EFFECT OF SUBINHIBITORY CONCENTRATIONS OF METHICILLIN AND VANCOMYCIN ANTIBIOTICS ON GROWTH OF STAPHYLOCOCCUS AUREUS

V. U. Thool[1], B. J. Wadher[2] and G. L. Bhoosereddy[3]
1Sardar Patel Mahavidyalaya, Chandrapur, M.S., India.
2P G Dept. of Microbiology, RTM Nagpur University, Nagpur, M.S., India.
3Sevadal Mahila Mahavidyalaya, Nagpur. M.S., India.
Corresponding author Email : vaishali.thool@gmail.com

Abstract:

Objectives: Methicillin and Vancomycin antibiotics exert potent bactericidal activity against Staphylococcus aureus. We investigated whether these antibiotics used at subinhibitory concentrations, produce any effect on the growth of S. aureus. Methods: Growth curves in the presence of subinhibitory concentration of Methicillin and Vancomycin antibiotics were studied. Results: The maximum growth rate curves with Methicillin and Vancomycin antibiotic for both MRSA and MSSA isolates were reduced in dose dependent manner. Conclusions: The results of the present study suggest that subinhibitory concentration of Methicillin and Vancomycin antibiotics modulates the growth of S. aureus, which might be especially helpful for the treatment of S. aureus infections.

Keywords:

growth, Methicillin, Vancomycin, S. aureus, subinhibitory concentration

Introduction:

S. aureus is a well armed pathogen and one of the most multitalented nosocomial pathogen worldwide. Rampant antimicrobial use and subsequent antimicrobial resistance among bacterial pathogens is creating menace. Apart from this with the emergence of multidrug resistant (MDR) strains, effective treatment of serious staphylococcal infections has become more difficult (Hiramatsu et al., 1997). Since 1961 with the dissemination of some highly epidemic clones, Methicillin resistant S. aureus (MRSA) have spread massively across the globe. The major factor in their dominance lies in their ability to acquire antibiotic resistant determinants. Due to the rampant use of antibiotics, over the table antibiotic use, poor handling of drugs on the part of doctors and patients, lead to selective pressure on the environment leading to emergence and dissemination of MRSA and is regarded as a case of accelerated evolution (Tomascz, 1998). The incidence rate of MRSA in India has increased drastically over the years and Linezolid resistance is also a concern of worry (Thool et al., 2012a). The situation is scary and hence the treatment option for MRSA infections is left with antibiotics like Vancomycin. Vancomycin antibiotic is a complex glycopeptide and is the antibiotic of choice for the treatment of infections caused by MRSA. More recently, Vancomycin resistant strains (VRSA) have been seen in India (Thool et al., 2012b). It also has been supposed that antibiotics at their subinhibitory concentrations can alter virulence factors of bacterial pathogens. This process may have clinical bearing because bacteria are exposed to subinhibitory concentrations of antibiotics at the start and end of an antibiotic regime, between the doses, or continuously during low-dose therapy (Odenholt, 2001). This may induce an assortment of changes in bacterial properties, including morphological or ultrastructure changes and inhibition or stimulation of enzyme and toxin production (Furneri et al., 2003). Looking at the MRSA menace, it is necessary to find a way out and hence the need to study the effect of subinhibitory concentrations of antibiotics, in the aim to see whether these antibiotic levels could determine any modification able to reduce the growth of MRSA.

