most recognized resistance mechanism in *S. aureus* strains is the methicillin resistance, which designates the MRSA (Methicillin-resistant *Staphylococcus*

aureus) strains. (Makgotlho *et al*., 2009); MRSA could gain extra resistance against multiple drugs and antibiotics, which increase their danger. MRSA strains were detected in 7.5% of the Danish imported pork and 18% of broiler meat (Agersø *et al*., 2012). MRSA became frequently resistant to further antimicrobials than methicillin, urging the necessity for novel effective antimicrobials from innovative sources, fluoroquinolone compounds (e.g. ciprofloxacin and norfloxacin) were proposed as effectual candidates to eradicate MRSA strains (Osman *et al*., 2017).

Commonly, the synthesis of metals nanoparticles (NPs) involved physical and chemical approaches. The disadvantages of the first methods (physical) include high energy necessity, low yields of produced nanomaterials, and elevated cost (Gahlawat & Choudhury, 2019). Also, most chemical methods are harmful ecologically due to involved hazardous chemicals in NPs synthesis. Promisingly, green biological synthesis (Biosynthesis) of metal NPs could overcome most of the abovementioned disadvantages because these methods are eco-friendly, cost-effective, and easily applicable (El Shafey, 2020). These methods generally exploit living organisms and/or their biological derivatives, e.g. plants, microbes, algae, animals' metabolites, biopolymers …etc., for NPs synthesis and production (Gahlawat & Choudhury, 2019; ElSaied *et al*., 2021). The biogenic/green synthesis of metals NPs, using secondary metabolites of various plants, were proved as are effectual methods for NPs production (Akhtar *et al*., 2013). The principal Phyto compounds in plants that assist in the reduction of metal ions are phenolic compounds, flavonoids, enzymes, amino acids, polysaccharides, citric acid, heterocyclic compounds, terpenoids, tannins, peptides, and saponins (El Shafey *et al*., 2020).

Plant extracts were enduringly exploited for fighting pathogenic organisms (De Britto *et al*., 2011; Manzoor *et al*., 2016**;** Arshad *et al*., 2019).

Amla "*Phyllanthus Emblica* L.; Syn. *Emblica Officinalis* Gaertn." was investigated for treating various disorders/diseases, including heart diseases, scurvy, and cancer; the key functions of plant leaves' constituent were the anti-platelet and anti-neutrophilic properties. Additionally, the Amla extracts exhibited numerous pharmacological characteristics, including their antimutagenic, anti-bacterial, antioxidant, anti-allergic, and anti-viral (HERPES VIRUS, HIV, CMV, AIDS) activities (Liu *et al*., 2008; Rahman *et al*., 2009; Poltanov *et al*., 2009). The usage of *P. Emblica* extract*s* (*Pe*E) for the biosynthesis of metals nanoparticles is illustrated due to the reducing powers of *Pe*E and its contents from bioactive phytochemicals (Gunti *et al*., 2019).

The necessity of Selenium (Se) human body was confirmed as a vital requirement for health maintenance "average daily requirement ranged from 40-300 mg as a dietary supplement for adults"; the Se exceptional physicochemical attributes include its chemical stability, biocompatibility, and minimal toxicity (Rayman, 2005). The Se deficiency is correlated with > 40 human disorders (Shirsat *et al*., 2015; Menon *et al*., 2019). The Se-NPs are much effective than native Se, regarding their bioactivities (Huang *et al*., 2016; Gunti *et al*., 2019). Even with higher doses, the human body can naturally degrade Se-NPs; the remaining residues in the body act as nutritional Se sources, which are nontoxic to humans Shirsat *et al*., 2015; Gunti *et al*., 2019; Menon *et al*., 2019). Se-NPs were effectually employed as functional drug-carriers, antimicrobial and anticancerous agents (Shirsat *et al*., 2015; Cremonini *et al*., 2016; Kokila *et al*., 2017; Menon *et al*., 2019; ElSaied *et al*., 2021)

Accordingly, the current investigation targeted the screening of MDR *S. aureus*

prevalence in marketed meat products in Saudi Arabia, phytosynthesis of selenium nanoparticles using *P. Emblica* extract, and evaluation of their capabilities to inhibit resistant bacterial strains.

2. Materials and Methods

2.1. Isolation and Identification of *Staphylococcus aureus* From Frozen Meat Samples

2.1.1. Bacterial isolation and identification:

A randomized collection of 120 raw meat products samples (whole meat, minced meat, sausages, and burger) was done from 27 butcheries in the middle and east regions of Saudi Arabia. Collected samples were upheld in ice-boxes until delivery to the laboratory, then meat samples (25 g) were transferred into 9 folds (w/v) from sterile PBS (phosphate-buffered saline, pH 7.4) and homogenized (Lab-Blender 400 stomacher, PBI, Milan, Italy) for 10 min.

Portions (25 mL) from homogenate were mixed with of Giolitti-Cantoni broth (10 ml) (Oxoid, Hampshire, UK), shaking incubated for 20–24 h at 37 °C then seeded onto supplemented Baird Parker medium (Oxoid) with an emulsion of egg yolk tellurite. Seeded plates were incubated again and individual colonies were streaked onto Tryptic Soy Agar (TSA, Sigma-Aldrich) with blood (TSA with 5% sheep blood), and re-incubated. Characterized staphylococci colonies (black, with or without a halo) were additionally identified via Gram staining, tube coagulase test, catalase assay, and additional tests for biochemical identification as stated in the standard diagnostic protocol (ISO 6888-1:1999/Amd.2:2018).

