Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

Analysis of Antibacterial Activity of Graded Concentrations of Teicoplanin Mixed With Acrylic Bone Cement in Staphylococcal (Methicillin-resistant Staphylococcus aureus) Infections and to Compare it with Vancomycin: An In Vitro Study

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Abstract

Background: Orthopedic infections by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming more frequent after device implantation, and often resistant to many commonly used antibiotics. The new line of antibiotics such as vancomycin, meropenem, and teicoplanin is being used for the treatment of such infections intravenously. However, these antibiotics can also be used along with bone cement as a local antibiotic spacer.

Aims: The aim of the study was to compare *in vitro* antibacterial activity of vancomycin and teicoplanin and to know the optimum concentration of teicoplanin at which there is maximum inhibition of bacteria.

Materials and Methods: Three different brands of bone cement discs (Palacos-R + G, surgical Simplex P, and CMW1) with vancomycin and teicoplanin of different concentration used. Inoculating media with bacterial isolates of *Staphylococcus aureus* (Methicillin-resistant) of strain ATCC 2593 with known minimum inhibitory concentration were used. In each media two discs of one formulation were placed and labeled accordingly. Readings were taken at 24 h, 48 h, and 6 days.

Results: All the cement brands eluted vancomycin equally well, but the zone of inhibition for palacos was marginally higher compared to the other two. Teicoplanin when increased from 400 mg to 1200 mg concentration showed a dose-dependent inhibition of MRSA with an increase in the zone of inhibition in all cements, with palacos being highest.

Conclusion: Teicoplanin in higher concentration is a better alternative to vancomycin in MRSA bone infection.

Key words: Arthroplasty, Bone cement, Methicillin-resistant *Staphylococcus aureus*, Teicoplanin, Vancomycin

INTRODUCTION

Orthopedic infections by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming more frequent after device implantation,[1] and these organisms are often resistant to many commonly used antibiotics.[2]

Access this article online

www.ijss-sn.com

Month of Submission : 02-2019
Month of Peer Review : 03-2019
Month of Acceptance : 03-2019
Month of Publishing : 04-2019

Treatment of infection without removal of the prosthesis is associated with a high probability of therapy failure.[3] Before implanting a new prosthesis, in addition to systemic antibiotics, some surgeons use antibiotic-impregnated cement spacers for local delivery of antibiotics to facilitate the revision surgery.[4]

Vancomycin or teicoplanin is usually used as first-line therapy for prosthesis infections because methicillin-resistant staphylococci remain sensitive to it. Since vancomycin can be stably incorporated into polymethylmethacrylate and elute well,[5,6] it is often loaded into cement spacers. Teicoplanin has also been studied as a local therapy.[7]

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In 1970, Buchholz and Engelbretz reported that penicillin, erythromycin, and gentamicin can be incorporated into the cement for treating orthopedic infections.[8] There is a large number of studies which evaluate gentamicin and vancomycin in bone cement. However, in this era of bacterial infections which are resistant and most of the age-old standard antibiotics such as penicilins, fluroquinolones, and cephalosporins. New class of antibiotic families such as carbapenemes and glycopeptide antibiotics has been invented for treatment. Today most of the hospital-acquired infections constitute extended-spectrum beta-lactamase and MRSA which over primarily resistant most of the first-line antibiotics. In orthopedics treatment of such infection with implants and prosthesis *in situ* is a difficult task for the surgeon. The new line of antibiotics such as meropenem and teicoplanin is being used for the treatment of such infections intravenously. However, these antibiotics can also be used along with bone cement as a delivery vehicle. The experience in terms of literature regarding the evaluation of meropenem and teicoplanin with bone cement as a local antibiotic spacer is sparse.

