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Exploring anti-MRSA activity of chitosan-coated liposomal dicloxacillin

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A B S T R A C T

One of the greatest disturbing global health problems is antibiotic-resistant bacterial infections, which have rendered numerous currently used antibiotics ineffective. Thus, the feasibility of chitosan-coated deformable liposomes (C-Lips) containing dicloxacillin (DLX) were evaluated for their efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) strains, which are resistant to beta lactam antibiotics. DLX-loaded liposomes (DLX-Lip) were prepared by a lipid film hydration method and then chitosan (CS) coated (C-DLX-Lip) by the electrostatic deposition method. Both DLX-Lips and C-DLX-Lips showed a particle size distribution with a nano-range and a narrow polydispersity index (PDI). After CS coating, the zeta potential was shifted from negative to positive value. The DLX entrapment efficiency (EE) and drug loading (DL) were 62% and 5.6% for C-DLX-Lips compared to 38% and 3.1% for DLX-Lip, respectively. The *in vitro* release profile of C-DLX-Lips possessed a slow release behavior. Moreover, the DLX-Lips and C-DLX-Lips demonstrated an enhanced anti-MRSA activity. These results revealed that DLX-Lips and C-DLX-Lips may serve as promising carriers for DLX to increase the efficacy against MRSA, which offers considerably clinical value for long-term use of DLX.

1. Introduction

Staphylococcus aureus (S. aureus) infections have historically resulted in hospital-acquired mortality and morbidity (Brakstad and Maeland, 1997). *S. aureus* has the ability to adapt and confer mechanisms of resistance to methicillin, a semisynthetic penicillinase-resistant beta lactam, with which usage was commenced upon emergence of penicillin resistance (Peacock and Paterson, 2015). However, possibly due to antibiotic misuse, poor infection control methods, and lack of antimicrobial stewardship processes, reports of resistant isolates have been confirmed (Shorr, 2007). These isolates, named methicillin-resistant *Staphylococcus aureus* (MRSA) have an intrinsic resistance to all beta lactams. There are two types of infections induced by MRSA. The first one is healthcare associated MRSA (H-MRSA) due to health care exposure causing pneumonia, bacteremia, and surgical site infections (Klevens et al., 2007). The other is community associated MRSA (C-MRA) attributable to the absence of such health care exposure resulting in skin and soft tissue infections. Nevertheless, MRSA infections are still the major cause of high mortality, health care costs, and longer hospital stays (Blot et al., 2002; Cosgrove et al., 2003; Gould, 2006). The antibacterial mechanisms of antibiotics against MRSA were associated with their actions. They have the ability to hamper the vital bacterial enzymes that hinder the function of ribosomes and the synthesis of proteins (Finberg et al., 2004). Moreover, they might interrupt cell wall or cell membrane function (Finberg et al., 2004). The intracellular action of antibiotics depends on agents and bacterium-related aspects such as permeation, accumulation, subcellular bioavailability, expression of activity and the sensitivity of the organisms (Bambeke et al., 2006).

MRSA has conferred susceptibility to glycopeptides including vancomycin and daptomycin (National Committee for Clinical Laboratory Standard, 2013). However, the decreased susceptibility to vancomycin gives rise to treatment failure (Lodise et al., 2008). Moreover, daptomycin treatment is limited due to its adverse events and lack of benefit in MRSA pneumonia (Durante et al., 2016). Hence, the discovery of new agents with activity against MRSA, to complete clinical utility is unclear in addition to the relatively high product-launching price.

