ORIGINAL ARTICLE

Resistance of Pseudomonas aeruginosa to gentamicin

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Summary: The resistance to gentamicin 4 μg./ml. of 250 *Pseudomonas aeruginosa* isolates was measured by a proportion method. Twenty-eight (11.2%) of the cultures fell into the most resistant group, in whose populations between 10 and 100% of the organisms were resistant. A relatively high percentage of urinary isolates and a comparatively low percentage of isolates from respiratory sources occurred in this group. Three of the 28 were resistant to carbenicillin 150 μg./ml. and 6 of 18 tested were as resistant to gentamicin 8 μg./ml. as they were to 4 μg./ml. The distribution of *Ps. aeruginosa* isolates between the different grades of resistance did not change significantly during the 10 months in which the survey was performed.

Résumé: *La résistance du* Pseudomonas aeruginosa *à la gentamicine*

Les chercheurs ont mesuré, par une méthode proportionnelle, la résistance à la gentamicine (4 μg/ml) de 250 isolats de *Ps. aeruginosa*. Vingt-et-une des cultures (soit 11.2%) étaient dans le groupe le plus résistant, parmi les populations dont les microbes étaient résistants dans la proportion de 10 à 100%. Dans ce groupe, on a découvert un pourcentage relativement élevé de spécimens urinaires et un pourcentage relativement faible d'isolats provenant de l'arbre respiratoire. Trois des 28 isolats étaient également résistants à la carbénicilline (150 μg/ml) et parmi les 18 spécimens essayés, 6 étaient aussi résistants à la concentration de 8 μg/ml de gentamicine qu'ils l'étaient à celle de 4 μg/ml. La distribution des isolats de *Ps. aeruginosa* entre les diverses catégories de résistance n'a guère changé durant les 10 mois de l'étude.

Pseudomonas aeruginosa is an opportunist pathogen of increasing concern in hospitals.[1-5] It is responsible for serious infections of burns and wounds, is one of several agents causing urinary infections in patients who have some structural or neurogenic abnormality in the urinary tract, and is sometimes responsible for pneumonia, meningitis, eye infections and septicemia. The organism frequently colonizes other sites such as tracheostomy wounds,[6] and remains a potential pathogen in this situation.

Control of *Ps. aeruginosa* infections is difficult, partly because of the inherent resistance of these organisms to most available antibiotics. At present, gentamicin, colimycin and carbenicillin are the agents most useful against *Ps. aeruginosa*, although tobramycin may well compete with these in the future.[7,8]

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Various workers have reported *Ps. aeruginosa* cultures resistant *in vitro* to concentrations of gentamicin in excess of those safely attainable in the blood.[9,10] In order to assess the current prevalence of gentamicin-resistant *Ps. aeruginosa* in one area, a survey of *Ps. aeruginosa* cultures isolated from specimens received by the Microbiology Laboratory of Kingston General Hospital was conducted from June 1972 until March 1973.

Materials and methods

Two hundred and fifty *Ps. aeruginosa* isolates were obtained from urine, sputum or tracheal secretions, burns, chronic skin ulcers, ear swabs and a few miscellaneous sites in patients. Only one isolate from any individual patient was included in the study. No distinction was made between *Ps. aeruginosa* isolates acting as pathogens or those merely colonizing a patient site. All cultures were identified as *Ps. aeruginosa* by a positive oxidase reaction, gram-negative morphology, oxidative breakdown of glucose in Hugh and Leifson medium, hydrolysis of arginine, oxidation of gluconate and reduction of nitrate. Cultures were pyocine-typed by Tripathy and Chadwick's modification[11] of the Gillies and Govan method.[12]

Gentamicin was obtained in powder form as gentamicin sulfate with a potency of 592 μg./mg. (Schering Corporation Limited, Pointe Claire, Quebec).

Gentamicin sensitivity was estimated by a quantitative method described by Chadwick,[13] based on the response of the organism to a critical concentration, 4 μg./ml. Parallel viable counts, by the Miles and Misra method,[14] were performed on six-hour Mueller-Hinton broth cultures of each isolate, (a) on agar made from Mueller-Hinton broth and Bacto-agar and (b) on the same medium containing gentamicin 4 μg./ml.

The response to gentamicin was expressed in terms of the proportion of resistant organisms in the total bacterial population. Different grades of response were defined as ranges rather than absolute figures, because previous investigations[13] had shown slight variation in response between replicate cultures set up from the same isolate.

Carbenicillin sensitivity was measured by an agar dilution method using 0.02-ml. drops from a 10^{-1} dilution of a six-hour broth culture as inoculum, and carbenicillin concentrations ranging through seven steps from 12.5 to 150 μg./ml. The basal medium, also used as a control agar, was made from Mueller-Hinton broth and Bacto-agar, as for gentamicin. Minimal inhibitory concentrations (MICs) of carbenicillin, in μg./ml., were defined as the lowest tested concentration causing significant reduction of growth on the agar.

