Silver Nanoparticle Impact as Antibacterial and Antibiofilm Agent Against MDR K.pneumonaie in Mosul City

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(Ann Coll Med Mosul 2024; 46 (2):159-169).
Received: 10th Febr. 2024; Accepted: 17th March 2024.

ABSTRACT

The prevalence of antibiotic resistance continues to rise rapidly, necessitating the development of innovative approaches to combat diseases caused by drug-resistant bacteria. One of these approaches is pharmaceutical nanotechnology, which has the potential to generate novel therapeutic compounds for biomedical applications. Of these substances, silver nanoparticles (AgNPs) exhibit promising antibacterial and anti-biofilm properties. Two hundred and thirty different clinical samples (urine, sputum, diabetic foot pus, pleural fluid, tracheostomy tube, cerebrospinal fluid, folly's catheter, ear swab, and blood) were collected looking for K.pneumonaie, which are multiple drug-resistant (MDR). Twenty-three K.pneumonaie clinical isolates were identified by conventional bacteriological media and confirmed by the VITEK-2GN system. Then, the biofilm-forming activity was assessed using qualitative methods such as Congo Red agar and quantitative methods such as microtiter plate assay that involved crystal violet staining. Conventional PCR detected fimH and mrkD biofilm gene frequency among biofilm-producing K.pneumonaie. The chemically synthesized AgNPs were used, and their Minimal Inhibitory Concentration (MIC) and Minimal Biofilm Inhibition Concentration (MIBC) were determined. Biofilm genes, namely fimH and mrkD gene, had been detected before and after treatment with silver nanoparticles in the clinical isolates. The findings indicated that all isolates resisted the tested antibiotics and could build biofilms to various degrees (19 had strong biofilm capability,3 were moderate biofilm producers, and only 1 had weak biofilm production capability). The susceptibility of all isolates to AgNPs was demonstrated by their antibacterial activity, with MIC and MIBC values being 50000 µg/ml or 25000 µg/ml according to the isolates. In addition, applying AgNPs resulted in a substantial decrease in biofilm formation, with 8 out of 23 isolates converting to non-biofilm producers. Biofilm genes fimH and mrkD were present in 100% and 78.26%, respectively, and not affected by silver nanoparticle treatment. There was a synergistic effect between silver nanoparticles and imipenem in compacting MDR K.pneumonaie.

***Keywords*:** Silver nanoparticle; MDR K. pneumonia; Minimal Inhibitory Concentration; Minimal Biofilm Inhibition Concentration; Biofilm genes.

تأثير جسيمات الفضة النانوية كعامل مضاد للبكتيريا ومضاد للأغشية الحيوية ضد بكتريا الكليبسيلا الرئوية المقاومة للعقاقير المتعددة في مدينة الموصل

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الخلاصة

يستمر انتشار مقاومة المضادات الحيوية في الارتفاع بسرعة، مما يستلزم تطوير أساليب مبتكرة لمكافحة الأمراض التي تسببها البكتيريا المقاومة للأدوية. إحدى هذه الأساليب هي تكنولوجيا النانو المتطورة التي لديها القدرة على توليد مركبات علاجية جديدة للتطبيقات الطبية الحيوية. من بين هذه المواد، وبالتحديد جسيمات الفضة النانوية التي تظهر خصائص واعدة مضادة للبكتيريا ومضادة للأغشية الحيوية. تم جمع مائتين وثلاثين عينة سريرية مختلفة من (البول، البلغم، صديد القدم السكري، السائل الجنبي، مسحة من أنبوب القصبة الهوائية، السائل النخاعي، مسحة من قسطرة فولي، مسحة الأذن والدم) للبحث عن بكتيريا الكليبسيلا الرئوية المقاومة للعقاقير المتعددة. تم تشخيص ثلاث وعشرون عزلة سريرية من بكتيريا الكليبسيلا الرئوية بواسطة الوسائط

