COMPARISON OF IN-VITRO EFFICACY OF VANCOMYCIN, LINEZOLID AND DAPTOMYCIN AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

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ABSTRACT

Objective: To compare the in-vitro efficacy of vancomycin, linezolid and daptomycin against *Methicillin Resistant Staphylococcus Aureus* (MRSA).

Study Design: Cross sectional study.

Place and Duration of Study: Department of Microbiology, Army Medical College Rawalpindi, from Jan 2012 to Jul 2012.

Methodology: *Staphylococcus Aureus* isolated from routine clinical specimens were subjected to Modified Kirby Bauer disc diffusion method for detection of MRSA as per Clinical and Laboratory Standards Institute guidelines. Minimum Inhibitory Concentration (MIC) of vancomycin, linezolid and daptomycin for each of 50 non-duplicate isolates of MRSA was determined by Etest, as per manufecturer's instructions (AB Biodisk, Solna, Sweden). The minimum concentration of the three antimicrobials required to inhibit fifty percent (MIC_{50}) and ninety percent (MIC_{90}) of the isolates were calculated by cumulative percentage.

Results: The Etest results revealed MIC_{90} of vancomycin, linezolid and daptomycin as 2µg/ml, 1µg/ml, and 0.5µg/ml respectively. Linezolid and daptomycin have better in-vitro efficacy based upon their lower MIC_{90} values. Moreover vancomycin MIC for one of the isolates was found to be in the non-susceptible range.

Conclusion: Compared to vancomycin, linezolid and daptomycin have better in vitro efficacy against MRSA but bactericidal action of daptomycin makes it superior over linezolid. Moreover, adoption of proper antiseptic measures and a judicious use of antimicrobial agents are the strongest weapons that we can develop against the multi-drug resistant organisms like MRSA.

Keywords: Daptomycin, Efficacy, Linezolid, Methicillin resistant, Staphylococcus aureus, Vancomycin.

INTRODUCTION

Since the discovery and the first use of antibiotics for the management of infections, the mankind is being challenged by newer resistance mechanisms in the bacteria[1]. Ever increasing resistance against antimicrobial agents is a matter of great concern for both the patients and the health care providers because they increase the mortality and morbidity of the patient[2]. *Methicillin Resistant Staphylococcus Aureus* (MRSA) is among the most frequently isolated multidrug resistant organisms[3]. MRSA is a type of *Staphylococcus aureus* which possesses mec-A gene rendering it resistant to all β-lactam drugs thus leaving us with limited treatment options[2]. It has been found to be responsible for blood stream, urinary tract, respiratory tract, endocardium, skin and soft tissue infections[4].

Vancomycin is a glycopeptide antibiotic which acts by inhibiting cell wall synthesis by binding with the D-alanyl-D-alanine of cell wall precursor[5]. It is mostly administered via parenteral route and orally only for the treatment of pseudo membranous colitis[5]. It belongs to Food and Drug Administration (FDA) pregnancy category C i.e. animal studies show toxicity, human studies are inadequate so can be used if benefit outweighs risk[6]. The main side effects are thrombophlebitis, red-man syndrome, erythema multiforme, toxic epidermal necrolysis, superinfection, thrombocytopenia and wet purpura[6]. Vancomycin intermediate and vancomycin

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resistant *Staphylococcus Aureus* (VISA and VRSA) strains are being increasingly reported world wide[3]. Slow antibacterial activity, low tissue penetration and infection relapse are other limitations of vancomycin[7].

Linezolid is a synthetic derivative of oxazolidinone which was approved for use for the first time in 2000[3]. It acts by inhibiting the protein synthesis by inhibiting formation of initiation complex. The oral administration is a big advantage for its use[3]. It is administered in a dosage of 600 mg twice daily. The main side effects are headache, diarrhea, nausea and if the treatment is prolonged for more than two weeks may lead to bone marrow suppression, thrombocytopenia, peripheral neuropathy, optic nerve damage and lactic acidosis. It also belongs to FDA pregnancy category C[6].