Dicloxacillin (DLX) can be administered by enteral or parenteral routes and its sodium salt has significant activity against gram-positive β-lactamase-producing microorganisms (Gravenkemper et al., 1965). Compared with other penicillins currently in clinical use namely oxacillin, cloxacillin, and flucloxacillin, DLX has shown activity against *S. aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae, Streptococcus epidermidis, Streptococcus viridans, Streptococcus agalactiae, and Neisseria meningitides* (Dimitrova et al., 1998). DLX is currently indicated in the treatment of bacterial infections such as bone, ear, skin, urinary tract infections and pneumonia (Blaszczyk and Kostanecka et al., 1998). The resistance mechanism is mediated by penicillin-binding protein 2a (PBP-2a), a protein with low affinity to beta lactams (Brakstad and

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Maeland, 1997; Peacock and Paterson, 2015). PBP-2a is encoded by the gene "mecA", located on the staphylococcal chromosome cassette (SCCmec). Thus far, there are six clones of MRSA worldwide encoded as SCCmec I to SCCmec VI. There was a concerning increase in the prevalence of MRSA resistance in the past years particularly within the hospital setting (Shorr, 2007). However, resistance patterns remain high and prevalence rates vary significantly among different countries in Asia and Europe (Sader et al., 2006). Several risk factors have been identified as contributors to the spread of MRSA (Klevens et al., 2007; Shorr, 2007). Therefore, it is required to improve the activities of the DLX using novel approaches to reduce the resistance of bacterial strains.

A lot of studies have proven the valuable combination of nanoparticles with antibiotics for improvement of their bactericidal efficiencies due to greater cellular uptake (Chen et al., 2016). Furthermore, the nano-carriers have the ability to restore the antibiotic-efficiency against resistant bacteria by facilitating drug-binding to bacteria. Liposomes are recognized as one of the fast employing nano-carriers due to their biocompatibility, biodegradability and reduced toxic effects of the loaded drug (Liu et al., 2015). It has been reported that antibiotic loaded-liposomes potentially enhance pharmacokinetics and pharmacodynamics of the drugs. However, the conventional liposomes (CL) are not better carriers for the drug delivery. Thus, a special form of liposome with edge activator (*e.g.* Tween 80) was used to create flexible liposomes (FL) to enhance drug delivery (Chen et al., 2016). Generally, these vesicles have the ability to cross pores of a size smaller than their size without disassembling due to their flexible membranes compared to CL (Perez et al., 2016).

Furthermore, surface modification of liposomes can impact the rate of cellular uptake with sustained drug release at a target site (Mady et al., 2009). In addition, the positively surface modified liposomes could increase the interaction with the cells efficiently (Laye et al., 2008). Chitosan (CS) is a cationic polymer, which has unique biological properties like bioadhesion, biocompatibility, biodegradability and low toxicity (Cho et al., 2016). CS has mucoadhesive properties, which enhances the absorption of hydrophilic drugs and promotes sustained drug release (Tseng et al., 2015). The positively charged liposomes interact with the negatively charged cell membrane, which can open the tight junctions between cells, resulting in enhanced permeation of hydrophilic drugs (Sunderland et al., 2006). In this study, DLX was loaded into FL as DLX-Lips, and CS coated liposomes (C-DLX-Lips); named chitosomes. The CS-based liposomes could both provide antibacterial activity and simultaneous delivery of DLX to MRSA cell membrane. Therefore, the objective of the current study was to evaluate the potential application of chitosomes as carriers for DLX in MRSA therapy. It has been previously reported that chitosan-coated liposomes improved the oral absorption of sorafenib (Zhao et al., 2018). Furthermore, the antifungal efficiency of chitosomes combining encapsulated metronidazole could offer enhanced ability in the treatment of vaginal infections (Andersen et al., 2017**)**. Likewise, chitosomes supported a sustained release delivery system of amoxicillin *in vitro*, which may improve drug activity (Menikarachch et al., 2016). Therefore, such chitosomes could offer a valuable approach to the antibacterial activity of DLX. This work was designed to increase the efficacy of DLX against bacterial strains of MRSA. The uncoated (DLX-Lips) and coated liposomes (C-DLX-Lips) were characterized in terms of particle size, zeta potential, polydispersity index (PDI), drug entrapment, *in vitro* drug release, and *in vitro* spot susceptibility test.

