

Chloramphenicol Resistance in *Pseudomonas cepacia* Because of Decreased Permeability

JANE L. BURNS,^{1,2*} LINDA A. HEDIN,¹ AND DENA M. LIEN¹

Division of Infectious Disease, Children's Hospital and Medical Center, Seattle, Washington 98105,^{1*} and Department of Pediatrics, University of Washington, Seattle, Washington 98195²

Received 1 July 1988/Accepted 31 October 1988

The mechanism of chloramphenicol resistance was examined in a high-level-resistant isolate of *Pseudomonas cepacia* from a patient with cystic fibrosis. We investigated potential resistance mechanisms, including production of chloramphenicol acetyltransferase, ribosomal resistance, and decreased permeability. This strain (MIC, 200 µg/ml) had no detectable chloramphenicol acetyltransferase activity. In *in vitro* translation experiments in which we compared the resistant isolate with a susceptible strain of *P. cepacia*, inhibition of amino acid incorporation was equivalent even in organisms that were preincubated with sub-MICs of chloramphenicol. A 21.9-kilobase (kb) fragment of DNA was cloned which coded for chloramphenicol resistance; this fragment was expressed in *P. cepacia* but not in *Escherichia coli*. Quantitation of chloramphenicol uptake in the isogenic pair of susceptible and resistant organisms revealed a nearly 10-fold decrease of drug entry into the resistant strain. Comparison of isolated outer membrane proteins and lipopolysaccharide patterns identified no significant differences between the isogenic pair of organisms. We concluded that the mechanism of chloramphenicol resistance in this strain is decreased permeability.

Pseudomonas cepacia is an important nosocomial pathogen and an organism that can cause severe pulmonary infections in children and young adults with cystic fibrosis (CF). Originally recognized as a plant pathogen in 1950 (5), the role of *P. cepacia* in hospital-acquired infections of the urine, lungs, and bloodstream was first reported in the early 1970s (12, 19, 22, 28, 35). Colonization with *P. cepacia* occurs late in the course of pulmonary disease in patients with CF, and in several CF centers, outbreaks of virulent strains have resulted in severe and overwhelming pneumonia in a subpopulation of patients (21, 37).

The major problem in the treatment of infections caused by *P. cepacia* is multiple antibiotic resistance. Isolates from patients with CF, in particular, are often highly resistant to the β-lactam agents and aminoglycosides that are used to treat *Pseudomonas aeruginosa* (4, 9, 15). Antibiotics with clinical efficacy against *P. cepacia* include chloramphenicol, trimethoprim, and the quinolones (12, 21, 28). However, more than half of clinical isolates may be resistant to these agents as well. Investigations of chloramphenicol resistance in *P. cepacia* strains from patients with CF consistently report that 45 to 50% of isolates are resistant (MIC, ≥10 µg/ml).

In this report we describe the characterization of chloramphenicol resistance in *P. cepacia*. We used an isolate of *P. cepacia* from a patient with CF; this isolate was resistant to chloramphenicol, trimethoprim, and ciprofloxacin, as well as all β-lactams and aminoglycosides tested, and was selected to characterize the resistance mechanism. We cloned a 21.9-kilobase (kb) DNA fragment that encoded resistance to chloramphenicol in *P. cepacia* but that was not expressed in *Escherichia coli*. Potential mechanisms of resistance, including enzymatic inactivation by chloramphenicol acetyltransferase (CAT), decreased ribosomal susceptibility, and

impermeability, were examined in both the clinical isolate and a strain expressing the cloned gene.

MATERIALS AND METHODS

Strains and plasmids. The *P. cepacia* and *E. coli* strains used in this investigation are listed in Table 1, as are the plasmid and cosmid vectors used in cloning and the plasmids constructed during these experiments.

Media. The liquid medium used in this study was L broth (27). Broth cultures were incubated at 37°C and shaken at 150 cycles per min. The solid medium used was L agar for *E. coli* strains and OFPBL (45) for *P. cepacia* strains, except for MIC determinations, for which we used MH agar (Difco Laboratories, Detroit, Mich.). Plate cultures were incubated at 37°C. Strains were stored at -70°C in skim milk and inoculated onto fresh agar medium 16 to 40 h prior to use.

