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Formulation of β-lactam Antibiotic Encapsulated Micro- and Multiple-Emulsions, and Evaluation of its Antibacterial Activity against β-lactamase Producing Uropathogens

Mobashshera Tariq[1], Swapnil Mohurle[2], Vandana B. Patravale[2] and K. Aruna[1]*

[1]Department of Microbiology, Wilson College, Mumbai 400 007, India
[2]Department of Pharmaceutical Science and Technology, Institute of Chemical Technology, Matunga, Mumbai 400 019, India
**Corresponding author*

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A B S T R A C T

Growing incidence of β-lactam resistance mediated by Extended Spectrum β-Lactamases (ESBLs) and Metallo-β-lactamases (MBLs) is a major concern due to their extreme resistance towards the commonly used antibiotics. The present study was undertaken to determine the in-vitro effect of antibiotic encapsulated micro/multiple emulsions on clinical isolates of 68 ESBL and 7 MBL-producing uropathogens by agar diffusion method. The water in oil (w/o) microemulsions of amoxicillin (particle size 11-15nm) and ceftazidime (particle size 17-19nm) did not exhibit antibacterial activity. However, water in oil in water (w/o/w) multiple-emulsion of both the drugs exhibited activity. Further, the effect of glyceryl dilaurate (GDL), a lipid emulgent having inherent antibacterial activity was studied. Ceftazidime multiple emulsions, with and without GDL showed zones of inhibition against all test uropathogens in the range of 11-32mm. Amoxicillin w/o/w multiple-emulsion without GDL was found to be ineffective. However, in presence of GDL, comparatively better activity was observed with zones of inhibition in the range of 10-13mm. All micro/multiple emulsions showed a PDI (Poly Dispersity Index) between 0.1-0.3. This study hereby indicates the potential of antibiotic micro/multiple emulsions to be used effectively for intracellular targeting.

Introduction

Urinary tract infections still remain the most commonly observed disease in any community settings. It is also one of the most frequently occurring nosocomial infections (Gastmeier, 1988). Indiscriminate use of antibiotics over the years has accompanied the evolution of antibiotic resistance (Gupta *et al.*, 2011; Talan *et al.*, 2008), which is now a worldwide phenomenon. The epidemiology of antibiotic resistant bacteria, however, varies from region to region and from one infection type to another. It also differs from one year to another (Wagenlehner and Naber, 2004).

Antibiotic resistance is an increasing

problem in urological practices, the main reason being the exhibition of resistance by the pathogens towards more than one antibiotic due to the presence of multiple resistance genes (Ruiz *et al*., 2013; Sirot *et al*., 1991; Charrakh *et al*., 2011).

The common problems faced in combating pathogens are their ability to evolve as a resistant mutant by either acquiring resistance by horizontal gene transfer (Cotton *et al*., 2000) or expressing inherent resistance genes which are vertically transferred (Platt *et al*., 1986). In such cases, the production of enzymes hydrolyzing the frequently encountered antibiotics remains the most commonly observed resistance mechanism by pathogens. Since, the antibiotics are hydrolyzed even before they reach the cell due to externally produced enzymes, they are rendered ineffective.

β-lactamases are one such enzymes that has almost ceased the use of most β-lactam antibiotics in clinical practices for countering UTIs. Amoxycillin, once used as a first-line therapy for UTIs, is now rarely effective in complicated cases of UTI. Third generation cephalosporins such as ceftazidime, cefotaxime and ceftriaxone which are now preferred for bacterial UTIs are also reported to be hydrolyzed by production of Extended spectrum β-lactamases (ESBLs) and Metallo-β-lactamases (MBLs). ESBLs are enzymes capable of hydrolyzing oxyimino-cephalosporins and are inhibited by β-lactamase inhibitors (Bush *et al.*, 1995). MBLs are bacterial zinc enzymes that are able to hydrolyze most β-lactam antibiotics, including the newer compounds like carbapenems, and are not inhibited by the available therapeutic β-lactamase inactivators (e.g. clavulanate and penicillanic acid sulphones). The incidence of ESBL and MBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options (Podschun *et al.*, 1998).