2. Materials and methods

2.1. Materials

Dicloxacillin sodium salt hydrate (DLX), low molecular weight CS, and cholesterol were purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). Sodium tripolyphosphate (TPP), tween 80 and glacial acetic acid were obtained from BDH, Organic, UK. Phospholipids, Lipoid S100 was purchased from Lipoid KG, Germany. Methanol and acetonitrile (HPLC grade) were obtained from Fisher Scientific Co., UK. PBS (pH 7.4) solution (8 g NaCl (137 mM), 0.2 g KCl (2.7 mM), 1.15 g $Na_2HPO_4.7H_2O$ (8.1 mM), 0.2 g KH_2PO_4 (1.47 mM) in 1000 mL deionized water) was selected as the release medium. All other chemicals were of analytical grade.

Table 1
Preparation of the liposomes and chitosan coated liposomes.

Codes/Ingredients (Molar ratios)	Lips	C-Lips	DLX-Lips	C-DLX-Lips
Lipoid S100	1.0	0.9	1.0	0.9
Cholesterol	0.3	0.3	0.3	0.3
Tween 80	0.1	0.1	0.1	0.1
CS (mg/mL)	–	0.5	–	0.5
DLX (mg/mL)	–	–	2.5	2.5

2.2. Preparation of DLX-Lips and C-DLX-Lips

DLX loaded liposomes (DLX-Lips) were prepared by using the thin film hydration method (Liu et al., 2015). The composition of the liposomes was presented in Table 1. The lipid (1 M), cholesterol (0.3 M) and tween 80 (0.1 M) were weighed precisely and dissolved in 5 mL of chloroform:methanol (9:1) to form lipid phase. The organic solvent was removed at 50 °C in a water bath under vacuum by the rotary evaporator. As an aqueous phase, DLX (2.5 mg/mL) was dissolved in PBS (pH; 7.4) by vortex mixing. DLX solution was added to hydrate the lipid film and agitated for 5 min under 50 °C. Then the liposomal suspension was sonicated using a probe-sonicator (Branson, USA) to have uniform dispersion with nano-size.

The C-DLX-Lips were prepared by the method of electrostatic deposition (Bian et al., 2015). CS was dissolved in PBS (pH; 7.4) containing acetic acid (0.5%, *v/v*) to form CS solution (1% *w/v*). Then, the liposomes containing DLX were added stepwise to the same volume of C solution under magnetic stirring for 45 min to improve CS coating. Then, the resulting C-DLX-Lips were collected from the suspension by centrifugation at 13000 rpm for 30 min and then resuspended in PBS (pH; 7.4). Liposomes (Lips) and CS coated liposomes (C-Lips) without DLX were prepared by thin film and electrostatic deposition, respectively as described above. The obtained formulations were freeze-dried. The freshly prepared formulations containing mannitol (1%, *w/v*) were pre-frozen in freezer for 24 h at -80 °C. Then, these samples were moved to the lyophilizer at -60 °C for 72 h. The lyophilized powder was collected for further experiments.

2.3. Measurement of particle size and zeta potential

The mean particle size and polydispersity index (PDI) of each formulation were measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, UK). The particle size of the prepared formulations was evaluated using Dynamic Light Scattering (DLS) mode at 25 °C after proper dilution. Zeta potential of each formulation was evaluated by Laser Doppler Velocimetry (LDV) mode using the same Nano ZS. All experiments were performed in triplicate. Each value reported was the average of five measurements.

2.4. Encapsulation efficiency (EE) and drug loading (DL)

In order to determine EE and DL, DLX-Lips and C-DLX-Lips were centrifuged using Optima™ Max-E, Ultra Centrifuge (Beckman Coulter, Pasadena, CA). To separate the free DLX, 1 mL of suspensions were centrifuged at 40000 rpm for 40 min. The solution in the supernatant was collected and detected by HPLC.

EE% and DL% were calculated according to the following equations:

$$EE\% = \frac{W_{total}\text{-}W_{free}}{W_{toal}} x100 \quad (1)$$

$$DL\% = \frac{Wtotal\text{-}Wfree}{Wmass} x100 \quad (2)$$

where W_{total}, W_{Free} and W_{mass} are the amount of DLX added, amount of DLX in the supernatant and the total weight of the formulation, respectively.