In an *in vitro* study conducted by Marks using palacos and Simplex P cement to test oxacillin, cefazolin, and gentamicin concluded that palacos elutes more antibiotics compared to Simplex P due to high porosity.[9]

In May, 2002, lsmael and Bleton studied teicoplanin cement spacers for treatment of prosthetic infections caused by *staphylococcus* and concluded that combined modality of treatment with both intravenous and local spacer gives best results when compared to individual modality.[10]

A study by Kinik and Karaduman evaluated treatment of chronic osteomyelitis (Cierney and Mader Type III-A) with culture septic antibiotic cement beads which are handmade. They have treated 26 patients (19 men and 7 women) with a mean follow-up of 36 years with a success rate of 100%. They Concluded that chronic osteomyelitis can be safely treated with this protocol [debridement followed by antibiotic cement beads].[11]

For treatment of established prosthetic joint infection commercially available antibiotic bone cement doses are inadequate because this condition needs high doses of specific antibiotics.[12]

In a study by Schurman *et al.* evaluated the elution kinetics of gentamicin from palacos. He concluded that the antibiotic concentration remained at a therapeutic level for 3 days where serum level was very low. 8% of antibiotic leached out of bone cement by 8 days. Most of it during the 1st day. The amount of antibiotic leaching out is directly proportional to the surface area of the cement.[13]

Penner *et al.* discussed the *in vitro* elution characteristics of CMW and Palacos-R cement for vancomycin and tobramycin antibiotics. It clearly showed that palacos is superior in eluting both antibiotics as compared to CMW1 and CMW3 cement. This study confirmed the superiority of palacos over other cement as shown in the previous studies.[14]

In the present study, we compared the efficacy of a teicoplanin-impregnated cement spacer and with that of vancomycin.

MATERIALS AND METHODS

This was a prospective observational *in vitro* laboratory study. This study did not involve any human/animal subjects. In this study, we analyzed three different brands of bone cement (Palacos-R+G, surgical Simplex P and CMW 1) with vancomycin and teicoplanin separately for antibacterial activity against *S. aureus* (Methicillin-resistant), respectively. All the bone cements used were in powder form. Both the antibiotics tested were also in powder form. Both the antibiotics are heat stable antibiotics and can be used with bone cement.

Based on literature reports and amount of antibiotics normally available in commercially available bone cement,

Table 1: Formulations

Formulations A	→	Antibiotic disc without bone cement
Formulations B	→	Palacos-R+G cement (40 g) with 1 g of vancomycin
Formulations C	→	CMW1 cement (40 g) with 1 g of vancomycin
Formulations D	→	Surgical Simplex *P* cement (40 g) with 1 g of vancomycin
Formulations E	→	PalacosR+G cement (40 g) with 400 mg of teicoplanin
Formulations F	→	CMW1 cement (40 g) with 400 mg of teicoplanin
Formulations G	→	Surgical Simplex *P* cement (40 g) with 400 mg of teicoplanin
Formulations H	→	PalacosR+G cement (40 g) with 800 mg of teicoplanin
Formulations I	→	CMW1 cement (40 g) with 800 mg of teicoplanin
Formulations J	→	Surgical Simplex *P* cement (40 g) with 800 mg of teicoplanin
Formulations K	→	PalacosR+G cement (40 g) with 1200 mg of teicoplanin
Formulations L	→	CMW1 cement (40 g) with 1200 mg of teicoplanin
Formulations M	→	Surgical Simplex *P* cement (40 g) with 1200 mg of teicoplanin

the following different formulations [Table 1] were prepared and analyzed for antibacterial activity.

To prepare these formulations, the cement powder was mixed with antibiotic powder under sterile conditions. Hand mixing of antibiotic with cement powder was done. After homogenous mixing of these powders monomers was added at controlled temperature of 23 ± 1°C and relative humidity of 50 ± 10%. Three discs of each formulation measuring 10 mm × 10 mm were prepared with the help of molds.

Bacterial isolates of *S. aureus* (Methicillin-resistant) of strain ATCC 2593 were procured from microbiology laboratory with known minimum inhibitory concentration.

The media used for inoculating were Muller-Hinton agar media. In each media, two discs of one formulation were placed and labeled accordingly. The bacteria were inoculated accordingly. All the media incubated at temperature 37°C and ambient air in an incubator. Readings were noted as the zone of inhibition in millimeter after 24 h, 48 h, 72 h, 96 h, 120 h, and 6 day's taking average of two different zones from each medium. To note bacterial growth inhibition in liquid media simulating serum nutrient broth of 5 ml was used, one discs of each formulation was put in 5 ml of liquid media containing 1.5×10^8 colony-forming units/ml of respective bacteria. Readings were taken at 24 h, 48 h, 72 h, 96 h, 120 h, and 6 days to know the bacterial colony count by subculture and inoculating the broth over a blood agar media. To count, the colonies measuring 0.5 mm in diameter minimum were considered. Readings were tabulated and compared.

