Application of INCOMYCIN Against Induced Skin Infection in Mice by METHICILLIN RESISTANT STAPHYLOCOCCUS (MRSA)

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Abstract

Skin and soft tissue infections are the most prevalent and frequent form of Methicillin-resistant Staphylococcus aureus (MRSA) infections. MRSA is characterized by resistance to most ß-lactam antibiotics. It can cause various infections including endocarditis, supportive pneumonia, and osteomyelitis. This study evaluates lincomycin activity against infection models induced by MERSA in induced neutropenic mice. The bacterium was isolated from one hundred and fifty samples of infected dog ears' exudate discharge from different locations in Baghdad governorate, Iraq during the period from October 2021 to march 2022, isolate achieved a probability of 99% belonging to Staphylococcus aureus by VITEK® 2 system identification The isolates showed high rate (75%) sensitive to Lincomycin, Mice rendered neutropenic by a total dose of cyclophosphamide 250 mg/kg intraperitoneal injections , Induction of Skin infection model by utilizing of a certified S. aureus isolate and assessing the incidence of infection through the development of clinical signs, inflammatory cell counting and counting of the recovered bacterial colonies. The treatment with Lincomycin (22mg/kg q 12 hrs.) orally was started immediately after diagnosis of the infection, and the bacteriological cure was assessed every 48 hrs. Lincomycin showed effective and satisfactory results within one week of treatment.

Keywords: *S. aureus, ß lactam, isolates, Vitek® 2, cyclophosphamide, neutropenic.*

Introduction

S. aureus is both a frequent and commensal bacterium that leading cause of skin and soft tissue infections (SSTI), endocarditis, bacteremia, and osteomyelitis (Kazimoto et al., 2018; Najwan et al.,2020).Staphylococcus aureus is a Gram-positive, non-motile, non-spore former; some strains are capsulated (Elhawy, 2021; Schmidt et al., 2015). Certain strains of Staphylococcus aureus are resistant to methicillin, which has been identified as MRSA and the creation of resistant strains of S. aureus is owing to the widespread and random use of antibiotics leading to the production of ß-lactamase that inactivated the β-lactam, additional mutations in target protein-coding genes, as well as the acquisition and accumulation of antibiotic resistance-conferring genes. (Santajit & Indrawattana , 2016). S. aureus are classified as nosocomial or community, depending on the source of infection (Rasheed et al., 2014, Khan et al., 2016).The most prevalent type of S. aureus infection is a skin infection like Small benign

boils, impetigo, folliculitis, cellulitis, and more serious, invasive soft-tissue infections are just a few examples of how this may present (Ghalehnoo, 2018; Kazimoto et al., 2018; Najwan et al., 2020). Burned patient are more susceptible to S. aureus infection because of their compromised immune system causing invasive types of infection (Anne et al., 2017). Pyoderma is a major clinical skin problem in dogs that is frequently caused by the administration of inappropriate antibiotics or inadequate antibiotic treatment (Sudhakara et al., 2014). Staphylococcus aureus is the most common pathogen found in surgical site infections, cutaneous abscesses, and purulent cellulitis, and it causes a wide range of SSTIs (Ibrahim et al., 2018; Malachowa et al., 2013; Malachowa et al., 2019), ranging from the benign (e.g., impetigo and simple cellulitis) to the potentially fatal (Tong et al., 2015). Skin and soft-tissue infections (SSTIs) refer to a group of pathological disorders that affect the skin and the underlying subcutaneous tissue, fascia, or muscle and can range from basic superficial infections to severe necrotizing soft-tissue infections (NSTIs) (Duane et al., 2021). MRSA strains have developed resistance to all beta-lactam antibiotics, including penicillin's (Santajit & Indrawattana, 2016). To cope with these multi-drug resistance problems, several anti-staphylococcal drugs such as Lincomycin, vancomycin linezolid, tedizolid, and daptomycin have been approved for treating the life-threatening infections caused by multi-drug-resistant S. aureus (Chen et al., 2020). Lincomycin was approved by the FDA on December 1964 and indicated for the treatment of serious bacterial infections caused by susceptible strains of streptococci, pneumococci, and staphylococci in patients who are allergic to penicillins or for situations in which penicillin is deemed inappropriate (Bottalico et al., 2022).

MATERIALS AND METHODS

Isolation and identification of S. aureus

One hundred and fifty dogs' ear swabs from October 2021 to December 2021, were obtained from various parts of Baghdad city included Baghdad veterinary hospital and private clinics, and the samples were cultured on a selected medium for 24 hours at 37°C before being suspected. Staph colonies were cultivated on blood agar for 24 to 48 hours at 37°C, and their morphology was assessed by shape, size, color, and microscopic inspection with gram stain (Prescott & Harley, (2002). VITEK®2 Compact (Biomerieux, USA) was used as an automated system as procedure for identifying Staphylococcus aureus isolates (Pincus, 2006).

