IN VITRO ACTIVITY OF TICARCILLIN, CARBENICILLIN AND AMPICILLIN AGAINST SOME GRAM-NEGATIVE BACILLI

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α-Carboxy-3-thienylmethyl penicillin (ticarcillin) is a relatively new semisynthetic penicillin which is more active than carbenillin against *Pseudomonas aeruginosa*. Among the strains tested, those isolated from the respiratory tract showed an increased susceptibility to carbenicillin and ticarcillin. As with carbenicillin, synergistic activity against *P. aeruginosa* could be demonstrated with ticarcillin in combination with gentamicin. Like other penicillins, the antibacterial activity was influenced by the inoculum size. The antibacterial activity of ticarcillin showed the compound to be almost equally active with carbenicillin and ampicillin against *Escherichia coli* and *Klebsiella aerogenes*, but less active than carbenicillin and ampicillin against indole-negative *Proteus* strains. Regarding the indole-positive *Proteus* species, at relatively low antibiotic concentrations the proportion of strains sensitive to ticarcillin was greater than to carbenicillin or ampicillin whereas at relatively high antibiotic concentrations the converse was the case. It is interesting to note that a high proportion of strains of *E. coli* and *K. aerogenes* were resistant to the three penicillins even at a concentration of 200 μg/ml, while 70% of *Proteus* strains were inhibited by these drugs at the same concentration. Disc susceptibility tests with ticarcillin were carried out according to BAUER-KIRBY method[3].

The incidence of infections caused by Gram-negative bacilli has increased during the last decades[2,10,19]. A substantial number of these infections especially in patients with leukemia, metastatic cancer, cystic fibrosis and external burns is caused by *Pseudomonas* and *Proteus*[11]. Colistin sulfate, polymyxin B sulfate and gentamicin have been proved effective against infections with *Pseudomonas* in patients with burns, but can not be used in patients with impaired host defences and blood dyscrasias. Moreover, they have potential nephrotoxicity.

Carbenicillin, a semisynthetic penicillin with antipseudomonal activity, is effective even in patients with impaired host defences and severe granulocytopenia[5,20]. However, very large doses are required for the treatment of systemic infections. This is a disadvantage for patients with marginal cardiac and renal functions, who are unable to tolerate a high sodium load[12]. Another penicillin 6-(D-α-sulfo aminophenyl-acetamido) penicillanic acid (BLP 1462) has undergone preliminary investigation and seems to be comparable in its action to carbenicillin[6]. A new semisynthetic penicillin, α-carboxyl-3-thienyl methyl penicillin (ticarcillin) appears to be slightly more active than carbenicillin *in vitro* and it has been suggested that this compound might be active clinically at lower doses than carbenicillin[4,15,21]. This study was, therefore, undertaken to compare the antimicrobial activity of carbenicillin and ticarcillin against *Pseudomonas aeruginosa*, *Proteus*, *Escherichia coli* and *Klebsiella aerogenes*.

Materials and Methods

Selection of strains: All the bacterial strains used in this study were clinical isolates ob-

Table 1. Patients' sites from which *Pseudomonas aeruginosa* strains were isolated.

Site	Number of strains
Sputum, bronchial washings	49
Urine	35
Wounds	16
Stools	8
Total	108

tained from a number of hospitals in Athens. In all instances, the organisms were considered as responsible for clinical infections or at least serious bacterial colonization. A total of 108 strains of *P. aeruginosa*, 60 strains of *Proteus*, 38 strains of *E. coli* and 20 strains of *K. aerogenes* were studied. The sites (in the patients) from which the 108 isolates of *P. aeruginosa* were taken are listed in Table 1. *Proteus* strains were obtained mostly from urine cultures, *E. coli* strains were all obtained from stool cultures while *K. aerogenes* strains were isolated from various sites. *P. aeruginosa* isolates were identified by previously published criteria[8] while enterobacterial isolates were classified according to the scheme proposed by COWAN and STEEL[11].

Antibiotics-Susceptibility Testing: The antibiotics used were α-carboxy-3-thienyl-methyl-penicillin (ticarcillin, 815 μg free acid/mg, Beecham; carbenicillin (790 μg free acid/mg, Beecham); ampicillin (845 μg free acid/mg, Beecham); gentamicin (587 μg base/mg, Schering). All the drugs were dissolved in nutrient broth (nutrient broth No. 2, Oxoid) to an original concentration of 1 mg/ml. These solutions were used on the day of preparation.