DNA preparation and analysis. Total cellular DNA was isolated from *P. cepacia* by the technique of Scordilis et al. (39) with spooling out the DNA on a glass rod. Plasmid DNA was purified from *E. coli* and *P. cepacia* by the alkaline lysis technique of Birnboim and Doly (2) followed by ethidium bromide-cesium chloride ultracentrifugation. Small amounts of less pure plasmid were prepared for analysis by a small-scale alkaline lysis technique (27). DNA was analyzed by 0.7% agarose horizontal slab gel electrophoresis in Tris borate buffer (27).

Cosmid cloning. *P. cepacia* K61-3 whole-cell DNA was partially digested with *Sall*, to produce a majority of fragments in the range of 15 to 30 kb. This DNA was electrophoresed on a preparative 0.5% low-melting-point agarose gel (SeaPlaque), the band between 15 and 30 kb was cut out, and the DNA was extracted (27). Cosmid pVK102 DNA was digested to completion with *Sall* and combined with insert DNA (*Sall*-cut whole-cell DNA from K61-3) in a ratio of 1:2. The DNA was ligated with T4 DNA ligase (27) and packaged with a lambda phage DNA packaging kit (Gigapak) from Vector Cloning Systems (San Diego, Calif.). *E. coli* HB101

* Corresponding author.

TABLE 1. Bacterial strains and plasmids used in this study

Strain or plasmid	Genotype or phenotype ^a	Source or reference
<i>P. cepacia</i>		
K61-3	CF isolate, Cm ^r Tmp ^r Cip ^r	D. Woods, Calgary, Alberta, Canada
249-2	Lys ⁻ Pen ^s	1
PC104	CF isolate, Cm ^s	R. Yogeve, Chicago, Ill.
<i>E. coli</i>		
HB101	<i>proA2 leuB6 thi-1 lacY recA13 rpsL20 hsdR hsdM</i>	3
DH5α	<i>endA1 hsdR17 supE44 thi-1 F⁻ recA1 gyrA96 relA1 λ⁻ φ80dlacZ ΔM15</i>	Bethesda Research Laboratories
C2110	<i>his rha polA1</i>	13
Plasmid		
pVK102	23 kb, BHR, mob ⁺ Tet ^r Km ^r	23
pLIN1	44.5 kb, BHR, mob ⁺ Km ^r Cm ^r	This study
pUC18	2.7 kb, Amp ^r	Bethesda Research Laboratories
pUC18-CM	2.5 kb, Cm ^r	M. Yanofsky, Seattle, Wash.
pTJS75	7.0 kb, BHR, mob ⁺ Tet ^r	38
pRK2013	48.0 kb, tra ⁺ Km ^r	13
pJLB1	3.7 kb, Amp ^r Cm ^r	This study
pJLB2	10.7 kb, BHR, mob ⁺ Amp ^r Cm ^r Tet ^r	This study

^a Abbreviations: Amp^r, ampicillin resistant; BHR, broad host range; Cip^r, ciprofloxacin resistant; Cm^r, chloramphenicol resistant; Cm^s, chloramphenicol susceptible; Km^r, kanamycin resistant; Lys⁻, lysine auxotroph; mob⁺, mobilizable; Pen^s, penicillin susceptible; Tet^r, tetracycline resistant; Tmp^r, trimethoprim resistant; tra⁺, transfer proficient.

was grown to the mid-log phase in L broth with 0.01 M MgSO₄ and 0.4% maltose and was used for transfection (20). Transfections were plated onto L agar containing 50 μg of kanamycin per ml. The resultant colonies were screened for inserts by placing them with a toothpick onto media containing 50 μg of tetracycline or 100 μg of chloramphenicol per ml.

Triparental mating experiments. Plasmid pRK2013 was used to mobilize pVK102 clones into *P. cepacia* 249-2. Matings were done overnight on filter paper (16) and were performed with 1 ml of mid-log-phase cultures of each organism. Following overnight incubation at 37°C on the filters, organisms were suspended in L broth and plated onto OFPBL agar containing 50 μg of kanamycin per ml, to determine whether the conjugation was successful, or 50 μg of kanamycin and 100 μg of chloramphenicol per ml, to screen for chloramphenicol-resistant transconjugants.

