Sulfamethoxazole-Trimethoprim-Polymyxin Therapy of Serious Multiply Drug-Resistant *Serratia* Infections

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Nonpigmented multiply drug-resistant *Serratia marcescens* caused an extensive outbreak of infection at the Nashville Veterans Administration Hospital. Isolates were of one serotype resistant to all currently available antimicrobial agents for therapy of systemic infections except for occasional susceptibility to chloramphenicol and kanamycin. Frequently strains were susceptible to nalidixic acid, and all were susceptible to amikacin (BB-K8). Drug-resistant strains caused 130 infections, 12 bacteremias, and 7 infection-associated deaths. Combinations of antimicrobial agents were evaluated for synergism against *Serratia* strains from infected patients. "Checkerboard" isobolograms indicated in vitro static synergism between sulfamethoxazole, trimethoprim, and polymyxin (STP). Killing curves using clinically achievable concentrations of STP verified the bactericidal effect of STP against these strains. In a daily dosage of 1,600 mg of sulfamethoxazole and 320 mg of trimethoprim orally in combination with 100 to 300 mg of colistimethate parenterally, serum cidal levels at 1:8 or greater were achieved in five of six patients. Clinical improvement or microbiological cure was effected in four of six patients. STP may be potentially useful for selected *Serratia* infections for which single agents are unavailable or ineffective.

Nonpigmented *Serratia marcescens* caused a formidable epidemic at the Nashville Veterans Administration (VA) Hospital during 1974. Multiply drug-resistant strains resulted in over 130 infections and 12 documented bacteremias and were associated with seven deaths. The majority of isolates were resistant to all commerically available antimicrobial agents for therapy of systemic infections including gentamicin. Over half the strains were susceptible to naladixic acid. Occasional susceptibility to chloramphenicol and kanamycin was observed, especially late in the epidemic. All strains were susceptible to amikacin (BB-K8) and resistant to tobramycin.

The combination of infectivity, pathogenicity, and multiple drug resistance prompted us to investigate several modes of therapy against resistant *S. marcescens*. Amikacin has proved efficacious for therapy of resistant *Serratia* infections (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 327, 1974). However, due to its investigational status, its usage was not practical in all patients. Furthermore, investigation of other modes of antimicrobial therapy was pursued due to concern over potential acquisition of resistance if amikacin were the only therapeutic agent used in a large epidemic. Having first confirmed in vitro reports of the efficacy of sulfamethoxazole-trimethoprim-polymyxin (STP) against *Serratia* (7, 14) in our laboratory, we proceeded to use this combination in the treatment of six acutely ill patients infected with multiply drug-resistant *S. marcescens*.

MATERIALS AND METHODS

Strains were isolated in the Microbiology Laboratory of the Nashville VA Hospital, and identification was confirmed and serotyping was performed by the Center for Disease Control. *Serratia* isolates, motile gram-negative rods forming nonpigmented colonies on nutrient agar, were differentiated from *Klebsiella* and *Enterobacter aerogenes* by failure to ferment lactose, arabinose, or rhamnose. By the Kirby-Bauer method (1), resistance in most instances was indicated by absence of any zone of inhibition. Fifty-microgram carbenicillin disks were used. Ten blood culture-derived isolates were tested for antibiotic susceptibility by serial twofold tube dilution assays in Mueller-Hinton broth (BBL, Bioquest), using an inoculum of 10^4 organisms per ml. Minimal inhibitory concentrations of gentamicin were greater than 25 μg/ml except for one strain with a minimal inhibitory concentration of 25 μg/ml. Isolates were frozen and stored at -20 C after over-

night growth in Mueller-Hinton broth until tested. Serum cidal levels were determined by a modification of the method of Schlicter et al. (17) after heat inactivation of complement at 56 C for 30 min as suggested by Klastersky et al. (10).

In vitro synergism studies. Trimethoprim and sulfamethoxazole (Burroughs Wellcome Co.), supplied separately as sterile powders, were dissolved in sterile water at a ratio of 1:20 to approximate the serum ratio achieved using commercially available combination tablets (4). A stock mixture containing 32 μg of trimethoprim and 640 μg of sulfamethoxazole per ml was diluted as indicated. This combination (designated undiluted drug) is treated as a single agent in further discussion. The polymyxin used was polymyxin E methane sulfonate, colistimethate (Warner Chilcott Laboratories), supplied as Coly-mycin M diagnostic powder initially dissolved in water. Several other combinations including rifampin-polymyxin and chloramphenicol-polymyxin were also initially examined for synergism.

