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In Vitro Antibiotic Susceptibility of Pseudomonads Other than *Pseudomonas aeruginosa* Recovered from Cancer Patients

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The increase in occurrence of infections due to opportunistic gram-negative bacilli in patients with impaired host defenses emphasizes the need for information on the antibiotic susceptibility of the organisms that colonize such patients. During a 20-month period, more than 100 pseudomonads which were not *Pseudomonas aeruginosa* were recovered from cancer patients at the Baltimore Cancer Research Center. These included *P. fluorescens*, *P. putida*, *P. multivorans* (*cepacia*), *P. maltophilia*, *P. stutzeri*, *P. alcaligenes*, and *P. pseudoalcaligenes*. Susceptibility tests with 12 antibiotics indicated that the intraspecies antibiograms for many of these species were more uniform than those of *P. aeruginosa*. The stability of susceptibility patterns allowed the antibiograms to be used as aids in the preliminary differentiation of these organisms. Variable antibiogram patterns were noted among certain species, i.e., *P. fluorescens*, *P. stutzeri*, and *P. multivorans*, whereas each of the other species had essentially one pattern. These in vitro studies showed that some of the *Pseudomonas* species other than *P. aeruginosa* were resistant to a number of antibiotics. Among these were antibiotics that are in general use for *P. aeruginosa* infections. Such differences in antibiotic susceptibilities emphasize the necessity for careful speciation of this group of microorganisms to assure proper epidemiological documentation of colonization and infection, as well as to ensure therapy with an antimicrobial agent to which the organism is susceptible in vitro.

The occurrence of infections due to gram-negative bacilli, some of which were previously considered to be of little infectious significance, has increased in patients with serious underlying diseases (4, 5). Although *Pseudomonas aeruginosa* and bacilli of the family *Enterobacteriaceae* have been the microorganisms most commonly responsible for this increase in infection, the incidence of patient colonization or infection, or both, by other less common gram-negative bacilli has also become more frequent (5). Among these bacilli is a heterogeneous group of bacteria which frequently has been classified only as *Pseudomonas* spp. other than *P. aeruginosa*. With the advent of more clearly defined criteria for the characterization of the genus *Pseudomonas* (7, 14–16, 22, 23), proper speciation is now possible in the diagnostic laboratory.

During a 20-month period, 131 strains of *Pseudomonas* other than *P. aeruginosa* were recovered from clinical materials collected from 52 cancer patients at the Baltimore Cancer Research Center. This group included strains identified as *P. multivorans* (*cepacia*), *P. maltophilia*, *P. putida*, *P. stutzeri*, *P. fluorescens*, *P. pseudoalcaligenes*, and *P. alcaligenes*. Unlike *P. aeruginosa*, most of these species are usually considered to possess a low degree of pathogenicity and invasiveness. However, many of these organisms were recovered from patients who were on broad-spectrum antibiotic regimens designed to treat gram-negative bacillary sepsis; such cancer patients generally have impaired host defenses and tend to become infected with their own endogenous flora. These factors emphasized a need for information on the antibiotic susceptibilities of "non-aeruginosa" pseudomonads so that immediate appropriate therapy could be instituted in the event that one of the organisms should cause clinical infection. The present study was undertaken to determine antibiograms for these species and to compare them with the susceptibility patterns found for *P. aeruginosa*.

MATERIALS AND METHODS

Antimicrobial susceptibility testing of 131 pseudomonads was performed by a slight modification of the Bauer-Kirby method (2). Three drops of an 18-hr

