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RESEARCH PAPER

Antibacterial activity of nickel and nickel hydroxide nanoparticles against multidrug resistance *K. pneumonia and E. coli* isolated urinary tract

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ABSTRACT

Objective(s): Antibacterial materials are so significant in the textile industry, water disinfection, medicine, and food packaging. Unfortunately, organic compounds for sterilization show toxicity to the human body; therefore, the interest in inorganic disinfectants such as metal oxide nanoparticles (NPs) is increasing.

Materials and Methods: Nickel and nickel hydroxide nanoparticles (NiNPs and Ni(OH)2-NPs) were prepared and characterized by DLS, SEM, AFM and ATR. Antibacterial activity assay was carried by Spot on lawn method against two selected standard pathogenic bacteria such as E. coli (as Gram negative), S. aureus (as Gram positive) and multidrug resistance K. pneumonia and E. coli.

Also the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined against two selected standard pathogenic bacteria and multidrug resistance K. pneumonia and E. coli.

Results: The formation of the NiNPs and $Ni(OH)_2$ NPs were confirmed by DLS, SEM, AFM and ATR. Antibacterial activity of nanoparticles were confirmed against two selected standard pathogenic bacteria such as E. coli and S. aureus. And also, NiNPs and $Ni(OH)_2$-NPs revealed fair antibacterial effect against multidrug resistance K. pneumonia and E. coli based on MIC and MBC data. As well, the experimental data presented that the antibacterial activity of NiNPs was more than $Ni(OH)_2$-NPs.

Conclusion: Based on the achieved results, NiNPs and $Ni(OH)_2$-NPs show antibacterial activity against clinical patients bacteria (multidrug resistance K. pneumonia and E. coli(. Finally, the NPs evaluated in this study have promising properties for applications as antiseptic agent for environment; however, further studies are warranted such as study toxicity NPs on normal human cell line and other clinical bacteria.

Keywords: *Antibacteial, NiNPs, $Ni(OH)_2$, Nanoparticles, Pathogen*

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INTRODUCTION

Antibacterial activity means either killing bacteria or slowing down their growth which are named as bactericidal or bacteriostatic respectively. It should be non-toxic to surrounding tissue [1]. Antibacterial agents are very useful in water disinfection, textile industry, medicine, and food packaging. Antibacterial compounds are divided into natural and chemically modified natural compounds. However, the development of bacterial resistance to antibacterial compounds is increasing and becoming a major problem [2]. Therefore, development of alternative compounds to treat bacterial diseases is necessary [3].

Nanoparticles (NPs) as a new alternative for antimicrobial compounds have developed. The

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main characteristic of NPs is high surface area to volume ratio which causes new properties such as chemical, mechanical, electrical, optical, and magnetic as different from their bulk properties [4]. In this case, NPs attracted the interest of Scientifics to apply them for combating bacteria [5]. The precise mechanisms of NP toxicity on the various bacteria have not been understood entirely. As it obvious, NPs would attach to the bacteria membrane by electrostatic interaction and disrupt the integrity of the bacterial membrane [6]. NPs with antibacterial activity included metallic and metal oxide NPs, semiconductors, polymers, and carbon-based materials against gram-positive and gram-negative bacteria [7]. Some NPs such as NiO nanoplates show good zone of inhibition against *B. subtilis, S. aureus, E. coli* and *P. vulgaris* [8]. And also NiNPs was reported to show potential antimicrobial activity against *E. coli, K. pneumoniae*, (Gram negative) *B. cereus and S. aureus* (Gram positive) [9]. Therefore, NiNPs and $Ni(OH)_2$-NPs have been revealed antibacterial activity. Recent data from the U.S. National Healthcare Safety Network indicate that gram-negative bacteria are responsible for more than 30% of hospital-acquired infections. Also, U. S. data indicate that *E. coli* is the most common etiologic gram-negative organism, followed in descending order of frequency by *P. aeruginosa*, klebsiella species, enterobacter species, and *A. baumannii* [10]. There is no report in calculating two significant parameters as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Finally, it is important to note that until now, the antimicrobial properties NiNPs and $Ni(OH)_2$-NPs have not been studied on multidrug resistance *K. pneumonia and E. coli*. Therefore, the purposes of this study were the synthesis and characterization of NiNPs and $Ni(OH)_2$-NPs as well as investigate their antimicrobial activity against two selected standard pathogenic bacteria and real clinical multidrug resistance *K. pneumonia and E. coli*.