Determination of antimicrobial susceptibility

Antimicrobial susceptibility determination carried out according to Kirby-Bauer

technique (Sachse et al., 2010) which is used to conduct the antimicrobial susceptibility test for 11 different antibiotics discs were chosen as examples of the main kinds of antibiotics used to treat S. aureus infections, the inhibitory zones of antibiotics measured by millimeter unite which demonstrated whether an isolate was considered to be sensitive, intermediate, or resistant to certain antibiotics CLS1 (2020).

Bacterial inoculation preparation

S. aureus isolates were stored in a frozen glycerol stock and inoculated in a flask-to-medium volume ratio of 5:1 to Trypticase Soy Broth (TSB) media. Bacteria should be grown at 37 °C with rotational shaking (225 rpm), Incubate bacteria in new TSB medium (1:200 dilution) at 37 °C with rotating shaking (225 rpm). Wash bacteria by dissolving pellets in an equivalent volume of PBS (phosphate buffered saline) and centrifuging for 10 minutes. Re-suspend the bacteria in sterile PBS until a final concentration of 1 ×10 7 colony-forming units CFU/ML is reached, then transfer the bacterial suspension to septum vials and stores them on ice until injection (Ibrahim et al., 2018).

Induction of skin infection

The mice used in the study were shaved at the infection site, typically the right and/or left flank, by pinching the skin around the prepared infection site, and inoculated subcutaneously with 0.1 mL of 1 × 10 7 live S. aureus or sterile saline. Keep mice in individual cages under observation until reading the results (Malachowa, et al., 2019).

Drug preparation

Fresh solution prepared by dissolving one capsule (500 mg /cap) of Lincomycin in 500 ml of distilled water to prepare the dose (22 mg/kg) the weight of experiment mice (35mg) so each mouse was dosed with 0.77ml/mice twice daily for 5-7 day (Rozalska & Wadström, (1993).

RESULTS AND DISCUSSION

The findings of the study were showed a significant reduction in values of leukocytes and neutrophils in mice after induction of neutropenia (2480 ± 95.22 and 43 ± 7.95, respectively) when compared to those of mice before induction of neutropenia (4680 ± 112.3 and 749 ± 46.65, respectively), (Table 1), Figure 2. These results are comparable to other results conducted before (Huyan et al., 2011) which proved the effect of several concentrations of cyclophosphamide on WBC count in tested mice. Another broach proved the decrease of total Leukocytes and Neutrophils in mice following I.P. injection of 150 and 100 mg/kg of cyclophosphamide on days (1st and 4th) respectively (Gudmundsson & Erlendsdottir (1999). Animals are frequently made neutropenia with cytotoxic drugs like cyclophosphamide in order to evaluate in vivo the intrinsic action of antibiotics without the interference of the immune system (Zuluaga et al., 2006).

Determination of Interleukin 6

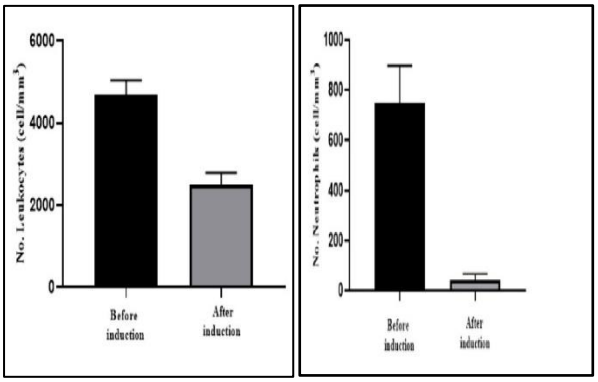
Regarding skin infection models, the findings of mice before experimental infection (15.86± 0.92) and after treatment with lincomycin (16.42± 0.29) were differed insignificantly (P□0.05) in their values; however, values of both groups were significantly (P□0.05) higher than those observed in mice of during experimental infection (38.21± 0.60 (Table 2), Figure (4).

Table 1. Numbers of Leukocytes and Neutrophils before and after induction of neutropenia by cyclophosphamide in mice.

