ABSTRACT

Objectives: Development of antimicrobial resistance in microorganism isolated from blood stream infection constitutes a major concern about their treatment. Teicoplanin is a glycopeptide antibiotic used in the treatment of infection caused by Gram-positive bacteria. This study was planned to determine Teicoplanin resistance in the Central India and recommend policy changes for prevention of the future resistance to the higher antibiotics.

Methods: A total of 1855 septicemia suspected blood samples were studied. The blood culture samples were processed and identified in the microbiology laboratory according to the Clinical and Laboratory Standards Institute guidelines. Antibiotic susceptibility test was done using Kirby-Bauer disk diffusion method.

Results: About 39.5% of blood culture samples showed positive growth for organism. We observed high teicoplanin resistance (29.5%) among Gram-positive isolates, predominantly (53%) in the Enterococcus species.

Conclusion: Teicoplanin resistance has emerged tremendously in the present study. Hence, attention is required about this serious issue otherwise very limited choice of antibiotics will be available for treating infections in the future.

Keywords: Teicoplanin, Blood culture, Sepsis, Gram-positive bacteria, Drug resistance.

INTRODUCTION

Teicoplanin is a glycopeptide antimicrobial agent which has almost similar antimicrobial spectrum as Vancomycin. It is active against infections caused by Coagulase Negative Staphylococcus (CONS), various Enterococcus species, Methicillin-Resistant Staphylococcus Aureus, and in some cases Vancomycin Resistance Staphylococci [1]. It is a bactericidal agent against susceptible Gram-positive bacterial strains and may be effective in Clostridium difficile-associated diarrhea and pseudomembranous colitis.

Teicoplanin binds with the D-Alanyl-D-alanine terminals of cell wall precursor units and thus inhibits bacterial cell wall synthesis. Teicoplanin does not penetrate the outer membranes of Gram-negative bacteria because of their large molecular size [2]. The Minimum Inhibitory Concentration (MIC) of teicoplanin for Staphylococcus, Streptococcus pneumoniae, viridians/non-viridians streptococci and Enterococci is 0.01–1 μg/ml whereas the MIC for Corynebacterium species, anaerobic Gram-positive cocci, and Listeria species is 0.25–2 μg/ml [3]. Teicoplanin has a fused ring structure contain a mixture of five major (A2-1-A2-5), four minor (RS-1-RS-4), and two carbohydrates (mannose and N-acetylglucosamine) compounds. Major and minor components contain a third carbohydrate moiety-Beta-D-glucosamine, all share same core of glycopeptides termed as teicoplanin A3-1 [4]. Antibacterial activity of Teicoplanin is affected by its protein binding capacity; it is highly bound by plasma proteins (90–95%). Teicoplanin has a long serum elimination half-life, (up to 100 h) with normal renal function in adult patients [5].

Vancomycin and Teicoplanin both have different antimicrobial activity, lipophilic activity, and pharmacokinetic properties with almost similar structures. Teicoplanin binds as a monomer whereas Vancomycin forms a dimer. Teicoplanin antibiotic susceptibility testing for CONS can be problematic because of poor diffusion of the molecule in solid media and effect of the medium used [6]. Glycopeptides intermediate S. aureus (GISA) isolates MIC range (4–16 μg/ml) have been recovered from patients after prolonged glycopeptides exposure in the recent years from most parts of the world. Glycopeptides resistance intrinsically arises due to frequent exposure, multiple mutations, and/or alterations in gene expression [7].

The purpose of our study was to determine the Teicoplanin-resistant among isolates from bacteremia or septicemia in the Central India and recommend strategy for the prevention of Teicoplanin resistance in the future.

METHODS

A cross-sectional observational study was conducted on 1855 blood culture isolates at Gandhi Medical College and associated Hamidiya Hospital, Bhopal, Madhya Pradesh, India, a 1000 bedded tertiary care hospital in the Central India.

All the blood samples were withdrawn and collected using strict aseptic measures and sent for culture and sensitivity testing to the clinical bacteriology laboratory. Blood culture was done by conventional blood culture method (BHI broth). Thereafter, bacterial isolates identification was performed from colony morphology, gram staining, and biochemical tests.

Teicoplanin sensitivity testing was performed by Kirby-Bauer’s disk diffusion method using Mueller Hinton agar plates as per Clinical and Laboratory Standards Institute guidelines [8]. Inoculums of 0.5 McFarland standards were poured on Mueller-Hinton Agar plates and a 30 μg Teicoplanin disk was applied. All plates were incubated for 16–20 h at 35–37°C temperature. A zone size more than 15 mm was taken as susceptible and <15 mm as resistance.
Data analysis
The proportion and percentage, confidence interval of the resistant isolates was calculated using graph pad software. The confidence intervals below are calculated using the so-called "exact" confidence intervals, computed by the method of Clopper and Pearson which are based on a relationship between the F distribution and the binomial distribution.

