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Antibacterial Activities of Selenium and Selenium Nano-particles (Products from *Lactobacillus acidophilus*) on Nosocomial Strains Resistant to Antibiotics

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Nosocomial infections are the major concern in the world. Today, with the emergence of antibiotic resistance among pathogenic bacteria, treatment of this category of infectious diseases is facing numerous problems. The present study investigation of antibacterial activities selenium and selenium nano-particles (synthesized by *Lactobacillus acidophilus*). *Lactobacillus acidophilus and Lactobacillus plantarum were* cultured in (MRS) broth medium. The 3-5% of inoculums culture and 10 ml of stock solution of selenium ($NaHSeO_3$) separately were added into two Erlenmeyer flasks containing (MRS) broth medium. The nano-particles were analyzed using scanning electron microscopy (SEM). In this study, the urine, blood, sputum and wound specimens of patients were collected. The isolates were identified and confirmed using the standard microbiological method and 50 antibiotic resistance strains from 436 samples were selected (80% *Escherichia coli* and 20% *Acinetobacter*). Antibacterial activities selenium and selenium nanoparticles on isolated bacterial strains were evaluated by disk diffusion and serial broth dilution methods to determine (MIC) and (MBC). The SEM results showed that *L.acidophilus* nano-particles are smaller than of *L. plantarum*, therefore it would be preferred for further investigation. The antibacterial activities of the selenium and selenium nano-particles demonstrated to be effective on 32% and 56% of the strains studied, respectively.

Key word: Nosocomial infections; antibiotics resistant bacteria; *Lactobacillus acidophilus; Lactobacillus plantarum;* Selenium; Selenium nano-particles.

Nosocomial infections are the major concern in the world. Since the bacteria discovery, human beings have always sought effective medicine against their infections, while bacteria found effective mechanisms to reject antibiotics. Today, with the emergence of antibiotic resistance among pathogenic bacteria, treatment of this category of infectious diseases is facing numerous problems[1,2] .

There are organisms that resists to the ordinary antibiotics. Long-term hospitalization of patients and the using devices such as urinary catheters and intravascular are other factors of increased drug resistance pattern in hospitals[3]. Some of bacteria with resistance genes can be noted are *Escherichia coli*, *Acinetobacter sp.*, *Klebsiella sp.*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Staphylococcus aureus*. *E. coli* is the

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most common bacterial causes of urinary tract, sepsis, wound infections, gastroenteritis and neonatal meningitis[3.4] . It is one of the most opportunistic pathogens in hospitals and due to the acquisition of the plasmids encoding, broad-spectrum beta lactamases have been resistant to beta-lactam antibiotics[5] . For this reason, treatments of infections caused by *E. coli* are difficult, and so *Acinetobacter.*sp has great potential for rapid development of antibiotic resistance which has led to multi-drug resistances[5.6]. *These* species are coccobacilli gram negative, aerobic, non-fermented and have been widely distributed in hospitals and are important opportunistic pathogens responsible for hospital infections[6]. In recent years using special abilities of microorganisms for the synthesis of nanoparticles of metal salts have drawn the attention of researchers[7].

The physical and chemical methods of nano-particle production in terms of energy and materials consumption are ineffective and result in low yield. On the other hand , biological synthesis techniques, have created special interest, due to high compatibility with the environment and reduction in energy consumption and costs[8].

Bacterial cells are constantly exposed to stress and they are still able to survive in this condition. Ability to grow microorganisms in the presence of high concentrations of metals may be due to specific mechanisms of resistance[9]. These mechanisms include the release system (efflux system), change in solubility and toxicity by changing the redox potential of metal ions, extracellular composition and sedimentation and imperfection in the transmission system of special metals[9.10]. Many bacteria have the ability to synthesize nanoparticles either extracellular or intracellular. Lactic acid bacteria such as Lactobacillus which are from probiotic family have also the ability to produce nano-particles with various sizes[11]. Many elements such as selenium in low concentrations are essential to the growth of micro-organisms and organisms, but are toxic in high concentrations[11].Micro-organisms including bacteria such as (*Lactobacillus, Bacillus and, E.coli)*For reducing the toxic effects, are able to produce metal nano-particles, such as nano-selenium That in the Lactobacillus bacteria has been observed that sediment nano-particles in intracellular form[12]. On the other hand, selenium can be used as an antimicrobial agent for control of bacterial infections resistant to due to its high anti-microbial properties especially in the form of nano particles in low dimensions[13.14.15].