broth culture of the test organism (an inoculum equivalent to the recommended standard) were added to 10 ml of tryptose phosphate broth. A Mueller Hinton plate (150 by 25 mm) with an agar depth of 5 mm was flooded with 2.5 ml of the resulting suspension. The plate was maintained in a slanted position for 5 min, the excess fluid was removed by suction, and the plate surface was allowed to dry for 15 min. Single high-potency discs of the following antibiotics were then placed on the agar surface: ampicillin (10 μg), cephalothin (30 μg), streptomycin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), kanamycin (30 μg), polymyxin B (300 units), colistin (10 μg), penicillin (10 units), gentamicin (10 μg), neomycin (30 μg), and carbenicillin (100 μg). After 30 min had been allowed for the antibiotics to diffuse into the medium, the plate was incubated for 18 hr at 30 C. Two plates with no more than eight discs per plate were used for each strain. Designations of responses as susceptible, intermediate, or resistant were made by measuring zone diameters (2). All strains were tested on at least two occasions.

RESULTS

A total of 64 strains of *P. multivorans*, 24 of *P. maltophilia*, 17 of *P. putida*, 13 of *P. fluorescens*, 10 of *P. stutzeri*, 2 of *P. pseudoalcaligenes*, and 1 of *P. alcaligenes* was recovered from the 52 patients. Because the small numbers of the last two species prevented adequate evaluation of their susceptibility patterns, they were not included in this study. A comparison of antimicrobial susceptibility patterns of the other five *Pseudomonas* species is shown in Table 1.

As expected, the five species were not affected by penicillin (data not shown) or by cephalothin. Four of the five species were usually resistant to ampicillin and streptomycin, as were three of them to tetracycline. Approximately half of the *P. stutzeri* strains were susceptible to streptomycin and tetracycline; *P. fluorescens* isolates were also usually susceptible to tetracycline. Chloramphenicol demonstrated little or no activity against *P. fluorescens*, *P. putida*, or *P. multivorans* but did inhibit *P. maltophilia* and *P. stutzeri*. The aminoglycoside antibiotics, kanamycin, gentamicin, and neomycin, were effective against most strains of *P. fluorescens*, *P. putida*, and *P. stutzeri* but had limited activity against *P. multivorans* and *P. maltophilia*. Carbenicillin failed to inhibit any of the strains of *P. putida* and *P. fluorescens* and more than half of the *P. maltophilia* and *P. multivorans* strains.

Polymyxin B and colistin were the most active of the antibiotics tested, as there were few or no resistant strains among four of the five *Pseudomonas* spp.; only among *P. multivorans* strains were the majority resistant to these two antimicrobial agents, 67.2 and 79.7%, respectively. Although these two antibiotics usually give similar results (19), unexpected differences were encountered in the present disc susceptibility tests. About half of the strains of *P. putida* that were susceptible to polymyxin B were only moderately so to colistin, and about 12% more of the *P. multivorans* strains were resistant to colistin than to polymyxin B.

As *P. aeruginosa* is the pseudomonad most frequently involved in patient infections at this hospital, a comparison of the most frequent antibiogram of this organism obtained at the Baltimore Cancer Research Center with those of the other species seemed of interest (Table 2). Antibiotic responses which all species shared with *P. aeruginosa* were resistance to ampicillin, cephalothin, and penicillin, and, with the excep-

TABLE 1. *Strain susceptibility responses of 5 Pseudomonas species to 11 antimicrobial agents*[a]