Ethical consideration
The study was approved by Institutional Ethical Committee of Gandhi Medical College and associated Hamidiya hospital, Bhopal M.P. Ethical guidelines given by the Declaration of Helsinki were adhered to throughout the study.

RESULTS
Table 1 shows geographical distribution of blood culture cases. Total of 1855 blood samples were received from different wards including medicine, pediatric, surgery, and burn. The maximum number of samples was received from pediatric wards followed by other department such as medicine, surgery, orthopedic, and burn. Out of 1855 blood sample received, 732 turn out to be positive for growth of bacteria (Table 2). The positive rate was found to be 39.4%.

Frequency of total blood isolates (Table 3 and Figure 1)
Of 732 positive culture Gram-negative strain accounts for 610 (83.33%), whereas Gram-positive strain was found to be 122 (16.66%). The isolated Gram-negative isolates include Klebsiella 317 (43.3%), E. Coli 121 (16.5%), Pseudomonas 76 (10.3%), the non-lactose fermenting Gram-negative bacteria 54 (7.3%), Citrobacter 36 (4.9%), and Acinetobacter 6 (0.8%). The Gram-positive bacteria isolated includes S. aureus 87 (11.8%), CONS 20 (2.7%), and Enterococcus 15 (2%).

Resistance to teicoplanin (Table 4)
The resistance to Teicoplanin among different Gram-positive bacterial isolates was found to be in staphylococcus 20 (23%), CONS 8 (40%), and Enterococcus 8 (53%).

DISCUSSION
Our study showed that 732 (39.4%) out of 1855 total samples were positive for presence of bacteria which is almost similar to Khanal et al. [9] and Sharma et al. [10], who reported positive blood cultures accounting for 44-33.9%, respectively but some other studies showed lower prevalence like Mehdinejad et al. [11], Vanitha et al. [12], Kalpesh Gohel et al. [13], and Mehta et al. [14] reported 5.6%, 8.3%, 9.2%, and 9.9%, respectively. Higher prevalence rate (39.4%) in our study may be due to emerging of multidrug-resistant bacterial strains and inadequate or rational use of antibiotic. Probable reasons for variation in blood culture positivity rate are amount of blood taken, administration of antibiotic therapy before blood collection, nature of population, epidemiological difference of the etiological agents, and different areas of study. Gram-negative bacteria were tremendous (83.3%) in the present study which was similar to Vaghela et al. [15], Paul et al. [16], and Santwana Pandey et al. [17]. However, this contrasts with other studies where Gram-positive organisms were predominant like Bebay et al. [18], Muley et al. [19], Pan et al. [20], and Sorsa et al. [21]. This variation of blood culture isolates may be due to various factors such as geographical location, seasonal variation, and endemcity of etiological agents.

In our study, we observed high prevalence of Teicoplanin resistance in CONS strain 40% which was concordance to Bertin et al. [22] and LaLlemard [23] but in discordance to our study many other observers showed very low prevalence to Teicoplanin resistance like Schlegel et al. [24] and Julie et al. [25].

Teicoplanin resistance in S. aureus strain was 23% reported in the present study in contrary to that other authors like Szymańsk-Majchrzak et al. [26] and Shuchi Kaushik et al. [27] showed very high resistance 76.6% and 66% respectively. Nidhi Pal et al. [28] and Vanitha et al. [29] studied resistance to Teicoplanin in S. aureus and Enterococcus respectively.

Table 1: Districts-wise distribution of positive and total blood culture cases

| District | Positive cases (%) | Total cases |
|----------|--------------------|-------------|
| Bhopal   | 415 (40.5)         | 1025        |
| Raigan   | 102 (35.6)         | 286         |
| Vidisha  | 74 (37.4)          | 198         |
| Sehore   | 86 (40.3)          | 213         |
| Raigarh  | 37 (43.5)          | 85          |
| Hoshangabad | 18 (37.5) | 48          |
| Total    | 732 (39.5)         | 1855        |

Table 2: Age-wise distribution of blood culture samples (n=1855)

| Age       | Growth | No growth | Total |
|-----------|--------|-----------|-------|
| Infant (<1 year) | 226    | 338       | 564   |
| Children (1-12)  | 165    | 242       | 407   |
| Adolescent (13-18) | 102   | 157       | 259   |
| Adult (18-49)    | 111    | 188       | 299   |
| Old (50 years)   | 128    | 198       | 326   |
| Total            | 732    | 1123      | 1855  |

