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Research Article

Vancomycin, Linezolid, and Ceftaroline *In vitro* Activity Against Methicillin susceptible *Staphylococcus aureus* (MSSA) and Methicillin-resistant *Staphylococcus aureus* (MRSA) Isolates

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ABSTRACT

MRSA-infected patients incur twice as many costs as MSSA-infected patients. Vancomycin, Linezolid, and, most recently, Ceftaroline are among Indonesia's several MRSA treatment options. Therefore, we sought to assess the efficacy of these three medications. The investigation was done at the Dr. Soetomo General Academy Hospital's Clinical Microbiology Laboratory in Surabaya. The bacterium ATCC 25923, ATCC 43300, MSSA clinical isolate, and MRSA clinical isolate of *Staphylococcus aureus* were studied. Vancomycin, Linezolid, and ceftaroline were administered at respective dosages of 1 MIC, 2 MIC, and 4 MIC. In addition, a time-kill test was performed, which consisted of counting the growth of colonies on solid media, generating a time-kill curve, and determining MBC. The number of colonies in the antibiotic groups at 4, 6, and 8 hours varied significantly, according to the study (Vancomycin, Linezolid, and Ceftaroline). In contrast, the number of bacteria did not differ significantly between Vancomycin and Linezolid until the fourth hour. Except at 6 and 24 hours, neither Vancomycin nor Ceftaroline significantly altered the number of bacteria. There was a significant difference in the number of colonies between Ceftaroline and Linezolid at 4, 6, and 8 hours. Vancomycin, Linezolid, and Ceftaroline against MSSA and MRSA isolates vary greatly.

Key words: Time-kill curve, MSSA, MRSA.

INTRODUCTION

Staphylococcus aureus is found on the skin and in the digestive and respiratory tract mucosa. This bacterium has been shown to colonize up to 30% of the world's population.[1] The mecA gene confers methicillin resistance on *Staphylococcus aureus*, resulting in MRSA resistance to methicillin. PBP2a is a gene that encodes a protein having a modest affinity for beta-lactam antibiotics.[2,3] Infections caused by MRSA increase morbidity, mortality, hospital length of stay, and treatment costs, especially antibiotic costs.

Furthermore, MRSA infection patients had worse outcomes than MSSA infection patients. In immunocompromised individuals, infections are a significant cause of mortality and morbidity, resulting in elevated mortality and morbidity rates. Due to these patients' weakened immune systems, bactericidal therapies are more effective than bacteriostatic ones. In addition, the most negligible bactericidal concentration can provide therapeutically helpful information regarding how bacteria and medications interact.[4]

MRSA infections cost twice as much as MSSA infections.[5] Therefore, in Indonesia, Vancomycin, Linezolid, and Ceftaroline are available as MRSA therapy alternatives. However, the three medicines' anti-MRSA properties and action mechanisms are distinct. The time-kill curve also indicates the efficacy of specific antimicrobial drugs against specific microorganisms.[6]

METHODS

This study examined the MSSA ATCC isolate (25923), the MRSA ATCC isolates (43300), the MSSA clinical isolate, and the MRSA clinical isolate. In addition, 1 MIC, 2 MIC, and 4 MIC concentrations of Vancomycin, Linezolid, and Ceftaroline were utilized as antibiotics.[7] In addition, a time-kill test was conducted, which included counting how many colonies were developed on solid media, constructing a time-kill curve, and calculating the MBC.

Ethical

This research has been assessed and authorized by the hospital's ethics committee (ethical certificate number: 0363/KEPK-I/2022).

Procedure

The medication concentrations were determined based on the CLSI cutoff value for the susceptible category of tested antibiotics. 1 MIC, 2 MIC, and 4 MIC stocks of antibiotics were created. Bacteria were subcultured on blood agar (Oxoid CM0055 Blood Agar Base containing 5% sheep blood) and incubated at 35°C for 24 hours. The bacterial suspension was prepared in cation-adjusted Mueller-Hinton broth (Oxoid CM0405 Mueller-Hinton Broth base) and then incubated for 4 hours at 35°C. A time-kill test was conducted with a starting inoculum of 6x105 CFU. After plating on Mueller-Hinton agar, bacterial counts were taken at 0, 1st, 2nd, 4th, 6th, 8th, and 24th hours (Oxoid CM 0337 Muelle-Hinton Agar base) (Oxoid CM 0337 Muelle-Hinton Agar base). The limit of detection (LOD) was 102 CFU/ml. The

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study quantified bactericidal activity as a 3 log10 decrease in CFU/mL. This investigation consisted of six replications.

