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ORIGINAL ARTICLE

Antibacterial & Antibiofilm Effect of Chitosan and Silver Nanoparticles against MDR *Klebsiella pneumoniae* isolated from Urinary tract infection

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ABSTRACT

This search was intended to study the Antibacterial ffect OfChitosanand Silver Nanoparticles against MDR Klebsiella pneumoniae isolated from urinary tract infections,in the province of AL-Najaf. Two hundred and twenty samples were carried out between (October , 2022 to January , 2023). were collected from patients with UTI infections from two separate hospitals Al-Hakam Hospital and Al- Zahraa Hospital as well as AL-Aman center for research AL-Najaf Governorate , isolates were identified based on the morphological and microscopic examination, and Vitek 2 system. Antibacterial susceptibility test was conducted for 60 K. pneumoniae isolates against 22 commonly used antibacterial agents by using the disk diffusion method , the highest rate of resistance was seen with Amoxicillin- clavulanic Acid 60/60 (100%)followed Trimethoprime - sulfamethaxazol 44/60(73.3%)and the low rate resistance was seen with impenem 4/60 (6.7%)and Nitrofurantoin was 3/60 (5 %). The capacity of some Klebsiella pneumoniae isolates to biofilm formation was detected by phenotypic method which included Congo Red Agar Method (CRA) , from the 60 (100%) isolates of K. pneumoniae 48 (80%) were biofilm producers when that appearance of black dry crystalline colonies on the CRA plates and 12 (20 %) were non - biofilm producers when the colonies of K. pneumonia remained pink or red colored. Molecular study of antibiotic resistance genes (gyrA,, Ant(2")-1a,,blaSHV-1) were detected in (MDR) K. pneumoniae isolates(20/20 (100%) , 11/20 (55%) , and 20/20 (100%) respectively. finally, the isolates were subjected to experiments with two Treatments Chitosan and silver nanoparticles. The results showed that ,inhibition zone of K. pneumoniae increased progressively with increase the Chitosan and silver nanoparticles concentrations in reaching a optimum inhibition in 400 µg/ml, also silver and chitosan's nanoparticles exhibit high antibiofilm activity via plate method against (MDR) K. pneumoniae isolates with increasing concentrations of silver and chitosan's nanoparticles.

Keywords: *Inhibition zones, Antibacterial effect, Silver nanoparticles*

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INTRODUCTION

Infections of the Urinary Tract are regarded as one of the most serious illnesses, ranking second among bacterial infections in the medical community, and they are prevalent, according to estimates of the number of individuals afflicted. Around 150 million individuals every year" The cost-estimation technique for identifying and treating urinary tract infections is critical since the expenses are substantial, and laboratory testing is required to achieve a state of recovery [1]**.** Urinary tract infections are dependent on the virulence of the causative bacteria and the host's sensitivity, because the infection occurs in a person whose urinary tract is anatomically and naturally normal, as well as the possibility of bacteria ascending automatically from the urethra to the bladder and, in some cases, the kidney [2]**.** *K. pneumoniae* is a member of the *Enterobacteriaceae* family and is one of the most significant opportunistic pathogens causing nosocomial and community acquired infections [3]**.**

MATERIAL AND METHODS

Chemicals: preparation Chitosan and Silver Nanoparticles, according of [4] and [5]**.**

Silver and chitosan nanoparticles as antibacterial and Antibiofilm agents.

Evaluation of the silver nanoparticles efficiency in the growth inhibition of MDR *K. pneumoniae* isolates

Antibacterial effects of Silver NPs were examined using agar well diffusion methods against MDR *K. pneumoniae* isolates, four isolated colony of fresh culture that suspended in five milliliters of (BHIB) and incubate in 37°C for 3 h. The turbidity produced by growth culture was calibrated with sterilized broth to achieve an optical density comparable to the 0.5 McFarland requirements (1.5 X 108 cells/ml the equivalent).Sterile cotton swab was dipped in the suspensions. The dipping cotton swabs was used to streak the whole surface of a Mueller Hinton Agar tray. Then, using a sterile cork Pore, Pores (7 mm diameter) were created and filled with Silver NPs (150 ul) in four concentrations (100, 200,300 and 400)µg /ml , the diameter of inhibition zones was measured using a meter ruler [6]**.**

Evaluation of the chitosan nanoparticles efficiency on the growth inhibition of MDR *K. pneumoniae* isolates

This test is agreed in the same manner described in paragraph (2.2.1) excluding the use of chitosan nanoparticles .