Daptomycin is a lipopeptide drug active against Gram positive bacteria[7,8]. FDA approved daptomycin, for the management of infections in 2003[9]. It acts by causing depolarization of cell membrane and loss of synthesis of proteins, RNA and DNA which causes bacterial cell death[8]. The main side effect of the drug is diarrhoea, rash, dizziness, dyspnoea, elevated serum creatinine phosphokinase (CPK) levels, myalgia and myopathy[6]. It belongs to FDA pregnancy category B i.e. Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women[8]. It is not approved for the treatment of meningitis and pneumonia because daptomycin has a poor penetration in cerebrospinal fluid and alveoli[5]. The FDA recommended dose of daptomycin is 6mg/kg intravenously however some authorities recommend 8-12 mg/Kg for bacteremia[6].

Due to multidrug resistance, infections due to MRSA are currently being mainly treated with vancomycin and linezolid however the emergence of resistance against our last resorts raises the need for evaluation of new antimicrobial agents for the management of such infections. Apropos above, this study was planned with the objective to compare the in-vitro efficacy of vancomycin, linezolid and daptomycin against methicillin resistant Staphylococcus aureus.

METHODOLOGY

This cross-sectional study was conducted in the Department of Microbiology, Army Medical College, Rawalpindi, from Jan 2012 to Jul 2012. The clinical specimens like pus, blood, urine, sputum, body fluids and catheter tips routinely received in the department of Microbiology, Army Medical College, Rawalpindi were inoculated on standard microbiological media like blood agar, mac Conkey agar and cystein lactose electrolyte deficient (CLED) agar. The isolates were identified as *Staphylococcus Aureus* by colony morphology, Gram staining, catalase, coagulase and DNA se tests. Antimicrobial susceptibility testing was carried out by Modified Kirby Bauer disc diffusion method using antibiotic discs of cefoxitin, erythromycin, clindamycin, rifampicin, fusidic acid, vancomycin, linezolid, chloramphenicol and gentamicin[10]. All those isolates showing a zone of inhibition of ≤21 mm around cefoxitin were considered as methicillin resistant *Staphylococcus Aureus* (MRSA)[10]. A total of 50 MRSA isolates were included in the study. MRSA isolated from the same patient during the same episode of illness were excluded. The sampling technique was non-probability consecutive.

A bacterial suspension of each isolate matching 0.5 McFarland turbidity standard was prepared and applied on three plates of Mueller Hinton agar (MHA). Etest strips of vancomycin, linezolid and daptomycin were applied on separate inoculated plates which were then incubated at 37°C for 16-24 hrs. Minimum inhibitory concentration (MIC) values were read and recorded according to the manufacturer's instructions (AB Biomeriux, Solna, Sweden)[11]. The percentage of the isolates exhibiting each MIC was calculated. The percentages and the respective MICs were then arranged in ascending order. Minimum concentration of the antimicrobials required to inhibit 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates were calculated by cumulative percen-

tages (table-I to III). MIC_{50} and MIC_{90} of the three antimicrobial agents were compared. Each isolate was considered susceptible to vancomycin, linezolid and daptomycin at MIC values of ≤ 2µg/ml, ≤4µg/ml and ≤1µg/ml respectively, as per Clinical and Laboratory Standards Institute (CLSI) guidelines[10]. The in vitro efficacy of the three antibiotics were compared descriptively on the basis of the lowest MIC_{90} value as a marker of better in vitro efficacy. Results were analyzed on the Statistical Package for Social Sciences (SPSS) version 20. The gender distribution and mean age of the patients with MRSA infection were calculated. Frequency and percentage were calculated for qualitative variables like wards, clinical specimens and antimicrobial susceptibility.

Table-I: Percentage representation of isolates showing respective MIC values against Daptomycin.

MIC (µg/ml)	No. of MRSA Isolates Having this MIC	Isolate Percentage (%)	MIC_{50} and MIC_{90}
0.016	-	-	
0.023	-	-	
0.032	1	2	
0.047	1	2	
0.064	1	2	
0.094	5	10	
0.125	11	22	
0.19	8	16	MIC_{50}
0.25	12	24	
0.38	5	10	
0.50	3	6	MIC_{90}
0.75	2	4	
1.0	1	2	
1.5	-	-	
2	-	-	

Table-II: Percentage representation of isolates showing respective MIC values against Linezolid.

