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Prevalence of Inducible Clindamycin Resistance and Nanotechnological Control of *Staphylococcus aureus* Clinical Isolates

Youssef E. Abdelmawgoud[(1)], Walaa Abd El- Latif[(2)], Nouran K. Fawzy[(1)], Sherif M. Elnagdy[(1)#]

[(1)] Department of Botany and Microbiology, Faculty of Science, Cairo University, Giza, Egypt; [(2)]Microbiology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

STAPHYLOCOCCUS aureus (*S. aureus*) is one of the pathogens that is proposed to cause various infections in humans. Clindamycin is therefore an important medication identified in treating these infections, especially those of the skin and soft tissue. Inducible clindamycin resistance (ICR) is a complication that however arises during the treatment of *S. aureus* infections in humans. Thus, it is difficult to detect *S. aureus* strains expressing ICR, using standard susceptibility test methods. Recently, an increasing need existed to find alternatives to antibiotics. Moreover, nanoparticles, especially silver nanoparticles, were previously reported as potential alternatives for traditional antibiotics (or in combination with traditional antibiotics) against the emergence of bacterial multidrug- resistance (MDR). Hence, this research studied the prevalence of ICR among *S. aureus* clinical isolates and investigated the antibacterial effects of AgNPs solely and combined with clindamycin against these isolates to evaluate the acute toxicity of intraperitoneally administrated silver nanoparticles (AgNPs). Of the one hundred *S. aureus* isolates under studied over a period of one year, 70 were identified as MRSA and 30 were MSSA. Results also revealed that the percentage of cMLSB, iMLSB, and MS phenotypes were 40%, 10%, and 9% respectively. Overall, 41% *S. aureus* isolates showed susceptibility to erythromycin. Additionally, both iMLSB and cMLSB phenotypes were the most predominated among MRSA isolates. Besides, AgNPs had strong antibacterial effects, with minimum inhibitory concentration (MIC)(1µg/ml) as well, in addition to a partial synergistic activity with clindamycin toward *S. aureus*. Based on these observations, the intraperitoneal administration of AgNPs was established as moderately toxic. Therefore, it may be recommended to use AgNPs as a potential treatment for infections caused by *S. aureus*.

Keywords: Clindamycin, Nanoparticles, Resistance, Silver, *Staphylococcus aureus*.

Introduction

Staphylococcus aureus (*S. aureus*) has been reported globally to be one of the riskiest pathogens causing several nosocomial and community-acquired infections (Lakshmi & Saikumar, 2018). These infections are therefore emerging rapidly, thereby posing as a grave threat to the public health due to the emergence of multidrug-resistant (MDR) isolates (Akram et al., 2016).

It has been reported that macrolide, lincosamide, and streptogramin B (MLSB) antibiotics are similar in their structure and have similar modes of action (Uzun et al., 2014). Hence, while lincosamide drugs, particularly clindamycin, are effective against some *S. aureus* infections (Thapa & Sapkota, 2016), clindamycin was noticed as the most frequently used drug due to its prominent pharmacokinetic properties. A 100% oral administration bioavailability, proper tissue penetration, tendency to accumulate in abscesses, tolerability, and low cost all represent these properties (Juyal et al., 2013).

Since the increase in staphylococcal infections, especially Methicillin-resistant *S. aureus* (MRSA)

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infections, an increase in the usage of macrolide-lincosamide-streptogramin B (MLSB) antibiotics to treat such infections has been observed. Sadly, the great use of these antibiotics has increased the number of resistant *S. aureus* strains to MLSB antibiotics (Singh et al., 2016). Thus, in proffering a solution, different mechanisms have been evolved to understand the mode of action of this resistance. Among these mechanisms is the active efflux mechanism, which the *msrA* gene encodes, thereby conferring resistance to macrolides and streptogramin B antibiotics (so called MS phenotype). Another mechanism is the modification of ribosomal targets, which is *erm* genes encode. This modification results in resistance to MLSB antibiotics; so, it is called MLSB resistance (Singh et al., 2016). The modification occurs through methylation of the 23S rRNA and production of methylase enzymes by *erm* genes, thereby reducing binding of the drug to the rRNA target (Mišić et al., 2017). The latter mechanism can either result in constitutive MLSB (cMLSB), in which the rRNA methylase is frequently produced, or inducible MLSB (iMLSB), where the methylase is produced only in the presence of an inducing agent (Ujwol et al., 2016). The most effective inducer of iMLSB resistance is erythromycin (Sasirekha et al., 2014).