Antimicrobial agent	*P. maltophilia* (24)[b]			*P. stutzeri* (13)			*P. fluorescens* (10)			*P. putida* (17)			*P. multivorans* (64)		
	S[c]	MS[c]	R[c]	S	MS	R	S	MS	R	S	MS	R	S	MS	R
Cephalothin	0	4.2[d]	95.8	0	0	100	0	0	100	0	0	100	4.7	0	95.3
Ampicillin	0	8.3	91.7	46.2	0	53.8	0	0	100	0	0	100	0	3.1	96.9
Streptomycin	8.3	0	91.7	53.8	46.2	0	20	0	80	0	25.3	76.5	7.8	4.7	87.5
Tetracycline	12.5	0	87.5	53.8	15.4	30.8	30	50	20	11.8	0	88.2	18.8	1.5	79.7
Chloramphenicol	79.2	20.8	0	92.3	7.7	0	0	20	80	0	0	100	25.0	7.8	67.2
Kanamycin	8.3	0	91.7	46.2	38.4	15.4	90	10	0	88.2	11.8	0	12.5	7.8	79.7
Polymyxin B	95.8	4.2	0	100	0	0	100	0	0	94.6	0	5.4	29.7	3.1	67.3
Colistin	95.8	4.2	0	100	0	0	100	0	0	52.9	47.1	0	17.2	3.1	79.7
Gentamicin	12.5	4.2	83.3	100	0	0	70	30	0	29.4	47.1	23.5	26.6	0	73.4
Neomycin	12.5	0	87.5	69.2	30.8	0	50	50	0	23.5	71.2	5.4	9.4	9.3	81.3
Carbenicillin	16.7	25.0	58.3	100	0	0	0	0	100	0	0	100	37.5	10.9	51.6

[a] All strains were resistant to penicillin.
[b] Number of strains.
[c] S = susceptible; MS = intermediate; R = resistant.
[d] Percent.

tion of *P. multivorans*, susceptibility to polymyxin B and colistin. Reactions to the other antimicrobial agents were varied. *P. aeruginosa* was resistant to tetracycline, as were strains of *P. maltophilia*, *P. putida*, and *P. multivorans*. On the other hand, susceptibility to gentamicin was common among strains of *P. aeruginosa*, *P. stutzeri*, and *P. fluorescens*, whereas strains of *P. putida* showed intermediate susceptibility to this antimicrobial agent. Although *P. aeruginosa* and *P. putida* demonstrated only intermediate susceptibility to neomycin, both *P. fluorescens* and *P. stutzeri* were susceptible. Three species, *P. aeruginosa*, *P. maltophilia*, and *P. multivorans*, were resistant to kanamycin, whereas the other three species were susceptible. Major differences between the antibiograms of *P. aeruginosa* and those of the other species were observed with streptomycin, chloramphenicol, and carbenicillin. All *Pseudomonas* spp. except *P. aeruginosa* were resistant to streptomycin and carbenicillin, whereas the latter was intermediately susceptible and susceptible. Similarly, all species were resistant to chloramphenicol except for *P. aeruginosa* and *P. maltophilia*.

Antibiotic susceptibility testing of different serological types of *P. aeruginosa* has shown that their antibiograms do not necessarily remain stable and that the method of using the antibiogram to establish similarity of infecting strains would not be feasible for *P. aeruginosa* (11). However, it would appear that the antibiograms of the 5 "non-aeruginosa" species are relatively stable and their antibiograms could be used to determine interspecies similarities (Table 3). Of the 24 strains of *P. maltophilia*, 20 gave a pattern in which only

TABLE 2. *Comparison of a typical P. aeruginosa antibiogram to antibiograms found for five other Pseudomonas species*

Antimicrobial agent[a]	*P. aeruginosa*	*P. maltophilia*	*P. putida*	*P. fluorescens*	*P. stutzeri*	*P. multivorans*
Streptomycin	MS[b]	R[b]	R	R	S[b]	R
Tetracycline	R	R	R	MS	S	R
Chloramphenicol	S	MS	R	R	S	R
Kanamycin	R	R	S	S	S	R
Polymixin B	S	S	S	S	S	R
Colistin	S	S	S	S	S	R
Gentamicin	S	R	MS	S	S	R
Neomycin	MS	R	MS	S	S	R
Carbenicillin	S	R	R	R	S	R

[a] All strains were usually resistant to ampicillin, cephalothin, and penicillin.
[b] R = resistant; MS = intermediate; S = susceptible.

