Comparative in-vitro activity of trovafloxacin and other related drugs against isolates of *streptococcus oralis*

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ABSTRACT

Objective: The in-vitro activity of trovafloxacin a new quinolone was compared with that of ciprofloxacin, erythromycin, various β-lactam and other appropriate antibiotics such as vancomycin, teicoplanin and clindamycin against 60 isolates of *S. oralis*.

Methods: Minimal inhibitory concentration was performed using the agar dilution technique and micro-titer method. In addition minimal bactericidal concentration and time-kill studies were carried out to estimate the bactericidal activity of trovafloxacin against *S. oralis* isolates.

Results: Trovafloxacin showed a four-fold increase in activity over ciprofloxacin, with a narrow minimal inhibitory concentration range, MIC_{50} and mode values of 0.25 mg/l. Although the minimal inhibitory concentrations of trovafloxacin for *S.oralis* were relatively high compared to all the antibiotics tested. The in-vitro bactericidal activity of Trovafloxacin was greater than all other antibiotics against *S. oralis*. Five out of 10 isolates showed there was a 100% kill within 6 hours of contact; in all other cases, the kill time was between 6 and 24 hours.

Conclusion: Overall, trovafloxacin showed superior minimal inhibitory concentration, bactericidal concentration and kill time when compared with different antibiotics.

Keywords: Trovafloxacin, ciprofloxacin, erythromycin, β-lactam, vancomycin, teicoplanin, clindamycin, *S. oralis*.

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Viridans streptococci continue to be the most frequent-cause of infective endocarditis but due to an increasing resistance to β-lactam antibiotics, its treatment has become more complicated.[1] Different therapeutic considerations govern the selection of optimal antimicrobial regimens for strains and species of these organisms. Amongst those agents that have been effective, quinolones are a class of antibiotics structurally related to nalidixic acid, which exhibit bactericidal activity primarily by inhibiting bacterial DNA gyrase. The new fluoroquinolones differ from their predecessors in their broad antibacterial spectrum including both Gram positive and Gram-negative bacteria.[2]

Trovafloxacin (CP-99, 219) a new fluoronaphthyridone substituted at C-7 position with a 3-azabicycclo [3.1] hexyl moiety and with a 2,4-difluorophenyl group at the N-1 position, possesses a broad spectrum of antibacterial activity, with enhanced potency against Gram-positive species.[3] Trovafloxacin has been shown to have excellent half-life (>10 hours in man). In order to enhance the solubility of the parent drug at physiological pH, several amino acid prodrugs of Trovafloxacin were synthesized and tested for their aqueous solubility at physiological pH and for their rate of hydrolysis in serum. The L-Ala-L-Alanyl amide, CP-116, 517, was rapidly hydrolyzed to Trovafloxacin both in-

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vitro and in-vivo and was shown to display excellent pharmacokinetics.[4] Trovafloxacin has been demonstrated to distribute extensively into the gastric mucosa of rats and mice,[5] while its main route of excretion is through the biliary tract with a mean of 61% of the dose being recovered in 96 hours.[2]

Methods. A total of 60 clinical isolates of *S. oralis* were collected from patients either with Endocarditis, neutropenia or from the normal oral flora of healthy individuals. Strains from blood culture were primarily isolated using BACTEC NR850 and identified using the API20 strep, and further confirmed by using laboratory methodology using further biochemical tests.[6]

Determination minimal inhibitory concentration. Sixty isolates were tested for their susceptibility to vancomycin (Sigma), teicoplanin, clindamycin (Upjohn), penicillin (Glaxo), amoxycillin (Sigma), quinolone Trovafloxacin (Pfizer), ciprofloxacin (Sigma) and erythromycin (Abbot). A standard agar dilution technique was performed with a multipoint inoculator (Denley Instruments Ltd.). A final inoculum of 104 cfu per spot was used. Iso-Sensitest agar (Unipath Ltd. Basingstoke, UK) was used for susceptibility studies throughout and was supplemented with 5% lysed horse blood (Unipath) for testing the organisms. The antibiotic containing plates which were then incubated aerobically at 37ºC for 18 hours. The MIC was defined as the lowest antibiotic concentration that completely suppressed visible growth (one colony being ignored). The Oxford *S. aureus* (NCTC6571) was used as a control.