Clindamycin is a good option because it has numerous advantages. However, it is proposed to cause therapeutic failures with isolates harboring *erm* genes due to the induced resistance toward clindamycin in patients (Nikbakht et al., 2017). Isolates have also been observed to acquire a high charge of spontaneous mutations, thereby allowing them to increase constitutive resistance to clindamycin at certain points of the therapeutic process (Kumurya, 2015). Hence, the prevalence of inducible resistance should be studied, as it varies according to geographical place, bacterial species, methicillin susceptibility, and even from one health center to another (Majhi et al., 2016). In some regional reports, the frequency of MRSA with macrolide resistance was even higher than that of Methicillin-Susceptible *S. aureus* (MSSA), which shows the severity of the issue at hand (Bouchiat et al., 2015).

Detection of isolates with ICR is a difficult problem because they appear to be resistant to erythromycin, but are falsely susceptible to clindamycin, using the disc diffusion method, thereby increasing the chance of therapeutic failures *in vivo* (Mansouri & Sadeghi, 2014; Adhikari et al., 2017). So, to detect ICR, the Clinical and Laboratory Standards Institute recommended the erythromycin-clindamycin disc diffusion (D-test) (Clinical and Laboratory Standards Institute (CLSI, 2017). Clindamycin susceptibility is then validated by a negative result for ICR through a D-test, which finally serves as a good therapeutic option (Mokta et al., 2015).

Many studies were conducted to discover alternatives for the treatment of MRSA infections. Among these alternatives, silver nanoparticles had a remarkable antibacterial activity that was proposed to be useful in suppressing different drug-resistant bacteria (Vazquez-Muñoz et al., 2019).

Silver showed more toxicity to microorganisms than other metals (Zhao & Stevens, 1998). Fortunately, bacteria were less expected to develop resistance toward silver compared to conventional antibiotics (Yuan et al., 2017). Moreover, silver shows low toxicity to mammalian cells (Krishnan et al., 2015).

If effective concentrations of AgNPs against various types of organisms were determined, its safe application in therapy can be proposed (Hwang et al., 2012). Currently, AgNPs are used broadly in medical applications, which include healing of wounds, diagnosis, and therapeutic treatments (Rineesh et al., 2018). Moreover, evidence for the synergistic antibacterial activity of AgNPs combined with traditional antibiotics was presented by several publications, particularly toward various multidrug-resistant bacteria, including *S. aureus* and *Escherichia coli* (Deng et al., 2016).

The unwanted side effects that happen either immediately or after a short time interval after a single or multiple administration of such particles within 24 h is defined as acute toxicity (Chinedu et al., 2013). The toxicity mechanism of AgNPs is associated with its ability to release free silver ions to the biological system. The quantity of silver ion released from silver nanoparticles depends on many factors, including size, shape, surface coating, and surface charge. Therefore, smaller-sized nanoparticles show better toxic effects due to increased surface (Elkhawass

et al., 2015). However, the toxicity assay is an essential issue that is normally considered before pharmacological agents can be sold in the market. These particles can result in chronic and acute toxicities that range from mild to severe according to their nature.

Hence, this study determined the prevalence of ICR among medical *S. aureus* isolates, using the D-test, to prevent misuse of the antibiotic. Afterward, we examined the antibacterial activity of AgNPs toward these isolates and its synergistic action with clindamycin to avoid antibiotic resistance, following the acute toxicity evaluation of AgNPs.

Materials and Methods

The isolation and identification of bacteria strains

Here, 100 isolates of *S. aureus* were collected from different clinical specimens of urine, pus, wound, wound swab, blood, and aspirates obtained from patients admitted to the Department of Microbiology, Kasr El-Ainy Hospital, Egypt, from February 2017 to February 2018. All specimens were subsequently inoculated on blood agar (BA-HiMedia, India), after which they were incubated at 37°C for 24–48h to obtain a single colony. Identification of isolates was then confirmed, using conventional methods, including colony morphology, Gram stain, catalase test, the slide and tube coagulase test, and the DNase test. To finally investigate methicillin resistance, cefoxitin disks (30mg) were used (using disc diffusion inhibition assay according to the guidelines of CLSI).

The D-test

CLSI guidelines recommended the D-test to detect ICR (Clinical and Laboratory Standards Institute, 2017). For the test, 15mg erythromycin and 2mg clindamycin disks (Oxoid Ltd, England) were placed 15mm apart on a Mueller Hinton plate inoculated with a staphylococcal isolate. After 24h, incubation at 37°C plates was conducted. Table 1 shows the interpretation of the inhibition zone diameter (mm) of the D-test.