TABLE 3. *Frequently occurring antibiotic susceptibility patterns among five Pseudomonas species*

Organism	Antimicrobial agent[a]											No. of occurrences
	AM	CF	S	TE	C	K	PB	CL	GM	N	CB	
P. maltophilia	R[b]	R	R	R	MS[b]	R	S[b]	S	R	R	R, MS, S	20/24
P. putida	R	R	R	R	R	S	S	S	MS	MS	R	13/17
P. fluorescens												
Pattern a	R	R	R	MS	R	S	S	S	S	S	R	5/10
Pattern b	R	R	S	S	MS	S	S	S	S	S	R	3/10
P. stutzeri												
Pattern a	S	R	S	S	S	S	S	S	S	S	S	6/13
Pattern b	R	R	MS	R	S	S	S	S	S	S	S	4/13
P. multivorans												
Pattern a	R	R	R	R	R	R	R	R	R	R	R	34/64
Pattern b	R	R	R	R	S	R	R	R	R	R	R	6/64
Pattern c	R	R	R	MS	S	R	S	R	S	R	R	8/64
Pattern d	R	R	R	R	S	MS	S	R	R	R	R	8/64

[a] Abbreviations of antibiotics are as used on discs obtained from Difco: AM = ampicillin; CF = cephalothin; S = streptomycin; TE = tetracycline; C = chloramphenicol; K = kanamycin; PB = polymyxin B; CL = colistin; GM = gentamicin; N = neomycin; CB = carbenicillin.
[b] R = resistant; MS = intermediate; S = susceptible.

susceptibility to carbenicillin varied. Two patterns ("a" and "b") were apparent for *P. fluorescens*; these could be distinguished from each other in that susceptibility to streptomycin and chloramphenicol always occurred in pattern "a" and resistance to these agents in pattern "b." Two types of antibiograms also were found among the *P. stutzeri* strains; 6 of 13 had a pattern "a" which showed susceptibility to ampicillin and tetracycline whereas pattern "b" did not.

Among *P. multivorans* strains, four patterns were evident. One pattern, "a," was clearly predominant, as it occurred in 34 of 64 strains tested; strains with this pattern were resistant to the 12 antibiotics under study. The second pattern, "b," differed from the predominant one only in susceptibility to chloramphenicol. Strains with the last two patterns, "c" and "d," were susceptible to polymyxin B, as well as chloramphenicol, and the patterns were distinguished from each other in that pattern "c" showed susceptibility to tetracycline and gentamicin whereas pattern "d" did not.

The antibiograms of the species studied at the Baltimore Cancer Research Center could be easily utilized as a presumptive means of distinguishing them from one another by their susceptibility to five antibiotics, i.e., chloramphenicol, kanamycin, tetracycline, colistin, and carbenicillin (Table 4). *P. maltophilia* was susceptible to chloramphenicol and colistin but resistant to kanamycin and tetracycline; its susceptibility to carbenicillin varied. Although the patterns "a" of *P. putida* and *P. fluorescens* were remarkably similar in their susceptibility to kanamycin and colistin and in their resistance to chloramphenicol and carbenicillin, *P. putida* was resistant to tetracycline whereas *P. fluorescens* was susceptible to this agent. Pattern "b" of *P. fluorescens* was susceptible to tetracycline but differed from pattern "a" in that it was also susceptible to chloramphenicol. *P. stutzeri* was susceptible to most of the antibiotics; only strains with pattern "b" showed resistance to chloramphenicol. All patterns of *P. multivorans* were characterized by resistance to colistin and carbenicillin. Strains with pattern "a" were also resistant to the other antibiotics as well. Strains with patterns "b," "c," and "d" were all susceptible to chloramphenicol. Moreover, pattern "c" was distinguished from pattern "d" by susceptibility to tetracycline in pattern "c" and kanamycin susceptibility in pattern "d."