Determination minimal bactericidal concentration. A total of 16 clinical isolates of *S. oralis* were used for this part of the study. Isolates were from patients with endocarditis, neutropenia and from normal oral flora and were designated as follows: AR3, AR12, AR13@, AR19, AR33, AR40, A 6, A 9, A10, 23, 24, 26, 93C 87, 92C 17, N4-1-4, T8-2-12. MIC/MBC values were determined for erythromycin, clindamycin, trovafloxacin, azithromycin, penicillin, teicoplanin, and vancomycin using a micro-titer method, with antibiotic concentrations ranging from 0.003-64 mg/ml. Eighteen hour cultures of strains grown on Columbia blood agar (CA) were used to inoculate 10 ml of brain heart infusion (BHI) which was incubated aerobically at 37ºC for 4 hours. The growth was adjusted in BHI to provide a final inoculum of 104 cfu in 100 ml in the wells of a microtitre tray. The inoculum used was determined by growing several isolates from each group of organisms under standard conditions and performing surface viable counts by the spread method. One hundred mls of diluted suspension was added to 100 ml volumes of the diluted antibiotic solutions in microtitre trays; the trays were then incubated at 37ºC for 18 hours in air. MIC was recorded as the highest antibiotic dilution showing no turbidity. MBC's were determined by transferring 100ml volumes from wells showing no growth to CA plates, the inoculum was allowed to dry before spreading and incubated for 18 hours at 37ºC in air. The MBC was taken as the lowest antibiotic concentration of antimicrobial that reduced the number of viable organisms by 100% kill after 18 hours incubation.

Time kill curves. The in-vitro bactericidal activities of quinolone Trovafloxacin, penicillin, and vancomycin, were compared against 10 isolates of *S. oralis*. Organisms were grown in BHI for 18 hours at 37ºC in air and 100 ml added to 100 ml of freshly prepared pre-warmed BHI. After a one-hour incubation at 37ºC on an aerobic shaker, 100 ml samples were taken and viable counts performed. Solutions of the antibiotics were then added to the culture to provide final concentrations of 4x the previously determined MIC's for each isolate of *S.oralis* under investigation and an antibiotic-free growth control. Samples were collected following incubation for 0, 1, 2, 4, 6 and 24 hours. Tenfold dilutions of each sample were prepared in peptone water and 20 ml volumes transferred in duplicate onto the surface of CA plates supplemented with 5% horse blood. The viable counts were determined by the method of Miles et al, with plates incubated for 48 hours in air at 35ºC before being read.[7]

Results. Table 1 demonstrates the relative activities of penicillin, amoxycillin erythromycin, azithromycin, clindamycin, vancomycin, teicoplanin, ciprofloxacin and trovafloxacin, against 60 isolates of *S. oralis* isolated from patients with infective endocarditis and neutropenia and from subjects with normal oral flora. Table 1 shows the MIC_{50}, MIC_{90} and mode MIC values for each of the antibiotics tested. Trovafloxacin was the most active of all agents tested, except with clindamycin and for some strains with erythromycin and azithromycin. Susceptibility to trovafloxacin, demonstrated a narrow band of uni-modal normal distribution with a narrow MIC range of 0.12-0.5 mg/l, substantially lower than the 1-8 mg/l range shown with ciprofloxacin. Although ciprofloxacin demonstrated a narrow MIC range for all isolates. MIC_{50}, mode and MIC_{90} values for ciprofloxacin were sixteen-fold greater than those for Trovafloxacin. MICs for Trovafloxacin were higher when compared against different antibiotics tested; the MIC_{90} values for trovafloxacin was 0.5 mg/l. This was much lower when compared with these for other antibiotics; only the MIC_{90} value for teicoplanin was lower than that for trovafloxacin. Two very distinct population of isolates could be distinguished for clindamycin with no isolates showing intermediate susceptibility. Clindamycin was the most active of the antibiotics

Table 1 - Percentage of S. Oralis (n=60) isolates susceptible to penicillin, amoxycillin, clindamycin, erythromycin, azithromycin, teicoplanin, vancomycin, ciprofloxacin, trovafloxacin, (MIC, mg/l).