Preparation of AgNPs

The chemical reduction method was used to prepare silver nanoparticles as reported by Gloria et al. (2017). The source of the Ag^+ ion precursor was obtained from a silver nitrate ($AgNO_3$) solution. Then, polyvinyl pyrrolidone (PVP) was used as a stabilizing agent, whereas borohydydrate was the mild reducing agent. Turning of the solution's color to grayish yellow slowly, indicated the reduction of Ag^+ ions to Ag nanoparticles. Antimicrobial effects were subsequently investigated after dilution of the resulting solution.

An Ocean Optics USB2000+VIS-NIR Fiber optics spectrophotometer was used to study the optical properties, using UV-Vis absorption spectra. Also, a JEOL JEM-2100 high resolution transmission electron microscope was used to examine its size and shape at an accelerating voltage of 200kV.

TABLE 1. Interpretation of zones of inhibition and D-test results in *S. aureus*

Drug	Sensitive (S) in mm	Intermediate (I) in mm	Resistant (R) in mm
Cefoxitin (30mg)	>22	-	<21
Erythromycin (15mg)	>23	14-22	<13
Clindamycin (2mg)	>21	15-20	<14
Cefoxitin and D-test result			
Cefoxitin-R		MRSA	
Cefoxitin-S		MSSA	
Erythromycin-R, Clindamycin-R		cMLSBphenotype	
Erythromycin-R, Clindamycin-S (Circular zone)		MS phenotype	
Erythromycin-R, Clindamycin-S (Flat zone)		iMLSB phenotype	

mm-Millimetre, R- resistant, S- sensitive, D-test: Double disk approximation test, iMLSB: Inducible macrolide-lincosamide-steptogramin B, MS: Macrolide –steptogramin B, cMLSB: Constitutive macrolide-lincosamide-streptogramin B, MSSA: Methicillin sensitive *S. aureus*, MRSA: Methicillin resistant *S. aureus*.

Antimicrobial assay

Isolates found to exhibit inducible MLSB resistance were subjected to susceptibility tests against AgNPs and clindamycin. Following the CLSI guidelines (2012), susceptibility tests were conducted, using the broth microdilution method in a 96-well microliter plate. The minimum inhibitory concentration (MIC) test of AgNPs used, comprised eight series of concentrations ranging from 16 to 0.125µg/mL (16; 8; 4; 2; 1; 0.5; 0.25; 0.125µg/mL). The MIC test for clindamycin used 10 series of concentrations, ranging from 256 to 0.5µg/ml (256; 128; 64; 32; 16; 8; 4; 2; 1; 0.5µg/mL). Afterward, of 100 µl Muller Hinton Broth (MHB) media was added to each well. Subsequently, 100µL test solution of AgNPs and clindamycin was added to the first well, to make a two-fold broth microdilution and this was repeated until the last well. Next, 10µL of the microbes having a concentration of 5×10^6CFU/mL was inoculated to each well. No antimicrobial agent was added to the negative control well. The minimum inhibitory concentration is defined as the lowest concentration of both antimicrobial agents that inhibited visible growth of bacteria after an overnight incubation at 37°C. Such inhibition of visible growth can then be determined through the appearance of turbidity that can be seen by an unaided eye, compared to the negative and positive control (CLSI, 2012). The MIC test was conducted in duplicate.

To determine the minimum bactericidal concentration (MBC) value, small aliquots (10–15µL) from each well showing negative growth (clear no turbidity), was streaked in the nutrient agar and incubated at 37°C for 24h under aerobic conditions, then observed for visible growth. The value of MBC was subsequently read as the lowest concentration that can inhibit 99.9% microbial growth. The MBC value was also obtained in duplicate.

Toxicity assay

The most straightforward way to obtain the acute toxicity of a solution is by determining its median lethal dose, LD_{50} (Erhirhie et al., 2018). The acute intraperitoneal toxicity of AgNPs was therefore evaluated in Swiss albino mice through determination of the median lethal dose, using a previously proposed method (Chinedu et al., 2013), based on the following formula:

$LD_{50} = (M_0+M_1)/2$.

where, M_0 symbolized the highest dose of the test substance that gave no mortality and M_1 symbolized the lowest test substance dose that gave a high mortality.

The evaluation of toxicity was then conducted, following the Hodge and Sterner scale, using the previously obtained LD_{50} (Table 2) (Hodage & Sterner, 2005). This method required four Swiss albino mice, which were divided into four groups of one mouse in each group. Then, different doses of AgNPs were administered intraperitoneally to the mice (10, 100, 300, and 600mg/kg). Animals were subsequently observed for 1 h after administration and for 10 min every 2h interval for 24h to record mortality. This assay was conducted in triplicates.