Table 3: Frequency of bacterial isolates obtained from blood sample (n=732)

| S. No | Bacteria isolated | Number | Percentage | Confidence interval |
|-------|-------------------|--------|------------|--------------------|
| 1     | Klebsiella        | 317    | 43.3       | 0.3968-0.4698      |
| 2     | E. coli           | 121    | 16.5       | 0.1391-0.1942      |
| 3     | Pseudomonas       | 76     | 10.3       | 0.0827-0.0952      |
| 4     | NLFGNB            | 54     | 7.3        | 0.0559-0.0952      |
| 5     | NLFGNB            | 36     | 4.9        | 0.0347-0.0674      |
| 6     | Acinabacter       | 6      | 0.8        | 0.0030-0.0178      |
| 7     | Staphylococcus    | 87     | 11.8       | 0.0963-0.1445      |
| 8     | CONS              | 20     | 2.75       | 0.0168-0.0419      |
| 9     | Enterococcus      | 15     | 2          | 0.0115-0.0336      |

Table 4: Resistance pattern to Teicoplanin

| Bacteria     | No. of resistant isolates | % of resistant isolates | Confidence interval |
|--------------|----------------------------|-------------------------|--------------------|
| Staphylococcus | 20 (n=87)                 | 23                      | 0.1464-0.3325      |
| CONS         | 8 (n=20)                   | 40                      | 0.1912-0.6395      |
| Enterococcus | 8 (n=15)                   | 53                      | 0.2659-0.7873      |
| Total        | 36 (n=122)                 | 29.5                    | 0.2180-0.3844      |

Figure 1: Growth profile of the blood culture samples
et al. [12] observed very low Teicoplanin resistance. The present study found that Enterococci were highly resistant to Teicoplanin 53% in discordance to that Palawar et al. [29] and Gupta et al. [30] reported very low resistance to Teicoplanin.

In the present study, we were reported 39.5% Teicoplanin resistance in overall Gram-positive isolates in the Central India which was clinically and statistically significant in because the majority of the previous studies in the Central India like Koksal et al. [31], Tripathi et al. [32], and Sodani et al. [33] reported 100% susceptibility to Teicoplanin.

The increasing Teicoplanin resistance in the present study could be due to unregulated or widespread use of the drug in the empirical treatment protocol, altered virulence factor expression, and altered autolytic properties of the resistant organism.

CONCLUSION

Teicoplanin is a reserve antibiotic for multi-drug-resistant staphylococci or Enterococci and therefore emergence of Teicoplanin resistance is a serious concern. This mandates judicious use of antimicrobials and a strict antibiotic policy on a large scale to decrease or prevents further Teicoplanin resistance.

RECOMMENDATIONS

Recommendation of policy changes in the future for prevention of antibiotics resistance are as under:

- Health-care professionals should be educated about antibiotics resistance and its consequences
- Antibiotics should be used judiciously
- Appropriate antibiotics should be used only after culture-sensitivity reports
- Control of substandard and counterfeit uses of antimicrobials
- All health-care facilities should have an antibiotic monitoring committee, a strict antibiotic policy which is updated periodically
- All health-care facilities should maintain detailed records regarding antibiotics use and resistance patterns.

CONFLICT OF INTEREST

Authors have no known conflicts of interest to declare.

SOURCES OF FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not for profit sectors.

