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In Vitro Antimicrobial Activity of Imipenem in Combination with Vancomycin or Teicoplanin against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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The interaction between imipenem and two glycopeptides against staphylococci was examined for potential synergy. Imipenem in combination with vancomycin or teicoplanin exerted a synergistic or additive effect against a majority of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates tested by the checkerboard method. Synergistic inhibitory effects were frequently accompanied by synergistic bactericidal effects. For a proportion of bacterial isolates of both species, the demonstration of synergy by the checkerboard method was confirmed by time-kill studies using antibiotic combinations at the MICs or at achievable serum antibiotic levels. Only with a single isolate of *Staphylococcus epidermidis* was antagonism with either antibiotic combination recorded.

Gram-positive bacterial infections are still a major cause of morbidity and mortality. In immunocompromised patients *Staphylococcus aureus* infections have an especially poor prognosis (1), and multiply antibiotic resistant coagulase-negative staphylococcal infections (2) are an increasing challenge with the use of new surgical procedures and the increasing use of inert materials as prostheses. In the treatment of severe staphylococcal infections when multiple antibiotic resistance is present, vancomycin is the drug of choice. Most recently a related glycopeptide, teicoplanin, has become available but resistance development with this drug may be more common than with vancomycin (3, 4). However, in these infections in immunocompromised patients, the response rate to either vancomycin or teicoplanin alone may not exceed 60–70 % (5).

In the empirical treatment of serious infections, especially in neutropenic patients, and in infections such as endocarditis where successful treatment requires a rapid bactericidal action, the use of drug combinations are most important (6).

Several recent studies have evaluated the in vitro interaction of a variety of drug combinations especially with respect to activity against gram-positive bacteria (7–10). Combinations have included either vancomycin or teicoplanin (11, 12). The novel carbapenem, imipenem, has a broad spectrum of activity, and in combination with either vancomycin or teicoplanin in empirical treatment of mixed infections or infections where the pathogen is unknown could yield good results. Some evidence suggests that combinations of teicoplanin and imipenem may indeed exert synergistic action on a range of gram-positive bacteria including *Staphylococcus aureus* and *Staphylococcus epidermidis* (11, 12). In this investigation we examined the in vitro antibiotic activity of imipenem, teicoplanin and vancomycin alone and of combinations of imipenem with the two glycopeptides against clinical isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Materials and Methods

Bacteria. Sixty isolates of staphylococci were selected from 60 different patients. All isolates were from clinically significant infections and were derived from blood or cerebrospinal fluid. The study population was selected to include 30 isolates of *Staphylococcus aureus* and 30 isolates of *Staphylococcus epidermidis*. The isolates were identified by an identification strip method (API Staph, API, France), and those identified as *Staphylococcus aureus* were confirmed to be tube coagulase positive. Representative strains of both species were known to be methicillin resistant on the basis of disc sensitivity testing at 30 °C or 37 °C on DST agar with 5 % salt added.

Media. Mueller-Hinton broth (CM405, Oxoid, UK) and Mueller-Hinton agar (152-03300M, Gibco, UK) were used as growth media throughout. One-quarter strength Ringer's solution (BR52, Oxoid, UK) was used as a diluent in the enumeration of bacteria.

Antibiotics. The following antibiotics were used in the study: imipenem, potency 964 µg/mg (Merck Sharpe and Dohme, USA) vancomycin, potency 950 µg/ml (Eli Lilly, USA) and teicoplanin, potency 853 µg/mg (Merrell Dow Pharmaceuti-

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cals, USA). Stock solutions of antibiotic were prepared in sterile distilled water on the day of use and filter sterilised. MICs of other antibiotics tested and reported were carried out by an automated fluoro-spectrophotometric method (Sensititre, UK).

