Cotrimoxazole-resistant *Escherichia coli* bacteremia in neutropenic patients at a regional oncology hospital

DANIEL B GREGSON, MD, ANNE G MATLOW, MD, ANDREW E SIMOR, MD, PETER G TUFFNELL, MD, DONALD E LOW, MD, RONALD FELD, MD, JAMES E BRUNTON, MD

DB GREGSON, AG MATLOW, AE SIMOR, et al. Cotrimoxazole-resistant *Escherichia coli* bacteremia in neutropenic patients at a regional oncology hospital. *Can J Infect Dis* 1991;3(1):14-18. In a regional oncology hospital using cotrimoxazole (trimethoprim-sulphamethoxazole) prophylaxis during chemotherapy-induced neutropenia, a single strain of *Escherichia coli* (indole negative) caused 15 of 27 episodes of Gram-negative rod bacteremia in 1987, and four of 32 such episodes in 1988. This biotype had not been recovered in 1986. Investigations during this 'outbreak' of bacteremias revealed enteric colonization with this strain of *E coli* in 37% of patients on leukemia or bone marrow transplant wards and in several staff members in July 1987. In 1988, 11 of 32 Gram-negative rod bacteremias were secondary to other strains of indole positive *E coli* of several different biotypes and plasmid profiles. Indole negative strains all exhibited low level trimethoprim resistance, whereas indole positive strains which subsequently appeared exhibited high level trimethoprim resistance. Failure of cotrimoxazole prophylaxis was initially due to the clonal dissemination of a single strain of *E coli* within the institution, with the subsequent appearance of multiple *E coli* strains with probable differing genetic bases for their resistance.

Key Words: Cotrimoxazole, *Escherichia coli*, Infections, Neutropenia, Septicemia, Trimethoprim resistance, Trimethoprim-sulphamethoxazole

Bactériémie à *Escherichia coli* résistant au cotrimoxazole chez les patients neutropéniques traités dans un centre régional anticancéreux

RESUME: Dans un centre régional anticancéreux utilisant le cotrimoxazole (triméthoprime-sulfaméthoxazole) à titre prophylactique durant une neutropénie provoquée par la chimiothérapie, une seule souche de *Escherichia coli* (ne produisant pas d'indole) a causé 15 des 27 accès de bactériémie à germe Gram négatif en 1987 et quatre cas sur 32 en 1988. Ce biotype n'a pas été retrouvé en 1986. Les enquêtes effectuées durant ces bactériémies "épidémiques" ont révélé une colonisation entérique avec cette souche d'*E coli* chez 37 % des patients atteints de leucémie ou ayant subi une transplantation de moelle osseuse et chez plusieurs membres du personnel en juillet 1987. En 1988, 11 des 32 bactériémies à germe Gram négatif étaient secondaires à d'autres souches d'*E coli* produisant de l'indole et présentant divers biotypes et profils plasmidiques. Les souches non productrices d'indole manifestaient toutes une résistance faible au triméthoprime contrairement aux souches productrices d'indole, qui lui semblaient hautement résistantes. L'échec du traitement par cotrimoxazole était initialement dû à la dissémination clonale d'une seule souche d'*E coli* au sein de l'institution, avec l'apparition subséquente de souches multiples d'*E coli* de bases génétiques probablement différentes quant à leur résistance.

Departments of Medicine and Microbiology, Mount Sinai Hospital and Princess Margaret Hospital; and University of Toronto, Toronto, Ontario

Correspondence and reprints: Dr DB Gregson, Department of Microbiology and Infectious Diseases, St Joseph's Hospital, PO Box 5777, London, Ontario N6A 4L6. Telephone (519) 439-3271 ext 4265, Fax (519) 439-1325