Marker	Before induction of neutropenia (Cell/mm^3)	After induction of neutropenia (Cell/mm^3)
Leukocytes	4680 ± 112.3 **A**	2480 ± 95.22 **B**
Neutrophils	749 ± 46.65 **A**	43 ± 7.95 **B**

Sample size = 10 mice difference in letters denote to significant difference between groups ($p \geqslant 0.05$) using of the Paired t-test

Figure 2. No. of Leukocytes and Neutrophils before and after injection of cyclophosphamide.



In this study, Interleukin 6 levels were elevated during the first two days following bacterial inoculation. Previous research has linked Interleukin 6 production to the severity of the infectious process (Rozalska & Wadström, (1993); Mölne et al., 2000). Another research

showed the invasion of S. aureus to epithelium in nasal polyposis capable to induce IL-6 synthesis in vitro (Wang et al., 2014).

Table 2. Concentration of interlukin-6 (pg/ml) during different periods of experimental infection.

Infection model	Before experimental infection	During experimental infection	After treatment with Lincomycin
Skin	15.86± 0.92 **A**	38.21± 0.60 **B**	16.42± 0.29 **A**

Sample size = 5 mice Difference in letters denote to significant difference between groups ($p \geqslant 0.05$) using of ANOVA (one-way)

It's worth noting that IL-6 is a multifunctional pro-inflammatory cytokine that is an important inflammatory mediator released in response to many different types of infections. In previous studies, the expression of IL-6 was increased in S. aureus infections (Yang et al., 2019).

Figure 3. Interlukin-6 value in skin infection

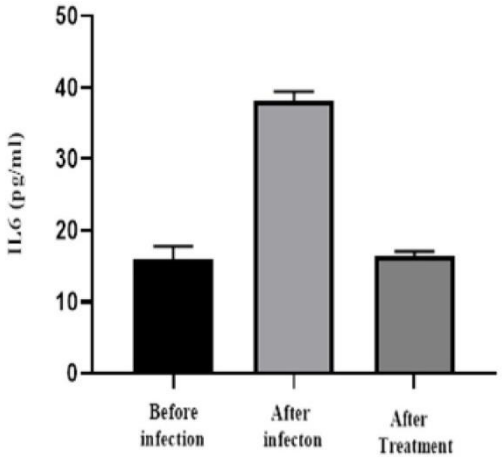


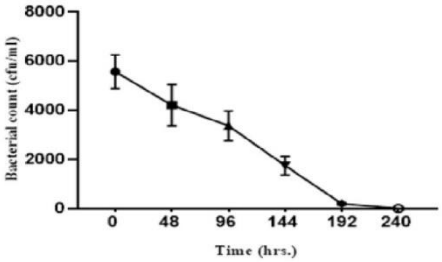
Table 3. Number of bacterial colonies (cfu/ml) during different periods of treatment with Lincomycin.

Infection model	Time (Hour) 0	48	96	144	192	240
Skin (cfu/m)	(55.6 ± 3.6) × 10^2 **A**	(42 ± 3.74) × 10^2 **B**	(33.6 ± 2.69) × 10^2 **BC**	(17.4 ± 1.69) × 10^2 **C**	(1.96 ± 0.27) × 10^2 **D**	0 ± 0* **D**

(*) There are no bacterial colonies below 30 cfu/ml

Sample size = 5 mice Difference in letters denote to significant difference between groups (p□0.05) using of ANOVA (one-way)

Figure 4. Show the bacterial counting of skin infection during 10 days of treatment.



The findings of skin infection model showed that the higher significant (P□0.05) value for the number of bacterial colonies was identified at 0 hour (55.6 ± 3.6) □ 102], while the lowered values were seen at 192 [(1.96 ± 0.27) □ 102] and 240 [0 ± 0] hours when compared to other times; 48 hours [(42 ± 3.74) □ 102], 96 hours [(33.6 ± 2.69) □ 102], and 144 hours [(17.4 ± 1.69) □ 102]. The results disclosed a clear variation in the number of bacterial colonies,

over the course of the 10-days treatment period. From the aforementioned, this study demonstrates unequivocally the effect of lincomycin on the methicillin-resistant S. aureus pathogen and reveals the pharmacological efficacy of lincomycin in the skin infection model, The satisfactory results of this study were obtained due to the optimal use of this antibiotic through the use of a sensitivity test according to VITEK® 2 system (Bemer et al., 2005) which showed the active efficacy of lincomycin toward S. aureus pathogen and considered an expert system for biological validation of susceptibility results and therapeutic interpretation provides also estimation of MIC method was used to determine the activity of lincomycin concentration (4 µg/mL) against isolates (asper NCCLS guidelines) (De Oliveira,2000). Many researches have suggested the effectiveness of lincomycin in the treatment of S. aureus MRSA due to mechanism of action, which inhibits microbial protein synthesis (Spížek & Řezanka, 2017).