TABLE 2. Hodge and Sterner Toxicity Scale

Toxicity rating	Commonly used term	LD50
1	Extremely toxic	Less than mg/kg
2	Highly toxic	1 - 50mg/kg
3	Moderately toxic	50 - 500mg/g
4	Slightly toxic	500 - 5000mg/kg
5	Practically non-toxic	5000 - 15000mg/kg

When the mortality dose was reached, it was confirmed or validated through a confirmatory test, which involved the administration of this dose to two animals. If, at least, a single animal from the two animals died, it was considered a confirmation and validation of the test result. The confirmatory test was conducted in triplicates.

Combination assay

The checkerboard titration method was used to evaluate the combination activity of both antimicrobial agents (Saiman, 2007). While clindamycin was added in concentrations between 64µg/mL and 4µg/mL, AgNPs was added at a range of 0.125–2µg/mL. The subsequent evaluation was based on the fractional inhibitory concentration (FIC), which can be calculated by dividing the MIC of the antimicrobial agent, combined with that of its MIC alone.

FIC clindamycin= MIC (CL) combined with AgNPs/ MIC CL alone

FIC of AgNPs= MIC (AgNPs) combined with

AgNPs/ MIC AgNPs alone

When the FIC of both clindamycin and AgNPs is summed up, the result is known as the FIC index (FICI), which can be interpreted as follows:

The combination is synergistic if FICI< 0.5 and partially synergistic when FICI ranges from 0.5 to 1. However, an additive combination is obtained when FICI =1, and an indifferent combination result when FICI ranges from 1 to 4. Finally, the combination is antagonistic when FICI >4 (Doern, 2014).

Statistical analysis

The Statistical Package for the Social Sciences was used to analyze data through the Chi-Square Test (v.17.0 software), and a statistical significance was considered when the *p* value was less than 0.05.

Results

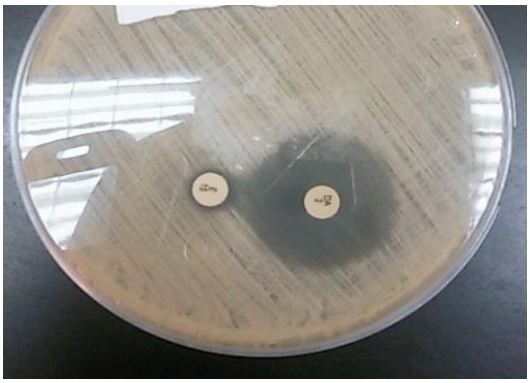
The isolation and identification of bacteria

The microscopic examination of the stained smear indicated that isolates were Gram positive and round shaped. Also, *S. aureus* isolates showed a positive response to catalase test, coagulase test, and DNase test.

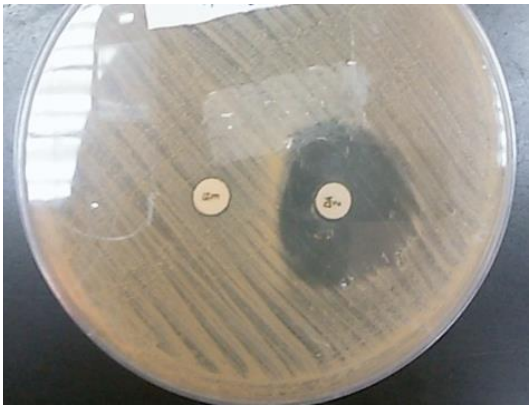
Of the 100 *S. aureus* isolates tested to determine ICR, 30 were MSSA and 70 s were MRSA. A statistically significant difference was also observed between the induction capabilities of MRSA and MSSA (P= 0.001). Most of the understudied isolates were from wound (45%), blood (17%), sputum (12%), body fluids/aspirates (11%), pus (9%), and urine (6%). The highest percentage of *S. aureus* was observed in samples isolated from wound. Furthermore, MRSA was significantly detected in wound isolates than MSSA (P= 0.001). The distribution of cases in our study is given in Table 3. Of the 100 *S. aureus* isolates, 55 isolates belonged to adult males and 45 to adult female patients. However, no significant difference was observed between the two genders (*P*= 0.20).

D-test

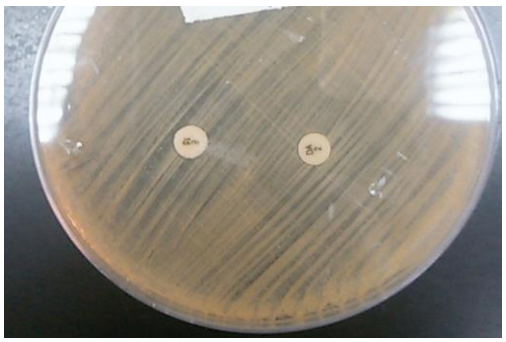
All isolates were subjected to the D-test. Results revealed that the percentage of cMLSB, iMLSB, and MS phenotypes were 40%, 10%, and 9% respectively. Overall, 41% isolates of *S. aureus* showed susceptibility to erythromycin. Phenotypic patterns of clindamycin resistance observed with MSSA and MRSA are illustrated in Fig. 1 and shown in Table 4.