Antibiotics	0.0037	0.0075	0.015	0.03	0.06	0.12	0.25	0.50	1	2	4	8	16	32	64
Penicillin	-	-	3	21	14	20	14	5	11	3	6	3	-	-	-
Amoxycillin	-	-	3	24	14	9	18	3	13	3	-	5	8	-	-
Clindamycin	43	14	13	13	3	-	-	-	-	2	7	5	-	-	-
Erythromycin	-	-	2	24	8	14	14	10	14	6	-	6	-	-	2
Azithromycin	-	-	21	6	32	5	3	18	3	3	-	2	2	-	5
Teicoplanin	-	-	-	-	14	40	41	2	2	-	1	-	-	-	-
Vancomycin	-	-	-	-	-	-	2	60	36	2	-	-	-	-	-
Ciprofloxacin	-	-	-	-	-	-	-	-	7	14	63	16	-	-	-
Trovafloxacin	-	-	-	-	-	14	72	14	-	-	-	-	-	-	-

tested against these isolates with MIC_{50} value of 0.00375 mg/l, in total clindamycin was active against 86% of the isolates in an MIC range of < 0.00375-0.06 mg/l. However 14 isolates showed a higher range of MICs of 2-8 mg/l. Table 2 shows the comparative in-vitro bactericidal activities of Trovafloxacin penicillin, clindamycin, erythromycin, *teicoplanin and vancomycin, against 15 isolates of S.oralis* using the micro dilution broth technique. MIC values of Trovafloxacin for the 15 isolates ranged from 0.25-1 mg/l. Eleven of these isolates showed a 100% kill at < 1 mg/l of Trovafloxacin, but four isolates showed MBCs of 2, 4, 32 and >64 mg/l. Trovafloxacin showed low MIC/MBC ratio of <2

Table 2 - Comparative MIC/MBC values (mg/l) for Trovafloxacin, Penicillin, Erythromycin, Teicoplanin, Vanocmycin, and Clindamycin against S. oralis.

Strains	Trovafloxacin		Penicillin		Erythromycin		Teicoplanin		Vancomycin		Clindamycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
AR3	0.25	4	0.12	0.12	0.06	>16	0.5	64	0.03	32	0.12	>4
AR12	0.25	2	0.12	16	0.06	4	2	>64	0.06	32	0.12	>4
AR13	0.25	0.5	0.12	0.12	0.12	>16	2	16	0.12	4	0.12	>4
AR19	0.5	1	0.25	2	0.5	>16	2	16	0.25	>32	0.25	4
AR40	0.25	>64	1	>16	0.5	>16	2	>64	0.03	>32	0.12	>4
23	0.5	32	0.25	>16	0.25	>16	4	>64	0	>32	0.25	>4
N4-1-4	1	1	0.25	0.25	2	4	2	1	0.03	>32	0.25	2
A9	0.5	1	0.25	0.5	0.03	>16	2	>128	0.06	>16	0.06	>1
24	0.5	0.5	0.06	<0.06	0.25	>16	1	4	0.12	>16	0.12	>1
26	0.5	0.5	0.06	8	0.12	1	2	32	0.12	>16	0.06	0.25
A6	0.25	1	0.06	<0.06	0.12	0.12	2	-	0.5	>16	0.12	0.12
A10	1	1	2	>2	0.12	0.5	2	>128	0.25	>16	0.06	0.5
92C17	1	1	0.06	4	0.12	>16	2	4	0.5	>16	0.12	>1
93C87	0.5	1	0.5	0.5	0.12	0.5	2	2	0.25	>16	0.12	>1
T8-2-12	0.5	1	0.25	>8	0.12	0.5	2	>128	0.5	>16	0.12	>1