CONCLUSION

1. Methicillin-resistant Staphylococcus aureus (MRSA) is the most prevalent cause of skin and soft tissue infections.

2. The practical application of skin infection revealed the efficacy of lincomycin as the optimum antibacterial agent against methicillin- resistant Staphylococcus aureus infections.

References

- Anderson, L. S., Reynolds, M. B., Rivara, K. R., Miller, L. S., & Simon, S. I. (2019). A mouse model to assess innate immune response to Staphylococcus aureus infection. JoVE (Journal of Visualized Experiments), (144), e59015.

- Anne M Lachiewicz, Christopher G Hauck, David J Weber, Bruce A Cairns, David van Duin. (2017). Bacterial Infections After Burn Injuries: Impact of Multidrug Resistance, Clinical Infectious Diseases, Volume 65, Issue 12, 15 December, Pages 2130–2136.

- Bemer, P., Juvin, M. E., Corvec, S., Ros, A., & Drugeon, H. (2005). Correlation of agar dilution and VITEK2 system for detection of resistance to macrolides, lincosamides and pristinamycin among Staphylococcus aureus and Staphylococcus epidermidis: association with genotypes. Clinical microbiology and infection, 11(8), 656-661.

- Bien, C.G.; Vincent, A.; Barnett, M.H.; Becker, A.J.; Blümcke, I.; Graus, F. and Sutton, I. (2012). Immunopathology of autoantibody-associated encephalitides: clues for pathogenesis. Brain, 135(5), 1622-1638.

- Bottalico, L., Charitos, I. A., Potenza, M. A., Montagnani, M., & Santacroce, L. (2022). The war against bacteria, from the past to present and beyond. Expert Review of Anti-infective Therapy, 20(5), 681-706.

- Chen, C. J., Huang, Y. C., & Shie, S. S. (2020). Evolution of multi-resistance to vancomycin, daptomycin, and linezolid in methicillin-resistant Staphylococcus aureus causing persistent bacteremia. Frontiers in microbiology, 11, 1414.

- De Oliveira, A. P., Watts, J. L., Salmon, S. A., & Aarestrup, F. M. (2000). Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis in Europe and the United States. Journal of dairy science, 83(4), 855-862.

- Delmas, J., Chacornac, J.P., Robin, F.,

Giammarinaro, P., Talon, R. and – Bonnet, R., (2008). Evaluation of the Vitek 2 system with a variety of Staphylococcus species. Journal of clinical microbiology, 46(1), pp.311-313.

- Duane, T. M., Huston, J. M., Collom, M., Beyer, A., Parli, S., Buckman, S., ... & Sanders, J. (2021). Surgical Infection Society 2020 updated guidelines on the management of complicated skin and soft tissue infections. Surgical infections, 22(4), 383-399.

- Elhawy, M. I. (2021). Role of the Low Molecular Weight Protein Phosphatases PtpA and PtpB on Infectivity of Staphylococcus aureus.

- Ghalehnoo, Z. R. (2018). Diseases caused by Staphylococcus aureus. Int. J. Med. Health Res, 4, 65-67.

- Gudmundsson, S., & Erlendsdottir, H. (1999). Murine thigh infection model. In Handbook of animal models of infection (pp. 137-144). Academic Press.

- Huyan, X. H., Lin, Y. P., Gao, T., Chen, R. Y., & Fan, Y. M. (2011). Immunosuppressive effect of cyclophosphamide on white blood cells and lymphocyte subpopulations from peripheral blood of Balb/c mice. International immunopharmacology, 11(9), 1293-1297.

- Ibrahim OM, Ayad ZM, Omar LW. Efficacy of silver nanoparticles, antimicrobial peptides and blue light combination on healing infected wound by methicillin resistance Staphylococcus aureus in rats. | Kufa Journal For Veterinary Medical Sciences. 2018; 9(2).

- Kazimoto T, Abdulla S, Bategereza L, Juma O, Mhimbira F, Weisser M, Utzinger J, von Müller L, Becker SL. Causative agents and antimicrobial resistance patterns of human skin and soft tissue infections in Bagamoyo, Tanzania. Acta tropica. 2018 Oct 1;186:102-6.

- Khan HA, Ahmad A, Mehboob R. Nosocomial infections and their control strategies. Asian pacific journal of tropical biomedicine. 2015 Jul 1;5(7):509-14.

- Malachowa, N., Kobayashi, S. D., Braughton, K. R., & DeLeo, F. R. (2013). Mouse model of Staphylococcus aureus skin infection. In Mouse Models of Innate Immunity (pp. 109-116). Humana Press, Totowa, NJ.