MATERIALS AND METHODS

Materials and apparatus

The materials were purchased and used as received without any further treatment. All materials including Nickel chloride ($NiCl_2 6H_2O$), sodium hydroxide (NaOH) ,polyvinilprolydon (PVP), ethanol (99%), H_2O_2 (30%), phenol, 4-Aminoantipyrine (4-AAP), trypic soy agar (TSA), trypic soy broth (TSB) were obtained from Merck (Germany). Hydrazine hydrate ($N_2H_4.H_2O$), 98%, was purchased from Roialex (India). Tetracycline (TE), ampicillin (AM) and medium were obtained from Sigma. The pH measurements were carried out with a pH meter Elmeiron model CP-501. Dynamic light scattering (DLS) (Brookhaven, USA) were used to measure size the nanoparticles. The Surface properties of the NiNPs were recorded using atomic force microscopy (AFM, Nanosurf easyScan, Swiss) in semi contact mode through silicon tip. SEM image of $Ni(OH)_2$-NPs were obtained by SEM (Phenom ProX, Netherlands). Attenuated total reflection (ATR) of nanoparticles was carried out using a Perkin-Elmer UATR Two (USA). All solutions were prepared in DI water [11, 12].

Table 1. The bacteria strains and groups of 8 real sample from clinical pathogens

Code	Strain	Group
1	*K. pneumoniae*	PDR
2	*K. pneumoniae*	XDR
3	*K. pneumoniae*	MDR
4	*K. pneumoniae*	NDR
5	*E. coli*	PDR
6	*E. coli*	XDR
7	*E. coli*	MDR
8	*E. coli*	NDR

Synthesis of NiNPs

The NiNPs were synthesized by reduction of $NiCl_2.6H_2O$ in an aqueous solution with hydrazine hydrate acting as the reductant [13]. Briefly, 0.5 g $NiCl_2.6H_2O$ was dissolved in 60mL distilled water. In a separate beaker, 0.2 g of PVP dissolved in 3mL of absolute ethanol and then added to 1.0 g of NaOH in 20mL DI water and then 20mL of hydrazine hydrate. This hydrazine hydrate solution then added to nickel solution. Subsequently, the combined solution stirred for 2 minutes until royal blue color appears, in a pH ~12.5. According to Chen and Zhou`s method, the reaction temperature needs to stay below 70 ^{0}C in order to prepare ultrafine NiNPs. The solution was sonicated for 15 minutes to ~55 ^{0}C, allowed to sit particles for 15 minutes in order to control the temperature, and then sonicated again for 15 minutes to ~65 ^{0}C, to activate the precipitation of the NiNPs. During the activation period, the solution initially became grey and coated along the side and bottom of the beaker and then precipitated out to the bottom as a black powder.

Table 2. Inhibition zone diameters of NiNPs at different concentration in presence of E. coli , S. aureus and 8 clinical pathogenic bacteria (based on Table 1) culture. Tetracycline (TE) antibiotic was used as positive control

NiNPs (µl)	*S. aureus* (mm)	*E. coli* (mm)	*1* (mm)	*2* (mm)	*3* (mm)	*4* (mm)	*5* (mm)	*6* (mm)	*7* (mm)	*8* (mm)
TE	20	13	-	-	-	-	-	-	-	-
2.5	12	23	15	13	14	14	12	15	23	16
5	20	30	19	16	15	17	18	19	31	23
10	22	35	24	18	16	21	21	24	37	25
20	30	36	27	26	20	23	24	27	45	29
40	32	40	29	28	22	25	20<	20<	20<	20<
60	34	40	33	31	30	30	20<	20<	20<	20<
80	35	46	40	36	35	35	20<	20<	20<	20<
100	37	47	50	40	39	40	20<	20<	20<	20<

Table 3. Inhibition zone diameters of Ni(OH)2-NPs at different concentration in presence of E. coli , S. aureus and 8 clinical pathogenic bacteria (based on Table 1) culture. Ampicillin (AM) antibiotic was used as positive control

$Ni(OH)_2$-NPs (µl)	*S. aureus*	*E. coli*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
AM	0	0	-	-	10	10	12	-	-	12
2.5	-	8	5	-	-	-	12	-	7	9
5	8	10	10	7	-	-	15	-	8	10
10	10	11	11	11	9	11	17	10	13	11
20	11	12	12	15	11	12	19	12	15	15
40	12	13	15	18	13	15	20	13	21	17
60	15	14	17	19	14	16	21	15	22	18
80	18	16	18	20	15	17	23	17	25	22
100	22	18	19	20	18	19	25	19	27	24

The nickel powder solution was centrifuged and washed with DI water and was allowed to dry at room temperature.