Construction of isobolograms. Initial static synergism studies were performed in Mueller-Hinton broth by a modification of the method of Sabath (16). A 0.25-ml twofold dilution of the first antimicrobial agent was placed vertically across a checkerboard pattern. A 0.25-ml twofold dilution of the second agent was placed horizontally across the grid. Addition of 0.5 ml of a 10^{-4} dilution of an 18-h broth culture of the Serratia strain to be tested brought the total volume to 1 ml. Final concentrations of antibiotics were recorded. Inhibition of visible growth of the Serratia inoculum after 18 to 24 h of incubation at 37 C provided the end point. Growth patterns indicated whether combinations were synergistic, indicated whether combinations were synergistic, in-

different, or antagonistic (8, 16).

Bactericidal studies. Killing curves were determined by the method of Rosenblatt and Stewart (14). Killing of Serratia-inoculated cultures was determined as a function of time by inoculation of 10^4 test organisms into Mueller-Hinton broth cultures containing each agent singly or in combination. Cidal antimicrobial effects were assessed by comparison of growth curves of the non-antimicrobial agent-containing culture. Concentrations of colistimethate (5 μg/ml) in combination with either trimethoprim-sulfamethoxazole (2 μg of trimethoprim and 40 μg of sulfamethoxazole per ml), rifampin (5 μg/ml), or chloramphenicol (5 μg/ml) were chosen to approximate readily attainable serum concentrations. Pour plates with 10 ml of Mueller-Hinton agar of appropriate dilutions from broth cultures were made at 0, 2, 4, 8, and 24 h. Incubation followed for 18 h at 37 C. Colony counts corrected for dilution indicated the number of viable organisms remaining in the initial broth cultures. Synergism was indicated when the combination resulted in a $10^{3.5}$ to 10^4 greater decrease in organisms in cultures containing the antimicrobial combination than cultures containing the most efficacious single agent.

Therapeutic trials. Six clinically ill, infected patients having at least two consecutive positive cultures for multiply drug-resistant nonpigmented S. marcescens were treated with STP. Three patients were bacteremic (Table 1). Administered dosages of polymyxin E were 2.0 to 5 mg/kg per day as colistimethate (usually 100 to 300 mg of base equivalent per day) parenterally. Trimethoprim (320 mg/day) and sulfamethoxazole (1,600 mg/day) were administered as two combination tablets (80 mg of tri-

TABLE 1. Clinical data: patients treated with STP

Pa-tient no.	Underlying disease	Serratia-positive culture	Therapy		Outcome
			Adequate duration	Cidal level	
1	Metastatic rectal carcinoma	Urine, blood	Yes	1:64	Improved: cultures sterilized; defervesced; became asymptomatic
2	Metastatic rectal carcinoma; epididymitis orchitis, prostatis	Urine	Yes	1:64[a]	Improved: urinary tract infection controlled; remained chronic urinary carrier
3	Metastatic pancreatic carcinoma, biliary obstruction due to tumor	Urine, blood, gall bladder, joint fluid, sputum	No	1:8	Failure: blood sterilized ante-mortem; positive blood culture post-mortem
4	Metastatic prostatic carcinoma with ureteral obstruction	Urine	Yes	1:8"	Failure: remained infected
5	Cerebrovascular accident; recurrent aspiration	Urine	Yes	1:4"	Improved: urine sterilized; defervesced
6	Metastatic epidermoid carcinoma, quadriplegia, indwelling catheter	Urine, blood, sputum	Yes	1:64	Improved: blood cultures sterilized; continued urinary carriage; defervesced; possible STP nephrotoxicity

[a] Cidal against non-blood culture isolates.

methoprim and 400 mg of sulfamethoxazole each) twice daily. Dosages of all agents were appropriately modified in the presence of renal insufficiency. Rising blood urea nitrogen or serum creatinine levels indicating possible drug-induced renal toxicity were regarded as cause for termination of combination drug therapy.

