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Original Article

Comparison of in vitro Efficacy of Quinopristin /Dalfopristin, Linezolid and Vancomycin for Methicillin Resistant Staphylococcus Aureus

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Abstract

Objectives: To compare the in vitro activity of quinopristin/dalfopristin, linezolid and vancomycin by determining their minimum inhibitory concentrations (MICs) for methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from various clinical samples received from LGH, Lahore.

Study Design : Comparative Study

Place and Duration of Study: Pathology Department, Post Graduate Medical Institute, Lahore from February 2013 to October 2013.

Methodology: The MIC of quinopristin/dalfopristin, linezolid and vancomycin for 50 MRSA strains were determined by using E-test strips (AB Biodisk, Biomeurix) and results were interpreted according to clinical and laboratory standards institute (CLSI) guidelines.

Results: All the isolates showed 100% susceptibility to linezolid and quinopristin/dalfopristin and currently no resistant strain was found for these drugs. Quinopristin/dalfopristin showed lowest MIC values than Linezolid and vancomycin.

Conclusion: The study provided the in vitro information in establishing the role of linezolid and quinopristin/dalfopristin as an alternative to vancomycin for the treatment of serious MRSA infections. It also gives information regarding linezolid and quinopristin/dalfopristin as the best therapeutic option for the treatment of hVISA, VISA and VRSA.

Key Words: Methicillin Resistant Staphylococcus aureus, Minimum Inhibitory concentration, Epsilometer Test, heteroresistant Vancomycin Intermediate Staphylococcus aureus, Vancomycin Intermediate Staphylococcus aureus, Vancomycin Resistant Staphylococcus aureus, Clinical Laboratory and Standard Institute.

Introduction

Resistance to antibiotics has increased noticeably over the past few years and now it has reached a level that places future patients in real danger. [1]

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Antibiotic resistance in bacterial species is becoming a serious problem and is a major concern throughout the world. The resistance of bacteria to many antimicrobial drugs is obvious and difficult to avoid because it represents a particular aspect of the general evolution of bacteria that cannot be stopped. [2]

Penicillin had been used as a drug of choice for treatment of serious Staphylococcus aureus infections. However, resistance to this drug developed early and soon after the introduction of benzyl penicillin into clinical use in the 1940s. ß-lactamase production by Staph.aureus which was under the plasmid control , made the organism resistant to penicillin only 2 years after its introduction [3]. Then, semisynthetic, ß-lactamase-stable penicillins (methicillin,1959) became the drug of choice for the treatment of infections due to penicillin resistant Staph.aureus. Again resistance to methicillin by Staph.aureus developed in early 1960's, and the strains were termed as methicillin-resistant Staphylococcus aureus. [4] Methicillin-resistant Staph.aureus (MRSA) strains are resistant to all ß-lactam agents including cephalosporins and carbapenems. [5]

Vancomycin has been considered as the treatment of choice for invasive MRSA infections for many years. [6] Since the mid-1980's, excessive use of vancomycin resulted in higher MIC values as well as emergence of heterogeneous Vancomycin-intermediate S. aureus (hVISA), Vancomycin-intermediate S. aureus (VISA) and vancomycin-resistant Staphylococcus aureus (VRSA).[7] Although, vancomycin resistant strains in Staph.aureus were rare in the beginning but now they are continuously rising in different parts of the world and are a real threat to mankind. [8]

Now it is the need of hour to characterize other treatment options not only for MRSA but also for the increasingly prevalent hVISA and VISA and VRSA isolates. [9]

Linezolid is the first licensed member of a new generation of antibiotics, synthetic oxazolidinone [10]. It has a great role in the treatment of necrotizing infections, including not only skin lesions, fascitis but also for pneumonia caused by community-associated MRSA. Recent studies show that linezolid has ability to reduce the production of toxic-shock syndrome toxin–1 and Panton-Valentine leukocidin i.e. a hemolysin. [6, 11] Linezolid is being used effectively for serious MRSA infections but the emerging resistance strains of linezolid resistance Staphylococcus aureus, although rare but is a real threat in near future. [10] New options should be kept in hand to cope with serious situations. [12]

Quinupristin/Dalfopristin (Synercid) is a new water soluble streptogramin antimicrobial agent. The preparation consists two constituents, quinupristin (a streptogramin B) and dalfopristin (a streptogramin A), in a fixed proportion (3:7). It has a potent activity against gram positive cocci. Synercid now appears to be one of those last reserve drugs for the clinician facing staphylococcal infections with such multiresistant isolates. [13]

Present study was planned to explore new therapeutic options like quinopristin/dalfopristin for MRSA, VISA and VRSA as well as a backup drug in rare cases of linezolid resistance. These resistance strains are a matter of serious concern in the present therapeutic scenario in the developing countries including Pakistan. So, the in vitro efficacy of new drugs was determined as a new therapeutic plan in near future in our setup.

