ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1977, p. 655-659

Vol. 12, No. 6
Printed in U.S.A.

Polymyxin B and Rifampin: New Regimen for Multiresistant *Serratia marcescens* Infections

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Received for publication 28 June 1977

Polymyxin B and rifampin were given to 12 patients with multi-drug-resistant nosocomial *Serratia marcescens* infections. Eight cures were achieved; drug hepatotoxicity occurred once; one fatal suprainfection was encountered; and two patients died during therapy of causes related to severe underlying illnesses. Polymyxin B and rifampin were uniformly synergistic in vitro against the infecting strains and against 40 additional clinical isolates of *S. marcescens*.

Serratia marcescens has become established as an important nosocomial pathogen in recent years (3, 8). The upsurge in infections due to this bacterium, previously considered to be of low virulence, is reminiscent of the phenomenon noted earlier in the antibiotic era with *Pseudomonas aeruginosa* because many nosocomial *Serratia* are resistant to antibiotics commonly used in hospital practice. An endemic involving multiresistant *Serratia* at the Little Rock Veterans Administration Hospital (LRVAH) necessitated development of a novel therapeutic regimen. Polymyxin B and rifampin are synergistic in vitro for this species (12, 14) but have not been used together in patients. This study was consequently undertaken to evaluate the extent of antibiotic resistance among *Serratia* at LRVAH, to determine their susceptibility to polymyxin B and rifampin, and to assess the efficacy and toxicity of therapy with polymyxin B and rifampin in patients seriously ill with infections due to multiresistant *Serratia*.

MATERIALS AND METHODS

Patient selection. During a 5-month period, from May through September 1976, all patients seen in consultation by the Infectious Disease Service at LRVAH who met all the following criteria were treated with polymyxin B and rifampin: (i) presence of a local infection characterized by symptoms and signs typical of bacterial infection in that site, a systemic response of fever, leukocytosis and change in general well-being, and isolation of *Serratia* in pure culture from involved tissue or fluid (except in respiratory tract infections, for which the criteria of Tillotson and Finland [13] were used); (ii) in vitro resistance of the infecting *Serratia* isolate to antibiotics available to treat systemic infections, or failure of previous antibiotic therapy to eradicate the infection; (iii) in vitro susceptibility, by standards set forth below, to polymyxin B-rifampin.

Therapy with polymyxin B and rifampin. Patients with normal renal function were given 2.5 mg of polymyxin B per kg per day intravenously in two doses spaced by intervals of 12 h. The dose of polymyxin B was reduced in the presence of renal insufficiency, according to previous recommendations (4). Twenty milligrams of rifampin per kilogram per day was given in divided doses by mouth 2 h before each infusion of polymyxin B. The total dose of rifampin did not exceed 1.2 g daily.

Complete blood count, urinalysis, and serum levels of creatinine, bilirubin, glutamic-oxaloacetic transferase, and alkaline phosphatase were obtained before therapy with polymyxin B and rifampin. Urinalysis, serum creatinine, and liver function studies were then obtained every other day during therapy. Any abnormality encountered among these studies was presumed to be a manifestation of drug toxicity and led to immediate cessation of therapy, unless an alternative explanation for the abnormality was apparent.

Evaluation of response to polymyxin B-rifampin. Responses to polymyxin B and rifampin were classified as favorable, if clinical and bacteriological cures were achieved, or adverse. Clinical and bacteriological cure was designated if *Serratia* was eradicated from cultures of infected tissue and if signs of infection disappeared during therapy. Included as adverse responses were cases of antibiotic failure, suprainfection, drug toxicity, or death during therapy. Antibiotic failure was diagnosed if *Serratia* persisted in cultures of infected tissue or if signs of infection persisted without an alternative cause. Drug toxicity was designated when signs related to the antibiotics, as delineated above, were encountered. Suprainfection was indicated when signs of infection reappeared during therapy after initial response to polymyxin B and rifampin and when cultures of involved tissue showed a new species of bacteria. Finally, in patients who died during therapy, evidence was sought of persisting infection, severe underlying disease, and drug tox-

icity. If the information available failed to permit a definite conclusion regarding the results of therapy with polymyxin B and rifampin, the patient was declared unsuitable for evaluation.