Construction of a broad-host-range plasmid encoding CAT. The 1.0-kb *Sau3A* fragment from pUC18-CM which encodes CAT production was inserted into the *Bam*HI site of pUC18, and this construct was used to transform *E. coli* HB101 (27). Plasmid DNA was purified from an ampicillin- and chloramphenicol-resistant clone. This plasmid, pJLB1, was digested with *Hind*III, as was the broad-host-range plasmid pTJS75; the two plasmids were ligated and used to transform *E. coli* C2110. An ampicillin-, chloramphenicol-, and tetracycline-resistant colony was selected; and the 10.7-kb plasmid that it

contained, pJLB2, was transferred into *P. cepacia* by triparental mating.

MICs. Determination of the MIC of chloramphenicol was performed by agar dilution with a Steers replicator (42) with an inoculum of 10⁵ CFU.

Assay for CAT. Cell extracts were prepared by sonication as described previously (6), and CAT activity was assayed by the spectrophotometric technique of Shaw and Brodsky (41). One unit of CAT activity was defined as 1 μmol of chloramphenicol-dependent 5,5'-dithiobis-2-nitrobenzoic acid reacted per min (with a millimolar extinction coefficient of 13.6 at 412 nm). The specific activity was calculated as milliunits per milligram of protein. Protein determination was done by the method of Lowry et al. (26) by using crystalline bovine serum albumin as a standard.

In vitro translation. Whole bacterial cells were made permeable with ether (6) and were used to examine the effect of chloramphenicol on the translation of endogenous mRNA. In some experiments, cells were preincubated with sub-MICs of chloramphenicol prior to extraction with ether. Extracted cells were incubated in buffer with an amino acid mixture containing [¹⁴C]valine (specific activity, 50 mCi/mmol). Chloramphenicol was added to each reaction in concentrations ranging from 1 to 100 μg/ml; control reactions were run without chloramphenicol.

Chloramphenicol uptake. The cellular permeability of chloramphenicol was determined as described previously (7) in whole bacterial cells which contained a plasmid encoding CAT production. The decrease in drug concentration in culture supernatants was quantitated by high-pressure liquid chromatography over a 20-h incubation period.

Outer membrane proteins. Outer membrane proteins were isolated from *P. cepacia* by a modification of the Triton X-100 solubilization technique described by Stull et al. (43). Cells were disrupted with a French pressure cell (Aminco) at 20,000 lb/in² in the presence of 20% sucrose, 50 μg of DNase per ml, 50 μg of RNase per ml, and 200 μg of lysozyme per ml in 30 mM Tris hydrochloride (pH 8.0). The membrane pellet which resulted from ultracentrifugation at 48,000 × *g* was extracted with 2% Triton X-100 in 30 mM Tris hydrochloride (pH 8.0) at 20°C for 30 min. Following washing twice in distilled water, suspended membranes were analyzed by sodium dodecyl sulfate (SDS)-10% polyacrylamide gel electrophoresis by the method of Laemmli (25). Proteins were visualized by staining them in 0.1% Coomassie brilliant blue (17).

Lipopolysaccharide. Lipopolysaccharide (LPS) was isolated from *P. cepacia* by the method of Darveau and Hancock (11). LPS extracts were analyzed by SDS-15% polyacrylamide gel electrophoresis in which 2 M urea was incorporated and were visualized by the silver staining technique of Tsai and Frasch (44).

RESULTS

Cloning of the chloramphenicol resistance gene. A total of 325 kanamycin-resistant *E. coli* colonies with *P. cepacia* insert DNA were screened, but none of them were resistant to chloramphenicol. To rule out the possibility that the resistance gene was not expressed in *E. coli*, the clones were scraped from the surface of agar plates and conjugated with *P. cepacia* 249-2 by triparental mating. Multiple kanamycin- and chloramphenicol-resistant colonies were identified, all of which appeared to contain the same 44.9-kb plasmid, pLIN1. After isolation and purification, pLIN1 was found to contain 23 kb of vector DNA (pVK102) and 21.9 kb of *P. cepacia* insert DNA.

