ISSN : 0974 - 7494 Volume 9 Issue 2

Nano Science and Nano Technology
An Indian Journal

Full Paper

NSNTAIJ, 9(2), 2015 [049-057]

Can penicillin-bound nanoparticles restore the activity of ß-lactam antibiotics against methicillin-resistant *staphylococcus aureus*?

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ABSTRACT

Loss of effectiveness of commonly used antibiotics such as penicillin and other β-lactam drugs against MRSA lead to calling for immediate need for improvement in drug design, discovery, and delivery. The application of nanotechnology to drug delivery system is widely expected to change the landscape of pharmaceutical and biotechnology industries for foreseeable future, where nanoparticles represent a very promising chitosan approach to this aim. The aim of this work was to assess whether penicillin-bound chitosan nanoparticles will display antibacterial activity against MRSA and to determine the bioactivity of penicillin-bound nanoparticles. Chitosan nanoparticles were prepared by iontogelation method and penicllin were loaded during processing of nanoparticles by incorporation method. Prepared nanoparticles were characterized using transmission electron microscope, particle size analyzer and drug encapsulation efficiency. *Staphylococcus aureus* isolates were subjected to oxacillin and cefoxitin disc diffusion method, and PCR for detection of mecA gene or identification of MRSA and MSSA isolates. Fifty MRSA and 30 MSSA were selected and further tested. Determination of penicillin and penicillin-bound chitosan nanoparticles MIC by broth microdilution method was performed. All MRSA isolates were resistant to penicillin using both methods. As for the MSSA isolates, using penicillin-bound chitosan nanoparticles, none displayed resistance at a dilution of e" 512 µg/ml (0.0%), ten (33%) revealed an MIC of e" 16 µg/ml, whereas 7 revealed an MIC of 16 µg/ml. It was observed that 3 isolates (10%) of MSSA turned sensitive to penicillin when performing the MIC broth microdilution test, using penicillin-bound chitosan nanoparticles. Though 27 MSSA isolate remained resistant; yet the MIC of penicillin-bound chitosan nanoparticles was significantly reduced. In conclusion, penicillin-bound chitosan nanoparticles was effective only with MSSA producing penicillinase in reducing MIC of penicillin or even making MSSA sensitive to penicillin. But with MRSA, penicillin-bound chitosan nanoparticles gave no effect.

KEYWORDS

MRSA;
Chitosan nanoparticles;
Pencillin.

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INTRODUCTION

The risk of nosocomial infection in ICU is 5–10 times greater than those acquired in general medical ward.[1] The most common pathogens are *Staphylococci*, *Pseudomonas*, *Escherichia coli*, *Mycobacterium tuberculosis*, *Candida* and *Aspergillus*. All are associated with increased morbidity and mortality[1].

According to the data issued by Nosocomial Infection Surveillance System (NNIS) of the Centers for Disease Control and Prevention (CDC) in 2002, methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 57.1% of all *S. aureus* isolates obtained from patients in more than 300 participating intensive care units throughout the United States[1].

Now loss of effectiveness of commonly used antibacterial antibiotics such as penicillin and other β-lactam drugs against MRSA lead to calling for immediate need for improvement in drug design, discovery, and delivery[1].

Nanotechnology is an enabling technology that deals with nanometer sized objects[1]. The application of nanotechnology to drug delivery system is widely expected to change the landscape of pharmaceutical and biotechnology industries for foreseeable future,[2] where nanoparticles (NP) represent a very promising approach to this aim[3, 4].

Penicillin could be incorporated into nanoparticle framework during preparation and be sheltered inside the matrix of nanoparticle from bacterial penicillinase degradation. If this could be achieved, the β-lactam antibiotics could potentially be rendered highly effective against β-lactamase producing MRSA[4].

The aim of this work was to assess whether penicillin-bound nanoparticles will display antibacterial activity against MRSA and to determine the bioactivity of penicillin-bound nanoparticles.