For this reason, it is not only important to design the most appropriate new drug for empirical therapy, but it is equally necessary to modify the drug delivery system in a way that makes the existing antibiotics useful. This can be done by studying the mechanism of antibiotic resistance and challenging the bacterial defense system with innovative mechanisms that can prove useful in combating infectious diseases such as UTI. Over the decades, the strategy for effective delivery of antibiotics has been gradually shifting towards the use of nano-carriers based on the fact that they can deliver the drug more efficiently inside the cell owing to their particle size and excipient properties (Nagahara *et al*., 1998).

A simple approach towards the problem of extracellular hydrolysis of antibiotics can be overcome by intracellular targeting of drugs. This can be done by encapsulating it in a stable nano-system that prevents the hydrolysis of drugs in the external vicinity and allows easy permeation through the cell membrane of the pathogens. Since micro-emulsions are thermodynamically stable solutions, with ultra-low interfacial tension, large interfacial area and inner structure of nanodroplets, they can solubilize a varied number of soluble as well as partly soluble drugs, as well as other biologically important components (Spernath and Aserin, 2006). Moreover, the ease of formulation of micro-emulsion favors its use in pharmaceuticals where the pathogens can be exposed to the optimum concentration of drugs using such systems (Fattal, 1992). Another important nano-system of interest is multiple emulsions which are complex poly-dispersed systems that provide addition stability to formulations especially designed

for oral delivery to prevent inactivation of drug in the acidic environment of the gastro-intestinal tract (Khan *et al*., 2006).

The current study was carried out with an attempt to investigate the effect of ceftazidime and amoxicillin loaded micro/multiple emulsions formulated with or without GDL, on ESBL and MBL producing uropathogens.

Materials and Methods

Chemicals

Capryol® 90 (Propylene Glycol Monocaprylate) obtained from Indchem International (Mumbai, India) was used as an emulsifying agent during the formulation. Transcutol® P, Diester (Gattefosse, Mumbai, India), Tween® 20 (S.D. Fine Chemicals, Mumbai, India) and Solutol HS 15 (BASF) were used as non-ionic surfactants in the preparation of micro/multiple emulsions. Other oils used in the formulation of multiple emulsions were labrafac, oleic acid, capmul MCM.

Micro/multiple Emulsions Formulation

The micro/multiple emulsions were prepared as per the method described by Uson et al (2004) and Porrasa et al (2004) previously. In the present study, since both the drugs were hydrophilic, w/o type micro-emulsion was prepared. However, many a times, the problem which arises due to such binary phase emulsion is that the drug may leach out due to single barrier and hence it may not serve our purpose to encapsulate the drug in the micro-emulsion formulation to protect it from the acidic environment (Constantinides and Scalart, 1997). Keeping this in mind w/o/w multiple-emulsion system was prepared to get the desired effect of the drug against the pathogens. Various surfactants were screened on the basis of HLB (hydrophilic lipophilic balance) value and solubility of the antibiotics.

Formulation of w/o type micro-emulsions: It was prepared by dissolving 1g of drug in 100ml double distilled water. To this mixture, transcutol P was added to stabilize the emulsion. Caproyl 90 was then added as the oil phase with continuous stirring at 750rpm to form the micro-emulsion system. Different ratios of water surfactant mixture to oil were studied to minimize the use of both.

Formulation of w/o/w type multiple-emulsion: It was prepared by solubilizing the antibiotic in the water/surfactant mixture which was then added to the previously optimized oil with continuous stirring. Solutol HS 15 was added to stabilize this system with the external water phase.

Formulation of w/o/w type multiple-emulsion with GDL: It was prepared as per the previous method except that during the first stage of formulation of water/surfactant mixture, GDL was added. This was done to explore the possibility of any further enhancement of antibiotic activity (Patravale, 2011; Joshi *et al*., 2008).

Characterization of Micro/multiple-emulsion Formulations

The formulations were optimized to get the nano size and good Poly Dispersity Index (PDI). The resultant micro/multiple emulsions were analysed for particle size at various time points and at various dilutions to check the thermodynamic and overall stability.

Determination of Mean Particle Size and Poly Dispersity Index (PDI)

The average size of the antibiotic micro/multiple emulsion was measured

using a Zeta-sizer model Nano-ZS, Malvern Instrument, which works on the principle of dynamic light scattering. The instrument has a 4 mW He-Ne red laser at 633nm. The light scattering is detected at 173° by non-invasive back-scatter (NIBS) technology with measuring range from approximately 0.6 nm to 6µm. The samples were placed in the folded capillary cells and the readings obtained for particle size and PDI were recorded (Elnaggar *et al*., 2009).

