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# Metallo-β-Lactamase-Producing *Bacteroides* Species Can Shield Other Members of the Gut Microbiota from Antibiotics

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**Antibiotics disrupt the intestinal microbiota, rendering patients vulnerable to colonization by exogenous pathogens. Intermicrobial interactions may attenuate this effect. Incubation with ceftriaxone-resistant, *ccrA*-positive, β-lactamase-producing *Bacteroides* strains raised the minimum bactericidal concentration of ceftriaxone required to kill a susceptible *Escherichia coli* strain (mean change, <0.25 to 29 mg/liter; *P* = 0.009); incubation with ceftriaxone-resistant but non-β-lactamase-producing *Bacteroides* strains had no effect. The production of β-lactamase by common members of the intestinal microbiota (*Bacteroides*) can protect susceptible fellow commensals from β-lactams.**

The indigenous anaerobic microbiota of the lower intestinal tract remains a crucial mammalian host defense against colonization by exogenous, potentially pathogenic microorganisms (1–3). This defense mechanism is termed colonization resistance and may be abolished in hospitalized patients by the administration of antibiotic therapy. Antibiotics, including β-lactam antibiotics, may disrupt the intestinal microbiota, rendering patients susceptible to colonization or infection with nosocomial pathogens.

We previously demonstrated in β-lactam-treated mice that oral recombinant proteolysis-resistant β-lactamase enzymes that inactivate β-lactams can preserve gut colonization resistance against multiple nosocomial pathogens, including vancomycin-resistant *Enterococcus* (VRE), extended-spectrum β-lactamase-producing *Klebsiella pneumoniae*, and *Clostridium difficile* (4–6), via intraintestinal degradation of excreted antibiotic by intraluminal β-lactamases. This strategy holds promise for the prevention of pathogen colonization in patients treated with parenteral antibiotics, as antibiotic degradation within the colonic lumen does not impact systemic concentrations (7). We also demonstrated that, despite their receipt of parenteral β-lactams, mice intestinally colonized with a β-lactamase-producing member of the commensal microbiota (*Bacteroides thetaiotaomicron*) preserved colonization resistance against VRE and *C. difficile* (8). However, the genetics of β-lactam resistance in the protective *Bacteroides* species in this study were not known, and a comparator anaerobe was not studied. Here, we hypothesized that ceftriaxone-resistant *Bacteroides* species producing the broad-spectrum metallo-β-lactamase CcrA would protect β-lactam-susceptible members of the microbiota from the β-lactam ceftriaxone *in vitro*, while ceftriaxone-resistant (but non-β-lactamase-producing) *Bacteroides* species would not.

(Portions of this study were previously presented in abstract form at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 17 to 20 September 2011.)

**Bacterial strains.** To test the protection of a clinical strain of ceftriaxone-susceptible *Escherichia coli* (chosen as a representative member of the human gut microbiome that can be grown on a medium different than that for *Bacteroides*) from ceftriaxone, four highly cephalosporin-resistant clinical *Bacteroides fragilis* group microorganisms were used (strain 1, *B. fragilis*; strain 2, *Bacteroides distasonis*; strain 3, *B. fragilis*; strain 4, *B. thetaiotaomicron*; all strains are part of a collection that was a kind gift from D. W. Hecht, Maywood, IL, and selection is described below). The MICs of ceftriaxone for the four *Bacteroides* strains were 500, 500, 250, and 500 mg/liter, respectively. The MIC of ceftriaxone for the *E. coli* strain was <0.25 mg/liter.

**Genetic and phenotypic characterization of β-lactamases.** Ceftriaxone-resistant clinical *Bacteroides* group organisms were assessed by PCR for the presence of the four common β-lactamase (*bla*) genes found in this genus (*cblA*, *cepA*, *cfxA*, and *cfiA*/*ccrA*). Primer sequences for the target genes were chosen based on either previously published primers or, if such were not available, flanking the entire gene sequence for the enzyme as reported in the literature (9–12). PCR was then performed according to the methods described by Hujer et al. (13).

The production of β-lactamase in all the isolates was also characterized phenotypically according to our previous methods (8) using a qualitative assay with the chromogenic cephalosporin substrate nitrocefin, which changes color within 1 min of undergoing hydrolysis of the amide bond by β-lactamase enzymes (14). As a result of these examinations, four *Bacteroides* strains were selected for the mixing studies (described below).