Received for publication September 19, 1990. Accepted November 30, 1990

PROPHYLACTIC COTRIMOXAZOLE (TRIMETHOPRIM-SULPHAMETHOXAZOLE) has been shown to reduce number of infections, episodes of bacteremia, days with fever, and need for parenteral antibiotics in leukemia patients with chemotherapy-induced neutropenia (1-6). Reports of infections with cotrimoxazole-resistant enterobacteriaceae associated with the use of this drug during chemotherapy-induced neutropenia have been infrequent (7-9). Since 1982 it has been policy to use prophylactic oral cotrimoxazole in a dose of two single strength tablets twice daily during chemotherapy-induced neutropenia and in bone marrow transplant patients at a 202-bed regional oncology hospital in Toronto, Ontario. In February 1987 a strain of indole negative, sucrose negative *Escherichia coli* which was also cotrimoxazole-resistant was recovered from the blood culture of a patient. The finding of this unusual biotype (less than 1% of clinical isolates of *E coli*) and its subsequent isolation in other cases of bacteremia suggested cross infection within the institution. Studies to determine the prevalence of enteric colonization with cotrimoxazole-resistant *E coli* and changes in organisms causing bacteremia within the hospital were thus initiated, and are the subject of this report.

MATERIALS AND METHODS

All positive blood cultures from 1986 to 1988 were reviewed retrospectively. Repeat blood isolates of the same organism from a single patient taken within 48 h of each other were classified as persistent or continuous bacteremia. The number of sets of blood cultures received each year was determined by review of the laboratory log. All *E coli* blood isolates from January 1986 to December 1988 had repeat identification and sensitivities performed using Microscan Gram-negative identification and susceptibility panels (Travenol Laboratories Inc, California).

To determine the distribution and risk factors for enteric colonization with cotrimoxazole-resistant *E coli*, the patients on five wards were used for a case control study. Three wards where bacteremias had occurred were defined as 'epidemic wards' and two wards where no clinical disease had occurred due to this strain were chosen as 'control wards'. The epidemic wards were used primarily for patients treated for hematological malignancies. One control ward was adjacent to an epidemic ward and the other was two floors away. Patients on the control wards were admitted for chemotherapy or radiotherapy of solid tumours. Fecal samples (stool or rectal swabs) were requested from all patients on the ward on a single day. Hand washes and fecal cultures were requested from hospital staff working on these wards. Six months following the initial prevalence screen, all patients admitted to two of the 'epidemic' wards had fecal cultures weekly to obtain prospective data. Point prevalence screening was repeated on these two wards six months later. Screen-

ing for enteric colonization in outpatients was done in two outpatient clinics.

Demographic data including age, sex, underlying diagnosis, previous admissions and antibiotics used were collected on all of the patients by chart and medication sheet review. Fecal specimens were inoculated onto selective (laked horse blood agar containing 64 µg/mL of trimethoprim) and nonselective media (MacConkey agar; Oxoid Ltd, United Kingdom). Facultative, aerobic, oxidase negative, Gram-negative rods were identified if they grew on the selective media. All oxidase negative, indole negative, Gram-negative rods on the nonselective media were also identified to allow the detection of cotrimoxazole-sensitive isolates of the epidemic strain if present. Hand washes of these personnel in 10 mL of trypticase soy broth were incubated overnight and inoculated onto selective and nonselective media to detect aerobic Gram-negative bacteria.

Ten separate isolates of indole negative, cotrimoxazole-resistant *E coli* (INTREC) were sent to the Laboratory Centre for Disease Control in Ottawa, Ontario for serotyping. Trimethoprim minimal inhibitory concentrations (MICs) were performed on all cotrimoxazole-resistant *E coli* by agar dilution technique following NCCLS guidelines (10). Plasmid extraction was done by the method of Birnboim and Doly (11), and extracts were run on 0.7% agarose gels.

Comparisons of proportions were done using Fisher's exact or χ^2 tests as appropriate.