RESULTS

Findings with Different Formulations were as Follow

First, the control disc (formulation A) showed a zone of inhibition of 16 mm on 24 h, 48 h, and 6 h day Figure 1.

With Formulations B, Formulations C, and Formulations D showed a zone of inhibition in millimeter and presence of growth on subcultures [Tables 2 and 3, Figure 2-4]

Table 2: MRSA growth inhibition with vancomycin 1 g

Antibiotic vancomycin 1 g	Zone of inhibition in mm					
Cement brand	**24th H**	**48th H**	**72th H**	**96th H**	**120th H**	**6th Day**
PalacosR+G	29.5	27	27	27	27	27
CMW1	26	26	26	26	26	26
Surgical Simplex P	27	25	24.5	24.5	24.5	24.5

Table 3: MRSA subcultures with vancomycin 1 g

Antibiotic vancomycin 1 g	Presence of growth on subcultures					
Cement brand	**24th H**	**48th H**	**72th H**	**96th H**	**120th H**	**6th Day**
PalacosR+G	+	–	–	–	–	–
CMW1	+	+	+	–	–	–
Surgical Simplex P	+	+	–	–	–	–

+ presence of growth, –No growth

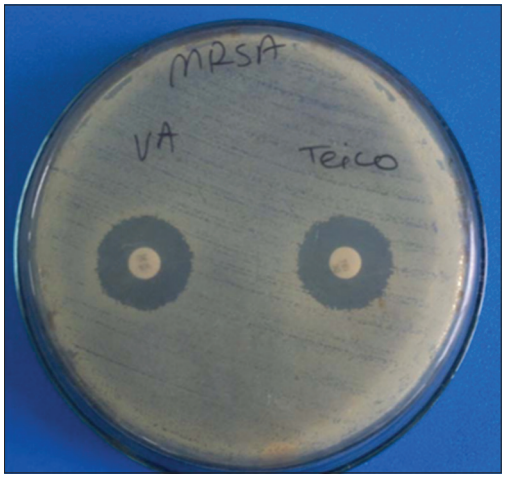

Figure 1: Formulation A

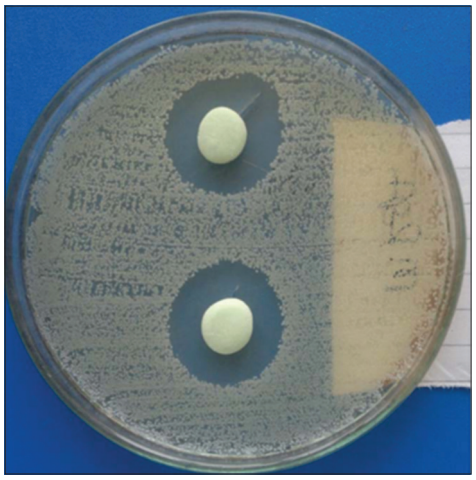

Figure 2: Formulation B

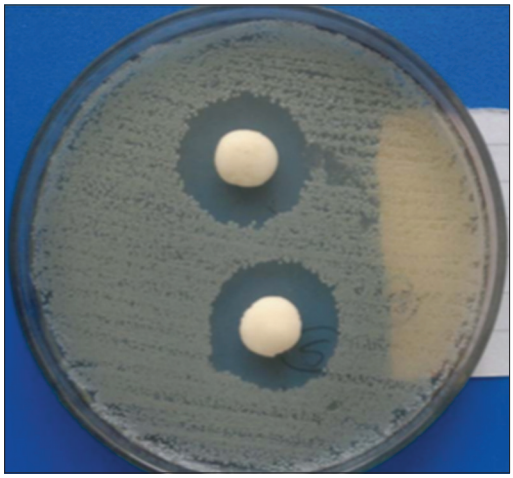

Figure 3: Formulation C

With Formulations E, Formulations F and Formulations G showed a zone of inhibition in millimeter and presence of growth on subcultures [Tables 4 and 5, Figures 5-7].

With Formulations H, Formulations I and Formulations J showed a zone of inhibition in millimeter and presence of growth on subcultures [Tables 6 and 7, Figure 8-10].

