Carbenicillin and gentamicin in the treatment of *Pseudomonas aeruginosa* infection

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Summary: The administration separately and sequentially of carbenicillin and gentamicin eradicated *Ps. aeruginosa* infections, during the period over which they were given, in all of 25 critically ill patients. Electron microscopy revealed differences in the action of these two antibiotics against *Ps. aeruginosa in vitro*. Culture studies showed synergism between them and destruction by gentamicin of the carbenicillin-induced long, filamentous form of the organism.

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In vitro and *in vivo* effects of carbenicillin on *Pseudomonas aeruginosa* have been reported by several British workers[1-6] and subsequently confirmed elsewhere.[7-9] Gentamicin also has been shown to exert *in vitro* and *in vivo* activity against *Ps. aeruginosa*.[10-15] Some encouraging results have been reported with their combined use,[4, 16-22] but other accounts deprecate this practice on the grounds that gentamicin is inactivated by carbenicillin in both laboratory and clinical tests.[23, 24] Furthermore, it has been stated[23] that "there are no convincing reports of the combination being superior to either drug alone for the treatment of patients with infections by a single organism". Such a conflict of opinion provokes an urgent need for accurate information about the use of these antibiotics singly or in combination, since it could pose a grave decision concerning medication of critically ill patients.

The present paper reports the results of laboratory and clinical studies that revealed synergism of action, and not antagonism, between these two antibiotics, in the following experiments.

1. Carbenicillin and gentamicin were tested *in vitro* against strains of *Ps. aeruginosa* derived from 32 clinical conditions.
2. The morphological effects of both agents, singly and in combination, were assessed by light and electron microscopy.
3. Observations on the clinical course were made in 25 critically ill patients with *Ps. aeruginosa* infections, treated with these agents in the intensive care units of three hospitals in Halifax.

Materials and methods

Laboratory studies

The strains of *Ps. aeruginosa*, the test organism, were isolated from patients in one Halifax hospital. Carbenicillin was supplied by Ayerst Laboratories and gentamicin by Schering Corporation, in commercial grades.

Tryptose phosphate broth was used as a medium in tube dilution tests to determine synergism or antagonism. The effect of gentamicin against *Ps. aeruginosa* was also studied by placing gentamicin discs on two agar plates; one contained carbenicillin (25 μg./ml.) and the other, control plate, was carbenicillin-free. Morphological changes induced in *Ps. aeruginosa* by treatment were studied in individual bacteria and in entire colonies. A strip of paper impregnated with gentamicin solution was

inserted under the agar surface, so that the antibiotic would diffuse upwards through the medium. This technique permitted observation of the effect of varying sublethal concentrations of each antibiotic tested in combination.

For securing electron microscope pictures, grids were placed on the surface of colonies on agar medium and then were picked off, fixed in formalin for 10 minutes, and examined at magnifications from x4750 to 20,400 with a Philips EM 300 electron microscope.

Laboratory tests on the action of carbenicillin and gentamicin in combination revealed three classes of effect: (1) The minimal inhibitory concentration (M.I.C) of each antibiotic in combination was one-quarter or less that of each antibiotic alone; this action was interpreted as indicative of definite synergism. (2) The M.I.C. of one antibiotic in combination was half that when alone, and the M.I.C. of the other antibiotic was a quarter or less than when used alone; this was designated moderate synergism. (3) Both antibiotics in combination showed no change in individual M.I.C. levels, indicating absence of antagonism.

Clinical studies. In three hospitals we observed a total of 25 critically ill patients being treated with carbenicillin or gentamicin or both. Many of them were in intensive care units, and all had underlying diseases with a poor long-term prognosis. The response to treatment was assessed by clinical and bacteriological criteria, including such features as improvement in general condition, amelioration of symptoms, decline of fever, and the results of repeated cultures of specimens for growth of *Ps. aeruginosa.*

Dosage of antibiotics. For a 65-kg. patient, the regimen of choice was an initial injection of carbenicillin 2 g., followed by 1 g. two-hourly for 12 hours, and then 1 g. three-hourly for three to seven days depending on results of bacteriological cultures. For critically ill patients the dosage was increased to 1 g. hourly for 24 hours or maintained until cultures became negative. The carbenicillin was administered as a single injection into intravenous tubing, or added to 100 ml. of 5% glucose and water in a microdrip cylinder and infused over half an hour. Gentamicin was administered separately as a single injection of 40 mg. given intravenously or intramuscularly eight-hourly for three to 10 (average five) days. Gentamicin was usually added 24 to 48 hours after the start of carbenicillin therapy. Thus the two antibiotics were not mixed; they were administered sequentially and separately at fixed intervals for two to seven days. In some cases gentamicin was continued for three to five days after cessation of carbenicillin therapy.