In vitro studies. The *Serratia* isolate from each patient was tested for susceptibility to polymyxin B and rifampin by a standard two-dimensional broth-dilution checkerboard technique (10). The studies were performed in brain heart infusion broth inoculated with 10^5 colony-forming units. Previous definitions for synergy in this system were used (15), but, in addition, an organism was considered susceptible only if the minimal inhibitory concentration (MIC) of the more active antibiotic in the combination occurred at a concentration less than one-half that of drug that could be expected in serum after administration of the doses used for treating patients.

Growth-curve experiments (5) were performed on the infecting *Serratia* strains from four patients treated with polymyxin B and rifampin. Portions of brain heart infusion broth containing no antibiotic or polymyxin B and rifampin (5 μg/ml, combined and separately) were inoculated with 10^5 to 10^6 colony-forming units/ml. The number of viable organisms per milliliter was then determined by pour-plate counting after 1, 2, 3, 6, and 24 h of incubation.

Forty additional *Serratia* isolates were collected from the clinical microbiology laboratory at LRVAH for further studies in vitro. This was done to determine the prevalence of polymyxin B-rifampin synergy toward *Serratia* at this hospital. A method was devised to test these isolates for polymyxin B-rifampin synergy, using a semiautomated microtiter system (Handititer 2, Ames Co., Elkhart, Ind.). Five longitudinal rows of wells on one microtiter plate (Autotray, Ames Co.) were used for successive twofold dilutions of five solutions in Mueller-Hinton broth: (i) 200 μg of polymyxin B per ml, added to well one, row one; (ii) 50 μg of rifampin per ml, added to well one, row two; (iii) 25 μg of polymyxin B per ml, and 12.5 μg of rifampin per ml, added to well one, row three; (iv) 25 μg each of polymyxin B and rifampin per ml, added to well one, row four; (v) 12.5 μg of polymyxin B per ml and 25 μg of rifampin per ml, added to well one, row five. After the dilutions were performed, a single *Serratia* strain was inoculated simultaneously into all the wells (inoculator apparatus for autotiter series, Ames Co.) to give a concentration of 10^5 colony-forming units/ml. The five inhibitory end points thus derived after overnight incubation were used to construct a modified isobologram consisting of the MIC for each antibiotic alone and those for the two drugs mixed together in concentration ratios of 2:1, 1:1, and 1:2. These isobolograms easily permitted an assessment of synergy by standard criteria. The microtiter system was also used to determine the susceptibility of these 40 *Serratia* isolates to gentamicin, amikacin, and chloramphenicol.

RESULTS

Outcome of therapy with polymyxin B and rifampin. Fourteen patients met the criteria for treatment with polymyxin B and rifampin; all were treated. Two cases were not evaluated because protocol for drug administration was not followed; in one instance, the patient was not cooperative, and, in the other, the supervising physician inadvertently amended the regimen. Limited follow-up of these two cases did not reveal evidence of treatment failure or drug toxicity.

The 12 *Serratia* infections we could evaluate included five that were bacteremic (Table 1). Eleven patients had significant underlying illnesses, and a like number received antibiotic therapy before treatment with polymyxin B and rifampin. Intravenous devices were present in 10 patients and bladder catheters in 7 when infection was diagnosed.

A total of 8 (67%) of the 12 cases, including all 5 patients with *Serratia* bacteremia, had favorable responses and were cured clinically and bacteriologically. These infections responded to polymyxin B and rifampin with prompt defervescence, with resolution of other signs of sepsis, and with appropriate amelioration of local infection. Therapy was terminated after 10 days in all responders except patient 11, who had osteomyelitis and received therapy for 6 weeks. Three patients died while receiving polymyxin B-rifampin therapy. Treatment of the infection was progressing satisfactorily in two (patients 8 and 10), but the patients succumbed to severe underlying illnesses. The other fatal case (patient 12) was related to suprainfection with *Proteus mirabilis* at the original *Serratia* infection site. Drug toxicity was encountered in one patient (case 7); jaundice and abnormal liver function studies were noted on day 3 of therapy and regressed promptly when antibiotics were discontinued.

