Bacteraemia due to a rifampicin-resistant strain of *Bacteroides fragilis*

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SUMMARY A strain of *Bacteroides fragilis* with high-level chromosomal resistance to rifampicin was isolated by blood culture from a patient with bacteraemia after gastrointestinal surgery. He had been receiving antituberculous therapy with rifampicin for nine months. This resistance led to some difficulty in the recognition and identification of the isolate by methods that depended upon antibiotic sensitivity patterns.

Bacteroides fragilis is the major cause of post-operative sepsis after abdominal surgery.^{1,2} Serious complications of these infections include bacteraemia. The commonest source of bacteroides bacteraemia is lower gastrointestinal perforation, surgery, or other serious pathology, and it commonly follows appendicitis, diverticulitis, or colon surgery, especially for carcinoma.³ The antibiotic sensitivity of the *Bacteroides* spp. isolated from these infections is important. The infections are serious and require treatment, but the antibiotic sensitivity patterns are also very useful in the preliminary recognition and identification of Bacteroidaceae.⁴⁻⁷

Case history

A 37-year-old man presented with a two-day history of abdominal pain and vomiting. His previous medical history included pulmonary tuberculosis, for which he had received three courses of chemotherapy since 1966, and Hodgkin's disease of mixed cellular type, diagnosed in 1974 and treated by splenectomy, total body irradiation, and cytotoxic chemotherapy. At the time of admission he had been receiving rifampicin and ethambutol for nine months.

On examination abnormal findings were: oral temperature 37.9°C, pulse rate 104/min, blood pressure 100/60 mm Hg, and generalised abdominal tenderness with guarding and board-like rigidity. A diagnosis of peritonitis due to rupture of the appendix was made. At emergency laparotomy the appendix was inflamed but not ruptured, but a 30-cm length of ileum was found to be infarcted; this was

resected, and an end-to-end anastomosis was performed. Antituberculous therapy was stopped and perioperative prophylactic cefazolin (one dose of 1 g by intramuscular injection) and metronidazole (1 g by rectal suppository) were given; metronidazole was continued until the seventh postoperative day. The patient was discharged home on the ninth postoperative day.

Four days later he was readmitted with severe abdominal pain, vomiting, and diarrhoea. At a second laparotomy the bowel anastomosis was found to have broken down and leaked. The anastomosis was resected and an ileostomy and mucous fistula were fashioned. Three doses of perioperative prophylactic cefazolin (1 g intramuscularly) and metronidazole (1 g per rectum) were again given.

Blood cultures were taken on the first, second, and third postoperative days because of episodes of pyrexia, hypotension, tachycardia, and rigors, and treatment with gentamicin and metronidazole was started on the fifth postoperative day. Approximately 5 ml of venous blood was seeded into each of two media: (a) 50 ml of brain-heart infusion broth (Gibco) with 10% sucrose and 1% sodium polyanethol sulphonate (Liquoid), and (b) 90 ml of brain-heart infusion broth with 0.1% sodium thioglycollate, 0.1% glucose, 0.05% agar, and methylene blue (for anaerobes). Both media were incubated at 37°C in air. After two days there was no macroscopic evidence of bacterial growth, and no micro-organisms were seen in Gram-stained smears from the cultures. First subcultures were made after incubation for four days on to 5% horse-blood agar incubated in air plus 5% CO₂ and 5% horse-blood agar with 0.05% cysteine and 0.5% yeast

extract, incubated anaerobically ('Gaspak' system). After 20 hours numerous small (<2 mm diam.), grey, lustreless colonies were found only on the anaerobic subculture from medium (b) of the cultures taken on the second postoperative day; a Gram-stained smear showed small Gram-negative bacilli. Disc sensitivity testing showed that the organism was sensitive to metronidazole, cefoxitin, clindamycin, erythromycin, and tetracycline; therefore gentamicin therapy was stopped. Metronidazole treatment was continued for 16 days, and the patient was discharged three weeks after the second operation. Attempts to identify the isolate by the Mastring system⁸ gave an anomalous result; it was sensitive only to erythromycin and resistant to colistin, kanamycin, benzyl penicillin, rifampicin, and vancomycin whereas typical strains of the *B. fragilis* group are sensitive to erythromycin and rifampicin.