(a) MS phenotype (D-test negative) [*S. aureus* isolate exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin with a Circular zone of inhibition around clindamycin]



(b) iMLS$_B$ phenotype (D-test positive) [*S. aureus* isolate showing resistance to erythromycin while being sensitive to clindamycin with D-shaped zone of inhibition around clindamycin with flattening towards erythromycin]



(c) cMLS$_B$ phenotype [*S. aureus* isolates, showed resistance to both erythromycin and clindamycin]

Fig. 1. D test showing susceptibilities to both clindamycin (2mg) and erythromycin (15mg)

TABLE 3. Distribution of clinical specimens according to their origin and methicillin susceptibility

Type of specimen	MRSA	MSSA	Total
Wound	34	11	45
Blood	11	6	17
Sputum	9	3	12
Body fluids/aspirates	6	5	11
Pus	6	3	9
Urine	4	2	6
Total	70	30	100

MRSA= Methicillin Resistant *S. aureus*; MSSA= Methicillin Susceptible *S. aureus*.

TABLE 4 . Phenotypic pattern of clindamycin resistance observed in MSSA and MRSA

Susceptibility pattern	MRSA	MSSA	Total
ER^S, CL^S	23 (32.9%)	18 (60%)	41(41%)
ER^R, CL^R (cMLSB phenotype)	34 (48.6%)	6 (20%)	40 (40%)
ER^R, CL^S(D+) (iMLSB phenotype)	9 (12.8%)	1 (3.3%)	10 (10%)
ER^R, CL^S(D-) (MSphenotype)	5 (7.14%)	4 (13.3%)	9 (9%)
Total	70 (70%)	30 (30%)	100 (100%)

MRSA= Methicillin Resistant *S. aureus;* MSSA= Methicillin Susceptible *S. aureus;* ER= Erythromycin; CL= Clindamycin; S= Susceptible; R= Resistant; D^-= D-test negative; D^+= D-test positive.

During result comparison between the 70 MRSA and 30 MSSA isolates, the MS phenotype, $iMLS_B$ phenotype, and $cMLS_B$ phenotype were detected among 5, 9, and 34 of the MRSA isolates and in 4, 1, and 6 of the MSSA isolates, respectively. Both iMLSB and cMLSB phenotypes were the most predominant among MRSA isolates.

Statistical results between MRSA and MSSA also showed a highly significant difference among MRSA strains harboring a higher proportion of $iMLS_B$ and $cMLS_B$ than MSSA ($p<0.05$). However, no significant difference in the MS phenotype between MRSA and MSSA was observed.

Ten *S. aureus* isolates having the iMLSB phenotype were isolated from blood (6), wound (2), and pus (2). Of the 10 isolates, 8 were from males.

Characterization of used silver nanoparticles (AgNPs)

A slow turning of the solution's color to grayish yellow, indicated a reduction in Ag+ ions to Ag nanoparticles. The measurement of the surface plasmon resonance band (SPR), using UV-visible spectroscopy, also confirmed the reduction of silver ions and synthesis of nanoparticles. Moreover, the UV-V's absorption spectrum exhibited a single plasmon band at approximately 401nm, which indicated the formation of spherical AgNPs, with diameter sizes ranging between 20 ± 5nm (Fig. 2). Additionally, TEM micrographs visibly showed that AgNPs have a spherical shape, with their sizes ranging between 20 ± 5nm (Fig. 3).