2.5. In vitro drug release

The *in vitro* release studies of DLX from DLX -Lips and C– DLX -Lips were carried out by the dialysis technique (Gu et al., 2012). An accurate quantity of the formulations was suspended in 2 mL of release medium (PBS; pH 7.4) in the dialysis tubing (cut off 14,000 kDa). This system was incubated in 50 mL beakers (PBS; pH 7.4). The dissolution medium was maintained at 37 °C ± 0.5 °C with shaking speed at 50 rpm. An aliquot of 5 mL sample was withdrawn at various time intervals and replenished with equal volumes of fresh dissolution medium to maintain the constant volume. The content of DLX in the samples was determined by HPLC. The mean calculated values were obtained from 3 replicates.

2.6. HPLC assay

DLX was analyzed using HPLC with some modification (Bhinge et al., 2014). A Waters HPLC system (Waters Co., Milford, MA) equipped with a reverse phase C-18 column (μBondapak™, 4.6 × 150 mm, 10 mm particle size), a pump (Waters 515), an automatic injector (Waters 717plus), and a UV/vis detector (Waters™ 2487 a Dual absorbance detector, USA) were used. The mobile phase consisted of 5 mM phosphate buffer (pH; 5.4):acetonitrile:methanol (42:55:03, *v*/v/v) and the eluent was monitored at 225 nm at a flow rate of 1.5 mL/min. The injection volume for DXT analysis was 10 μL.

2.7. Bacterial strains and bacterial growth media

Bacterial strains of MRSA used in this study were gifted from King Khalid University Hospital. According to the Clinical and Laboratory Standards Institute (CLSI) (Wayne et al., 2007) *S. aureus* strains exhibiting growth on MHA supplemented with 2% NaCl and ≥ 4 μg/mL Oxacillin were considered methicillin-resistant while *S. aureus* strains only exhibiting growth on MHA supplemented with 2% NaCl when Oxacillin-levels are ≤2 μg/mL were considered methicillin-susceptible.

2.7.1. In vitro spot susceptibility test

Using aseptic technique, several well isolated colonies of *S. aureus* from the streak plate were transferred into 1 mL of PBS, contained in a 1.5 mL microfuge tube. The transferred *S. aureus* was re-suspended thoroughly by vortexing the mixture or pipetting up and down repeatedly. The turbidity of the *S. aureus* suspension was adjusted to that of a 0.5 McFarland Standard using a spectrophotometer (Sambrook et al., 1989). Adjusted Mcfarland (50 μL) of *S. aureus* was added to 5 mL of 0.7% agarose and poured on the surface of MHA-salt plate and allowed to dry (MacPherson, 1973). The plates were then filled with 10 μL of diluted DLX solution as a control (1), Lips (2), DLX-Lips (3), C-Lips (4) and C-DLX-Lips (5) and allowed to diffuse at room temperature. Allowed spots in the plates were dried and then incubated at 37 °C for 24 h. After incubation, plates were examined to detect and measure any zone of inhibition. This was interpreted as an indication of susceptibility of MRSA to various tested formulae.

2.8. Statistical data analysis

Data analysis was carried out with the software package, Microsoft Excel, Version 2010 and origin software, version 6.1. Results were expressed as mean ± standard error ($n = 3$ independent samples).

Table 2
The characterization of the liposomes and chitosan coated liposomes.

Codes	Particle size (nm)	PDI	Zeta Potential (mV)
Lips	117.3 ± 6.4	0.211 ± 0.042	−6.4 ± 0.4
C-Lips	224.2 ± 16.2	0.352 ± 0.074	8.2 ± 1.16
DLX-Lips	178.5 ± 13.6	0.247 ± 0.008	−12.7 ± 1.2
C-DLX-Lips	263.4 ± 19.1	0.411 ± 0.082	15.7 ± 0.8

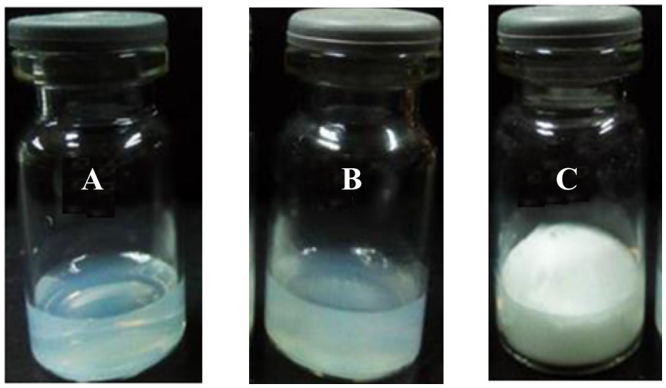

Fig. 1. Photographs of (A) DLX-Lips (B) C-DLX-Lips, (C) freeze-dried.

