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Influence of Antibiotics on Intestinal Tract Survival and Translocation of Environmental *Pseudomonas* Species

S. ELIZABETH GEORGE,* MICHAEL J. KOHAN, DOUGLAS A. WHITEHOUSE, JOHN P. CREASON, AND LARRY D. CLAXTON

Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711

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The environmental release of microorganisms has prompted the investigation of potential health effects associated with their release. In this study, survival and translocation to the spleen and liver of several environmental *Pseudomonas* spp. were investigated in antibiotic-treated mice. *Pseudomonas aeruginosa* BC16 and *P. maltophilia* BC6, isolated from a commercial product for polychlorinated biphenyl degradation; *P. aeruginosa* AC869, a 3,5-dichlorobenzoate degrader; and *P. cepacia* AC1100, an organism that metabolizes 2,4,5-trichlorophenoxyacetic acid were examined for their survival capabilities in the intestines of mice dosed with clindamycin, kanamycin, rifampin, or spectinomycin. A mouse intestinal isolate, strain PAMG, was included in the study. Following antibiotic pretreatment (1 mg twice daily for 3 days), mice were dosed by gavage with 10^9 CFU of each *Pseudomonas* strain. At the end of the 5-day test period, strains AC869 and PAMG survived in kanamycin-, rifampin-, spectinomycin-, and clindamycin-treated animals. A statistically significant ($P < 0.05$) increase in survival of strain PAMG was observed in clindamycin-, kanamycin-, and spectinomycin-treated mice for the test period. Treatment with clindamycin or rifampin increased ($P < 0.05$) survival of strain BC6, an organism resistant to both antibiotics. However, strain BC6 was detected only in rifampin-treated mice at the end of the 5-day test period. Strain BC16, a clindamycin-resistant strain, was detected in clindamycin-treated mice and the untreated control animals. Rifampin had a negative effect ($P < 0.05$) on strain AC869 and PAMG survival. Translocation to the spleen was observed in spectinomycin- and clindamycin-treated mice but was not detected in kanamycin- or rifampin-treated animals. Depending on the dosed strain, translocation to the liver was observed in animals treated with all four antibiotics and in the untreated mice. However, detection in the liver was generally observed at later time points in antibiotic-treated animals.

Recombinant, mutant, and naturally occurring *Pseudomonas* species have a variety of commercial environmental applications. Pseudomonads degrade environmental contaminants such as 2,4,5-trichlorophenoxyacetic acid (22), chlorobenzoates (6, 7), and trichloroethylene (26). Members of this same genus have been engineered for use as pesticides (27) and for the prevention of ice nucleation on plants (24). *Pseudomonas* spp. have long been recognized as opportunistic pathogens. The organisms have been shown to be involved in serious infections in immunosuppressed patients (18), those on antimicrobial agent chemotherapy (1), and leukemia patients (4, 30). Colonization studies are done generally with clinical isolates (19). Because environmentally released microorganisms are primarily isolated from environmental sources, the colonization potential of environmentally used pseudomonads may be different from their better-adapted clinical counterparts.

Through pseudomonad environmental release, there are several routes of potential exposure, including the gastrointestinal (GI) tract, skin, and oropharynx (23). It has been well documented that the GI tract serves as a reservoir for potential pathogens (8, 21, 31). If the normal microbiota of the GI tract are altered, potential pathogens can colonize or resident pathogens can multiply, having an infectious effect on the host (11). Previous work in our laboratory, using environmental *Pseudomonas* spp., has shown that not only can the strains survive in mice for 14 days, but the normal flora can be altered in the presence of the dosed environmental pseudomonads (16). Antibiotic treatment of mice has been shown to increase the colonization potential of *Pseudomonas* spp. (17a, 23, 33).

The mouse has been used extensively to study intestinal tract colonization by opportunistic and pathogenic microorganisms. It was used as a model for *Klebsiella pneumoniae* (33), *Salmonella typhimurium* (28), *Candida albicans* (9), and *Escherichia coli* (10, 15) in GI tract colonization. The mouse also was used to study antibiotic-induced translocation from the GI tract to the spleen, mesenteric lymph nodes, and liver (2, 19, 36). For example, clindamycin promotes translocation of the gram-negative enteric bacilli (2) and the enterococci (36) to the mesenteric lymph nodes of mice. Streptomycin has a similar effect on *S. typhimurium* and also causes translocation to the spleen and liver.