TABLE 2. MICs for strains examined in this study

Strain ^a	MIC ($\mu\text{g/ml}$)			CAT activity (mU/mg of protein)
	Chloramphenicol	Trimethoprim	Ciprofloxacin	
K61-3	200	40	20	<10
249-2	20	0.1	1	<10
249-2(pVK102)	20	0.1	1	ND ^b
249-2(pLIN1)	200	40	10	<10
249-2(pJLB2)	ND	ND	ND	126
249-2(pLIN1, pJLB2)	ND	ND	ND	131

^a Plasmid designations are as follows: pVK102 is the cosmid vector, pLIN1 is the vector plus insert DNA from K61-3, and pJLB2 is the plasmid coding for CAT production.

^b ND, Not determined.

MIC. The MICs of chloramphenicol, trimethoprim, and ciprofloxacin for strains K61-3 (the isolate from a patient with CF), 249-2 (the laboratory recipient strain), 249-2(pVK102) (the recipient plus vector), and 249-2(pLIN1) (the recipient with the vector plus insert DNA) are listed in Table 2. The cloned fragment encoded resistance to trimethoprim and ciprofloxacin, in addition to resistance to chloramphenicol.

CAT activity. There was no detectable CAT production by strains 249-2, K61-3, or 249-2(pLIN1); the two strains into which pJLB2 was conjugated, 249-2(pJLB2) and 249-2(pLIN1, pJLB2), produced equivalent amounts of CAT (Table 2). No CAT activity was detected in culture supernatants of 249-2(pJLB2) or 249-2(pLIN1, pJLB2).

In vitro translation. The results of in vitro translation experiments are shown in Fig. 1. The activity of strain K61-3 was compared with that of a chloramphenicol-susceptible strain, PC104 (MIC, 5 $\mu\text{g/ml}$). Because no differences in ribosomal susceptibility were seen in these two strains, comparison of isogenic organisms is not included here. Incorporation of [¹⁴C]valine into trichloroacetic acid-precipitable material was equivalently inhibited by chloramphenicol in the two strains.

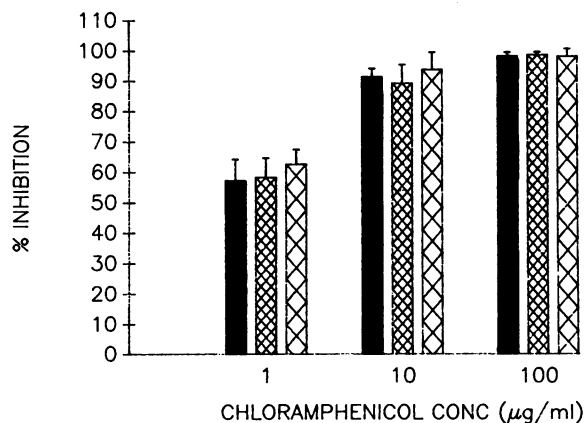


FIG. 1. In vitro translation in the presence of chloramphenicol. Percent inhibition of [¹⁴C]valine incorporation into trichloroacetic acid-precipitable material is plotted versus the chloramphenicol concentration. Zero percent inhibition is defined as the incorporation in the absence of chloramphenicol. Solid bars are results for PC104, a chloramphenicol-susceptible strain; narrow cross-hatched bars are results for strain K61-3; and wide cross-hatched bars are results for strain K61-3 preincubated with 20 μg of chloramphenicol per ml. Values represent the mean \pm standard deviation of four experiments.

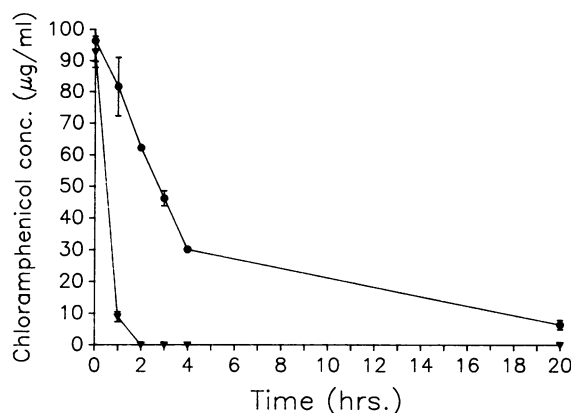


FIG. 2. Chloramphenicol loss from culture supernatants over time. Results are of high-pressure liquid chromatographic quantitation of the chloramphenicol concentration remaining in the culture medium after incubation for 1, 2, 3, 4, and 20 h. The starting concentration was 100 $\mu\text{g/ml}$. Symbols: ∇ , 249-2(pJLB2); \bullet , 249-2(pLIN1, pJLB2). Values represent the mean \pm standard deviation of three experiments.