One patient (case 1 and 9) was treated for two distinct *Serratia* infections. He developed *Serratia* pneumonia and was cured, but 2 weeks later acquired, and was successfully treated for, septic thrombophlebitis, a bacteremic illness associated with an intravenous catheter. In the interim period, *Serratia* was not present in any culture.

In vitro synergy of polymyxin B and rifampin against *Serratia*. All infecting strains were moderately resistant to rifampin (all MIC values were greater than 6.25 μg/ml) and highly resistant to polymyxin B (all MIC values were greater than 100 μg/ml). However, when combined in the checkerboard studies, the antibiotics acted synergistically against all 12 strains from infected patients. An isobologram derived from a representative study is depicted in Fig. 1. These results were confirmed by those of growth-curve studies. Antibiotic concentra-

TABLE 1. *Characteristics of patients with multiresistant Serratia infections and outcome of therapy with polymyxin B and rifampin*

Infection type	Patient data			Primary infection	Underlying disease	Preceding antibiotics	Length of PB-R therapy[a] (days)	Outcome of PB-R therapy
	Case	Age	Sex					
Bacteremic infections								
	1	50	M	Septic thrombophlebitis	Meningitis	Ampicillin	10	Clinical and bacteriological cure
	2	61	M	Septic thrombophlebitis	COPD[b]	Cephalothin	10	Clinical and bacteriological cure
	3	65	F	Subclavian catheter tip	Ca tongue[c]	Gentamicin	10	Clinical and bacteriological cure
	4	64	M	Urinary tract	Stroke	Gentamicin	10	Clinical and bacteriological cure
	5	78	M	Urinary tract	Prostatic hypertrophy	Gentamicin	10	Clinical and bacteriological cure
Nonbacteremic infections								
	6	65	M	Urinary tract	Parkinson's disease	Chloramphenicol	10	Clinical and bacteriological cure
	7	80	M	Urinary tract	Ca prostate	None	3	Drug toxicity
	8	65	M	Pneumonia	*Staphylococcus aureus* endocarditis	Nafcillin Gentamicin	7	Death due to underlying disease
	9	50	M	Pneumonia	Meningitis	Gentamicin Tobramycin TMP-SMZ[d] Chloramphenicol	10	Clinical and bacteriological cure
	10	60	F	Wound	Acute myelogenous leukemia	Chloramphenicol Gentamicin	8	Death due to underlying disease
	11	38	M	Osteomyelitis	None	Gentamicin	6 weeks	Clinical and bacteriological cure
	12	62	M	Peritonitis	Acute renal failure	Gentamicin	5	Death due to suprainfection

[a] PB-R, Polymyxin B and rifampin.
[b] COPD, Chronic obstructive pulmonary disease.
[c] Ca, Carcinoma.
[d] TMP-SMZ, Trimethoprim-sulfamethoxazole.

tions attainable in serum (5 μg/ml) sterilized broth cultures of four infecting strains within 24 h, whereas that concentration of each drug alone had no influence on growth of the bacteria (Fig. 2).

Results of susceptibility testing of 52 *Serratia* strains from LRVAH (12 treated patients and 40 additional isolates) to polymyxin B and rifampin are shown in Table 2. Fifty-one of the 52 strains were inhibited by a solution containing 3.1 μg of both drugs per ml, concentrations easily achievable in serum. In addition, all isolates had at least a fourfold decrease in the MIC for rifampin, the more potent of the two drugs, in the presence of an equal concentration of polymyxin B.