Evaluation of the silver nanoparticles efficiency in the biofilm formation inhibition of MDR *K. pneumoniae*

Microtiter plate method was used for in *vitro* antibiofilm activity, four concentrations(100, 200, 300 and 400) µg/ml of silver NPs , 0.1ml of *K. pneumoniae* suspension having 0.5 O.D at 630nm have been inoculated in 1.9 ml BHIB broth medium, 150 ul of the cultured BHIB broth then transferred into each well of 96- well microtiter plate in use , an amount of 50ul of each 4X concentration was added to the corresponding wells to obtain the final concentrations , an amount of 50ul of BHIB broth was added one well corresponding to *K. pneumoniae* isolate used as control to confirm production of biofilm by bacteria and inhibition of Biofilm formation by silver NPs , an amount of 200µl of autoclaved distilled water was added in peripheral wells (to reduce the water loss) , Microtiter plate was incubated for 16 h at 37°C, Planktonic cells then aspirated, and fixed with 99% methanol , plates then Washed twice with phosphate buffer saline or sterile saline water and air-dried , about 200 µl of crystal violet solution (0.2%) then added to all wells , after 5 min, excess crystal violet was removed and washed twice , after that the plate was air dried and the cell bound crystal violet was dissolved in 33% acetic acid , The optical density (O.D.) at 630nm was recorded [7].

Evaluation of the chitosan nanoparticles efficiency in the biofilm formation inhibition of MDR *K.pneumonia*

This test is agreed in the same manner described in paragraph (2.2.3) excluding the use of chitosan nanoparticles .

RESULTS AND DISCUSSION

The effect of different concentrations of sliver nanoparticles against multidrug-resistant (MDR) *K. pneumoniae* isolates growth

The results indicated that inhibition zone of resistance *K. pneumoniae* isolates bigger progressively with increased the silver nanoparticles concentrations. *K. pneumoniae* has been reported to have the a finest inhibition zone (26 mm) at concentrations 400µg/ml picture (1).A additional of science reports suggests that the antibacterial mode of action of silver nanoparticles is similar to the antimicrobial effects of silver ions, due to the life cycle of silver nanoparticles and their change to silver ions [8], Silver ions have ability connection for the specific transporter proteins and enzymes residing in the microbial plasma membrane , the sequence of proteins and enzymes carriage electrons also concurrently transfer protons from the cytoplasm for the periplasmic area making a concentration decline called the proton motive force, the electron transport scheme above the plasma membrane is the main producer of ATP through aerobic breathing in microbes, in addition the manner is named chemiosmosis, the one place protons have ability diffuse into the cytoplasm again is through the ATP formation compound, which effects in the creation of ATP in a redox response amid ADP and inorganic phosphate, also the electrons reaching the ending electron acceptor place, the last electron acceptor is melted oxygen in the situation of aerobic respiration, also mainly results in water or in minor concentrations of cytotoxic reactive oxygen species, which specific enzymes method [9, 10].