Synthesis of $Ni(OH)_2$-NPs

The synthesis of $Ni(OH)_2$-NPs was done base on previously reported method [13]. The $Ni(OH)_2$-NPs was prepared by two steps: firstly, solution A was prepared by dissolving 1.19 g $NiCl_2 6H_2O$ in 50mL DI water; then 200mL of absolute ethanol was added to it, forming a green solution. The solution was transferred into a 500mL conical flask. Secondly, solution B was prepared in a 200mL volumetric flask by dissolving 0.4 g of sodium hydroxide in 100mL of DI water; 1.5mL of hydrazine hydrate was added and then diluted up to 200 ml with DI water. Subsequently, solution B was slowly dropped into solution A at a rate of ~0.2mL per minute and mixed with a magnetic stirrer. The resulting solution became cloudy green with precipitate forming as soon as the pH reached 6.8 and then turned to a cloudy blue green. The mixed solution finally became a grey blue with the addition of 80–90mL of solution B at a final pH of ~10. The solution was sonicated for 15 minutes to 55 ^{0}C, allowed to sit for 15 minutes in order to control the temperature, and then sonicated again for 15 minutes to ~ 65^{0}C. The precipitated solution was centrifuged. The Nickel Oxide powder solution was centrifuged and washed with DI water and was allowed to dry at room.

Preparation of nanoparticle suspensions

The synthesized Ni and $Ni(OH)_2$ NPs were added to 1mL of double distilled water to obtain a final concentration of 20 mg/ml and shaken vigorously and dispersed by ultrasonication (BANDELIN, Germany).

Spot on the lawn test

The Antimicrobial propertices of NiNPs and $Ni(OH)_2$-NPs were investigated using *E.*

coli (*PTCC1394*as gram negative) and *S. aureus* (*PTCC1431* as gram positive) and 8 clinical multidrug resistance K. pneumonia and E. coli (Isolated urinary tract, Milad Laboratory, Yazd, Iran, Table 4) by the spot on the lawn method [14]. The bacterial suspension was rubbed uniformly on the surface of a Tryptic Soy Agar (TSA, Merck, Germany) plate at a concentration of 10^7 CFU/ml. For antibacterial investigation, Antibacterial activity of NiNPs and $Ni(OH)_2$-NPs were tested by spotting from 2.5 to 100 µl (presented in Table 1 and 2) of nanoparticle suspensions (20 mg/ml) on TAS plate seeded with 10^7 cell/mL *E. coli* , *S. aureus* and 8 clinical pathogenic samples respectively. Antibiotic disks including Tetracycline (TE) and Ampicillin (AM) were used as positive control for to comparing the inhibition of growth of bacteria with NiNPs and $Ni(OH)_2$-NPs respectively. For negative control water was used. The control and sample plates were incubated at 37°c for 24h, after which the average diameter of the inhibition zone surrounding the spot was measured with a ruler.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

The MIC is defined as the minimum inhibitory concentration of antibacterial material sufficient to prevent bacterial growth in vitro. The MICs were determined using the broth dilution technique as recommended by CLSI. Briefly, serial dilutions of Ni and Ni $(OH)_2$-NPs (13- 0.5 of mg/ml) in TSB (Trypic Soy Broth, Merck, Germany) were prepared. Bacterial suspensions were then added to each tube to achieve final inoculums of 8 logs CFU/mL. Tubes containing growth media alone or bacterial culture without Ni and $Ni(OH)_2$-NPs suspension were included as negative and positive controls, respectively. The cultures were inspected for bacterial growth after incubation at 37°C for 24 h and the MICs of Ni and $Ni(OH)_2$-NPs suspension were recorded by measuring optical density at 620 nm in a spectrophotometer. The amount of NPs that lead to growth inhibition of bacteria was regarded as MIC.

The MBC (*minimum bactericidal concentration*) was defined as the lowest concentration of Ni and Ni $(OH)_2$-NPs suspension that resulted in more than 99.9% reduction of the initial inoculums. MBC was assessed by sub-culturing 50 µl on to TSA, from each tube in which no growth was viewable. After incubation for 24 h at 37°C, the number of grown colonies will be enumerated. The growth of one colony showed a 99.9% fall for in viable numeration [15].