MATERIAL AND METHODS

Clinical specimens submitted to the routine laboratory of the Microbiology Department at Alexandria Main University Hospital, starting from 4/2010 to 6/2011 were enrolled in the study. Each clinical specimen was inoculated onto both Columbia blood agar (Oxoid), MacConkey's agar (Oxoid) and Mannitol salt agar (MSA) (Oxoid) and incubated at 37°C for 24-48 hours aerobically. *S. aureus* isolates identified and confirmed according to standard techniques, were subjected to oxacillin (1 μg), cefoxitin (30μg)[9] disc diffusion method, and Polymerase Chain Reaction (PCR) for detection of mecA gene for identification of MRSA and MSSA isolates. Fifty MRSA and 30 MSSA were selected and further tested.

Antimicrobial susceptibility testing was done using disc diffusion method for penicillin (10 units), oxacillin and cefoxitin according to CLSI recommendation[5].

All *S. aureus* isolates were tested for the presence of mecA gene. One mecA positive strain (ATCC43300) and one mecA negative strain (ATCC 25923) were included as positive and negative control respectively. The oligonucleotide primers (Geno-Mechanix) were designed to amplify 310 base pairs (bp) fragment of the mecA gene[6]. The forward primer sequence was TGG CTA TCG TGT CAC AAT CG and the reverse primer sequence was CTG GAA CTT GTT GAG CAG AG. The reaction was carried out using (Techne Genius, Cambridge, UK) thermal cycler under the following conditions : Initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds, followed by final extension at 72°C for 3 min.[10]

PCR products were electrophoresed (100 volts, for 45 min) through a 2% agarose gel (conda, pronadisa) containing 0.5 μg/ml ethidium bromide and amplified PCR product bands were visualized on an ultraviolet transilluminator (UVP Dual Intensity Transilluminator, USA). Molecular size of the amplicons were determined by comparison to a 100 bp DNA ladder[10].

A positive result was inferred by detection of 310 bp band.

Determination of Penicillin MIC by broth microdilution method performed according to CLSI recommendations[9]. A standard MSSA strain

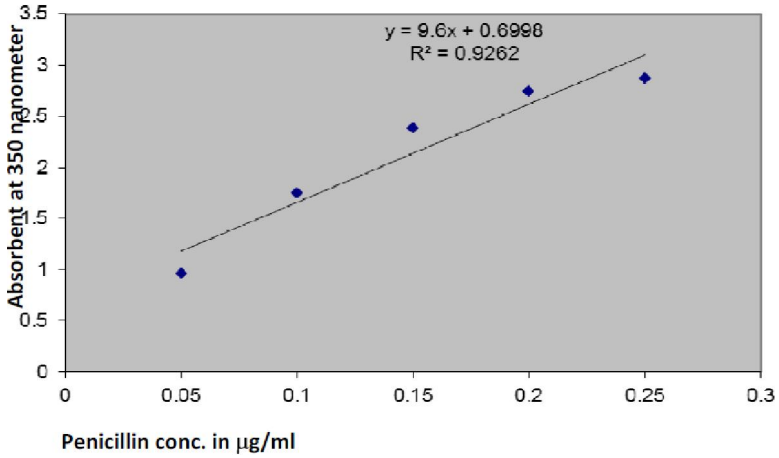

Figure 1 : Penicillin standard curve

(ATCC29213) was used to monitor antibiotic potency for quality control. (supplied by NAMRU-3). Cation-adjusted Muller-Hinton broth (CAMHB) (oxoid) with 2% NaCl and penicillin G powder (supplied by Pharco pharmaceutical company) was used. The antibiotic stock solutions were prepared using the formula: 1000/PxVxC =W (where P is the potency given by the manufacturer in relation to the base, V is the volume, C is the final concentration of solution in multiples of 1000, and W is the weight of the antibiotic in milligram to be dissolved in the volume). The range of penicillin G dilution used was: 0.06-256 mg/L. An antibiotic-free growth control was included. The results were read for turbidity. Penicillin MIC was defined by the least concentration of antibiotic which inhibits visible growth of the organism.

Equivalent MIC breakpoints for *S. aureus* as recommended by CLSI: ≥ 0.25 µg/ml is resistant and d" 0.12 µg/ml is sensitive

Determination of Penicillin-bound nanoparticles Minimum Inhibitory Concentration (MIC) by broth microdilution method: Preparation of penicillin-bound nanoparticles was done by preparation of the Chitosan nanoparticles (CSNPs)[11],ionic gelation[11] and lyophilization techniques[12, 13].