- Malachowa, N., Kobayashi, S.D., Lovaglio, J., DeLeo, F.R. (2019). Mouse Model of Staphylococcus aureus Skin Infection. In: Allen, I. (eds) Mouse Models of Innate Immunity. Methods in Molecular Biology, vol 1960. Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-9167-9_12.

- Markey, B.K.; Leonard, F.C.; Archambault, M. Cullinane, A. and Maguire, D. (2014). Clinical Veterinary Microbiology. 2nded. Mosby Elsevier. Pp.161.

- Mölne, L., Verdrengh, M., & Tarkowski, A. (2000). Role of neutrophil leukocytes in cutaneous infection caused by Staphylococcus aureus. Infection and immunity, 68(11), 6162-616.

- Najwan Sadeq Shareef, Raed Taha Yaseen Alneama, Lamyaa Kadhim Baqer. In vitro and In vivo antimicrobial effect of coconut oil against Methicillin Resistant Staphylococcus aureus from wound infections. Al-Qadisiyah Journal of Veterinary Medicine Sciences 2020, Volume 19, Issue 1, Pages 1-8.

- Papich, M. G. (2020). Papich Handbook of Veterinary Drugs-E-Book. Elsevier Health Sciences.

- Pincus DH. Microbial identification using the bioMérieux Vitek® 2 system. Encyclopedia of Rapid Microbiological Methods. Bethesda, MD: Parenteral Drug Association. 2006:1-32.

- Prescott, L. M., & Harley, J. P. (2002). Harley Prescott: Laboratory Exercises in Microbiology, Fifth Editio.

- Rasheed, R. Y., Abdulamir, A. S., & Raziq, A. H. (2014). Studying the Frequency of Methicillin-Resistant Staphylococcus aureus Through the Molecular Detection of mecA. Iraqi Journal of Medical Sciences, 12(3).

- Rozalska, B., & Wadström, T. (1993). Interferon-γ, interleukin-1 and tumour necrosis factor-α synthesis during experimental murine staphylococcal infection. FEMS Immunology & Medical Microbiology, 7(2), 145-152.

- Sachse, F., Becker, K., Von Eiff, C., Metze, D., & Rudack, C. (2010). Staphylococcus aureus invades the epithelium in nasal polyposis and induces IL‐6 in nasal epithelial cells in vitro. Allergy, 65(11), 1430-1437.

- Santajit, S., & Indrawattana, N. (2016). Mechanisms of antimicrobial resistance in ESKAPE pathogens. BioMed research international, 2016.

- Schmidt T, Kock MM, Ehlers MM. Antimicrobial Resistance in Staphylococciatthe Human–-Animal Interface. Antimicrobial Resistance: An Open Challenge. 2015 Nov 26;85.

- Spížek, J., & Řezanka, T. (2017). Lincosamides: Chemical structure, biosynthesis, mechanism of action, resistance, and applications. Biochemical pharmacology, 133, 20-28.

- Sudhakara Reddy, B., Nalini Kumari, K., Vaikunta Rao, V., & Rayulu, V. C. (2014). Efficacy of cefpodoxime with clavulanic acid in the treatment of recurrent pyoderma in dogs. International Scholarly Research Notices, 2014.

- Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews. 2015 Jul;28(3):603-61.

- Wang, T., Guo, M., Song, X., Zhang, Z., Jiang, H., Wang, W., … & Zhang, N. (2014). Stevioside plays an anti-inflammatory role by regulating the NF-κB and MAPK pathways in S. aureus-infected mouse mammary glands. Inflammation, 37, 1837-1846.

- Wesson, C.A., J. Deringer, L.E. Liou, K.W. Bayles, G.A. Bohach, and W.R. Trumble. 2000. Apoptosis induced by Staphylococcus aureus in epithelial cells utilizes a mechanism involving caspases 8 and 3. Infection and Immunity 68: 2998–3001.

- Yang, X., Wang, D., Zhou, Q., Nie, F., Du, H., Pang, X., … & Xu, Y. (2019). Antimicrobial susceptibility testing of Enterobacteriaceae: determination of disk content and Kirby-Bauer breakpoint for ceftazidime/avibactam. BMC microbiology, 19(1), 1-7.

- Zuluaga, A. F., Salazar, B. E., Rodriguez, C. A., Zapata, A. X., Agudelo, M., & Vesga, O. (2006). Neutropenia induced in outbred

mice by a simplified low-dose cyclophosphamide regimen: characterization and applicability to diverse experimental models of infectious diseases. BMC infectious diseases, 6(1), 1-10.