البكتريولوجية التقليدية وتم تأكيدها بواسطة نظام الفايتك تو. ثم تم تقييم نشاط تكوين الأغشية الحيوية باستخدام الطريقة النوعية مثل أجار الكونغو الأحمر والطريقة الكمية مثل الطبق ذو المعيار الصغير والذي يتضمن صبغة الكريستال البنفسجية وكما وتم استخدام تفاعل البلمرة المتسلسل التقليدي لقياس مدى انتشار مورثات ال fimH وmrkD في بكتريا الكليبسيلا الرئوية المكونة للأغشية الحيوية. و كذلك تم تحديد الحد الأدنى من التركيز المثبط والحد الأدنى من تركيز تثبيط الأغشية الحيوية لجسيمات الفضة النانوية. ثم تم الكشف عن مدى انتشار مورثات الأغشية الحيوية وهي مورث fimH ومورث mrkD وتمت معالجتها بجزيئات الفضة النانوية في العزلات السريرية. أشارت النتائج إلى أن جميع العزلات أظهرت مقاومة للمضادات الحيوية التي تم اختبارها، وكذلك أظهرت جميع العزلات القدرة على بناء الأغشية الحيوية بدرجات مختلفة حيث كانت ١٩ عزلة بنسبة ٨٢.٦٠% لديها قدرة قوية على إنتاج الأغشية الحيوية، ٣ عزلات بنسبة ١٣.٠٤ % منتجة للأغشية الحيوية المعتدلة وواحدة فقط بنسبة ٤.٣٤ % لديها قدرة ضعيفة على إنتاج الأغشية الحيوية. تم إثبات حساسية جميع العزلات لـ جسيمات الفضة النانوية من خلال نشاطها المضاد للبكتيريا، حيث كانت قيم التركيز الادنى المثبط لنمو البكتريا وكذلك المثبط لتكوين الغشاء الحيوي هي ٥٠٠٠٠ ميكروجرام / مل و ٢٥٠٠٠ ميكروجرام / مل تبعا لنوع العزلة. بالإضافة إلى ذلك، أدى تطبيق جسيمات الفضة النانوية إلى انخفاض كبير في تكوين الأغشية الحيوية، حيث تحولت ٨ من أصل ٢٣ عزلة من منتجي الاغشية الحيوية إلى غير منتجين للأغشية الحيوية، وكانت مورثات الأغشية الحيوية fimH و mrkDموجودة بنسبة ١٠٠% و٧٨.٢٦% على التوالي ولم تتأثر بالمعالجة بجسيمات الفضة النانوية و كان هناك تأثير تآزري بين جسيمات الفضة النانوية وعقار الايميبينيم ضد بكتريا الكليبسيلا الرئوية المقاومة للعقاقير المتعددة.

الكلمات المفتاحية : جسيمات الفضة النانوية; بكتريا الكليبسيلا الرئوية المقاومة للعقاقير المتعددة; الحد الأدنى من التركيز المثبط. الحد الأدنى من تركيز تثبيط الأغشية الحيوية؛ جينات الأغشية الحيوية.

INTRODUCTION

Microbial infections are constantly adapting and developing resistance to both novel and well-established therapeutic strategies. This has increased the breadth of antimicrobial resistance (AMR), its adverse effects on global healthcare, and a marked decline in antibiotic research [1]. In Mosul city, after the crisis that had suffered and the last war, many infectious diseases had increased caused by multiple drug-resistant pathogens. Klebseiella pneumoniae (K.pneumonaie) [2].

K.pneumoniae is a noteworthy opportunistic bacteria that is classified as one of the ESKAPE pathogens, which include Enterococcus faecium, Staphylococcus aureus, K.pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp. [3]. The group above has been recognized for its tendency to induce opportunistic infections. It is distinguished by a concerning degree of resistance to antibiotics, primarily due to the acquisition of mobile genes associated with antimicrobial resistance by horizontal gene transfer [4]. The capacity of K.pneumoniae to form biofilms plays a crucial role in protecting strains from the immunological response of the host as well as antibiotics, particularly within multidrug-resistant (MDR) isolates [5].

Biofilms consist of organized groups of microorganisms that have increased resistance to antimicrobial substances and the immunological responses of the host, including the complement system, antimicrobial peptides, and phagocytosis [5]. Biofilms are composed of bacteria surrounded by diverse architecture of an extracellular matrix. This matrix comprises proteins, carbohydrates, and genetic material from bacteria and the host [6]. Biofilm production impedes the effectiveness of antibiotics and can lead to resistance through cell-to-cell interactions and the acquisition of DNA from neighboring bacterial communities [6]. Various genes associated with the production of biofilms, such as mrkD (type 3 fimbriae) and fimH (type 1 fimbrial adhesion), are known to have significant implications in facilitating this biological process" [7,8]. Physicians face difficulties in selecting the appropriate course of treatment since, despite the development of new generations of antibiotics, none of them have yet proven to be 100% effective against bacteria that form biofilms or are multidrug-resistant (MDR) like K.pneumoniae [9]. It is consequently imperative to find an alternative therapy [10].

Silver nanoparticles (AgNPs) have proven incredibly efficient against various infections, including multidrug-resistant ones like MDR-K.pneumonaie [11]. AgNPs have antibacterial properties because they can release silver ions that interact with the internal components and

membranes of bacteria, ultimately causing the bacterium to die. The work of Pragati Rajendra et al. in 2023 serves as an example of how modern research is still illuminating this issue [12]. The basis of the AgNPs' antibiofilm activity is their ability to damage the biofilm matrix and prevent it from progressing [13]. Moreover, AgNPs aid in producing reactive oxygen species, which cause oxidative stress in bacteria enclosed in biofilms. Mimi Seo et al.'s 2021 study shed light on the complex interactions between AgNPs and biofilm constituents and showed how they could be used to treat infections associated with biofilm formation [14]. A few studies have also demonstrated AgNPs' efficacy in preventing biofilm development. AgNPs' high surface capacity rate inhibits biofilm components' growth, which is necessary to shield bacteria from antibiotics [15,16].