During preparation of CSNPs by ionic gelation mechanism, Chitosan (Sigma–Aldrich [Catalogue No. MMW448877] degree of deacetylation was 84.7%.) and Sodium Tripolyphosphate (Sigma–Aldrich Chemical Co. Ltd.) were used. All other reagents used were of analytical grade[7].

During lyophilization of nanoparticles[8, 9], penicillin loaded CSNPs were frozen by vacuum Freeze Drying Machine (Model/CRYODOS-50) which worked at 230V, 50 Hz.

Characterization of prepared CSNPs was done by particle size by particle size analyzer[10] on a Beckman Coulter Particle Size Analyzer (N5 submicron particle size analyzer, Japan).

Determination of shape of dried nanoparticles was done by using transmission Electron Microscope (TEM) (Jeol, JSM-6360LA, Japan) after mounting them on carbon coated copper grid and stained with uranyl acetate (SPI-Module TM sputter coater, Japan).

The bioactivity of penicillin-bound nanoparticles was done by determination of Encapsulation Efficiency of CSNPs[14]

A standard curve from known concentrations of penicillin was constructed as shown in Figure 1.

The drug loading efficiency (DLE) and entrapment efficiency (EE) were presented by equation (1)-(2), respectively.

$$\mathbf{DLE} = \frac{\text{Weight of drug in nanoparticles}}{\text{Weight of nanoparticles}} \quad \textbf{(1)}$$

$$\mathbf{EE} = \frac{\text{Weight of drug in nanoparticles (actual concentration)}}{\text{Weight of drug added (therotical concentration)}} \quad \textbf{(2)}$$

This was followed by the determination of Penicillin-bound nanoparticles MIC which was performed according to CLSI recommendations[9] as mentioned before.

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RESULTS

Antimicrobial susceptibility testing

Results of Oxacillin (1μg) and Cefoxitin (30 μg) disc diffusion tests are shown in TABLE 1.

Antimicrobial susceptibility testing results of the MRSA, illustrated that all 50 MRSA isolates (100%) were resistant to penicillin, while antimicrobial susceptibility testing results of the MSSA illustrated that 10 (33.3%) out of 30 were sensitive to penicillin.

PCR amplification of the mecA gene

After DNA amplification, the 310 bp mecA fragment was obtained from 50 *S. aureus* isolates (62.5%), hence proven to be MRSA by PCR. On the other hand, no 310 bp mecA fragment has been detected among 30 *S. aureus* isolates (37.5%), and thus identified as MSSA.

All the 50 mecA +ve isolates and 30 mecA-ve were correctly identified by oxacillin and cefoxitin disc diffusion test as resistant and susceptible isolates respectively, resulting in 100% sensitivity. The oxacillin disc diffusion test identified the 30 mecA -ve isolates as oxacillin susceptible resulting in 100% specificity. Therefore, the test had a positive predictive value (PPV) of 100% and negative predictive value (NPV) of 100%. The result is shown in TABLE 2.

CSNPs preparation

Particle size distribution that was carried out by particle size analyzer showed preparation of CSNPs with mean particle sizes of 53.3nm with baseline error 1.34% and at diffraction angle of 11.1°. Polydispersity index was 0.7 (i.e. less than 1), which indicates homogeneous nature of the formulation (Figure 2).

The findings obtained from transmission electron microscopy examination revealed the presence of completely spherical CSNPs with smooth surfaces, and no agglomeration formed. These data were obtained for the colloidal nanosuspension prepared by ionic gelation method using chitosan as polymer for matrix material and sodium tripolyphosphate as cross linking (Figure 3).

The percentage of DLE was 44.5% and the percentage of EE was 66.8%. The drug loading was 445 μg penicillin in each 1 mg CSNPs containing penicillin.

The in vitro release data (Figure 4) indicate that the drug-loaded formulation obtained after ionic gelation method exhibited sustained release behavior with a steady rise in cumulative drug release (> 70 %) up to the 5th hour. Thereafter, there was no further significant release of the drug (i.e. an initial burst release followed by slow sustained drug release).