It was identified as *B. fragilis* (formerly *B. fragilis* ss. *fragilis*) by a combined set of tolerance, antibiotic disc resistance, biochemical, and fermentation tests (Table)^{6,7} and resistance to rifampicin was confirmed. There was no zone of inhibition around the rifampicin 15 µg disc, and the minimum inhibitory concentration of rifampicin for this strain by the plate dilution method was 64 µg/ml; all other results were typical of *B. fragilis*. Resistance to rifampicin was the result of chromosomal mutation. The resistance could not be transferred in mating experiments with sensitive strains of *B. fragilis* and *Escherichia coli* that were competent recipients. The methods used for the transfer experiments were a centrifugation technique and a modification of the membrane filter technique of Brefort *et al.*;⁹ transfer of clindamycin and erythromycin resistance and chloramphenicol, erythromycin, and tetracycline resistance from clinical isolates of *B. fragilis* to sensitive *B. fragilis*, *B. distasonis*, and *E. coli* recipients was demonstrated in parallel experiments. The rifampicin resistance was not cured by subinhibitory concentrations (16 µg/ml) of acriflavine, acridine

orange, or ethidium bromide in broth cultures held for 21 days; the control strains of *B. fragilis* were cured of their plasmid-mediated multiple transferable resistance after 24 hours. Plasmid DNA was not detected in the rifampicin-resistant strain by agarose gel electrophoresis but was demonstrated in control strains of *B. fragilis* and *E. coli*.¹⁰

Discussion

Resistance to rifampicin led to some problems of recognition and identification of the organism isolated from this patient although *B. fragilis* is the most common cause of postoperative sepsis after lower gastrointestinal surgery.^{1,2} Patterns of resistance with a selected set of antibiotic discs is very useful in the preliminary identification of Gram-negative anaerobic bacilli. The Mastring identification system is based on the work of Sutter and Finegold;⁴ it depends upon the pattern of resistance of non-sporing anaerobes to six antibiotic discs: erythromycin (60 µg), colistin (10 µg), kanamycin (1000 µg), benzyl penicillin (2 units), rifampicin (15 µg), and vancomycin (5 µg). Similar disc resistance tests form part of other schemes,^{6,7} and tests with rifampicin are particularly useful for the separation of most *Bacteroides* spp. (sensitive) from *Fusobacterium* spp. and *B. corrodens* (mostly resistant).⁵ The experience described here reinforces the concern that antibiotic sensitivity patterns are not permanent and should not be relied upon as the sole basis for identification.

Resistance to rifampicin is rare in strains of the *B. fragilis* group isolated from clinical infections or from the normal human flora. However, early work with rifampicin established that large bacterial populations of many species may contain a few resistant mutants,^{11,12} and resistance appears readily during the treatment of urinary tract infections with rifampicin.¹¹ Plasmid-free, rifampicin-resistant *B. fragilis* strains have been obtained *in vitro* by culture on selective media containing rifampicin.^{13,14} The

Results obtained in tests for the identification of the Gram-negative anaerobic isolate

<i>Mastring</i> ⁸		<i>Combined set of conventional tests</i> ^{6,7}			
		<i>Antibiotic resistance tests</i>		<i>Biochemical tests</i>	
Erythromycin (60 µg)	S	Metronidazole (5 µg)	S	Indole	—
Colistin (10 µg)	R	Neomycin (1000 µg)	R	Aesculin hydrolysis	+
Kanamycin (1000 µg)	R	Kanamycin (1000 µg)	R	Fermentation of:	
Penicillin (2 units)	R	Penicillin (2 units)	R	Glucose	+
Rifampicin (15 µg)	R	Rifampicin (15 µg)	R	Lactose	+
Vancomycin (5 µg)	R	<i>Tolerance tests</i>		Sucrose	+
		Taurocholate	+	Rhamnose	—
		Victoria blue 4R	+	Trehalose	—
		Gentian violet	—	Mannitol	—
				Xylose	+

resistant strain isolated from this patient presumably arose by selection of resistant mutants during anti-tuberculous therapy with rifampicin. The failure to demonstrate transfer of rifampicin resistance, the absence of plasmid DNA, and the failure to cure resistance with aminoacridine dyes or ethidium bromide by methods that consistently demonstrated plasmid-mediated resistance to clindamycin, erythromycin, chloramphenicol, and tetracycline in other strains of *B. fragilis*¹⁰ confirm that the rifampicin resistance was not plasmid-mediated.

The resistance to rifampicin did not compromise the treatment of this patient. At present rifampicin is rarely used in the United Kingdom for non-tuberculous infection because of fears for the development of resistance in *Mycobacterium tuberculosis*. However, this view has been challenged, and more widespread use may be advocated in the future.^{15 16} If rifampicin usage became more widespread it would be reasonable to assume that this problem would become more common.

This patient developed a bacteroides septicaemia despite peroperative prophylaxis with metronidazole, to which the *B. fragilis* strain was sensitive; the infection eventually responded to therapy with metronidazole. The failure of prophylaxis was probably due to the presence of an established focus of infection in the abdomen at the time of operation. Short-term peroperative prophylaxis is designed to prevent infection as a result of operative contamination of previously healthy tissue but may be insufficient to overcome established infection which requires a therapeutic course of treatment.

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