Susceptibility Testing. MICs and MBCs were determined in plastic 96-well microtitre plates with round-bottomed wells (U24ARTL, Sterilin, UK) containing 0.05 ml amounts of the antibiotic in doubling dilutions in Mueller-Hinton broth. Bacterial suspensions of log phase cultures in Mueller-Hinton broth were adjusted by turbidity to yield an inoculum of 10^5 CFU/ml, and 0.05 ml volumes added to individual wells as required. Appropriate controls for individual antibiotics without inocula were included. End-points for MIC testing were read as the lowest concentration of antibiotic that completely prevented visible turbidity in individual microtitre wells after 24h of incubation at 37 °C. Samples of 0.02 ml were subcultured onto Mueller-Hinton agar for MBC testing and plates incubated for 48h at 37 °C. A reduction in colony count of > 99.9 % was used as the end-point for MBC testing.

Checkerboard Technique. Synergy testing was performed by the checkerboard method in microtitre plates using the same inocula as used for MIC testing. The fractional inhibitory concentration (FIC) (MIC in combination/MIC alone) for each component as well as the sum of the FICs (ΣFIC) for each checkerboard combination was calculated. For individual strains and antibiotic combinations the observed interaction was recorded as synergistic (ΣFIC < 0.5), additive (ΣFIC 0.5–0.7), indifferent (ΣFIC 0.7–2.0) or antagonistic (ΣFIC > 2.0) (8). Bactericidal synergy was evaluated in the same way to yield FBC values for individual strains and antibiotic combinations. The observed interaction was recorded in the same bands as the FIC. All determinations were carried out in duplicate.

Time-Kill Studies. For selected strains time-kill curves were determined. Inocula were prepared by overnight incubation in Mueller-Hinton broth and added to broth to yield an inoculum at zero time of 10^7 CFU/ml. Broth contained individual antibiotics at the respective MIC or a concentration of 5 mg/l alone or in combination with each antibiotic at the respective MIC or at a concentration of 5 mg/l. Samples (0.1 ml) were taken after incubation for 0h, 6h and 24h at 37 °C, appropriate dilutions prepared and counts determined by the method of Miles and Misra (9) on Mueller-Hinton agar. All time-kill tests were carried out in duplicate. For time-kill curves, synergy was defined as a > 2 $\log_{10}$ decrease in CFU/ml between the drug combination and its most active constituent (8). Results are presented as synergy if decreases in CFU/ml between 2 $\log_{10}$ and 3 $\log_{10}$ were obtained, and pronounced synergy if > 3 $\log_{10}$ decreases in CFU/ml were recorded. Synergy determinations were made after incubation for 6h and 24h.

Results

Antibiotic Sensitivity Tests. The MICs of a range of anti-staphylococcal antibiotics for the *Staphylococcus aureus* and *Staphylococcus epidermidis* strains tested are shown in Table 1. Eleven methicillin-resistant *Staphylococcus aureus* isolates and 13 methicillin-resistant *Staphylococcus epidermidis* isolates were included. For methicillin the MIC50 and range of MIC values were the same for both species. A majority of isolates of both species were resistant to

Table 1: MICs of antibiotics tested against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

	MIC (mg/l)		
	MIC 50	MIC 90	Range
S. aureus (n = 30)			
Methicillin	4.0	> 16.0	1.0 – > 16.0
Gentamicin	0.5	16.0	< 0.12 – > 16.0
Fusidic acid	0.5	8.0	0.12 – > 8.0
Erythromycin	0.25	> 32.0	0.25 – > 32.0
Clindamycin	0.12	> 16.0	0.12 – > 16.0
Chloramphenicol	8.0	32.0	2.0 – > 32.0
Tetracycline	1.0	> 16.0	0.25 – > 16.0
Rifampicin	< 1.0	< 1.0	< 1.0 – 2.0
S. epidermidis (n = 30)			
Methicillin	4.0	16.0	1.0 – > 16.0
Gentamicin	> 16.0	> 16.0	0.12 – > 16.0
Fusidic acid	0.12	8.0	0.06 – 8.0
Erythromycin	0.25	32.0	0.25 – > 32.0
Clindamycin	0.12	8.0	0.12 – > 16.0
Chloramphenicol	4.0	> 32.0	2.0 – > 32.0
Tetracycline	0.5	16.0	0.25 – > 16.0
Rifampicin	< 1.0	< 1.0	< 1.0 – < 1.0

Table 2: MICs and MBCs of vancomycin, teicoplanin and imipenem against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