3. Results and discussion

3.1. Particle size and zeta potential measurements

DLX-Lips and C-DLX-Lips were prepared for the purpose of cellular uptake to enhance their antibacterial efficiency. The obtained formulations were characterized in terms of particle size, PDI and zeta potential (Table 2). The photographs of fresh-prepared DLX-Lips and C-DLX-Lips and the freeze-dried powder of C-DLX-Lips are shown in Fig. 1. It was observed that the suspension of DLX-Lips and C-DLX-Lips were translucent and showed light blue opalescence. The lyophilized formulation appeared as a white fluffy powder.

It was observed that all investigated dispersions exhibited particle sizes in the nano-range with low PDI. The reduction of the particle size of Lips could be attributed to the insertion of edge activators (tween 80) into the bilayers. This behavior could increase the flexibility and cause a decrease in the radius of the Lips (Alomrani et al., 2014). The results showed that the particle sizes of Lips and DLX-Lips formulations were 117.3 and 178.5 nm, respectively. It was noticed that the particle size was increased after addition of DLX, as in DLX-Lips.

Moreover, the particle sizes of C-Lips and C-DLX-Lips were significantly increased (mean of 224.2 and 263.4 nm, respectively) compared to uncoated ones. Thus, the presence of CS led to an increase in particle size of the coated liposomes (Table 2, Fig. 2). The CS produced a highly viscous dispersed phase leading to a bigger particle size of the formation (Karn et al., 2011). This increase in the particle size confirmed the presence of the CS coated layer to the liposomal surface. The recorded values of PDI were < 0.5, which represent an acceptable particle size distribution indicating the narrow distribution (Table 2).

The zeta potential values of uncoated liposomes were − 6.4 and − 12.7 mV for Lips and DLX-Lips, respectively. The negative surface charges were due to the presence of the charge carried by lipid. The high negative zeta potential of DLX may be due to the negative charge resulting from the carboxyl of DLX. After addition of CS to get chitosomes, the zeta potential values were measured to be 8.4 and 15.7 mV for C-Lips and C-DLX-Lips, respectively. The surface charge inversion indicates the electrostatic interaction of positively charged CS with the