Only ten (19%) of these 52 *Serratia* strains were inhibited by 6.3 μg of gentamicin per ml, a concentration attainable in serum. Susceptibility to chloramphenicol among these isolates was also low (12%), but all 52 isolates were susceptible to 6.3 g of amikacin per ml.

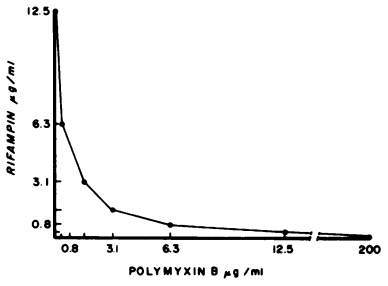

FIG. 1. *Synergistic inhibition of S. marcescens (patient 6) by polymyxin B and rifampin. Isobologram was derived by the broth-dilution checkerboard method (10), and the points correspond to antibiotic concentrations, individually and combined, at which growth inhibition occurred.*

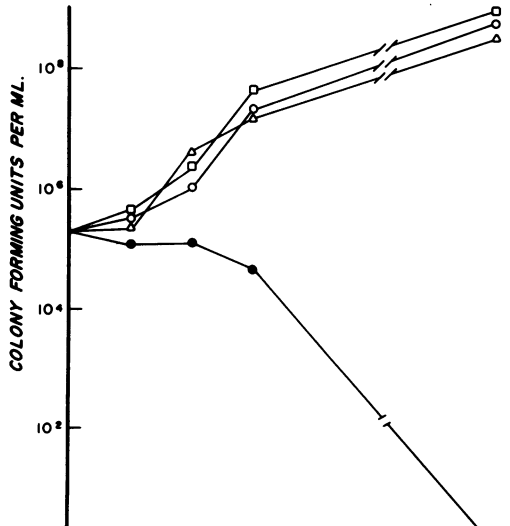

FIG. 2. *Effect of polymyxin B and rifampin, individually and combined, on growth of S. marcescens (patient 6) in brain heart infusion broth. Symbols: (○) no added antibiotic; (□) 5 μg of rifampin per ml added; (△) 5 μg of polymyxin B per ml added; (●) 5 μg each of polymyxin B and rifampin per ml.*

DISCUSSION

The present study confirms previous reports of polymyxin B and rifampin synergy against *Serratia* (12, 14) and extends those observations by demonstrating the efficacy of polymyxin B and rifampin in serious *Serratia* infections. Three of the four patients who failed to respond to polymyxin B and rifampin had severe underlying illnesses, an established risk factor in the treatment of gram-negative bacillary infections (6). Drug toxicity was a major concern in the use of polymyxin B and rifampin in elderly patients with a variety of preexisting illnesses, but careful monitoring revealed only one instance, and in that case the signs of hepatic dysfunction regressed promptly upon withdrawal of drugs.

Gentamicin has been considered the drug of choice for infections due to *Serratia*. However, recent nosocomial outbreaks have been characterized by resistance to gentamicin and to most other antibiotics appropriate to treat systemic infection. The current rate of *Serratia* resistance to gentamicin at LRVAH is 81%. Accordingly, it would no longer be wise to choose gentamicin empirically in a nosocomial infection due to *Serratia* unless in vitro testing showed that the isolate was susceptible.

Amikacin, a new aminoglycoside antibiotic, has excellent potential for use in infections such as those treated in the present study. Most aerobic gram-negative bacilli that are resistant to gentamicin are susceptible to amikacin, and clinical trials have proved the value of this drug in infections caused by organisms resistant to gentamicin, including *Serratia* (7, 11). We presently consider amikacin as the drug of choice for multiresistant *Serratia* infections. However, three recent articles report resistance emerging to amikacin during treatment of *Serratia* and *P. aeruginosa* infections with that antibiotic and concomitant clinical failure to cure the infection (1, 2, 9). Consequently, the availability of amikacin does not obviate the need for effective alternative therapy for infections ascribable to multiresistant nosocomial pathogens. Based on the encouraging results in this small, uncontrolled study, further evaluation of polymyxin B and rifam-