With Formulations K, Formulations L and Formulations M showed a zone of inhibition in millimeter and presence of growth on subcultures [Table 8 and 9, Figures 11-13].

DISCUSSION

Our study demonstrated the *in vitro* efficacy of vancomycin and teicoplanin against *S. aureus* (Methicillin-resistant), as the study does not involve any animal or human subjects. It also showed the differential ability of different bone cement brands to elute the same antibiotic used in similar conditions.

Considering vancomycin control disc (formulation) at 24 h, 48 h, and 6 h days had a zone of inhibition 16 mm (16, 16, and 16 mm, respectively) maintained over 6 days.

Table 4: MRSA growth inhibition with Teicoplanin 400 mg

Antibiotic teicoplanin 400 mg	Zone of inhibition in mm					
Cement brand	24th H	48th H	72th H	96th H	120th H	6th Day
Palacos-R+G	20.5	20	20	20	19.5	19
CMW1	20	20	20	19.5	19	19
Surgical Simplex P	18	17.5	17.5	17.5	17.5	17

Table 5: MRSA subcultures with Teicoplanin 400 mg

Antibiotic teicoplanin 400 mg	Presence of growth on subcultures					
Cement brand	24th H	48th H	72th H	96th H	120th H	6th day
Palacos-R+G	+	+	–	–	–	–
CMW1	+	+	+	–	–	–
Surgical Simplex P	+	+	+	–	–	–

+ presence of growth, –No growth

Table 6: MRSA growth inhibition with Teicoplanin 800 mg

Antibiotic teicoplanin 800 mg	Zone of inhibition in mm					
Cement brand	24th H	48th H	72th H	96th H	120th H	6th day
Palacos-R+G	20	19	19	19	19	19
CMW1	20	19	19	19	18.5	18.5
Surgical Simplex P	21	19	19	19	19	19

Table 7: MRSA subcultures with Teicoplanin 800 mg

Antibiotic teicoplanin 800 mg	Presence of growth on subcultures					
Cement brand	24th H	48th H	72th H	96th H	120th H	6th day
PalacosR+G	+	+	–	–	–	–
CMW1	+	+	+	–	–	–
Surgical Simplex P	+	+	–	–	–	–