In this study, we describe the effects of rifampin, clindamycin, spectinomycin, and kanamycin on the GI tract survival and translocation of environmentally isolated *Pseudomonas* spp. Two of the strains, *Pseudomonas aeruginosa* BC16 and BC6, were isolated from a commercial product for polychlorinated biphenyl biodegradation (17). The organisms examined in this study are representative of the types of pseudomonads that have the potential to be released into the environment in high numbers.

MATERIALS AND METHODS

Chemicals. All chemicals used in this study were reagent grade. Clindamycin hydrochloride, spectinomycin dihydrochloride, rifampin, kanamycin sulfate, L-cysteine, vitamin K_1, and hemin were obtained from Sigma Chemical Co., St. Louis, Mo. Mercuric chloride was purchased from Mallinck-

* Corresponding author.

rodt, Paris, Ky. Irgasan was provided by Ciba-Geigy Co., Inc., Greensboro, N.C.

Bacterial strains. *P. aeruginosa* BC16 and *P. maltophilia* BC6 were isolated from a commercial product for polychlorinated biphenyl degradation as described previously (17). *P. aeruginosa* AC869, a 3,5-dichlorobenzoate degrader, was obtained from A. M. Chakrabarty (6). *P. cepacia* AC1100, which uses 2,4,5-trichlorophenoxyacetic acid as sole source of carbon and energy, was obtained from A. M. Chakrabarty through P. H. Pritchard at the U.S. Environmental Protection Agency-Environmental Research Laboratory, Gulf Breeze, Fla. (22).

Media and buffers. *Pseudomonas* isolation agar (PIA; Difco, Detroit, Mich.) was prepared by the manufacturer's instructions. When the commercial source was unavailable, PIA was prepared as follows, per liter: 20 g of peptone; 1.4 g of $MgCl_2$; 10.0 g of K_2SO_4; 25 mg of Irgasan; 20 ml of glycerol; 13.6 g of agar; and 980 ml of deionized water (12). Kanamycin, 50 μg/ml, and mercuric chloride, 30 μg/ml, were added to PIA when required. Yeast extract-tryptone medium without NaCl, with or without 1.5% (wt/vol) agar (Difco), was used for strain growth (25). In the microbial antibiotic resistance experiments, kanamycin, spectinomycin, clindamycin, kanamycin and rifampin were added at a final concentration of 10 or 50 μg/ml as indicated.

Bacterial strains were centrifuged and suspended in Dulbecco phosphate-buffered saline (GIBCO Laboratories, Grand Island, N.Y.) prior to animal dosing. The intestines, spleen, and liver were homogenized in buffer containing 2.0 g of gelatin, 0.5 g of cysteine, 500 ml of salts solution (0.1 g of $CaCl_2$ [anhydrous], 0.1 g of $MgSO_4$ [anhydrous], 0.5 g of K_2HPO_4, 0.5 g of KH_2PO_4, 5.0 g of $NaHCO_3$, 1.0 g of NaCl), and 500 ml of deionized water (20).

Animals. Sixty-day-old male CD-1 mice (Crl:CD-1 [ICR]BR, COBS; Charles River Laboratories Kingston, Stone Ridge, N.Y.) were used in this study. Animals were provided water and sterilized chow (autoclaved in aluminum foil-covered 250-ml polypropylene beakers for 30 min at 121°C with a 15-min drying period; Wayne Sterilizable Rodent Blox [#8656], Continental Grain Co., Chicago, Ill.) ad libitum. Animals were housed in polycarbonate cages with pine shavings. Unless otherwise indicated, each data point is an average of four animals per treatment-time combination.

Treatment of CD-1 mice with antibiotics and microorganisms. Mice were dosed by gavage individually with 1 mg of each antibiotic (0.1 ml of a 10.0-mg/ml aqueous stock solution) twice (6-h interval) a day for 3 days. On day 4, mice were gavaged individually with the *Pseudomonas* sp. Bacteria were grown overnight in 25 ml of yeast extract-tryptone broth, centrifuged, and suspended in 5 to 10 ml of phosphate-buffered saline. Dilutions of the dosing suspension were made in phosphate-buffered saline and CFU per milliliter were determined by extrapolation from a standard curve. A 0.1-ml sample (10^9 CFU) of the bacterial suspension was administered to mice by gavage. Daily antibiotic treatment (1.0 mg/animal per day) continued on days 4 to 8.