Aim of the Work

1. Examine the antimicrobial and antibiofilm effects of silver nanoparticles (AgNPs) on multidrug-resistant (MDR) K.pneumoniae isolated from different clinical samples from hospitalized patients in Mosul City.
2. Detect the frequency of fimH and mrkD genes among biofilm-producing K.pneumonaie.

MATERIALS AND METHODS

Ethical Approval

Ethical approval for this study was obtained from the Iraqi Ministry of Health/Mosul Health Department, with an assigned approval letter Number 9295 on Feb 19, 2023.

Study Design

A cross-sectional study was conducted at Al-Salam and Al-Jumhoree Teaching Hospital in Mosul City from February 2023 to August 2023.

Patients

Patients from the ages of three months to 80 years of both sexes are included in this study, and the aim is to report each patient's medical history, including their name, age, gender, and the type of specimen obtained. Additionally, it was ensured that patients included in the study were not under treatment, specifically no antibiotic intake for three days before specimen collection for culture.

Sample Collection:

A total of 230 different clinical samples were collected, as shown in (Table 1)

Table (1) Types and numbers of clinical samples

Sample type	No.
Urine	72
Pus (from diabetic foot)	56
Sputum	40
Pleural fluid	20
High Vaginal Swab (HVS)	12
Cerebral Spinal Fluid (CSF)	12
Tracheostomy tube swabs	10
Foleys catheter swabs	4
Ear swab	3
Blood	1
Total	230

Bacterial Strain Identification:

All those specimens were cultured on MacConkey, Eosin Methylene Blue, Blood agar, and Brain Heart Infusion broth (BHI) and then incubated overnight at 37 ^{0}C. Colonies that appeared were tested for oxidase, catalase, and urease production, as well as biochemical reactions for exact strain identification, as confirmed by the VITEK2 GN ID card .

Antimicrobial Susceptibility Testing Test:

The Kirby-Bauer disc diffusion method tested the susceptibility of isolated bacterial strains to various antibiotics according to CLSI 2022 guidelines [17]. Antibiotic discs produced by Oxoid in England were utilized. These antibiotic discs came in the following types and concentrations: 10µg/disc of imipenem, 30µg/disc of augmentin, 10 mg/disc of amikacin, ten µg/disc of tetracycline, ten µg/disc of cefotrixone, and ten µg/disc of gentamycin, chloramphenicol ten µg/disc, cefotaxime ten µg/disc, ceftazidime ten µg/disc, and colistin ten µg/disc.

Biofilm Qualitative and Quantitative Assessment Methods:

According to Badawy MSEM, 2020 qualitative assessment of biofilm by Congo red agar, MDR K. pneumonia is cultured on Congo red agar that contains (BHI agar with sucrose and congo red stain within estimated concentrations), for 24h at 37 C^{0}, and the black-colored colonies are considered as strong biofilm producer while the pink colored are moderate and the red colored are non biofilm producer.

After being cultured on Congo red agar, the microtiter plate method estimated the biofilm, which is the quantitative measurement of biofilm formation. A loop full of the examined organisms'

overnight cultures was added to 5 mL of brain heart infusion (Lab M Ltd, UK) containing 1% glucose, and the mixture was then cultured for 24 hours at 37 °C. A sterile 96-well flatbottom polystyrene tissue culture plate added 180 µL of the bacterial solution (Sigma-Aldrich Co. LLC, USA) to each well. As a negative control, broth without cultured microorganisms was used. The plates were incubated at 37 °C for a whole day. Following incubation, each well's contents were carefully tapped out, and the wells were then cleaned three times using 300 L of sterile saline. The residual germs that adhered were fixed by adding 180 ml of methanol to each well for 15 minutes. Then, each well received 180 ml of 1% crystal violet stain. After 15 minutes, 95% ethanol, 150 mL of volume, was added to each well to remove any leftover dye. The optical densities (OD) at 630nm were measured in half an hour using a tissue culture plate. The results of the test were averaged after it was run three times. After calculating the OD values for each tested strain and the negative controls, the cut-off value (ODc) was determined and equal to 0.139. The results were interpreted using the strain classifications listed below[18] :

Non-biofilm maker (0): OD ≤ ODc,

ODc < OD ≤ 2 × ODc for weak biofilm producers (1+)

2 × ODc < OD ≤ 4 × ODc for moderate biofilm producers (2+)

4 × ODc < OD for strong biofilm producers (3+)

After adding AgNPs / Sky Spring Nanomaterial USA company, the previously outlined procedure was used to test the antibiofilm impact of AgNPs. The following equation was utilized to determine the prevention of biofilm formation formula[19] :

(1-(OD treatment /OD non treated control)*100) is the inhibition rate.