Further confirmation of bacterial identities was conducted using API® Staph system (bioMérieux SA, l'Etoile – France), according to manufacturer' instructions

2.1.2. Phenotypic determination of the antibiotic resistance profile:

Using the disk diffusion method, the antibiotic resistance phenotype was examined in the 32 *Staphylococcus* spp. isolates that were recovered from meat products on Mueller–Hinton agar plates as described (CLSI, 2007). *Escherichia coli* ATCC-25922 and *S. aureus* ATCC-25923 were employed as negative and positive controls, respectively. The examined antibiotics were selected from 2 groups, as follows:

(i) Extremely important antibiotics: methicillin (5 μg), oxacillin (1 μg), ampicillin-sulbactam (20 μg), penicillin (10 μg), erythromycin (15 μg), ciprofloxacin (5 μg), vancomycin (30 μg), and gentamicin (10 μg).
(ii) Very important antibiotics: clindamycin (2 μg), tetracycline (30 μg), chloramphenicol (30 μg), and sulfamethoxazole/trimethoprim (25 μg).

2.1.3. Molecular characterization:

DNA from the 32 *Staphylococcus* sp. isolates was extracted from overnight culture using the boiling protocol described by Sowmya *et al*., (2012). Tris-EDTA buffer was used in appropriate volume to resuspended DNA precipitates. The preparation of cell lysates to be used as DNA templates required taking overnight cultures of all *Staphylococcus* isolates and treated them with boiling water. Genus-specific validation was conducted through PCR using the 16S rRNA gene *Staphylococcus*-genus-specific primers and cycling conditions as described by Zhang *et al*., (2004).

All confirmed *Staphylococcus* isolates were subjected to molecular assaying for *mecA* gene detection, which is the gold standard for confirmation of MRSA strains (Paterson *et al*., 2014).

The extra antimicrobial resistance indicators, that are frequently presenting in *S. aureus*, were screened by PCR, i.e. the *gyrA* and *gyrB* genes (responsible for quinolone resistance) and the *cfr* gene (responsible for chloramphenicol-florfenicol resistance), conferring resistance to numerous classes of antibiotics (lincosamides, phenicols, pleuromutilins, oxazolidinones, and streptogramin A, which known as the PhLOPSA phenotype. PCR amplifications were performed using GeneAmp 9700 System (Perkin-Elmer, Germany) using the described primers and the cycling conditions of Osman *et al*., (2016).

2 µL of DNA template solution were combined with 23 µL of master mix solution that included 1 µM of each primer and 3U of Taq polymerase. Reference *S. aureus* strains (ATCC BAA-2312 and ATCC-25923) were employed as positive controls for the *mecA* and *cfr* genes, respectively.

2.2. Plant Extract Preparation

Dried fruits of Amla (*Phyllanthus Emblica*) were attained from the ARC (Agricultural Research Center- Giza- Egypt), Deionized water (DIW) was used for washing plant materials, which were re-dried and ground into fine powder. 100 g of fruits' powder were soaked into 850 mL of 70% ethanol and agitated at 270 x *g* for 36 h at 26±2 °C then filtered to eliminate fruits residues. The extract of *Phyllanthus Emblica* (*Pe*E) was vacuum dried at 43 °C and dark-kept at 4 °C.

2.3. Selenium Nanoparticles (Se-NPs) Synthesis

For the phytosynthesis of Se-NPs, many concentrations from sodium selenite (Na_2SeO_3, Sigma-Aldrich, St. Louis, MO), i.e. 1, 10, 25, 50 and 100 mM, in extract solution, and from *Pe*E (0.5, 1.0, 1.5, 2.0 and 2.5 %, in DIW) were preliminary assessed for Se-NPs phytosynthesis. The optimal concentrations (for obtaining the least Se-NPs homogenous size with least *Pe*E concentration) were 10 mM from Na_2SeO_3 in a 1.0 % *Pe*E solution. The practical conditions for synthesis were stirring of composite solution for 18 h at 170 x *g* at 25±2 °C in dark. The synthesis of Se-NPs was proved by changed solution color to brownish orange, which was observed visually and with analysis of their UV-vis spectrum using spectrophotometer UV-2450 model, Shimadzu, Japan. The *Pe*E phytosynthesized Se-NPs (*Pe*E/Se-NPs) were separated through centrifugation at 12.000 x *g* for 25 min. For separating the pure Se-NPs, samples were washed 5 times with 25 folds from DIW and centrifuged after each time.

2.4. Characterization of phytosynthesized *Pe*E /Se-NPs

The nanoparticles' size (PS), distribution, and charges of *Pe*E /Se-NPs were appraised via PCS (photon correlation spectroscopy) with Zetasizer (Nano ZS Malvern™, UK), after dissolving and sonication of NPs. Furthermore, the *Pe*E /Se-NPs size, topography, and dispersity were investigated via TEM (transmission electron microscopy JEOL JEM 2100, Japan)

2.5. Antibacterial Property Assessment

The valuation of *Pe*E, Se-NPs, and *Pe*E /Se-NPs antibacterial performance was conducted (via qualitative and quantitative assays), targeting various *S. aureus* isolates

and standard strains, i.e. ATCC BAA-2419, isolate WR-3, and isolate MR-4. The bacterial assaying was conducted using TSA and TS Broth, aerobically incubated at 37±1 °C. The inspected Se-NPs were acquired via repeated centrifugation/washing with DIW to eliminate most of the attached *Pe*E with NPs.