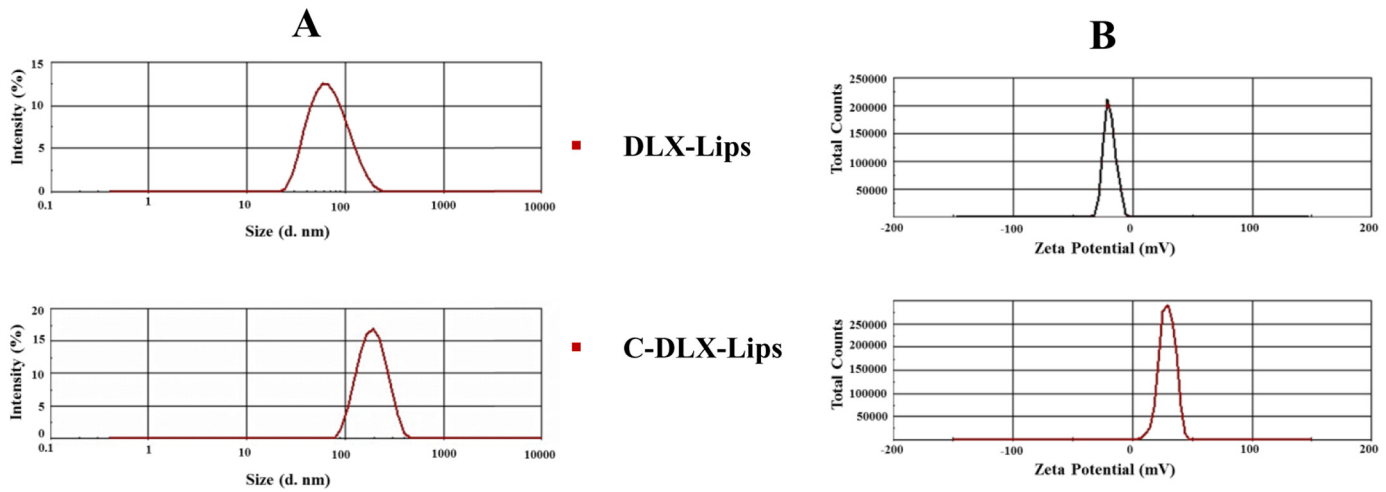

Fig. 2. Particle size (A) and zeta potential (B) of DLX-Lips and C-DLX-Lip.

negatively charged liposome, which is considered the main impact in the formation of chitosomes (Table 2, Fig. 2). This behavior can be clarified by two mechanisms. One is the electrostatic interaction between negatively charged Lips and CS with positive charge (amine groups). While, the other mechanism is the hydrogen bonding between the polysaccharide (CS) and glyceride head groups (Lip) (Song et al., 2011). After coating, CS carried by liposomes may cause the shielding effect on the liposomal surfaces. So, the positively charged liposomes supported the conclusion that CS coating onto the liposomal surfaces occurred (Wang and Kong, 2013).

3.2. Entrapment efficiency (EE%) and drug loading (DL%)

An important aspect of effective treatment of MRSA infections is assuring that an appropriate amount DLX exists at the site of action for enough period of time. Then, the high EE and DL of these formulations are a key aspect of successful therapy. The ability of the Lips and C-Lips to entrap DLX was studied. Significant differences between Lips were detected as displayed in Fig.3. The encapsulation of the C-Lips was increased compared with that of the Lips. Thus, after coating with CS, the EE% and DL% reached 62% and 5.6% for C-DLX-Lips compared to 38% and 3.1% for DLX-Lip. This behavior is due to the absorption saturation influence of CS on the surface of the Lips (Chen et al., 2016). In addition, the increase of EE% might be due to the surface properties of C-DLX-Lips, which created the ionic interaction between positive CS and negative DLX in solution producing high drug loading (Chen et al., 2016). The coated CS concentration combined with the particle Size and surface charge need to be examined further.

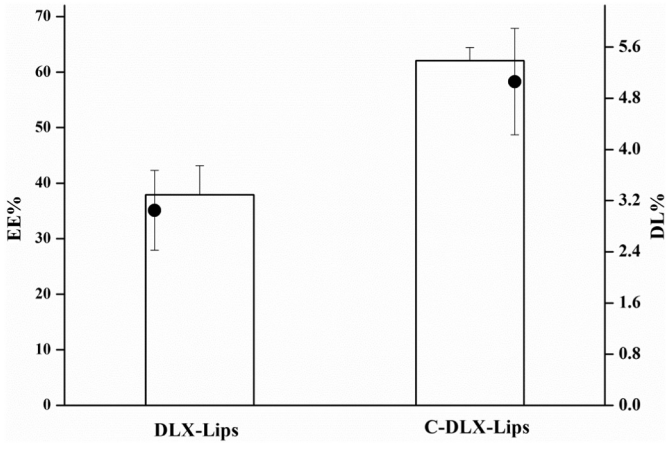

Fig. 3. EE% and DL% of DLX loaded liposomes and chitosan coated liposomes.