RESULTS

Figures 1 and 2 demonstrated that ATCC MSSA and clinical MSSA isolates exhibited comparable time-kill curves. Negative controls could include growth controls. At 0, 1, 2, 4, 6, 8, and 24 hours, colony counts (log 10 CFU/mL) were determined for all groups depending on the bacterium and antibiotic. At the 8th hour, Vancomycin had a bactericidal effect, as seen by a drop in CFU/mL of more than 3 logs relative to the initial number of bacteria, although growth on solid NA media was unaffected. Since the log CFU/mL declined over time, Linezolid had a more significant bacteriostatic effect, albeit it did not reach a decrease of 3 logs 10. All isolates were killed by ceftaroline, as evidenced by a decrease of more than 3 logs in CFU/mL relative to the initial number of bacteria and the formation of 0 colonies on NA solid media, indicating that MBC could not be obtained.

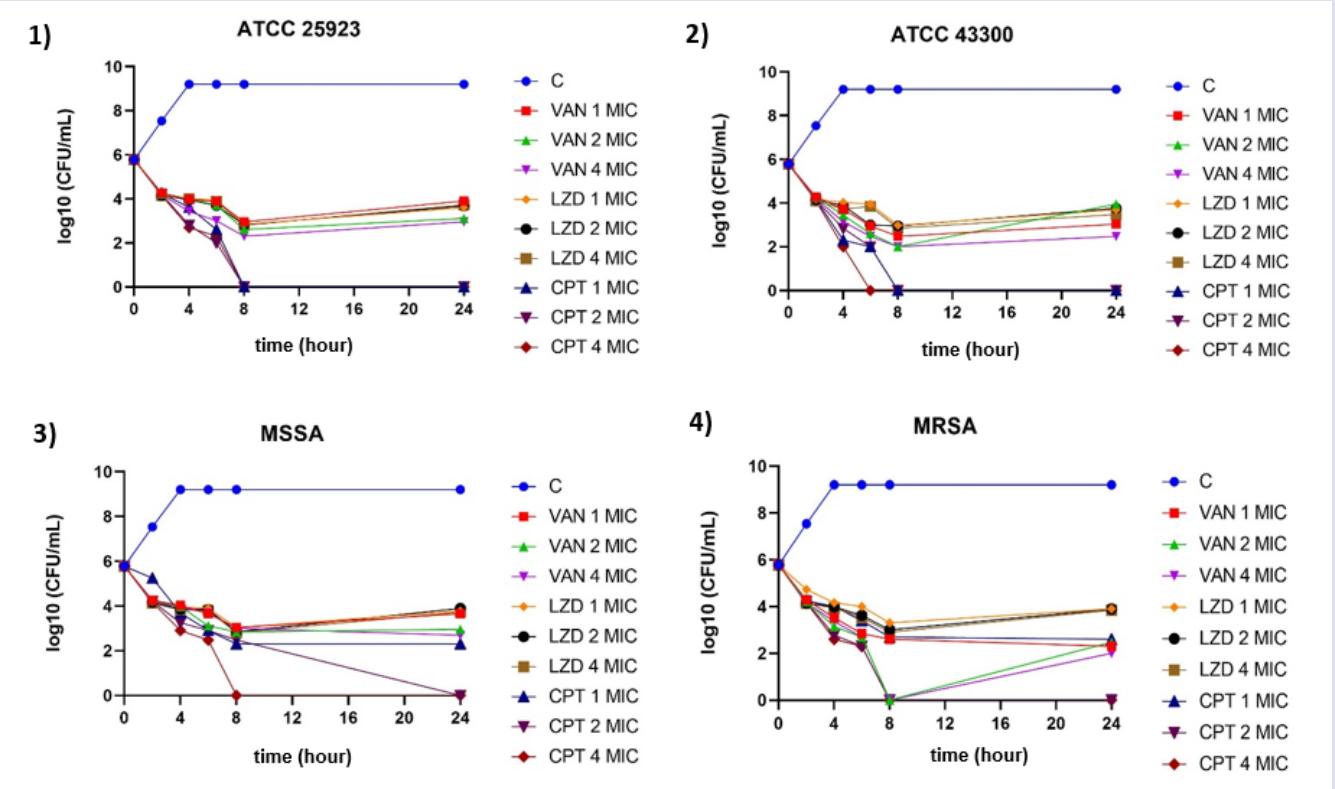
Similar tendencies characterize the ATCC MRSA and MRSA time-kill curves. Negative controls could include growth controls. Depending on the bacterium and antibiotic used, colony counts (log 10 CFU/mL) were calculated for all groups at 0 hours, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 24-hour intervals (log 10 CFU/mL). At 8 hours, Vancomycin had a bactericidal effect, as evidenced by a decrease in CFU/mL by more than 3 logs relative to the original number of bacteria; however, ATCC MRSA isolates continued to thrive on NA solid media. Since log CFU/mL reduced over time, Linezolid had a more significant bacteriostatic impact, albeit it did not reach a 3 log reduction. All isolates were killed by ceftaroline, as indicated by a fall of more than 3 logs in CFU/mL relative to the original number of bacteria and the inability to achieve MBC with the growth of zero colonies on NA solid media (Figures 3 and 4).

