Therapeutic Efficacy of Linezolid and Rifampicin against Experimentally Induced MRSA Mediastinitis in Rabbits

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ABSTRACT
Methicillin-resistant Staphylococcus aureus (MRSA) remains a significant challenge for human and veterinary health professions especially in the developing nations. Resistance to wide array of antibiotics is a major hurdle in treating MRSA infections including mediastinitis. In present study, therapeutic efficacy of linezolid alone and in combination with rifampicin was evaluated in experimentally induced MRSA mediastinitis in rabbits. Experimental upper median sternotomy was performed on 56 rabbits divided into seven groups. Out of these seven groups, one was taken as uncontaminated control group; one as untreated contaminated group; two groups were given different doses of linezolid, one group was treated with rifampicin alone whereas remaining two groups were given combinational therapy of linezolid and rifampicin. Both 50 mg/kg and 100 mg/kg dose rates of linezolid were found to be effective whereas no added benefit was observed in rabbits given a combination of rifampicin and linezolid. Linezolid can be used in serious MRSA infections especially in those patients not responding to conventional antimicrobial therapy but the selection of correct dose of linezolid is important in each patient.

INTRODUCTION
Methicillin resistant Staphylococcus aureus (MRSA) infections remain to be a significant challenge for human healthcare profession (Dulon et al., 2011; David et al., 2012; Khan et al., 2013). During the past two decades, pockets of methicillin resistant Staphylococcus aureus (MRSA) infections have been recognized in both developed (Brugnaro et al., 2009, Jarvis et al., 2012; Aidah et al., 2014) and developing countries including Pakistan (Assma et al., 2009). MRSA infections have been associated with high mortality, and morbidity with high therapeutic costs (Cosgrove et al., 2003; Engemann et al., 2003). To date, scanty data are available regarding prevalence of MRSA infections in Pakistan (Hakim et al., 2007). Antibiotics continue to play an iconic role in the prevention and cure of staphylococcal infections but resistance to conventional antibiotics can lead to treatment failure as may be seen in patients with MRSA infections. Patients undergoing cardiothoracic surgery are at higher risk of developing postoperative mediastinitis and MRSA mediastinitis is not uncommon among these patients. Higher mortality rates have been reported due to MRSA mediastinitis after open-heart surgery procedures (Braxton et al., 2000; Miralem et al., 2004; Memon et al., 2013).

Vancomycin (a glycopeptides antibiotic) has been the first line therapy for the treatment of MRSA infections. However, increase in minimum inhibitory concentration (MIC) of vancomycin against MRSA isolates and notable association between these isolates with higher rates of treatment failure and mortality has prompted investigators to research for other treatment options. Linezolid offers the promise of an ideal antibiotic for the treatment of MRSA infections (Watkins et al., 2012). It is a synthetic oxazolidinone antibiotic endowed with an excellent antimicrobial activity against Staphylococcus aureus resistant to methicillin and vancomycin (Rybak et al., 2000, Jinjian et al., 2013), while rifampicin has also been used in combinational therapy against MRSA (Forrest and Tamura, 2010). The present study was planned to evaluate the efficacy of two frontline antibiotics viz. linezolid and rifampicin against MRSA infection in a rabbit model.

MATERIALS AND METHODS
Two frontline drugs viz., linezolid and rifampicin were evaluated for their therapeutic efficacy against experimentally induced MRSA mediastinitis in rabbits.
Confirmation of MRSA isolate and susceptibility testing: The MRSA isolate used in the present study was recovered from the human patient with surgical site infection and was confirmed by the composite of Gram staining, catalase reaction (using freshly prepared 3% hydrogen peroxide solution), β hemolysis on blood agar, growth on CHROMagar MRSA (CHROMagar Microbiology, Paris, France), tube coagulase test (using lypholyzed rabbit plasma), clumping factor (bound coagulase), protein A (Staphytec Plus, Oxoid Ltd, Basingstoke, UK) and analytical profile index (api® Staph, bioMérieux Sa, France). Further confirmation was based on latex agglutination test (PBP2' test kit; Oxoid Ltd, Basingstoke, UK), resistance to oxacillin on Mueller-Hinton agar plates (by disc diffusion method) and molecular detection of mecA gene as per Brown et al. (2005). MIC of the strain was determined by E-test (AB Biodisk, Solna, Sweden) in accordance to Anonymous (2006).

Experimental rabbits and grouping: A total of 56 adult male rabbits weighing between 1.5-2 kg were randomly divided into 7 groups viz., A, B, C, D, E, F and G, having 8 rabbits each. The rabbits of each group were housed in separate cages and were provided with water and feed ad libitum. Group A and B were regarded as uncontaminated and contaminated untreated controls respectively. The subsequent groups (C, D, E, F and G) were subjected to MRSA contamination in order to induce mediastinitis. Rabbits in group C and D were treated with linezolid @ 50 and 100 mg/kg, IV, b.i.d respectively whereas rabbits in group E received rifampicin @ 40 mg/kg orally b.i.d. Group F and G received a combination therapy (linezolid 50 mg/kg + rifampicin 40 mg/kg) and (linezolid 100 mg/kg + rifampicin 40 mg/kg) twice a day, respectively.