2.5.1 Qualitative assay (Inhibition zone, IZ) :

To have qualitative values of produced agents' activity toward S. aureus strains, the IZ assay (using disc diffusion method) was directly accomplished on spread bacteria onto TSA plates. Sterile discs (Whatman No.1 filter paper, 6 mm diameter) impregnated with 100 µg from *Pe*E, Se-NPs, or *Pe*E /Se-NPs, after dissolving in DIW. The emerged IZ after plates' incubation (for 24 h at 37±1 ^{o}C) were appraised and the means of triplicated trials' were calculated.

2.5.2. Quantitative assay (Minimum inhibitory concentration, MIC):

The microdilution technique was followed to assess the MICs of *Pe*E, Se-NPs, or *Pe*E /Se-NPs against challenged *S. aureus* strains (Tayel *et al.*; 2011). In sterile microplates (96 wells tissue-culture plates), bacterial suspension (~3 X 10^7 CFU/mL) were challenged with serial concentrations from examined agents (2.5–100 µg/mL in TSB), then bacterial viability, after incubation of microplates for 24h, were screened by chromogenic staining by INT (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride, 4% w/v), as this dye is transforming to red- color formazan by viable cells' activity. For authorizing the bactericidal action, aliquots (100 µL) from treated wells were later plated onto plain TSA plates and incubated, the growth-free plates confirmed bactericidal activity. The minimum concentrations that banned microbial development (in microplates and on TSA plates) were identified as MIC.

The growth rate of exposed *S. aureus* isolates to the MIC of *Pe*E /Se-NPs in TSB, was conducted at 2 h intervals, compared to untreated standard strain.

2.5.3 SEM (Scanning electron microscopy) Imaging

For elucidating the possible action modes of phytosynthesized NPs, the micrographs of SEM (Hitachi S-500, Tokyo, Japan) were

captured to screen the structural and morphological alterations in *S. aurus* cells (from MDR and standard strains), after exposure to *Pe*E /Se-NPs. The cells' suspensions from MDR strain (WR-3) and standard sensitive strain (ATCC BAA-2419) were aseptically treated with 22.5 µg/mL from *Pe*E /Se-NPs (in TSB) for 4 h and 8 h, compared to untreated (control) cells. The cells were then collected, washed with DIW, dehydrated, and prepared for SEM imaging. The captured SEM micrographs are based on the cells' morphological alterations after interaction with NPs.

2.6. Statistical Analysis

Triplicated trials were performed; their means and SD (Standard Deviation) were calculated (using Microsoft Excel 2010). Statistical significance was considered at $p \leq 0.05$ using MedCalc software V. 18.2.1 (MedCalc, Mariakerke, Belgium) and applying the one-way ANOVA test.

Table (1): Prevalence of *Staphylococcus* spp. in imported meat products, including the numbers of total and drug-resistant *Staphylococcus aureus* isolates

Meat Samples	Samples No	*Staphylococcus* spp. isolates No (%)	*Staphylococcus aureus* isolates No (%)	MDR *S. aureus* isolates No (%)
Whole meat	58	14 (24.1%)	7 (12.1%)	5 (8.6%)
Minced meat	36	11 (30.6%)	6 (16.7%)	4 (11.1%)
Sausages	15	4 (26.7%)	2 (13.3%)	2 (13.3%)
Burger	11	3 (27.3%)	2 (18.2%)	0 (0.0%)
Total	**120**	**32 (26.7%)**	**17 (14.2%)**	**11 (9.2%)**

3. Results

The screening of *Staphylococcus* spp. prevalence in meat samples revealed that bacteria were detected in 26.7% from screened samples and that *S. aureus* isolates were 52.18 % from these isolates, with a prevalence percentage (PP) of 14.2% in meat samples (Table 1). The highest PP of *Staphylococcus* spp. and *S. aureus* isolates were attained from minced meat samples, whereas the highest PP for multidrug-resistant (MDR) *S. aureus* isolates were recorded from sausages samples. The highest number of MDR *S. aureus* isolates was obtained from whole and minced meat (5 and 4 isolates, respectively), whereas no MDR *S. aureus* was isolated from burger samples (Table 1).

The occurrence of antibiotic-resistant (AR) genes among *S. aureus* isolates is illustrated in the table (2); the *gyrA* and *mecA* genes recorded the highest occurrence rate (10 and 9, respectively, out of 11 isolates), whereas the *cfr* gene was detected in 3 isolates only (Table 2). The complete set of screened genes was detected in two isolates, i.e. WR-3 from whole meat and MR-2 from the minced sample. Only one AR gene (*mecA* or *gyrA*) was detected in individual isolates, where four isolates had 2 AR genes and three isolates had 3 AR genes.