Statistical analysis of all isolates demonstrated that Vancomycin and Linezolid were equally effective ($p>0.05$) in lowering the number of bacteria. Except at 6 and 24 hours, there was no significant difference ($p>0.05$) in bacterial decline between Vancomycin and Ceftarolin. However, Ceftarolin and Linezolid reduced the number of germs at 4, 6, 8, and 24 hours in significantly different ways ($p<0.05$). In addition, the number of bacteria in the statistical analysis of four bacterial isolates did not differ substantially ($p>0.05$). Similarly, the number of bacteria did not substantially differ ($p > 0.05$) across the three tested MIC dosages.

DISCUSSION

The antibacterial activities against certain microorganisms were analyzed using time-kill curves. Antibiotics' pharmacokinetics and pharmacodynamics are determined by their eradication activity, defined as concentration- and time-dependent bacterial death. The bactericidal activity can be determined from the time-kill curve if the original bacterial population is reduced by more than 3 log10-fold, corresponding to a 99.9% inoculum kill. The time-kill test is used to evaluate the efficacy of antimicrobial medicines against distinct bacterial strains. Additionally, it can assess if a drug inhibits or kills microorganisms over time.

Vancomycin is less effective against MRSA than beta-lactam antistaphylococcal medicines against S. *aureus* infection. *In vitro* data demonstrating Vancomycin's delayed bactericidal efficacy against S. aureus relative to antistaphylococcal beta-lactams lends this theory. Vancomycin is a time-dependent antibiotic whose tissue distribution, inoculum quantity, and establishment of resistance are crucial clinical aspects.[8] Vancomycin is bactericidal against a 106-cell S. *aureus* inoculum. However, at inoculum 109, Vancomycin revealed no bactericidal activity.[9] Vancomycin has a half-life of 6 to 8 hours.



Figures 1-4: Demonstrated that ATCC MSSA and clinical MSSA isolates exhibited comparable time-kill curves and all isolates were killed by ceftaroline, as indicated by a fall of more than 3 logs in CFU/mL relative to the original number of bacteria and the inability to achieve MBC with the growth of zero colonies on NA solid media.

With increasing concentration at 2-4 MIC, the post-antibiotic effects (PAE) of Vancomycin on S. *aureus* have grown from 0.2 h to 2 h at 2-4 MIC. Thus, the AUC/MIC ratio is an essential measure of vancomycin action.[8]

Linezolid has little bactericidal activity against staphylococci but is very effective against streptococci and bacteriostatic against enterococci. The quantity of *Staphylococcus aureus* inoculum does not affect the bacteriostatic activity of Linezolid.[9] Linezolid has a half-life between 3.4 and 7.4 hours. Therefore, AUC/MIC and T > MIC are suitable PK/PD measurements.[10] In addition, Linezolid is as effective as vancomycin in the laboratory against MRSA.[11]