To rule out the possibility that an inducible ribosomal resistance was operating, K61-3 cells were preincubated with 20 μg of chloramphenicol per ml prior to extraction with ether; again, no differences were seen between susceptible and resistant strains (Fig. 1).

Chloramphenicol uptake. Chloramphenicol penetration into whole bacterial cells was compared in the isogenic strains 249-2 and 249-2(pLIN1) containing pJLB2, which codes for the production of CAT. CAT modified the chloramphenicol within the cell to maintain the concentration gradient across the membrane. The results of uptake experiments are shown in Fig. 2. A marked difference in drug uptake was seen at all time points. At 1 h there was nearly a 10-fold difference between 249-2(pJLB2) and 249-2(pLIN1, pJLB2): 8.9 ± 1.55 versus 81.64 ± 9.36 $\mu\text{g/ml}$, respectively, remaining in culture supernatants.

Outer membrane protein profiles. A Coomassie blue-stained SDS-polyacrylamide gel comparing outer membrane protein profiles of strains 249-2, 249-2(pVK102), 249-2(pLIN1), and K61-3 is shown in Fig. 3. There were no consistent differences identified between the chloramphenicol-susceptible or -resistant isogenic strains. However, on some gels (including the one in Fig. 3) an 18-kilodalton protein was visible in strain 249-2(pLIN1) that was not identified in the other isogenic strains. Strain K61-3 was included for comparison, although since it was not isogenic, differences in the outer membrane protein pattern were difficult to interpret. Its pattern did not show the 18-kilodalton protein.

LPS patterns. Figure 4 shows the pattern of LPS extracted from the isogenic chloramphenicol-susceptible and -resistant strains 249-2 and 249-2(pLIN1). Again, no significant differences in LPS patterns were detected.

DISCUSSION

Bacterial resistance to chloramphenicol may be caused by inactivation by CAT (40, 41), ribosomal resistance (33), or impermeability (6, 7, 14, 29). In *P. aeruginosa*, 50 to 85% of resistant strains produce CAT (29, 32). In the remainder, resistance is caused by decreased outer membrane permeability (7, 24, 29), possibly because of the absence of porins

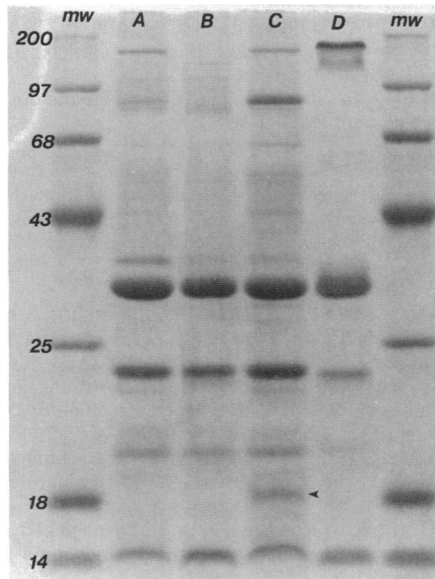


FIG. 3. Outer membrane protein profiles. Coomassie blue-stained SDS-polyacrylamide gel of outer membrane preparations of strains 249-2 (lane A), 249-2(pVK102) (lane B), 249-2(pLIN1) (lane C), and K61-3 (lane D). All major bands were the same in the isogenic strains, with the exception of the 18-kilodalton band seen in 249-2(pLIN1) (arrow), which was not reproducibly present (see text). Sizes of molecular weight (mw) standards (10^3) are indicated on the left.

(7). Although ribosomal alterations conferring chloramphenicol resistance have been reported in *Bacillus* species (33), gram-negative organisms have not exhibited this resistance mechanism.