RESULTS

Description of epidemic. The epidemic has been in part described previously (D. R. Schaberg, W. E. Stamm, R. H. Alford, W. Schaffner, and J. V. Bennett, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 155, 1975; F. E. Thomas, J. M. Leonard, and R. H. Alford, Clin. Res. 23:53A, 1975). Briefly, both variably susceptible and multiply antibiotic-resistant *Serratia* were isolated from over 500 clinical specimens from patients at the Nashville VA Hospital during 1974. More than half of these isolates were resistant by the Kirby-Bauer disk susceptibility method to all commercially available antibiotics, including gentamicin, useful for systemic infections.

The epidemic curve indicating number of patients having newly acquired resistant *Serratia* infections is indicated in Fig. 1. Gentamicin-resistant *Serratia* did not occur in our hospital before October 1973. Patients having resistant *Serratia* isolates were detected occasionally thereafter, with a sudden increase in incidence in February 1974. From that time until August 1974, approximately 10 to 20 patients having newly acquired gentamicin-resistant *Serratia* isolates were detected monthly in the Nashville VA Hospital, declining to three to six monthly in the last 4 months of 1974. Infections occurred in patients in all service areas of the hospital. A total of 12 patients were identifiably bacteremic. Infections varied in severity from asymptomatic long-term urinary carriage to especially serious and virulent infections in patients having underlying immunological defense failures, structural abnormalities of the urinary tract, indwelling urethral catheters (Foley), pyelostomy, or complicated abdominal surgical procedures. Eighty percent of all isolates were of urinary origin. A significant correlation existed between indwelling urinary tract catheterization or prior antimicrobial therapy and acquisition of multiply drug-resistant nonpigmented *Serratia* (Schaberg et al., Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 1975).

Resistant *Serratia* strains were all of one serotype, O1:H7, the same serotype of resistant strains recovered from three other hospitals with which the Nashville VA Hospital shares housestaff (Schaberg et al., Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 1975). Three discrete R factor-mediated patterns of drug resistances were identified by Eric E. M. Moody (University of Texas Medical School, San Antonio) using several of our O1:H7 *Serratia* strains to infect susceptible *Escherichia coli*. Transfer of R factor-mediated gentamicin resistance could not be demonstrated in vitro, suggesting inherent non-R factor-mediated resistance to gentamicin.

Bacteria. Static synergism was demonstrated with three antibiotic combinations—STP, rifampin-polymyxin, and chloramphenicol-polymyxin. Isobolograms constructed from checkerboard assays indicating significant synergism are depicted in Fig. 2. STP bactericidal effects against *Serratia* were striking. Killing curves by STP of multiply drug-resistant *Serratia* strains from blood culture isolates are depicted in Fig. 3. The curves shown used organisms from the two bacteremic patients treated in this study. Synergy of sulfamethoxazole, trimethoprim, and polymyxin was confirmed by a $10^{3.5}$
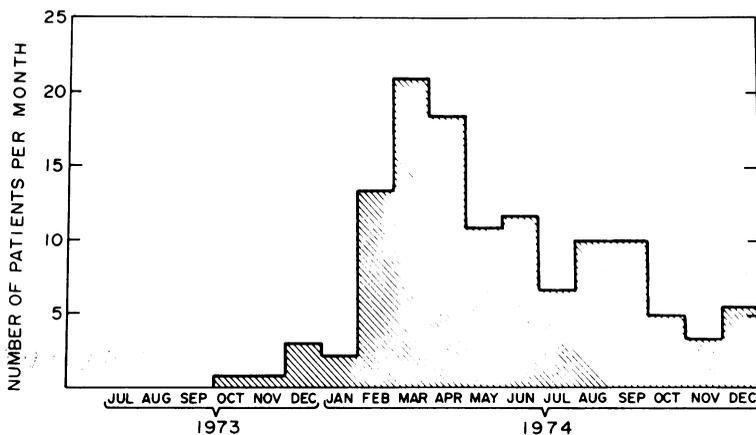