RESULTS

The numbers and species of aerobic Gram-negative bacilli causing bacteremia in the authors' institution from 1986 to 1988 are shown in Table 1. In all years, *E coli* bacteremia occurred as frequently or more frequently than all other enterobacteriaceae and *Pseudomonas aeruginosa* bacteremias combined. Although the number of *E coli* bacteremias was increased by 50% in the two years following 1986, the frequency of *E coli* bacteremias per set of blood cultures received in the laboratory was not statistically significant. There was a significantly higher rate of recovery of cotrimoxazole-resistant *E coli* in the latter two years ($P=0.03$). Thirty-nine episodes of cotrimoxazole-resistant *E coli* bacteremia occurred in 35 patients during these three

TABLE 1
Aerobic Gram-negative bacilli blood isolates 1986-88

Organism	1986	1987	1988
<i>Escherichia coli</i> (cotrimoxazole-resistant)	23 (6)	34 (15)	36 (18)
Other enterobacteriaceae	17	16	24
<i>Pseudomonas aeruginosa</i>	6	14	9
Other Gram-negative bacilli	4	4	2
Mixed	5	7	4
Total (episodes)	48	67	65
Sets of blood cultures	3154	3160	3184

TABLE 2
***Escherichia coli* bacteremias in neutropenic patients**

Organism	1986	1987	1988
Indole (-), TMP-SMX-resistant <i>E coli</i>	0	14	4
Indole (+), TMP-SMX-resistant <i>E coli</i>	4	1	11
TMP-SMX-sensitive <i>E coli</i>	4	6	4
Total	8	21	19

TMP-SMX Cotrimoxazole

years. Patients were febrile and granulocytopenic in 34 of these 39 episodes. Four of the five non-neutropenic patients with cotrimoxazole-resistant *E coli* bacteremia had a urinary source, and one had small bowel obstruction. Thirty of 35 patients with cotrimoxazole-resistant bacteremia had received prior cotrimoxazole, compared to only four of 37 who had bacteremia with sensitive strains ($P < 0.001$). Forty of 54 (74%) cotrimoxazole-sensitive *E coli* bacteremias were from non-neutropenic patients.

The number of episodes of *E coli* bacteremia in granulocytopenic patients during these three years are summarized in Table 2 according to indole reaction and susceptibility to cotrimoxazole. Fourteen episodes of INTREC bacteremia occurred in febrile neutropenic patients in 1987. During the preceding year only four bacteremias with other biotypes of indole positive, cotrimoxazole-resistant *E coli* had occurred. Five bacteremias with INTREC occurred in 38 patients during initial allogeneic bone marrow transplantation done in 1987, whereas no *E coli* bacteremias occurred during any of the 31 bone marrow transplants performed in 1986 ($P < 0.05$). Of the 14 neutropenic patients with cotrimoxazole-sensitive *E coli* bacteremia, 10 (six with non-Hodgkin's lymphoma, one with breast cancer, one with cervical cancer and one with renal cell carcinoma) had not received any prior cotrimoxazole. Three of the other four patients had acute leukemia, but only one was taking oral cotrimoxazole at the time of the bacteremia. The last patient with cotrimoxazole-sensitive *E coli* bacteremia had cervical cancer with an indwelling Foley catheter, and had received one week of cotrimoxazole one month before the bacteremia. In 1988, indole positive, cotrimoxazole-resistant *E coli* predominated as the bacteremic pathogen in this patient population.

During the initial screen for enteric colonization, fecal samples were received from 61 of 76 patients (80%) targeted for screening. Patients were missed only if they were discharged from hospital prior to a sample being obtained. Enteric colonization with INTREC was present in 16 of 43 patients (37%) on the epidemic wards, and only three of 24 (13%) on the control wards ($P < 0.05$). One of the patients on a control ward who was colonized had been on an epidemic ward the preceding week. The other two patients on nonepidemic wards who were colonized had never been on epidemic wards