Methodology

The present study was carried out on fifty consecutive isolates of methicillin resistant Staphylococcus aureus recovered from various clinical specimens including pus, blood, urine, sputum, wound swabs, aspirates, CSF, high vaginal swabs, central venous lines from the patients admitted in Lahore General Hospital. These specimens were processed according to standard operating procedures at Microbiological lab of PGMI, Lahore.

Preliminary identification of Staphylococcus aureus was done by noting the colony morphology on blood agar plates, Gram stain and catalase tests. Further biochemicals like coagulase and

DNase were performed for the confirmation of organism. The phenotypic resistance to methicillin was determined using cefoxitin disk 30µg (oxoid Ltd) on Mueller Hinton agar, inoculated with the organism suspension adjusted according to 0.5McFarland turbidity standards. The plates were incubated at 35°C for 24 hrs and interpretation was done according to the clinical and laboratory institutes (CLSI) guideline. [14] All isolates that showed cefoxitin resistance were tested for *mecA* gene product (PBP2a) using latex agglutination kit (Slidex, Biomeurix) for confirmation of MRSA.

A bacterial suspension was prepared by direct colony suspension method from an overnight culture, adjusted to 0.5 McFarland turbidity standard was plated on Mueller-Hinton agar and E-strips (AB Biodisk, BioMerieux) of quinopristin/dalfopristin, linezolid and vancomycin were applied over it. The plates were incubated at 35˚C for 24 hours aerobically. The MICs were read directly from a scale on the top of the strip at a point where ellipse of growth inhibition intercepted the strip. MIC results were interpreted according to the criteria set by Clinical Laboratory Standards Institute. [14] Control strains of MRSA ATCC 33591 and MSSA ATCC 25923 were used as positive and negative control respectively.

Statistical Analysis Data was analyzed using SPSS Version 17.0 (Statistical Package for Social Sciences). MIC values of the Vancomycin, Linezolid and Quinopristin Dalfopristin were compared by using Kruskal-Wallis Test and Pair wise comparison of three drugs was done by using Bonferroni Test**.**

Results

The frequency distribution of MIC values of vancomycin for 50 isolates of methicillin resistant *Staphylococcus aureus* is shown in table 1. MIC 1.5 µg/ml of vancomycin was observed for 7 (14%) isolates of methicillin resistant *Staphylococcus aureus*. 18 (36%) isolates of MRSA were inhibited at MIC 2 µg/ml. While MIC 3 µg/ml and MIC 4 µg/ml of vancomycin was observed for 21 (42%) and 4 (8%) isolates of MRSA respectively.

Table 1. Frequency Distribution of MICs of Vancomycin for Methicillin Resistant Staphylococcus aureus isolates (n = 50)

Vancomycin MIC values (µg/mL)	Frequency	Percentage
1.50	7	14
2.00	18	36
3.00	21	42
4.00	4	8
Mean±SD	2.5100±0.7249	

The results of Table 2 show the frequency distribution of MIC values of linezolid for 50 isolates of methicillin resistant *Staphylococcus aureus*. Two (4%) and nine (18%) isolates of MRSA were inhibited at MIC 0.25 µg/ml and MIC 0.38 µg/ml respectively. While MIC 0.5 µg/ml and MIC 0.75 µg/ml of linezolid was observed for 14 (28%) and 16 (32 %) isolates of MRSA. Moreover, 7 (14%) and 2 (4%) isolates of MRSA were inhibited at MIC 1 µg/ml and MIC 1.5 µg/ml respectively.

Table 2. Frequency Distribution of MICs of Linezolid for Methicillin Resistant Staphylococcus aureus isolates (n = 50)

Linezolid MIC values (µg/mL)Linezolid (µg/mL)	Frequency	Percentage
0.25	2	4
0.38	9	18
0.50	14	28
0.75	16	32
1.00	7	14
1.50	2	4
Mean±SD	0.6584±0.2766	

Table 3 depicts the frequency distribution of MIC values of quinopristin/dalfopristin for 50 isolates of methicillin resistant *Staphylococcus aureus*. MIC 0.19 µg/ml and MIC 0.25 µg/ml of quinopristin/dalfopristin was observed for 3 (6%) and 12 (24 %) isolates of MRSA respectively. 14 (28%) and 16 (32%) isolates of MRSA were inhibited at

MIC 0.38 µg/ml and MIC 0.5 µg/ml respectively. While MIC 0.75 µg/ml of quinopristin/dalfopristin was observed for 2 (4%) isolates of methicillin resistant Staphylococcus aureus.