MIC (µg/ml)	No. of MRSA Isolates Having this MIC	Isolate Percentage (%)	MIC_{50} and MIC_{90}
0.016	-	-	
0.023	-	-	
0.032	-	-	
0.047	-	-	
0.064	-	-	
0.094	-	-	
0.125	2	4	
0.19	3	6	
0.25	2	4	
0.38	5	10	
0.50	6	12	
0.75	17	34	MIC_{50}
1.0	12	24	MIC_{90}
1.5	3	6	

RESULTS

During the period of our study, a total of 55 MRSA were isolated but after excluding 5 isolates as per exclusion criteria, 50 strains of MRSA were included in the study. The mean age of the patients with MRSA infection was 35.2 ± 19.7 years. The male to female ratio was 1.7 to 1. The highest percentage of MRSA was isolated from out-patient departments (22%) followed by dermatology (16%), Male medical ward (14%), Pediatric ward (14%), Intensive care unit (12%), female medical and gynecology ward (10%), surgical post operation ward (6%) and only 2% were isolated from rehabilitation ward, high nursing care and psychiatry unit each. Seventy four percent of the MRSA were isolated from pus (representing skin and soft tissue infection) followed by blood (10%) (indicating bacteremia), sputum (8%) (representing pneumonia), urine (4%) and least commonly from pleural fluid

Table-III: Percentage representation of isolates showing respective MIC values against Vancomycin.

MIC (µg/ml)	No. of MRSA Isolates with this MIC	Percentage of MRSA Isolates with this MIC	Cumulative Percentage	MIC_{50} and MIC_{90}
0.016	-	-	-	
0.023	-	-	-	
0.032	-	-	-	
0.047	-	-	-	
0.064	-	-	-	
0.094	-	-	-	
0.125	-	-	-	
0.19	-	-	-	
0.25	-	-	-	
0.38	-	-	-	
0.5	2	4	4	
0.75	3	6	10	
1.0	14	28	38	
1.5	24	48	86	MIC_{50}
2.0	6	12	98	MIC_{90}
3.0	1	2	100	

(2%) and double lumen catheter tip (2%). Their antimicrobial susceptibility pattern revealed 100% susceptibility to linezolid and daptomycin, 98% to vancomycin, 88% to minocycline and they were least susceptible to ciprofloxacin (12%) (fig-1). The Etest results revealed MIC ranges of vancomycin, linezolid and daptomycin as 0.5-3.0 µg/ml, 0.125-1.5 µg/ml, and 0.125-1.0 µg/ml respectively. A comparison of the MIC_{50} and MIC_{90} values of the three drugs revealed that as compared to vancomycin and linezolid, daptomycin had lowest MIC_{90} value and thus it has the best in vitro efficacy against MRSA (fig-2).