Results

Clinical results.

Twenty-five patients suffering from a range of serious clinical conditions complicated by *Ps. aeruginosa* infection have been treated with carbenicillin plus gentamicin and 20 recovered (Table I). In 17 patients, cultures for *Ps. aeruginosa* became negative after treatment. Three patients, cultures from whom showed very light growth of *Ps. aeruginosa,* had a good clinical response.

Table II

Combination antibiotic dosages that show definite synergism

	M.I.C.		Effective combination
Ps. aeruginosa strains	carbenicillin (μg./ml.)	gentamicin (μg./ml.)	Carbenicillin + gentamicin
1	250	5	3.9 + 1.25
2	125	20	3.9 + 1.25
3	125	1.25	7.8 + 0.31
4	125	0.62	31.25 + 0.078
5	62.5	2.5	7.8 + 0.62
6	31.25	2.5	3.9 + 0.31
7	31.25	1.25	7.8 + 0.31

Table III

Combinations of carbenicillin and gentamicin* which show moderate synergism

	M.I.C.		Effective combination
Ps. aeruginosa strains	carbenicillin (μg./ml.)	gentamicin (μg./ml.)	Carbenicillin + gentamicin
8	250	5	62.5 + 2.5
9	250	2.5	62.5 + 1.25
10	250	2.5	15.5 + 1.25
11	250	2.5	15.5 + 1.25
12	250	2.5	15.5 + 1.25
13	125	0.31	31.25 + 0.15
14	125	1.25	7.8 + 0.62
15	125	2.5	15.5 + 1.25
16	125	2.5	31.25 + 1.25
17	125	1.25	15.5 + 0.62
18	125	2.5	31.25 + 1.25
19	62.5	1.25	15.5 + 0.62

*In these combinations the M.I.C. of gentamicin was half that when used alone.

Table I

Summary of the patients treated with carbenicillin and gentamicin combined

Infection	No. of patients	Micro-organism isolated	No. of (−) cultures after treatment	No. of (+) cultures after treatment	No. of cases with good clinical response	No. of cases with poor clinical response
Lower respiratory tract infections (including empyema)	11	*Ps. aeruginosa*	7	4	9	2
Skin, bone and soft tissue infections (including osteomyelitis, pelvic abscess)	10	*Ps. aeruginosa*	8	2	9	1
Septicemia	2	*Ps. aeruginosa*	—	2	0	2
Urinary tract infections	2	*Ps. aeruginosa*	2	—	2	—

Five patients showed no response and died. Four of these were cases of leukemia receiving immunosuppressive drugs and the fifth was a case of gas gangrene.

Laboratory.

The *in vitro* susceptibility of *Ps. aeruginosa* to carbenicillin, to gentamicin and to both agents combined is shown in Tables II, III and IV.

The combination of carbenicillin and gentamicin had a definite synergistic effect on seven of 32 strains (21.7%) of *Ps. aeruginosa* and a moderate synergistic effect on 18 of 32 strains (56%) (Tables III and IV).

The inhibition zone for *Ps. aeruginosa* of a gentamicin disc was 24 mm. on a carbenicillin-free agar plate, whereas it was 32 mm. for the same strain on a carbenicillin-containing medium.

Morphological studies.

In these experiments the effect of treating *Ps. aeruginosa* with carbenicillin has been compared with morphological changes after similar treatment of the organism with gentamicin. Further, these effects have also been compared with those following treatment of *Ps. aeruginosa* with a combination of both antibiotics. The normal appearance of *Ps. aeruginosa* as seen under the electron microscope is shown in Fig. 1. Carbenicillin induced the formation of long, filamentous, convoluted and spheroblastic forms (Figs. 2 and 3) exhibiting disintegrated cell walls containing granules and vacuoles, with apparent absence of dividing septa. Carbenicillin appears thus to affect the cell wall. Subcultures from colonies exhibiting such changes frequently produced growth with reversion of *Ps. aeruginosa* to its original bacillary form.[25]

The action of gentamicin on *Ps. aeruginosa* appeared to differ from that of carbenicillin in several respects. Thus, no convoluted filamentous forms, spheroblasts or conspicuous membranes were evident (Figs. 4 and 5). Single bacillary forms showing varying degrees of cytoplasmic disintegration with vacuole-containing cell walls were noticed instead. The action of gentamicin would therefore seem to be against the cytoplasm rather than on the cell wall. Attempts to obtain subcultures from colonies showing such changes frequently yielded no growth, suggesting that the organisms were not viable.