Detection of pseudomonads in the intestinal tract. At 3, 24, 48, 72, and 120 h after the pseudomonad dose, animals were sacrificed by CO_2 asphyxiation. A lateral pneumothorax was performed, and the GI tract was removed. Preparation of the GI tract homogenate was done by a method described previously (17). Dilutions of the homogenate were made in buffer, and duplicate platings were done on PIA media supplemented with antimicrobial agents as indicated. Strain BC16 was enumerated on PIA containing mercuric chloride. Kanamycin and mercuric chloride were used for the selection of strain PAMG. Strain AC1100 was enumerated on PIA with no antimicrobial agents added, and strains BC6 and AC869 were grown in the presence of kanamycin. Strains AC1100 and AC869 were incubated at 30°C for 48 h. The others were grown at 37°C for 48 h. After growth, individual colonies were isolated. Antibiotic sensitivities, using antibiotic disks (BBL Microbiology Systems, Cockeysville, Md.), were determined for each isolate prior to identification as original dosed strain.

Determination of pseudomonad translocation. The liver and spleen were removed from the treated animals at 3, 24, 48, 72, and 120 h after the initial pseudomonad dose and weighed. The tissues were placed into 5 ml of buffer described above and homogenized with a Polytron tissue homogenizer (Brinkmann Instruments, Westbury, N.Y.). Homogenates were plated onto PIA medium, using the same supplements, incubation conditions, reisolation, and identification techniques as described above for the GI tract.

Statistical analysis. To determine whether there was a significant antibiotic treatment effect on *Pseudomonas* survival, a two-factor (antibiotic and time) analysis of variance model, including interaction, was used. Logarithms of all responses (counts) were analyzed, since application of a procedure described by Box and Cox (5) had indicated in earlier similar experiments that such a transformation of scale was desirable to satisfy analysis of variance assumptions. Prior to transformation, an arbitrary constant of 0.0001 was added to each response to preclude problems with zero values. The analyses of variance were performed by using the general linear model procedure of the Statistical Analysis System (30). The *P* values cited in the study are those associated with the analyses of variance and post hoc comparisons of the logarithmic data as detailed below.

For those strains for which significant treatment or treatment-time interactions were indicated in the initial analysis, a post hoc two-tailed Dunnett test procedure (13, 14), implemented through the Statistical Analysis System, was

TABLE 1. Clindamycin, spectinomycin, rifampin, and kanamycin resistance of *P. maltophilia* BC6, *P. aeruginosa* BC16, PAMG, and AC869, and *P. cepacia* AC1100[a]

Antibiotic	Resistance[b]				
	BC6	BC16	PAMG	AC869	AC1100
None	+	+	+	+	+
Clindamycin					
10 μg/ml	+	+	+	+	+
50 μg/ml	+	+	+	+	+
Spectinomycin					
10 μg/ml	+	+	+	+	−
50 μg/ml	+	+	+	+	−
Rifampin					
10 μg/ml	+	+	+	+	+
50 μg/ml	+	−	−	−	+
Kanamycin					
10 μg/ml	+	−	+	+	−
50 μg/ml	+	−	+	+	−

[a] Yeast extract-tryptone medium with or without the supplemented antibiotics was inoculated with the indicated strains. The plates were incubated at 30°C. Microbial resistance to the antibiotics was determined after 24 and 48 h of incubation. No change was observed at 48 h.

[b] +, Growth (resistant); −, inhibited growth (not resistant).