RESULTS

Characterizations of NiNPs and Ni $(OH)_2$-NPs

The formation of the NiNPs was confirmed by studying two dimensional AFM image. The AFM image for NiNPs at glass plate, is shown in Fig 1. SEM image of Ni $(OH)_2$-NPs is presented in Fig 2. ATR would be useful for investigation of the synthesized NiNPs and $Ni(OH)_2$-NPs. ATR spectra for NiNPs in KBr disc and that gotten for free PVP were compared and shown in Fig. 3.

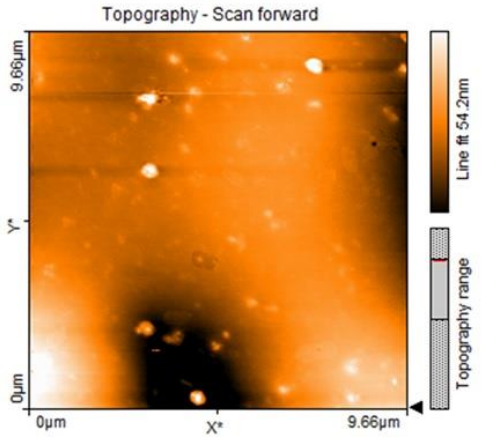

Fig. 1. AFM image of NiNPs

The ATR spectrum of and Ni $(OH)_2$-NPs was illustrated in Fig. 5. The ATR results showed a strong peak at 1654 cm^{-1} which is related for the carbonyl stretch of pure PVP. The C=O stretch was made weaker and also red shifts from pure

Table 4. Minimum bactericidal and inhibitory concentrations (MIC and MBCs) for Ni and Ni(OH)2 NPs on E. coli , S. aureus and 8 clinical pathogenic (based on Table 1) bacteria

Nanoparticles	*E. coli PTCC1394*		*S. aureus PTCC1431*		*8 clinical pathogenic samples*	
	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)
NiNPs	3.25	1.62	1.62	0.81	1.75	0.75
$Ni(OH)_2$-NPs	6.5	5.85	13	6.5	6.5	3.25

PVP to 1581 cm^{-1} as nickel is protected with PVP. Additionally the peaks around 1280 to 1180 cm^{-1} indicated the C–N stretch in PVP. As it could be seen, the peaks are strong with three different peaks for each of the C–N bonds in pure PVP. But as NiNPs was synthesized, this peak became weaker in the region around 1214 cm^{-1}. Hence, these changes in ATR spectra revealed that NiNPs synthesized by PVP (16).

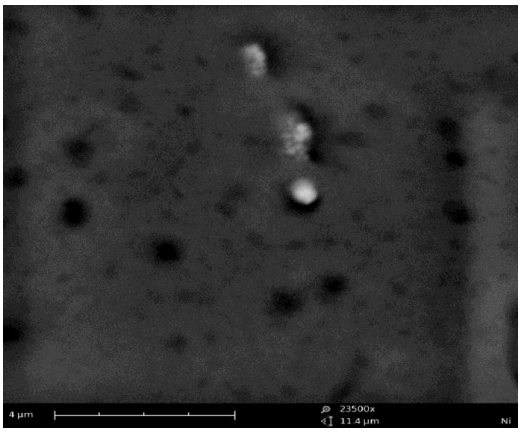

Fig 2. SEM image of Ni(OH)2-NPs

The ATR spectrum of Ni $(OH)_2$-NPs in Fig 4 was demonstrated a hydroxyl stretching band related to $Ni(OH)_2$ lattice at 3300 cm^{-1}. Also, an H–O–H bend is observed at 1635 cm^{-1} from the vibration of free water molecules. The spectrum also shows a sharp O–H stretch at 608 cm^{-1} from the hydroxyl lattice vibration and a weak peak around 480 cm^{-1} indicating a Ni–O lattice vibration [13]. As a whole, based on data obtained from ATR spectra for NiNPs and Ni $(OH)_2$-NPs and compared in literature, it would be resulted that NiNPs and $Ni(OH)_2$-NPs were synthesized completely.

DLS technique was applied for measuring the average sizes of NiNPs and Ni $(OH)_2$-NPs. The results showed in Fig 5. It can be seen that the average diameter for in deionized water were 5 and 75 nm respectively.