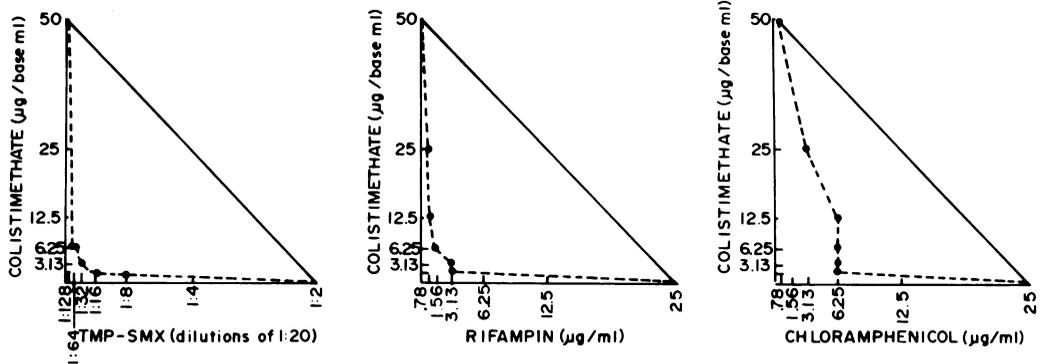FIG. 1. *Epidemic curve indicating patients newly infected with resistant Serratia marcescens.*

FIG. 2. *Representative isobolograms indicating bacteristatic synergism against multiply drug-resistant Serratia marcescens. The solid diagonal line indicates the values that would have been expected from a theoretical addition interaction. The broken line connecting observed values by virtue of its location far below the theoretical line indicates synergism. See text for methodology.*
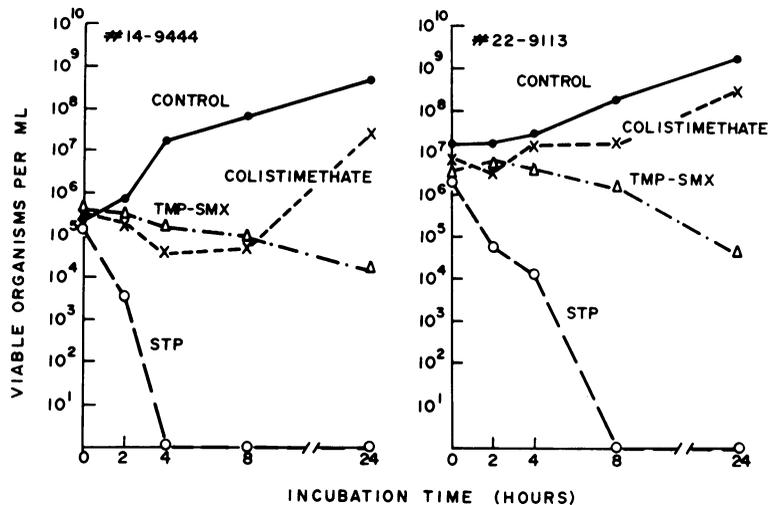

FIG. 3. *Bactericidal effects (killing curves) of STP and the antimicrobial components of the combination against resistant Serratia marcescens obtained from the blood of two bacteremic, STP-treated patients. Cidal synergism was indicated by 10^4 fewer organisms at 24 h in cultures containing the combination than in cultures containing the component antibiotics.*

to 10^4 decrease in *Serratia* colony count at 24 h. Thirteen strains were tested for synergy. A definite synergistic effect was demonstrated against 11 strains, with an indifferent effect detected against two strains not originating from STP-treated patients. Cidal effects of the potentially useful rifampin-polymyxin E combination are indicated in Fig. 4. Chloramphenicol-polymyxin, though showing static synergism in isobolic studies, failed to show cidal synergism in killing-curve determinations in the concentrations used (Fig. 4).

