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EFFECT OF TOPICAL RECOMBINANT LYSOSTAPHIN AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTION IN A MOUSE BURN WOUND MODEL

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ABSTRACT

Increased incidence of antibiotic resistant *S. aureus* infections has spurred the need for new antimicrobial agents. Lysostaphin is an antistaphylococcal agent, which cleaves the pentaglycine cross bridges of *S. aureus* cell wall leading to its lysis. Antimicrobial activity of r-lysostaphin was tested against 73 MRSA isolates by the disc diffusion assay and minimum inhibition concentration. Burn wound MRSA infected mice were treated with mupirocin and r-lysostaphin for a period of 5 days. All the MRSA isolates were sensitive to r-lysostaphin (50 µg/disc) with a zone of inhibition ranging from 14 to 17 mm and the MIC ranged between 0.25 to 2 µg/ml. The MRSA count in untreated group was 10^8 CFUs/g tissue. Mupirocin showed 3 log reduction (10^5 CFUs/g tissue) in bacterial count and r-lysostaphin significantly reduced >5 log (10^3CFUs/g tissue) in bacterial count as compared to the untreated group. Hence, r-Lysostaphin can be used as an effective alternative treatment for MRSA infections.

KEYWORDS: **Antibiotic resistance, Methicillin Resistant *Staphylococcus aureus* (MRSA), Recombinant Lysostaphin (r-lysostaphin), Burn wound infection**

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INTRODUCTION

CDC and WHO are concerned about emergence of drug resistant microbe across the globe and described it as a global threat. The lack of new antibiotics and increased antibiotic resistance is a major public health problem for the foreseeable future[1, 2]. One such pathogen is methicillin resistant *Staphylococcus aureus* (MRSA). Currently vancomycin is the drug of choice for MRSA infections. However, the efficacy of vancomycin in the treatment of MRSA infections in compromised hosts was found to be less than satisfactory. The rate of mortality from invasive MRSA infections is reported to be 10–30% even after treatment with vancomycin[3]. Linezolid is a bacteriostatic agent, which has potent activity against MRSA isolates, vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* and penicillin-resistant *Streptococcus pneumoniae*[4]. However, mutations in the central loop of domain V of 23S rRNA have been associated with development of resistance to linezolid among both *Enterococcus* and *Staphylococcus* species[5, 6]. The continuing emergence of antibiotic resistant pathogens necessitates the need for development of new antimicrobial agents. Scientists are showing a renewed interest in enzymatic action of lysostaphin to treat staphylococcal infections. Lysostaphin is produced by *Staphylococcus simulans* biovar *Staphylolyticus*, is a glycylglycine endopeptidase zinc-containing metallo-enzyme which causes the rapid lysis of the bacteria by hydrolyzing a pentaglycine cross-bridge of the staphylococcal cell wall. Previous studies from 1960s to 1970s have demonstrated lysostaphin as an effective anti-staphylococcal agent[7, 8]. The studies on lysostaphin were discontinued due to lack of homogenous preparations of lysostaphin and the availability of other effective antibiotics. Decreasing effectiveness of routine antibiotics and the availability of r-lysostaphin, have rekindled the interest in using lysostaphin as a therapeutic agent for staphylococcal infections[9, 10]. r-Lysostaphin has been found to be an effective agent in various topical application studies, it has been reported to clear nasal colonization and keratitis[11,12]. The present study evaluates the effect of topical r-lysostaphin treatment against MRSA infection in a mouse burn wound model.

MATERIALS AND METHODS

r-lysostaphin (Sigma Aldrich L9043), vancomycin (Sigma Aldrich 861987), linezolid (Sigma Aldrich PZ0014) and oxacillin (Hi-Media CMS5372-5G) mupirocin (Bactroban, 2% Mupirocin calcium ointment) were used in the present study.

(i) Bacterial isolates

A total of 213 *S. aureus* isolates were collected from various clinical specimens like pus, burn wound, ET aspirate, sputum and blood from the patients visited to Kasturba Hospital affiliated to Manipal University, India. Isolates were identified as *S. aureus* by conventional methods and stored at -80ºC in soybean casein digest broth containing 20 % of glycerol.