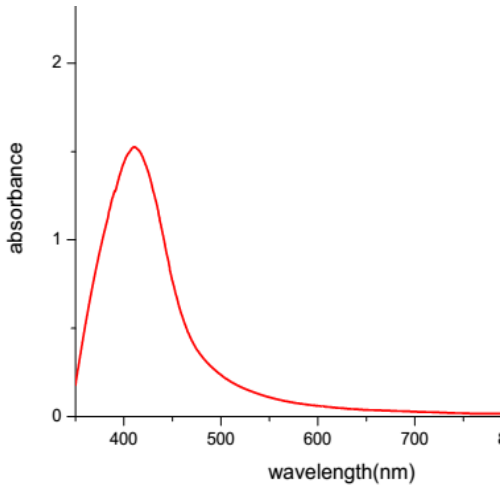


Fig. 2 . UV-Vis absorption spectrum of AgNPs of diameter of 25nm

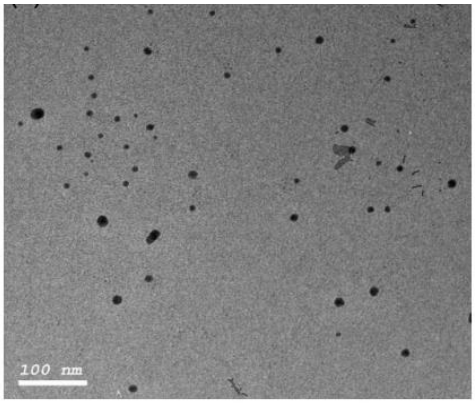


Fig. 3. JEOL JEM-2100 high resolution transmission electron microscope (TEM) micrograph of AgNPs

Antimicrobial assay

A typical serial microdilution assay was used to evaluate the inhibitory effect of AgNPs and clindamycin on the growth of $iMLS_B$ phenotype *S. aureus* isolates. Results from this test showed that AgNPs at a concentration of 1µg/mL and clindamycin at a concentration 32µg/mL, inhibited pathogen growth. So, the MIC of AgNPs and clindamycin were therefore determined as 1µg/mL and 32µg/mL, respectively. Below the MIC value, bacterial suspensions also showed a normal growth that was similar to the negative control. However, the MBC value of clindamycin was 128µg/mL, showing no bacterial growth.

Toxicity assay

It was observed that the highest dose of silver nanoparticles that gave no mortality in rat (M_0) was 100mg/kg, whereas the lowest dose that gave mortality (M_1) was 300 mg/kg. According to the formula used, the LD_{50} of silver nanoparticles was 200mg/kg, which was validated through a confirmatory test to ensure that the given dose was the cause of mortality. As observed, silver nanoparticles were moderately toxic, according to Hodge and Sterner scale.

Combination assay

The checkerboard method was used to investigate the synergistic effects of AgNPs combined with clindamycin against $iMLS_B$ isolates. The synergistic test was based on the MIC result obtained from the combination treatment, compared with that from the single treatment. The AgNPs concentration range was set at 1–0.125µg/mL, whereas that of clindamycin was set at 64–4µg/mL. Results from microorganism growth is illustrated in Table 5. The combination assay results showed a decline in MIC of clindamycin from 32µg/mL to 8µg/mL, in contrast to the MIC of AgNPs, which ranged from 1µg/mL to 0.25 µg/mL. The fractional inhibitory concentration index (FICI) evaluated this effect. As indicated, 0.5 was as the point of partial synergistic interaction (FICI of AgNPs + clindamycin= 0.5). Figure 4 showed the values obtained from MIC results.

Discussion

Of the 100 isolates of *S. aureus* understudied over a period of one year, 70 isolates were identified as MRSA, whereas 30 were MSSA.

In this study, iMLSB resistance was observed in 10% of the isolates. Our findings agree with the results of Adhikari et al. (2017), where iMLSB was 11.48%. In contrast, a higher 37.5% incidence of inducible resistance was observed from India and 90% from Japan (Lall et al., 2014; Shoji et al., 2015). The occurrence of iMLSB phenotypes varies substantially with geographical areas, age ranges, antibiotic prescription patterns, methicillin susceptibility, and infection rates from hospital to hospital (Majhi et al., 2016).

TABLE 5. The checkerboard method investigating the synergistic effects of AgNPs, combined with clindamycin against iMLSB isolates [Microbial growth in the AgNPs and clindamycin combination is represented by the gray boxes]

Clindamycin (µg/mL)	Silver nanoparticles (µg/mL)				
	0	0.125	0.25	0.5	1
0					
4					
8			FIC		
16					
32					
64					

Growth No growth

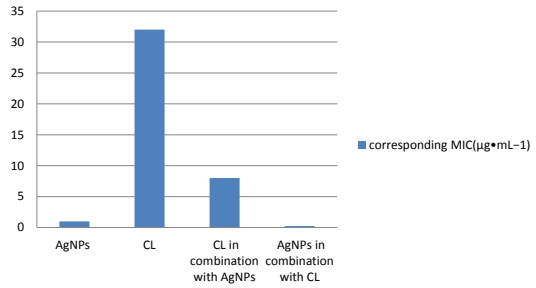


Fig. 4. The minimum inhibitory concentration (MIC) of both clindamycin (CL) and AgNPs alone and their combination against the iMLSB phenotype of *S. aureus* isolates

In our study, the occurrence of iMLSB was higher in MRSA samples 9(12.8%) than in MSSA 1(3.3%). Similar observations were made by Kavitha, 2020, who showed 18.70% in MRSA and 11.89% in MSSA. We also observed a higher percentage of the iMLSB phenotype in MRSA infections compared to MSSA infections, indicating that clindamycin treatment was successful in many cases in MSSA infections. However, it can lead to treatment failure in MRSA infections. Results also showed that the occurrence of cMLSB was high (40%), similar to those obtained by Sedaghat et al. (2017) (32.1%). In contrast, Mokta et al. (2015) and Mittal et al. (2013) discovered that cMLSB was 17.1% and 6.1%, respectively. Additionally, the cMLSB phenotype in our study was higher in MRSA 34 (48.6%), compared to MSSA 6 (20%). A similar observation was made by Goudarzi et al. (2020), who reported 30.2% in MRSA and 24.4% in MSSA.