	MIC (mg/l)			MBC (mg/l)		
	MIC 50	MIC 90	Range	MBC 50	MBC 90	Range
S. aureus (n = 30)						
Imipenem	0.03	4.0	0.007 – 8.0	0.03	16.0	0.007 – 32.0
Vancomycin	2.0	4.0	1.0 – 8.0	2.0	8.0	1.0 – > 8.0
Teicoplanin	2.0	4.0	0.25 – 4.0	2.0	8.0	0.25 – > 8.0
S. epidermidis (n = 30)						
Imipenem	0.015	0.25	< 0.002 – 2.0	0.06	4.0	0.015 – 8.0
Vancomycin	2.0	4.0	1.0 – 8.0	4.0	8.0	1.0 – 8.0
Teicoplanin	4.0	8.0	0.25 – > 8.0	4.0	8.0	1.0 – > 8.0

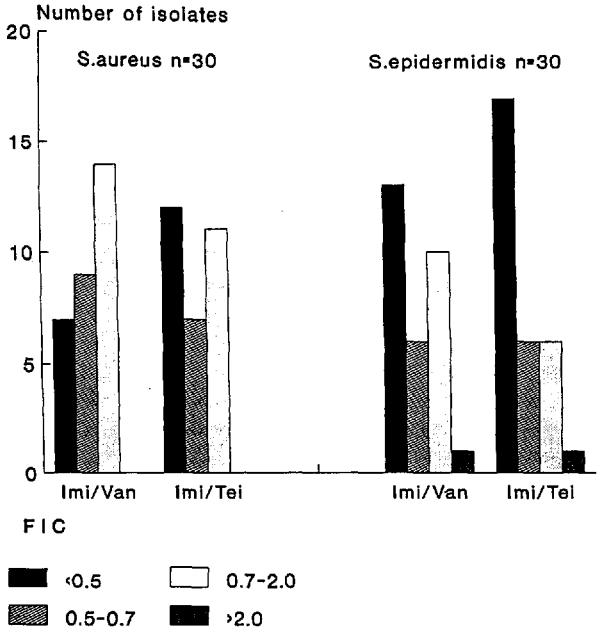

Figure 1: The sum of FICs (ΣFIC) of imipenem in combination with vancomycin (Imi/Van) or teicoplanin (Imi/Tei) for *Staphylococcus aureus* (n = 30) and *Staphylococcus epidermidis* (n = 30).

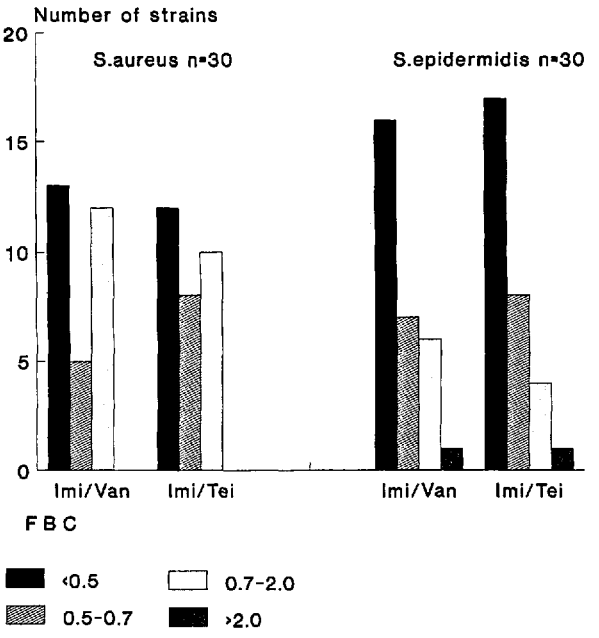

Figure 2: The sum of FBCs (ΣFBC) of imipenem in combination with vancomycin (Imi/Van) or teicoplanin (Imi/Tei) for *Staphylococcus aureus* (n = 30) and *Staphylococcus epidermidis* (n = 30).

chloramphenicol. Gentamicin resistant isolates of both species were present but *Staphylococcus epidermidis* isolates were more often resistant to gentamicin (MIC50 > 16 mg/l) than *Staphylococcus aureus* isolates (MIC50 0.5 mg/l). Multiple resistance, including resistance to the macrolides, fusidic acid and tetracycline, was characteristic of a minority of isolates of both species. All isolates were uniformly sensitive to rifampicin.