(ii) Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by Kirby Bauer disk diffusion method as recommended by Clinical Laboratory Standard Institute[13]. Ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), chloramphenicol (30 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), tetracycline (30 µg), linezolid (30 µg) and vancomycin (30 µg) were used for disc diffusion assay. ATCC 43300 (MRSA) and ATCC 25923 (MSSA) were used as quality controls strains. The isolates which showed a zone diameter of ≤19 mm for cefoxitin disc (30 µg) were identified as MRSA. Inducible clindamycin resistance was tested for all the MRSA isolates obtained from clinical specimens. D-zone test was adopted to determine this inducible clindamycin resistance. An erythromycin disc (15 µg) was placed 15 mm from a clindamycin disc (2 µg) in a Kirby Bauer disc diffusion method. A flattening of the zone of inhibition in the area between the discs where both drugs have diffused after 16 hours of incubation was considered to be inducible clindamycin resistance.

(iii) Determination of in-vitro bactericidal activity of r-lysostaphin

Disc diffusion assay

Six-millimeter-diameter sterile discs (Whatman No.1; Whatman International Ltd.) were impregnated with two different concentrations of r-lysostaphin (50µg and 25µg/disc) in phosphate-buffered saline (PBS). Discs were dried at room temperature for 1 hour and stored at -20°C in a sealed container until used. MRSA isolates (with an inoculum at a McFarland standard of 0.5, corresponding to cell count was ~1x10^8 CFUs/ml) were plated on cation-adjusted Mueller Hinton agar and r-lysostaphin discs were placed on the agar surface. Following incubation at 37° C for 18 hours, the zones of inhibition was measured[14]. ATCC 25923 was used as quality control strain.

Minimum Inhibitory Concentration (MIC)

To determine MIC, microbroth dilution method was performed as per CLSI guidelines[13]. The concentrations of r-lysostaphin ranged from 0.06 to 16 µg/ml. Two fold dilutions of lysostaphin were prepared in cation-adjusted Mueller Hinton broth supplemented with 0.1% bovine serum albumin (BSA). Diluted drug was added to 96-well polystyrene plate. Wells of a 96-well polystyrene plate were inoculated with 5x10^5 CFUs/ml bacteria. A positive control for bacterial growth was included without lysostaphin in each assay. Microtiter plates were incubated at 37°C for 16 hours. ATCC 29213 (MSSA) and ATCC 43300 were tested as a quality control strains. 0.1% BSA was incorporated to inhibit nonspecific lysostaphin adherence to the polystyrene plate. MIC is expressed as the lowest concentration of lysostaphin required to inhibit the visible bacterial growth in the microtiter well.

Minimum bactericidal concentration (MBC)

MBC was determined as per CLSI guidelines[13]. Briefly, a 10 µL of 10 mg/ml proteinase K (Sigma) in PBS was added to each well of microbroth dilution assay to inhibit the residual activity of r-lysostaphin. 100 µL from the MIC, MICx2 and MICx4 wells were plated soybean casein digest agar and incubated at 37°C overnight. MBC was defined as minimum concentration required achieving and maintaining > 99.99% killing of the bacteria up to 24 hours.

(iv)Evaluation of topical treatment r-lysostaphin in a mouse burn wound model

Preparation of r-lysostaphin gel (0.25 % w/w)

Carbopol 934 (2 %) was dispersed in deionized water with constant stirring using mechanical stirrer at 1200 rpm for 2 hours. Lysostaphin (0.25 %) and propylene glycol (5 %) were added to the Carbopol gel base and stirred until homogeneous mixture was obtained. The dispersion was then allowed to hydrate and swell for 1 hour. The Carbopol dispersion was neutralized with 98 % triethylamine (TEA) to obtain a pH value approximately 6.8±0.2. Gel was allowed to equilibrate for 24 hours at room temperature prior to use in treatment of burn wound infection[15].