Like other beta-lactam antibiotics,[12-15] Ceftaroline exhibits pharmacologic activity that corresponds well with the time the free plasma concentration surpasses the target bacteria's minimum inhibitory concentration (MIC).[16] Plasma concentrations of 31% can decrease the number of S. *aureus* colonies by one log 10.[17] Ceftaroline has a higher affinity for PBPs than other beta-lactams, necessitating a lower MIC for bactericidal activity. Antibiotic side effects Ceftaroline has respective half-lives of 0.8 and 7.2 hours, while its persistence in S. *aureus* is substantially longer. The cumulative percentage of free Ceftaroline concentrations in 24 hours that surpassed the MIC under static conditions revealed that 24.8% was related to a bacteriostatic effect, 27.8% was associated with a 1 log drop, and 32.1% was associated with a bacteriostatic effect. A decline in colony numbers by two logs. Regrowth was less than 50% after 96 hours of exposure to four MICs. Macgowan and his colleagues discovered in 2013 that, *in vitro*, Ceftaroline was as effective as Vancomycin and more effective than Linezolid.[18] Additionally, it can reach its destination quickly.[19]

All of the time-kill curves for the microorganisms tested followed a similar pattern. At 8 hours, Vancomycin displayed a bactericidal effect, as indicated by a decrease in CFU/mL of more than 3 logs relative to the original number of bacteria; however, ATCC MRSA isolates continued to thrive on NA solid media. Linezolid had a more significant bacteriostatic action, as the log CFU/mL declined over time without reaching a reduction of 3 logs 10. In contrast, ceftaroline eliminated all of the isolates, as demonstrated by a fall of more than 3 logs in the number of CFU/mL relative to the initial number of bacteria and the formation of zero colonies on NA solid media. The number of colonies significantly differed across antibiotic groups (Vancomycin, Linezolid, and Ceftaroline) at four, six, and eight hours. Except until after six hours, there was no significant difference in the number of bacteria between Vancomycin and Linezolid. Similarly, except at the 6th and 24th hours, there was no statistically significant difference in the amount of bacteria between Vancomycin and ceftaroline. The number of colonies at the 4th, 6th, 8th, and 12th hours varied considerably between Ceftarolin and Linezolid.

Most *in vitro* studies using Vancomycin revealed a concentration-independent time-kill curve.[9] According to one investigation.[20] According to Hendry *et al*., concentrations of ceftaroline beyond the minimum inhibitory concentration (MIC) have a bactericidal effect, and concentrations twice the MIC inhibit all PBP molecular masses.[21] During the second, fourth, sixth, eighth, and twenty-fourth hours, the number of colonies in the MIC group did not differ significantly (1 MIC, 2 MIC, and 4 MIC).

ATCC 25923 and ATCC 43300 are common laboratory bacteria that preserve their original genotypic and phenotypic features. In the meantime, bacteria isolated from patient samples (MSSA and MRSA) may be able to change their characteristics based on their nutritional supply, adapting specifically to low nutrient levels. Microorganisms can diversify into subpopulations that differ genotypically and phenotypically under varied host environments.[22] Linezolid is equally effective against MSSA and MRSA. The 2020 study by Alfouzan *et al*. demonstrates that ceftaroline can inhibit the growth of MRSA and MSSA in the laboratory.[18] The number of colonies at the first, second, fourth, sixth, eighth, and twenty-fourth hours following Vancomycin, Linezolid, and Ceftaroline exposure did not differ significantly between MSSA (ATCC 25923: MSSA clinical isolate) and MRSA (ATCC 43300: MRSA clinical isolate).

CONCLUSION

Vancomycin, Linezolid, and Ceftaroline perform differently against isolates of *Staphylococcus aureus*.

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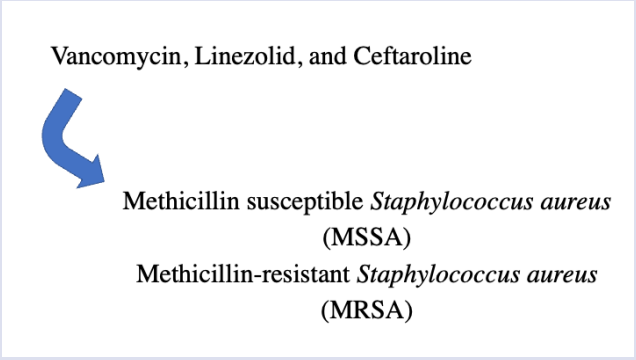
GRAPHICAL ABSTRACT



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