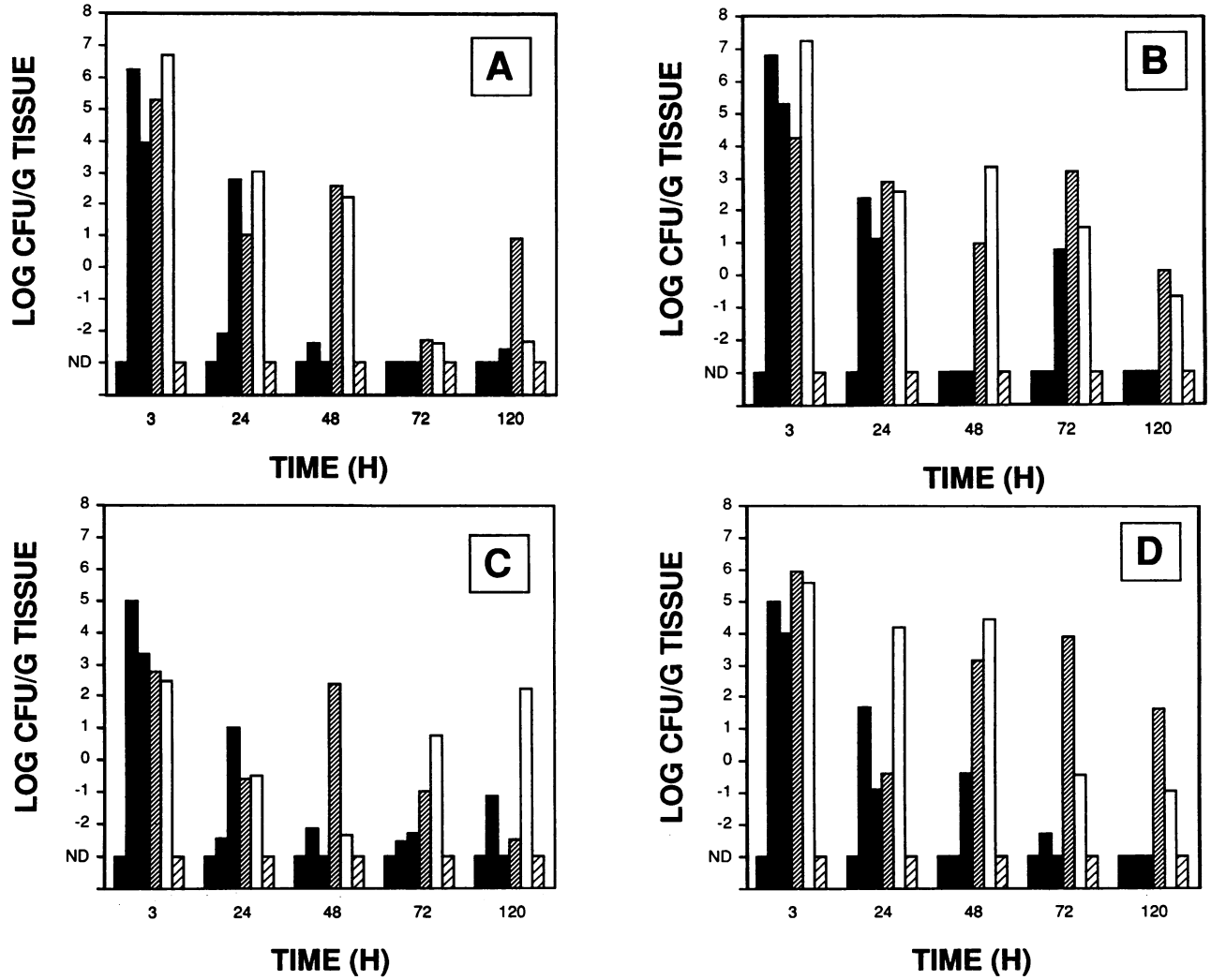

FIG. 1. Survival of environmental pseudomonads in the GI tract of antibiotic- and untreated mice. Mice were administered 1 mg of the indicated antibiotic twice a day for 3 days. Next, mice were dosed individually by gavage with 10^9 CFU of *P. aeruginosa* BC16 (▨), AC869 (□), or PAMG (▨), *P. maltophilia* BC6 (▨), or *P. cepacia* AC1100 (▨) or with phosphate-buffered saline control solution (■). On the following experimental days, mice received a single 1-mg dose of the antibiotic. At 3, 24, 48, 72, and 120 h after the bacterial dose, animals were sacrificed and the intestines were homogenized and plated on selective media described in Materials and Methods for enumeration of the dosed strain. Shown is survival of the pseudomonads in (A) untreated animals and in (B) kanamycin-treated, (C) rifampin-treated, (D) spectinomycin-treated, and (E) clindamycin-treated mice. When the dosed strains were not detectable in the homogenate, the time point was plotted at the ND point on the graph.

used for comparing the four antibiotic treatments with the matching control treatment at each of the time points.