Preparation of bacterial inoculum

The growth of control strain (ATCC 43300) and clinical isolate (SA6) in soybean casein digest broth was centrifuged at 10,000 rpm for 5 minutes. The pellet was washed and suspended in physiological saline to attain a concentration of ~1×10^7 CFUs/ml (Colony Forming Units). Colony count was confirmed by plating on soybean casein digest agar and mannitol salt agar supplemented with 6 µg/ml of oxacillin[16].

Burn wound infection in mouse model

Swiss albino mice (Adult male) weighing 40-45 gms, bred locally in the animal house Manipal University, Manipal, were selected for the study. They were housed under controlled conditions of temperature (23 ± 2°C), humidity (50 ± 5%) and 10-14 hours of light and dark cycles. Partial thickness burn wounds were inflicted[17], after sedating animals with ketamine (50mg/kg/i.p) by pouring hot molten wax at 80° C into a metal cylinder of 200 mm^2 circular opening placed on shaven back of the mouse. Animals were randomly divided in to two groups. Animals in group A was infected with ATCC 43300 and group B with a clinical isolate (SA6). 100 µl of bacterial suspension was injected subcutaneously. On the 4^{th} day of post-infection, 3 animals from each group were sacrificed to measure bacterial counts and confirm MRSA infection. To evaluate the effect of topical treatment of r-lysostaphin, animals with partial thickness burn wounds were randomly divided into 4 subgroups (n=6). Group 1 without receiving any drug served as an untreated/control group. Group 2 received placebo gel, Group 3 and Group 4 received topical cream of mupirocin (Bactroban, 2% mupirocin), r-lysostaphin (0.25 % lysostaphin gel) respectively twice a day for 5 days.

Cell count evaluation

After 5 days of treatment, animals were euthanized by exposing to ether and wound tissue was harvested. Extracted tissues were homogenized in 0.8 ml of PBS. Supernatant was serially diluted and 100 µl was plated on soybean casein digest agar and mannitol salt agar supplemented with 6 µg/ml oxacillin. Plates were incubated at 37°C for 18 hours. Number of CFUs recovered from treated group was compared with untreated group to see the effect of treatment on burn wound infection[16].

STATISTICAL ANALYSIS

Results were analyzed by Oneway anova (SPSS 16.0). Wound tissue evaluation data are presented as the mean ± standard error of the mean (SEM). Differences among the experimental group in bacterial counts were considered to be statistically significant at a P value of <0.05.

RESULTS

A total of 213 *S. aureus* isolates were collected over the period of 3 months from November 2011 to January 2012. They were tested for antibiotic susceptibility testing, out of which 73 isolates (34.2 %) were found to be resistant to cefoxitin disc with zone of inhibition <19 mm. 32.8 % (24/73) isolates were found to be inducible clindamycin resistance (iMLSb phenotype), 9.6 % (7/73) isolates were resistant to both clindamycin and erythromycin (constitutive resistance, cMLSb phenotype), 32.8 % (24/73) isolates were resistant only to erythromycin (MS phenotype) and 24.6 % (18/73) isolates were sensitive to both antibiotics. All the MRSA isolates were sensitive to vancomycin, linezolid and teicoplanin (Fig. 1). 88% (64/73) of isolates showed MIC value between 8 to 128 µg/ml to oxacillin and 12% (9/73) were highly resistant to oxacillin with MIC of >256 µg/ ml.

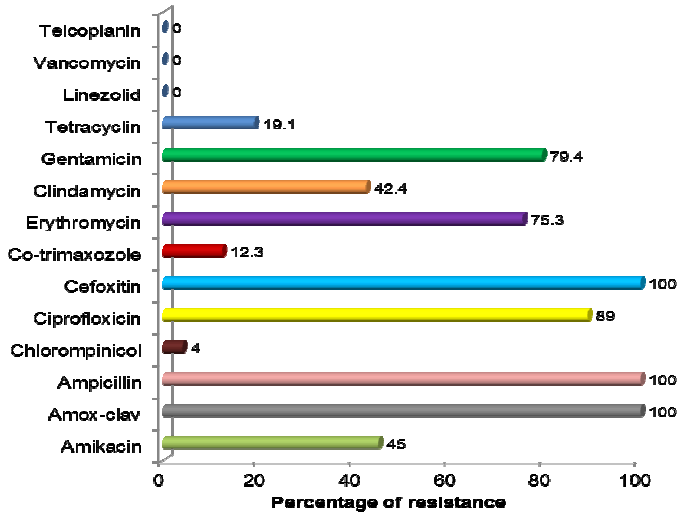

Figure 1
Antibiotic susceptibility profile of MRSA isolates from clinical specimens