Antibacterial assay based on spot on the lawn method

The antibacterial activity of nanoparticles was taken on the basis of diameter of zone of inhibition of bacterial growth which was measured at cross-angles after 24hr of incubation. The results are shown in Tables 2 and 3 illustrated the inhibition of the *E. coli, S. aureus and* 8 clinical multidrug resistance *K. pneumonia and E. coli* on the agar plates by using different concentrations of NiNPs and $Ni(OH)_2$-NPs respectively. As it shown, in the presence of NPs the zone of inhibition of bacterial growth was increased which is related to increasing antibacterial activity. Based on decreasing the bacterial growth in present of nanoparticle, it would be concluded that these nanoparticles show antibacterial activity on *E. coli*, *S. aureus* and 8 clinical multidrug resistances *K.*

pneumonia and E. coli

MBC and MIC assay

MBC and MIC concentration which is related to power of nanoparticles to settle down the *E. coli* , *S. aureus* and 8 clinical multidrug resistance *K. pneumonia and E. coli* growth determined and demonstrated in Table 4. It could be seen that the lower concentration of NiNPs is enough for prevention *S. aureus* bacterial growth than *E. coli*. But for $Ni(OH)_2$-NPs almost the same concentration was needed for inhibiting the *E. coli* and *S. aureus* bacteria growth.

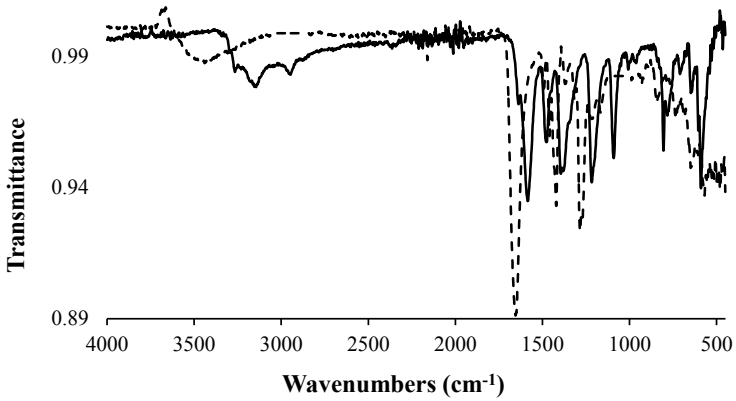

Fig 3. ATR absorption spectra of NiNPs (solid line) and PVP (dotted line line)

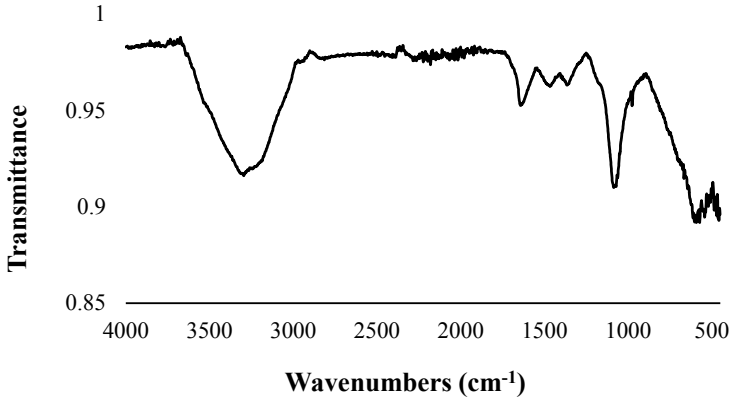

Fig 4. ATR absorption spectrum of Ni(OH)2-NPs

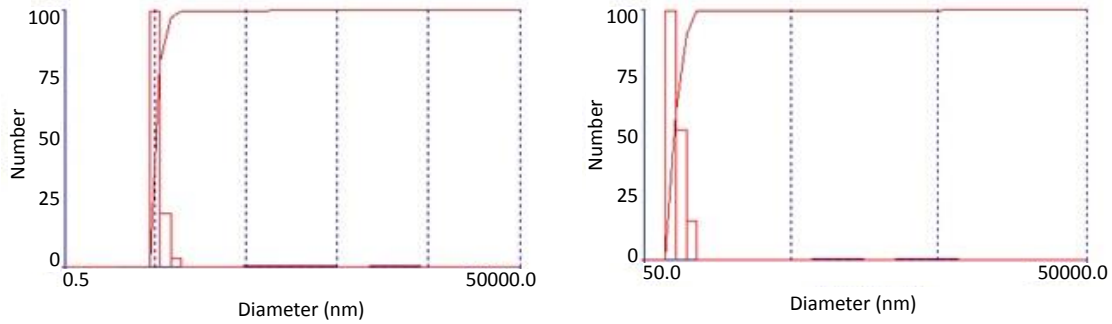

Fig 5. Size distribution of NiNPs (A) and Ni(OH)2-NPs (B). The poly disparity are 0.475 and 0.368 respectively

These data confirm that NiNPs and $Ni(OH)_2$-NPs presence to have an antimicrobial activity on *E. coli* and *S. aureus*. Among the NiNPs and $Ni(OH)_2$-NPs , the treatment of NiNPs was the most effective in inhibition of the *E. coli* and *S. aureus* bacteria growth. Additionally, MBC and MIC concentration for 8 clinical multidrug resistance *K. pneumonia and E. coli* showed that NiNPs and $Ni(OH)_2$-NPs have good antibacterial activity against four groups as categorized: sensitive (NDR), sensitive (MDR), resistant (XDR) and completely resistant (PDR).