Effect of Dilution on Particle Size and PDI

Different dilutions of the micro/multiple emulsions were prepared using saline and the resultant particle size and PDI were measured to check the compatibility with large volume intravenous fluids.

Test Organisms

Drug resistant gram negative uropathogens were collected from local pathological laboratories and hospitals and characterized for ESBL and MBL production in our laboratory in a previous study (Aruna and Tariq, 2012; Tariq and Aruna, 2015). Sixty eight ESBL and seven MBL-producing uropathogens were used in the study as listed in table 1.

These isolates were maintained on Luria-Bertani (LB) agar slants supplemented with 100µg/ml of ampicillin and stored at refrigerated conditions.

Qualitative In-vitro Evaluation of Drug Solubilized Micro/multiple Emulsion by Agar well Diffusion Method

The effect of drug solubilized micro/multiple emulsion on the test pathogens, were assayed by agar well diffusion method as described by Toda et al (1989). A loopful of the test isolates were inoculated in 10ml of Brain Heart infusion (BHI) broth and incubated at 37°C for 24h in order to obtain actively growing log phase cultures. Sterile 20ml of molten nutrient agar was cooled to approximately 40°C and 0.4ml test culture (0.1 O.D. at 540nm) was seeded and poured into sterile petri plates. Using a sterile cork-borer (8mm in diameter), wells were punched in each plate after solidification of the medium. 50µl of different concentrations of micro/multiple emulsion was then added to the wells and incubated at 37°C for 24h to observe the zones of inhibition. Control wells were also set up using 50µl of micro/multiple emulsions prepared without antibiotics to check for antibacterial activity of the surfactants if present.

Statistical Analysis

All studies were carried out in triplicates and results were recorded as mean.

Results and Discussion

Use of micro/multiple emulsions in targeting epithelial cells and macrophages to get rid of intracellular pathogens is well known for decades (Tulkens and Trouet, 1978; Tomioka *et al*., 1991; Price *et al*., 1994). However, recently the studies are more focused on targeting the pathogens itself for delivering the antibiotics (Saranya *et al*., 2012; Karthikeyan *et al*., 2012). This study was carried out to study the in-vitro effect of antibiotic micro/multiple emulsions on 68 ESBL and 7 MBL producing uropathogens.

Micro/multiple Emulsion Formulation

In the present study, amoxicillin and ceftazidime encapsulated micro/multiple emulsions were prepared aiming at the formulation of thermodynamically stable

drug delivery systems which can protect the drug from degradation in the systemic and local environment produced by the resistant bacterial strains.

The system was prepared on the basis of the nature of solubility of the drug. Since both the antibiotics used in the present study were highly hygroscopic in nature and hence highly water soluble, the W/O system was prepared using caproyl 90 as the oil phase. Other oils which were used for the micro/multiple emulsions formulation were capmul MCM, oleic acid and labrafac which fall under the GRAS (generally regarded as safe) category of oils. Traditionally thermodynamically stable micro/multiple emulsions have been prepared on this principle (Trotta, 1999; Panayiotis *et al*., 1994). The systems containing oils, other than capmul MCM however, turned turbid on the addition of saline to the systems. This is an indication of unstable system. Other studies have also been carried out that shows formulation of more stable micro/multiple emulsions in presence of capmul MCM (Khoo *et al*., 1998; Constantinides *et al*., 1996).

Surfactants were selected on the basis of their respective HLB values. Surfactants with higher HLB value are used in formulation of micro-emulsion, since, low HLB value surfactants will solubilize the oils and thus prevent the formation of separate phases for trapping the drug molecules (Constantinides *et al*., 1996). Since oil was used as the continuous phase for w/o system, Transcutol P was used as the surfactant. Similar principles were used while formulating w/o/w multiple-emulsion where Transcutol P and Solutol HS 15 were used as the internal and external surfactants respectively. In this case, oil was acting as a continuous phase internally and water was acting as a continuous phase externally.

The emulgent i.e GDL was further added to see if it helps in enhancing the overall activity of the antibiotics loaded multiple emulsions. The interesting observation found was the multifold reduction in emulsion size by addition of GDL. This may be due to the additional surfactant property provided by GDL, which causes the formation of compact and stable nano structures.