Results of penicillin MIC by broth microdilution method

TABLE 1 : Results of oxacillin and cefoxitin disc diffusion test of the 80 *S. aureus* isolates

	Sensitive (s)	Intermediate (I)	Resistant (R)
Oxacillin disc (1 μg)	30	0	50
Cefoxitin disc (30 μg)	30	-	50

Oxacillin disc diffusion interpretation: S ≥ 13, I = 11-12, R ≤ 10 mm; Cefoxitin disc diffusion interpretation: S ≥ 20, R ≤ 19 mm.

TABLE 2 : Comparison of mecA gene detection by PCR with Oxacillin and Cefoxitin disc diffusion test of the 80 *S. aureus* isolates

Test	PCR for mecA gene				Sensitivity	Specificity	PPV	NPV
	MecA +ve (n=50)		MecA –ve (n=30)					
	True +ve	False +ve	True -ve	False -ve				
OX DD (R)	50	0	0	0	100.0	100.0	100.0	100.0
OX DD (S)	0	0	30	0				
FOX DD (R)	50	0	0	0	100.0	100.0	100.0	100.0
FOX DD (S)	0	0	30	0				

OX DD: oxacillin disc diffusion, FOX DD: cefoxitin disc diffusion, PPV: positive predictive value, NPV: negative predictive value, mecA +ve: mecA positive, mecA –ve: mecA negative, R: resistant, S: sensitive.

11.1°, Repetition 1 Unimodal Distribution

Unimodal Results Summary

Angle	Mean (nm)	P.I.	Diff.Coef (m^2/s)	Counts/s	Baseline Error	Overflow
11.1°	53.3	-5.635	8.04e-12	2.47e+06	1.34%	0

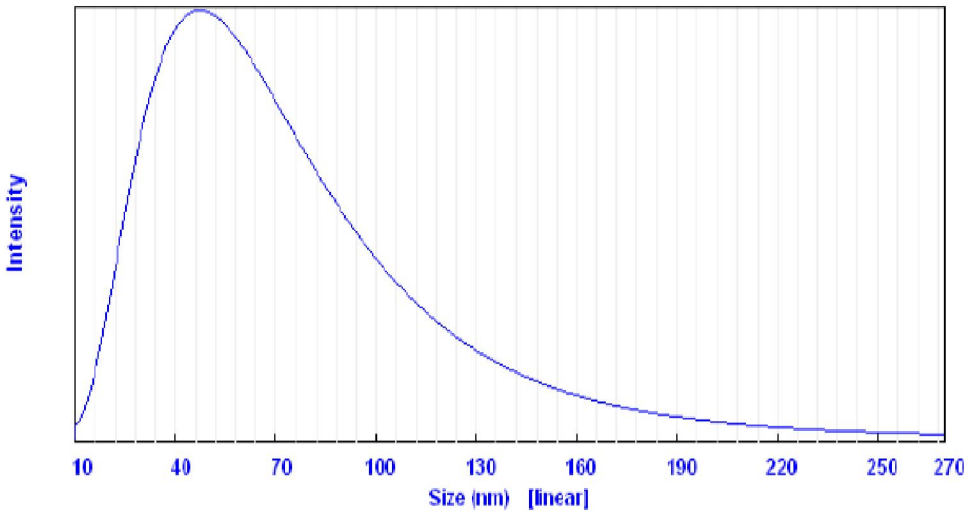

Figure 2 : Particle size distribution of prepared penicillin-loaded CSNPs showing mean particle size of 53.3nm at diffraction angle of 11.1°.