Table 2: Prevalence of antibiotic-resistant genes among *Staphylococcus aureus* isolates

Antibiotic-resistant Gene	Primer sequences (5′-3′)	PCR Amplion size (bp)	No of positive *S. aureus* isolates
mecA	**F:** GTAGAAATGACTGAACGTCCGATAA **R:** CCAATTCCACATTGTTTCGGTCTAA	**310**	9
gyrA	**F:** ATGGCTGAATTACCTCAATC **R:** CATCATAGTTATCGATGAAATC	**399**	10
gyrB	**F:** CAGCGTTAGATGTAGCAAGC **R:** CCGATTCCTGTACCAAATGC	**250**	4
cfr	**F:** TGAAGTATAAAGCAGGTTGGGAGTCA **R:** ACCATATAATTGACCACAAGCAGC	**746**	3

The application of *Pe*E for Se-NPs phytosynthesis from Na_2SeO_3 was successfully achieved as evidenced through from visual color change of Se-NPs solution from bale yellow to intense brownish-orange. The maximum UV-vis absorbance of Se-NPs solution was detected at 261 nm (Figure 1)

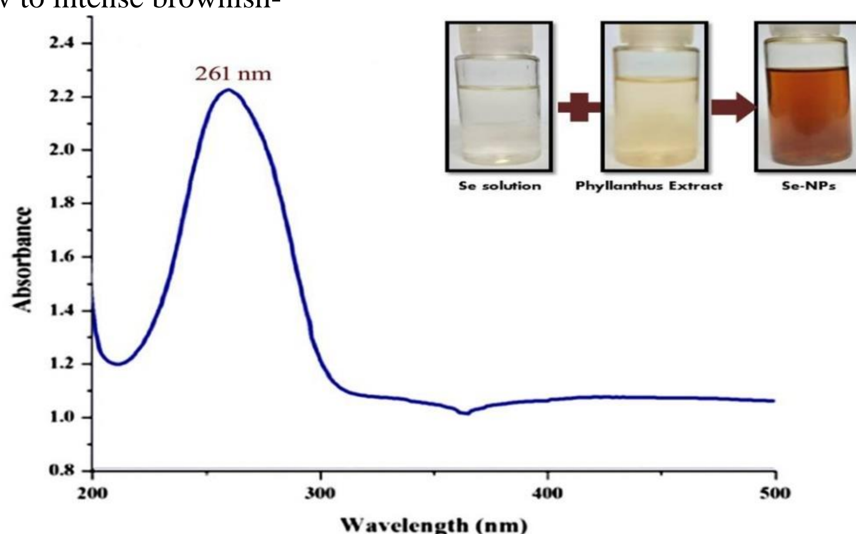

Fig. 1: Visual appearance and UV-vis spectrum of phytosynthesized Se-NPs using 10 mM from Na_2SeO_3 in 1.0 % *Phyllanthus Emblica* extract

The PCS analysis of *Pe*E-synthesized Se-NPs indicated their sizes' range around 4.38-17.62 nm, with median and mean diameters of 11.43 nm and 11.98 nm, respectively (Figure 2-A).

The *Pe*E-synthesized Se-NPs were negatively charged with a strong zeta potential of -33.6 mV (Figure 2-B); which could increase their stability and dispersity.

The microstructures of phytosynthesized *Pe*E/Se-NPs were predicted usinTEM imaging, which designated their uniform distribution and PS (at 5.62-15.88 nm range). The phytosynthesized Se-NPs were not aggregated and had spherical shapes (Figure 2-C). Miniature *Pe*E particles were shown in a matrix with Se-NPs. The elemental analysis of NPs indicated the presence of Se, O, and C elements (Figure 2-D) as an indicator for phytosynthesis of Se-NPs.