Determination of Mean Particle Size and Poly Dispersity Index (PDI)

Figure 1 shows the particle size distribution and Figure 2 shows the PDI of the various micro/multiple emulsions. The particle size was found to be below 25nm in case of w/o emulsions while w/o/w multiple emulsions were found to be in the range of 150 to 200nm. Interesting observation was made on the addition of GDL to w/o/w multiple emulsions where the particle size was reduced by multiple times to less than 30nm.

The PDI was found to be less than 0.3 in all cases indicating uniform particle size distribution.

The effect of dilution on particle size and PDI was carried out using Malvern particle sizer in order to check the thermodynamic stability of the system. Each formulation was diluted 5X, 10X and 50X times. Figures 3 and 4 indicate the effect of dilution on the particle size and PDI respectively.

The extent of dilution in case of amoxicillin loaded w/o micro emulsion did not show significant effect till 10 fold dilution. But it showed dramatic effect on the particle size upon 50 fold. As shown in figure the particle size increased from 20 nm to 50 nm on 50 fold dilution.

In case of w/o/w and w/o/w with GDL however, the effect was not much significant at all the dilutions. No phase separation was seen in any of the multiple emulsion prepared. This shows an excellent thermodynamic stability of the system.

Usually drug delivery by w/o micro-emulsions for oral or parenteral route is difficult by the fact that they are destabilized extensively by an aqueous phase (Narkhede *et al*., 2014). This can be seen in our study too when w/o microemulsion is diluted beyond 50 times. Due to the increase in the volume, fraction of the aqueous phase to surfactant is also increased which also causes change in the ratio of water to oil. This ultimately leads to the droplet size increase and percolation eventually. If continuous dilution takes place then phase inversion or separation may occur which may results in load dumping (Muzaffar *et al*., 2013).

Phase inversion phenomenon which might take place in case of w/o micro emulsion, on dilution with the aqueous phase does not happen in o/w or w/o/w kind of micro/multiple emulsions.

Also both the drugs in the current studies are hydrophilic so increase in the volume fraction of the aqueous phase to the surfactant doesn't result in the dramatic or major changes in the particle size. This particular property of these micro/multiple emulsions prepared will be helpful post-delivery of the formulation as it will result in the controlled release in the in vivo environment.

Qualitative In-vitro Evaluation of Drug Solubilized Micro/multiple Emulsions by Agar well Diffusion Method

Amoxicillin w/o micro-emulsion as well as w/o/w multiple-emulsions did not show antibacterial activity against the test pathogens. This may be explained by the fact that all test isolates used in the study were resistant to amoxicillin as well as ceftazidime and, drug encapsulated micro-emulsions can be used for enhancement of antibacterial activities only, if present. Inherent resistance in pathogens may occur due to reasons like presence of efflux systems, intracellular enzymes, alteration of drug binding proteins on their surface etc. (Wagenlehner and Naber, 2004). This allows proliferation of pathogens even in presence of high concentrations of antibiotics. Since amoxicillin was used as a first line drug for treatment of infections since a very long time, it is apparent to experience resistance of pathogens towards this antibiotic. In a longitudinal study carried out in Mumbai for a period of 15 years has shown development of resistance towards amoxicillin and similar antibiotics among 75% of uropathogens by 1980s (Acharya *et al*., 1989). Most of ESBL and MBL producing uropathogens are completely resistant to amoxicillin as observed in recent studies (Aruna and Tariq, 2012; Tariq and Aruna, 2015).

Contrary to amoxicillin, ceftazidime is a third generation cephalosporin antibiotic, introduced during 1985 in the Indian markets (Acharya *et al*., 1989). The resistance to this antibiotic is not as widespread as amoxicillin. However, most of ESBL and MBL producers exhibit resistance towards this antibiotic (Aruna and Tariq, 2012; Tariq and Aruna, 2015). In our study, ceftazidime w/o micro-emulsion was found to be equally ineffective as compared to amoxicillin. However, w/o/w multiple-emulsions of ceftazidime showed appreciable zones of inhibition (in the range of 11-32 mm) against the test pathogens (Table 2).