All the 73 MRSA isolates showed zone of inhibition ranging from 14 to 17 mm with r-lysostaphin 50 µg/disc, however 25 µg/disc showed smaller zone of inhibition of 10 to 13 mm when tested by disc diffusion method. The size of inhibition zone continued to increase by 3 to 4 mm upon incubation of further 24 hours. ATCC 25923 had 17 mm zone of inhibition around lysostaphin disc. There was no presence of small colonies inside the zone of inhibition. MIC of r-lysostaphin ranged from 0.25 to 2 µg/ml (Fig. 2), whereas MIC50 and MIC90 was <1 µg/ml. Minimum bactericidal concentration of r-lysostaphin ranged between 0.25 to 2 µg/ml, MBC50 and MBC90 were found to be <1 µg/ml. ATCC 29213 and ATCC 43300 had MIC of 0.5 µg/ml and 1 µg/ml respectively.

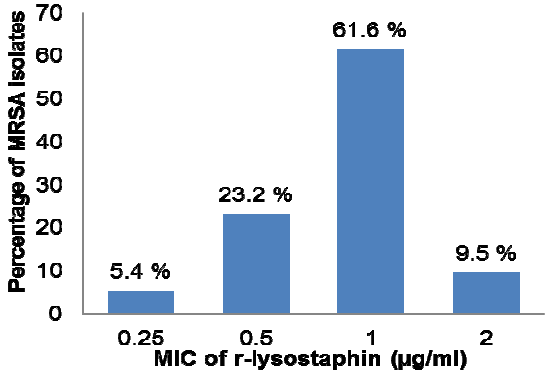

Figure 2
Graph showing percent wise distribution of MRSA isolates with MIC (µg/ml) of r-lysostaphin

Effect of topical application of r-lysostaphin against MRSA infection in a mouse burn wound model was evaluated. The mean of tissue extracted from 200 mm^2 area was 0.2 grams ±0.02. After inoculation with 10^6 CFUs of MRSA in burn wound, all the groups showed an active infection with the symptoms of serous fluid discharge. Mean bacterial count in untreated group was significantly high ($4.2\times10^8 \pm 1.5\times10^8$ CFUs/g tissue) as compared to treatment groups. The untreated and placebo group did not show any significant difference in bacterial count (p 0.76). After 5 days of drug treatment, the group treated with mupirocin gel showed 3 log reduction ($2.9\times10^5 \pm 1.3\times10^5$ CFUs/g tissue) in count compared to the untreated group. The most significant reduction in quantitative bacterial count of excised tissue was seen in mice receiving r-lysostaphin topical gel ($3.2\times10^3 \pm 1.2\times10^3$ CFUs/g tissue), in which there was a 5 log reduction in counts ($p<0.0001$, Fig. 3). Lysostaphin activity on ATCC 43300 and Clinical isolate (SA6) found to be similar. SA6 isolate had high tolerance to oxacillin with the MIC of >256 µg/ml, where as ATCC 43300 was borderline resistant to oxacillin and MIC was found to be 4 µg/ml. The drug treated groups showed recovery from the infection with decrease in serous fluid discharge (Fig. 4).