+ presence of growth, –No growth

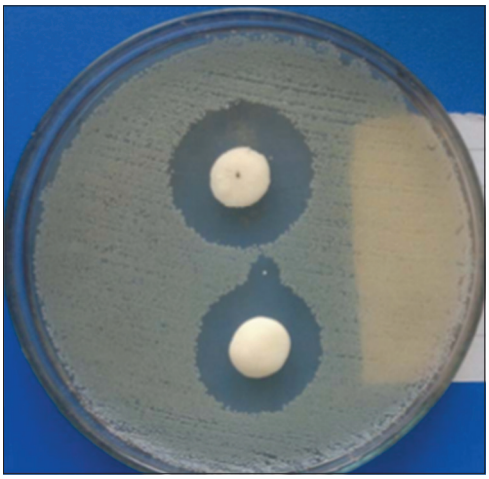

Figure 4: Formulation D

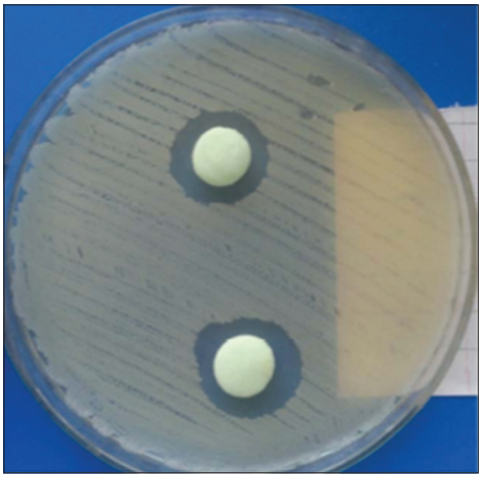

Figure 5: Formulation E

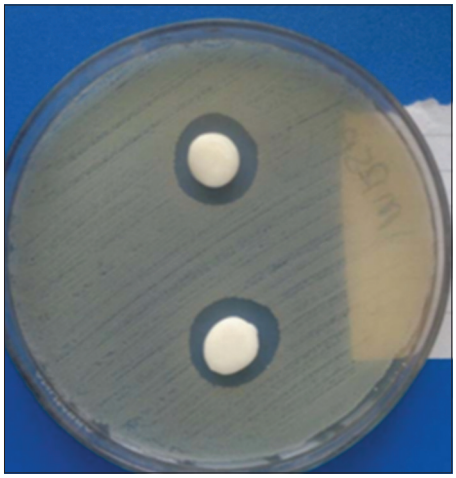

Figure 6: Formulation F

Table 8: MRSA growth inhibition with Teicoplanin 1200 mg

Antibiotic teicoplanin 1200 mg	Zone of inhibition in mm					
Cement brand	**24th H**	**48th H**	**72th H**	**96th H**	**120th H**	**6th day**
Palacos-R+G	25	24.5	24.5	24.5	24.5	24.0
CMW1	21	20.5	20.5	20.5	20.5	20.5
Surgical Simplex P	23	22.5	22.5	22.5	22.5	22.5

Table 9: MRSA subcultures with Teicoplanin 1200 mg

Antibiotic teicoplanin 1200 mg	Presence of growth on subcultures					
Cement Brand	**24th H**	**48th H**	**72th H**	**96th H**	**120th H**	**6th day**
Palacos-R+G	+	–	–	–	–	–
CMW1	+	+	–	–	–	–
Surgical Simplex P	+	+	–	–	–	–

+ presence of growth, –No growth

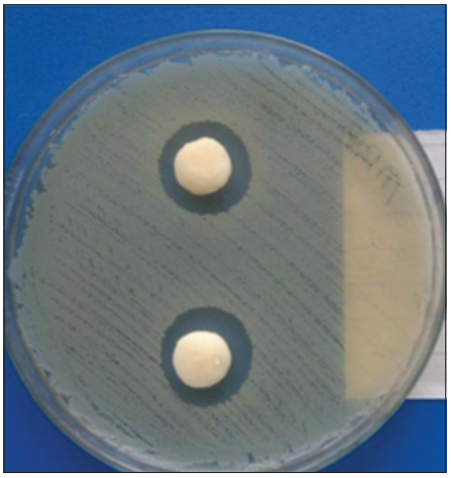

Figure 7: Formulation G

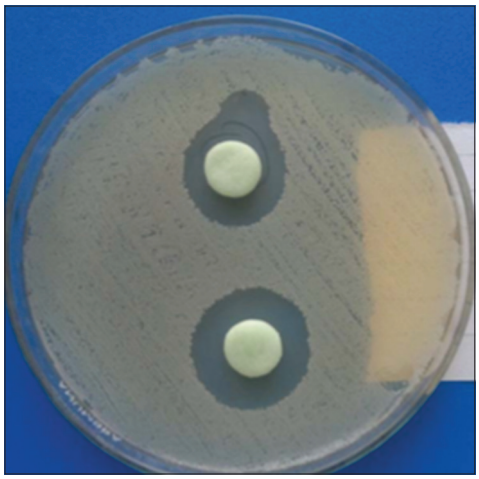

Figure 8: Formulation H

Formulations (B, C, and D) at 24 h, 48 h, and 6 h days, the average of the zone of inhibition remained almost same