Material and Method:

Bacterial strains: SA28 (Methicillin sensitive) and SA63 (Methicillin resistant) selected for the study on growth curves at subinhibitory concentration, are clinical isolates obtained from dirty wounds of orthopaedic patients. The strains were reconstituted from glycerol stock cultures and propagated on Luria Bertani broth and agar. Antimicrobial susceptibility testing: Methicillin and Vancomycin (Hi-Media, India) minimal inhibitory concentration for SA28 and SA63 were determined by Ezy MIC test. All MICs were determined according to the instructions given by the manufacturer (Hi- Media Laboratories, Pvt Ltd, India). Growth curves: One to two colonies of S. aureus SA28 and SA63 were inoculated into approximately 10 mL BHI broth and were incubated at 35°C with shaking for 24 hrs. One mL of this overnight culture was then diluted to 50 mL of fresh BHI broth containing subinhibitory concentrations of Methicillin and Vancomycin antibiotics. A control flask was also included containing no antibiotics. After the flasks were inoculated, samples were taken at 0,1,2,3,4,5,6,7,8,9 and 24 hrs. The OD of each sample was determined at 540 nm, and was considered as an estimation of growth. The OD values were then plotted against time. (Hammer et al., 2005; Starner et al., 2008).

Result and Discussion:

Results The growth curves obtained for SA63 and SA28 isolates, for Methicillin antibiotic (fig 1A and 1C) were similar with each other and growth curves obtained for SA63 and SA28 isolates for Vancomycin antibiotic (fig 1B and 1D) were same. The maximum growth rate curves with Methicillin antibiotic for both the isolates were reduced in dose dependent manner suggesting that Methicillin antibiotic was effective in changing the specific growth rate of both MRSA and MSSA isolate. But this was not the case with Vancomycin antibiotic in which the 1/2 MIC of Vancomycin was effective in changing the specific growth rate of both MRSA and MSSA isolate and concentrations below 1/2 MIC was unable to modulate the growth curves. On the whole Methicillin antibiotic was more effective in modulating the growth curves as compared to Vancomycin antibiotic for both MRSA and MSSA isolates. Discussion S. aureus produces a variety of different virulence factors in a growth phase-dependent manner. Also the expression of staphylococcal genes is generally regulated in response to fluctuations in cell population density through quorum sensing. S. aureus virulence gene expression is mainly controlled by at least two global regulatory element - the accessory gene regulator agr that suppresses the post exponential phase expression of cell surface binding proteins and enhances the expression of secreted protein and the sar locus that activates the synthesis of both extracellular and surface bound proteins in S. aureus (Novick and Jiang, 2003). In this study the growth curves were studied for Methicillin and

Vancomycin antibiotics at subinhibitory concentrations as according to Hammer et al., 2005 and Starner et al., 2008. Growth curves were studied in flow cell media by some authors (Starner et al., 2008). Also a different method was followed by Gerber et al., 2008 in which bacterial growth was measured by counting plated cells at 0, 4, 8, 12, 16, 20 and 24 hr. The maximum growth rate curves with Methicillin and Vancomycin antibiotic for both the MRSA and MSSA isolates were reduced in dose dependent manner suggesting that they are effective in changing the specific growth rate at subinhibitory concentrations. Similar results were given for Methicillin by Bernardo et al., 2004; Cummins et al., 2009; Hammer et al., 2005; Koszczol et al., 2006 and for Vancomycin by Joo et al., 2010; Qiu et al., 2011. We can say that at the subinhibitory concentrations the agr and the sar locus are suppressed along with the suppression in growth. Leng et al., 2011, studied the growth curves of S. aureus in the presence of subinhibitory concentrations of allicin and documented that it had little influence on the growth. However with Vancomycin antibiotic the growth in the presence of 1/4 MIC and 1/8 MIC was similar to that of control suggesting that only the 1/2 MIC of Vancomycin is effective in changing the specific growth rate of both MRSA and MSSA isolate. Therefore, we here ascertained that the used antibiotic concentrations below the 1/2 MIC of Vancomycin antibiotic did not cause considerable growth defects over the entire growth curve (Joo et al., 2010; Qiu et al., 2011). This represents a noteworthy finding, as agr and sar locus controls a series of key virulence factors of S. aureus, and Vancomycin is frequently used for the treatment of MRSA infections. These infections may thus be severely exacerbated at subinhibitory concentrations, such as when not following the given treatment regime.