Results of a study in which chloramphenicol resistance in a *P. cepacia* isolate was investigated suggested that resistance was caused by a block in transport (31). This study also found decreased susceptibility to chloramphenicol in the presence of EDTA. Further examination of chloram-



FIG. 4. LPS patterns. Silver-stained SDS-polyacrylamide gel of LPS preparations from strains 249-2 (lane A) and 249-2(pLIN1) (lane B).

phenicol resistance mechanisms in *P. cepacia*, or in closely related species such as *Pseudomonas mallei* and *Pseudomonas pseudomallei*, has not been undertaken.

In this study we demonstrated that decreased permeability is a mechanism of chloramphenicol resistance in *P. cepacia*. We were unable to detect either production of CAT or ribosomal resistance to chloramphenicol in the high-level-resistant strain that we investigated. After cloning the resistance marker into a susceptible *P. cepacia* strain and conjugating a plasmid encoding CAT production into the same strain, we were able to demonstrate a 10-fold difference in chloramphenicol uptake between the susceptible and resistant strains. The MICs for the organisms also differed by 10-fold.

Despite the fact that we identified no structural alterations in outer membrane proteins (including porins) or LPS, the difference in chloramphenicol uptake between the susceptible and resistant strains was likely caused by outer membrane impermeability. In addition, several studies of antibiotic resistance in *P. cepacia* have suggested that decreased outer membrane permeability plays a key role. Chemical antibacterial agents such as quaternary ammonium compounds are effective against *P. cepacia* only at high concentrations. Resistance to these compounds is increased even further by the addition of the chelating agent EDTA (36) and by depletion of magnesium in the culture medium (10). This is opposite from the effects of divalent cations on the outer membrane of other gram-negative bacteria including *P. aeruginosa*, *E. coli*, and *Salmonella* species (18). In these organisms, the presence of a chelating agent increases permeability and thereby increases susceptibility to antimicrobial agents.

Recent data from Moore and Hancock (30) confirm that *P. cepacia* is resistant to the outer membrane-permeabilizing effects of EDTA. They reported that although LPS in intact *P. cepacia* cells lacks binding sites for polycations (such as the aminoglycosides, polymyxin, and a fluorescent polycation, dansylpolymyxin), purified LPS from the same strains had an affinity for dansylpolymyxin equal to that of *P. aeruginosa*. In addition, Parr et al. (34) measured the permeability coefficient of *P. cepacia* K61-3. The porin-mediated permeability of this strain to the β -lactam nitrocefin was low, comparable to that of *P. aeruginosa*. However, strain K61-3 was not compared with an antibiotic-susceptible strain of *P. cepacia*.

Although the unique organization of the outer membrane of *P. cepacia* makes it a likely site for a permeability barrier, decreased permeability across the inner membrane is also possible. Active transport of chloramphenicol across the cytoplasmic membrane has been demonstrated in *Haemophilus influenzae* (8). Alterations in this cytoplasmic membrane pump could be a mechanism of chloramphenicol resistance in *P. cepacia*. However, the cloned DNA fragment codes for cross-resistance to trimethoprim and ciprofloxacin, in addition to chloramphenicol, suggesting that the permeability barrier is nonspecific and therefore not at the level of the cytoplasmic membrane.

We conclude that the mechanism of chloramphenicol resistance in isolate K61-3, which was obtained from a patient with CF, is decreased membrane permeability. Although no structural differences were detected between the isolated outer membrane proteins and the LPS of isogenic susceptible and resistant strains, the most likely resistance mechanism is structural or functional differences that are present in intact outer membranes. Conversely, a decrease in the inner membrane pumping of chloramphenicol could be

responsible for the decrease in drug entry into the cell. Further studies, including the construction of membrane vesicles, are required to fully examine these possibilities.

ACKNOWLEDGMENTS

This study was supported by grants (to J.L.B.) from the Cystic Fibrosis Foundation and the National Institutes of Health.

