ORIGINAL ARTICLE

Susceptibility of methicillin-resistant staphylococcus aureus and enterococci to teicoplanin in Pakistan: The MRSET study

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Abstract

Objective: To evaluate the susceptibility pattern of ***S. aureus*** and ***enterococci*** to teicoplanin using an in vitro method.

Methods: Between February and November 2011, valid bacteriological samples were collected at three hospitals in three cities in Pakistan and the organism was isolated. Only samples containing ***S. aureus*** or ***enterococci*** were tested for their sensitivity to teicoplanin and various other standard antimicrobials in therapy, using the disc diffusion testing by the Kirby-Bauer method. SPSS 18 was used for statistical analysis.

Results: Of the 401 isolates collected, a majority 293 (59.6%) were methicillin-sensitive ***S. aureus***, while 136 (33.9%) were methicillin-resistant ***S. aureus***; and 26 (6.5%) were ***enterococci***. All isolates were sensitive to teicoplanin and vancomycin.

Conclusion: Teicoplanin had the same in vitro sensitivity as vancomycin against methicillin-sensitive ***S. aureus***, methicillin-resistant ***S. aureus*** and ***enterococci*** in clinical isolates.

Keywords: Teicoplanin, Methicillin-resistant, Staphylococcus aureus, Enterococci, In vitro, Pakistan. (JPMA 64: 256; 2014)

Introduction

Staphylococcus aureus (***S. aureus***) is a common cause of infections in hospitalised patients. Back in the early 1940s, isolates of ***S. aureus*** were found to be resistant to penicillin due to the production of β-lactamase.[1] Later, in 1961, the first isolate of methicillin-resistant ***S. aureus*** (MRSA) was reported from the UK.[1] Since then, MRSA has become a leading cause of invasive infections worldwide and a serious health concern.[1-3] The proportion of MRSA isolates among inpatients in the United States is between 48% and 57%, while in many European countries the prevalence of MRSA in hospital settings is around 30%.[4-6] Between 1991 and 2003, the overall prevalence of MRSA saw a dramatic doubling from 29% to 59%.[4] The statistics reported from previous studies in Pakistan vary between 29% and 43%.[7-9] The other major pathogen that is fast becoming a menace in hospital settings and is responsible for nosocomial infections is antibiotic-resistant enterococcus with about 28% of all enterococci isolates in the hospital setting in the United States being resistant to vancomycin.[4,10]

The first-line antimicrobial agent of choice for treatment against invasive MRSA infection is a glycopeptide antibiotic.[11] Vancomycin and teicoplanin are naturally occurring antibiotics that hinder the peptidoglycan synthesis that is necessary for the formation of Gram-positive bacterial cell wall and thus demonstrate bactericidal effects.[3] The spectrum of activity of both vancomycin and teicoplanin is similar except in the case of vancomycin-resistant enterococci (VRE) against which teicoplanin is active.[12-14] Besides antibiotic-resistance, there is still uncertainty regarding the nephrotoxic effects of vancomycin. Vancomycin accumulates in the proximal tubular cells in the kidney where it inhibits key enzymes like sphingomyelinase resulting in vacuolisation and necrosis.[15] Studies have shown an increased risk of kidney failure after vancomycin treatment, but it is also speculated that this could be because of older antibiotic purification techniques.[16] Vancomycin usage is also associated with 'red man syndrome', a unique hypersensitivity reaction.[17] The main advantages of teicoplanin are once-a-day bolus dose, intramuscular route that does not require serum monitoring, and possibly lower nephrotoxicity than that caused by vancomycin.[18] In a previous meta-analysis comparing the efficacy of teicoplanin to vancomycin, the investigators concluded that both agents were equally efficacious. However, the observed nephrotoxicity was at 10.7% in the vancomycin group in comparison to 4.8% in the teicoplanin group.[19] But it is argued by Cavalcanti, et al that the results of this meta-analysis cannot be relied upon given that the methods employed were poorly reported.[20] In a more recent systematic review, these

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results were recapitulated in terms of equivalent efficacy and a better safety profile for teicoplanin.[20]

With a broad range of antimicrobial agents available and an increasing resistance due to inappropriate antibiotic usage, selecting a correct agent is challenging, whether in empiric therapy or for infection by a known pathogen. In vitro antimicrobial susceptibility testing can be a useful guide to selection of the appropriate therapeutic agent.

The purpose of this study was to estimate in vitro sensitivity of ***S. aureus*** and ***enterococci*** to teicoplanin across three major cities of Pakistan.

Methods

The MRSET (Susceptibility of Methicillin Resistant Staphylococcus aureus and Enterococci to Teicoplanin in Pakistan) study was an investigation to evaluate the susceptibility of ***S. aureus*** and ***enterococci***, two of the leading causes of nosocomial infections worldwide, to teicoplanin and other antimicrobials in contemporary use. This was an in vitro study, and valid bacteriologic samples were collected from three study sites - Indus Hospital, Karachi; Pakistan Institute of Medical Sciences, Islamabad; and Mayo Hospital,Lahore - across Pakistan within a span of nine months between February and November 2011.