This discrepancy between different studies on the prevalence of cMLSB can be due to variations in the consumption of macrolides in community and hospital settings, which account for the observed differences in bacterial susceptibility based on geographical distribution.

In this study, 9% of erythromycin-resistant staphylococcal isolates showed the MS phenotype (isolates were susceptible to clindamycin). So, clindamycin is proposed for use in treating patients with infections without the emergence of resistance during therapy.

Studies by Kavitha (2020) and Sedaghat *et al.* (2017) showed that the MS phenotype was 12.26% and 6%, respectively. In this study, silver, which is considered for its antimicrobial activity, was evaluated in the form of nanoparticles against the iMLSB phenotype *S. aureus* isolates. The antimicrobial activity of AgNPs was inversely proportional to the particle size and form. The smaller the particle size, the higher the active or specific surface area to come in contact with the microbial cells, thereby increasing interaction and penetration into the cell membrane (Wady et al., 2014). The antimicrobial assay showed that 25nm AgNPs at a concentration of 1µg/mL, inhibited pathogen growth. Another study by Wady et al. (2014) suggested a powerful growth inhibition of *S. aureus* and MRSA at concentration of 1.95µg/mL, using 9nm AgNPs. Also, MIC value of AgNPs toward *S. aureus* was determined to be 2µg/mL, using 30nm AgNPs, as reported by Youn et al. (2017).

AgNPs are proposed to enhance antibacterial outcomes of traditional antibiotics as reported by many studies. Combining amoxicillin and nano-Ags toward *E. coli* showed synergistic effects compared with when they were applied separately (Li et al., 2005), Also nano-Ags and polymyxin B showed synergistic effects against Gram negative bacteria (Ruden et al., 2009). Recently, combining gentamicin and chloramphenicol with AgNPs has a greater antibacterial effect on MDR in E. faecalis than both antibiotics alone (Katva et al., 2017).

In this study, a combined treatment of AgNPs with clindamycin acted synergistically against the tested isolates. It led to a decline in MIC of clindamycin from 32µg/mL to 8µg/mL and AgNPs from 1 to 0.25µg/mL (MIC decreased by four-fold). Therefore, this mixture improved the efficiency of both clindamycin and AgNPs. Complex formation between antibiotics and AgNPs is proposed as the possible mechanism illustrating their synergistic effects. The antibiotic's active and functional groups like hydroxyl and amino groups were bonded to the large surface area of AgNPs through chelation (Fayaz et al., 2010; Rahim & Mohamed, 2015). Accordingly, synergism can decrease the MIC of AgNPs since it will be used in less concentrations than when it is used alone. Therefore, its toxicity can be decreased to some extent since it is dose-dependent.

Haryanvi & Rosana (2020) reported partial synergistic effects of AgNPs that were functionalized with clindamycin. It was proven

that the antibacterial impact of antibiotics was enhanced against several animal and human pathogens, in addition to resistant bacterial strains, when combined with AgNPs (Paná˘cek et al., 2016; Smekalova et al., 2016).

The proposed acute toxicity test method (Chinedu et al., 2013) had some advantages over other methods. It was simple, accurate, less time consuming, and required a few animals. Also, it can be used by both individuals and organizations because it is inexpensive. Most *in vivo* studies on AgNPs selected routes of administration like inhalation, oral, and intra tracheal instillation (Elkhawass et al., 2015). In this study, the intraperitoneal path was preferred to introduce agents into the circulation quicker than the oral route (Thamer & AL-Mashhady, 2016). After administering the acute toxicity doses, no mortality was observed after 1h and for 24h. Moreover, the LD_{50} of AgNPs in mice was 200mg/kg. Therefore, based on the Hodge and Sterner toxicity scale, the obtained LD_{50} value classified AgNPs as moderately toxic during a single dose (Hodage & Sterner, 2005).