Table.1 Test Organisms used in the Study

Pathogens	ESBL producer	MBL producer
Escherichia coli	39	3
Klebsiella pneumoniae	8	2
Proteus mirabilis	4	-
Proteus vulgaris	3	-
Pseudomonas aeruginosa	3	1
Citrobacter amalonatius	1	-
Citrobacter diversus	9	1
Enterobacter aerogenes	1	-
Total	68	7

Table.2 Effect of Micro/multiple Emulsions on Test Pathogens

Isolates	**Zone of inhibition range in mm (n=75)**					
	500 µg/ml ceftazidime multiple-emulsion		**500 µg/ml Amoxicillin nano-emulsion**		**Emulsion control**	**Antibiotic Control**
	w/o/w	**w/o/w with GDL**	**w/o/w**	**w/o/w with GDL**		
ESBL producers						
E. coli	11.33-30.28	11.67-32.82	-	10.52-11.66	-	-
P. aeruginosa	17.28-22.67	12.66-20.92	-	-	-	-
K. pneumonia	17.52-38.84	16.33-22.58	-	12.67-12.82	-	-
Citrobacter spp.	13.92-36.33	12.68-24.33	-	10.75-12.58	-	-
Proteus spp	12.67-23.33	12.33-26.33	-	10.66-12.72	-	-
MBL producers						
E. coli	15.33	13.66	-	-	-	-
E. coli	32	12.33	-	-	-	-
P. aeruginosa	-	16	-	-	-	-
E. coli	30.66	24.33	-	11.66	-	-
P. aeruginosa	22.66	22	-	12	-	-
K. pneumonia	21.33	17.33	-	-	-	-
C. amalonatus	-	23	-	-	-	-