DISCUSSION

P. aeruginosa infections in man have proven to be particularly recalcitrant to antibiotic therapy even though in vitro tests show these organisms to be susceptible to certain antibiotics, e.g., streptomycin and chloramphenicol. More recently, therapy with carbenicillin and gentamicin, singly or in combination, (18) has proved to be clinically efficacious, and another new drug, nebramycin (17), holds promise. As the incidence of patient colonization with "non-aeruginosa" pseudomonads has increased, a comparison of the antibiotic susceptibilities of these organisms to those of *P. aeruginosa* was indicated. In vitro studies at the Baltimore Cancer Research Center showed that five "non-aeruginosa" species, i.e., *P. multivorans* (*cepacia*), *P. maltophilia*, *P. fluorescens*, *P. putida*, and *P. stutzeri*, were resistant to a variety of antibiotics, including those presently known to be useful for clinical infection due to *P. aeruginosa*. *P. maltophilia*, a species

TABLE 4. *Susceptibility patterns for five antibiotics useful for interspecies differentiation*

Organisms	Antimicrobial agent[a]				
	C	K	TE	CL	CB
P. maltophilia	MS[b]	R[b]	R	S[b]	S, MS, R
P. putida	R	S	R	S	R
P. fluorescens (a)	R	S	MS	S	R
(b)	MS	S	S	S	R
P. stutzeri (a)	S	S	R	S	S
(b)	S	S	R	S	S
P. multivorans (a)	R	R	R	R	R
(b)	S	R	R	R	R
(c)	S	R	S	R	R
(d)	S	MS	R	R	R

[a] Abbreviations of antibiotics are as used on discs obtained from Difco: C = chloramphenicol; K = kanamycin; TE = tetracycline; CL = colistin; CB = carbenicillin.

[b] R = resistant; MS = intermediate; S = susceptible.

reported to be of occasional medical importance (6), was usually resistant to 9 of 12 antibiotics tested. The incidence of colonization by *P. multivorans* (*cepacia*), a species recovered at other institutions from cases of endocarditis (20), pneumonitis (3), and other infectious processes (1, 9, 10, 13, 21, 24), has increased in patients at the Baltimore Cancer Research Center. The majority of strains from this hospital were resistant to 11 antimicrobial agents and over 50% were resistant to all 12 antibiotics. *P. putida* and *P. fluorescens* were both resistant to more than half of the antibiotics. In comparison, *P. aeruginosa* was typically resistant to only five antimicrobial agents and was, except for *P. stutzeri*, susceptible in vitro to more antibiotics than were the other species investigated.

Moody et al. (11) reported that the antibiograms of different serological types of *P. aeruginosa* do not necessarily remain stable in strains of the same types isolated from different sites or from the same site over time, and that susceptibility patterns could not be used as a method of establishing identity of or similarity of infecting strains. However, the antibiograms of these five *Pseudomonas* species appear to be quite stable as compared with those of *P. aeruginosa*. This investigation and others (8, 12, 25) indicate that the susceptibility patterns of these five species can be used as an aid in their identification.

The change noted in *P. multivorans* from susceptibility to resistance to carbenicillin during the investigation may reflect the introduction of carbenicillin-gentamicin therapy for *P. aeruginosa* infections (18). For approximately 1 year after initiation of such therapy in our hospital, only a few resistant strains were encountered. It is assumed that selection of resistant strains has occurred as only these strains were recovered from patients during the last 8 months of the study. An increased incidence of recovery was also noted. During this latter period, two carbenicillin-susceptible strains were recovered from the environment. Although *P. multivorans* has not caused proven infection thus far in our patients (it was recovered from the lungs of a patient at autopsy), it has been reported to cause human infection (1, 3, 9, 10, 13, 18, 21, 24). The spread of this relatively resistant microorganism among high risk patients could conceivably be creating the appropriate circumstances needed for an opportunistic microorganism to become a new "hospital strain" capable of causing nosocomial infections.

The antibiograms of the *Pseudomonas* spp. studied clearly show great differences in their in vitro antibiotic susceptibilities. From the data presented, careful speciation of pseudomonads, especially those recovered from human clinical materials, in addition to antibiotic susceptibility testing, is essential for the effective control of these potentially infectious agents.

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