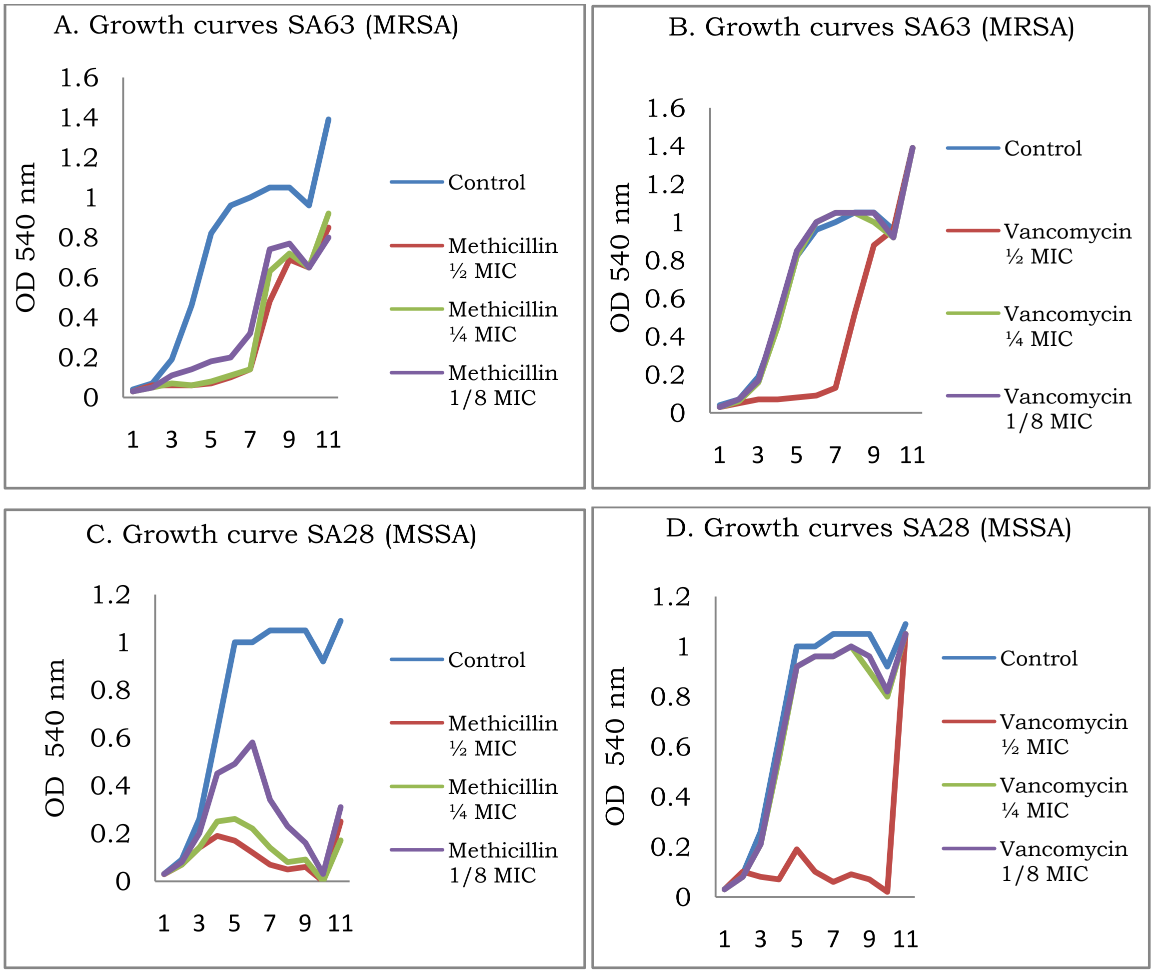

Fig 1. Representative growth curves of SA63 (A and B) and SA28 (C and D) in the presence of subinhibitory concentrations of Methicillin and Vancomycin antibiotic.

Conclusion:

The results presented here offer an important contribution to the field of subinhibitory concentrations of antibiotics and have clear implications for the treatment of MRSA and MSSA infections with Methicillin and Vancomycin antibiotics.

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