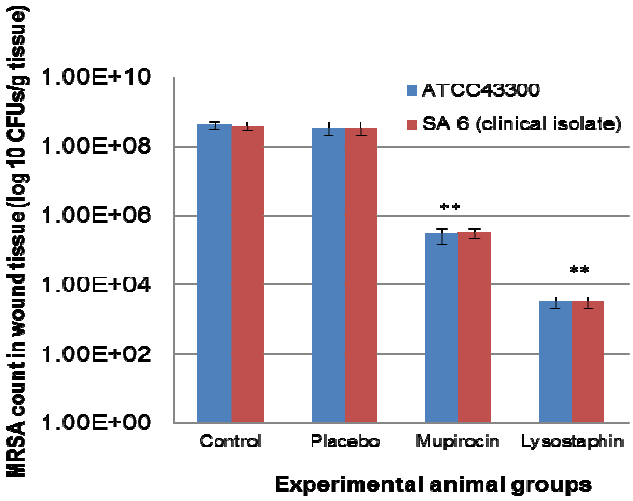

Figure 3
Effect of topically administered mupirocin and r-lysostaphin against MRSA infection in a mouse burn wound model. Graph shows the bacterial count recovered from untreated group ($4.2\times10^8 \pm 1.5\times10^8$CFUs/g tissue), placebo group ($3.4\times10^8 \pm 1.4\times10^8$CFUs/g tissue), mupirocin treated ($2.9\times10^5 \pm 1.3\times10^5$ CFUs/g tissue) and lysostaphin treated ($3.2\times10^3 \pm 1.2\times10^3$ CFUs/g tissue) **P <0.0001

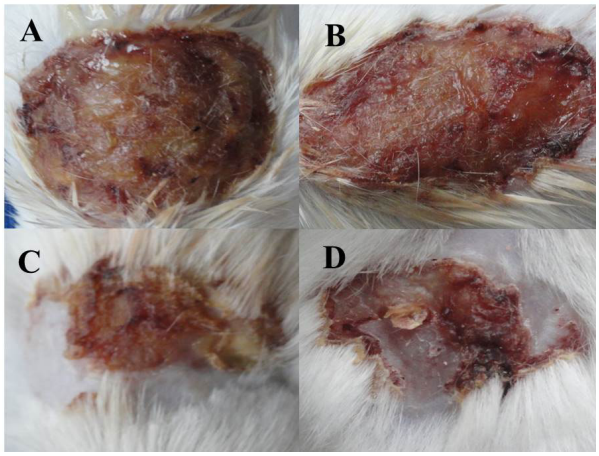

Figure 4
Burn wound infection in mice, untreated (A) and placebo (B) group showing serous fluid discharge; 2 % mupirocin (C) and 0.25 % r-lysostaphin (D) treated group showing recovery from burn wound infection with scaling of the skin and decreased serous fluid discharge.

DISCUSSION

Recent surveillance report from a network of microbiology laboratories (Indian Network for Surveillance of Antimicrobial Resistance- INSAR) at premier medical colleges and hospitals in India, found that the overall prevalence of MRSA infections was 41 % with majority of *S. aureus* isolated from patients with skin and soft tissue infections followed by blood stream and respiratory infections[18]. In the present study we isolated MRSA in 34% of cases, majority being from skin and soft tissue infections. Detection of clindamycin inducible resistance among clinical isolates plays a crucial role in choosing macrolides for the treatment. Thirty three percent of our MRSA clinical isolates were positive for inducible clindamycin resistance. The emergence of macrolide resistance in *Staphylococcus* is mainly mediated by two mechanisms; MLS type B (MLSb) or efflux mechanism phenotypes. Expression of MLSb phenotype can be either constitutive or inducible in presence of low levels of inducers such as erythromycin. The later results in increased resistance to MLS class antibiotics such as clindamycin which is responsible for treatment failure[19]. Development of multiple-drug resistant strains has frequently caused treatment failure of MRSA infections[3]. These findings have encouraged researchers to renew their interest in developing new antibiotics against MRSA infections. *S. aureus* cell wall