REFERENCES

1. Reynolds PE. Structure, biochemistry and mechanism of action of glycopeptide antibiotics. Eur J Clin Microbiol Infect Dis 1989;8:943-50.
2. Pacifici, G. Clinical pharmacology of teicoplanin in neonates: Effects and pharmacokinetics. Int J Pediatr 2016;4:3669-84.
3. de Lalla F, Nicolin R, Rinaldi E, Scarpellini P, Rigoli R, Manfrin V, et al. Prospective study of oral teicoplanin versus oral vancomycin for therapy of pseudomembranous colitis and Clostridium difficile-associated diarrhea. Antimicrob Agents Chemother 1992;36:2192-6.
4. Bernaseggi A, Borgh A, Borgonov M, Cavenaghi L, Ferrari P, Vékey K, et al. Teicoplanin metabolism in humans. Antimicrob Agents Chemother 1992;36:1744-9.
5. MacDougall C, Chambers HF. Protein synthesis inhibitors and miscellaneous antibacterial agents. In: Brunton L, Chabner, Knollman B, editors. Goodman and Gilman’s. The Pharmacological Basis of Therapeutics. New York: Mc Graw Hill; 2011. p. 1539-41.
6. Beauregard DA, Williams DH, Gwynn MN, Knowles DJ. Dimerization and membrane anchors in extracellular targeting of vancomycin group antibiotics. Antimicrob Agents Chemother 1995;39:781-5.
7. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in Staphylococcus aureus. Emerg Infect Dis 2001;7:327-32.
8. CLSI Performance Standard for Antimicrobial Susceptibility Testing: Twenty Fourth Informational Supplement CLSI Document M100-S24. Wayne, PA: Clinical Laboratory Standard Institute; 2014.
9. Khanal B, Harish BN, Sethuraman KR, Srivinasa S. Infective endocarditis: Report of a prospective study in an Indian hospital. Trop Doct 2002;32:83-5.
10. Parmar PP, Haider D, Dutta AK, Dutta R, Bhatnagar S, Baji A, et al. Bacteriological profile of neonatal septicemia. Indian Pediatr 1987;24:1011-7.
11. Mehdinejad M, Khosravi AD, Morvariadi A. Study of prevalence and antimicrobial susceptibility pattern of bacteria isolated from blood cultures. J Biol Sci 2009;9:249-53.
12. Rani NV, Gopal K, Narendra MV, Vishwakantan D, Nagesh VR, Yogita M, et al. A retrospective study on blood stream infections and antibiotic susceptibility patterns in a tertiary care teaching hospital. Int J Pharm Pharm Sci 2012;4:543-8.
13. Pandey S, Raza S, Bhatta CP. The aetiology of the bloodstream infections in the patients who presented to a tertiary care teaching hospital in Kathmandu, Nepal. J Clin Diagn Res 2013;7:638-41.
14. Verma S, Bollampally S, Kallur A, Sanjeev V, Kataria V, Pandey A. A prospective study on blood stream infections in neonates in a tertiary care hospital of a tertiary care teaching hospital in Kathmandu, Nepal. J Clin Diagn Res 2013;7:638-41.
15. Muley VA, Ghadage DP, Bhide AV. Bacteriological profile of neonatal septicemia in a tertiary care hospital from Western India. J Glob Infect Dis 2015;7:57-5.
16. Pan F, Zhao W, Zhang H. Value of time to positivity of blood culture in children with bloodstream infections. Can J Infect Dis Med Microbiol 2019;2019:597837.
17. Sorsa A, Früh J, Stötter L, Abbissa S. Blood culture result profile and antimicrobial resistance pattern: A report from neonatal intensive care unit (NICU), Assela teaching and referral hospital, Assela, South East Ethiopia. Antimicrob Resist Infect Control 2019;8:42.
18. Berti M, Muller A, Bertrand X, Cornette C, Thouvery M, Talon D. Relationship between glycopeptide use and decreased susceptibility to teicoplanin in isolates of coagulase-negative staphylococci. Eur J Clin Microbiol Infect Dis 2004;23:375-9.
19. Lallierand S, Thouvery M, Boisson K, Talon D, Bertrand X. Bacteraemia caused by coagulase-negative staphylococci exhibiting decreased susceptibility to teicoplanin. J Hosp Infect 2002;51:207-14.
20. Schlegel L, Saliba F, Mangeney N, Mathieu D. Pulsed field gel electrophoresis typing of coagulase-negative staphylococci with decreased susceptibility to teicoplanin isolated from an intensive care unit. J Hosp Infect 2001;49:62-8.
21. Crenniter J,SSLASSI A, Quincaux L, Vaidya-Gundu V, Bajrangi P, Porcher R, et al. Decreased susceptibility to teicoplanin and vancomycin in coagulase-negative Staphylococci isolated from orthopedic-device-associated infections. J Clin Microbiol 2010;48:1428-31.
22. Szymańczak-Majchrzak K, Mlynarzczycy A, Mlynarzczycy G. Characteristics of glycopeptide-resistant Staphylococcus aureus strains isolated from inpatients of three teaching hospitals in Warsaw, Poland. Antimicrob Resist Infect Control 2018;7:105.
23. Kauszki S, Tomar R, Tiwari U, Saraswat A, Shrivastava A. Antimicrobial investigation of different medications against nosocomial infections causing Staphylococcus aureus. Int J Dev Res 2014;2:215-7.
24. Pal N, Ramamurthy S. Microbiological profile and antimicrobial resistant pattern of blood culture isolates, among septicemia suspected patients. Natl J Lab Med 2016;5:17-21.
25. Palwar M, MadhuDikar S, Dohe V, Kagal A, Karyakarte R. Bacteriological profile and antibiogram of blood cultures isolated from a tertiary care hospital of Western India. J Datta Meghe Inst Med Sci Univ 2020;15:261-5.
26. Gupta S, Kashyap B. Bacteriological profile and antibiogram of blood cultures isolated from a tertiary care hospital of North India. Trop J Med Res 2016;19:94-9.
31. Koksal F, Yasar H, Samasti M. Antibiotic resistance patterns of coagulase-negative *Staphylococcus* strains isolated from blood cultures of septicemia patients in Turkey. Microbiol Res 2009; 164:404-10.

32. Tripathi A. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* in central India. MedPulse Int Med J 2015;2:45-8.

33. Sodani S, Hawaldar R. Bacteriological profile and antimicrobial sensitivity pattern of blood cultures in a diagnostic Centre of Central Madhya Pradesh: A retrospective study. J Microbiol Relat Res 2020;6:15-25.