Following cultivation of *Ps. aeruginosa* in carbenicillin-containing medium for three hours, the susceptibility of the elongated forms to the action of sublethal doses of gentamicin was next investigated. It was evident that the carbenicillin-induced form of *Ps. aeruginosa* was highly vulnerable to the action of gentamicin. Indeed, the disintegration of elongated forms of *Ps. aeruginosa* caused by treatment with gentamicin was so thorough that it was difficult to obtain satisfactory photomicrographs to demonstrate the phenomenon (Fig. 6). Thus, the action of carbenicillin followed by gentamicin appears to be more pronounced than the effect of gentamicin alone.

Table IV

Moderate synergism between carbenicillin* and gentamicin

	M.I.C.		Effective combination
Ps. aeruginosa strains	carbenicillin (μg./ml.)	gentamicin (μg./ml.)	Carbenicillin + gentamicin
20	250	1.25	125 + 0.039
21	125	1.25	62.5 + 0.039
22	125	2.5	62.5 + 0.039
23	125	2.5	62.5 + 0.039
24	125	10	62.5 + 0.078
25	62.5	0.62	31.25 + 0.078

*In these combinations, the M.I.C. of carbenicillin was half that when used alone.

Discussion

The incidence of *Ps. aeruginosa* infections has shown an increase in recent years because of world-wide use of antibiotics, and it is well recognized that *Ps. aeruginosa* is among the most resistant micro-organisms to commonly used antibiotics. Three potent anti-pseudomonas antibiotics exist, namely, carbenicillin, gentamicin and the polymyxins (B and E). Their toxic side effects, especially those of the last named, limit the use of gentamicin and polymyxins in high doses and in certain circumstances. Carbenicillin therefore seems to be the only antibiotic which can be safely used in high doses and in almost every patient. Unfortunately *Ps. aeruginosa* rapidly develops resistance to carbenicillin as well as to gentamicin if dosage is unduly prolonged. There is no clear evidence of the development of cross-resistance following simultaneous administration, and it is quite possible that their use in combinations may reduce the period of therapy and delay the development of resistance. Moreover, *in vitro* tests have shown that synergism exists between the action of these antibiotics on 25 out of 32 strains of *Ps. aeruginosa*.

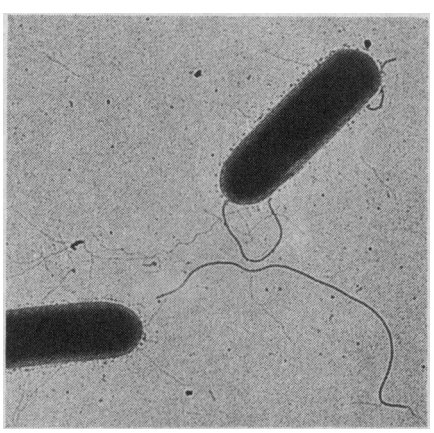

FIG. 1—Electron photomicrograph of normal *Ps. aeruginosa* bacilli with flagella and fimbriae unstained. (x 20,400.)

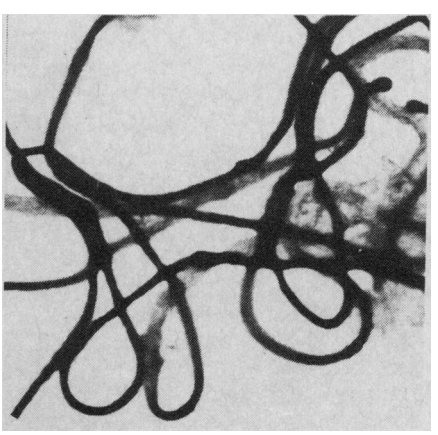

FIG. 2—Electron photomicrograph of *Ps. aeruginosa* after 4 hours' growth on media containing sublethal concentration (50 μg./ml.) of carbenicillin. Note the long, convoluted, filamentous forms with bulbous swellings and dilations. (Unstained, x 4750.)

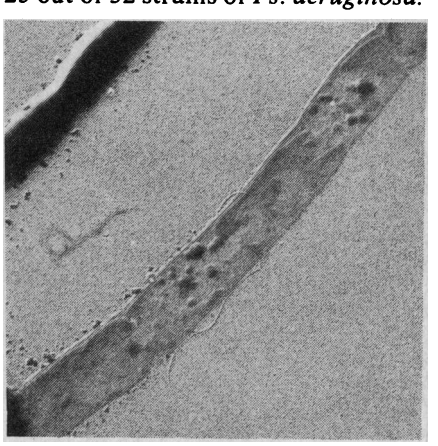

FIG. 3—*Ps. aeruginosa* grown on carbenicillin-containing medium showing swelling, absence of cell wall and dividing septa. (x 19,800.)