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Lactobacillus acidophilus (ATCC10711) and *Lactobacillus plantarum* (ATCC10817) were originally obtained from American Type Culture Collection (ATCC). Bacteria strains were cultured in MRS broth medium (Proteose peptone 10g, Beef extract 10g, Yeast extract 5g, Dextrose 20g, Polysorbate 80; 1g, Ammonium citrate 2g, Sodium acetate 5g, Magnesium sulphate 0.1g, Manganese sulphate 0.05, Dipotassium phosphate 2g, Agar 12g, distilled water 1000ml and incubated at 37°C in 50rpm shaking condition for 24-72 hours.

Preparing Basic Solution of Selenium

In order to provide a basic solution of selenium, 10mg sodium hydrogen selenite was dissolvedin 1000ml dionized water to obtain the stock solution. Then 10 ml of this solution was added to 490 ml of the sterilize 200mg/l MRS broth (11).

Bacterial Inoculum Cultures

Lactobacillus acidophilus (ATCC10711) and *Lactobacillus plantarum* (ATTC10817) inoculums culture was performed in MRS broth medium and incubated at 37°C for 48-72h. Bacterial cell density was adjusted to 0.257 at 600nm (equal to 3×108 CFU/ml) by UV-VIS scan-ning spectrophotometer, UV 2101pc, Shimadzu. Then 3-5% of this inoculums culture -and 10 ml of basic solution of selenium separately were added into two Erlenmeyer- flasks containing MRS broth medium, incubated for 24- 72 at 37°C 50rpm shaking condition. At the end of the fermentation process, the culture medium became red. Subsequently, the medium was centrifuged at 11rpm for 15-20 minutes and the supernatant was discarded. The sediment was suspended in the purified water. In lactic acid bacterium, the mechanism of formation of selenium element is mainly intracellular; therefore; the cells have to be digested with high concentration of an acid such as HCl in order to in order to release the nanoparticle from bacteria. The solution was prepared concentrated HCl (37%) and selenium

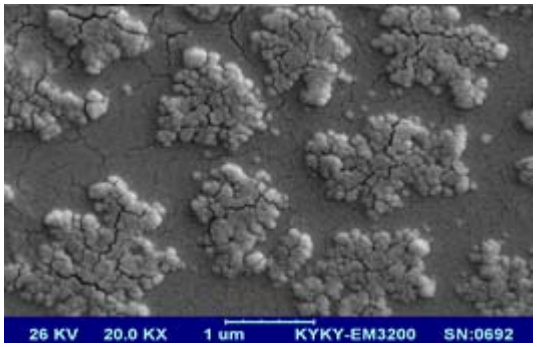
nanoparticles (1.5:1V/V).The acidic hydrolysis took an approximate six days at room temperature. Then the sample was centrifuged at 10rpm for 15minutes and sedimentary was washed many times with purified water until its pH reached to neutral, followed by ultrasonication for 15-20 minutes in order to disintegrate the cohesive selenium spheres. The nanoparticles produced were visualized using scanning electron microscope (SEM).

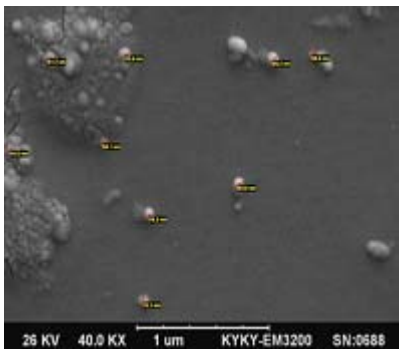
Specimen Collection and Isolation of Bacteria

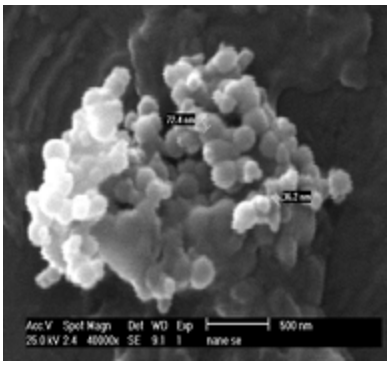
The urine, blood, sputum and wound specimens collected from patients, were cultured on 5% blood agar, Mac Conkey, and Eosin-

a) MRS broth culture with 10ml of selenium-based solution includes, (NaHSeO3), and inoculated strains of *L. acidophilus* and *L. plantarum* is equal to half McFarland before incubation b) Selenium nanoparticles production and color change after 48-72 hours incubation at 37 °C medium with 50rpm in MRS broth culture.