Experimental contamination of mediastinum with MRSA in rabbits: Each rabbit was anesthetized with combination of medetomidine (@ 0.15-0.25 mg/kg) and ketamine (@ 10-15 mg/kg) intramuscularly as per Meredith and Flecknell (2006). After surgical preparation of cranial thorax of each rabbit, the incision was made over cranio-dorsal area of sternum. Both skin and pre-sternal layers were incised and partial upper median sternotomy was performed without opening the pleural space (Sacar et al, 2008). All rabbits (n=48; group B thru G) received 0.5 ml of 10^8 colony forming units (CFU)/ml of MRSA directly in to the wound (mediastinal and sternal layers). Following inoculation, the skin layers were immediately closed with surgical silk (2-0). All subjects (n=56) were examined twice daily for physical parameters (temperature, pulse rate, respiration rate).

Evaluation of the infection: Twelve hour post-therapy, all rabbits (n=48) of groups B-G were euthanized by injecting 120 mg of pentobarbital as per guidelines of Ethics Committee, University of Agriculture, Faisalabad Pakistan. Sternotomy was performed on each rabbit and swabs were obtained from mediastinum. Swabs samples were placed in tubes containing 1 ml of phosphate buffered saline and vortexed for 3 minutes. In order to quantitate MRSA isolates, serial 10-fold dilutions (0.1 ml) of bacterial suspensions were streaked on sheep blood agar plate, incubated for 37°C for 48 hours. All plates were evaluated for presence of MRSA growth and number of CFUs per plate was counted.

Statistical analysis: Data were analyzed with SPSS for windows 17.0. Arithmetic mean±SD of CFU was calculated for quantitative culture results. One way analysis of variance (ANOVA) was used to calculate the difference among the groups. Difference were recorded significant if P<0.05.

RESULTS

The clinical isolate used in the present study was found to be susceptible to linezolid and rifampicin using the microdilution broth method. The minimal inhibitory concentrations (MICs) for both rifampicin and linezolid were found to be 0.01 µg/ml and 2 µg/ml, respectively. All (n=80) rabbits in groups receiving antibiotic therapy, except 4 (group C), 6 (group D), 1 (group E), 5 (group F) and 6 (group G) rabbits showed evidence of mediastinitis. Culture negative rates and mean bacterial counts are shown in Table. The 50 mg/kg dose of linezolid alone or in combination with rifampicin was found effective in comparison with the contaminated untreated group (P>0.05). Quantitative mean bacterial counts were found significantly lower in comparison with contaminated untreated group receiving 50 mg/kg linezolid alone or in combination with rifampicin (group C and F). Gross difference in overall mean bacterial count could be observed in groups receiving 100 mg/kg linezolid alone or in combination with rifampicin (group D and G) in comparison with untreated contaminated group (P<0.05). However, in group E where 40 mg/kg rifampicin was used, the results were not as promising as in groups receiving linezolid alone in combination with rifampicin. On the other hand, in terms of mean bacterial count, no statistically significant difference was found between groups receiving linezolid alone or in combination with rifampicin.

DISCUSSION

The aim of the present study was to evaluate the efficacy of linezolid alone or in combination with rifampicin in the treatment of post-sternotomy mediastinitis in experimentally induced infections of MRSA in rabbit model. Moreover, efficacy of rifampicin along with linezolid was also evaluated accordingly. Mediastinitis model were selected in rabbits with experimental induction of MRSA since analogous infections in human are not uncommon and generally need in time aggressive treatment and long-term antimicrobial therapy. In present study authors used both 50 mg/kg and 100 mg/kg doses of linezolid as 25 mg/kg dose has been reported to be ineffective in such infections (Sacar et al., 2008). Similarly one report (Oramas-Shirey et al., 2001) documented that 25 mg/kg IV dose of linezolid given thrice a day was found to be ineffective in treating S. aureus infections. Post-cardiovascular surgery infections are life threatening and add up to post-surgical complications leading to increased rates of morbidity and
mortality. It has been reported that MRSA infections are often related with worse clinical outcome (Mekontso-Dessap et al., 2001). Vancomycin has commonly been used in patients with serious post-cardiovascular surgery MRSA infections. However, since there are numerous reports concerning vancomycin resistant MRSA isolates (Sakoulas et al., 2007; Van Hal and Fowler, 2013) vancomycin is not widely being used in treating MRSA infections.

In the present study, linezolid both at dose rate of 50 mg/kg and 100 mg/kg was found to be effective as significant (P<0.05) reduction was observed in overall bacterial count in mediastinum. Moreover, combinational therapy using linezolid and rifampicin was found to carry no advantage over use of linezolid alone. Our results are in line with those described by Sacar et al. (2008) where they reported that linezolid alone or in combination with rifampicin was effective against MRSA strain without any added benefit of using rifampicin. This study has shown indifference regarding combinational therapy using linezolid and rifampicin versus linezolid alone. This finding in our results is in line with that described by Dailey et al. (2003). Linezolid both at 50 mg/kg and 100 mg/kg found to be effective alone and in combination with rifampicin. Nevertheless, there are numerous reports (Diekema and Jones, 2001; Meka et al., 2004; Gu et al., 2013) of development of resistance against this agent and therefore its use should be restricted only for patients with serious life-threatening infections. Linezolid alone or in combination with rifampicin was found to be effective in treating the experimentally induced MRSA mediastinitis in experimental rabbits. However, adding the rifampicin with linezolid did not show any beneficial response in treating experimentally induced MRSA mediastinitis in experimental rabbit infection model.

Conclusion: Linezolid both at 50 and 100 mg/kg dose was found effective in reducing the overall bacterial count in MRSA mediastinitis in experimental rabbits. However, addition of rifampicin did not show any additional benefits thus use of linezolid alone in serious MRSA infections might be more judicious to avoid unnecessary use of rifampicin and also to prevent the development of rapid resistance against rifampicin.

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