Vancomycin MIC creep in Staphylococcus aureus collected over a period of four years in a tertiary care centre

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Abstract:
Title Vancomycin MIC creep in Staphylococcus aureus collected over a period of four years in a tertiary care centre

Introduction S. aureus is one of the well-established and successful pathogens worldwide causing pyogenic as well as toxin mediated diseases. Methicillin resistant Staphylococcus aureus (MRSA) is currently one of the important pathogens responsible for many hospital acquired infections, especially in the Western population. Vancomycin is the drug of choice for MRSA infections. Complete vancomycin resistance of S. aureus has been established only in few circumstances. However, there are studies and meta-analysis which has shown that MRSA strains with higher MICs to vancomycin are more difficult to treat even though the MIC falls in the susceptible range. Retrospective analyses of vancomycin MIC over years in many institutes have observed a phenomenon where the numbers of isolates with higher MICs have been rising over the years. This phenomenon has been termed MIC creep.

Aim To determine the existence of vancomycin MIC creep in S. aureus in our tertiary care centre

Methods Consecutive clinical MRSA isolates from blood, sputum, pus, CSF, urine and body fluids of patients admitted to the hospital from January 2010 to December 2013 were examined. Cefoxitin resistance was determined using Kirby Bauer disc diffusion and interpretation was based on CLSI guidelines. Vancomycin MIC was determined using agar dilution technique. The geometric mean MIC was calculated using STATA IC 10.1 software.

Results A total of 6641 isolates of MRSA was included in the study. The percentage rise of MIC over four years show an initial increase in isolates with MIC of 1 microgram per ml in the year 2011 compared to 2010. However, there was an isolated significant fall in the percentages of isolates with an MIC of one in the past two years (p 0.01). No consistent trend was observed in the mean MICs over four years.

Conclusion The geometric mean
MIC over four years does not show any evidence of an MIC creep. Continuous monitoring of vancomycin MIC should be carried out to detect any variation in the trend.

**Keyword:** Staphylococcus aureus, Vancomycin, MIC creep, geometric mean MIC

**Introduction:**

Staphylococcus aureus is one of the well-established and successful pathogens worldwide and is associated with a number of pyogenic as well as toxin mediated diseases (1). The discovery of penicillin by Alexander Fleming and the chance observation of the inhibition of S.aureus by the compound ushered in an era of antimicrobials, one of the landmarks in the history of medicine (2). However, within four years of introduction of the drug S.aureus responded with the production of an enzyme penicillinase that rendered the antibiotic ineffective (3). Scientists quickly came forward with a counter attack in 1960 with the production of Methicillin, a prototype drug of the lactamase resistant class of drugs. However, methicillin resistant S.aureus (MRSA) strains emerged within one year of introduction of the antibiotic and the mechanism was due to an altered penicillin binding protein PBP2a. PBP2a was a novel penicillin binding protein with no affinity for methicillin and its class of drugs. It was coded for by mecA gene carried on a mobile genetic element known as staphylococcal cassette chromosome/SCCmec (4). MRSA emerged as a worldwide epidemic, quickly becoming the foremost pathogen that was isolated from hospital acquired infections in the United States. In a meta-analysis published in 2010, the prevalence of MRSA in 590 hospitals in USA was 66.4 per 1000 hospitalised patients (5). In this hospital MRSA roughly accounts for 40% of the S.aureus isolates from patient samples. In addition to increasing the mortality and morbidity of patients, it increases the healthcare expenses (6). Moreover, the drugs that are currently available with anti-MRSA activity is limited and the organism has already started developing resistance against agents such as linezolid, daptomycin, quinupristin, dalfopristin and tigecycline (7). Further, tigecycline is largely reserved for multi-drug resistant gram negative pathogens. The drug of choice for MRSA remains vancomycin, an old drug which currently regained importance in treatment of MRSA infections (8). The emergence of S.aureus strains showing resistance to vancomycin has been slow. The first strains showing intermediate resistance to glycopeptides were isolated approximately 40 years after the introduction of the drug (9). The mechanism responsible for this intermediate resistance has been proposed to be the mutations that cause an overproduction of D-Ala-D-Ala residues in the cell wall, which serve as dead-end binding sites for the antibiotic and prevent the further entry of vancomycin molecule into the bacterial cell (10). Till today only 13 cases of completely resistant strains have been isolated. The mechanism responsible for vancomycin resistant S.aureus (VRSA) is the production of D-Ala D-Lac residues in place of D-Ala D-Ala in the peptidoglycan cell wall, making it an ineffective binding site for the antibiotic. This is emerged into a worldwide epidemic, quickly coded by gene vanA, which was acquired by S.aureus through a lateral transfer from Enterococcus spp. The isolated VRSA strains were mostly from Michigan, USA with very few reports from outside USA (11). The MIC of vancomycin for MRSA isolates should be regularly monitored. No disc diffusion criteria exists for vancomycin as the molecule is big and its diffusion across the agar plate is not uniform enough to warrant standard conditions for...
resistance monitoring with this method. MIC can be measured using agar dilution technique, broth microdilution (BMD), E-test or automated systems such as Vitek 2, the CLSI reference method being BMD (12). The rising incidents of clinical failure of vancomycin in isolates with higher MICs in the susceptible ranges of MIC was a cause of concern among the physicians, while treating MRSA infections. This led them to closely observe the MIC values for vancomycin over the years and revision of the Clinical and Laboratory Standards Institute (CLSI) guidelines in 2006 lowered the MIC values of susceptible ranges from 4 µg/ml to 2 µg/ml (13). The current vancomycin MIC interpretive criteria for S.aureus as per CLSI is that isolates with MIC less than or equal to 2 µg/ml is considered susceptible to the antibiotic, MICs between 4 µg/ml and 8 µg/ml is interpreted as intermediate resistance and isolates with MIC more than or equal to 16 µg/ml are termed as resistant. Heteroresistance (hVISA/hGISA) is seen within MIC range of 1 to 2 µg/ml and consist of subpopulations (10^6) that may grow in media containing more than 2 µg/ml of vancomycin. Retrospective analysis of literature on MIC values over several years has raised the possibility of a possible increase in MRSA isolates with higher MICs in the susceptible range than reported earlier (14,15). This phenomenon has been termed the ‘MIC creep’ and the current analysis was carried out to determine the presence or absence of MIC creep from MRSA MIC data generated real time in our hospital from 2010 to 2013.