In 18 of these the M.I.C. value for one antibiotic was halved when used in combination with the other. From the results it appeared more than an additive effect, because the M.I.C. of the second antibiotic decreased in the range of 4- to 250-fold. For instance, as seen in Table IV, the M.I.C. of gentamicin alone in strain No. 24 was found to be 10 μg./ml. for one strain of *Ps. aeruginosa* and for carbenicillin used alone it was 250 μg./ml. When carbenicillin was present in a concentration of 125 μg./ml., the M.I.C. of gentamicin decreased to 0.078 μg./ml. Such a decrease is not explicable as a simple additive effect and was duly designated as indicating moderate synergism.

The suggestion that gentamicin inactivates carbenicillin when "the daily doses of both drugs were mixed in dextrose-saline and given over 24 hours" has been commented on. In our patients the antibiotics were not mixed but were administered separately over a period of time varying from 20 to 60 minutes. Our clinical results suggest that the effect of administering carbenicillin and gentamicin as described is beneficial rather than detrimental.

The electron microscope photographs are the work of Mr. James Boutilier, A.R.T., to whom we owe our thanks.

Résumé

La carbénicilline et la gentamicine dans le traitement des infections à Pseudomonas aeruginosa

L'administration en séquence (mais séparée) de carbénicilline et de gentamicine a permis d'éliminer des infections à *Ps. aeruginosa* pendant la période où ces antibiotiques ont été administrés à 25 malades dont l'état était critique. Des études au microscope électronique ont révélé des différences notables dans le mode d'action *in vitro* des deux antibiotiques contre *Ps. aeruginosa*. L'étude des cultures a mis en évidence l'existence d'une synergie entre ces antibiotiques et la destruction par la gentamicine de la forme longue et filamenteuse du microbe, créée par la carbénicilline.

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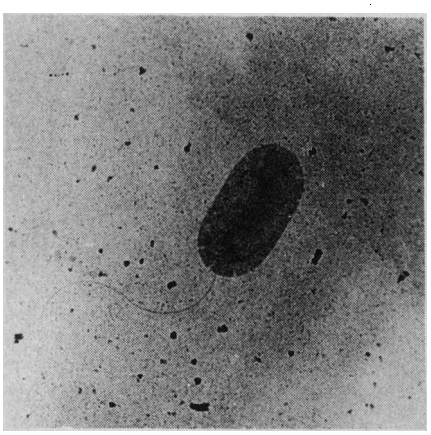

FIG. 4—*Ps. aeruginosa* after cultivation on media with inserted gentamicin filter paper strip. Note swelling of the organism with wedge-shaped indentations of the cell wall. (x 19,500.)

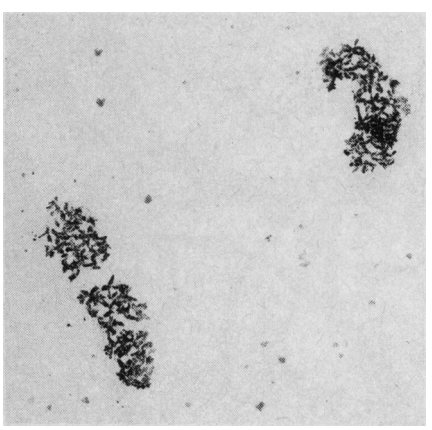

FIG. 5—*Ps. aeruginosa* cultivated in media with paper strip containing gentamicin. (x 19,500.) Note complete disintegration of bacteria.

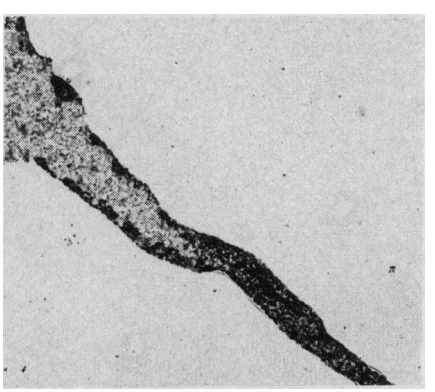

FIG. 6—The filamentous form of *Ps. aeruginosa* induced by growth on carbenicillin-containing media for three hours followed by exposure to gentamicin for three hours. Observe marked destruction of the bacterial filament with partial absence of cell wall. (x 7350)