Samples were collected from pus, blood, sputum, tracheal aspirate, urine, ear, wound, abscess, tissue, central venous pressure (CVP) line, catheter tip, vagina, hydatid cyst fluid, throat, and scrotal swab. Activities carried out at the sample collection sites included isolation and identification of indigenous strains of S. aureus and enterococci based on cultural, morphological and biochemical properties.

The Indus Hospital Laboratory served as the central point to confirm the findings of the sample collection sites in addition to determining the antibiotic susceptibility of the bacterial isolates using the disc testing by Kirby-Bauer method. Only those isolates that contained either S. aureus or enterococci were tested for susceptibility. Susceptibility breakpoints were based on the recommendations made by the Clinical and Laboratory Standards Institute (CLSI) in 2010.[21] Control strains of bacteria were S. aureus ATCC 25923 and Enterococcus ATCC29212 (ATCC™, Virginia, USA). Bacterial culture and subsequent testing was carried out in Mueller Hinton agar medium.

Competitor/reference antimicrobial agents in addition to teicoplanin for staphylococcus were vancomycin, penicillin, oxacillin, cefazolin, cefuroxime, gentamicin, linezolid, ofloxacin, erythromycin, clindamycin, sulphamethoxazole-trimethoprim (SMZ-TMP), fusidic acid and amikacin. Similarly, antimicrobial agents for enterococci were vancomycin, ampicillin, nitrofurantoin, ciprofloxacin, high concentration gentamicin (120 µg/disc) and linezolid.

Teicoplanin discs were purchased from Oxoid (Oxoid Limited, United Kingdom). In the event of teicoplanin sensitivity, result of 'intermediate' (zone diametre 11-13mm) or 'resistant' (zone diametre <10 mm), the strains were required to be collected and kept in glycerol agar tube (<-80ºC) and the results verified by estimating the minimum inhibitory concentration by the microbroth dilution method.

Assuming sensitivity of ***S. aureus*** isolates to teicoplanin is 99%[9] and assigning a 1% margin of error for an acceptable 95% confidence interval, 381 samples were required to meet the study objective. It was proposed to study 400 isolates to account for missing information.

All categorical variables were analysed as frequencies and percentages while all continuous variables were reported as means with standard deviations. Statistical analyses were performed using SPSS version 18.

Results

Of the total 401 isolates, 237 (59%) were recovered from male patients. Overall, 239 (59.6%) isolates were methicillin-sensitive S. aureus (MSSA), followed by 136 (33.9%) MRSA ; and 26 (6.5%) enterococci (Table-1). Most of the isolates (244/401; 60.8%) were detected from pus samples.

All bacterial isolates of S. aureus, MRSA and MSSA, were sensitive to teicoplanin as well as vancomycin (Table-2). High level of resistance was seen against penicillin in the 133 (97.8%) MRSA isolates as well as 239 (90.8%) MSSA isolates. Linezolid was the only other antimicrobial agent that demonstrated a high level of MRSA sensitivity in 134 (98.5%) although this did not match the universal sensitivity of teicoplanin and vancomycin. As per the protocol, minimum inhibitory concentration estimation

Table-1: Distribution of isolates from study sites.

Hospital	MSSA	MRSA	Enterococci	Total
Indus	154	66	26	246
PIMS	69	58	0	127
Mayo	16	12	0	28
Total (%)	239 (59.6)	136 (33.9)	26 (6.5)	401

Abbreviations: MSSA: methicillin-sensitive Staphylococcus aureus. MRSA: methicillin-resistant Staphylococcus aureus. PIMS: Pakistan Institute of Medical Sciences.

Table-2: Antibiotic susceptibility pattern of MRSA and MSSA.

Antimicrobial	MRSA isolates (N=136)			MSSA isolates (N=239)		
	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)
Teicoplanin	136 (100.0)	0 (0.0)	0 (0.0)	239 (100.0)	0 (0.0)	0 (0.0)
Vancomycin	136 (100.0)	0 (0.0)	0 (0.0)	239 (100.0)	0 (0.0)	0 (0.0)
Penicillin	2 (1.5)	0 (0.0)	133 (97.8)	20 (8.4)	0 (0.0)	217 (90.8)
Oxacillin	0 (0.0)	0 (0.0)	136 (100.0)	239 (100.0)	0 (0.0)	0 (0.0)
Cefazolin	56 (41.2)	0 (0.0)	80 (58.8)	236 (98.7)	0 (0.0)	2 (0.8)
Cefuroxime	30 (22.1)	0 (0.0)	106 (77.9)	223 (93.3)	0 (0.0)	16 (6.7)
Gentamicin	54 (39.7)	1 (0.7)	81 (59.6)	235 (98.3)	0 (0.0)	4 (1.7)
Linezolid	134 (98.5)	0 (0.0)	1 (0.7)	239 (100.0)	0 (0.0)	0 (0.0)
Ofloxacin	59 (43.4)	0 (0.0)	77 (56.6)	235 (98.3)	0 (0.0)	4 (1.7)
Erythromycin	46 (33.8)	0 (0.0)	90 (66.2)	215 (90.0)	0 (0.0)	24 (10.0)
Clindamycin	83 (61.0)	0 (0.0)	53 (39.0)	231 (96.7)	0 (0.0)	8 (3.3)
SMZ-TMP	76 (55.9)	0 (0.0)	59 (43.4)	191 (79.9)	1 (0.4)	47 (19.7)
Fusidic acid	116 (85.3)	0 (0.0)	19 (14.0)	229 (95.8)	1 (0.4)	9 (3.8)
Amikacin	94 (69.1)	9 (6.6)	33 (24.3)	238 (99.6)	1 (0.4)	0 (0.0)