The MICs and MBCs of imipenem, vancomycin and teicoplanin for *Staphylococcus aureus* and *Staphylococcus epidermidis* are shown in Table 2. MIC50, MIC90, MBC50 and MBC90 of both vancomycin and teicoplanin were very similar for both *Staphylococcus aureus* and *Staphylococcus epidermidis*. The range of MICs and MBCs for both species tended to be slightly wider with teicoplanin than with vancomycin. However, for both species and both antibiotics the MBC/MIC ratio was low. With imipenem the MIC90 and MBC90 were higher for *Staphylococcus aureus* than for *Staphylococcus epidermidis*. MIC and MBC ranges were wider than with either vancomycin or teicoplanin, and for a number of isolates MBC/MIC ratios were higher than seen with either of the glycopeptide antibiotics.

Checkerboard Studies. Using the checkerboard method combinations of imipenem with both vancomycin and teicoplanin were shown to result in synergistic inhibitory or bactericidal interaction with some isolates (Figures 1 and 2).

With *Staphylococcus aureus* significant synergistic inhibitory interaction (Figure 1) was more commonly seen with a combination of imipenem and teicoplanin. Both drug combinations showed an additive effect for a number of isolates and for a substantial number of isolates indifference was seen. Both drug combinations produced a synergistic bactericidal effect with a similar number of isolates (Figure 2). An additive or indifferent effect was shown with the remaining isolates. Antibiotic antagonism was not noted with any isolates.

With *Staphylococcus epidermidis* significant synergistic inhibitory effects were seen with both drug combinations. With teicoplanin and imipenem a synergistic reaction was noted for 17 (57 %) isolates. An additive reaction with both drug combinations was noted for six isolates (20 %). Antagonism (ΣFIC > 2.0) was noted for one isolate with both combinations. ΣFBC indices indicated that for a majority of isolates there was a significant synergistic bactericidal effect with both drug combinations. An additive effect was seen for seven isolates (23 %) with vancomycin and imipenem and for eight isolates (26 %) with teicoplanin and imipenem. For a number of isolates indifference was noted with both drug combinations and antagonism was noted for a single isolate.

Interaction against methicillin resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* was found to fall into all categories, for some strains synergy being seen and for some strains indif-

ference, as with methicillin sensitive strains. Synergistic inhibitory and bactericidal effects were recorded for a number of methicillin-resistant isolates of both species.

Comparison of Antibiotic Combinations. Vancomycin plus imipenem and teicoplanin plus imipenem exhibited a greater synergistic effect for a larger percentage of strains of *Staphylococcus epidermidis* than of *Staphylococcus aureus*. Teicoplanin and imipenem in combination showed synergistic or additive effects for a greater number of strains than vancomycin plus imipenem. Little difference in antibiotic interaction at the inhibitory level (ΣFIC indices) or bactericidal level (ΣFBC indices) was seen with teicoplanin. However, with vancomycin and imipenem in combination synergistic bactericidal effects (ΣFBC indices) were more frequently noted than synergistic inhibitory effects (ΣFIC indices).

With a majority of the strains for which inhibitory or bactericidal synergy was seen between vancomycin and imipenem, as judged by ΣFIC and ΣFBC indices, similar inhibitory or bactericidal synergy was also seen between teicoplanin and imipenem. However, with *Staphylococcus aureus* (2 isolates) and *Staphylococcus epidermidis* (1 isolate) bactericidal synergy (ΣFBC index < 0.5) was noted between vancomycin and imipenem without a similar bactericidal synergistic effect between teicoplanin and imipenem.