Figure.1 Particle Size Distribution of Formulations

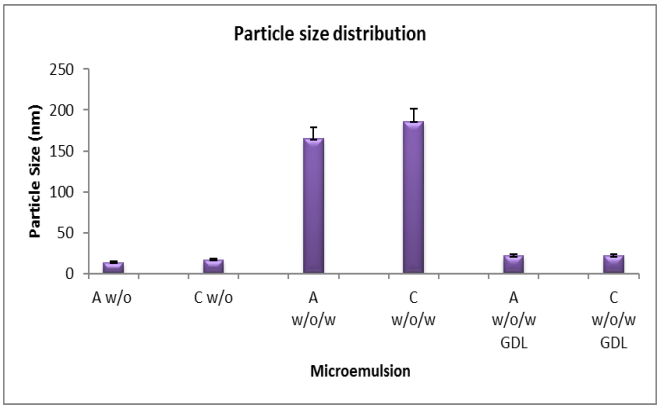


Figure.2 Poly Dispersity Index Distribution of Formulations

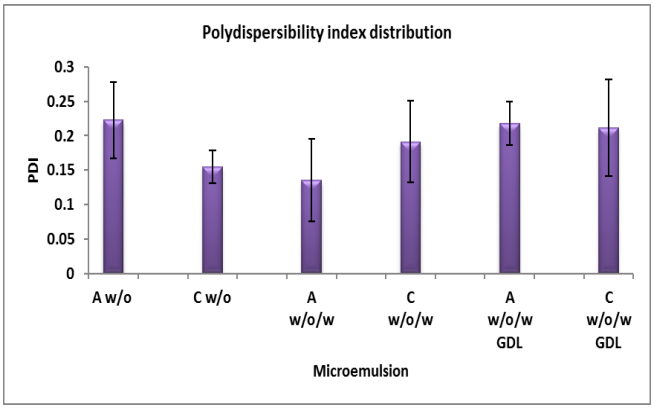


Figure.3 Effect of Dilution on Particle Size of Formulations

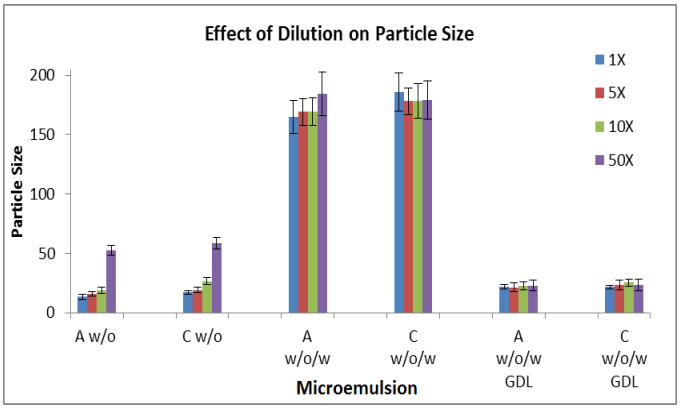
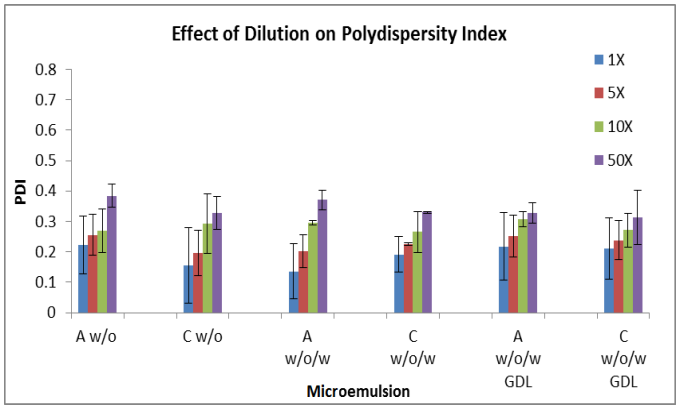


Figure.4 Effect of Dilution on Poly Dispersity Index of Formulations



In addition to inherent resistance, ineffective antibacterial activity may be a result of reduced stability of drugs carriers, less than optimum drug concentration etc. The higher activity of w/o/w multiple emulsions may be due to their higher kinetic stability owing to the presence of many surfactants and hence higher exposure of the pathogens (Shinoda and Lindman, 1987). Unlike similar antibacterial studies carried out (Al-Adham *et al*., 2012; Teixiera *et al*., 2007), where solubilization of hydrophobic drugs was the key aim, this study was carried out to prevent the hydrolysis of β-lactam antibiotics by extracellular β-lactamase enzymes. This is possible only in presence of a kinetically as well as thermodynamically stable system as observed in w/o/w multiple-emulsions of ceftazidime. To the best of our knowledge, this is the first study carried out to evaluate the enhancement of antibacterial efficacy of antibiotic loaded micro- and multiple-emulsions against uropathogens, by preventing extracellular hydrolysis of the same.

The antimicrobial activity of ceftazidime loaded w/o/w multiple-emulsions was comparatively lower but appreciable zones of inhibition (in the range of 11-25 mm) were observed on addition of GDL. Reduction of particle size in presence of GDL may be due to the additional surfactant property provided by it. Decrease in particle size with or without enhancement of antimicrobial activity can help in the preparation of specific emulsions where particle size is of prime importance.

In conclusion, the study shows the possible use of antibiotic encapsulated micro/multiple emulsions in targeting the pathogens that produce extracellular enzymes like β-lactamases. With the use of such micro/multiple emulsions the existing antibiotics can be useful in combatting infections by drug resistant bacteria, which are otherwise ineffective or requires extremely high dose for its activity.

References

Acharya, V.N., Almeida, A., Jadav, S.K., Sharma, S.D. 1989. Netilmicin in multiple resistant nosocomial urinary tract infections in patients with normal and impaired renal function. *Indian Practitioner*., 1: 853-7.

Al-Adham, I.S., Al-Nawajeh, A.S., Khalil, E., Collier, P.J. 2012. The Antimicrobial Activity of Oil-in-Water Microemulsions is predicted by their position within the

Microemulsion Stability Zone. *Int Arabic J Antimicrob Agents*., 2: 1-8.

Aruna, K., Tariq, M. 2012. Prevalance of extended spectrum beta-lactamase production among uro-pathogens in south Mumbai and its antibiogram pattern. *EXCLI*., 11: 363-373.

Borhade, V., Nair, H., Hegde, D. 2008. Design and evaluation of self-microemulsifying drug delivery system (SMEDDS) of tacrolimus. *AAPS Pharm Sci Tech*., 9: 13-21.

Bush, K., Jacoby, G.A., Medeiros, A.A. 1995. A functional classification scheme for β- lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*., 39: 1211-1233.