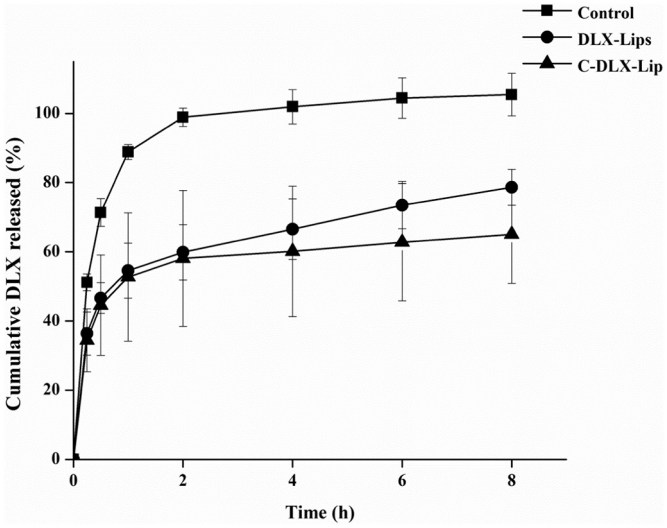

Fig. 4. *In vitro* release profile of DLX loaded liposomes and chitosan coated liposomes.

3.3. In vitro release studies

The *in vitro* release study was performed to investigate the influence of liposomes coating on DLX release. Fig. 4 shows the release profiles of two different dosage forms, DLX-Lips and C-DLX-Lips compared to drug solution as a control. DLX was released rapidly from the dialysis bag within the first hour by using the drug solution, which implied that the diffusion was not affected by dialysis bag. DLX-Lips and C-DLX-showed an initial rapid DLX release followed by slow release. It was observed that the drug release is strongly influenced by the properties of liposomes. The rapid release (2 h) of about 55% and 52% of DLX from DLX-Lips and C-DLX-Lips were detected, respectively. An initial rapid release may be related to the immediate release of the un-encapsulated and surface-associated DLX (Fig. 4). Furthermore, the slow release could be caused by the diffusion of the entrapped drug from the coated and uncoated Lips up to 8 h, as 78%, and 66% DLX for DLX-Lips and C-DLX-Lips, respectively. After CS coating, the release of DLX from CS coated Lips was further suppressed compared to the uncoated one. In contrast, the high release of uncoated Lips was probably due to the swelling or disruption of the vesicles (Beenken et al., 2014). Moreover, the slow release of C-DLX-Lips could be attributed to CS envelop of the lipid

Table 3
Measurement of inhibition zone.

Drug/polymer	Zone diameter (mm) ± SD
DLX	33.0 ± 0.89
C-DLX-Lips	34.3 ± 0. 51
DLX-Lips	55.0 ± 1.70
Lips	0
C-Lips	0

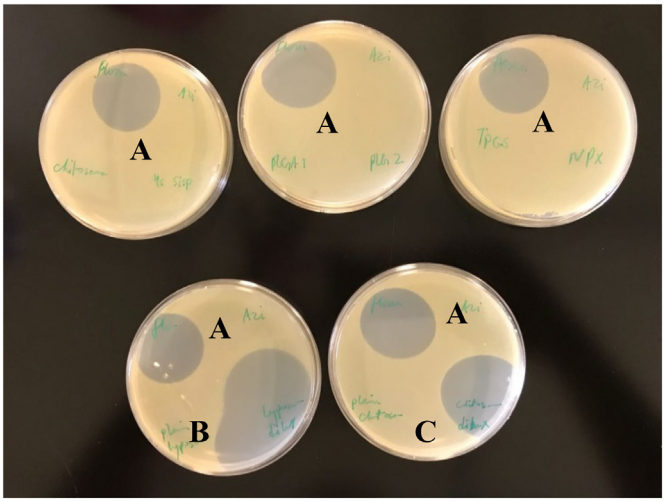

Fig. 5. *In vitro* susceptibility test using top-soft agar diffusion method. (A) is the control DLX, (B) the DLX-Lips, and (C) C-DLX-Lips.

matrices. The coated liposomes could enhance the stability and slow release of liposomes (Beenken et al., 2014).