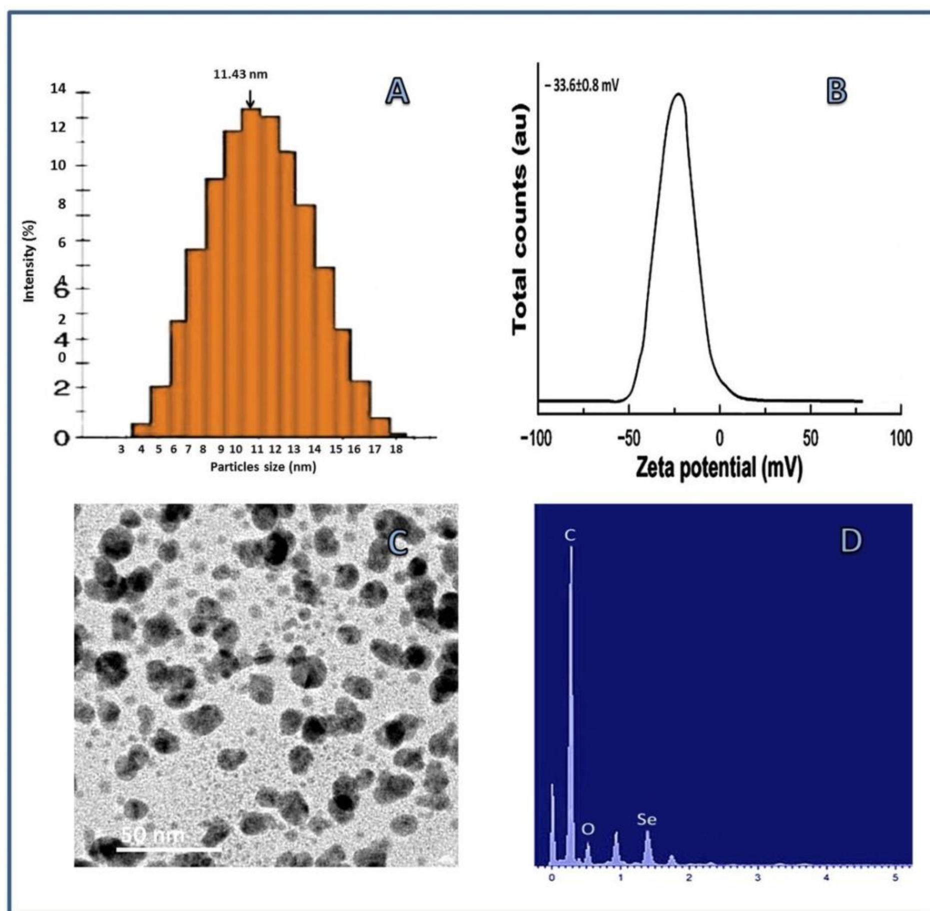

Fig. 2: The microstructure attributes of phytosynthesized selenium nanoparticles using *Phyllanthus Emblica* extract, indicating the distribution of particles' size (A), their zeta potential (B) the appearance of Se-NPs using TEM imaging (C), and the elemental analysis of phytosynthesized Se-NPs (D).

The anti- *S. aureus* activities of *Pe*E, phytosynthesized Se-NPs, and their composite (*Pe*E/Se-NPs), against the different standard and drug-resistant strains, are illustrated, using IZ qualitative assay and MIC quantitative assay (Table 3). It was observed that *Pe*E/Se-NPs activities were significantly stronger than individual examined agents (*Pe*E and Se-NPs), regarding the *Pe*E/Se-NPs wider IZ diameters (Figure 3) and lesser MIC values. Additionally, Se-NPs had significant action as anti- *S. aureus* agents that *Pe*E, although the extract had also a remarkable antibacterial action against the entire *S. aureus* strains. The standard *S. aureus* strain (ATCC BAA-2419) was significantly more susceptible to the entire examined antimicrobials, whereas the MDR strain (WR-3) that had the complete set of

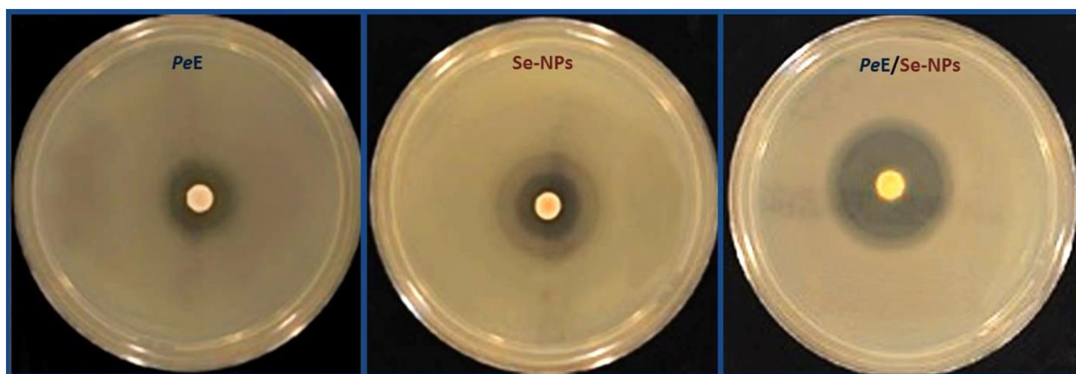

Fig. 3: Appeared inhibition zones after *Staphylococcus aureus* (ATCC BAA-2419) exposure to *Phyllanthus Emblica* extract (*Pe*E), phytosynthesized Selenium nanoparticles (Se-NPs), and their composite (*Pe*E/Se-NPs).

Table (3): The anti- *Staphylococcus aureus* activities* of *Phyllanthus Emblica* extract (*Pe*E), phytosynthesized Selenium nanoparticles (Se-NPs), and their composite (*Pe*E/Se-NPs) against the different standard and drug-resistant strains

Antimicrobial agent	***Staphylococcus aureus* strain**					
	ATCC BAA-2419		**WR-3**		**MR-4**	
	IZ (mm)	**MIC (µg/ml)**	**IZ (mm)**	**MIC (µg/ml)**	**IZ (mm)**	**MIC (µg/ml)**
*Pe*E	13.4 ± 0.9^{a1}	35.0	10.1 ± 0.7^{a2}	42.5	11.8 ± 0.9^{a2}	37.5
Se-NPs	19.6 ± 1.2^{b1}	22.5	16.8 ± 1.4^{b2}	30.0	17.3 ± 1.3^{b2}	25.0
*Pe*E/Se-NPs	24.8 ± 1.4^{c1}	15.0	20.4 ± 1.3^{c2}	22.5	21.9 ± 1.4^{c2}	22.5
Oxacillin	18.7 ± 1.1^{b}	20.0	Not detected	> 100	Not detected	> 100

* Different superscript characters (letters within the same column and numbers within the same raw) indicate significant differences ($p \leq 0.05$)

antibiotic-resistance genes, was the most resistant strain toward all anti- *S. aureus* agents. The growth patterns of treated *S. aureus* strains (ATCC BAA-2419, WR-3 and MR-4), with 22.5 µg/ml from PeE/Se-NPs, compared with untreated standard culture, are illustrated in Figure 4.While the untreated culture tended to sharply increase in their number with incubation prolongation, the treated cells' numbers decreased dramatically after treatment with NPs. The decrement was more obvious insensitive strain (ATCC BAA-2419) than in MDRSA strains (WR-3 and MR-4). The cells numbers declined to zero after 6, 8, and 10 h, for the screened strains ATCC BAA-2419, MR-4, and WR-3,respectively.