Fig. 1. Synthesis of selenium nanoparticles by two strains of *L.acidophilus* and *L. plantarum*, including the change of the medium color







a) Far view of selenium nano-particles produced by *L.acidophlilus* activity
b) Far view of selenium nano-particles produced by *L.plantarum*
c) Selenium nano-particles (40KX)

Fig. 2. Images of selenium nano-particles by electron microscope (SEM) (26kV, 20KX)

Table 1. Resistant strains of bacteria isolated from hospitalized patients samples and reports of types of resistant antibiotics

S. No	Bacterial detection form	Baeteria	Antibictics			Antibictics					
1	urine	*E .coli*	NAL30	CO . 10	PI+Tz	CIPR5	CTX30	CTR30	CFM5	NI300	CFO30
2	urine	*E .coli*	CTX30	CO . 10	CFO30	NAL30	PI+TZ	CIPR5	CTR30	CFM5	NI300
3	urine	*E .coli*	IMI10	CO . 10	AMI30	CTR30	CTX30	CFM5	SXT25	*	*
4	urine	*E .coli*	AMI30	IMI10	CFO30	MRP10	SXT25	NI30	CAZ30	*	*
5	urine	*E .coli*	SXT25	IMI10	CIPR5	PI+TZ	CFO30	CAZ30	AMI30	*	*
6	urine	*E .coli*	CIPR5	FEP30	MRP10	CFM5	AMI30	CF030	CO .10	*	*
7	Blood	*Acineto-bacter*	CB100	FEP30	PI+TZ	GEN10	CFM5	CAZ30	CFO30	MRP10	*
8	urine	*E .coli*	CTX30	CTR30	MRP10	CO.10	MRP10	CFM5	NI300	*	*
9	urine	*E .coli*	AMP10	CTX30	NAL30	CTPR5	CFM5	AMI30	NI300	*	*
10	urine	*E .coli*	CFO30	IMI10	CAZ30	MRP10	AMI30	NI300	CFM5	*	*
11	urine	*E .coli*	CO.10	SXT25	NAL30	NI300	CAZ30	CFO30	CTR30	*	*
12	urine	*E .coli*	CIPR5	CTR30	PI+TZ	CAZ30	IMI10	CTX30	*	*	*
13	Urine	*E .coli*	CTX30	FEP30	PI+TZ	CTR30	SXT25	CTR30	CAZ30	*	*
14	urine	*E .coli*	CIPR5	CFO30	PI+TZ	MRP10	CO.10	IMI10	*	*	*
15	urine	*E .coli*	CIPR5	CAZ30	MRP10	CFM5	CTR30	PI+TZ	*	*	*
16	urine	*E .coli*	CIPR5	DOX30	IMI10	CO.10	AMI30	FEP30	MRP10	*	*
17	urine	*E .coli*	AMI30	FEP30	CTX30	SXT25	CTR30	PI+TZ	CAZ30	*	*
18	urine	*E .coli*	MRP10	CFM5	CTR30	CAZ30	PI+TZ	CIPR5	*	*	*
19	sputum	*Acineto-bacter*	CFM5	MRP10	CTR30	TET30	CB100	CO.10	FEP30	SXT25	CTX30
20	sputum	*Acineto-bacter*	CB100	CO . 10	FEP30	AMI30	CTX30	SXT25	CFO30	IMI10	CEGN10
21	urine	*E .coli*	CIPR5	IMI10	CAZ30	PI+TZ	CTR30	CIPR5	CTX30	*	*