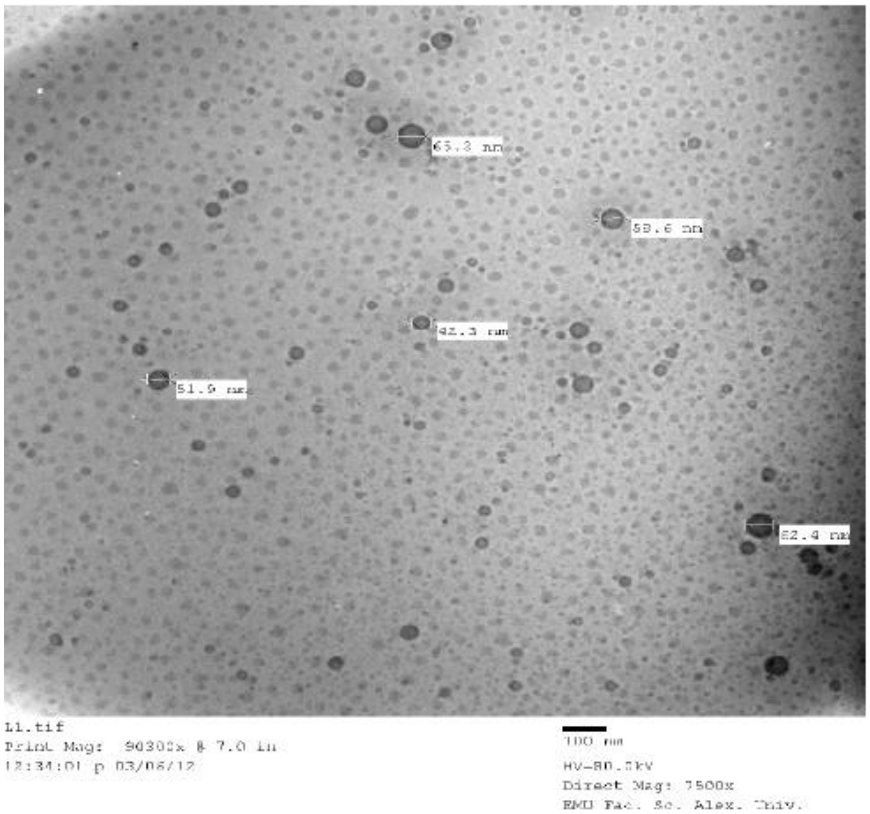

Figure 3 : TEM of spherical CSNPs with an average size of 10 nm. (Magnification 7500x)

All MRSA isolates were resistant to penicillin by broth microdilution method; where thirty one iso-

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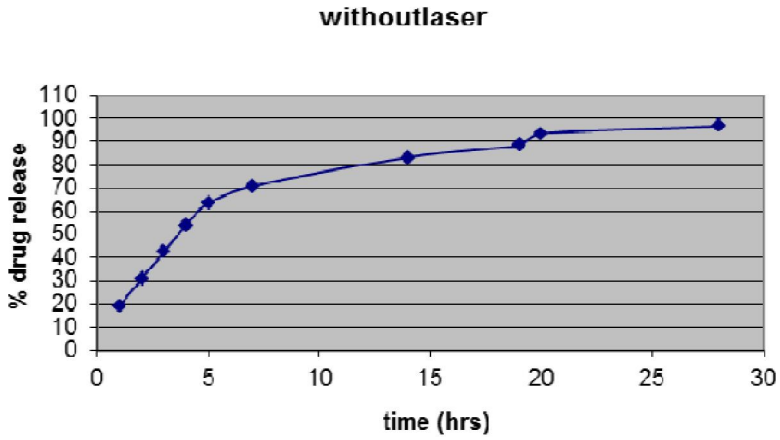

Figure 4 : Drug release from penicillin-loaded CSNPs

TABLE 3 : Comparison between MRSA and MSSA in MIC before treatment with nanoparticles

Unit in µg/ml	MRSA		MSSA	
	No.	%	No.	%
< 16	0	0.0	10	33.3
16	0	0.0	7	23.3
32	0	0.0	5	16.7
64	0	0.0	4	13.3
128	1	2.0	2	6.7
256	12	24.0	2	6.7
512	6	12.0	0	0.0
> 512	31	62.0	0	0.0
X^2		105.6		
p		0.0001*		

MIC sensitive ≤ 0.12 µg/ml; MIC resistant ≥ 0.25 µg/ml

late (62%) displayed an MIC of ≥ 512 µg/ml, while the remaining 19 MRSA strains possessed an MIC that varied from 128-512 µg/ml.