Results

Distribution of responses to gentamicin 4 µg./ml. and relation to source of isolates

The distribution of responses for 250 isolates of *Ps. aeruginosa* is shown in Table I. Each grade of response was represented by a considerable number of isolates, although the largest number occurred in the 10^{-6} to 10^{-5} and the 10^{-5} to 10^{-4} groups. The distribution did not change significantly over the 10-month survey period. The most resistant group, in whose populations 10 to 100% of the bacteria were resistant, included 28 cultures and made up 11.2% of the total.

The table also shows the distribution, according to source, of isolates with different grades of response. With the exception that none of the ear cultures fell into the most sensitive group, all grades of response were represented by isolates from all sources. Urinary isolates were relatively more frequent in the more resistant groups, and those from the respiratory tract showed a reverse trend, 39 of the 54 (72%) falling into the three most sensitive groups. Of the isolates classified under septic lesions, 11 of 40 (27.5%) were in the most sensitive group, while the remainder were distributed haphazardly between the more resistant grades. The sources in this group were varied and included superficial septic lesions such as cellulitis or infected ulcers, postoperative wound infections, inflammatory lesions in deeper tissues, and infected burns. However, a breakdown of isolates into these different subcategories did not show any distribution of responses different from the main group.

Carbenicillin sensitivity of the 250 isolates

In Table II is shown the carbenicillin sensitivity of the *Ps. aeruginosa* isolates with different grades of gentamicin response. The groups most sensitive and most resistant to gentamicin showed a higher percentage of their members resistant to carbenicillin 50 µg./ml. than did the intermediate groups. This was also true for the cultures resistant to more than 150 µg./ml. of carbenicillin, although the numbers involved were smaller.

Features of the most resistant isolates

Some of the associated features of the 28 isolates in the most resistant group may be derived from Tables I and II. Seventeen of the 28 (61%) were obtained from urine, in contrast with 115 of the 250 in the whole series, or 46%. Three isolates (11%) were resistant to carbenicillin at a level of 150 µg./ml. and 11 (39%) were resistant to 50 µg./ml. of this antibiotic. Corresponding percentages for the whole series were 6 and 20 respectively.

Seven of the 28 isolates showed complete resistance to gentamicin 4 µg./ml., in that their viable counts on gentamicin and control media were virtually identical. Eighteen of the 28 were tested for their response to gentamicin 8 µg./ml. by the same method as used for measuring the response to 4 µg./ml.; six showed more than 10% of their bacterial populations resistant to the higher concentration.

Only three of the 28 patients from whom these resistant isolates were obtained had received gentamicin therapy prior to isolation of the *Ps. aeruginosa.*

Twelve distinguishable pyocine patterns were found among the 28 cultures.

Discussion

Garrod and O'Grady[15] recommended that for effective antibiotic therapy the concentration of drug in the blood should exceed the MIC for that organism by several-fold, for most of the time between one dose and the next. Darrell and Waterworth[16] have pointed out that a considerable number of *Ps. aeruginosa* isolates have an MIC of at least 4 µg./ml. Garrod and O'Grady's[15] principle could only be

Table I—Distribution of 250 *Ps. aeruginosa* isolates in terms of response to gentamicin 4 µg/ml. and source of organism

Proportion of resistant organisms in bacterial population	Number of isolates from different sources					Total isolates	Percentage
	Urine	Respiratory tract	Septic lesions	Ear	Other		
$<10^{-6}$	13	11	11	0	4	39	15.6
$10^{-6}-10^{-5}$	20	14	4	8	3	49	19.6
$10^{-5}-10^{-4}$	23	14	8	6	2	53	21.2
$10^{-4}-10^{-3}$	10	2	5	1	2	20	8.0
$10^{-3}-10^{-2}$	14	7	3	4	2	30	12.0
$10^{-2}-10^{-1}$	18	4	6	2	1	31	12.4
$10^{-1}-10^{-0}$	17	2	3	4	2	28	11.2
Totals	115	54	40	25	16	250	100.0

Table II—Carbenicillin sensitivity of 250 *Ps. aeruginosa* isolates in relation to response to gentamicin 4 µg/ml.