Patient studies. Results of six clinical trials of STP therapy for multiply drug-resistant *Serratia* infections are recorded. Table 1 summarizes the clinical and therapeutic data from

our patients. The seriousness of their underlying illnesses is indicated by the fact that all died from their underlying diseases or complications thereform. Five of six had malignant neoplasms, but none had received antitumor chemotherapy. Indwelling urethral catheters that were subsequently removed from patients 1 and 2 were the likely portal of entry of infection in all six treated patients. Eradication of *Serratia* was achieved in two patients (patients 1 and 5). Temporary suppression of infection enabled patient 2 to undergo two genitourinary surgical procedures without complication but with subsequent relapse after discontinuation of STP after only 10 days of therapy. Without further antimicrobial medica-
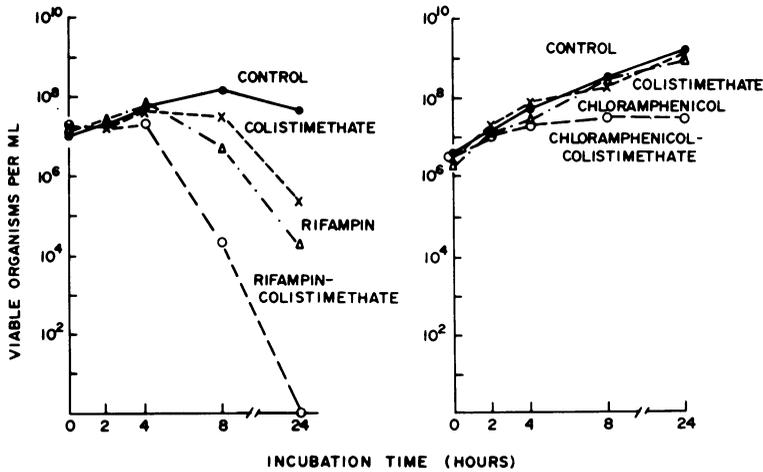
FIG. 4. *Representative killing curves of rifampin-colistimethate and chloramphenicol-colistimethate combinations. Synergistic rifampin-colistimethate effects contrast to the lack of effect of the chloramphenicol-colistimethate combination.*

tion, he remained an asymptomatic carrier of the organism until subsequently dying of non-infectious causes. Patient 3 died with continuing *Serratia* sepsis after only 4 days of STP therapy. The STP combination failed to sterilize the urine of patient 4, who had carcinoma of the prostate with total obstruction of one ureter and partial obstruction of the other due to metastatic tumor requiring pyelostomy. Drug failure in that patient probably resulted from obstructive uropathy with stasis plus uremia, which necessitated marked STP dosage reduction. Patient 6 was cleared of sepsis but remained a urinary carrier of *Serratia* in the face of continuous indwelling urethral catheterization.

Serum cidal levels recorded in Table 1 indicated blood levels of STP apparently adequate for systemic therapy in five of six patients treated. Patient 5, though not having high serum cidal levels, was cured of his urinary tract infection. Unfortunately, control sera were not assayed for bactericidal activity in all our cases. Study sera were obtained from patients who had not received antimicrobial agents other than STP for at least 72 h before tests. Furthermore, study sera were inactivated at 56 C (10). In our laboratory, sera from un-infected and *Serratia*-infected patients not receiving antimicrobial agents often have low-titer, heat-labile bactericidal activity against *Serratia*. Similar complement-dependent bactericidal activity has been pointed out as a potential hazard in interpretation of low-dilution titers in serum cidal tests with other gram-negative bacteria (2). Non-antimicrobic cidal effects against *Serratia* were obviated by heating

sera that had no or at most a single twofold dilution effect upon cidal titers of STP-receiving patients. Titers thus measured appear to offer a valid guide to levels of effective systemic STP therapy.

DISCUSSION

Nonpigmented *S. marcescens* has become one of the leading causes of outbreaks of hospital-acquired multiply drug-resistant gram-negative infections (Thomas et al., Clin. Res. 23:53A, 1975). Factors favoring non-point source noso-comial epidemics include: (i) resistance of the organisms to multiple antibiotics; (ii) relatively nonfastidious growth requirements; (iii) persistence or growth at 25 or 37 C; (iv) a reservoir of patients with poor host defenses and complicated illnesses often requiring prolonged hospitalization with indwelling Foley catheterization; and finally (v) survival on the hands of personnel sufficient to facilitate patient-to-patient spread (3, 5, 6, 13, 20, 22; Schaberg et al., Prog. Abst. Intersci. Conf. Antimicrob. Agents Chemother., 1975; Thomas et al., Clin. Res. 23:53A, 1975). The recorded *Serratia* epidemic was unique in magnitude (500 isolates, 130 patients), in involvement of virtually every area of a 480-bed hospital, and in its tenaciousness in the face of usually prescribed control measures (Schaberg et al., Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 1975).