22	urine	*E .coli*	FEP30	AMI30	SXT25	GB100	CO.10	CFM5	MRP10	GEN10	*
23	urine	*E .coli*	CFO30	CIPR5	IMI10	MRP10	CO.10	PI+TZ	*	*	*
24	urine	*E .coli*	CTR30	NI300	CTX30	PI+TZ	AMI30	SXT25	CTR30	*	*
25	urine	*E .coli*	MRP10	NAL30	CAZ30	CIPR5	CFM5	FEP30	IMI10	CFO30	
26	wound	*Acineto-bacter*	FEP30	CTX30	CFO30	CAZ30	CIPR5	PI+TZ	GEN10	MRP10	IMI10
27	urine	*E .coli*	MRP10	FEP30	CFO30	PI+TZ	CTPR5	CAZ30	CTX30	IMI10	*
28	urine	*E .coli*	CFM5	NT300	CTX30	AMI30	SXT25	CTR30	CFM5	PI+TZ	*
29	urine	*E .coli*	CFM5	NAL30	AMP10	CFO30	CO.10	PI+TZ	CAZ30	NT300	*
30	sputum	*Acineto-bacter*	CF030	PI+TZ	CAZ30	CFM5	GEN10	CO.10	CB100	*	*
31	sputum	*E .coli*	CTX30	PI+TZ	NAL30	CO.10	CTR30	AMI30	*	*	*
32	sputum	*Acineto-bacter*	AMI30	CO . 10	SXT25	PI+TZ	CFM5	FEP30	CTX30	CTR30	*
33	urine	*E .coli*	PI+TZ	SXT25	CO.10	AMI30	CTX30	FEP30	CFM5	CTR30	*
34	urine	*E .coli*	NAL30	NI300	CIPR5	CAZ30	AMP10	MRP10	IMI10	CFO30	*
35	urine	*E .coli*	CFM5	SXT25	AMI30	NI300	CTR30	CTX30	CFO30	CO.10	*
36	wound	*E .coli*	CFM5	CTR30	CTX30	CFO30	SXT25	AMI10	CO.10	*	*
37	urine	*E .coli*	NAL30	CO . 10	CTR30	CAZ30	CTX30	PI+TZ	AMI30	*	*
38	wound	*Acineto-bacter*	MRP10	GEN10	PI+T2	CO.10	CAZ30	CB100	CFM5	*	*
39	Blood	*Acineto-bacter*	CB100	GEN10	MRP10	CO.10	CAZ30	PI+TZ	*	*	*
40	Blood	*Acineto-bacter*	GEN10	TET30	DOX30	CB100	CAZ30	CO.10	MRP10	PI+TZ	*
41	urine	*E .coli*	FEP30	CF030	PI+TZ	CTPR5	CAZ30	CTX30	MRP10	IMI10	*
42	urine	*E .coli*	CTR30	AMI30	PI+TZ	NAL30	NI300	CAZ30	FEP30	*	*
43	urine	*E .coli*	SXT25	MRP10	CTPR5	CFM5	CF030	IMI10	CTX30	CO.10	NI300
44	urine	*E .coli*	CTR30	AMI30	PI+TZ	CAZ30	NI300	NAL30	FEP30	*	*
45	wound	*E .coli*	CTR30	FEP30	CFM5	IMI10	SXT25	CAZ30	CFO30	CIPR5	*
46	urine	*E .coli*	CTR30	IMI30	CO.10	SXT25	CFM5	CTX30	MRP10	PI+TZ	*
47	wound	*E .coli*	CTX30	PI+TZ	CTR30	CAZ30	AMI30	FEP30	CO .10	*	*
48	urine	*E .coli*	SXT25	CFM5	IMI10	CIPR5	NAL30	MRP10	CFO30	NT300	*
49	urine	*E .coli*	CTX30	PI+TZ	CTR30	CAZ30	AMI30	FEP30	CO .10	*	*
50	sputum	*Acineto-bacter*	GEN10	CB100	IMI10	CIPR5	AMI30	FEP30	CO .10	CTR30	*