Proportion of organisms in bacterial population esistant to gentamicin 4 µg./ml.	Number of isolates for which MIC of carbenicillin was			Total isolates	Percentage of isolates resistant to carbenicillin	
	12.5 — 50 µg./ml.	75 — 150 µg./ml.	>150 µg./ml.		50 µg./ml.	>150 µg./ml.
$<10^{-6}$	28	5	6	39	28	15
$10^{-6}-10^{-5}$	42	3	4	49	14	8
$10^{-5}-10^{-4}$	45	7	1	53	15	2
$10^{-4}-10^{-3}$	19	1	0	20	5	0
$10^{-3}-10^{-2}$	26	4	0	30	13	0
$10^{-2}-10^{-1}$	23	7	1	31	26	3
$10^{-1}-10^{-0}$	17	8	3	28	39	11
Totals	200	35	15	250		

MIC = minimal inhibitory concentration

realized if the serum gentamicin level were maintained at a minimum of 8 μg./ml. for much of the time between doses. Gentamicin concentrations in the serum of the order of 10 μg./ml. are considered toxic.[17] Therefore the margin between effective therapeutic and toxic concentrations is small.

In this study, 11.2% of 250 *Ps. aeruginosa* isolates showed a degree of resistance to gentamicin 4 μg./ml. such that 10% or more of their bacterial populations grew normally in the presence of this drug concentration. The observations presented in this paper suggest that about one third of the isolates showing this grade of resistance would be similarly resistant to 8 μg./ml. susceptibility.

Whether a quantitative measure of bacterial sensitivity to an antibiotic is expressed in terms of minimal inhibitory concentration or proportional resistance, definition of a critical level of resistance beyond which no clinical response can be expected is difficult. The outcome of antibiotic therapy depends not only on the *in vitro* susceptibility of the organism but also on the penetration of adequate quantities of antibiotic to the site of infection, the virulence of the organisms and the efficiency of host defence mechanisms such as phagocytosis. The expression of microbial resistance in terms of proportion, as done here, allows the definition of several grades of resistance to a critical antibiotic concentration, and may facilitate a study of clinical response in terms of resistance level. Meanwhile, it would not be unreasonable to suppose that resistance of 10% or more of the bacterial population to gentamicin 4 μg./ml. may preclude successful therapy with this agent, with the reservation that lower grades of resistance, e.g. involving 1% of the population, may also be significant, especially if the infecting organisms are relatively inaccessible to the antibiotic.

The proportion method for measuring antibiotic sensitivity has been used by Canetti *et al*[18] for *Mycobacterium tuberculosis*. These workers considered that the critical proportion of resistant organisms would be 1, 10 or 50%, depending on the drug concentration used for testing.

Interpretation of the resistance level may require modification in the case of urinary infection since the concentration of gentamicin attained in the urine after therapeutic doses is much higher than that in the blood. Riff and Jackson[19] reported that the gentamicin concentration in the urine of 40 patients with normal renal function, after a dose of 1.5 mg./kg./day, ranged from 10 to 125 μg./ml. Clearly if the object of therapy is to eradicate organisms from the urine, measurement of resistance to 4 μg./ml. has no clinical relevance. However, patients with *Ps. aeruginosa* infections of the urinary tract may have, at some stage, renal involvement, and are also candidates for septicemia due to invasion of the bloodstream by organisms in the primary urinary focus. In either event the degree of resistance of *Ps. aeruginosa* to attainable blood and tissue concentrations of gentamicin again becomes important. It is interesting that, in this investigation, a relatively high proportion of the 28 isolates in the most resistant group were obtained from patients with urinary infections. This may lessen the importance of resistance but does not eliminate it.

Besides gentamicin, carbenicillin and polymyxins B and E are available for therapy of *Ps. aeruginosa* infections. Carbenicillin is a nontoxic drug which can be given in doses of up to 30 g. daily, and will in many instances be the antibiotic of choice for a *Ps. aeruginosa* infection. Although there are several reports[20,21] of resistance of *Ps. aeruginosa*, isolated from patients, to high concentrations of carbenicillin, other workers[22,23] have reported a synergistic effect between gentamicin and carbenicillin, suggesting that significant resistance to gentamicin or carbenicillin may be overcome by using a combination of these two agents. This combination, however, will not be appropriate for a patient with a history of allergy to penicillins. The choice here may be between a polymyxin or tobramycin.

A point of great concern to those involved in the management of infectious disease is the possibility of increasing prevalence of antibiotic-resistant pathogenic bacteria. This is already a problem with certain types of enterobacteria resistant to many antibiotics by virtue of their transmissible R-factors.[24-26] *Ps. aeruginosa* has not been prominent in papers on infectious drug resistance but in a few instances R-factor-determined resistance in this organism has been reported.[27,28] It is important at the present time to be aware of trends towards increasing resistance, so that suitable modification of antibiotic policies may be made. This investigation demonstrates that there is, in this area, a small focus of gentamicin-resistant *Ps. aeruginosa* which does not appear to be increasing in extent, but which may be a cause for concern in future years.