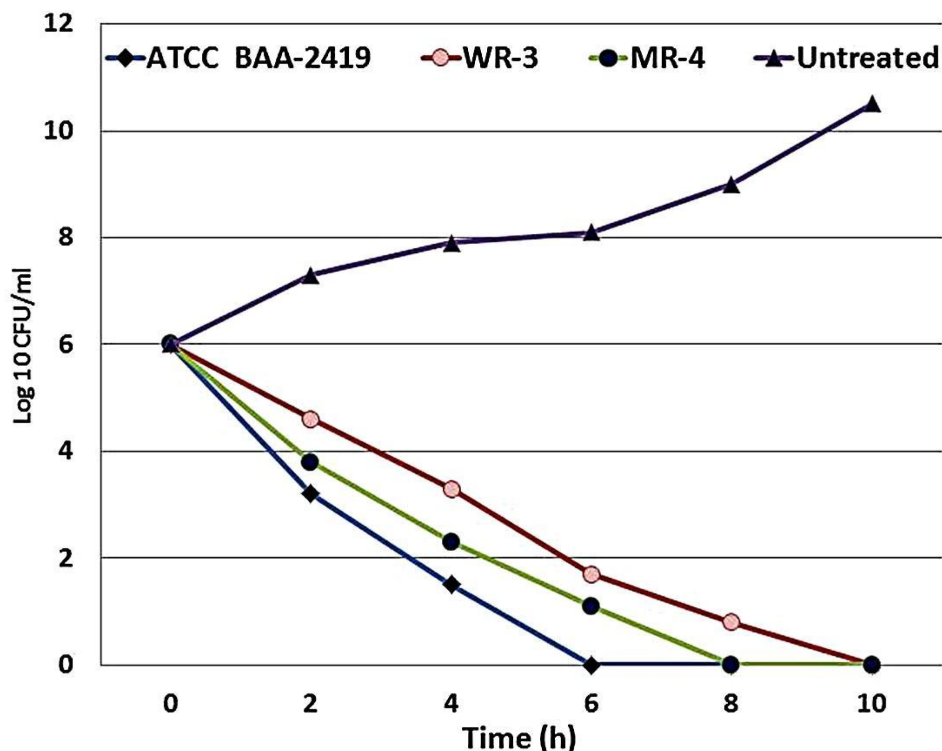

Fig. 4: Growth and survival rate of treated *Staphylococcus aureus* strains (ATCC BAA-2419, WR-3, and MR-4) with 22.5 µg/ml from *Pe*E/Se-NPs, compared with untreated standard culture.

The consequences of exposure to *Pe*E/Se-NPs on *S. aureus* cells from MDR strain (WR-3) and standard sensitive strain (ATCC BAA-2419) are illustrated in **Figure (5)**. At the initiation of treatment (zero time), both strain cells appeared with healthy shapes, uniformed round surfaces, and smooth cell walls (**Figure 5-R0 and 5-S0**). The exposed cells to *Pe*E/Se-NPs were combined and partially lysed after 4 h of treatment; many NPs attached the outer membranes of lysed cell surfaces, which indicates the potential interactions between them (Figure 5-R4 and 5-S4). With the *Pe*E/Se-NPs exposure prolongation (for 8 h), the lyses signs became very detectable; the cells of drug-sensitive strain (ATCC BAA-2419) were entirely lysed and released their interior components that were intermixed with NPs (Figure 5-S8). Also, the cells of MDR *S. aureus* strain (WR-3) were mostly lysed, and many residual cell walls could be detected in attachment with *Pe*E/Se-NPs (Figure 5-R8).

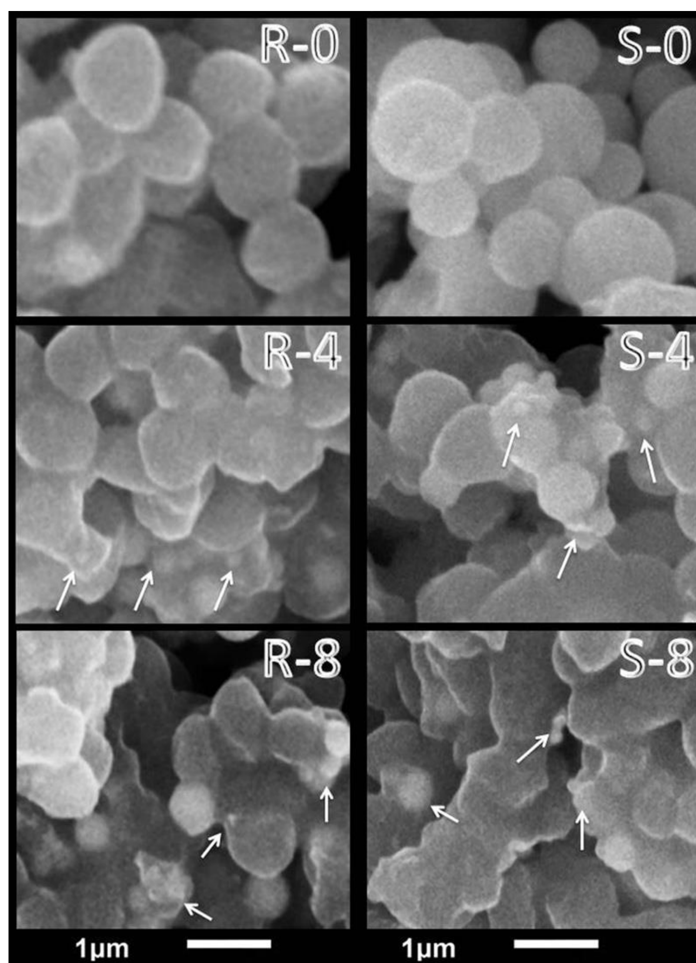

Fig. 5: SEM micrographs indicating the consequences of exposure to phytosynthesized selenium nanoparticles* with *Phyllanthus Emblica* for 0, 4, and 8 h on the cells of drug-resistant (R) and sensitive (S) strains from *Staphylococcus aureus*