Silver Nanoparticle MIC Determination:

The MIC of AgNP was estimated according to Saginur, R et al. 2006 and Al-Rashidy MAAM 2023 [20,21] by serial twofold dilutions from formally prepared AgNP stock solution, which have a concentration equal to 200000 microgram /2ml, so had different concentrations in 10 tubes which are (50000, 25000, 12500, 6250, 3125, 1562.5, 781.25, 390.625,195.3, 97.65) then few colonies from fresh culture media of K.peumonaie inoculated in each 10 test tubes and incubated for 24 hours. The concentration of the last tube with no obvious growth is considered the MIC of AgNPs for that bacteria.

Silver Nanoparticle MIBC Determination:

As mentioned above in the microtiter plate method and according to Siddique, M. H et al. 2020 [19], 20µl of each bacterial suspension was introduced into 96-well flat-bottomed microtiter plates containing 80µl of BHI broth with 2% sucrose. Additionally, 80 µl of silver nanoparticles at varying concentrations starting with sub-MIC was added to each well. The contents were thoroughly mixed and, after that, incubated for 24 hours at a temperature of 37°C and then washed three times with normal saline and continued as the previous microtiterplate method of washing then adding methanol, crystal violet, and ethanol added in the same quantities and concentrations and then read OD at 630 nm.

DNA Extraction for fimH and mrkD biofilm Genes Detection Using Conventional PCR:

According to the manufacturing procedure, DNA was first extracted from bacteria using a commercial kit (Geneaid company, Taiwan). The primer sequence was selected as in (Table 2). Primer conditions were as follows: for both fimH and mrkD gene, Initial denaturing at 94 °C for 5 min followed by 30 cycles, each cycle contained 1 min at 94 °C for denaturation, 30 s for annealing and 60 s for extension steps, and finally one cycle for the final extension at 72 °C for 10 min.

Table (2): Primers information .

Name of the gene	Sequence	Annealing temperature	Amplicon size	Reference
fimH	F AAATAATCCCCCTGTTCACC R GGTAAGAGGTGCCGTTATATT	45°C	306	This article
mrkD	F CCACCAACTATTCCCTCGAA R TGGAACCCACATCGACATT	50 °C	226	[22]

Statistical Analysis

The statistical analyses were conducted using IBM SPSS statistics software version 25.0 (IBM Corp., Armonk, NY, USA). Data were presented as tables, charts, and diagrams. Statistical significance was defined as p-values less than 0.05. The t-test values were utilized to compare categorical variables.

RESULTS

In this study, out of these 230 clinical isolates, only 142 (61.73%) clinical isolates showed positive growth for different kinds of bacteria. From this 142 bacterial growth, 33 (23.2%) samples contained Klebsiella spp 11 (33.3 %) were isolated from urine, 7(30.3%) were isolated from pus, 10(21.2%) were isolated from sputum, 2(6.06%) were isolated from tracheostomy, 1(3.03%) were isolated from pleural fluid, 1(3.03%) were isolated from CSF, and 1(3.03%) were isolated from Foley's catheter, as shown in Figure (1)

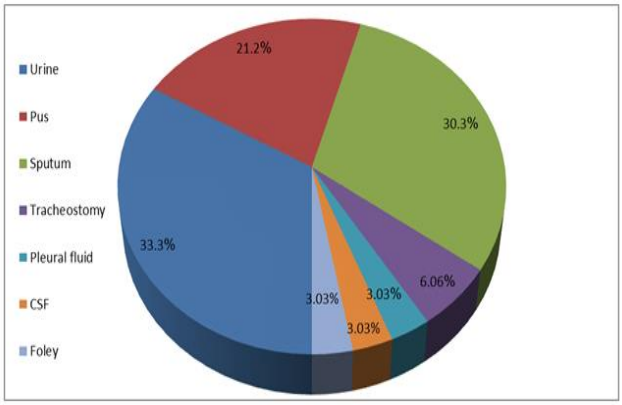

Figure (1): Percentage of Klebsiella Spp isolates from different clinical samples

From 33 Klebsiella spp clinical isolates, female predominance was obvious in urine, pus, and Foleys catheter samples. In contrast, male patients had more Klebsiella spp in sputum, tracheostomy tube, pleural fluid, and CSF samples than female patients, as shown in (Table 3).

Table (3): Male and female distribution among different clinical samples

Sex	Urine	Pus	Sputum	Tracheostomy tube	Foleys catheter	Pleural fluid	CSF	Total
Male	4	2	8	2	0	1	1	18
Female	7	5	2	0	1	0	0	15
Total	11	7	10	2	1	1	1	33

Forty bacterial samples that exhibited a pink color on MacConkey agar (lactose fermentor) and lacked a bright, green metallic appearance on EMB agar were subjected to identification using the vitek-2 system. Using Gram-Negative Identification cards (GNB- ID), ten isolates were K. oxytoca, 2 were Enterobacter aregenosa, 1 Citrobacer kosiri, and 4 were E.coli. These 17 isolates were excluded from this study, and only the remaining 23 were found to be K. pneumoniae sub-SPP pneumonia with different degrees of efficacy in the vitek-2 system ranging from (94% to 100%).