Minimal inhibitory concentrations were determined by the two-fold serial tube dilution method. Five ml volumes of nutrient broth having the appropriate antibiotic concentration were inoculated with a drop (0.03 ml) of an overnight culture. This inoculum yields $10^6 \sim 10^7$ cells per ml. In certain assays with *P. aeruginosa* a 1:1,000 dilution of this inoculum was also used.

Growth was recorded after 18 hours of incubation at 37°C. The minimal inhibitory concentration (MIC) of the antibiotic was defined as the lowest concentration which inhibited development of visible turbidity. With *P. aeruginosa* the trace growth observed in tests with the undiluted inoculum was disregarded because this "tailing" phenomenon is not due to the presence of resistant variants[21].

Disc susceptibility tests were performed with discs containing 100 μg of ticarcillin; all the conditions of the test were standardized according to the method of BAUER *et al*[8] using MÜLLER-HINTON agar. Three selected strains of *P.aeruginosa* being inhibited by a concentration of 200 μg/ml of ticarcillin were used to test the synergism of ticarcillin with gentamicin.

Results

The activity of ticarcillin against *P. aeruginosa* is shown in Fig. 1. The drug was most active against the strains isolated from the respiratory tract; nearly 80 % of these isolates having an MIC of 100 μg/ml or less, while about 55 % of the strains isolated from urinary infections, stools and wounds were inhibited by ticarcillin at that concentration.

The activity of carbenicillin against the same strains is presented in Fig. 2. Again it is observed that strains isolated from the respiratory tract were more sensitive than those isolated from urine.

The activity of ticarcillin and carbenicillin against the *P. aeruginosa* strains independently of their origin is shown in Fig. 3. It is observed that ticarcillin is more active than carbenicillin: 68 % of the strains were inhibited by 100 μg/ml of ticarcillin, while 43 % of the strains were inhibited by carbenicillin at the same concentration.

Figure 4 shows that the activity of ticarcillin against *P. aeruginosa* is greatly enhanced when a diluted inoculum is used. Nearly 97 % of the strains were inhibited by a concentration

Fig. 1. Activity of ticarcillin against *Pseudomonas aeruginosa* strains isolated from different clinical sources.

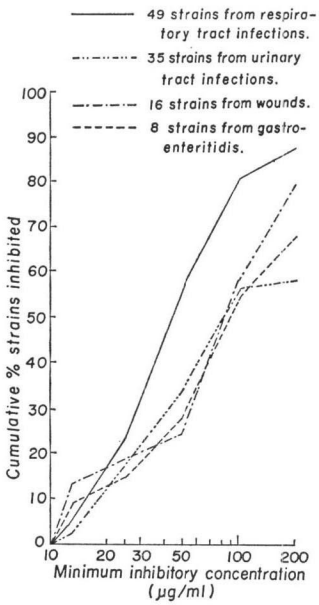


Fig. 2. Activity of carbenicillin against *Pseudomonas aeruginosa* strains.

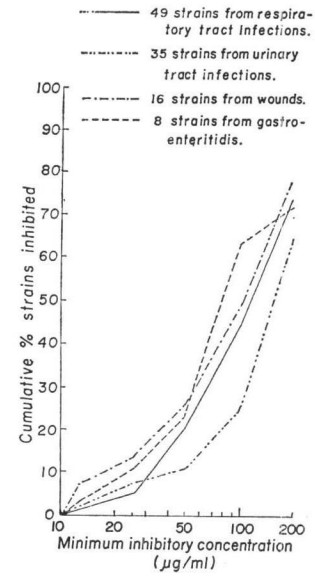


Fig. 3. Activity of ticarcillin and carbenicillin against 108 strains of *Pseudomonas aeruginosa*. Number and origin of strains as in Fig. 1.

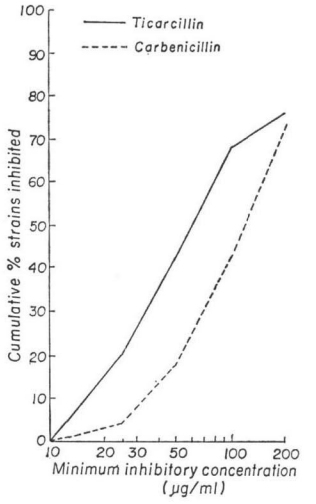
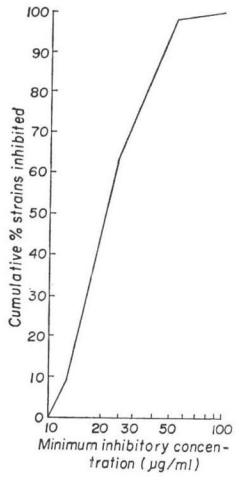