and had not received cotrimoxazole. Thirteen of 19 patients (68%) colonized with INTREC were taking oral cotrimoxazole at the time the prevalence study was performed. Fecal samples were provided by only 24 of 79 hospital personnel (30%) working on the epidemic wards. INTREC were recovered from two of these samples (8%). Hand washes from these 79 hospital personnel were positive for aerobic Gram-negative bacilli in 40 instances (50%), but no cotrimoxazole-resistant *E coli* were recovered. Six months later the repeat survey of two epidemic wards for INTREC revealed 18 of 47 patients (38%) admitted and followed on these wards over two months were colonized with INTREC ($P = 0.17$). One year after the initial screen, screening of inpatients on two of the epidemic wards revealed only two of 19 patients (10%) were colonized with INTREC. This was a significantly lower rate of enteric colonization than that at the time of the initial screening done the preceding year ($P = 0.02$). The rate of colonization with other cotrimoxazole-resistant *E coli* during all of these periods ranged from 11 to 14%. Nine of 33 outpatient leukemia or bone marrow transplant patients (27%) who had been admitted to hospital in 1987 were colonized with INTREC, whereas none of 15 chronic leukemia outpatients who had never been admitted to the hospital was colonized with INTREC ($P = 0.03$). Two of three bone marrow transplant patients screened six months after discontinuation of oral cotrimoxazole were still colonized with this strain. Three patients admitted for bone marrow transplantation and followed prospectively with stool cultures became colonized with the INTREC strain after a mean of 20 days in hospital.

The isolates of INTREC were resistant to trimethoprim, sulphamethoxazole, ampicillin and cefazolin, and were sensitive to all aminoglycosides, third generation cephalosporins and norfloxacin. In the INTREC blood isolates the trimethoprim MICs were 512 µg/mL, except for five isolates with MICs of 1024 µg/mL. All of the INTREC were of a single Microscan biotype (5310401-2), and the 10 isolates serotyped were all 01:H6:K0. Plasmid profiles of ten random INTREC blood isolates were all of the same pattern (data not shown). Investigations of the indole positive, cotrimoxazole-resistant isolates from 1988 has shown four different antibiotic sensitivity patterns, five different biotypes and three different whole cell DNA restriction patterns on four strains examined, indicating that several different strains of *E coli* are involved (data not shown). Trimethoprim MICs were greater than 2048 µg/mL in all but one of these isolates.

DISCUSSION

This report details the largest number of bacteremias due to cotrimoxazole-resistant *E coli* in an institution using this agent prophylactically during iatrogenic neutropenia. Wells *et al* (8) described widespread enteric colonization with cotrimoxazole-resistant organisms in

a transplantation unit with subsequent bacteremias. Enteric colonization rates for resistant organisms in their study were similar to those found in the authors' point prevalence screen in 1987. In that study, however, several different species of cotrimoxazole-resistant enterobacteriaceae were involved. It is of interest that four of the eight documented bacteremias in Wells's report were *E coli* bacteremias in bone marrow transplant patients. These isolates were of several different biotypes. In the authors' hospital, a single strain of *E coli* predominated as a bacteremic pathogen and enteric colonizer. Investigators from Spain have also reported on failure of cotrimoxazole prophylaxis in pediatric leukemia patients, and suggested that such prophylaxis was not indicated when such bacteremias occurred (9).

By the time these cotrimoxazole-resistant strains were recognized to be a significant clinical problem, widespread enteric colonization had already occurred. As expected, enteric colonization with this strain was strongly associated with admission to specific wards where cotrimoxazole was used prophylactically. Nosocomial acquisition of this strain was documented prospectively in three patients. The absence of enteric colonization in outpatients never admitted to the authors' institution was also in keeping with a nosocomial source for this organism. The mechanism of spread of this organism was not documented in the present study, but enteric colonization of health care personnel and isolates on distant wards suggests the possibility of dissemination by health care workers.

Interestingly, other indole positive, cotrimoxazole-resistant strains of *E coli* predominated as bacteremic pathogens in febrile neutropenic patients in 1988. Plasmid profiles, 'antibiograms' and biotyping showed that these isolates represented multiple strains which differed substantially from the INTREC strain prevalent in 1988. Resistance to cotrimoxazole could be transferred to laboratory strains from an INTREC isolate (data not shown). However, INTREC strains and transconjugants exhibited low level trimethoprim resistance (MIC less than 1024 µg/mL), whereas all but one isolate of the indole positive strains exhibited high level trimethoprim resistance.

These findings suggest that trimethoprim resistance was mediated by the type I or II dihydrofolate reductase genes in the indole positive strains, and by a different gene in the INTREC strains (12).