Charrakh, A.H., Yousif, S.Y., Al-janabi, H.S. 2011. Occurrence and detection of extended-spectrum - lactamases in Klebsiella isolates in Hilla, Iraq. *Afr J Biotechnol.*, 10: 657-665.

Constantinides, P., Scalart, P. 1997. Formulation and physical characterization of water-in-oil microemulsions containing long- versus medium-chain glycerides. *Int J Pharm.*, 158: 57-64.

Constantinides, P.P., Welzel, G., Ellens, H., Smith, P.L., Sturgis, S., Yiv, S.H., Owen, A.B. 1996. Water-in-oil microemulsions containing medium-chain fatty acids/salts: formulation and intestinal absorption enhancement evaluation. *Pharm Res.*, 13: 210-215.

Cotton, M.F., Wasserman, E., Pieper, C.H., Theron, D.C., Tubberght, V.D., Campbell, G., Fangs, F.C., Barnes, J. 2000. Invasive disease due to extended spectrum β-lactamase-producing *Klebsiella pneumoniae* in a neonatal unit: the possible role of cockroaches. *J Hospital Infect*., 44: 13-17.

Elnaggar, Y.S., El-Massik, M.A., Abdallah, O.Y. 2009. Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization. *Int J Pharm*., 380: 133-141.

Fattal, E. 1992. Intracellular targeting of antibiotics by means of biodegradable nanoparticles. *J Control Rel*., 19: 259-267.

Gastmeier, P., Kampf, G., Wischnewski, N., Hauer, T., Schulgen, G., Schumacher, M., Daschner, F., Ruden, H. 1998. Prevalence of nosocomial infections in representative German hospitals. *J Hosp Infect*., 38: 37-49.

Gupta, K., Hooton, T.M., Naber, K.G., Wullt, B., Colgan, R., Miller, R.G., et al. 2011. International Clinical Practice Guidelines for the source of origin of carbapenem resistance. A Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin Infect Dis.*, 52: 103-120.

Joshi, M., Pathak, S., Sharma, S., Patravale V. 2008. Design and in vivo pharmacodynamic evaluation of Nanostructured Lipid carriers for parenteral delivery of Artemether: Nanoject. *Int. J. Pharm.* 364: 119-126.

Karthikeyan, R., Bennett, T., Amaechi., Rawal, H., Ralph., Valerie, A., Lee. 2012. Antimicrobial activity of nanoemulsion on cariogenic planktonic and biofilm organisms. *Arch oral Biol.*, 57: 15-22.

Khan, A.Y., Talegaonkar, S., Iqbal, Z., Ahmed, F.J., Khar, R.K. 2006. Multiple emulsions: an overview. *Curr Drug Deliv*., 3: 429-443.

Khoo, S.M., Humberstone, A.J., Porter, C.J., Edwards, G.A., Charman W.N. 1998. Formulation design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. *Int J Pharm.*, 167: 155-164.

Lawrence, M.J., Rees, G.D. 2012. Microemulsion-based media as novel drug delivery systems. *Advan Drug Del Rev.*, 64: 175–193.

Lee, P.J., Langer, R.V., Shastri, P. 2003. Novel Microemulsion Enhancer Formulation for Simultaneous Transdermal Delivery of Hydrophilic and Hydrophobic Drugs. *Pharm Res.*, 20: 264-269.

Muzaffar, F., Singh, U.K., Chauhan, L. 2013. Review on microemulsion as futuristic drug delivery. *Int J Pharm Sci*., 5: 39-53.

Nagahara, N., Akiyama, Y., Tada, M., Nakao, M., Kitano, M., Ogawa, Y. 1998. Mucoadhesive microspheres containing amoxycillin for clearance of *Helicobacter pylori*. *Antimicrob Agents Chemother*., 42: 2492-2494.

Narkhede, R.S., Gujar, K.N., Gambhire, V.M. 2014. Design and evaluation of self-nano emulsifying drug delivery systems for nebivolol hydrochloride. *Asian J Pharm*., 8: 200-209.

Panayiotis, C., Jean, P.S., Cindy, L., Joseph, M., Gary, M., Harma, E., Philip, S. 1994. Formulation and Intestinal Absorption Enhancement Evaluation of Water-in-Oil Microemulsions Incorporating Medium-Chain Glycerides. *Pharma Res.*, 11: 1385-1390.