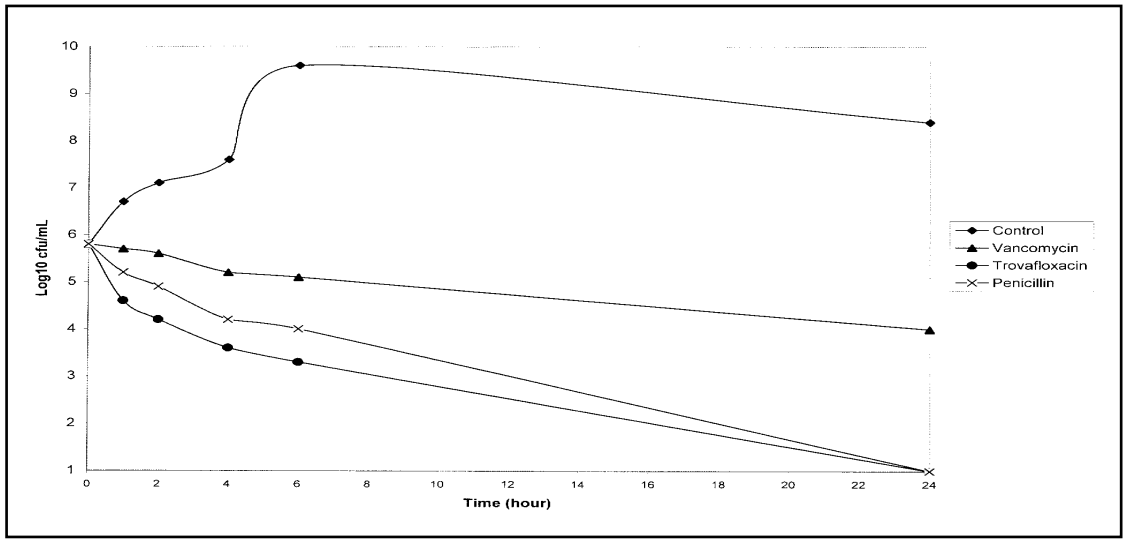

Figure 1 - Bacterial activity of Trovafloxacin (CP99, 219), vancomycin and penicillin at 4 x MIC against S. oralis from endocarditis in patient 1.

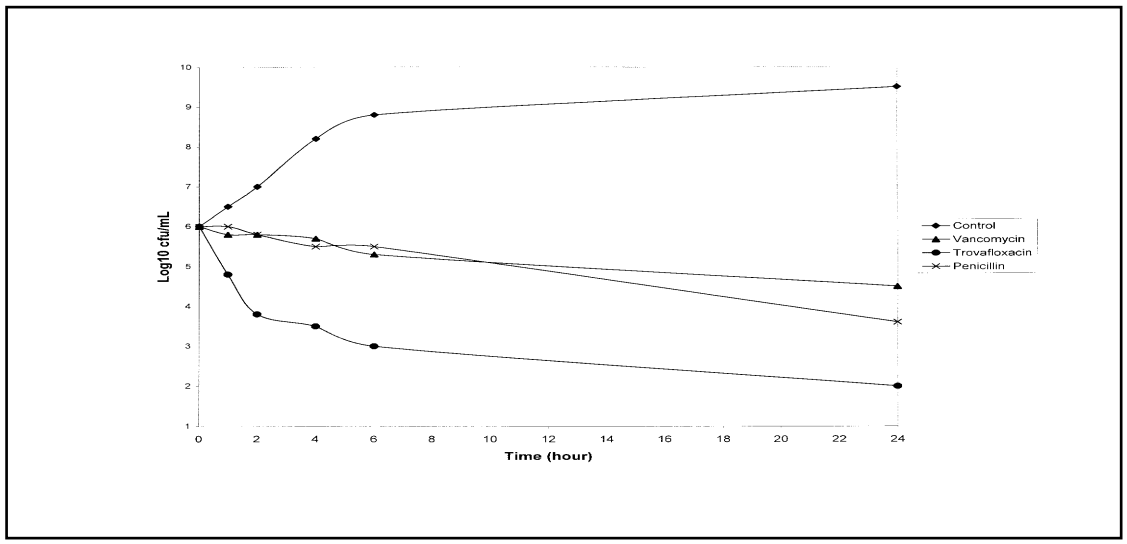

Figure 2 - Bacterial activity of Trovafloxacin (CP99, 219), Vancomycin and penicillin at 4 x MIC against S. Oralis from endocarditis patient 2.

