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 anti-
biotic 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 bacteroi des bacteraemia is
lower gastrointestinal perforation, surgery, or other
serious pathology, and it commonly follows appendici-
tis, diverticulitis, or colon surgery, especially for
carcinoma.3 The antibiotic sensitivity of the Bacter-
oides 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 chem-
otherapy 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 peroperative 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 post-
operative 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 mucus fistula
were fashioned. Three doses of peroperative prophy-
lactic cefazolin (1 g intramuscularly) and metronida-
zone (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 thio-
glycollate, 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 macro-
scopic 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

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extract, incubated anaerobically (‘Gaspack’ 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 system 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) 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 Befort et al.; 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, acriflavine 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.

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. 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; 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 and tests with rifampicin are particularly useful for the separation of most Bacteroides spp. (sensitive) from Fusobacterium spp. and B. corrodens (mostly resistant). 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 and resistance appears readily during the treatment of urinary tract infections with rifampicin. Plasmid-free, rifampicin-resistant B. fragilis strains have been obtained in vitro by culture on selective media containing rifampicin. The

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<tr>
<th>Results obtained in tests for the identification of the Gram-negative anaerobic isolate</th>
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<tr>
<td><strong>Mastring</strong></td>
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<td>Erythromycin (60 μg)</td>
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<td>Colistin (10 μg)</td>
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<td>Kanamycin (1000 μg)</td>
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<td>Penicillin (2 units)</td>
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<td>Rifampicin (15 μg)</td>
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<td>Vancomycin (5 μg)</td>
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<td><strong>Antibiotic resistance tests</strong></td>
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<td>Metronidazole (5 μg)</td>
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<td>Neomycin (1000 μg)</td>
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<tr>
<td>Kanamycin (1000 μg)</td>
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<td>Penicillin (2 units)</td>
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<td>Rifampicin (15 μg)</td>
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<td><strong>Fermentation tests</strong></td>
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resistant strain isolated from this patient presumably arose by selection of resistant mutants during antituberculous 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 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 nontuberculous 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. 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.

References

8. Mast Laboratories Ltd, 38 Queensland Street, Liverpool, L7 3JG.

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