All K. pneumonia sub-spp pneumonia in this study is multiple drug-resistant isolates with the highest degree of resistance occurring against tetracycline, colistin, and ceftriaxone with a rate of 100%. On the other hand, all isolates were sensitive to imipenem. The pattern of antibiotic resistance exhibited by the isolates to 10 antibiotics (using disc diffusion Kirby Bauer method on Muller Hinton agar measuring the diameter of inhibition zone and compared it to CLSI,2021) is illustrated in (Table 4).

Table (4): Antibiotic susceptibility test results against K. pneumoniae

Antibiotics	R*	I**	S***
	NO. (%)	NO. (%)	NO. (%)
Augmentin AMC (30) µg	19 (82.61%)	2(8.69 %)	2(8.69%)
Amikacine AK (10) µg	15(65.21%)	1 (4.34%)	7(30.43%)
Ciprofloxacin CIP (10) µg	3(13.04%)	2(8.69%)	18(78.26%)
Tetracycline TE (10) µg	23(100%)	0(0%)	0(0%)
Chloramphenicol C (10) µg	3 (13.04%)	4(17.39%)	16(69.56%)
Colistin CL (30) µg	23(100%)	0 (0%)	0(0%)
Cefotaxime CTX (10) µg	9(39.13%)	5(21.73%)	9(39.13 %)
Imipenem (10) µg	0(0%)	0(0%)	23(100%)
Ceftriaxone CRO (10) µg	23(100%)	0(0%)	0(0%)
Ceftazidime CAZ (30) µg	8 (34.78%)	9(39.13%)	6(26.08%)

*R (Resistant), **I (Intermediate sensitivity),***(Sensitive)

Biofilm Production Estimation:

By the congo red agar method, all isolates seem to be strong biofilm producers, except one is the non-biofilm producer. In contrast, by the microtiter plate method, 19 (82.6%) were strong biofilm producers, 3 (13.04%) were moderate biofilm producers, and the one that seemed to be biofilm producer was weak biofilm producer assessed by microtiter plate method, as shown in following Figure 2 and Table 5.

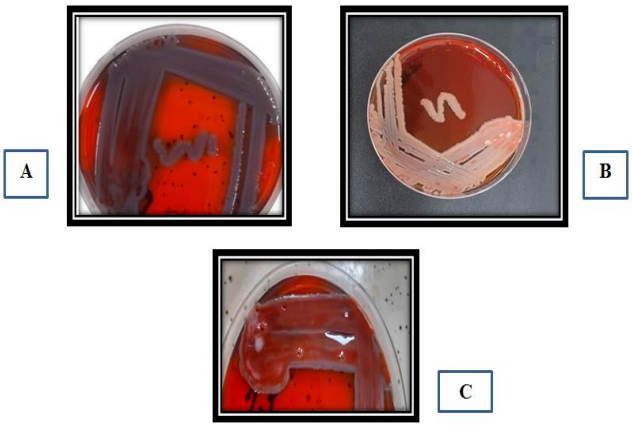

Figure (2): Congo red agar with biofilm producer K. pneumoniae sub-SPP pneumonia showing (A) strong biofilm producer blacked colored colonies (B) moderate biofilm producer pink colored colonies (C) negative control red colored colonies

Table (5) Biofilm distribution according to different clinical samples

Sample type	Strong adherent NO. (%)	Moderate adherent NO. (%)	Weak adherent NO. (%)
Sputum	7 (30.43%)	0	0
Urine	6 (26.08%)	1 (4.34%)	0
Tracheostomy tube swab	2 (8.69%)	0	0
Pus swab	1 (4.34%)	2 (8.69%)	1 (4.34%)
Foleys catheter	1 (4.34%)	0	0
Pleural fluid	1 (4.34%)	0	0
CSF	1 (4.34%)	0	0
Total	19 (82.60%)	3 (13.04%)	1 (4.34%)

Determination of MIC of silver nanoparticles

The lowest concentration of silver nanoparticles that is necessary to inhibit visible growth by using the serial dilution tube method was variable against different isolates, but its value ranged from 50000 µl to 25000 µl as shown in the following Figure (2)

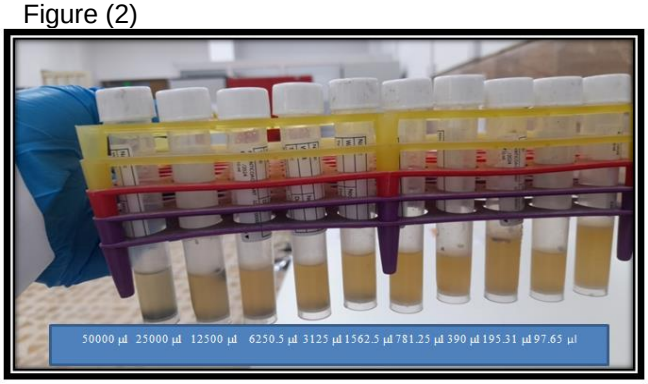

Figure (3): Serial dilution method for detection of the MIC of silver nanoparticles against K. pneumoniae sub-Spp pneumonia

