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-
licity. 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 incul-
ated with 10^5 colony-forming units. Previous defi-
nitions for synergy in this system were used (15),
but, in addition, an organism was considered sus-
ceptible only if the minimal inhibitory concentra-
tion (MIC) of the more active antibiotic in the combi-
nation 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 10^6 to 10^8
colony-forming units/ml. The number of viable or-
ganisms 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 deter-
mine the prevalence of polymyxin B-rifampin syn-
ergy toward Serratia at this hospital. A method
was devised to test these isolates for polymyxin B-
rifampin synergy, using a semiautomated microti-
ter 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 (inocu-
lator apparatus for autotiter series, Ames Co.) to
give a concentration of 10^6 colony-forming units/ml.
The five inhibitory end points thus derived after
overnight incubation were used to construct a modi-
fied 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 sys-
tem 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 supervis-
ing physician inadvertently amended the regi-
men. 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 ill-
nesses, 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 re-
sponded to polymyxin B and rifampin with
prompt defervescence, with resolution of other
signs of sepsis, and with appropriate ameliora-
tion 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 suc-
cumbed 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); jaun-
dice 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 bacteri-
emic illness associated with an intravenous
catheter. In the interim period, Serratia was
not present in any culture.

In vitro synergy of polymyxin B and rifam-
pin against Serratia. All infecting strains were
moderately resistant to rifampin (all MIC val-
ues were greater than 6.25 μg/ml) and highly
resistant to polymyxin B (all MIC values were
greater than 100 μg/ml). However, when com-
bined in the checkerboard studies, the antibiot-
ics 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-

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<th>Infection type</th>
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<th>Primary infection</th>
<th>Underlying disease</th>
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<td>1  50 M</td>
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<td>Meningitis</td>
<td>Ampicillin</td>
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<td>2  61 M</td>
<td>Septic thrombophlebitis</td>
<td>COPD(^a)</td>
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<td>Subclavian catheter tip</td>
<td>Ca tongue(^c)</td>
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<td>7  80 M</td>
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<td>Ca prostate</td>
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<td>8  65 M</td>
<td>Pneumonia</td>
<td><em>Staphylococcus aureus</em> endocarditis</td>
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<td>Death due to underlying disease</td>
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<td>9  50 M</td>
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<td>10 60 F</td>
<td>Wound</td>
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<td>11 38 M</td>
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<td>Gentamicin</td>
<td>5</td>
<td>Death due to suprainfection</td>
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\(^a\) 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.

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

ACKNOWLEDGMENTS

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

LITERATURE CITED


