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Emergence of Metronidazole-Resistant *Bacteroides fragilis*, India

To the Editor: Members of the *Bacteroides fragilis* group are the most commonly isolated anaerobic pathogens in humans. Metronidazole has been the drug of choice for preventing and treating such infections for 40 years. Although *B. fragilis* exhibits the broadest spectrum of recognized resistance to antimicrobial agents among anaerobes, the worldwide rate of metronidazole resistance remains low, <5% (1,2). We report here the first metronidazole-resistant strain of *B. fragilis* from India.

A 34-year-old man with myelodysplastic syndrome was admitted to our hospital with a short history of myalgia, general malaise, and bleeding gums. Bone marrow examination showed evidence of severe aplastic anaemia, for which he was treated with cyclophosphamide and blood transfusions. Ceftazidime and amikacin were also administered empirically for febrile neutropenia. The patient remained in the intensive care unit of our medical oncology ward and was given repeated courses of chemotherapy and blood transfusions. He also had repeated episodes of febrile neutropenia, which resolved with a combination of vancomycin, aminoglycosides, and third-generation cephalosporin. After 4 months in the hospital, during an episode of febrile neutropenia, the patient's condition started to deteriorate, and high-grade fever developed. Physical examination showed temperature of 38°C, heart rate 80/min, blood pressure 100/70 mmHg, and marked pallor. Laboratory investigations showed a hemoglobin level of 4g/dL and marked neutropenia (absolute neutrophil count 320/mm³). Liver and renal function test results were within normal limits. Peripheral blood smears were negative for malarial parasites. Culture of urine revealed no growth, and the Widal test was negative. Two blood samples were collected in Wampole isolator tubes (Wampole Laboratories, Cranbury, NJ), for isolation of aerobic and anaerobic bacteria. Subsequently, intravenous antimicrobial therapy with vancomycin, metronidazole, and ceftazidime was started. The patient died a day after collection of blood for culture.

Antemortem blood cultures grew *Pseudomonas aeruginosa* and *B. fragilis*. The isolate of *B. fragilis* was identified by conventional tests and Rap ID ANA II system (Innovative Diagnostic System, Norcross, GA). *P. aeruginosa* was sensitive to piperacillin but resistant to amikacin, ceftazi-

dime, cefotaxime, and ciprofloxacin. *B. fragilis* was resistant to metronidazole (MICs, 256 µg/mL) by both standard broth dilution method and E-test (AB Biodisk, Solne, Sweden). The isolate was also resistant to cefotaxime and ceftazidime. However, it was sensitive to chloramphenicol, clindamycin, and imipenem.

Primary bacteremia caused by anaerobic organisms accounts for <5% of septicemia in cancer patients (3). Chemotherapy is a known predisposing factor for anaerobic bacteremia because it causes gastrointestinal ulceration, which permits anaerobes to enter circulation (4).

Anaerobic bacteremia is usually polymicrobial in etiology and has a high death rate (4). In this case, both bacterial isolates were resistant to the empirical treatment. Delay in initiating appropriate therapy was perhaps a major contributor to the patient's death.

Metronidazole is the drug of choice for empirical coverage of anaerobic infections. The precise incidence of resistance to metronidazole in *B. fragilis* isolates is difficult to estimate (5), since routine antimicrobial sensitivity testing of anaerobes is not being done by most laboratories in the world. Published articles reveal only a few reported cases of *B. fragilis* that were resistant to metronidazole (6-10). Although the incidence of resistance to penicillin, cephalosporins, and clindamycin is increasing dramatically, no resistance to metronidazole in *B. fragilis* was found in some large-scale studies done throughout the world (11,12).

The true incidence of metronidazole resistance in India too is possibly underestimated since antimicrobial sensitivity testing is not being done routinely. However, we are conducting antimicrobial susceptibility testing of all anaerobic isolates in our institute. In a previous study we conducted (13), contrary to this report, none of 32 clinical isolates belonging to the family *Bacteroidaceae* obtained over a 5-year period were resistant to metronidazole.

Recently, the anaerobic reference unit in the UK noted a possible increase in the incidence of metronidazole resistance in *B. fragilis*, an observation that would have major implications for clinical microbiology laboratories, as well as for prophylactic and treatment regimens (5).

There is now a growing debate whether in vitro susceptibility testing should be performed for all *Bacteroides* isolates to guide antimicrobial therapy. The acquisition of metronidazole resistance by *B. fragilis* reported here from India emphasizes the need for a study to assess more accurately the susceptibilities of clinical isolates of *Bacteroides* spp.

Diagnostic microbiology laboratories and clinicians should be aware that the incidence of metronidazole resistance in clinically significant anaerobes may be increasing (5). Since antimicrobial resistance in anaerobes varies from one hospital to another and between different geographic locations, all hospitals should survey their sensitivity patterns and report any emerging resistance.

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Proper Nomenclature for the Human Granulocytic Ehrlichiosis Agent

To the Editor: In their recent article, "Antigenic variations in vector-borne pathogens," Barbour and Restrepo discuss the outer membrane protein components of *Anaplasma marginale* and related bacteria (1). Citing a reference by Zhi et al. (2), they state that *Ehrlichia granulocytophila* is the agent of human granulocytic ehrlichiosis (HGE).

The use of new names and combinations not widely recognized for genera and species lends increasing confusion to a group of bacteria already in taxonomic disarray. Several other species names have been suggested for the HGE agent since the initial description of the clinical illness caused by this agent and the in vitro technique used to isolate the agent in blood samples (3,4). Both *E. phagocytophila* and *E. equi* are genetically nearly identical to the HGE agent, and the three are probably conspecific. Thus, most scientists in the field today would support use of the name *Ehrlichia phagocytophila* to describe these bacteria.

Recent phylogenetic analyses show that *E. phagocytophila* strains align into a clade that includes *Anaplasma marginale*, the historical precedent in this grouping. Such phylogenetic analyses, which are also supported by comparative antigenic and biological studies, have resulted in a proposal for reclassification of several *Ehrlichia* spp., including *E. phagocytophila*, into the genus *Anaplasma* (5). Until a cogent reclassification based on objective criteria is firmly accepted, the creation and use of new scientific name combinations for a single bacterium yield clinical and laboratory confusion and should be avoided.

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Single Nucleotide Polymorphisms in *Mycobacterium tuberculosis* Structural Genes

To the Editor: A recent article by Fraser et al. (1) discussed the frequency of single nucleotide polymorphisms (SNPs) in two genomes of *Mycobacterium tuberculosis*, strains H37Rv (2) and CDC1551 (unpublished). The article contains an inaccurate representation of our published *M. tuberculosis* data on SNP frequency. The authors state that "detailed comparison of strains H37Rv and CDC1551 indicates a higher frequency of polymorphism, approximately 1 in 3,000 bp, with approximately half the polymorphism [sic] occurring in the intergenic regions. In other words, 50% of the polymorphisms are in 10% of the genome. While this rate is higher than that suggested (3), it still represents a lower nucleotide diversity than found in limited comparisons from other pathogens."

On the basis of comparative sequence analysis of eight *M. tuberculosis* structural gene loci (open reading frames [orf]), we initially published an estimated average number of synonymous substitutions per synonymous site (K_s value) that indicated that this pathogen had, on average, approximately 1 synonymous difference per 10,000 synonymous sites (4). This finding was unexpected given the relatively large population size of *M. tuberculosis* and paleopathologic