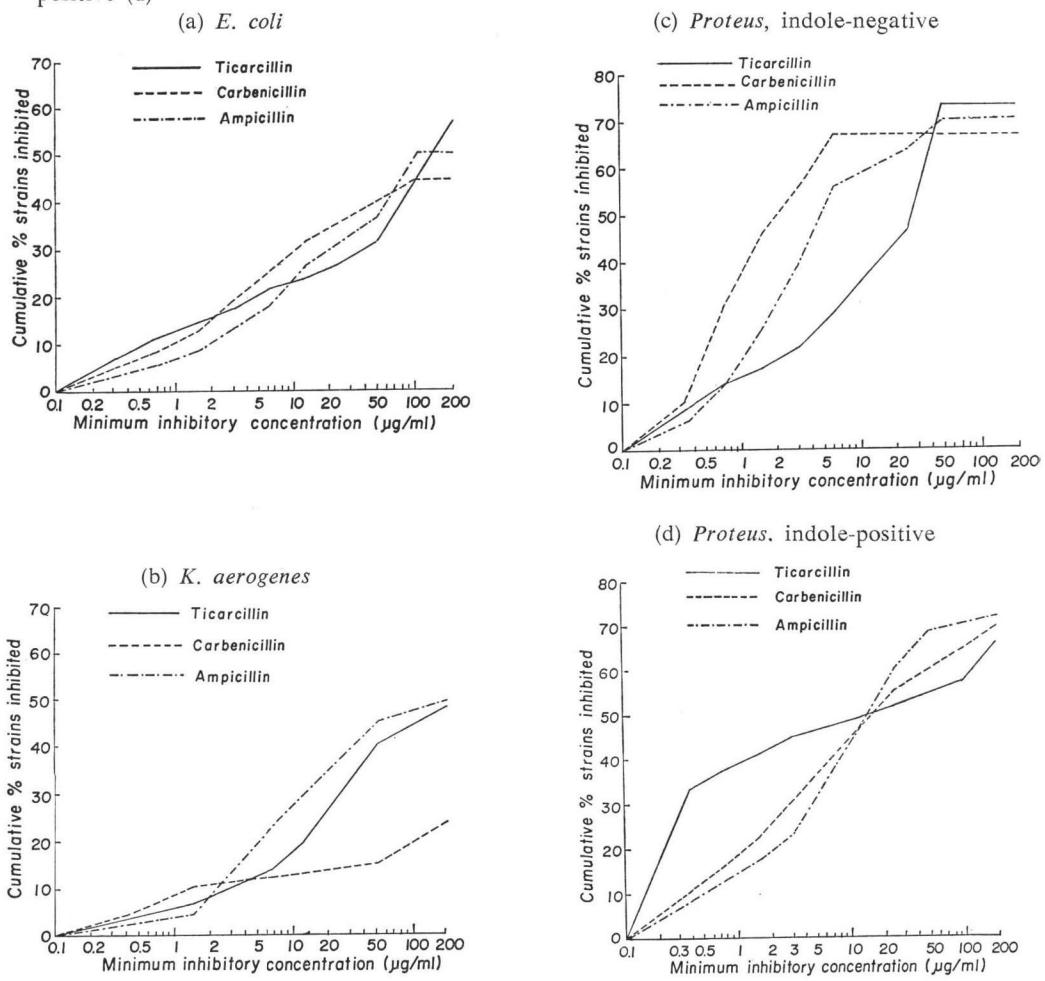
Fig. 4. Activity of ticarcillin against 41 clinical isolates of *Pseudomonas aeruginosa* of the following origin. 14 from urinary tract; 19 from respiratory tract; 5 from wounds and 3 from gastroenteritis. Inoculum: one drop of 1:1,000 dilution of overnight culture in 5 ml of medium.



of 50 μg/ml of the drug, while only 45 % were sensitive when the undiluted inoculum was used (Fig. 3).

The relative activities of ticarcillin, carbenicillin and ampicillin against clinical isolates of *E. coli*, *K. aerogenes* and *Proteus* are shown in Fig. 5 (a~d). All three penicillins showed similar activity against *E. coli* (Fig. 5a), 42~50 % of the strains being inhibited by a concen-