* Examples of some attached NPs to bacterial cells are distinguished with arrows

4. Discussion

The MDR *S. aureus* (MDRSA) spreading, including MRSA and VRSA strains, is a continuing public health-threatening, regarding humanoid or veterinary medicines. Because of the wide existence of MRSA strains, the β-lactamic antibiotics became somewhat useless for clinical treatments, leading to the usage of the "MRSA" term to designate most resistant *S. aureus* strains that are harboring *mecA* gene and have β-lactamase-resistance (Paterson *et al*., 2014)

The misuse of antibiotics from varied classes in clinical practices led to further resistance of MRSA to these classes and the emergence of many MDRSA strains (Lowy, 2003); this enforced the investigations for more effective anti-MRSA agents from innovative sources (Zhang *et al*., 2017).

Herein, the total percentages of isolated *Staphylococcus* spp. (26.7%) and *S. aureus* (14.2%) were higher than those obtained from a previous study conducted in Egypt (23 and 3 %, respectively) (Osman *et al*., 2017), which could be attributed to the variations in the sources of imported meats, the environmental conditions during storage and processing. This is further supported by the high PP highest PP of *Staphylococcus* spp. and *S. aureus* isolates that attained from

minced meat samples, which are subjected to more handling and processing steps that could be additional sources for their contamination.

The prevalence of MDRSA isolates (have resistance to ≥3 antibiotic classes) in meat samples was comparable or slightly higher than some former investigations (Chan *et al*., 2008; Buyukcangaz *et al*., 2013; Osman *et al*., 2016; 2017); which is assumingly attributed to the different antibiotic treatment strategies during animals rearing, e.g. for growth-promoting and/or treating animals mastitis, before exportation and some lack of hygienic protocols during meat processing (Karam *et al*., 2016).

The detection of high frequencies from MDRSA was associated with the isolates expression of MDR genes in the current study. The conferring gene for methicillin resistance (*mecA*) is encoding for a binding protein to penicillin (a2) with fewer affinities for beta-lactams [Lee, 2003]; this frequently leads to MDR, mainly against ciprofloxacin, methicillin, oxacillin, gentamycin, and vancomycin (Chan *et al*., 2008; Karam *et al*., 2016). Other investigations also, carried on MDRSA isolates, have appointed that *the mecA* gene was directly associated with *S. aureus* resistance to these antibiotic classes (Lee, 2003, Osman *et al*., 2016; 2017).

The other genetic determinants for *S. aureus* antibiotic-resistance (*gyrA*, *gyrB*, and *cfr* genes) were also screened due to their frequent usage as indicators for MDRSA strains. The occurrence of the MDR gene (*cfr*) in staphylococcal isolates is of excessive global importance to designate MDRSA in humanoid and veterinary medicines (Wang *et al*., 2013) which encoded for lincosamide and phenicol resistance (Witte & Cuny, 2011).

The phytosynthesis (PSn) of Se-NPs was effectually achieved, as was firstly proved visually from the color change of NPs solution. This change in Se-NPs color, after reduction by *Pe*E, is caused by the SPR (Surface Plasmon Resonance) effect, as results from *Pe*E reducing and stabilizing effects during Se-NPs synthesis (Menon *et al*., 2019; Mulla *et al*., 2020).

The nanocomposite *Pe*E/Se-NPs carried comparatively high negative charges (-33.6 mV) that could suggest their increased capability regarding electrostatic stability and aggregates prevention (Cremonini *et al*., 2016).

The TEM micrographs and the distribution of NPs size appointed well-dispersity and miniature sizes of PSn Se-NPs, which indicates the advanced *Pe*E stabilizing and reducing activities. The antioxidant and reducing powers of *Pe*E are assumingly to its bioactive constituents; mainly from active tannoids (including emblicanin A and B, punigluconin and pedunculagin) (Bhattacharya *et al*. 1999), flavonoids and polyphenolic compounds (including gallic and ellagic acids and corilagin) (Liu *et al*. 2008), and the elevated content from ascorbic acid and other bioactive phytoconstituents (Saito *et al*., 2008). These *Pe*E pytocompounds were mainly the reliable factors for forcing the NPs formation from Se ions, stabilizing these phytosynthsized Se-NPs, and prevent their aggregation (Khopde *et al*., 2001; Poltanov *et al*., 2009; Chalise *et al*., 2010). The *Pe*E high potentiality for Se-NPs synthesis (with mean PS diameter of 11.98 nm) comparably exceeded former biogenic agents used for Se-NPs biosynthesis, e.g. fenugreek seed extract (50–150 nm) (Ramamurthy *et al*., 2013), *Bougainvillea spectabilis* flowers (18–35 nm) (Ganesan, 2015), and microbial cultures (102–170 nm) (Cremonini *et al*., 2016); this superior capability of *Pe*E is assumingly attributed to its elevated reducing powers generated from its phytoconstituents.