Patravale, V.B. 2011. Lipidic nanoparticulate based dosage forms of antiparasitics and antiinfectives. Indian patent 248977.

Platt, D.J., Chesham, J.S., Kristinsson, K.G. 1986. R-plasmid transfer in vivo: a prospective study. *J Med Microbiol*., 21: 325-330.

Podschun, R., Ullmann, U. 1998. Klebsiella spp. as Nosocomial Pathogens: epidemiology taxonomy, typing methods and pathogenicity factors. *Clin Microbiol Rev*., 11: 589-603.

Porrasa, M., Solansb, C., Gonzaleza, C., Martıneza, M., Guinarta, A., Gutierreza, J.M. 2004. Studies of formation of W/O nano-emulsions. *Colloids and Surfaces A: Physicochem Eng Aspects*., 249: 115–118.

Price, C.I., Horton, J.W., Baxter, C.R. 1994. Liposome encapsulation: a method for enhancing the effectiveness of local antibiotics. *Surgery*., 115: 48-487.

Ruiz, B., Vinué, L., Román, E. J., Guerra, B., Carattoli, A., Torres, C., Martínez, M.L. 2013. Molecular characterization of multiresistant *Escherichia coli* producing or not extended-spectrum β -lactamases. *BMC Microbiol.*, 13: 84-95.

Saranya, S., Chandrasekharan, N., Mukherjee, A. 2012. Antimicrobial Activity of Eucalyptus oil nanoemulsion against *Proteus mirabilis. Int J Pharm Pharm Sc.*, 4: 668-671.

Shinoda, K., Lindman, B. 1987. Organised surfactant systems: microemulsions. *Langmuir*., 3: 135-149.

Sirot, D., Champs, C.D., Labia, R., Michaud, A.D., Perroux, R., Sirot, J. 1991. Extended-Spectrum B-Lactamase between Conjugative Plasmids of *Klebsiella pneumoniae* and *Escherichia coli*. *Antimicrob. Agents Chemother*., 35: 1576-1581.

Spernath, A., Aserin, A. 2007. Microemulsions as carriers for drugs and nutraceuticals. *Advances in*

colloidal and interface science., 130: 47-64.

Talan, D.A., Krishnadasan, A., Abrahamian, F.M., Stamm, W.E., Moran, G.J. 2008. Prevalence and risk factor analysis of trimethoprim-sulfamethoxazole- and fluoroquinolone- A worldwide spread of Enterobacteriaceae resistant *Escherichia coli* infection among emergency department patients with pyelonephritis. *Clin Infect Dis*., 47: 1150-1158.

Tariq, M., and Aruna, K. 2015. Phenotypic and Molecular characterization of MBL genes among uropathogens isolated in Mumbai city. *BMRJ*., 5: 368-383.

Teixeira, P.G., Leite, G.M., Domingues, R.J., Silva, J., Gibbs, P.A., Ferreira, J.P. 2007. Antimicrobial effects of a micro-emulsion and a nano-emulsion on enteric and other pathogens and biofilms. *Int J of Food Microbiol*., 118: 15-19.

Toda, M., Okubu, S., Hiyoshi, R., Shimamura T. 1989. The bactericidal activity of tea and coffee. *Lett appl Microbiol*., 8: 123-125.

Tomioka, H., Saito, H., Sato, K., Yoneyama, T. 1991. Therapeutic efficacy of liposome-encapsulated kanamycin against *Mycobacterium intracellulare* infection induced in mice. *Am Rev Respir Dis*., 144: 575–579.

Trotta, M. 1999. Influence of phase transformation on indomethacin release from microemulsions. *J Control Rel.*, 60: 399-405.

Tulkens, P., Trouet, A. 1978. The uptake and intracellular accumulation of aminoglycoside antibiotics in lyso-somes of cultured rat fibroblasts. *Biochem Pharmacol.*, 27: 415-424.

Uson, N., Garcia, M.J., Solansa, C. 2004. Formation of water-in-oil (W/O) nano-emulsions in a water/mixed non-ionic surfactant/oil systems prepared by a low-energy emulsification method. *Colloids and Surfaces A: Physicochem. Eng Aspects*., 250: 415-421.

Wagenlehner, F.M., Naber, K.G. 2004. Antibiotics and Resistance of Uropathogens. *EAU update series*., 2: 125-135.

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