Identification of R factors capable of in vitro transference of multiple drug resistance to susceptible *E. coli* strains caused us concern that in vivo transference to other susceptible gram-negative rods might occur, but to date such

transference has not been detected with certainty. The multiple antibiotic resistance marker in this hospital has been limited to *S. marcescens* with the exception of a transient outbreak of *Pseudomonas aeruginosa* strains and some strains of *Klebsiella pneumoniae* having the same pattern of antibiotic resistance (Leonard and McGee, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 1974; Thomas et al., Clin. Res. 23:53A, 1975).

Synergistic effects of STP in vitro against a number of gram-negative bacilli including *S. marcescens* have been previously recorded (7, 14, 15, 18, 19), yet few clinical trials have been forthcoming. In vitro static and cidal tests in our laboratory confirmed the synergistic effect of this three-drug combination against multiply drug-resistant *S. marcescens* obtained from clinical isolates. The rifampin-polymyxin combination, which also revealed in vitro cidal synergism but was not tried clinically, could provide another useful drug regimen when multiply drug-resistant *Serratia* outbreaks occur.

Clinically, the STP combination was well tolerated in most instances. Possible renal toxicity occurred in two patients, although continuing infection and obstructive uropathy probably produced azotemia in one. In the other terminally ill patient, azotemia without other demonstrable etiology accompanied by an increase in serum creatinine from 1 to 2.7 occurred during STP therapy. Colistimethate potential for nephrotoxicity is well described (11), and trimethoprim-sulfamethoxazole nephrotoxicity, though apparently less frequent, may also be a hazard (9). Accordingly, the combination STP should be used cautiously, with routine every-other-day determinations of blood urea nitrogen and serum creatinine. Components of the STP combination should be reduced appropriately (12, 21) in the face of preexisting renal failure with discontinuation of the combination should further renal decompensation occur. Reasoning from isobolograms indicating significant synergism as little as 1.56 μg of colistimethate per ml (Fig. 3), low-dosage colistimethate (2.5 mg/kg per day) should provide effective synergism with minimal nephrotoxic effects in patients with normal renal function. Further reduction in colistimethate dosage could be entertained when high serum cidal levels are demonstrated.

Therapy with STP was apparently efficacious in four of six patients. Two of four improving patients were bacteriological cures. The third benefited patient's urine was sterilized during two genitourinary operations, but relapse of the

urinary tract infection subsequently occurred. The fourth patient was relieved of fever and sepsis, though urinary *Serratia* carriage persisted due to the presence of an indwelling catheter.

Two patients (3 and 4, Table 1) were considered therapeutic failures. However, patient 3 died after only 4 days of therapy and patient 4 had tumor-induced obstructive uropathy, which would be expected to overwhelmingly compromise the efficacy of any drug or combination of antimicrobial agents. We view improvement of these very ill patients on STP therapy as probably significant in light of their underlying disease.

In vitro confirmation of adequacy of serum levels of a three-drug antimicrobial combination for therapy of serious infections is mandatory. Assay of each of the three drugs individually is impractical in most hospitals. Biological assays for antibiotics utilizing growth inhibition of organisms of known susceptibilities, which are used in the majority of special infectious diseases laboratories, cannot discriminate between three antibiotics. Efficacy of multi-drug therapeutic regimens for bacterial endocarditis may be guided by the serum bactericidal test introduced by Schlicter et al. (2, 17). Though not directly relevant to the management of our patients' infections, especially of the urinary tract, the serum bactericidal test appears to provide a practical guide to efficacy of therapy with STP. STP levels of 1:8 or greater were obtained in five of six patients, indicating effective systemic anti-*Serratia* therapy.

Despite the small number of patients treated, we conclude that our results are encouraging enough for further trials of STP in patients having multiple resistant *S. marcescens* infections. Clinical investigation of STP is indicated to provide alternative therapy for acute urinary tract infection and sepsis due to resistant *Serratia* when single agents are ineffective or unavailable.

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