Fig. 5. Comparison of the activity of ticarcillin, ampicillin and carbenicillin against clinical isolates of *Escherichia coli* (a), *Klebsiella aerogenes* (b) and *Proteus*: indole-negative (c) and indole-positive (d)



tration of 100 μg/ml. Ampicillin was the most active against *K. aerogenes* isolates (Fig. 5b), 45 % of the strains being inhibited at a concentration of 50 μg/ml while carbenicillin was the least active with only 15 % of the strains inhibited at the same concentration. However, rare *Klebsiella* isolates were sensitive to all three penicillins. Regarding the *Proteus* isolates, ampicillin was most active against the indole-negative strains (Fig. 5c) followed by carbenicillin with ticarcillin the least active. At a concentration of 6.25 μg/ml, 66 % of these strains were inhibited with ampicillin, while at the same concentration 54 % and 26.5 % of the strains were inhibited by carbenicillin and ticarcillin respectively. With indole-positive *Proteus* (Fig. 5d), *e.g. P. rettgeri*, *P. morganii* and *P. vulgaris*, ticarcillin was most active of the three penicillins in relatively low concentration; 44 % of the strains being inhibited with a concentration of 3 μg/ml, while carbenicillin and ampicillin inhibited 30 % and 23 % of the strains respectively at the same concentration. It is worth noting that a high proportion of strains of *E. coli* and *K. aerogenes* were resistant to the three penicillins even at a concentration of 200 μg/ml and the same was true for about 30 % of the strains of *Proteus*.

Table 2. Synergism between ticarcillin and gentamicin against 3 strains of *Pseudomonas aeruginosa*[a]

Concentration of ticarcillin (μg/ml)	Minimal inhibitory concentration (μg/ml) gentamicin		
	1[b]	2	3
0	2	2	3
12.5	0.5	1	1
25	0.05	0.2	0.2

(*a*) Minimal inhibitory concentration of ticarcillin, 200 μg/ml (*b*) Numerals correspond to three different strains (clinical isolates)

Fig. 6. Activity of ticarcillin (MIC values and zones of inhibition around 100 μg discs) against 86 clinical isolates of *Pseudomonas aeruginosa*.

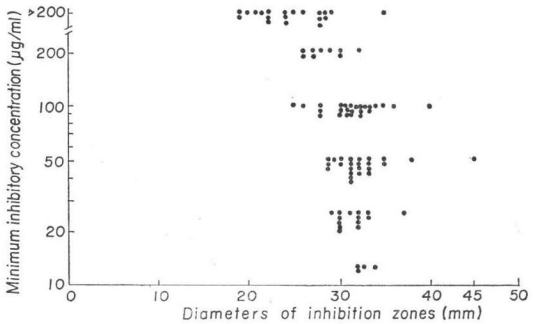
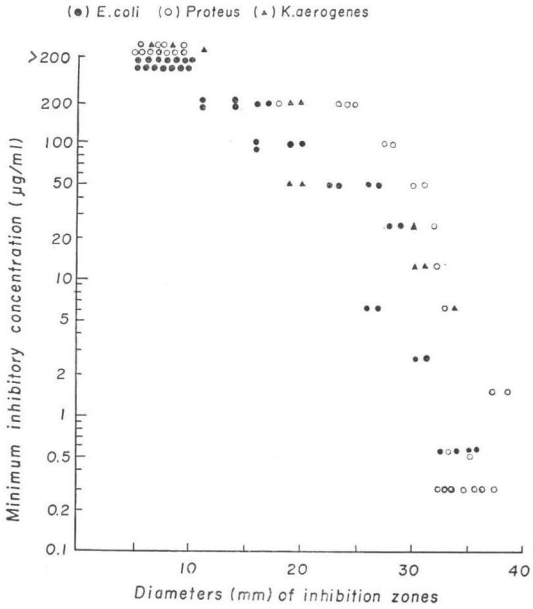


Fig. 7. Activity of ticarcillin (MIC values and zones of inhibition around 100 μg discs) against 38 strains of *Escherichia coli*, 35 strains of *Proteus*, and 11 strains of *Klebsiella aerogenes* strains.



Significant synergism between ticarcillin and gentamicin was seen with the three strains of *P. aeruginosa* tested (Table 2). The addition of 12.5 μg/ml of ticarcillin increased three to fourfold the activity of gentamicin, while the activity of the latter was raised fifteen to fourtyfold by the addition of 25 μg/ml of ticarcillin.

Using 100 μg ticarcillin discs the correlation between the MIC and size of inhibition zone for *P. aeruginosa* is shown in Fig. 6. The majority of sensitive strains, namely those requiring 100 μg/ml or less for inhibition, gave inhibition zones 25 mm or more in diameter. Nine of the isolates were inhibited by 200 μg of the drug per ml; the corresponding zone diameters measured 26~32 mm. Eleven out of the twenty strains which proved resistant at a concentration of 200 μg/ml gave inhibition zones which measured 25 mm or less, while the remainder gave a spectrum of inhibition diameters lying in the range of sensitive strains. The frequency distribution of the size of inhibition zones showed that over 80 % of the sensitive strains gave inhibition zones 29~33 mm in diameter while in 4 % of the strains the inhibition zone was 25~28 mm in diameter.