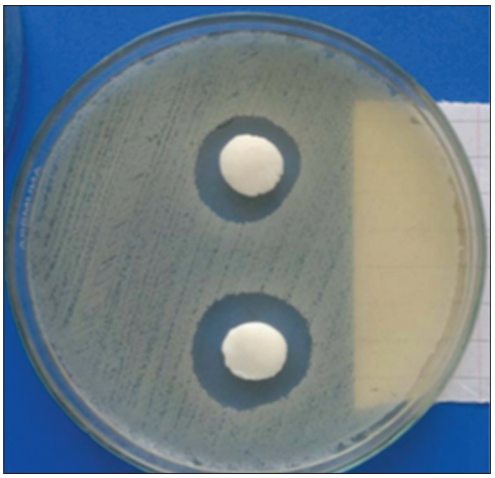

Figure 9: Formulation I

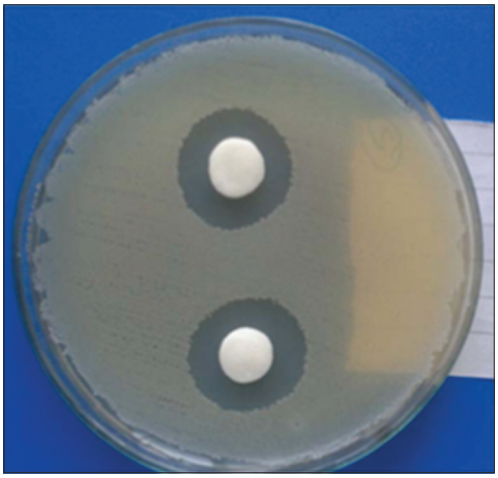

Figure 10: Formulation J

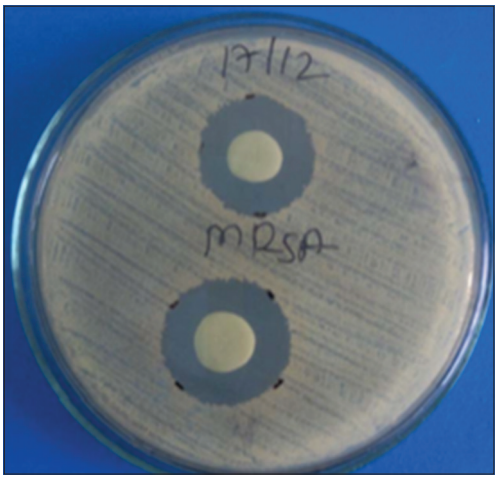

Figure 11: Formulation K

over 6 days for each of the cement brands, i.e., 27.83 for palacos (29.5, 27, and 27, respectively), 26.0 mm for CMW 1 (26.0, 26.0, and 26.0, respectively), and 25.5 mm (27, 25, and 24.5, respectively) for surgical Simplex P cement. Bacterial colony counts were done by subculturing the aliquots of

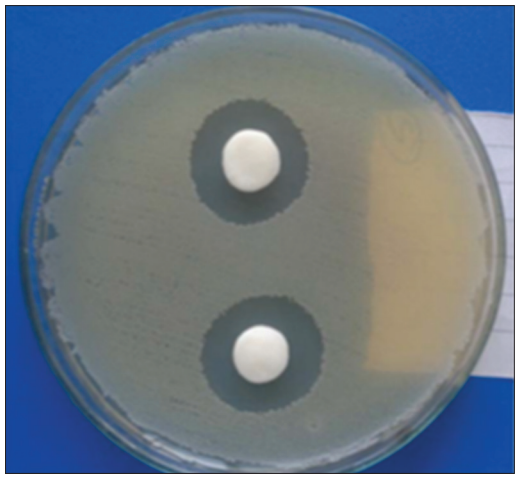

Figure 12: Formulation I

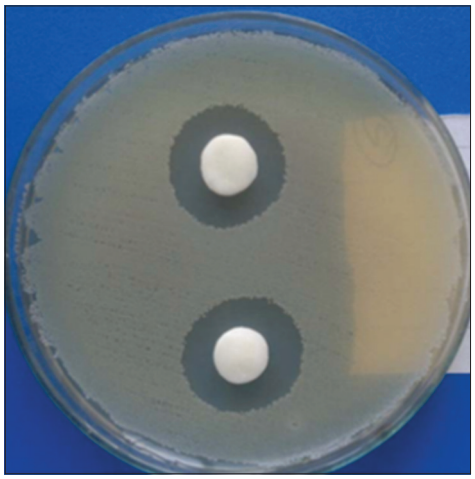

Figure 13: Formulation M

liquid media in which the discs were immersed, at periodic intervals of 24 h, 48 h, and 6 days. All the formulations (B, C, and D) showed no growth of bacteria including control at the end of 6 h day indicated that the antibiotic is eluted at a concentration well above the inhibitory for MRSA.

Interestingly complete inhibition with no growth on subculture was observed within 48 h in palacos, 72 h in surgical simplex, and 96 h in CMW1 cement.

As inference, we can state that all the cement brands eluted vancomycin equally well, but the zone of inhibition for palacos was marginally higher compared to the other two cements. All the cement discs eluted vancomycin well and maintained it above the zone of inhibition of control discs over the period of 6 days, but antibiotic in palacos inhibited the bacteria 2nd day followed by Simplex P followed by CMW1.