Regarding the MSSA isolates, none displayed resistance at a dilution of ≥ 512 µg/ml (0.0%), ten MSSA (33%) revealed an MIC of ≥ 16 µg/ml, whereas 7 revealed an MIC of 16 µg/ml. The difference proved to be highly statistically significant, (P=0.0001). (TABLE 3)

On the other hand, it was observed that 3 isolates (10%) of MSSA turned sensitive to penicillin when performing the MIC broth microdilution test, using penicillin bound nanoparticles. Though 27 MSSA isolate remained resistant; yet the MIC of penicillin bound nanoparticles was significantly reduced; where 25 isolate (83%) displayed an MIC of ≤ 16 µg/ml when compared to 10 isolates (33.3%) before adding nanoparticles. The difference was proven to be statistically significant (P=0.000) (TABLE 4)

The binding to nanoparticles did not alter the resistance of MRSA isolates to penicillin, and the majority of isolates (62%) revealed the same MIC of ≤ 512 µg/ml to penicillin when retested with penicillin bound nanoparticles (P=1) (TABLE 5)

DISCUSSION

During the past 50 years, *Staphylococcus aureus* has been a dynamic human pathogen that has gained the deepest respect of clinicians. MRSA is especially troublesome in hospitals, where acquisition of antimicrobial resistance make MRSA infection more difficult to treat. Great development is expected in medicine with the explosion of nanotechnology and the use of nanoparticles for treatment of disease.

TABLE 4 : MIC of Penicillin-bound nanoparticles for MSSA group

Unit in µg/ml	Before		After	
	No.	%	No.	%
Sensitive	0	0.0	3	10.0
< 16	10	33.3	25	83.3
16	7	23.3	2	6.7
32	5	16.7	0	0.0
64	4	13.3	0	0.0
128	2	6.7	0	0.0
256	2	6.7	0	0.0
512	0	0.0	0	0.0
X^2		45.65		
p		0.0001*		

MIC sensitive ≤ 0.12 µg/ml; MIC resistant ≥ 0.25 µg/ml

TABLE 5 : MIC of Penicillin-bound nanoparticles for MRSA group

Unit in µg/ml	Before		After	
	No.	%	No.	%
128	1	2.0	1	2.0
256	12	24.0	12	24.0
512	6	12.0	6	12.0
> 512	31	62.0	31	62.0
X^2		0.00		
p		1.0		

MIC sensitive ≤ 0.12 µg/ml; MIC resistant ≥ 0.25 µg/ml

In our study, the oxacillin 1µg and the cefoxitin 30 µg disc diffusion correctly identified both 50 *S. aureus* isolates as MRSA being oxacillin resistant and cefoxitin resistant and 30 *S. aureus* isolates as MSSA isolates as proven to be oxacillin and cefoxitin sensitive. Thornsberry et al.[15] stated that the disc diffusion test was very reliable if the incubation temperature did not exceed 35°C. Felten et al.[12], however, reported absolute discrimination of 83 MRSA strains from 69 MSSA strains by the use of cefoxitin disc test at 37°C, but by oxacillin disc diffusion test the sensitivity was only 41% for the detection of MRSA.

The detection of methicillin resistance in *Staphylococcus aureus* by oxacillin disc diffusion test has always presented problems for diagnostic laboratories. Hence, the detection of the mecA gene or PBP2a is considered the reference methods. In our study, the comparison of mecA gene detection by PCR with oxacillin and cefoxitin disc diffusion test of the 80 *S. aureus* isolates has been performed. The tests had correctly identified all the 50 mecA positive (MRSA) and 30 mecA negative (MSSA) strains resulting in 100% sensitivity and 100% specificity.

In the present study, CSNPs containing penicillin G were prepared using ionic gelation method. In principle, they were designed in the size range 40–60 nm with mean particle size of 53.3 nm to allow cell internalization process according to endocytosis pathway. For this purpose, the ionic gelation technique was adopted to formulate the nanosystems because the convenience of such approach mainly relies on the simple mixing of oppositely charged aqueous solutions without any organic solvent or covalent cross-linking agent (i.e., glutraldehyde)[13]. Our work was in accordance with that of Saha et al.[14]

In our study, in vitro MIC experiments were conducted to determine if penicillin bound nanoparticles displayed antibacterial activity against MRSA and MSSA.

The results of these assays showed that penicillin-bound nanoparticles displayed significant anti-

bacterial activity against MSSA isolates, where P was 0.0001, but not against the MRSA isolates (P=1.0). Hence, suggesting the detection of penicillinase production among MSSA.