Materials and methods:
Consecutive clinical MRSA isolates from blood, sputum, pus, CSF, urine and body fluids of patients admitted to the hospital from January 2010 to December 2013 were included in the study. Only the first isolate of the patient was taken for the analysis. The isolates were tested for cefoxitin resistance (surrogate marker for MRSA) using the disk diffusion method. The zone of inhibition shown by the isolate to cefoxitin (30µg) on Mueller Hinton agar after overnight incubation was measured and interpreted based on the CLSI breakpoints (M100-S20). A zone size lesser than or equal to 21mm was considered resistant to cefoxitin. The MIC was determined using agar dilution for vancomycin for all these isolates. S.aureus broth was prepared from fresh subcultures in normal saline adjusted to turbidity standard McFarland 0.5. It was then spot inoculated onto multiple agar plates with doubling dilutions of vancomycin using a 20 point inoculator. The results were read after 24 hours incubation at 37 ºC. ATCC control strains with known MIC were included for quality control with each batch. The percentage of isolates with each MIC was calculated for all the four years. Geometric mean MIC for each year was calculated as it was a more sensitive indicator of MIC changes over the years (15). The geometric mean MIC for each year was calculated using STATA I/C 10.1 software (StataCorp LP, Texas, USA).

Results:
A total of 6641 isolates of MRSA was included in the study and it comprised of 25.4% (n=1,689) isolates in 2010, 25.6% (n=1,706) isolates in 2011, 24.8% (n=1,648) isolates in 2012, and 24.1% (n=1,598) isolates in 2013. The lowest MIC observed was 0.031 µg/ml for only one isolate in 2013. No isolate with MIC more than or equal to 2 µg/ml was observed. The number of isolates with each MIC is represented in Table1 with the per-
Discussion:
The percentage rise of MIC over four years show an initial increase in isolates with MIC of 1 µg/ml in the year 2011 as compared to 2010. However, there was an isolated significant fall in the percentages of isolates with an MIC of one in the past two years (p = 0.01). No consistent trend was observed in the mean MICs over four years. The geometric mean MIC over four years does not show any evidence of an MIC creep. Studies conducted in small centres such as by Ho et al in Hong Kong on 247 sepsis MRSA isolates from 1997 to 2008 were able to demonstrate a distinct vancomycin MIC creep (16). Similarly, Yeh et al were able to demonstrate an increase in the geometric mean MIC of MRSA isolates from 140 patients over 10 years (17). However, in a multicenter study conducted by Sader et al on 1,800 MRSA bloodstream isolates from nine hospitals across the United States from 2002 to 2006, the mode MIC remained stable at 0.625 mg/L during the study period with no evidence of an MIC creep (18). Likewise, a population analysis by Jones et al on 35,458 S.aureus strains over 6 years (1998 – 2003)
collected through the SENTRY antimicrobial surveillance program database was unable to demonstrate an MIC creep (19). The inference that can be drawn from the literature available on MIC creep is that large multicentric studies are unable to demonstrate a creep while single centre studies on fewer isolates were able to demonstrate an MIC creep. The observation of discrepancy in MIC creeps between large and single centre studies may be in part due to the various other factors that may play a role such as the MIC determination methods, the storage of isolates and statistical methods used for evaluation of the MIC creep. Although CLSI recommends the use of both broth microdilution and agar dilution for MIC measurement, it prefers BMD as the reference standard. BMD is cumbersome to perform and may not be a feasible option in laboratories with high sample turnovers. E-test is relatively simple to perform and has intermediate dilutions of concentrations of antibiotic in addition to the traditional concentrations, giving more accurate MICs. However, studies have proven that E-test overcalls MIC with results predicting MIC breakpoints higher than BMD. Further E-test strips are expensive making it a limiting factor in low resource settings (9,12). Studies have also shown that MIC testing on the isolate at the time of isolation and after storage yield discrepant results (20). Percentage increases of isolates with each MICs may also not be an accurate indicator of MIC creep. Geometric mean calculation and comparison over years is considered to be more useful in predicting an MIC creep (15). The above mentioned factors and the non-uniform nature of the data make the phenomenon of MIC creep a difficult entity to prove. The limitation of the present study is that it included only past four year’s data which may not be adequate enough to observe an MIC creep. A study over a longer study period may be more reliable in identifying the presence or absence of MIC creep.

**Conclusion:**
The collation of MIC data over 4 years in the tertiary care institution was unable to reveal an evident MIC creep for vancomycin in MRSA isolates. Further no difference in the clinical response to the antibiotic over the years is observed. Hence in this institution vancomycin still remains the drug of choice for MRSA infections with continuous monitoring of the variations in vancomycin MIC of MRSA.

**References:**