Time-Kill Studies. Time-kill curves were used to detect synergy of the two antibiotic combinations. Ten strains of *Staphylococcus aureus* and 14 of *Staphylococcus epidermidis* for which synergy was shown by the checkerboard method were selected for study (Figures 3 and 4). No strain with any antibiotic combination showed an increase in colony count above that shown with individual antibiotics alone at either concentration (MIC or 5 mg/l) after 6h or 24h. Synergy was rarely shown after 6h of incubation (Figure 3), although for three isolates of *Staphylococcus epidermidis* there was synergy between vancomycin and imipenem. After 24h of incubation (Figure 4), synergy was seen with at least one antibiotic combination for a majority of strains.

For 16 of 24 isolates (*Staphylococcus aureus* and *Staphylococcus epidermidis*) synergy (> 2 $\log_{10}$ reduction in CFU/ml) was seen with vancomycin and imipenem and for 13 of 24 isolates with teicoplanin and imipenem. Altogether, only 5 of 24 isolates did not show a > 2 $\log_{10}$ or greater reduction in CFU/ml with some antibiotic combination (4 *Staphylococcus aureus* and 1 *Staphylococcus epidermidis*).

As shown in Figure 4, time-kill curves showed synergy for a maximum of 50 % of isolates of *Staphylococcus aureus* with teicoplanin and imipenem. With

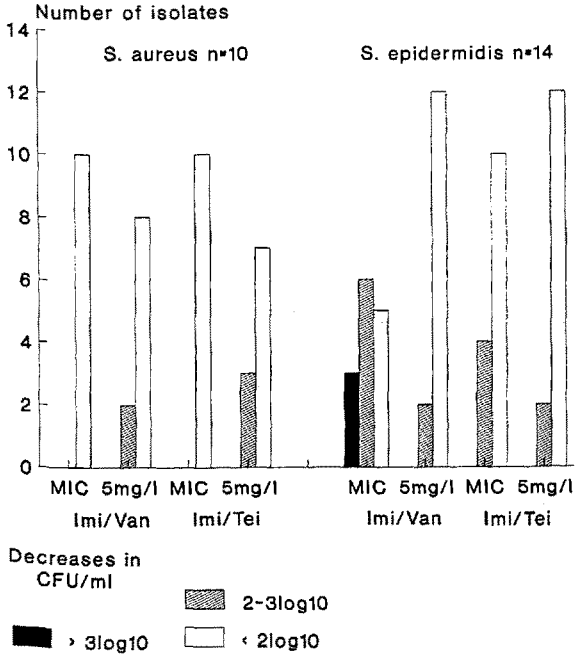

Figure 3: The in vitro interaction of imipenem with vancomycin (Imi/Van) or teicoplanin (Imi/Tei) at MICs or 5 mg/l in a time-kill assay at 6 h for *Staphylococcus aureus* (n = 10) and *Staphylococcus epidermidis* (n = 14).

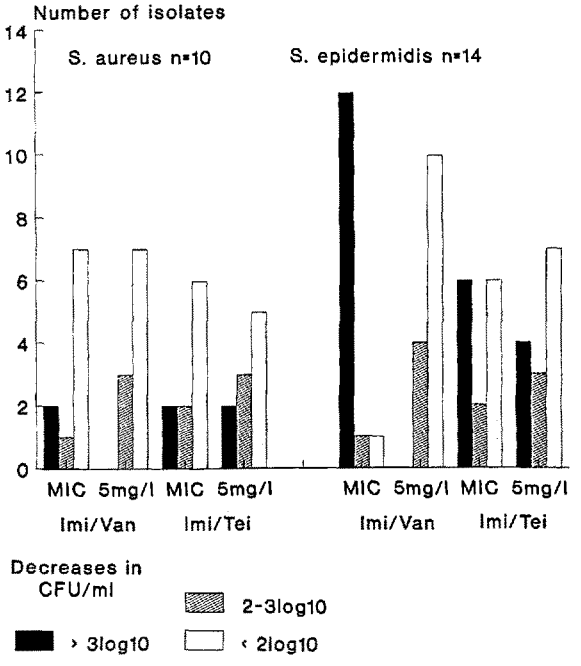

Figure 4: The in vitro interaction of imipenem with vancomycin (Imi/Van) or teicoplanin (Imi/Tei) at MICs or 5 mg/l in a time-kill assay at 24 h for *Staphylococcus aureus* (n = 10) and *Staphylococcus epidermidis* (n = 14).