The anti-biofilm effect of silver nanoparticles on 23 clinical isolates of K. pneumonia was clear in 8 (34.78%) isolates with a concentration of 50000 µl was done by using the microtiter plate method as shown in the following Figure 4

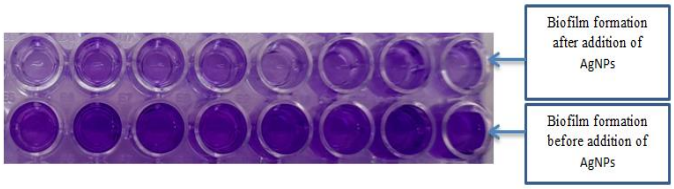

Figure (4): Microtiter plate method showing the antibiofilm effect of silver nanoparticle on 8 K. pneumoniae sub-spp pneumonia

There was a significant difference in biofilm production before and after the addition of AgNPs detected by t-test, and the p-value was 0.001, as shown in Figure 5. The inhibition of biofilm was evident in 8 clinical isolates (3,4,5,6,7,8,9,10), and the ratio of the anti-biofilm effect of AgNPs was 81.98%.

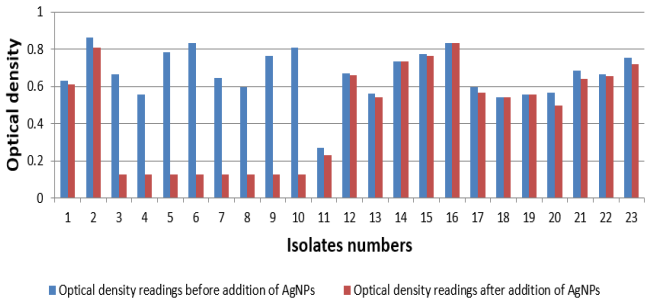

Figure (5): Antibiofilm effect of silver nanoparticles in 8 isolates

The inhibitory effect of imipenem was increased when silver nanoparticle-loaded filter paper with a concentration of 25000 µg or 50000 µg was added near to it. This was visualized when the diameter of the inhibitory circle around the imipenem was increased by 5 mm around the studied isolates, than each item used alone as shown in the following Figure 6.

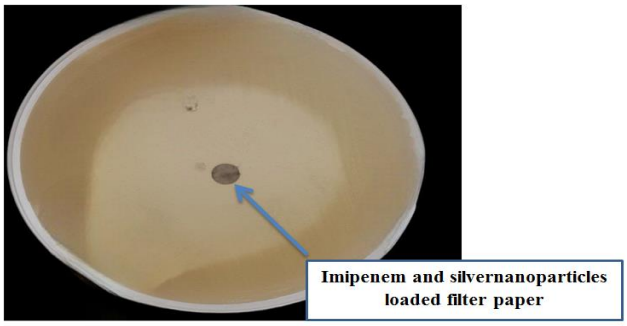

Figure (6): The synergistic effect between the silver nanoparticle and imipenem against K. pneumoniae sub-spp pneumonia

Molecular methods for detection of biofilm genes fimH and mrkD before and after treatment with silver nanoparticles:

DNA of K.pneumoniae isolates was analyzed by using conventional PCR. Amplification of genes for 23 isolates of K.pneumoniae sub-spp pneumonia to identify two biofilm-associated genes: mrkD and fimH before and after silver nanoparticles treatment. Results for biofilm-associated genes before treatment with silver nanoparticles showed that all isolates (100%) were positive for fimH gene and 18 (78.26%) out of 23 isolates were positive for mrkD, and only 5 (21.73%) were negative for mrkD as shown in the following (Figures 7,8,9,10,11)

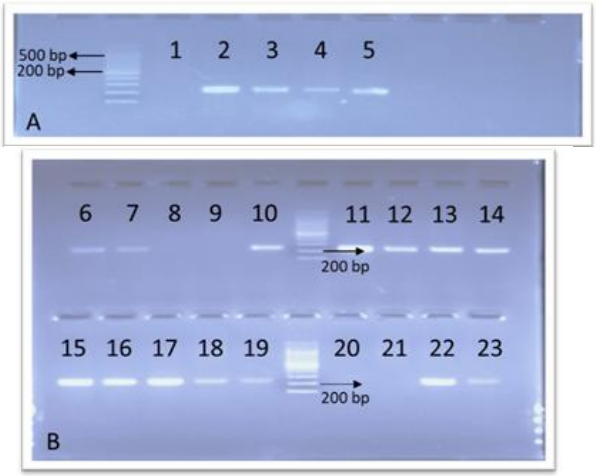

Figure 7 (A) and Figure 8 (B): 2% agarose gel electrophoresis at 75 volts for 50 minutes was run for PCR products of mrkD gene in 23 K.pneumoniae sub-spp pneumonia isolates its length 226bp