Turos et al.[15] reported equipotent in vitro antibacterial properties using MIC method against MRSA and MSSA and indefinite stability towards β-lactamase. Through personal communication with Dr. Turos[19], inquiring about an explanation of the difference of his results in contrast to ours, he stated that; they have no evidence that the penicillin binding proteins in the MRSA strains they used in their test are structurally altered to the extent that they are completely unable to be targeted by penicillin G. Instead, penicillin resistance is due to the large amount of ß-lactamase these microbes generate extracellularly. Thus, penicillin G shows very little bioactivity in vitro against these strains. Conversely, the recovered activity of the penicillin-attached nanoparticles against MRSA suggests that the nanoparticles deliver the antibiotic into the cell, or at least to the cellular membrane, where the antibiotic is then released (presumably by esterase activity) from the nanoparticle matrix. In this way, the penicillin can react freely with the penicillin binding proteins in the membrane, without being degraded by penicillinases on the outside of the cell. This is what they believe to have happened in their case.

Khadri et al.[16] demonstrated that 83% and 86% of MRSA and methicillin resistant coagulase negative staphylococci isolates respectively, were β-lactamase producers. Norris al.[17] also declared that MRSA strains produce β-lactamase.

McDougall et al.[18] stated that though the acquisition of mecA by *S. aureus* is usually essential for expression of high level methicillin resistance there are other mechanisms that explain low-level methicillin resistance found in some clinical isolates of *S. aureus* strains that lack mecA gene i.e. over expression of β-lactamase in some *S. aureus*.

Greenhalgh et al.[23] reported first in-vivo study of penicillin conjugated nanoparticles emulsion in murine model. Penicillin analogues were incorporated into nanoparticles system and analyzed for activity against MRSA. Favorable results were observed in vivo.

Abeylath et al.[20], show that the glyconanoparticles containing N-thiolated β-lactam and ciprofloxacin exhibited significant antibacterial activity against MRSA and *Bacillus anthracis*, while the penicillin-bound glyconanoparticles and non-drug containing nanoparticles were completely inactive against these microbes.

Brown et al.[21], constructed silver nanoparticles (AuNP), gold nanoparticles (AgNP) and then functionalized them with ampicillin, as a drug delivery system. AuNP and AgNP functionalized with ampicillin were comparable as bactericides and killed MRSA, E. coli, *Vibrio cholera*, and multidrug resistant bacteria such as *Pseudomonas aeruginosa*, and *Enterobacter*.

In conclusion, penicillin-bound nanoparticles was effective only with MSSA producing penicillinase in reducing MIC of penicillin or even making MSSA sensitive to penicillin. But with MRSA, penicillin-bound nanoparticles gave no effect.

This finding needs further studies particularly with the rapid promising progress in nanotechnology.

REFERENCES

[1] J.David, A.William; Nosocomial infections in the ICU, Chest J, **115**, 34-41 **(1999)**.

[2] M.Jan, R.Bikha, Z.Syed, A.Tauseefullah, B.Ishrat; Frequency, pattern and etiology of nosocomial infections in ICU, J Ayub.Med.Coll.Abbotabad, **20(4)**, 37-40 **(2008)**.

[3] National nosocomial infections surveillance system, National nosocomial infections surveillance (NNIS) system report, Data summary from January 1992 through June 2003, Issued August 2003, Am J Infect Control, **31**, 481-98 **(2003)**.

[4] T.Edward, G.K.Suresh, G.Kerriann, R.Praveen, C.Sampath, J.Seyoung et al.; Penicillin-bound polyacrylate nanoparticles: Restoring activity of β-lactam antibiotics against MRSA, Bioorg Med Chem Lett., **17(12)**, 3468-72 **(2007)**.

[5] O.V.Salata; Application of nanoparticles in biology and medicine, J Nanobiotechnology, **2**, 3-15 **(2004)**.

[6] C.Omid, L.Robert; Impact of nanotechnology on drug delivery, ACSNANO, **3**, 16-20 **(2009)**.

[7] P.Maincent, P.Thouvenot, C.Amicabile, M.Hoffman, J.Kreuter, P.Couvreur et al.; Lymphatic

targeting of polymeric nanoparticles after intraperitoneal administration in rats, Pharm Res., **9**, 1534-9 **(1992)**.

[8] Y.Nakada, E.Fattal, M.Foulquier, P.Couvreur; Pharmacokinetics and biodistribution of oligonucleotide adsorbed onto poly (isobutylcyanoacrylate) nanoparticles after intravenous administration in mice, Pharm Res., **13**, 38-43 **(1996)**.