RESULTS

Antibiotic sensitivities. To determine whether antibiotic resistance had an effect on survival, clindamycin, spectinomycin, rifampin, and kanamycin resistance was determined for the strains used in this study. Two concentrations, 10 and 50 μg/ml, were tested. All of the strains examined were resistant to clindamycin (Table 1). *P. maltophilia* BC6 was resistant to all four antibiotics tested. The results are summarized in Table 1.

Survival of pseudomonads in the GI tract of antibiotic-treated mice. Mice were dosed individually by gavage with 10^9 CFU of *P. aeruginosa* BC16, PAMG, and AC869, *P. maltophilia* BC6, or *P. cepacia* AC1100. Survival in the GI

TABLE 2. Translocation of environmental pseudomonads to the spleen[a]

Antibiotic treatment of mice	Dosed strain	Time detected (h)	CFU/mg of tissue
No treatment	None detected		
Spectinomycin	PAMG	72	1.75 (1)[b]
	BC6	3	1.25 (1)
Clindamycin	AC869	3	0.50 (1)
	Control	48	0.25 (1)[c]
		120	0.25 (1)[c]
Kanamycin			ND[d]
Rifampin			ND

[a] Mice were dosed with the indicated antibiotic as decribed in Materials and Methods. The spleen was removed and homogenized in VPI buffer, and 0.1 ml was plated on PIA containing 30 μg of $HgCl_2$ per ml (BC16), 50 μg of kanamycin per ml (AC869 and BC6), $HgCl_2$ and kanamycin (PAMG), or no antimicrobial agent (AC1100). Homogenates from animals that received no pseudomonad were plated on all media for use as controls. Plates were incubated for 48 h, and colonies were enumerated. Four animals per treatment-time combination were used.

[b] Number in parentheses indicates number of animals whose spleen contained detectable dosed pseudomonads.

[c] Microorganism isolated was similar to strain PAMG, a normally occurring member of the GI microbiota.

[d] ND, None detected.

TABLE 3. Translocation of environmental pseudomonads to the liver[a]

Antibiotic treatment of mice	Dosed strain	Time detected (h)	CFU/mg of tissue
No treatment	BC6	3	8.21 (1)[b]
	PAMG	3	0.13 (2)
		24	0.01 (1)
	AC869	3	4.77 (1)
Spectinomycin	BC16	3	0.01 (1)
		48	0.58 (1)
	PAMG	3	0.02 (1)
		72	0.85 (1)
	AC869	48	0.01 (1)
		72	0.02 (1)
Clindamycin	BC6	3	0.04 (1)
	BC16	3	0.04 (2)
	PAMG	3	0.06 (1)
		120	0.03 (1)
	AC869	3	0.03 (2)
		24	0.02 (1)
		120	1,800.00 (1)
Kanamycin	Control	48	0.01 (1)[c]
	BC16	3	0.44 (1)
	PAMG	3	0.73 (1)
	AC869	3	0.14 (1)
		48	4,300.00 (1)
Rifampin	BC16	3	0.06 (1)
		72	0.03 (1)
	PAMG	3	0.24 (2)
	AC869	3	0.29 (1)

[a] Mice were dosed with the indicated antibiotic as described in Materials and Methods. The liver was removed and homogenized in VPI buffer, and 0.1 ml was plated on PIA containing 30 μg of $HgCl_2$ (BC16), 50 μg of kanamycin (AC869 and BC6), $HgCl_2$ and kanamycin (PAMG), or no antimicrobial agent (AC1100). Homogenates from animals that received no pseudomonad were plated on all media for use as controls. Plates were incubated for 48 h, and colonies were enumerated. Four animals per treatment-time combination were used.

[b] Number in parentheses indicate number of animals whose liver contained detectable dosed pseudomonads.