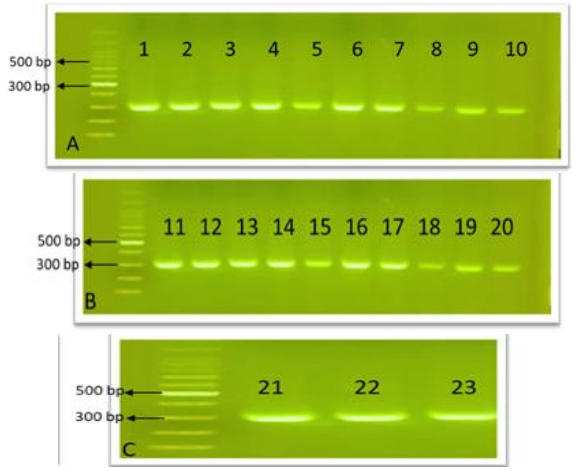

Figure 9 (A), Figure 10 (B), and Figure 11(C):2% agarose gel electrophoresis at 75 volte for 50 minutes had run for PCR products of fimH gene in the 23 isolated K.pneumonaie sub-spp pneumonia its size 306bp

Biofilm-associated genes after treatment with silver nanoparticles

The anti-biofilm effect of silver nanoparticles was visualized only in 8 isolates by tissue culture plate method phenotypically. Still, by PCR, the biofilm-associated genes, which are fimH and mrkD, were not affected and were positive in the eight isolates even after treatment with silver nanoparticles, as shown in Figures (12) and Figure (13).

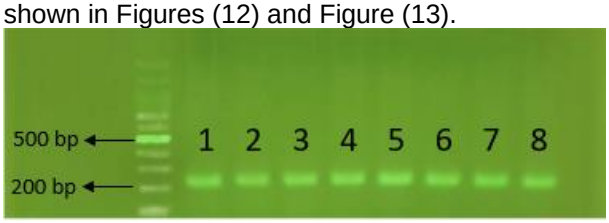

Figure (12): 2% agarose gel electrophoresis at 75 volte for 50 minutes was run for PCR products of mrkD gene in 8 K.pneumonaie sub-spp pneumoniae isolates after treatment with silver nanoparticle its size 226bp.

Figure (13): 2% agarose gel electrophoresis at 75 volte for 50 minutes had run for PCR products of fimH gene in 8 K.pneumonaie sub spp pneumonaie isolates after treatment with silver nanoparticle size 306bp.

DISCUSSION

The World Health Organization has classified K.pneumoniae as a "priority pathogen," recognizing its critical role in the intensifying fight against antibiotic resistance. K.pneumoniae is a major contributor to nosocomial infections, emphasizing its prominence in healthcare-associated infections [23]. Since K. pneumoniae is a common infection and is involved in many other infections, treating K.pneumoniae infections effectively is essential to preventing the emergence of antibiotic resistance. Addressing K.pneumoniae's role is crucial to maintaining the effectiveness of antibiotics as resistance mechanisms continue to develop and spread [24].

The most frequent sample that contains K.pneumonaie in this study was urine 33.3%. This result is generally consistent with numerous studies conducted locally. A study done in Baghdad city by Al-Saady O.M.F [25] showed that the most frequent sample containing Klebsiella spp was from urine 37(37%) out of 108 isolates.

Another study in Duhok city and Erbil agreed with this study in which the most frequent sample containing Klebsiella was urine samples 66% and 56%, respectively (26,27), whereas other studies done in different countries like in China, Iran, and Indonesia by [28-30] showed that upper respiratory tract samples were the most frequent sites of K.pneumonaie infections.

The current research findings reveal that the frequency of K.pneumoniae isolated from various clinical samples was more significant in female patients (78%) compared to males (22%) in relation to gender, regarding urine, pus, and Foleys catheter samples. However, in the case of sputum, tracheostomy tube swabs, pleural fluid, and CSF samples, the frequency of the isolates was higher in males than females. This finding aligns with previous research indicating that K.pneumoniae was more commonly found in female than male patients [26]. However, this contrasts with a study conducted by Elsaid E.M et al. in Egypt [32] and another study by Nirwati and colleagues in Indonesia [31], who found that male patients tended to acquire Klebsiella infection more than female patients. The male predominance of K.pneumonaie infections in these studies has been linked to unhealthy lifestyle habits like smoking, which may decrease the strength of the immune system and enhance opportunistic infections [30]. The observed variance is challenging to explain and may result from differences in sample collection, study design, sample population, environmental factors, and personal cleanliness.

The elderly age group was the most predominant group who had K.pneumonaie infections, and the median age was 68 years; this agrees with other studies which showed a higher frequency of K.pneumonaie infection in older people than young individuals [33]

The efficacy of presently accessible antibiotics is diminishing due to the rising prevalence of resistant strains responsible for illnesses and the improper prescription and usage of antibiotics [34]. The therapeutic options for multiple drug-resistant strains of pathogens are greatly restricted [35]. The current study found that K. pneumoniae isolated from different clinical samples had varying levels of susceptibility to commonly used antibiotics, and all isolates were multiple drug resistant. The most significant resistance level is observed against tetracycline, colistin, and ceftriaxone, with a 100% rate. Also, resistance to penicillin and amikacin was 82.6% and 65.21%, respectively. However, all isolates exhibited 100% sensitivity to imipenem. This may be due to the over usage of previously mentioned drugs even without a doctor's prescription, especially in Mosul city, where imipenem usage is low in comparison to other medications, so lesser resistance compared to other drugs due to its high price, therefore not accessible to all people. This agreed with other studies done in Iraq and abroad, especially in developing countries [36,37,26].