No data from this institution are available showing that prophylactic cotrimoxazole reduced the incidence of Gram-negative bacteremia when this policy was first introduced in 1982. The data from 1986 to 1987, however, do show that the incidence of *E coli* bacteremias increased at a time when colonization with resistant isolates was frequent. The tertiary care nature of the institution undoubtedly promoted the problem. Patients admitted to the 'epidemic' wards in this hospi-

tal commonly required repeated hospitalizations for intensive chemotherapy and repeated courses of prophylactic cotrimoxazole. The inability to segregate bone marrow transplant patients from those undergoing remission induction chemotherapy for leukemia would expose the latter population to a group likely to be colonized with cotrimoxazole-resistant organisms. Also, it was found that some patients other than bone marrow transplant patients were receiving oral prophylaxis before the onset of neutropenia, or that oral cotrimoxazole was continued after the institution of parenteral antibiotics for fever. The increased awareness among hospital staff and the enforcement of hand-washing practices reduced the incidence of bacteremia and prevalence of enteric colonization with the INTREC strain which was predominant in 1987. However, other cotrimoxazole-resistant strains increased as bacteremic pathogens in 1988. The authors therefore conclude that cotrimoxazole prophylaxis during chemotherapy-induced neutropenia is no longer effective in their institution, due to nosocomially acquired enteric colonization with cotrimoxazole-resistant *E coli*.

ACKNOWLEDGEMENTS: The authors thank the staff of the leukemia and bone marrow transplant service for their cooperation, Ruby Bailey for assistance with data collection, and Kay Herbage for technical assistance.

REFERENCES

1. Kauffman CA, Leipman MK, Bergman AG, Mioduszewski J. Trimethoprim/sulfamethoxazole prophylaxis in neutropenic patients. *Am J Med* 1983;74:599-607.
2. Guiot HFL, van den Brock PJ, van der Meer JWM, van Furth R. Selective antimicrobial modulation of the intestinal flora of patients with acute nonlymphocytic leukemia: A double-blind placebo-controlled study. *J Infect Dis* 1983;147:615-24.
3. Gurwith MJ, Brunton JL, Lank BA, Harding GKM, Ronald AR. A prospective controlled investigation of prophylactic cotrimoxazole in hospitalized granulocytopenic patients. *Am J Med* 1979;66:248-56.
4. Gualtieri RJ, Donowitz GR, Kaiser DL, Hess CE, Sande MA. Double blind randomized study of prophylactic trimethoprim/sulfamethoxazole in granulocytopenic patients with hematologic malignancies. *Am J Med* 1983;74:934-40.
5. Karp JE, Merz WG, Hendricksen C, et al. Oral norfloxacin for the prevention of Gram-negative bacterial infections in patients with acute leukemia and granulocytopenia. *Ann Intern Med* 1987;106:1-6.
6. Bodey GP. Antimicrobial prophylaxis for infection in neutropenic patients. In: Remington JS, Swartz MN, eds. *Current Clinical Topics in Infectious Diseases*. New York: McGraw-Hill Book Co, 1988:1-43.
7. Wilson JM, Guiney DG. Failure of oral trimethoprim-sulfamethoxazole prophylaxis in acute leukemia. *N Engl J Med* 1982;306:16-20.
8. Wells CL, Podzorski RP, Peterson PK, Ramsay NK, Simmons RL, Rhame FS. Incidence of trimethoprim-sulfamethoxazole-resistant Enterobacteriaceae among transplant recipients. *J Infect Dis* 1984;150:699-706.

9. Esquembre C, Ferris J, Verdeguer A, Castel V. Failure of trimethoprim/sulfamethoxazole as prophylactic therapy of Gram-negative sepsis in pediatric patients with cancer. *Pediatrics* 1986;78:719. (Lett)
 10. National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. NCCLS publication M7-A. Villanova: NCCLS, 1985.
 11. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* 1978;7:1513-23.
 12. Huovinen P. Trimethoprim resistance. *Antimicrob Agents Chemother* 1987;31:1451-6.
-