The antibacterial powers of *Pe*E, Se-NPs, and their composite (*Pe*E/Se-NPs) were proved toward MDRSA and standard *S. aureus* strains; this indicates the individual and synergistic action of examined agents and suggests their practical applications for eliminating various *S. aureus* strains.

The *Pe*E antimicrobial activity was documented against *Aeromonas hydrophila* and *Xanthomonas campestris*, with comparable results to those reported in this study against *S. aureus* strains (De Britto *et al*., 2011). These observations were reported also for the extracted *Pe*E with chloroform and methanol against many Gram-negative (G^-) and Gram-positive (G^+) pathogenic bacteria (Rahman *et al*., 2009). Additionally, the *P. Emblica* essential oils exhibited potent broad-spectrum antimicrobial activity against several pathogenic bacterial and fungal species (Liu *et al*., 2009); the G^+ bacteria were highly sensitive than G^- to this essential oil.

The Se and Se-NPs antimicrobial actions were repeatedly documented toward many G^- and G^+ bacterial pathogens; the potential toxicity from Se ions somewhat hindered their wide applications for human purposes (Huang *et al*., 2016). However, the biosynthesis of Se-NPs was applied to diminish the Se toxicity and enhance its biofunctionality through bio-transforming their particles to nano-form (Gunti *et al*., 2019). The PSn Se-NPs, using plant extracts and constituents, were reported as promising antimicrobials agents against several bacterial and fungal human pathogens (Kokila *et al*., 2017; Gunti *et al*., 2019).

The size and shape of PSn Se-NPs could influence their microbicidal action; it was suggested that Se-NPs with spherical shapes and tinier size could readily access bacterial membranes and hinder cells' biological activities (Menon *et al.,* 2019). The principal actions of Se-NPs, as antimicrobial particles, were attributed to their capability for attachment, absorption then penetration into microbial cells; these actions frequently derive shrinkage cells' cytoplasm membrane and inhibition of their bioactivities (Cremonini *et al*., 2016; ElSaied *et al*., 2021).

Although the exact mechanisms of Se-NPs microbicidal actions are still somewhat indistinct, several reports claimed that free radicals and ROS "reactive oxygen species" production are the main bases for destructing bacterial cells by Se-based composites (Yan *et al.,* 2013; Zhao *et al.,* 2018).

The PSn Se-NPs antimicrobial potentialities were also reported to be synergistically augmented by their combining with the reducing agents from plant derivatives (Mittal *et al*., 2014; Kokila *et al*., 2017); the synergistic antimicrobial actions from the Se-NPs and phytoconstituents were effective for inhibitions of numerous microbial pathogens, even the MDR and superbugs strains (Huang *et al*., 2016).

Regarding Se-NPs applicability in the food system, Se ions are crucial trace minerals for the maintenance of health, with an advised daily intake of 40 to 300 mg (Rayman, 2015). Se deficiency is correlated with $\geq$ 40 diverse diseases (Gunti *et al*., 2019). The human body can naturally degrade Se-NPs; the excess amounts are the nutritional source for the element, which have minor or no toxicity to the human body (Shirsat *et al*., 2015; ElSaied *et al*., 2021).

The harsh morphological alteration after treatment of *S. aureus* with *Pe*E/Se-NPs, as detected by SEM imaging, could assumingly attribute to synergistic actions of both *Pe*E and Se-NPs. The nanometals, e.g. Se-NPs exhibited forceful inhibitory actions for Gram-positive species, e.g. *S. aureus*, which is presumably attributed to the composition of their cell walls, principally containing lipoteichoic and teichoic acids. The NPs

could interfere with these constituents to hinder their synthesis or actions for regulating walls autolytic enzymes, which could lead to cells lysis and/or mislay their protective membranes (Zonaro *et al*., 2015, ElSaied *et al*., 2021).

Many findings reported the Se-NPs antibacterial actions toward *S. aureus* strains among further bacterial pathogens, the electron microscopic imaging of exposed *S. aureus* suggested the inhibition of biofilm/micro-colony formation after Se-NPs treatment; the PSn Se-NPs were recommended as alternate approaches for preventing *S. aureus* biofilm infections (Sonkusre & Cameotra, 2015).

The Se-NPs, in pure form or conjugation with quercetin and acetylcholine, induced observable morphological alterations in exposed *S. aureus* cells, most cell walls were sunken and/or damaged after Se-NPs exposure (Huang *et al*., 2016); these damages were assumed as results from cell membranes disorganization and cytoplasmic release. The nanocomposites, e.g. Se-NPs, were proposed to kill bacteria via penetration into the bacterial cell and their disordered membranes and stimulating ROS production. However, the main proposed key causes for Se-NPs antibacterial actions were the NPs ability to (1) affecting the integrity of compromised cell membranes, (2) penetrating cell walls/membranes, interacting and disrupting DNA structure, and (3) increasing intracellular ROS production inside bacterial cells; these combined actions could drive microbial cell lysis and death (Tran & Webster, 2011; Beheshti *et al*., 2013; Huang *et al*., 2016; ElSaied *et al*., 2021).

Conclusion:

The prevalence of *S. aureus* contamination, including the MDR strains, was considerably high in frozen meat products in Saudi markets. The nanocomposites from phytosynthesized Se-NPs with *P. emblica* fruits extract proved to act as a powerful antibacterial agent to control both standard and MDR-*S. aureus*, which recommends their future practical applications for preventing meat contamination with resistant pathogenic bacteria.

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