[9] Clinical and laboratory standards institute, Performance standards for antimicrobial susceptibility testing, fifteenth informational supplement, CLSI document M100-S15, Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA, **25(l)**, **(2005)**.

[10] P.Vannuffel, J.Gigi, H.Ezzedine, B.Vandercam, M.Delmee et al.; Specific detection of methicillin-resistant *Staphylococcus* species by multiplex PCR, J.Clin.Microbiol., **33**, 2864-7 **(1995)**.

[11] A.Trapani, M.Garcia-Fuentes, M.J.Alonso; A new drug nanocarrier combining hydrophilic cyclodextrins and chitosan, Nanotechnology,**19**, 185101/1–1185101 **(2008)**.

[12] C.Kirby, G.Gregoriadis; Dehydration-rehydration vesicles: A simple method for high yield drug entrapment in liposomes, Biotechnology, **2**, 979–8 **(1984)**.

[13] M.U.Uhumwangho, R.S.Okor; Current trends in the production and biomedical applications of liposomes: A review JMBR, Journal of Biomedical Sciences, **4**, 9-21 **(2005)**.

[14] A.Trapania, J.Sitterbergb, U.Bakowskyb, T.Kissel; The potential of glycol chitosan nanoparticles as carrier for low water soluble drugs, Int.J.Pharm., **375**, 97–106 **(2009)**.

[15] C.hornsberry, J.Q.Caruthers, C.N.Baker; Effect of temperature on the in vitro susceptibility of *Staphylococcus aureus* to penicillinase-resistant penicillins, Antimicrob Agents Chemother, **4**, 263-9 **(1973)**.

[16] A.Felten, B.Grandry, P.H.Lagrange, I.Casin; Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disc diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-Screen latex agglutination test, J.Clin.Microbiol., **40**, 2766-71 **(2002)**.

[17] X.Gao, J.Chen, W.Tao, J.Zhu, Q.Zhang, H.Chen et al.; UEA Ibearing nanoparticles for brain delivery following intranasal administration, Int.J.Pharm., **340**, 207–15 **(2007)**.

[18] P.Saha, A.K.Goyal, G.Rath; Formulation and evaluation of chitosan-based ampicillin trihydrate nanoparticles, Trop J Pharm.Research, **9(5)**, 483-8 **(2010)**.

[19] E.Turos, S.K.Reddy, K.Greenhalgh, P.Ramaraju, S.C.Abeylath, S.Jang et al.; Penicillin-bound polyacrylate nanoparticles: Restoring activity of β-lactam antibiotics against MRSA, Bioorg Med.Chem.Lett., **17(12)**, 3468-72 **(2007)**.

[20] H.Khadri, M.Alzohairy; Prevalence and antibiotic susceptibility pattern of methicillin-resistant and coagulase-negative *Staphylococci* in a tertiary care hospital in India, Int.J.Med.Med.Sci., **2(4)**, 116-20 **(2010)**.

[21] S.R.Norris, C.W.Stratton, D.S.Kernodle; Production of A and C variants of *staphylococcal* β-Lactamase by Methicillin-resistant strains of *Staphylococcus aureus*, Antimicrob Agents Chemother, **38**, 1649-50 **(1994)**.

[22] L.K.McDougall, C.Thornsberry; The role of β-lactamase in *Staphylococcal* resistance to penicillin-resistant penicillins and cephalosporins, J.Clin.Microbiol., **23**, 832-9 **(1986)**.

[23] K.Greenhalgh, E.Turos; In vivo studies of polyacrylate nanoparticle emulsions for topical and systemic applications, Nanomedicine, NBM, **5**, 46-54 **(2009)**.

[24] S.C.Abeylath, E.Turos, S.Dickey et al.; Glyconanobiotics: Novel carbohydrate nanoparticles antibiotics for MRSA and *Bacillus anthracis*, Bioorg.Med.Chem., **16**, 2412–8 **(2008)**.

[25] A.N.Brown, K.Smith, A.Tova, T.A.Samuels et al.; Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of *Pseudomonas aeruginosa* and *enterobacter aerogenes* and methicillin-resistant *Staphylococcus aureus*, Appl.Environ.Microbiol., **78**, 2768-74 **(2012)**.