LITERATURE CITED

- Beckman, W., and T. G. Lessie. 1979. Response of *Pseudomonas cepacia* to β -lactam antibiotics: utilization of penicillin G as the carbon source. *J. Bacteriol.* **140**:1126-1128.
- Birnboim, H. C., and J. Doly. 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* **7**:1513-1523.
- Bolivar, F., and K. Backman. 1979. Plasmids of *Escherichia coli* as cloning vectors. *Methods Enzymol.* **68**:245-267.
- Bosso, J. A., B. A. Saxon, and J. M. Matsen. 1987. In vitro activity of aztreonam combined with tobramycin and gentamicin against clinical isolates of *Pseudomonas aeruginosa* and *Pseudomonas cepacia* from patients with cystic fibrosis. *Antimicrob. Agents Chemother.* **31**:1403-1405.
- Burkholder, W. H. 1950. Sour skin, a bacterial rot of onion bulbs. *Phytopathology* **40**:115-117.
- Burns, J. L., P. M. Mendelman, J. Levy, T. L. Stull, and A. L. Smith. 1985. A permeability barrier as a mechanism of chloramphenicol resistance in *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* **27**:46-54.
- Burns, J. L., C. E. Rubens, P. M. Mendelman, and A. L. Smith. 1986. Cloning and expression in *Escherichia coli* of a gene encoding nonenzymatic chloramphenicol resistance from *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **29**:445-450.
- Burns, J. L., and A. L. Smith. 1987. Chloramphenicol accumulation by *Haemophilus influenzae*. *Antimicrob. Agents Chemother.* **31**:686-690.
- Chiesa, C., P. H. Labrozzi, and S. C. Aronoff. 1986. Decreased baseline β -lactamase production and inducibility associated with increased piperacillin susceptibility of *Pseudomonas cepacia* isolated from children with cystic fibrosis. *Pediatr. Res.* **20**:1174-1177.
- Cozens, R. M., and M. R. W. Brown. 1983. Effect of nutrient depletion on the sensitivity of *Pseudomonas cepacia* to antimicrobial agents. *J. Pharm. Sci.* **72**:1363-1365.
- Darveau, R. P., and R. E. W. Hancock. 1983. Procedure for isolation of bacterial lipopolysaccharide from both smooth and rough *Pseudomonas aeruginosa* and *Salmonella typhimurium* strains. *J. Bacteriol.* **155**:831-838.
- Ederer, G. M., and J. M. Matsen. 1972. Colonization and infection with *Pseudomonas cepacia*. *J. Infect. Dis.* **125**:613-618.
- Figurski, D. H., and D. R. Helinski. 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided in *trans*. *Proc. Natl. Acad. Sci. USA* **76**:1648-1652.
- Gaffney, D. F., E. Cundliffe, and T. J. Foster. 1981. Chloramphenicol resistance that does not involve chloramphenicol acetyltransferase encoded by plasmids from gram-negative bacteria. *J. Gen. Microbiol.* **125**:113-121.
- Gold, R., E. Jin, H. Levison, A. Isles, and P. C. Fleming. 1983. Ceftazidime alone and in combination in patients with cystic fibrosis: lack of efficacy in treatment of severe respiratory infections caused by *Pseudomonas cepacia*. *J. Antimicrob. Chemother.* **12**(Suppl. A):331-336.
- Goldberg, J. B., and D. E. Ohman. 1984. Cloning and expression in *Pseudomonas aeruginosa* of a gene involved in the production of alginate. *J. Bacteriol.* **158**:1115-1121.
- Hames, B. D. 1981. An introduction to polyacrylamide gel electrophoresis, p. 1-91. In B. D. Hames and D. Rickwood (ed.), *Gel electrophoresis of proteins: a practical approach*. IRL Press Ltd., Oxford.
- Hancock, R. E. W. 1984. Alterations in outer membrane permeability. *Annu. Rev. Microbiol.* **38**:237-264.
- Hardy, P. C., G. M. Ederer, and J. M. Matsen. 1970. Contamination of commercially packaged urinary catheter kits with the pseudomonad E0-1. *N. Engl. J. Med.* **282**:33-35.
- Hohn, B. 1979. *In vitro* packaging of lambda and cosmid DNA. *Methods Enzymol.* **68**:299-309.
- Isles, A., I. Maclusky, M. Corey, R. Gold, C. Prober, P. Fleming, and H. Levison. 1984. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J. Pediatr.* **104**:206-210.