Table 2. Antibacterial activites of selenium on antibiotices resistant bacteria

Sample	Anti Bacteria inhibition Zone	MIC/ML	MIC/ML
1	6	700	1000
2	-	-	-
3	-	-	-
4	5	800	1000
5	7	800	1000
6	-	-	-
7	-	-	-
8	-	-	-
9	8	600	800
10	-	-	-
11	-	-	-
12	-	-	-
13	-	-	-
14	8	800	1000
15	-	-	-
16	10	500	800
17	-	-	-
18	-	-	-
19	-	-	-
20	-	-	-
21	8	700	900
22	-	-	-
23	8	600	800
24	10	600	850
25	-	-	-
26	-	-	-
27	-	-	-
28	-	-	-
29	6	700	1000
30	-	-	-
31	-	-	-
32	-	-	-
33	8	500	800
34	-	-	-
35	10	600	800
36	-	-	-
37	8	800	1000
38	-	-	-
39	-	-	-
40	-	-	-
41	-	-	-
42	8	600	800
43	-	-	-
44	10	500	800
45	-	-	-
46	-	-	-
47	-	-	-
48	-	-	-
49	10	600	900
50	-	-	-

Table 3. Table3. Antimicrobial effect of nanoselenium synthesized by L.acidophillus

Sample	Anti Bacteria inhibition Zone	MIC/ML	MIC/ML
1	10	500	800
2	-	-	-
3	-	-	-
4	-	-	-
5	10	600	1000
6	-	-	-
7	6	800	1000
8	8	600	800
9	9	600	850
10	-	-	-
11	10	500	800
12	8	600	800
13	-	-	-
14	12	500	700
15	-	-	-
16	10	500	800
17	-	-	-
18	8	700	900
19	-	-	-
20	6	800	1000
21	10	800	800
22	-	-	-
23	8	600	1000
24	12	500	800
25	6	600	1000
76	-	-	-
77	-	-	-
78	-	-	-
79	6	700	1000
80	-	-	-
81	-	-	-
82	-	-	-
83	8	500	800
84	-	-	-
85	10	600	800
86	-	-	-
87	8	800	1000
88	-	-	-
89	-	-	-
90	-	-	-
91	-	-	-
92	8	600	800
93	-	-	-
94	10	500	800
95	-	-	-
96	-	-	-
97	-	-	-
98	-	-	-
99	10	600	900
100	-	-	-

Methylene blue (EMB) and incubated in both aerobic and anaerobic conditions for 24 hours at 37°C. Positive samples with colony counts equal or more than 10 CFU/ ml were selected. The isolates were identified and confirmed using standard microbiological methods.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Merck, Germany) using disk diffusion (Kirby Bauer's) technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines The following: Cefotaxime 30ìg, NalidixicAcid 30ìg, Trimethoprim-sulphamethoxazole 30ìg, Ceftazidime 30ìg, piperacillin-tazobactam , Co-Amoxiclav 10ìg, Nitrofurantoin 300ìg, Imipenem 10ìg, Meropenem 10ìg, Ampicillin 30ìg, Cefoxitin 30ìg, Gentamicin10ìg, Cefixime 5ìg, Cefepime 30ìg, Carbenicillin 100g, Cefprozil 30ìg and Tetracycline 30ìg.Among 436 samples tested, 50 samples of bacteria were resistant to antibiotics which were isolated.

Antibacterial Tests

Antimicrobial activities of selenium prepared in Section (3.2) and selenium nanoparticle (produced with *L. acidophilus* on isolated bacterial strains)were evaluated by Disc diffusion and se=rial broth dilution methods to determine minimum in=hibitory concentration (MIC) and minimum bactericidal concentration (MBC), as given in the following section.

Disc Diffusion Method

Blank discs were immersed in selenium and selenium nanoparticle and placed on Muller Hinton agar medium (Merck, Germany); discs with isolated bacteria inoculums culture, incubated at 37°C for 24h, and finally inhibition zone around the discs determined**.** ***Serial Broth Dilution Method***

Serial dilutions of selenium nanoparticles were prepared from 100 to 1000ìl/mL in Muller-Hinton broth medium (Merck, Germany), then 1ml of each isolated bacteria inoculums sample was added into Muller- Hinton broth medium and then incubated at 37°C for 24h.

RESULT

The results of Erlenmeyer flask containing MRS broth medium and *L. acidophilus* or *L. plantarum* inoculums culture showed, as a selenium culture medium, the culture medium becomes red; the color change indicates conversion of sodium hydrogen selenite to selenium red element and red sediment is visible (Figure1).

Surveying images of nanoparticles synthetized by *L. acidophilus* and *L. plantarum* using electron microscope showed nanoparticles with an average size of 64.41 nm and 66.44 nm, respectively (Figure2).

Among samples of urine, sputum, blood and wound of hospital patients and after microbiological tests, 50 strains of antibiotics bacteria resistant that had the highest resistance to antibiotics were used in order to investigate the antibacterial activities of selenium and selenium nanoparticles (synthesized by *L. acidophilus*).

The results of surveying 50 strains of antibiotic-resistant strains isolated showed that in 80% of cases *E.coli* and in 20% of cases *Acinetobacter*. (table 1).