DISCUSSION

NiNPs and $Ni(OH)_2$-NPs were prepared and characterized by DLS and spectroscopic technique. Experimental investigations have shown encouraging results about antibacterial activity of NiNPs and $Ni(OH)_2$-NPs on *E. coli* and *S. aureus* bacteria. The inhibition zone diameters of bacterial growth and MBCs and MIC parameters determined. Furthermore, 8 clinical bacteria from two different bacteria strain as *E. coli* and *K. pneumoniae* in 4 groups such as sensitive (NDR), semi-sensitive (MDR), resistant (XDR) and completely resistant (PDR) was investigated for confirming antibacterial activity of NiNPs and $Ni(OH)_2$-NPs. As reported in literature, NiNPs (20-25nm diameter) showed antibacterial activity against gram positive and negative bacteria. And also the MBC factor was 100 $mg.mL^{-1}$ but the MCI factor has not been determined yet [18]. In another study the effect of NiNPs (25-100 nm) on different bacteria and fungi was investigated. These studies showed the inhibition zone diameters of bacterial growth by NiNPs against *E. coli* and *K. pneumoniae* was 15.3-25.1 and 9.5-23.3 mm respectively. And also, the antibacterial effect of NiNPs was approved based on MBC and MIC parameters. The MBC parameter was determined for NiNPs against *E. coli* and *K. pneumoniae* were 25±0.3 and 25±2.8 $mg.mL^{-1}$. And the MIC parameters were 25±2.4 and 25±0.7 $mg.mL^{-1}$ for *E. coli* and *K. pneumoniae* respectively [19]. Santhoshkumar et al. reported

the antibacterial activity of $Ni(OH)_2$-NPs (96 nm in diameter) against *K. pneumoniae*. The results showed that inhibition zone diameters of bacterial growth was 23 mm [20]. Up to now there is no report for calculating two significant parameters as MIC and MBC for NiNPs and $Ni(OH)_2$-NPs. In this study we reported inhibition zone diameters of bacteria growth, MIC and MBC parameters for two standard gram negative and positive bacteria as *E. coli* and *S. aureus* bacteria respectively. The MBC parameter for NiNPs and $Ni(OH)_2$-NPs against *E. coli* were 3.25 $mg.mL^{-1}$ and 6.5 $mg.mL^{-1}$ respectively. These results demonstrated that the MBC parameter was so lower compare to literature. Additionally the MIC parameter for *E. coli* was lower compare to literature too. It would be concluded that the antibacterial effect of nanoparticles depends on their size and morphology. Since, in this study the size of NiNPs and was 5 nm which was lower than reported in literature.

And for confirming the antibacterial activity of NiNPs and $Ni(OH)_2$-NPs, 8 pathogenic clinical were tested. For these clinical pathogens three parameters as inhibition zone diameters of bacterial growth, MBCs and MIC showed that NiNPs and $Ni(OH)_2$-NPs possess good antibacterial activity.

CONCLUSION

NiNPs and $Ni(OH)_2$-NPs were synthesized and characterized by AFM, SEM, ATR, DLS. NiNPs and $Ni(OH)_2$-NPs show an antibacterial activity against *S. aureus*, *E. coli* and 8 clinical bacteria from two different bacteria strain as *E. coli* and *K. pneumoniae* in 4 groups such as NDR, MDR, XDR and PDR. The recommended daily amounts of nickel are not fixed, but it is suggested that it is enough to intake about 100 micrograms per day. However, some studies show that it can be consumed daily about 200-750 micrograms of nickel (21). So, this study reveals the potential of NiNPs and $Ni(OH)_2$-NPs as an antimicrobial agent at as antimicrobial coatings on surface of materials for various environmental and as the antiseptic agent for environment.

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CONFLICT OF INTEREST

Author has no received research grants. The author declares that he has no conflict of interest.

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