TABLE 2. *Effect of polymyxin B on susceptibility to rifampin of 52 clinical isolates of S. marcescens*

Drug	Cumulative strains inhibited at (μg/ml): (%)						
	≤0.4	0.8	1.6	3.1	6.3	12.5	25
Rifampin alone	0	0	2	10	40	85	100
Rifampin plus polymyxin B[a] (in equal concentrations)	31	58	92	98	100	100	100

[a] MIC values of polymyxin B alone were all equal to or greater than 100 μg of drug per ml.

pin is warranted in infections due to multiresistant *Serratia*.

ACKNOWLEDGMENTS

We gratefully acknowledge the advice of Robert S. Abernathy in preparation of this manuscript, and the valuable secretarial assistance of Inelle Reynolds.

LITERATURE CITED

1. Amirak, I. D., R. J. Williams, P. Noone, and M. R. Wiles. 1977. Amikacin resistance developing in a patient with *Pseudomonas aeruginosa* bronchopneumonia. Lancet i:537–538.
2. Craven, P. C., J. H. Jorgensen, R. L. Kaspar, and D. J. Drutz. 1977. Amikacin therapy of patients with multiple antibiotic-resistant *Serratia marcescens* infections. Am. J. Med. 62:902–910.
3. Farmer, J. J., III, B. R. Davis, F. W. Hickman, D. B. Presley, G. P. Bodey, M. Negut, and R. A. Bobo. 1976. Detection of *Serratia* outbreaks in hospitals. Lancet ii:455–459.
4. Hoeprich, P. D. 1970. The polymyxins. Med. Clin. North Am. 54:1257–1265.
5. Jawetz, E., and J. B. Gunnison. 1952. Studies on antibiotic synergism and antagonism. Antibiot. Chemother. (Basel) 2:243–247.
6. McCabe, W. R., and G. G. Jackson. 1962. Gram-negative bacteremia. II. Clinical, laboratory, and therapeutic observations. Arch. Int. Med. 110:856–864.
7. Meyer, R. D., R. P. Lewis, E. D. Carmalt, and S. M. Finegold. 1975. Amikacin therapy for serious gram-negative bacillary infections. Ann. Int. Med. 83:790–800.
8. Meyer, R. D., R. P. Lewis, J. Halter, and M. White. 1976. Gentamicin-resistant *Pseudomonas aeruginosa* and *Serratia marcescens* in a general hospital. Lancet i:580–583.
9. Overturf, G. D., B. E. Zawscki, and J. Wilkins. 1976. Emergence of resistance to amikacin during treatment of burn wounds: the role of antibiotic susceptibility testing. Surgery 79:224–228.
10. Sabath, L. D. 1968. Synergy of antibacterial substances by apparently known mechanisms, p. 210–217. Antimicrob. Agents Chemother. 1967.
11. Tally, F. P., T. J. Louie, W. M. Weinstein, J. G. Bartlett, and S. L. Gorbach. 1975. Amikacin therapy for severe gram-negative sepsis. Ann. Int. Med. 83:484–488.
12. Thomas, F. E., J. M. Leonard, and R. H. Alford. 1976. Sulfamethoxazole-trimethoprim polymyxin therapy of serious multiply drug-resistant *Serratia* infections. Antimicrob. Agents Chemother. 9:201–207.
13. Tillotson, J. R., and M. Finland. 1969. Bacterial colonization and clinical superinfection of the respiratory tract complicating antibiotic treatment of pneumonia. J. Infect. Dis. 119:597–624.
14. Traub, W. H., and I. Kleber. 1975. In vitro additive effect of polymyxin B and rifampin against *Serratia marcescens*. Antimicrob. Agents Chemother. 7:874–876.
15. Weinstein, R. J., L. S. Young, and W. L. Hewitt. 1975. Comparison of method for assessing *in vitro* antibiotic synergism against *Pseudomonas* and *Serratia*. J. Lab. Clin. Med. 86:853–862.