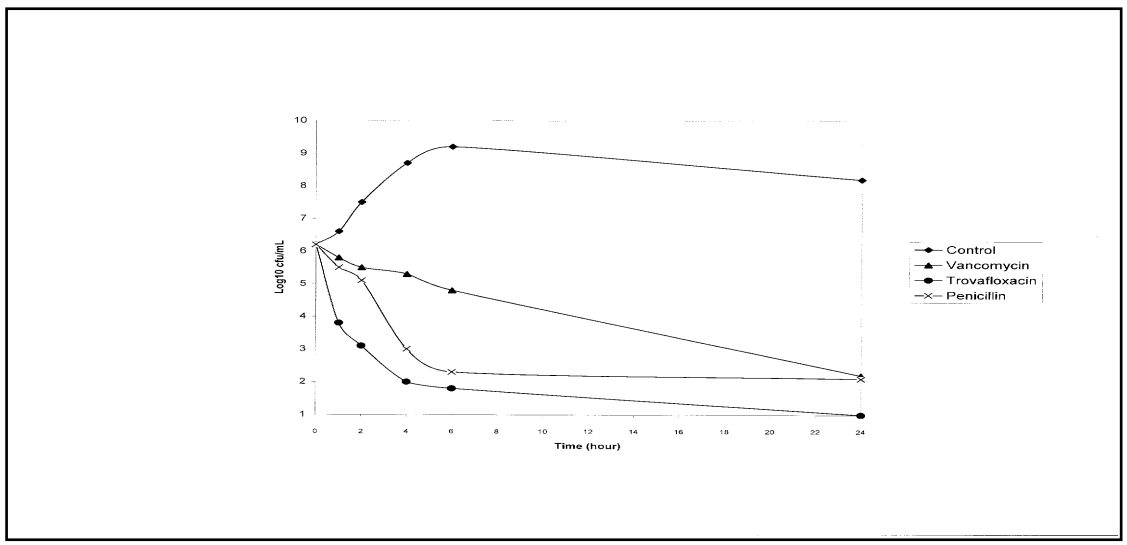

Figure 3 - Bacterial activity of Trovafloxacin (CP99, 219), vancomycin and penicillin at 4 x MIC against S. oralis from septicaemia patient.

with 11/15 of the isolates and only 4 strains showed tolerance to trovafloxacin. Three strains showed moderate penicillin resistance (>0.25 mg/l), but all 15 isolates were inhibited by < 2-mg/l penicillin. Seven of these were penicillin tolerant (MIC/MBC ratio > 1:8) and required > 2 mg/l of penicillin for a 100% kill. The MIC's for Vancomycin (range 0.03-0.25 mg/l) against these isolates were lower than those of teicoplanin (range 0.5-2 mg/l), but the bactericidal activity of teicoplanin was lower than that of vancomycin recorded over the 24 hour time period. An MBC range of 16>32-mg/l vancomycin was needed for 14 strains, whilst the MBC for one isolate was 4 mg/l. All isolates showed a high tolerance to vancomycin with MIC/MBC ratio of >32. With teicoplanin, MBC values for 10 of the 15 isolates ranged from 16-128 mg/l and 1-4 mg/l for 5 isolates. The majority (10/15) of the isolates showed a high tolerance to teicoplanin with an MIC/MBC ratio >8. All but one of the 15 strains were inhibited by < 0.5 mg/l erythromycin but 8 strains were not killed by 16 mg/l of erythromycin. Erythromycin showed much lower bactericidal activity than Trovafloxacin. Eight of the 15 isolates required > 16 mg/l of erythromycin for a 100% kill. Although clindamycin demonstrated lower MIC values (range 0.06-0.25 mg/l) than those for trovafloxacin, with 12 of the isolates inhibited by < 0.12 mg/l of clindamycin, the MBC values for 10 of the 15 isolates were between 1-4 mg/l of clindamycin for a 100% kill, 5 isolates required >4 mg/l. All isolates showed a relatively high tolerance to clindamycin, with 12/15 having an MIC/MBC ratio of >8.

Time kill curve. Killing curve was performed on 10 isolates from endocarditis, neutropenic and normal oral flora strains. The pattern of kill was similar and not much variation was seen from these different sources of strains. Figure.1, 2 and 3 shows the comparative bactericidal activity at 4 x MIC of Trovafloxacin, vancomycin and penicillin against an isolate of *S.oralis*. Figure 1 isolate from endocarditis patient 1, achieved three-log reduction by Trovafloxacin and penicillin between 6 and 24 hours. Vancomycin showed less than two a log reduction over 24 hours. Figure 2 isolate from patient 2 with endocarditis showed a three-log reduction after 6 hours in contact with trovafloxacin. Penicillin showed poor bactericidal activity against this isolate with a two-log reduction in viable count occurring between 6 and 24 hours. Vancomycin also showed low bactericidal activity against this isolate with a reduction in viable count of 1.5 logs between 6 and 24 hours. Figure 3. *S.oralis* an isolate from a septicemic patient showed a three-log reduction in viable count within 2 hours of the organism coming into contact with Trovafloxacin. A three-log reduction was achieved with penicillin after four hours exposure, whereas vancomycin showed less than a 1.5 log reduction in viable count over 6 hours.