Table 3. Frequency Distribution of MICs of Quinopristin/Dalfoprsitin for Methicillin Resistant Staphylococcus aureus isolates (n = 50)

Quinopristin/Dalfopristin MIC values (µg/mL)	Frequency	Percentage
Quinopristin/Dalfopristin (µg/mL)		
0.19	3	6
0.25	12	24
0.38	24	48
0.50	9	18
0.75	2	4
Mean±SD	0.3720±.11867	

The MIC 50 and MIC 90 of all three drugs were calculated and compared, as shown in Figure 1.

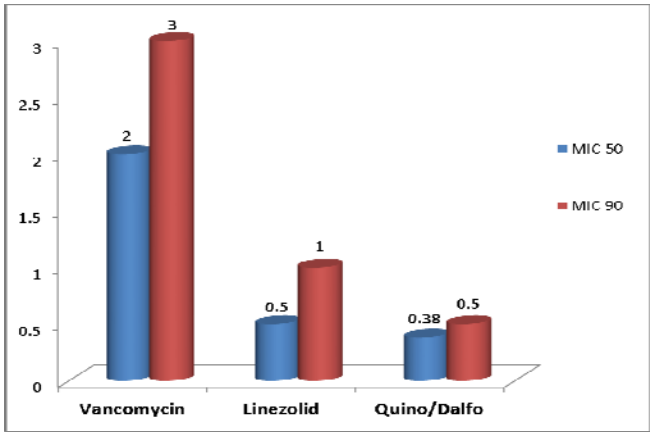

Figure 1: Graph Showing Comparison of MIC 50 & MIC 90 Values of Vancomycin, Linezolid and Quinopristin/Dalfopristin isolates for Methicillin Resistant Staphylococcus aureus (n = 50)

MIC 50 of vancomycin, linezolid and quinopristin/dalfopristin were 2 ug/ml, 0.5 ug/ml and 0.38 ug/ml respectively whereas 3 ug/ml, 1 ug/ml and 0.5 ug/ml were calculated as MIC90 of vancomycin, linezolid and quinpristin/dalfopristin respectively.

The range of MIC values of vancomycin for MRSA isolates was 1.5ug/ml to 4ug/ml whereas the MIC values of linezolid and quinopristin/dalfopristin were in the range of 0.25ug/ml to 1.5ug/ml and 0.19 to 0.75ug/ml respectively. The most frequently detected MIC value for Methicillin Resistant Staphylococcus aureus was 3µg/ml (42%) for vancomycin, 0.75µg/ml (32%) for linezolid and 0.38µg/ml (48%) for quinopristin/dalfopristin.

Kruskal-Wallis Test implies that mean rank of vancomycin is highest followed by the mean ranks of linezolid and quinpristin/dalforpristin respectively .P- Value < 0.05 indicated that highly statistically significant difference was present between MICs of vancomycin, linezolid and quinopristin/dalfopristin. Pair-wise of means of three different drugs on the basis of Bonferroni Post Hoc test .There was statistically high significant difference among MICs of vancomycin and linezolid, vancomycin and quinopristin/dalfopristin and linezolid and quinopristin/dalfopristin.

Discussion

The development of antimicrobial resistance is a serious situation all over the world and is worsening day by day. It is considered as the result of their excessive use since their introduction [15]. In future, there is danger that many infections may remain untreatable because of antibiotic resistance to already present compounds together with the continuous decline in the success rate of the discovery of new ones. [16] This issue has produced a major demand for new antimicrobial agents that can fight those resistance strains that cause infections not only in health care sector but also those that arise in community. [17]

In our study, all isolates of MRSA showed 100% susceptibility to linezolid and quinopristin/ dalfopristin. These two drugs showed better invitro efficacy than vancomycin. MIC50, MIC90 values and MIC range of linezolid and quinopristin/dalfopristin were lower than vancomycin.

Results of our study are in concordance with a study done by Kaleem et al. [18] In their study Linezolid showed better invitro efficacy than vancomycin. The MIC50, MIC 90 values as well as MIC range of Linezolid was lower than vancomycin. Similarly, Hannan et al [19] in 2010 found that linezolid has an outstanding activity for serious MRSA infection. However, its use as an empirical treatment without proper laboratory evaluation must be avoided. Chitnis et al [20] and Ranjan et al [21] in India found that Linezolid remain good therapeutic alternative to vancomycin for the treatment of infections caused by MRSA. However, a study was carried out by Kohno et al [22] in Japan in 2007. In their study, it was concluded that linezolid was equally effective to vancomycin for the treatment of patients with pneumonia, cSSTI and sepsis caused by MRSA and it showed better efficacy achieving microbiological eradication. Similar results were reported by Henwood et al [23] in UK and Beibei et al [24] in China.