contains high proportions of pentaglycine cross bridges, making lysostaphin highly effective against both actively growing and quiescent bacteria[7, 8]. Yang et. al. have studied in-vitro bactericidal activity of r-lysostaphin against *S. aureus* and observed the MIC ranging from 0.03 to 2 µg/ml[20]. In the present study all MRSA isolates were sensitive to r-lysostaphin, when tested with both disc diffusion assay and microbroth dilution assay. MIC of r-lysostaphin ranged from 0.25 to 2 µg/ml. Disc diffusion assay was the simplest method to test in-vitro susceptibility of lysostaphin. Data obtained from the previous studies and current study indicated continued increase in the size of inhibition zone when the plates were incubated for 48 hours shows that lysostaphin is stable at 37° C[14, 21]. In-vivo bactericidal activity of topically administered r-lysostaphin on MRSA burn wound infection was evaluated against commercially available 2 % mupirocin ointment. In comparison to mupirocin, r-lysostaphin is found to be very effective in reducing the bacterial load, serous fluid discharge and improving the wound healing process. Wound infections leads to economic loss, increase in patient morbidity and mortality and many wound infections are caused by *Staphylococci* that are capable of developing antibiotic resistance[22]. Previous in-vivo studies have shown lysostaphin is an effective anti-staphylococcal agent. Lysostaphin immobilized in biomaterial cellulose fiber has shown bactericidal activity against *S. aureus* with low toxicity towards keratinocytes[23]. Chitosan-collagen hydrogel incorporated with lysostaphin (CCHL) gauze used for the treatment of MRSA infected third-degree burn wounds, is found to be effective in decolonizing bacteria at the site of infection after two weeks of treatment [24]. r-lysostaphin cream (~120 and 150 µg) has shown >3 log reduction in bacterial load in skin abrasion infected with MRSA in mouse model[9]. Although commonly used anti-staphylococcal antiseptics and topical agents have bactericidal activity against MRSA, a significant number of these organisms are not decolonized. In the present study we observed lysostaphin was effective in reducing the bacterial count in burn wound model.

CONCLUSION

Use of topical antimicrobial agents for the treatment of burn wound infection is of importance for a successful therapy. Our study demonstrated that the topical application of r-Lysostaphin is beneficial for reducing the bacterial load in infected burn wound and accelerated healing process. No change was observed in the susceptibility of the bacteria to lysostaphin between the beginning and the end of the treatment. Hence, r-lysostaphin can be a potential therapeutic agent in treating MRSA infections however; further investigations are required in this field.

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CONFLICT OF INTREAST

Conflict of interest declared none.

ETHICAL COMMITTEE APPROVAL

Institutional ethical committee approval was obtained from Kasturba Hospital, Manipal. (Reference number – IEC 197/2011). The Institutional Animal Ethical Committee approval was taken before conducting burn wound infection in mice (IAEC/KMC/79/2011-2012).

REFERENCES

1. Centers for Disease Control and Prevention, “Threat Report 2013: Antibiotic/Antimicrobial Resistance” 3 March 2015. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf
2. World Health Organization, “Antimicrobial Resistance: Global Report on Surveillance 2014” 3 March 2015. http://www.who.int/drugresistance/documents/surveillancereport/en
3. Ruef C, Epidemiology and clinical impact of glycopeptide resistance in *Staphylococcus aureus*, Infection, 32: 315-327, (2004).
4. Zurenko GE, Yagi BH, Schaadt RD, Allison JW, Kilburn JO, Glickman SE, Hutchinson DK, Barbachyn MR, Brickner SJ, In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents, Antimicrobial Agents and Chemotherapy, 40(4):839-845, (1996).
5. Johnson AP, Tysall L, Stockdale MV, Woodford N, Kaufmann ME, Warner M, Livermore DM, Asboth F, Allerberger FJ, Emerging linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium* isolated from two Austrian patients in the same intensive care unit, European J Clinical Microbiology and Infectious diseases, 21(10): 751-754, (2002).
6. Swaney SM, Shinabarger DL, Schaadt RD, Bock JH, Slightom JR, Zurenko GE, Oxazolidinone resistance is associated with a mutation in the peptidyltransferase region of 23S rRNA. Abstracts of the Thirty-eighth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, *CA, USA,* Abstract, C-104: 98, (1998).
7. Bastos MCF, Coutinho BG, Coelho MLV, Lysostaphin: A Staphylococcal Bacteriolysin with Potential Clinical Applications, Review, Pharmaceuticals, 3: 1139-1161, (2010).

