ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1974, p. 853-855

Vol. 6, No. 6
Printed in U.S.A.

NOTES

Morphological Alterations of *Pseudomonas aeruginosa* by Ticarcillin: a Scanning Electron Microscope Study

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Received for publication 6 September 1974

Pseudomonas aeruginosa was exposed to 0.1, 1.0, 10, 100, and 1,000 times the minimal inhibitory concentration of ticarcillin in vitro and subsequently examined with the scanning electron microscope. The morphological alterations observed were filamentation, mid-cell defects, and spheroplast formation, and these alterations were dependent upon the drug concentration.

Ticarcillin, a new semisynthetic penicillin, has an antibacterial spectrum similar to that of carbenicillin and is reported to be significantly more active against *Pseudomonas aeruginosa* (1, 2, 5, 8). In the present investigation, *P. aeruginosa* was exposed to various concentrations of ticarcillin and subsequently examined with the scanning electron microscope to observe the morphological alterations induced by this drug.

P. aeruginosa (minimal inhibitory concentration [MIC] of ticarcillin, 25 μg/ml, as determined by a microdilution procedure [3, 4]) was cultured in Trypticase soy broth (BBL) for 4 h, and then 1-ml portions were transferred to 9 ml of Trypticase soy broth containing various concentrations of ticarcillin. Final ticarcillin concentrations equivalent to 0.1, 1.0, 10, 100, and 1,000 times the MIC were studied. The antibiotic-containing cultures were incubated at 37 C for 4 h. Control cultures of *P. aeruginosa* exposed to drug-free diluent were treated in an identical manner. The specimens were then prepared for scanning electron microscopy as follows (7). The cells were centrifuged at 800 × *g* for 10 min, and the pellets were immediately resuspended in 2% glutaraldehyde with 0.05 M phosphate buffer and 4% sucrose, pH 7.3. Fixation was obtained overnight at 4 C. The specimens were then centrifuged at 800 × *g* for 10 min, washed four times in distilled water, placed on aluminum foil disks, air-dried, gold-coated, and examined with a Cambridge Mark II Stereoscan electron microscope.

Effects of ticarcillin on the surface morphology of *P. aeruginosa* are shown in Fig. 1. Untreated control organisms appeared as normal rods approximately 2.5 μm long and 0.6 μm wide (Fig. 1A). Exposure of the organism to the equivalent of 0.1 × MIC ticarcillin resulted in minimal elongation, and the organisms generally appeared as the untreated control organisms (Fig. 1B). However, exposure to 1.0 × MIC of ticarcillin caused marked morphological alterations of the organisms (Fig. 1C). Some organisms appeared to have incomplete septum formation, whereas others appeared as long filamentous forms with no evidence of transverse septum formation or cell division (Fig. 1C). Exposure to 10 × MIC of ticarcillin also resulted in marked filamentation of the organisms with no evidence of transverse septum formation or cell division (Fig. 1D). Exposure to 100 × MIC of ticarcillin resulted in filamentation as well as induced mid-cell defects and spheroplast formation (Fig. 1E). At exposure to 1,000 × MIC of ticarcillin, the organisms appeared primarily as spheroplasts (Fig. 1F). Occasionally, a short rod form was observed at the 1,000 × MIC level, but no filamentous forms or mid-cell defects were observed.

These different morphological alterations at different concentrations of ticarcillin were also observed with two other strains of *P. aeruginosa*. Also, exposure of these strains to 0.1, 1.0, 10, 100, and 1,000 times the MIC of carbenicillin (MIC, 50 μg/ml) resulted in morphological alterations similar to those previously reported (6) and to those observed with equivalent MICs of ticarcillin. Exposure of *Escherichia coli* (MIC, 12.5 μg/ml) and *Proteus mirabilis* (MIC, 1.56 μg/ml) to ticarcillin using