[c] The microorganism isolated was similar to strain PAMG, a normally occurring member of the GI microbiota.

tract was monitored over a 5-day period. Figure 1A shows the survival of tested strains in the GI tract of untreated animals. By the end of the test period, only the *P. aeruginosa* strains were detectable. *P. aeruginosa* AC869 and PAMG survived in the GI tract in all of the antibiotic-treated animals (Fig. 1B to E). Strain BC16 was detectable for 5 days in clindamycin-treated animals (Fig. 1E), and *P. maltophilia* BC6 survived the test period in rifampin-treated mice (Fig. 1C). Kanamycin (Fig. 1B), spectinomycin (Fig. 1D), and clindamycin (Fig. 1E) enhanced the overall survival of *P. aeruginosa* PAMG at 72 h ($P < 0.05$). Rifampin had a negative effect ($P < 0.05$) on the survival of strains AC869 (48 h) and PAMG (3 h) (Fig. 1C). Clindamycin (24 h) (Fig. 1E) and rifampin (120 h) (Fig. 1D) had a selective effect ($P < 0.05$) on strain BC6 survival.

Translocation of pseudomonads to the spleen and liver of antibiotic-treated mice. Translocation of the dosed pseudomonads from the intestinal tract to the spleen or liver was determined by enumeration of colonies on selective medium from tissue homogenates. In untreated mice, no translocation to the spleen was observed (Table 2). Also, no translocation of the dosed pseudomonads to the spleen was evident in animals dosed with kanamycin or rifampin (Table 2). *P. aeruginosa* PAMG and *P. maltophilia* BC6 were detected in the spleen of spectinomycin-treated animals, and *P. aeruginosa* AC869 was detected in the spleen of clindamycin-treated mice. Translocation to the liver did occur in untreated mice at 3 h after dosing with strains BC6 and AC869 and in the mouse isolate, strain PAMG (Table 3). Strain PAMG was also detected in the liver 24 h after dosing. Translocation of the dosed pseudomonads to the liver occurred in animals treated with each antibiotic tested (Table 3).

DISCUSSION

It is well established that antibiotic treatment alters the resident microbiota of the GI tract. Colonization resistance is then reduced, and the colonization by "foreign" microbes can occur more readily (34). Translocation from the GI tract to the mesenteric lymph nodes, spleen, or liver occurs during antibiotic treatment when the intestinal epithelium is physically damaged or the immune system is functioning inadequately (11). Stress or trauma helps facilitate translocation (11). In the case of antibiotic treatment, the intestinal ecology is disrupted and either invading or "suppressed but harbored" pathogens can colonize and cause disease.

The mouse has been used as a model to examine colonization and translocation of clinically isolated pseudomonads as well as other microorganisms (9, 10, 15, 28, 32, 33). Therefore, the mouse was chosen to examine the effect of antibiotics on the survival and translocation of environmentally isolated pseudomonads. Previous work in our laboratory investigated the effect of antibiotics on the microbiota of CD-1 mice. From these results, four antibiotics were chosen that altered different members of the GI microbial populations. Van Furth and Guiot (35) surmised that reduction in anaerobic microorganism numbers, more so than the facultative species, plays an important role in the introduction and colonization of foreign microorganisms. The antibiotics used in this study were chosen because of the population(s) they altered (S. E. George and M. J. Kohan, unpublished data). Clindamycin and spectinomycin decreased both the

TABLE 4. Summary of pseudomonad antibiotic resistance and survival in tissues of antibiotic-treated CD-1 mice

Test	Control	BC6	BC16	PAMG	AC869	AC1100
Control (no antibiotic)						
Resistance	NA[a]	NA	NA	NA	NA	NA
Detection						
GI (120 h)	−[b]	−	+	+	+	−
Spleen	−	−	−	−	−	−
Liver	−	+	−	+	+	−
Clindamycin						
Resistance	NA	+	+	+	+	+
Detection						
GI (120 h)	−	−	+	+	+	−
Spleen	+[c]	−	−	−	+	−
Liver	−	+	+	+	+	−
Spectinomycin						
Resistance	NA	+	+	+	+	−
Detection						
GI (120 h)	−	−	−	+	+	−
Spleen	−	+	−	+	−	−
Liver	−	−	+	+	+	−
Rifampin						
Resistance	NA	+	W[d]	W	W	+
Detection						
GI (120 h)	−	+	−	+	+	−
Spleen	−	−	−	−	−	−
Liver	−	−	+	+	+	−
Kanamycin						
Resistance	NA	+	−	+	+	−
Detection						
GI (120 h)	−	−	−	+	+	−
Spleen	−	−	−	−	−	−
Liver	+[c]	−	+	+	+	−

[a] NA, Not applicable.
[b] +, Growth; −, no growth.
[c] *P. aeruginosa* strain, identical in antibiotic resistance pattern to that of the mouse isolate strain PAMG. This organism is commonly found in the mouse GI tract.
[d] W, Week: growth on 10 μg of rifampin per ml; no growth on 50 μg of rifampin per ml.

obligately anaerobic gram-negative rods and lactobacilli. Rifampin increased total anaerobic or facultative gram-positive counts while significantly decreasing the lactobacilli. Kanamycin increased the obligately anaerobic gram-negative rods and the lactose-fermenting enterobacteria.