Nanoparticles are considered an excellent and applicable alternative to antibiotics, which may solve the MDR evolution problem among bacteria [38]. Among the different Nano-sized antibacterial agents, AgNPs have proved to be a highly efficient particles against a wide range of microbes. This effect is significant in Gam-negative bacteria, especially K. pneumonia e[39]. In this regard, the growths of K.pneumoniae were examined after applying different concentrations of AgNPs, and data depicted that most of the assigned concentrations of AgNPs caused growth inhibition of K.pneumoniae, and increased concentration resulted in more inhibition with a MIC ranging from 25000 µg/mL to 50000 µg/mL. This high MIC was approximate to the MIC used by other studies done in Mosul city [21,40]. Whereas lower concentration is needed to inhibit bacterial growth like that study of Elsaid, E. M, 2023, done in Egypt [32], which needs 16-128 µg/mL against K.pneumonaie bacteria and by using biologically synthesized silver nanoparticle characterized by its lower surface volume and higher efficacy in lower concentration compared to the higher surface volume in chemically synthesized silver nanoparticle that is used in this study.

The present study found that all isolates are biofilm producers with various degrees of biofilm production capability. Nineteen isolates were categorized as strong biofilm producers, whereas 3 were moderate biofilm, and only 1 had weak biofilm ability. Another study done in Egypt showed that most K.pneumoniae were biofilm producers, accounting for 44% of the samples, whereas 32% were moderate adherent and 24% were weak biofilm producer strains [32]. The various ability of isolates to form biofilms is influenced by multiple parameters, including physicochemical traits, the nature of the surface to which the biofilm attaches, the physical interactions between different components, pH, temperature, and sample type [32]. In this study, the strains obtained from sputum samples showed a greater capacity to generate biofilm than other specimens. This demonstrates the significance of biofilm in facilitating the colonization of microbes in the lungs [41]. Karimi et al. [42] found that 20.4% of K.pneumoniae isolates exhibited significant biofilm development, indicating a high rate of biofilm formation compared to other tested isolates. In addition, Yang and Zhang (2014) [29] conducted a study to assess the capacity of K. pneumoniae strains to generate biofilm.

These strains were obtained from blood samples, wound swabs, sputum samples, and urine. Their investigation revealed that 62.5% of all isolates formed biofilms.

AgNPs exert their activity remains uncertain, although various possibilities have been postulated. According to one hypothesis, AgNPs interact with oxygen, interfering with the cellular respiratory chain. Additionally, AgNPs engage with the cell membrane, resulting in cellular death. Another hypothesis is that AgNPs may exhibit antibacterial effects by inhibiting the unwinding of DNA. Furthermore, the antibacterial effectiveness of AgNPs may be attributed to the oxidative harm induced by reactive oxygen species (ROS), which could be responsible for the antibacterial properties of AgNPs [49].

The mean ratio of silver nanoparticle antibiofilm was 81.98 % in this study, and there was a significant difference in biofilm production capability before and after adding this nano agent. This was in agreement with other studies like that of Corticata G,2020 et al. [43], who showed the antibiofilm effect of silver nanoparticles reached 88% against K.pneumonaie through in vitro and in vivo studies, another study of biologically synthesized silver nanoparticles against different gram negative biofilm producing microorganism including K.pneumonaie showed that reduction in biofilm capability occurred by 1.5, and 1.2 fold against biofilm producing K.pneumonaie and E.coli respectively [44].

Synergy is manifested when the combined impact of two or more distinct products exceeds the total impact of each product. When the total impact is equivalent to the sum of the separate outcomes, they are considered additive. The phenomenon of items being less effective when combined than when used alone is referred to as antagonism [45]. The results of this study demonstrated an apparent enhancement in the combined effectiveness of antibiotic imipenem when silver nanoparticles (AgNPs) are present, in contrast to using antibiotics alone against MDR K.pneumonaie. This agrees with other studies like that of Abo-Shama UH et al. in 2020, who showed synergistic effect between silver nanoparticles and different antibiotics against gram negative bacteria [46]. This synergistic effect was shown in other studies that used other nanoparticles in combination with imipenem antibiotics against K.pneumonaie [47,48].

CONCLUSION

The current study proposes that AgNPs can be employed as effective antibacterial and antibiofilm agents against multidrug-resistant K.pneumoniae. There is a synergistic effect between silver nanoparticles and antibiotics against various microorganisms. Both fimH and mrkD are common genes among biofilm-producing K.pneumonaie.

Conflict of Interest

There are no conflicts of interest regarding the publication of this manuscript.

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