The antibiotics used to determine the resistance of examined strains are reported in Table (1).

The study of isolated 50 strains resistant to an antibiotic contains, 72%, 12%, 10%, 6% from urine, sputum-, wounds and blood; respectively.

The nanoparticles produced by *L.acidophillus* are smaller than nanoparticles synthesized with *L.plantarum*. The nanoparticles synthesized by *L.acidophillus* with antimicrobial property with an average size of 64.41 were used.

The antibacterial activities of the selenium and selenium nanoparticles demonstrated that they were effective on 32% and 56% of the strains studied, respectively. Selenium was only effective on urine samples and selenium nanoparticles most effective on 43% of urine samples, 7.84% of sputum samples, 3.9% of wound samples and 1.68 % of blood samples (Tables 2,3).

The most effective antimicrobial inhibition zone and minimum bactericidal concentration (MBC) on bacteria resistant antibiotics (*E.coli*) were evaluated by selenium nanoparticles with 12mm in diameter and 1000ìl/L, respectively (Chart1).

The results of the minimum inhibitory concentration of bacteria MIC and minimum bacteria concentration MBC has been shown in Chart (2,3).

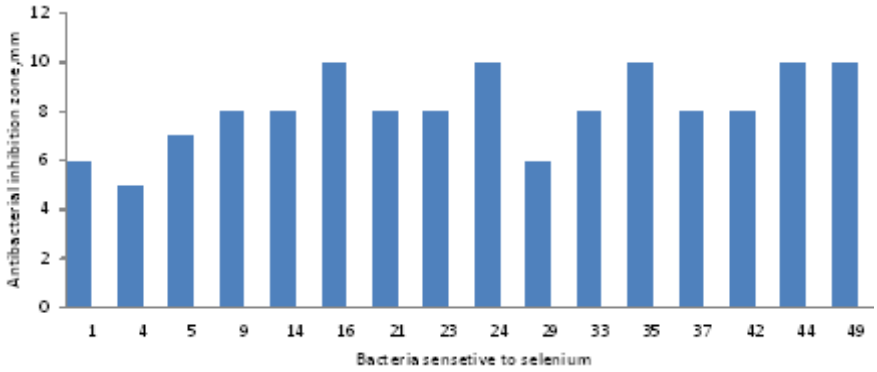


Chart 1. Antibacterial inhibition zone of bacteria sensitive to selenium

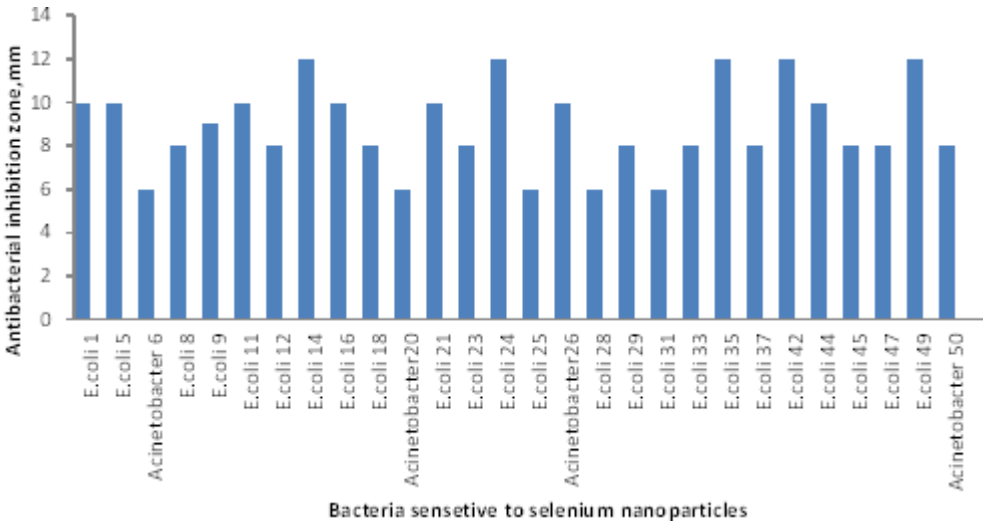


Chart 2. Antibacterial inhibition zone of bacteria sensitive to selenium nanoparticles

a) MRS broth culture with 10ml of selenium-based solution includes, ($_{NaHSeO3}$), and inoculated strains of *L. acidophilus* and *L. plantarum* is equal to half McFarland before incubation b) Selenium nanoparticles production and color change after 48-72 hours incubation at 37 °C medium with 50rpm in MRS broth culture.