vancomycin and imipenem at the MICs or at 5 mg/l synergy was recorded for only 3 of 10 *Staphylococcus aureus* isolates. However, with *Staphylococcus epidermidis* both antibiotic combinations showed synergy at MICs for a majority of isolates; synergy was recorded at MICs with vancomycin and imipenem for 13 of 14 isolates and with teicoplanin and imipenem for 8 of 14 isolates. In both cases synergy was less often recorded when antibiotics were used in combination at 5 mg/l. The difference in synergy recorded between combinations at MICs and 5 mg/l was more marked with vancomycin than with teicoplanin. However, this was not due to relative resistance to individual antibiotics since for no strain included in the time-kill study was the MIC of vancomycin > 5 mg/l, although four strains of *Staphylococcus epidermidis* were included for which the MIC of teicoplanin was > 5 mg/l. Indeed, synergy was recorded for three of these four strains with a combination of imipenem and teicoplanin.

Discussion

Combinations of imipenem with vancomycin or teicoplanin were shown in this study to exhibit a synergistic inhibitory effect for the majority of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates tested by the checkerboard method. Clear synergy for a majority of strains of both species was shown with combinations of teicoplanin and imipenem. The results are in agreement with those of previous studies which examined the interaction of imipenem and teicoplanin (11, 12), and provide further evidence that beta-lactam antibiotics may act synergistically with vancomycin (10). However, for a substantial number of strains of both species the result of interaction of both antibiotic combinations was indifference, contrary to the findings of Debbia et al. (10). Antagonistic effects of antibiotic combinations were shown only for one isolate of *Staphylococcus epidermidis*. Time-kill studies were carried out on strains which demonstrated synergy by the checkerboard method. For few strains was there failure to show synergy by this method with some antibiotic combination and concentration. However, the high correlation between the two methods shown by Debbia et al. (11) was not upheld: substantial disparity between the two methods was noted for both species and both antibiotic combinations. The response of *Staphylococcus epidermidis* at the two antibiotic concentrations studied (MIC or 5 mg/l for both components) demonstrates that synergy is highly concentration dependent and that the study of a mixed population of strains at selected antibiotic concentrations has severe limitations. A comprehensive study of a range of concentrations would give a more meaningful result but would be very demanding in terms of experimental time (13).

The synergistic inhibitory effects of the antibiotic combinations described here were paralleled by synergistic bactericidal effects (ΣFBC indices, Figure 2). As with the inhibitory effects, synergistic bactericidal effects were found for a greater number of *Staphylococcus epidermidis* than *Staphylococcus aureus* isolates. However, this difference was less pronounced and no difference in synergistic bactericidal effect was noted between the two antibiotic combinations for both species. The major difference between synergistic bactericidal and inhibitory effects was the higher number of *Staphylococcal aureus* isolates showing synergistic bactericidal effects than synergistic inhibitory effects with combinations of imipenem and vancomycin (Figures 1 and 2). If it is of importance in the treatment and prophylaxis of some conditions such as endocarditis (14) that bactericidal antibiotic concentrations are achieved, then the synergistic bactericidal effects noted here may have some relevance when evaluating those antibiotic combinations for use in the treatment of particular conditions.

The results presented here confirm and expand previous findings of synergism between beta-lactam and glycopeptide antibiotics. The checkerboard system indicated predominantly synergistic interaction with both antibiotic combinations. The rate of confirmation of synergy in time-kill studies was not as high as reported by Debbia et al. (11), which may however only reflect the characteristics of individual isolates tested and the antibiotic concentrations used in the studies. The enhanced inhibitory and bactericidal activity of the antibiotic combinations may be of relevance in antistaphylococcal therapy especially where effective bactericidal concentrations may be important as in infections in immunocompromised patients, or where prostheses are in situ. The combinations may also be of value in the empirical treatment of infections in compromised patients where imipenem may provide broad spectrum antibacterial activity as well as specific enhancement of the antistaphylococcal activity of teicoplanin or vancomycin.

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