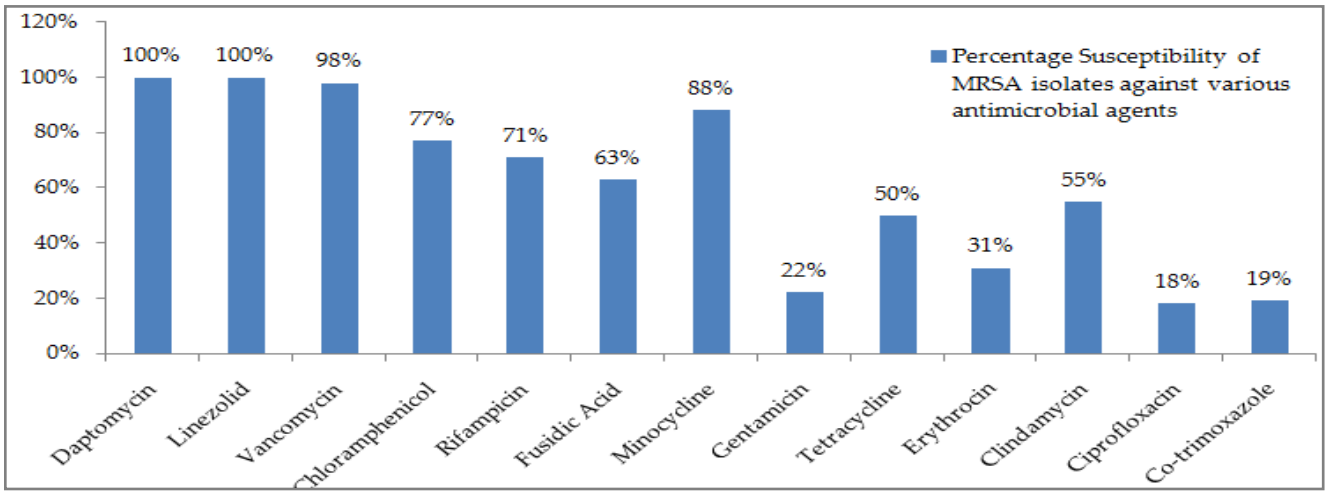

Figure-1: Percentage susceptibility of MRSA isolates (n=50) against various antimicrobial agents.

DISCUSSION

Since the discovery of MRSA in 1961, Infections due to MRSA are being increasingly reported worldwide[12]. Frequency of MRSA has been variously reported to range from 24-50% in different cities of Pakistan[12]. Vancomycin is the mainstay of treatment of such infections. With the emergence of first case of VISA from Japan and VRSA from United States the management of such infections has become even more difficult[13]. In this scenario, evaluation of a newer antimicrobial like daptomycin was of paramount importance. So a comparison of daptomycin with linezolid and vancomycin, the two currently used antimicrobials against MRSA was carried out in this study.

The colonization of MRSA in anterior nares, axilla and skin is likely to play a key role in subsequent MRSA infections in the person himself when the immunity is compromised e.g. in trauma, surgery or catheterization[14]. The carriers can be either persistent or intermittent[14]. Therefore the decolonization of MRSA especially before the planned surgical procedures can be a very important strategy to successfully reduce the rate of MRSA infections[14]. The decolonization success rate has been reported to range from 25% to 95% depending upon the protocol adopted[15]. Local application of 2% mupirocin ointment three times a day for four to seven days along with daily bathing with chlorhexidine gluconate soap has been recommended for successful decolonization of MRSA[14]. However increasing resistance in MRSA against mupirocin is being reported in

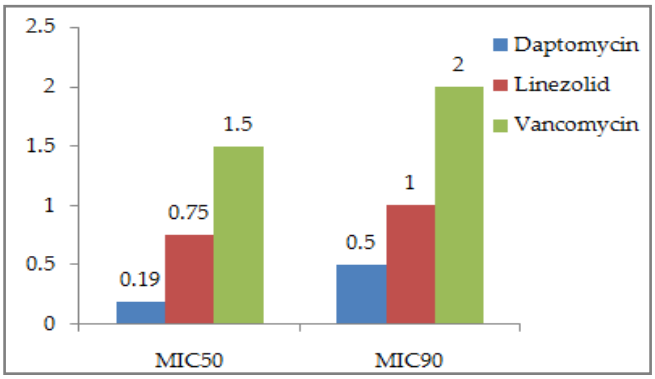

Figure-2: Comparison of MIC_{50} (µg/ml) and MIC_{90} (µg/ml).

various studies[16]. Chadha *et al* have reported mupirocin resistance of 15% while Dardi has reported 5.99% high level mupirocin resistance and 15.35% low level mupirocin resistance in MRSA[16,17].

Our study revealed 98% susceptibility of MRSA against vancomycin which is higher as compared to a study conducted in Karachi, by Hakim *et al*, reporting frequency of isolation of VISA of 13%[18]. In a study conducted in Lahore in 2015, 8% of the isolates were found to be VISA[18]. Saleem *et al*, however reported a 100% susceptibility of MRSA to vancomycin which is in contrast to our observation[19]. Regional study from India revealed 7 VISA strains[11].