DISCUSSION

Nosocomial infections followed by, long term hospitalization of patients in hospital, use of devices such as urinary and intravascular catheters as well as high use of various antibiotics are the factors in increase of multidrug resistance[16]. Bacteria which cause urinary and hospital infections such as *E. coli, Acinetobacter, Pseudomonas aeruginosa, Proteus*, etc. have antibiotics resistance genes[17]. Due to the increase in multidrug resistance to antibiotics, extensive researches have been done on nano-particles with antimicrobial activities to deal with resistant bacteria. In a recent study after surveying samples from hospitalized patients and doing the necessary microbiological tests, 50 strains with the highest resistance from 436 samples were used to investigate the antibacterial activities of selenium nano-particles synthesized by *L. acidophilus* which results showed that in 80% of cases *Ecoli* and in 20% of cases *Acinetobacter.*

In a study that Fazely and et al, did on 278 clinical isolate of *E.coli* strains in 2008, 62% of the resistant samples taken from urine sample of the patients[18]. In this study from 50 strains

resistant to antibiotics of *E.coli* and *Acinetobacter,*72% of strains isolated from urine sample.

In a study performed by Ana Kaftandzhieva and et al on 212 strains of *E.coli* isolated from samples of hospitalized patients in 2009 showed that 11.8% of E.coli strains demonstrate resistance to beta-lactam antibiotics of these, 7.2% of the cases were related to urinary tract infections[19]. The survey results show that high proportion of hospital infections accounted for urinary tract infections and in current research the results showed 80% of samples were collected from patient was *E.coli* .

Due to the rise of antibiotic-resistant strains, a large part of research studies allocated to access to effective anti-microbial materials, In the meantime the use of microorganisms for the synthesis of metal nano-particles because of their high efficiency and lower costs compared to the physical and chemical methods, is of particular importance[20].

In recent study for the synthesis of nano-particles of selenium were used two strains of *Lactobacillus* that are from probiotic bacteria names *L.acidophilus* and *L.plantarum.* Electron microscopy studies showed that respectively the mean particle size of nano-particles were 64.41nm and 66.44nm.

In a study conducted by Nidhi singh et al in 2014 for synthesis selenium nanoparticles used from *Bacillus* Sp.JAPK2 and nano-particles scale reported 29.1[21]. As well in a study carried out by Santanu sasidharan and colleagues in 2014, for Selenium nano-particles synthesis used *L.acidophilus* and *L.helveticus* strains and reported nanoparticles dimensions between 50-500 nm That surveying the results of synthesis selenium nano-particles to bacteria shows studied bacteria possess the ability to synthesize nano-particles with dimensions less than 100 nanometers[11]. In this study, the effect of synthesized selenium nano-particles were studied on 50 strains of antibiotic-resistant bacteria from 436 samples were collected from hospitalized patients, The antibacterial activity of the selenium and selenium nano-particles demonstrated were effective on 32% and 56% respectively of the strains studied, of which 43% of the urine samples, 7.84% of sputum samples and 3.9% of the wounds and 1.68% was the blood samples.

During study of Nidhi singh and his et al on the bacteria, *Pseudomonas* SP and *E.coli* and *Staphylococcus aureus* and *Klebsiella* in 2014, have reported the greatest impact of nano-selenium respectively on *Pseudomonas* SP and after that *Staphylococcus aureus* and the least impact on *E.coli* and *Klebsiella*[20].In current research showed the greatest effect of nanoselenium respectively on *E.coli* and *Asinetobacter.*

In recent years, however, a variety of nano structured metals has evidenced very promising antibacterial properties. The uses of these metal nanoparticles have potential advantages over conventional antimicrobial agents due to their high surface to volume ratios that allow a higher area of interactions with biological systems. In the study that Emanuele zonaro and et al in 2015 performed, showed selenium nanoparticles exhibite dantimicrobialand anti-biofilm activity against *E.coli*, *P.aeruginosa* and *S.aureus*[21].In current research that showed selenium nanoparticles have most effective on *E.coli.*

The use of metal nanoparticles of microbial activity against antibiotic-resistant bacteria could be a good option to deal with this group of bacteria.

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