Linezolid has an effective invitro activity for MRSA, VISA and VRSA. Linezolid is available as both oral and intravenous administration. Parenteral therapy should only be given to those patients who have problems with gastrointestinal absorption or if the patient is not able to take oral medications for any kind of reasons. Linezolid resistance is also very rare. [25]

A number of studies have demonstrated that patients with complicated skin and soft tissue infections treated with linezolid shows higher clinical cure rates and reduced lengths of hospitalization as compared to vancomycin. Higher clinical cure rates and higher survival rates were found in a number of analyses of clinical trials comparing linezolid to vancomycin in the treatment of MRSA pneumonia. The reason may be that linezolid provides much better tissue penetration especially lung penetration as compared to vancomycin .[26, 17]

A great number Staphylococcus aureus with intermediate and reduced susceptibility to vancomycin are emerging as reported by several researchers. More worryingly, is the emergence of linezolid reistance that have been reported in United states, Uk, Italy, Greece and other parts of the world. [27, 10, 28] Eventhough, linezolid resistance is extremely rare in Asian countries but now some cases have been reported from India and Iran. [29, 30]

Regarding this grave situation of rising resistance strains , it was the need of hour to do determine invitro efficacy of recently licensed new drugs like quinopristin/dalfopristin as a future therapeutic plan in Pakistan as it have not yet been in clinical use in our country. In our study, Quinopristin/dalfopristin, showed lowest MIC 50 and MIC 90 values i.e. 0.38 and 0.5 µg/ml respectively as compared to vancomycin and linezolid.

Quinopristin/Dalfopristin has been used as an effective salvage therapy for the treatment of invasive MRSA infections in cases of vancomycin treatment failure in adults and children. [26] Quinupristin/dalfopristin offers great advantages in the treatment of deep-seated infections such as osteomyelitis, endocarditis, meningitis and infections in the neutropenic host, where bactericidal activity is believed to be of special importance. The outstanding antibacterial activity of quinopristin/dalfopristin has indicated a great role of this novel agent in the treatment of severe and multidrug resistant infections in hospitalized patients. [31] A number of recent in-vitro studies of quinupristin/dalfopristin have proved that the antibiotic is showing excellent activity against S aureus isolates investigated in different parts of the world. [32, 33]

The results of our study are comparable with a study carried out in Turkey in 2006 by Baysallar et al [34]. They determined the in vitro antimicrobial activity of quinupristin/dalfopristin and linezolid for MRSA strains. The results showed that all MRSA strains were fully susceptible to both new compounds. Similarly, Limoncu et al [35] did a study on investigation of the bactericidal effects of vancomycin and quinupristin/dalfopristin on Staphylococcus aureus isolates in Turkey in 2003. The quinupristin/dalfopristin MIC range of the isolates was 0.12-2 µg/ml, MIC50 and MIC90 values were1 µg/ml.

Another study was carried out in United Kingdom by Griethuysen et al [36] in 2003. In their study, the MIC range for quinopristin/dalfopristin was 0.038-1.0 and MIC 50 and MIC 90 values were 0.38 and 0.5 respectively. Similarly, Baudoux et al [13] in Belgium (2010) concluded that quinupristin/dalfopristin now appears to be one of those last resource drugs for the clinician facing multidrug resistant Staphylococcal infections.

Data regarding minimum inhibitory concentration (MICs) of drugs and culture and sensitivity of isolates varies according to different geographical areas from country to country and even in the different cities and hospitals of the same country. That is why, nationwide surveillance programs must be carried out periodically to record the susceptibility pattern of drugs. Such types of study should regularly be conducted in every hospital to detect any change in MIC values (i.e. MIC shift) so that each hospital could formulate its own antibiotic policy.

Conclusion

Results of our study concluded that linezolid and quinopristin/dalfopristin showed excellent invitro efficacy for isolates of MRSA and currently no resistant strain was found for these drugs i.e. all the isolates were sensitive to these two drugs. Thus the study not only presents the invitro information in establishing the role of linezolid and quinopristin/dalfopristin as an alternative to vancomycin for the treatment of serious MRSA infections but it also provides the information regarding linezolid and quinopristin/dalfopristin as the best therapeutic option for the treatment of hVISA, VISA and VRSA. MRSA infections are very serious and a major cause of increased mortality and health care cost. It is the need of hour that we should carefully establish the diagnosis, test for antimicrobial susceptibility and MIC determination to ensure adequate dosing of drug. There is need of randomized clinical trials in this field to compare the traditional therapeutic agents with potential new therapeutic options.

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