In the current study MIC_{90} values of vancomycin, linezolid and daptomycin were found to be 2µg/ml, 1µg/ml and 0.5µg/ml respectively. Comparing our findings with other studies conducted in different parts of the world reveals varied results. A study conducted by Munera *et al* revealed MIC_{90} values of vancomycin, linezolid and daptomycin as 1.5, 3 and 0.5µg/ml respectively[20]. The MIC_{90} of daptomycin in this study was same however the MIC_{90} of vancomycin was lower and of linezolid was higher as compared to our isolates[20]. In a previous study by Niveditha *et al* the MIC_{90} value of vancomycin (2µg/ml) was same as our study but of daptomycin (0.38 µg/ml) was lower and that of linezolid(1.5 µg/ml) was higher as compared to our findings[11]. In both studies daptomycin revealed a better in vitro efficacy against MRSA as compared to vancomycin and linezolid. Another study by Nandakumar *et al*, also revealed better in vitro efficacy of daptomycin (0.09 µg/ml) as compared to vancomycin (1.3µg/ml)[21]. Maraconescu *et al* also reported better in vitro activity of daptomycin as compared to linezolid and vancomycin[22]. It can also be well appreciated that linezolid revealed higher MIC_{90} values as compared to our study. In contrast to this, a study by Chadha *et al* revealed, better in vitro efficacy of linezolid (100% susceptibility, MIC range: 0.047 - 4.0 µg/ml) against MRSA as compared to vancomycin (99% susceptibility, MIC range: 0.19 - 3.0 µg/ml) and daptomycin (99% susceptibility, MIC range: 0.032 - 1.5 µg/ml)[16].

A study by Kumari *et al* revealed 100% susceptibility of MRSA to daptomycin in contrast to 96.4% to vancomycin[9]. Four isolates in this study were VISA[9]. Despite 100% susceptibility of MRSA against daptomycin, the author concluded that daptomycin cannot be used as an alternative to vancomycin for MRSA infections because the four VISA isolates also showed high MIC of daptomycin (1µg/ml)[9]. In contrast to this, a study by Moore *et al*, revealed that as compared to vancomycin, daptomycin was more effective in bacteremia due to MRSA with high vancomycin MIC values[1]. Comparing these two studies with ours, we find that although the isolate showing vancomycin MIC of 3 µg/ml, in our study, also showed daptomycin MIC of 1µg/ml but is still within the susceptible range of daptomycin. Therefore unless any in vivo study proves daptomycin ineffective in managing infections with VISA isolates with high susceptible daptomycin MIC, it still remains the alternative treatment for management of infections due to VISA. Small sample size and no clinical correlation were the limitations of our study. We recommend further large scale clinical studies to evaluate the efficacy of daptomycin against these high daptomycin MIC isolates.

The difference in the in vitro efficacy of the three antimicrobial agents can be attributed to the level of exposure of the strains to these agents. This also suggests that an appropriate selection of antimicrobial agents for the management of various infections may prevent this rapid emergence of resistance against them. This would help save the broad spectrum antimicrobial agents for the emergency use.

Disclosure

The study was presented in 36th Annual PAP/1st Joint Conference of Societies of Pathology in 2012.

CONCLUSION

Compared to vancomycin, linezolid and daptomycin have better in vitro efficacy against