Now we consider the second antibiotic teicoplanin, the average zone of inhibition was measured in two different zones of each medium.

The control disc had a zone of 16 mm (16, 16, and 16 mm, respectively) maintained over 6 days.

Taking first 400 mg of concentration in each packet of cement it remained almost same for palacos and CMW1, i.e., 19.83 mm (20.5, 20, and 19, respectively) and 19.67 mm (20.0, 20.0, and 19.0 mm, respectively) but the zone for gradually decreased from 20.5 mm at 24 h to 20.0 mm at 48 h and 19.0 mm at 6 days indicating gradual diminution of antibiotic concentration at the periphery as the time passed in palacos. Surgical simplex showed a slight smaller zone of inhibition 18 mm on 24 h to gradual diminution to 17 mm at 6th day.

Hence, there was a minute decrease in an antibiotic release from all the cements over the period of 6 days in all the cements

When inoculated in the broth all the cements containing teicoplanin inhibited the MRSA, palacos inhibiting it at 72 h and other at 96 h in 400 mg of concentration. There was no regrowth of bacteria at 6 days in culture tubes, and subculture was negative at 6 h day.

Now taking 800 mg of teicoplanin in consideration zone of inhibition by palacos cement disc was 19.33 on average with a slight decrease in zone from 20 mm to 19 mmover 6 days, other cement showed similar response with 19.16 mm for CMW 1 and 19.67 mm for Simplex p cement.

Hence, when we increased concentration from 400 mg to 800 mg of teicoplanin, it was an almost the same zone of inhibition with palacos and CMW1 as compared to 400 mg of teicoplanin. However, Simplex p showed a higher the zone of inhibition. Clinical significance of this is uncertain. It may be related to the fact that teicoplanin is supplied in a powder form that forms small aggregates like small pebbles. At the time of mixing, it may not uniformly distribute in all the cements with hand mixing technique this tendency is more so with cement with high viscosity. Samples of low viscosity showed consistent results with this experimental method, for example, of Simplex p.

In liquid broth when the concentration was increased to 800 mg of teicoplanin, there was the presence of growth up to the same time period as of 400 mg of teicoplanin, but Simplex p inhibited growth 1 day prior.

Now when 1200 mg of teicoplanin was mixed with all the cements, the zone of inhibition was average 24.5 mm for palacos and 20.67 for CMW 1 and 22.67 for Simplex p cement. All discs showed time-dependent decrease in the zone of inhibition with the highest zone of inhibition in first 24–48 h. It decreased from 25 to 24 mm in case of

palacos and 21 to 20.5 mm in case of CMW1 and 23 mm to 22.5 in case of Simplex p cement.

As inference, we can state that even though the zone of inhibition of all formulations was comparable to control disc without cement. All of the cements showed an increase in inhibition when the concentration was increased from 800 to 1200 with palacos showing highest inhibition followed by Simplex p followed by CMW1.

The control disc did not grow any bacterial colonies after serial subcultures. In tubes containing broth, there was a inhibition of growth in all tubes, but palacos started to clear the growth within 48 h, but other tubes showed clearance only at 72 h.

Hence, teicoplanin when increased from 400 mg to 1200 mg concentration showed a dose-dependent inhibition of MRSA with an increase in the zone of inhibition in palacos, CMW1, and Simplex p cement. Furthermore, in tubes when concentration of antibiotics is increased there was inhibition of growth gradually within a smaller time period was observed with earliest in palacos.

CONCLUSION

Vancomycin is a better antibiotic to be used with bone cement, when organisms are sensitive to vancomycin. When organisms are virulent and resistant to vancomycin or a patient-related factors that hypersensitivity or Red man syndrome, teicoplanin is a better alternative. It has a dose-dependent inhibition of the growth of MRSA bacteria and elution is comparable to vancomycin in a concentration of 1200 mg so it can be used as an antibiotic cement spacer.

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How to cite this article: Goyal V, Agrawal P, Desai MM. Analysis of Antibacterial Activity of Graded Concentrations of Teicoplanin Mixed With Acrylic Bone Cement in Staphylococcal (Methicillin-resistant *Staphylococcus aureus*) Infections and to Compare it with Vancomycin: An *In Vitro* Study. Int J Sci Stud 2019;7(1):91-97.

Source of Support: Nil, **Conflict of Interest:** None declared.