- Kaslow, R. A., D. C. Mackel, and G. F. Mallison. 1976. Nosocomial pseudobacteremia. Positive blood cultures due to contaminated benzalkonium antiseptic. *J. Am. Med. Assoc.* **236**:2407-2409.
- Knauf, V. C., and E. W. Nester. 1982. Wide host range cloning vectors: a cosmid clone bank of an agrobacterium Ti plasmid. *Plasmid* **8**:45-54.
- Kono, M., and K. O'Hara. 1976. Mechanism of chloramphenicol-resistance mediated by KR102 factor in *Pseudomonas aeruginosa*. *J. Antibiot.* **29**:176-180.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* **227**:680-685.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265-275.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Martone, W. J., C. A. Osterman, K. A. Fisher, and R. P. Wenzel. 1981. *Pseudomonas cepacia*: implications and control of epidemic nosocomial colonization. *Rev. Infect. Dis.* **3**:708-715.
- Mitsuhashi, S., H. Kawabe, A. Fuse, and S. Iyobe. 1975. Biochemical mechanisms of chloramphenicol resistance in *Pseudomonas aeruginosa*, p. 515-523. In S. Mitsuhashi and H. Hashimoto (ed.), *Microbial drug resistance*. University of Tokyo Press, Tokyo.
- Moore, R. A., and R. E. W. Hancock. 1986. Involvement of outer membrane of *Pseudomonas cepacia* in aminoglycoside and polymyxin resistance. *Antimicrob. Agents Chemother.* **30**:923-926.
- Nielsen, P. A., and J. Close. 1982. Edetate disodium mediated chloramphenicol resistance in *Pseudomonas cepacia*. *J. Pharm. Sci.* **71**:833.
- Nitzan, Y., and N. M. Rushansky. 1981. Chloramphenicol acetyltransferase from *Pseudomonas aeruginosa*—a new variant of the enzyme. *Curr. Microbiol.* **5**:261-265.
- Osawa, S., R. Takata, K. Tanaka, and M. Tamaki. 1973. Chloramphenicol resistant mutants of *Bacillus subtilis*. *Mol. Gen. Genet.* **127**:163-173.
- Parr, T. R., Jr., R. A. Moore, L. V. Moore, and R. E. W. Hancock. 1987. Role of porins in intrinsic antibiotic resistance of *Pseudomonas cepacia*. *Antimicrob. Agents Chemother.* **31**:121-123.
- Rapkin, R. H. 1976. *Pseudomonas cepacia* in an intensive care nursery. *Pediatrics* **57**:239-243.
- Richards, R. M. E., and J. M. Richards. 1979. *Pseudomonas cepacia* resistance to antibacterials. *J. Pharm. Sci.* **68**:1436-1438.
- Rosenstein, B. J., and D. E. Hall. 1980. Pneumonia and septicemia due to *Pseudomonas cepacia* in a patient with cystic fibrosis. *Johns Hopkins Med. J.* **147**:188-189.
- Schmidhauser, T. J., and D. R. Helinski. 1985. Regions of broad-host-range plasmid RK2 involved in replication and stable maintenance in nine species of gram-negative bacteria. *J. Bacteriol.* **164**:446-455.
- Scordilis, G. E., H. Ree, and T. G. Lessie. 1987. Identification of transposable elements which activate gene expression in *Pseudomonas cepacia*. *J. Bacteriol.* **169**:8-13.
- Shaw, W. V. 1967. The enzymatic acetylation of chloramphenicol by extracts of R factor-resistant *Escherichia coli*. *J. Biol. Chem.* **4**:687-693.
- Shaw, W. V., and R. F. Brodsky. 1968. Characterization of

- chloramphenicol acetyltransferase from chloramphenicol-resistant *Staphylococcus aureus*. J. Bacteriol. **95**:28–36.
42. Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. **4**:307–311.
 43. Stull, T. L., K. Mack, J. E. Haas, J. Smit, and A. L. Smith. 1985. A comparison of techniques for isolation of outer membrane proteins of *Haemophilus influenzae* type b. Anal. Biochem. **150**:471–480.
 44. Tsai, C.-M., and C. E. Frasch. 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
 45. Welch, D. F., M. J. Muszynski, C. H. Pai, M. J. Marcone, M. M. Hribar, P. H. Gilligan, J. M. Matsen, P. A. Ahlin, B. C. Hilman, and S. A. Chartrand. 1987. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. J. Clin. Microbiol. **25**:1730–1734.