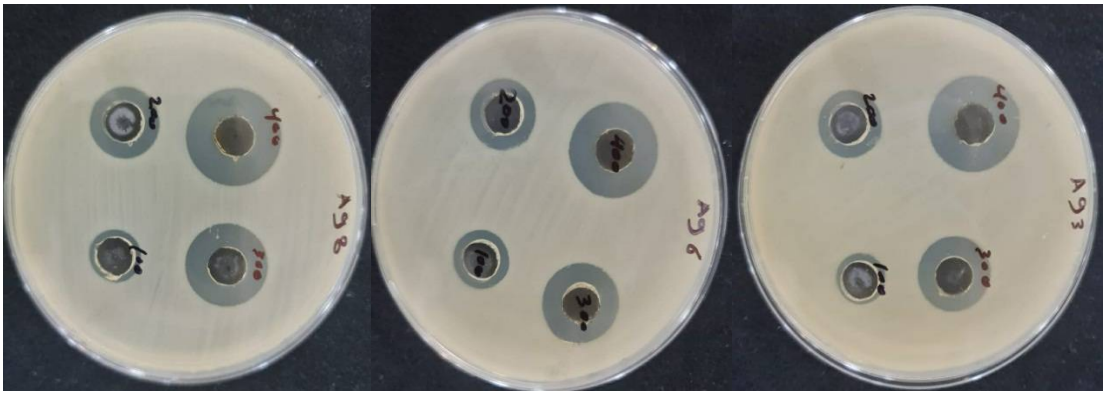

Figure (1) : Effect of different concentrations of silver nanoparticles (100,200,300 and 400) µg/ml on the growth of MDR *K. pneumonia*

Anti-biofilm activity of different concentrations of sliver nanoparticles MDR *K.pneumoniae* **isolates**.

The results showed that silver nanoparticles expressed high antibiofilm activity via plate method against MDR *K.pneumoniae* isolates using different concentrations of silver NPs (100 , 200 ,300 and 400) µg/ml Table [1] .

Several mechanisms proposed to interpret the variations in the extant of and antibiofilm activity of AgNPs: .Inhibition of biomass production, Inhibition of the gene expression of quorum sensing genes (QS genes), Inhibition of secretion of biofilm, alteration Biofilm structure and reduce adhesively**(11).**

Table 1: Antibiofilm activity of silver NPs at four concentrations by absorbance at 630 nm against MDR *K.pneumoniae* isolates

No. of *K.p.* isolates	Absorbance of silver NPs (µg/ml)			
	100	**200**	**300**	**400**
*K. p*1	0.395	0.275	0.175	0.087
*K. p*2	0.240	0.214	0.150	0.064
*K p*3	0.290	0.253	0.156	0.032
*K. p*4	0.380	0.324	0.159	0.047
*K p*5	0.362	0.286	0.236	0.181
*K. p*6	0.337	0.276	0.115	0.096
*K. p*7	0.295	0.253	0.125	0.086
*K. p*8	0.374	0.297	0.163	0.075
*K. p*9	0.394	0.361	0.173	0.062
*K. p*10	0.375	0.294	0.143	0.025
*K. p*11	0.311	0.253	0.135	0.042
*K. p*12	0.397	0.311	0.132	0.046
*K. p*13	0.285	0.233	0.126	0.051
*K. p*14	0.327	0.302	0.138	0.075
*K. p*15	0.284	0.256	0.114	0.063
*K. p*16	0.348	0.276	0.190	0.089
*K. p*17	0.368	0.321	0.244	0.132
*K. p*18	0.285	0.271	0.225	0.137
*K. p*19	0.311	0.295	0.266	0.183
*K. p*20	0.284	0.265	0.214	0.165

The effect of different concentrations of Chitosan nanoparticles against MDR *K.pneumoniae* isolates growth :

The results indicated that inhibition zone of MDR *K. pneumoniae* isolates bigger progressively with increase the chitosan's nanoparticles concentrations in reaching a maximum inhibition in 400 µg/ml.*K. pneumoniae*has been reported to have the higher inhibition zone (20 mm)atconcentrations400 µg/ml, figure (2).

The exact mechanism of antibacterial activity is yet to be fully understood. It is known that chitosan's antimicrobial activity is influenced by a number of factors that act in an orderly and independent fashion. The most prevalent proposed antibacterial activity of chitosan is by binding to the negatively charged bacterial cell wall causing disruption of the cell, thus altering the membrane permeability, followed by attachment to DNA causing inhibition of DNA replication and subsequently cell death [12]**.**

Another possible mechanism is that chitosan acts as a chelating agent that electively binds to trace metal elements causing toxin production and inhibiting microbial growth [13].