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References

1. Fierer J, Taylor PM, Gezon HM: *Pseudomonas aeruginosa* epidemic traced to delivery-room resuscitators. *N Engl J Med* 276: 991, 1967
2. Jellard CH, Churcher GM: An outbreak of *Pseudomonas aeruginosa* (pyocyanea) infection in a premature baby unit, with observations on the intestinal carriage of *Pseudomonas aeruginosa* in the newborn. *J Hyg (Camb)* 65: 219, 1967
3. Ayliffe GAJ, Lowbury EJL, Hamilton JG, et al: Hospital infection with *Pseudomonas aeruginosa* in neurosurgery. *Lancet* II: 365, 1965
4. Dexter F: *Pseudomonas aeruginosa* in a regional burns centre. *J Hyg (Camb)* 69: 179, 1971
5. Tinne JE, Gordon AM, Bain WH, et al: Cross-infection by *Pseudomonas aeruginosa* as a hazard of intensive surgery. *Br Med J* IV: 313, 1967
6. Lowbury EJL, Thom BT, Lilly HA, et al: Sources of infection with *Pseudomonas aeruginosa* in patients with tracheostomy. *J Med Microbiol* 3: 39, 1970
7. Meyer RD, Young LS, Armstrong D: Tobramycin (Nebramycin factor 6): in vitro activity against *Pseudomonas aeruginosa*. *Appl Microbiol* 22: 1147, 1971
8. Traub WH, Raymond EA: Evaluation of the in vitro activity of tobramycin as compared with that of gentamicin sulphate. *Appl Microbiol* 23: 4, 1972
9. Klein JO, Eickhoff TC, Finland M: Gentamicin; activity in vitro and observations in 26 patients. *Am J Med Sci* 248: 528, 1964
10. Weinstein MJ, Drube CB, Moss EL, et al: Microbiologic studies related to bacterial resistance to gentamicin. *J Infect Dis* 124 (suppl): 811, 1971
11. Tripathy GS, Chadwick P: The effect of mitomycin C on the pyocine typing patterns of hospital strains of *Pseudomonas aeruginosa*. *Can J Microbiol* 17: 829, 1971
12. Gillies RR, Govan JRW: Typing of *Pseudomonas pyocyanea* by pyocine production. *J Pathol Bacteriol* 91: 339, 1966
13. Chadwick P: An approach to quantitative antibiotic sensitivity testing for some gram-negative pathogens. *Can J Microbiol* 18: 1145, 1972
14. Miles AA, Misra SS: The estimation of the bactericidal power of the blood. *J Hyg (Camb)* 38: 732, 1938
15. Garrod LP, O'Grady F: *Antibiotics and Chemotherapy*, third ed. Edinburgh and London, E&S Livingstone, 1971, p 268
16. Darrel JH, Waterworth PM: Dosage of gentamicin for Pseudomonas infections. *Br Med J* II: 535, 1967
17. Gingell JC, Waterworth PM: Dose of gentamicin in patients with normal renal function and renal impairment. *Br Med J* I: 19, 1968
18. Canetti G, Fox W, Khomenko A, et al: Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. *Bull WHO* 41: 21, 1969
19. Riff LJ, Jackson GG: Pharmacology of gentamicin in man. *J Infect Dis* 124 (suppl): 98, 1971
20. Bell SM, Smith DD: Resistance of *Pseudomonas aeruginosa* to carbenicillin. *Lancet* I: 753, 1969
21. Lowbury EJL, Kidson A, Lilly HA, et al: Sensitivity of *Pseudomonas aeruginosa* to antibiotics, emergence of strains highly resistant to carbenicillin. *Lancet* II: 448, 1969
22. Yuce K, van Rooyen CE: Carbenicillin and gentamicin in the treatment of *Pseudomonas aeruginosa* infection. *Can Med Assoc J* 105: 919, 1971
23. Klastersky J, Swings G, Daneau D: Antimicrobial activity of the carbenicillin/gentamicin combination against gram-negative bacilli. *Am J Med Sci* 260: 373, 1970
24. Gardner P, Smith DH: Studies on the epidemiology of resistance (R) factors. *Ann Intern Med* 71: 1, 1969
25. Hinshaw V, Punch J, Allison MJ, et al: Frequency of R factor-mediated multiple drug resistance in *Klebsiella* and *Aerobacter*. *Appl Microbiol* 17: 214, 1969
26. Bergfors S, Burman LG, Eklof P, et al: Transferable and non-transferable drug-resistance in enteric bacteria isolated from urinary specimens in Northern Sweden. *Acta Pathol Microbiol Scand [B]* 80: 511, 1972
27. Roe E, Jones RJ, Lowbury EJL: Transfer of antibiotic resistance between *Pseudomonas aeruginosa, Escherichia coli* and other gram-negative bacilli in burns. *Lancet* I: 149, 1971
28. Sykes RB, Richmond MH: Intergeneric transfer of a B-lactamase gene between *Ps. aeruginosa* and *E. coli*. *Nature* 226: 952, 1970