8. Schindler CA, Schuhardt VT, Lysostaphin: a new bacteriolytic agent for the *Staphylococci*. Proceedings of the National Academy of Sciences of the United States of America, 51: 414-421, (1964).
9. Kumar A, Khan IA, Sharma PR, Sumathy K, Krishna ME, Evaluation of activity of recombinant lysostaphin against isolates of meticillin-resistant *Staphylococcus aureus* from Indian hospitals, J Med Microbiol., 63(5):763-766, (2014).
10. Kumar JK. Lysostaphin: an anti-staphylococcal agent, Appl. Microbiol. Biotechnol., 80(4):555-61, (2008)
11. Dajcs JJ, Hume EB, Moreau JM, Caballero AR, Cannon BM, O'Callaghan RJ, Lysostaphin treatment of methicillin-resistant *Staphylococcus aureus* keratitis in the rabbit, Investig. Opthalmol. Vis. Sci., 41:1432–1437, (2000).
12. Kokai-Kun JF, Walsh SM, Chanturiya T, Mond JJ, Lysostaphin cream eradicates *Staphylococcus aureus* nasal colonization in a cotton rat model, Antimicrob. Agents Chemother., 47:1589–1597, (2003).
13. CLSI M02-A10, performance standards for antimicrobial susceptibility testing, Approved Standards, 10th edition, CDC, USA, (2009).
14. Kusuma CM, Kokai-Kun JF, Comparison of four methods for determining lysostaphin susceptibility ofvarious strai ns of *Staphylococcus aureus*, Antimicrob Agents Chemother., 49(8): 3256–3263, (2005).
15. Marwa HS, Ghada FM, Evaluation of topical gel bases formulated with various essential oils for antibacterial activity against methicillin- resistant *Staphylococcus aureus*, Tropical Journal of Pharmaceutical Research, 12 (6): 877-884, (2013).
16. Bairy KL, Somayaji SN, Rao CM, An experimental model to produce partial thickness burn wound, Indian J Exp Biol., 35:70-2,(1997).
17. Jacobsen F, Fisahn C, Sorkin M, Thiele I, Hirsch T, Stricker I, Klaassen T, Roemer A, Fugmann B, Steinstraesser L, Efficacy of topically delivered moxifloxacin against wound infection by *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*, Antimicrobial agents and chemother., 5: 2325–2334, (2011).
18. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India, Joshi S, Ray P, Manchanda V, Bajaj J, Chitnis DS, Methicillin resistant *Staphylococcus aureus* (MRSA) in India: Prevalence & susceptibility pattern. The Indian Journal of Medical Research, 137(2):363-369, (2013).
19. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, Emekdas G, Inducible clindamycin resistance in *Staphylococci* isolated from clinical samples, Jpn J Infect Dis.,58:104–106, (2005).
20. Yang XY, Li CR, Lou RH, Wang YM, Zhang WX, Chen HZ, Huang QS, Han YX, Jiang JD, You XF, In-vitro activity of recombinant lysostaphin against *Staphylococcus aureus* isolates from hospitals in Beijing, China, J Med Microbiol.,56: 71-76, (2007).
21. Von Eiff C, Kokasi Kun JF, Becker K, Peters G, In vitro activity of recombinant lysostaphin against *Staphylococcus aureus* isolates from anterior nares and blood, Antimicrob Agents Chemother., 47:3613–3615, (2003).
22. Simonetti O, Cirioni O, Lucarini G, Orlando F, Ghiselli R, Silvestri C, Brescini L, Offidani A, Tigecycline accelerates staphylococcal-infected burn wound healing through matrix metalloproteinase-9 modulation, Journal of Antimicrobial Chemother., 67(1):191-201, (2012).
23. Miao J, Pangule RC, Paskaleva EE, Hwang EE, Kane RS, Linhardt RJ, Dordick JS, Lysostaphin-functionalized cellulose fibers with antistaphylococcal activity for wound healing applications, Biomaterials 32(36): 9557-9567, (2011).
24. Cui F, Li G, Huang J, Zhang J, Lu M, Lu W, Huan J, Huang Q, Development of chitosan-collagen hydrogel incorporated with lysostaphin (CCHL) burn dressing with anti-methicillin-resistant *Staphylococcus aureus* and promotion wound healing properties, Drug Delivery, 18(3): 173-180, (2011).