Conclusion

Conclusively, the D-test should be conducted for detecting ICR to prevent failures that can occur during treatment against *S.* aureus infections. Results also revealed the potential usage of silver nanoparticles against these isolates. So, if AgNPs can replace traditional antibiotics or the combined AgNPs with antibiotics treatment, considering the suitable toxicity level and estimated cost, a promising agent to kill bacteria is proposed to exist without the development of antibiotic resistance. Furthermore, it was observed that the intraperitoneal administration of AgNPs was relatively moderately toxic. Therefore, AgNPs can be used with a certain degree of safety that should be studied further during *in vivo* toxicity testing studies.

Conflicts of interest: Authors declare no conflict of interest.

Author contributions: YA, WA, NF and SE conceived the research. WA and NF performed the experiments. NF and SE drafted the manuscript. SE revised the first draft. All authors read and approved the final version of the manuscript.

Ethical approval: Not applicable

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انتشار مقاومة الكليندامیسن المستحثة والمكافحة النانوتكنووجية للعزلات السريرية للاستافيلوكوكس اوريس

يوسف الإمام عبد الموجود[1]، ولاء عبد اللطيف[2]، نوران خالد فوزى[1]، شريف محمد النجدي[1]
[1]قسم النبات والميكروبيولوجي - كلية العلوم - جامعة القاهرة - الجيزة - مصر، [2]قسم الميكروبيولوجي - كلية الطب- جامعة عين شمس - القاهرة - مصر.

الاستافيلوكوكس اوريس هي واحدة من مسببات الأمراض التي يُقترح أن تسبب عدوى مختلفة في البشر. لذلك فإن الكليندامیسن هو دواء مهم تم تحديده في علاج هذه العدوى، وخاصة تلك التي تصيب الجلد والأنسجة الرخوة. مقاومة الكليندامیسن المستحثة هي من المضاعفات التي تنشأ أثناء علاج عدوى الاستافيلوكوكس اوريس في البشر. وبالتالي، من الصعب اكتشاف سلالات الاستافيلوكوكس اوريس التي تعبر عن مقاومة الكليندامیسن المستحثة، باستخدام طرق اختبار الحساسية القياسية. في الآونة الأخيرة، توجد حاجة متزايدة لإيجاد بدائل للمضادات الحيوية. علاوة على ذلك، تم الاشارة سابقًا الي الجسيمات النانوية، وخاصة الجسيمات النانوية الفضية، كبدائل محتملة للمضادات الحيوية التقليدية (أو بالاشتراك مع المضادات الحيوية التقليدية) ضد ظهور المقاومة البكتيرية للأدوية المتعددة. ومن ثم، فقد درس هذا البحث انتشار مقاومة الكليندامیسن المستحثة بين عزلات الاستافيلوكوكس اوريس الإكلينيكية وفحص التأثيرات المضادة للبكتيريا لجسيمات الفضة النانوية بشكل منفصل وأيضا مع دمجها مع الكليندامیسن ضد هذه العزلات لتقييم السمية الحادة لجسيمات الفضة النانوية داخل الصفاق. من بين مائة عزلة من الاستافيلوكوكس اوريس التي خضعت للدراسة على مدار عام واحد، تم تعريف 70 عزلة على أنها الاستافيلوكوكس اوريس المقاومة للميثيسيلين (MRSA) و30 عزلة من الاستافيلوكوكس اوريس الحساسة للميثيسيلين (MSSA). كما أوضحت النتائج أن النسبة المئوية للأنماط الظاهرية ماكرولايد-لينكوساميد-ستربتوجرين ب التكويني (iMLSB) و ماكرولايد-لينكوساميد-ستربتوجرين ب المستحث (cMLSB) والمقاوم للماكرولايد-وستربتوجرين ب (MS) كانت 40٪ و 10٪ و 9٪ على التوالي. بشكل عام، أظهرت 41٪ من عزلات الاستافيلوكوكس اوريس حساسية تجاه الاريثروميسين. بالإضافة إلى ذلك، كانت كل من الطرز الظاهرية iMLSB وcMLSB هي الأكثر انتشارًا بين عزلات الاستافيلوكوكس اوريس المقاومة للميثيسيلين. إلى جانب ذلك، كان لجسيمات الفضة النانوية تأثيرات قوية مضادة للجراثيم، مع أدني تركيز مثبط (MIC) (1 ميكروغرام / مل) أيضًا، بالإضافة إلى نشاط تآزري جزئي مع الكليندامیسن تجاه الاستافيلوكوكس اوريس. بناءً على هذه الملاحظات، تم تحديد أضافة جسيمات الفضة النانوية داخل الصفاق على أنها معتدلة السمية. لذلك، من الممكن التوصية باستخدام جسيمات الفضة النانوية كعلاج محتمل للعدوى التي تسببها الاستافيلوكوكس اوريس.