Discussion. Susceptibility studies demonstrated that both penicillin and amoxycillin showed similar activities against 60 isolates of *S.oralis*. These strains showed a wide range of susceptibilities but the MIC_{50} and the Mode MIC values for both antibiotics were 0.125 mg/l and 0.03 mg/ml. Erythromycin was marginally less active than Azithromycin with MIC_{50} of 0.25 mg/l compared to 0.06 mg/l for azithromycin. The results in this study are in agreement with other studies.[8] Teicoplanin demonstrated greater in-vitro activity than vancomycin, 95% of the isolates were inhibited by 0.25 mg/l of teicoplanin, whereas 96% of the isolates required 1 mg/l of vancomycin for inhibition. Although teicoplanin and vancomycin showed similar MIC ranges of 0.06-8 mg/l, the overall MIC_{50}, mode and MIC_{90} values for teicoplanin were two-fold lower than those for vancomycin. Therefore teicoplanin appears to be more effective than vancomycin, at least in this study. Trovafloxacin showed a four-fold increase in activity over ciprofloxacin, with a narrow MIC range, of MIC_{50} and mode values of 0.25 mg/l, where as MIC_{50} and mode values for ciprofloxacin were both 4 mg/l. This correlates well with studies where Trovafloxacin has been compared with ciprofloxacin against *S. pneumoniae* and others Gram-positive organisms.[3, 8-12] Although the MIC's of Trovafloxacin for *S.oralis* were relatively high compared to all the antibiotics tested. The in-vitro bactericidal activity of Trovafloxacin was greater then the rest of the antibiotics tested against *S. oralis*. Eleven of these isolates showed a 100% kill at < 1 mg/l of Trovafloxacin, whereas the other isolates showed MBCs of 2, 4, 32 and >64 mg/l. Trovafloxacin showed low MIC/MBC ratio of <2 mg/l with 11/15 of the isolates and only four strains which show tolerance to CP 99,219. MBC values for clindamycin and erythromycin were much higher. Eight of the 15 *S. oralis* tested required > 16 mg/l of erythromycin and the remaining seven required < 4 mg/l. Similarly for clindamycin the MBC varied, for three isolates the MBC was 0.5 mg/l, whereas for the remaining 12 isolates the MBC ranged between 1-> 4 mg/l. The in-vitro MBC values of <16 mg/l for vancomycin were shown with 6 isolates whereas an MBC of > 128 mg/l was shown for the remaining nine isolates. From these findings it was clear that trovafloxacin has superior bactericidal activity against S. oralis when compared to these antibiotics. For all isolates selected, MBC values for penicillin were at least four times the MIC. Trovafloxacin at 4 x MIC showed demonstrated superior bactericidal activity when compared to penicillin and vancomycin against all ten isolates of S. oralis Trovafloxacin was bactericidal (100% kill) 6-24 hours of contact. Interestingly with Trovafloxacin only five out of ten isolates showed a 100% kill within six hours of contact, the remaining isolates requiring between 6

and 24 hours. Penicillin at 4 x MIC showed superior bactericidal activity against S. oralis compared to vancomycin, but not when compared to Trovafloxacin. With vancomycin only three isolates showed a 100% kill of which two were from the normal oral flora. This demonstrates the inability of vancomycin to show bactericidal activity against S. *oralis* in-vitro on its own. This study correlates with the findings of Shanson and Tadayon.[13] In summary Trovafloxacin was the most bactericidal against *S. oralis* of all the antibiotics tested. With penicillin only four of the six infective endocarditis isolates showed a three-log reduction in viable colony count within 6-24 hours. Only three out of the 10 isolates showed a three-log reduction in viable counts with vancomycin. Whereas 5 out of 10 isolates showed a 100% kill within six hours of contact with trovafloxacin, in all other cases, 100% kill was achieved between 6 and 24 hours.

There is an increasing prevalence of resistance to Viridans streptococci, methicillin resistant *S. aureus* (MRSA) and *S. epidermidis* in the USA and Europe.[14-15] Hence the need for an agent with excellent activity against macrolide resistant strains of Gram-positive organisms is becoming more urgent. Superior activity of trovafloxacin against gram positive organisms have been demonstrated,[16-19] thus making it a potential candidate for the empiric treatment of patients with suspected bacteraemia, septicaemia and complicated cases of infective endocarditis.

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