MRSA but bactericidal action of daptomycin makes it superior over linezolid. Moreover, adoption of proper antiseptic measures and a judicious use of antimicrobial agents are the strongest weapons that we can develop against the multi-drug resistant organisms like MRSA.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Oksuz L, Gurler N. Susceptibility of clinical methicillin-resistant Staphylococci isolates to new antibiotics. J Infect Dev Ctries 2013; 7(11): 825-31.
2. Ullah A, Qasim M, Rahman H, Khan J, Haroon M, Muhammad N, Khan A, Muhammad N. High frequency of methicillin resistant Staphylococcus aureus in Peshawar Region of Pakistan. Springer Plus 2016; 5(1): 600-05.
3. Khalid A, Usman J, Faiz U, Kaleem F, Hassan A, Omair M. In-vitro susceptibility of linezolid against methicillin resistant Staphylococcus aureus at a tertiary care hospital in Pakistan. J Microbiol Infect Dis 2013; 3(4): 203-06.
4. Hsieh Y, Lin Y, Huang Y. Vancomycin, teicoplanin, daptomycin, and linezolid MIC creep in methicillin-resistant Staphylococcus aureus is associated with clonality. Medicine 2016; 95(41): e5060.
5. Lewis JS, Bush K. Antibacterial Agents. In: Jorgensen JH, Pfaller MA, Carroll KC, Landry ML, Funke G, Richter SS, Warnock DW (ed), Manual of clinical microbiology 11th ed. Washington, DC: American Society for Microbiology; 2015: 1171-1211.
6. Gilbert DN, Chambers HF, Eliopoulos GM, Saag MS. The Sanford guide to antimicrobial therapy. Antimicrobial Therapy. 45th ed. USA: INC; 2015.
7. Dilworth TJ, Ibrahim O, Hall P, Sliwinsky J, Walraven C, Mercier R. β-Lactams Enhance Vancomycin Activity against Methicillin-Resistant Staphylococcus aureus Bacteremia Compared to Vancomycin Alone. Antimicrob. Agents Chemother 2014; 58(1): 102-9.
8. Chitnis S, Katara G, Hemnavi N, Pareek S, Chitnis DS. In vitro activity of daptomycin & linezolid against methicillin resistant Staphylococcus aureus & vancomycin resistant enterococci isolated from hospitalized cases in Central India. Indian J Med Res 2013; 137(1): 191-6.
9. Kumari J, Shalini SM, Chakrapani M, Vidyalakshmi K, Gopalkrishna BK. In vitro activity of vancomycin and daptomycin against healthcare-associated Methicillin-Resistant Staphylococcus aureus isolated from clinical specimens. Asian J Pharm Clin Res 2016; 9(3): 44-6.
10. Clinical and Laboratory Standards Institute (CLSI)/NCCLS. Performance standards for antimicrobial susceptibility testing; twenty fifth informational supplement (M100-S23). Vol.33. Wayne, PA: CLSI/NCCLS; 2013.
11. Niveditha N, Sujatha S. Worrisome trends in rising minimum inhibitory concentration values of antibiotics against methicillin resistant Staphylococcus aureus – Insights from a tertiary care center, South India. Braz J Infect Dis 2015; 19(6): 585-9.
12. Taj R, Muhammadzai I, Ahmad J, Khan A, Syed F, Khan Z. Frequency and antibiotic susceptibility pattern of methicillin resistant Staphylococcus aureus in Abbottabad city of Pakistan. Khyber Med Univ J 2015; 7(4): 157-61.
13. Chen CJ, Huang YC. New epidemiology of Staphylococcus aureus infection in Asia. Clin Microbiol Infect 2014; 20(7): 605-23.
14. Abad CL, Pulia MS, Safdar N. Does the nose know? An update on MRSA decolonization strategies. Curr Infect Dis Rep 2013; 15(6): 455-64.
15. Sai N, Laurent C, Strale H, Byl B. Efficacy of the decolo-nization of methicillin-resistant Staphylococcus aureus carriers in clinical practice. Antimicrob Resist Infect Control 2015; 4(1): 56-63.
16. Chadha P, Mariano N, LaBombardi V, Segal-Maurer S, Urban C. In vitro activities of mupirocin, tigecycline, ceftaroline, vancomycin, linezolid and Daptomycin in clinical isolates of Methicillin-resistant Staphylococcus aureus by E-test methodology. Open J Med Microbiol 2015; 5(1): 12-6.
17. Dardi CK. Mupirocin resistance in clinical isolates of methicillin-resistant Staphylococcus aureus from a tertiary care rural hospital. Int J Adv Med Health Res 2014; 1(2): 52-6.
18. Arshad F, Javed I, Mushtaq S, Anwer S. Emergence of Vancomycin Intermediate Staphylococcus aureus (VISA) in a Tertiary Care Hospital in Lahore: An Alarming Situation. Int J Pathol 2015; 13(3): 89-94.
19. Saleem F, Fasih N, Zafar A. Susceptibility pattern of methicillin resistant Staphylococcus aureus to vancomycin and other alternate agents: report from a private sector hospital laboratory. J Pak Med Assoc 2017; 67(11): 1743-6.
20. Munera JMV, Rios AMO, Urrego DM. In vitro susceptibility of methicillin-resistant Staphylococcus aureus isolates from skin and soft tissue infections to vancomycin, daptomycin, linezolid and tedizolid. Braz J Infect Dis 2017; 21(5): 493-9.
21. Nandakumar A, Kumar A, Dinesh KR, Karim S. Vancomycin creep and daptomycin minimum inhibitory concentration in methicillin-resistant staphylococcus aureus. Ibnosina J Med BS 2014; 6(4): 180-84.
22. Maraconescu P, Graviss EA, Musher DM. Rates of killing of methicillin–resistant Stapylococcus aureus by ceftaroline, daptomycin, telavancin compared. Scand J Infect Dis 2012; 44(8): 884.