Abbreviations: MSSA - methicillin-sensitive Staphylococcus aureus, MRSA - methicillin-resistant Staphylococcus aureus, SMZ-TMP - sulphamethoxazole-trimethoprim.

Table-3: Antibiotic susceptibility pattern of Enterococci.

Antimicrobial	Enterococci isolates (N=26)		
	Sensitive, n (%)	Intermediate, n (%)	Resistant, n (%)
Teicoplanin	26 (100.0)	0 (0.0)	0 (0.0)
Vancomycin	26 (100.0)	0 (0.0)	0 (0.0)
Ampicillin	19 (73.1)	0 (0.0)	7 (26.9)
Nitrofurantoin	24 (92.3)	0 (0.0)	2 (7.7)
Ciprofloxacin	16 (61.5)	0 (0.0)	10 (38.5)
Gentamicin, high concentration	21 (80.8)	0 (0.0)	5 (19.2)
Linezolid	25 (96.2)	0 (0.0)	1 (3.8)

was not performed, as all isolates were sensitive to teicoplanin.

As seen in the case of ***S. aureus***, all ***enterococci*** were sensitive to teicoplanin and vancomycin (Table-3). Linezolid was effective in 25 (96.2%) of the 26 isolates.

Discussion

The MRSET study clearly demonstrated the equivalence of teicoplanin to vancomycin against ***S. aureus*** and ***enterococci*** in samples collected from three major hospitals in Pakistan when probed by an in vitro (Kirby-Bauer disc diffusion) method.

Teicoplanin and vancomycin demonstrated a complete in vitro sensitivity with all isolates, be it MRSA, MSSA or enterococci. Linezolid also demonstrated a good sensitivity profile in >95% of the isolates. Besides these three antibiotics (teicoplanin, vancomycin, and linezolid), the rest demonstrated poor sensitivity to MRSA, especially those of the beta-lactam class. Our findings, indicating complete susceptibility of MRSA to teicoplanin and vancomycin, are in line with the results published by earlier studies conducted in Pakistan as well as other parts of the world like South Africa, Brazil and Turkey.[9,22-24] However, in a study conducted by Kaleem et al, all 139 MRSA isolates were susceptible to vancomycin and linezolid, but only 130/139 (94%) isolates were susceptible to teicoplanin.[25]

Another point that needs consideration is the utility of in vitro testing in selecting an appropriate antibiotic, especially in hospital-acquired infections. We recognise that in vitro susceptibility test is not a perfect predictor of patient outcome. Certain technical factors like the type of the test method used, the inoculum size of the microorganism and its growth phase and characteristics, and other laboratory variables can affect the outcome of the test. Clinical factors like the virulence of the microorganism, the burden of infection, pharmacokinetics parameters and individual patient characteristics will affect whether the drug will provide therapy. For this, the CLSI formulates guidelines, based on consensus from the clinic, which in turn will help the physician in prescribing the appropriate antimicrobial therapy and reducing the chances of antibiotic resistance.[21]

Though our study assessed the sensitivity of teicoplanin in an in vitro setting, two considerations need to be made. Firstly, a comprehensive meta-analysis of clinical data has shown comparative efficacy for teicoplanin and vancomycin.[3] Secondly, we have shown that all ***S. aureus*** strains isolated from three geographically diverse centres in Pakistan were sensitive to teicoplanin. Hence, in the

absence of any data from studies in Pakistan that correlate in vitro sensitivity to clinical outcome, these results could serve to support the use of teicoplanin for hospital-acquired MRSA and enterococcal infections. Other factors, like estimation of minimum inhibitory concentration, especially while prescribing teicoplanin against a particular strain, will also help the physician in choosing an appropriate line of treatment against nosocomial infections.[26,27]

Conclusion

For MSSA, MRSA and enterococcal strains isolated and tested during the study, teicoplanin was as sensitive as vancomycin. Therefore, we suggest that in the current situation teicoplanin can be an alternative and suitable drug for the treatment of highly-resistant gram-positive infections. We also recommend that antimicrobial resistance surveillance should be performed routinely.

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