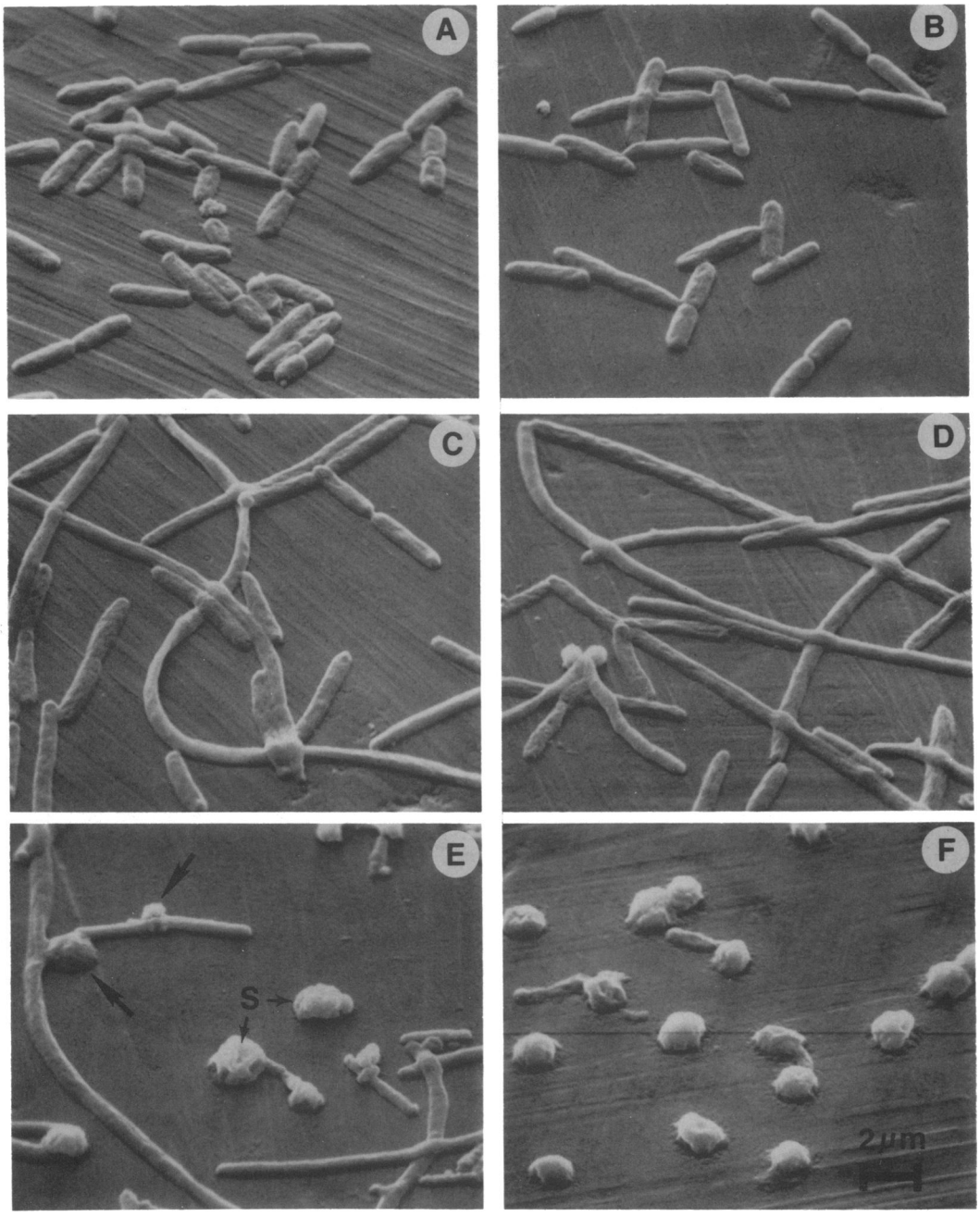

FIG. 1. *Scanning electron micrograph of P. aeruginosa exposed to different concentrations of ticarcillin. (A) Untreated control organisms. (B) Exposure to 0.1× MIC showing minor elongation and evidence of cell division. (C) Exposure to 1.0 × MIC showing marked filamentation. (D) Exposure to 10 × MIC also showing marked filamentation. (E) Exposure to 100 × MIC showing filamentation, mid-cell defects (arrows), and spheroplast formation (S). (F) Exposure to 1,000 × MIC showing predominantly spheroplast formation.*

these same experimental conditions also resulted in similar morphological alterations.

The present study demonstrated that the mode of action of ticarcillin is similar to that of carbenicillin, since low concentrations of ticarcillin caused filament formation due to inhibi-

tion of cell division and high concentrations inhibited cell growth and resulted in spheroplast formation.

We thank Robert L. Vann, Beecham-Massengill Pharmaceuticals, for kindly supplying us with the ticarcillin.

LITERATURE CITED

1. Alder, J. L., J. P. Burke, C. Wilcox, and M. Finland. 1971. Susceptibility of proteus species and *Pseudomonas aeruginosa* to penicillins and cephalosporins, p. 63–67. Antimicrob. Ag. Chemother. 1970.
2. Bodey, G. P., and B. Deerhake. 1971. In vitro studies of α-carboxyl-3-thienylmethyl penicillin, a new semisynthetic penicillin. Appl. Microbiol. **21**:61–65.
3. Conrath, T. B. (ed.) 1972. Handbook of microtiter procedures, p. 37–39. Dynatech Corp., Cambridge, Mass.
4. Gardner, W. G., R. B. Prior, and R. L. Perkins. 1973. Fluid and pharmacological dynamics in a subcutaneous chamber implanted in rats. Antimicrob. Ag. Chemother. **4**:196–197.
5. Neu, H. C., and E. B. Winshell. 1971. In vitro studies of a semisynthetic penicillin, 6-[D(-)-α-carboxy-3-thienylacetamido] penicillanic acid (BRL 2288), active against *Pseudomonas*, p. 385–389. Antimicrob. Ag. Chemother. 1970.
6. Perkins, R. L., and A. S. Klainer. 1971. Carbenicillin-induced alterations in the surface morphology of bacteria: a scanning-beam electron microscope study, p. 100–104. Antimicrob. Ag. Chemother. 1970.
7. Prior, R. B., and R. L. Perkins. 1974. Artifacts induced by preparation for scanning electron microscopy in *Proteus mirabilis* exposed to carbenicillin. Can. J. Microbiol. **20**:794–795.
8. Sutherland, R., J. Burnett, and G. N. Rolinson. 1971. α-Carboxy-3-thienylmethylpenicillin (BRL 2288), a new semisynthetic penicillin: in vitro evaluation, p. 390–395. Antimicrob. Ag. Chemother. 1970.