All pseudomonads used in this study were resistant to clindamycin. However, only three were recoverable at the end of 120 h in clindamycin-treated animals. To interpret the data better, a statistical analysis (two-tailed Dunnett test) was done. Clindamycin was selective for *P. maltophilia* BC6 and the mouse isolate, strain PAMG, which is better adapted to the murine intestinal tract. Kanamycin (Fig. 1B) and spectinomycin (Fig. 1D) enhanced ($P < 0.05$) survival of the spectinomycin- and kanamycin-resistant strain PAMG. Rifampin had a positive effect ($P < 0.05$) on strain BC6 (rifampin resistant) survival but decreased ($P < 0.05$) survival of *P. aeruginosa* AC869 and PAMG (Fig. 1C). Strain BC6 is resistant to rifampin, whereas strains PAMG and AC869 are sensitive to the drug at 50 μg/ml (Table 1). These findings support the initial hypothesis that antibiotic resistance would tend to enhance survival.

Translocation to the mesenteric lymph nodes is thought to be the first step in the spread of microorganisms from the GI tract to the spleen and liver (11, 32). Clindamycin promoted translocation of the gram-negative enteric microorganisms (2, 36). No gram-positive enterococci were observed in the mesenteric lymph nodes in clindamycin-treated animals (36). In gnotobiotic or mono-, di-, and triassociated mice, translocation to the mesenteric lymph nodes also occurs (3, 32).

Translocation of strains PAMG, AC869, and BC6 to the spleen was observed in spectinomycin- and clindamycin-treated mice. At the time points at which translocation to the spleen was detected, the dosed pseudomonads were recovered from 25% of the mice. In clindamycin-treated animals not administered pseudomonads, a PAMG-like pseudomonad was detected. Hentges et al. (19) reported translocation of a clinical *P. aeruginosa* isolate to the spleen in streptomycin-treated mice 1 week after dosing with 10^8 CFU. Streptomycin also promoted translocation of *S. typhimurium* to the spleen (28). The kanamycin results supported those of Hentges et al. (19), who found no translocation to the spleen in kanamycin-treated animals. In this study, translocation to the spleen was not dependent on detection of the administered pseudomonads in the liver.

Translocation to the liver in mice has been reported in streptomycin-treated animals for *P. aeruginosa* (20) and *S. typhimurium* (28). However, no translocation of *P. aeruginosa* to the liver was observed 1 week after dosing in clindamycin-, kanamycin-, ampicillin-, or metronidazole-treated animals (14). In this study (Table 3), translocation to the liver in animals treated with spectinomycin, clindamycin, kanamycin, or rifampin was observed. Translocation occurred in 25 to 50% of positive treatment groups. However, the observation period after dosing was considerably shorter than that reported by Hentges et al. (19), which may explain the difference in translocation results. It has been reported that antibiotic activity is present in the ceca of mice treated with kanamycin and clindamycin (19). This may have maintained an altered population longer, thereby promoting translocation more readily. When translocation to the liver or spleen was observed, the translocated organism was detectable in the intestinal tract.

In strain AC869-dosed mice, high numbers of organisms were recovered in one animal in the liver 48 (clindamycin) and 120 (kanamycin) h after dosing. It should be noted that the clindamycin-treated animal exhibited poor health after treatment, losing 9.5 g of body weight in 7 days. However, the kanamycin-treated animal only lost 1.5 g in 3 days and appeared in good health.

The results from this study are summarized in Table 4. *P. aeruginosa* AC869 and PAMG were resistant to most of the tested antibiotics, survived in the intestinal tract for 5 days in the presence of the antibiotics, and translocated to the liver and spleen. On the other hand, *P. cepacia* AC1100 was not detectable in any of the tissues even though it was resistant to two of the antibiotics. In the future, this model will be used to study other environmental strains or microbe-containing products.

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