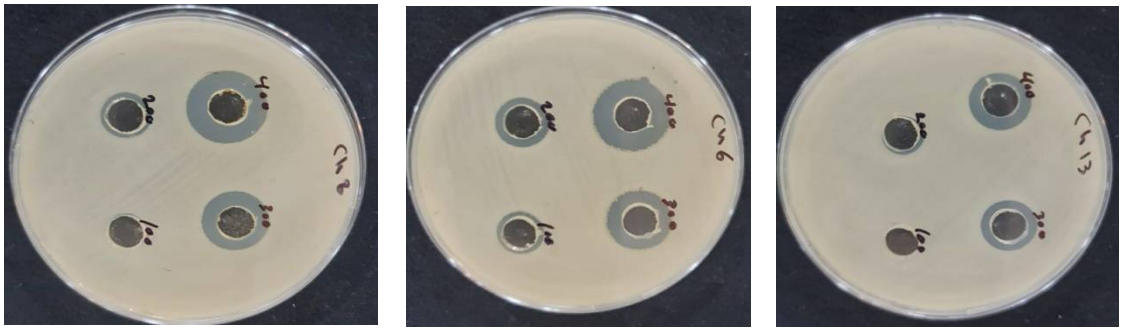

Figure (2) : Effect of different concentrations of chitosan's nanoparticles (100, 200, 300, 400) µg/ml on the growthMDR of *K. Pneumonia*

Anti-biofilm Activity of Chitosan NPs against MDR *K. Pneumoniae* isolates.

The results showed that chitosan NPs expressed high antibiofilm activity via plate method against *K. pneumoniae* isolates with increasing concentrations of chitosan NPs (100 ,200 ,300 and 400) µg/ml Table (2).

According to some reports, the mechanism of the chitosan NPs anti-biofilm property is mainly attributed to the polycationic nature of the N-acetylglucosamine unit's functional amino groups , in low pH settings, grants chitosan biological activity, namely through interaction with the negative biofilm elements such as proteins, polysaccharides , phospholipids and DNA, which inhibit bacterial biofilm [14].

Chitosan NPs was mixed with other substances in experiments to determine its efficacy in preventing and treating biofilm formation , The antibiofilm effects of chitosan on *Bacillus cereus, Staphylococcus aureus, Salmonella enterica* and *Cryptococcus neoformans* have been investigated [15] .

Table(2): Antibiofilm activity of chitosan NPs at four concentrations by absorbance at 630 nm against *K. pneumoniae* isolates

No.of *K. p.*isolates	Absorbance of chitosan NPs			
	100	**200**	**300**	**400**
*K.p*1	0.345	0.228	0.168	0.065
*Kp*2	0.340	0.211	0.150	0.055
*K.p*3	0.382	0.253	0.155	0.031
*K.p*4	0.382	0.323	0.156	0.032
*K.p*5	0.363	0.289	0.238	0.185
*K.p*6	0.337	0.275	0.111	0.091
*K.p*7	0.298	0.251	0.124	0.087
*K.p*8	0.371	0.298	0.165	0.077
*K.p*9	0.366	0.232	0.175	0.064
*K.p*10	0.335	0.251	0.125	0.020
*K.p*11	0.310	0.251	0.131	0.041
*K.p*12	0.345	0.212	0.131	0.047
*Kp*13	0.365	0.212	0.116	0.030
*K.p*14	0.310	0.203	0.110	0.042
*K.p*15	0.264	0.232	0.111	0.031
*K.p*16	0.320	0.222	0.130	0.049
*K.p*17	0.323	0.315	0.222	0.110
*K.p*18	0.255	0.232	0.213	0.113
*K.p*19	0.310	0.233	0.232	0.152
*K.p*20	0.344	0.211	0.111	0.020

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