The correlation between MIC values and size of inhibition zones of ticarcillin with enterobacterial isolates is shown in Fig. 7. It is evident that all enterobacterial isolates characterized by MIC values of 100 μg of the drug per ml or less yielded inhibition zones of 16 mm or more in diameter. Twelve isolates (six isolates of *E. coli*, four isolates of *Proteus* and two isolates of *K. aerogenes*) required 200 μg/ml of ticarcillin for inhibition; however, the diameters of the zones of inhibition varied widely. Those enterobacterial isolates that were not inhibited by 200 μg/ml of ticarcillin gave zones of inhibition that meas-

ured 11 mm or less in diameter. The frequency distribution of the sizes of inhibition zones of the sensitive strains, namely those characterized by MIC value of 100 μg/ml or less, showed that 79 % of the *Proteus* isolates gave inhibition zones which ranged from 30 to 37 mm in diameter. With *K. aerogenes*, 70 % of the sensitive isolates had inhibition zones of 30~34 mm in diameter.

Sensitive *E. coli* isolates had a wide distribution in the size of inhibition zones and ranged between 18 to 35 mm in diameter.

Discussion

The activity of ticarcillin was found to be comparable to that of carbenicillin and ampicillin against *E. coli* isolates. This is in agreement with previous reports[4,21,22]. However, we found a higher percentage (about 50 %) of strains resistant to these antibiotics. The isolates resistant to ticarcillin were mostly resistant to the other two penicillins. The higher incidence of resistance observed in this study may be related to the fact that our strains were all isolated from stools, whereas in most of the previously published studies the isolates were mainly from urine. With regard to *K. aerogenes* isolates, our results are in agreement with published reports showing resistance of these bacteria to penicillins. Their difference of susceptibility to the drugs is probably due to the differential activity of their β-lactamases[18]. However, SELIGMAN[17] supports that, besides the production of β-lactamase, a mechanism of intrinsic resistance for *Klebsiella* may operate.

Ticarcillin and carbenicillin were less active than ampicillin against the indole-negative *Proteus* isolates. The finding of NEU and WINSHELL[15] that the affinity of β-lactamase of *Proteus* strains to ticarcillin and carbenicillin is lower to that of ampicillin does not explain our results. On the other hand, ticarcillin was relatively stable to β-lactamase activities of the indole-positive *Proteus* isolates and inhibited their growth at relatively low concentrations.

In general, the number of resistant enterobacterial isolates demonstrated by us is greater than that reported by others[1,4,21,22]. This difference together with the existing cross resistance may be attributed to the widespread use in recent years of carbenicillin and ampicillin.

Ticarcillin exhibited greater activity than carbenicillin against *P. aeruginosa* isolates. This increased activity was more pronounced in the case of isolates from the respiratory tract. This activity would appear to be related to the replacement of N-phenyl group by a thienyl moiety. The observed higher activity of ticarcillin and carbenicillin against the isolates obtained from the respiratory tract compared with the other *P. aeruginosa* isolates is difficult to explain. The special environmental conditions may play some role.

The synergy of the combination of ticarcillin with gentamicin is similar to that of carbenicillin[19]. This synergy may be of help in preventing the development of resistance to ticarcillin of *P. aeruginosa*. However, this does not appear to have been the case with the combined use of carbenicillin and gentamicin[19].

The marked inoculum effect with ticarcillin and *P. aeruginosa* isolates may appear to suggest that these strains produce some inactivating enzymes. However, NEU and WINSHELL[15] support the view that the destruction of ticarcillin by β-lactamases is not the defence mechanism in most *P. aeruginosa* strains.

The activity of ticarcillin against *P. aeruginosa* is considerably lower than that of the polymyxins and aminoglycosides (gentamicin, tobramycin). However, the use of these antibiotics is limited by their toxicity while ticarcillin appears to be non-toxic in animal studies. Carbenicillin has been proved effective in the treatment of severe infections caused by *P. aeruginosa*[16], but as a result of the level of activity of carbenicillin against *P. aeruginosa*, large doses are

required for the treatment of such infections. Such a high dosage is undesirable in renal and cardiac failure[12]. On the other hand high dosage results more readily in the maintenance of a bactericidal level of the drug. In this connection, the greater activity of ticarcillin compared with carbenicillin on a weight basis, is of considerable interest.

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