3.4. Spot susceptibility test

The top-soft diffusion method is a simple method for determining antimicrobial resistance due to its convenience, efficiency and the cost. A diluted MRSA isolate at a standard concentration was mixed with 5 mL of 0.7% agarose and poured onto Mueller-Hinton agar, then allowed to dry. The agarose was diffused throughout the growth medium forming a thin seeded layer, which was then spotted with a particular antibiotic that evenly dispensed onto the surface. After an overnight incubation, the bacterial growth against each formula was observed. If the test isolate was susceptible to a particular antibiotic, a clear area of "no growth" was observed around that particular formula. Table-3 shows the average measurement of the inhibition zone ± SD. Fig. 5 shows the inhibition zone of the control DLX compared to DLX-Lips and C-DLX-Lips. It is clear that plain Lips were not effective as no clear zone presented (Fig. 5). Although C-Lips showed faint inhibition DLX-Lips and C-DLX-Lips showed reasonable zone. Loaded formulae showed double zone in size compared to the control (corresponding to DLX solution). Different mechanisms have been proposed for enhanced MRSA cell membrane permeation of drugs delivered from liposomes. These supposed cell membrane permeation mechanisms can be categorized into four categories. Firstly, the free drug might permeate through the membrane independently after liberating from the liposomes (Rukavina et al., 2016). Secondly, the high drug permeation could be attributed to exchanges in the phospholipid monolayer of the membrane with similar lipid components of liposomes (Strathmann et al., 2002). Thirdly, liposomes could fuse with the surface of cell membrane leading to increased drug partitioning into the membrane (Sachetelli et al., 2000). Fourthly, the fused liposomes might engulf by endocytosis mechanism and transport directly to the lysosomes, releasing entrapped drug into the surrounding cytoplasm (Gao et al., 2013). The antibacterial action of CS by interfering with microbial virulence factors is highly expected to be related to the construction of bacterial biofilm (Kandimalla et al., 2013). CS alone is limitedly used due to its potential cytotoxicity, which could be overcome by the fabricated nano-carriers, like physiologically suitable phospholipids. Then, nano-carriers could be attained by combining liposomes-entrapped DLX and CS with augmented antimicrobial results. The enhanced MRSA cellular uptake of DLX was due to the subsequent cell internalization by positive charged CS and the negative glycocalyx on the cell membrane (Liu et al., 2015). Fig. 6 is a repeat test of only effective formulae, DLX-Lips and C-DLX-Lips.

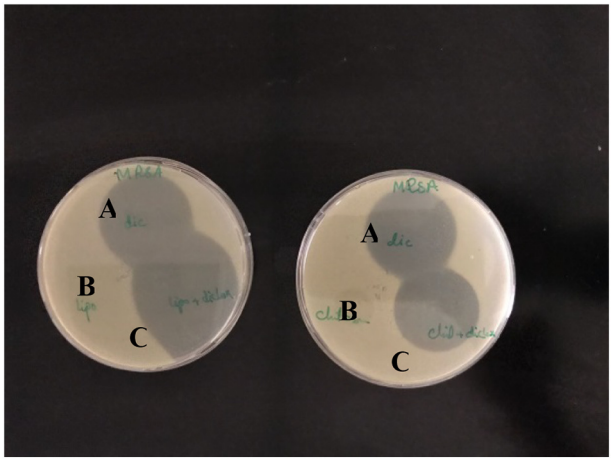

Fig. 6. *In vitro* susceptibility test using top-soft agar diffusion method; (A) the control DLX, (B) empty liposomes, and (C) DLX loaded formula. Right is for the DLX-Lips and left is for C-DLX-Lips.

4. Conclusion

The C-Lips were developed due to their efficiency as a carrier system for treatment of MRSA isolates. C-DLX-Lips have significantly improved EE% and DL% of DLX compared to DLX-Lips. The liposomal size of coated Lips was increased in comparison to uncoated Lips. However, the increase of vesicle size may limit the antibacterial activities of C-DLX-Lips, despite having a similar drug release profile of DLX-Lips. *In vitro* spot susceptibility test on MRSA revealed that DLX-Lips exhibit significantly wider zone of inhibition in comparison to DLX or C-DLX-Lips. This may be attributed to the flexibility of the Lips and relatively small size compared to C-DLX-Lips. On the basis of these results it could be suggested that the Lips may enhance overall efficacy of DLX against MRSA. Nevertheless, testing C-DLX-Lip *in vivo* on an MRSA infected animal model is needed to draw a clinically relevant conclusions.

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