**Minimum bactericidal concentrations.** We obtained the broth microdilution minimum bactericidal concentrations (MBCs) of ceftriaxone for all five organisms according to standard microbiologic methods (15, 16). We used supplemented brucella broth (Becton-Dickinson, Cockeysville, MD) for determinations of *Bacteroides* susceptibilities and Mueller-Hinton broth (Becton-Dick-

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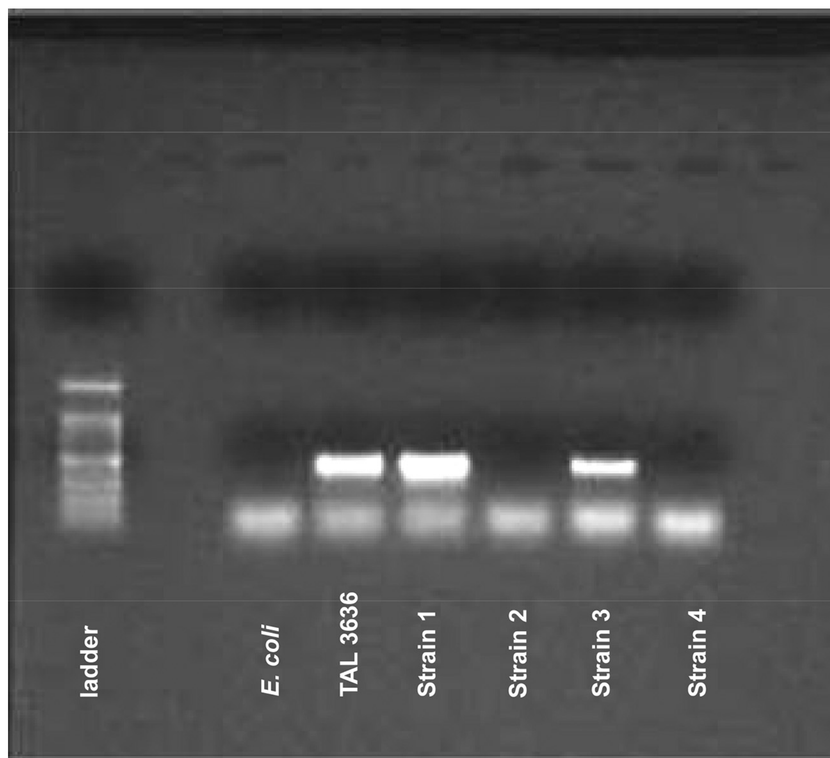


**FIG 1** *ccrA* β-lactamase background in the four *Bacteroides* sp. PCRs for the *ccrA* metallo-β-lactamase gene in 4 carbapenem-resistant clinical *Bacteroides* strains show that isolates 1 and 3 possess this gene, while isolates 2 and 4 do not. Isolates 1 and 3 also displayed good hydrolytic activity against the chromogenic cephalosporin substrate nitrocefin, while 2 and 4 did not. *E. coli* was run as a negative control that does not possess the *ccrA* gene. TAL3636 was used as the positive control as it is a *Bacteroides* sp. known to possess the *ccrA* gene (18).

inson) to determine the *E. coli* strain susceptibilities. Subsequently, *in vitro* mixing studies, and the measurement of broth microdilution MBCs with *E. coli* in the presence of each of the 4 *Bacteroides* isolates, were performed in brucella broth. We obtained MBCs, rather than MICs, as the visual inspection of antibiotic-containing microtiter plates does not indicate whether it is the ceftriaxone-susceptible or the ceftriaxone-resistant organism that has survived. For the mixing studies, standard concentrations (1:100 dilution of the 0.5 McFarland standard) of the five organisms were prepared. We prepared antibiotic dilutions of ceftriaxone according to CLSI methods (15) for MBC determinations in microtiter plates. We then inoculated ceftriaxone-containing wells of the microtiter plates first with the appropriate concentration of each specified *Bacteroides* strain and then with the McFarland dilution of *E. coli*. After they were incubated overnight at 37°C in an anaerobic chamber (Coy Laboratories, Grass Lake, MI), MBCs for the susceptible *E. coli* strain were determined by plating samples from all of the wells onto agar selective for facultative Gram-negative bacteria (MacConkey, Becton-Dickinson, Sparks, MD). The plates were subsequently incubated aerobically at 37°C overnight to determine *E. coli* growth. We defined bactericidal activity as a $\geq$3-$\log_{10}$ reduction in the initial inoculum (99.9% killing) after 24 h of incubation (17).

**Statistical analysis.** The paired *t* test was used to compare differences in the effects of ceftriaxone on the MBCs for the *E. coli* strain that had been incubated with ceftriaxone-resistant *ccrA*-positive versus that incubated with *ccrA*-negative *Bacteroides* strains.

***Bacteroides* strain choice.** After assaying for known *Bacteroides* β-lactamase genes among our collection, four *Bacteroides* strains were chosen to test our hypothesis. PCR for *ccrA* (a broad-spectrum Ambler class B metallo-β-lactamase that hydrolyzes carbapenems and cephalosporins [18]) in 4 cephalosporin-resistant *Bacteroides* strains showed that strains 1 and 3 possessed this gene, while strains 2 and 4 did not (Fig. 1). All four strains were PCR negative for the other known *Bacteroides* β-lactamase genes (*cblA*, *cepA*, and *cfxA* [other gels not shown]). Additionally, strains 1 and 3 exhibited very rapid hydrolysis of nitrocefin, while strains 2 and 4 did not hydrolyze nitrocefin (i.e., their mechanism of resistance to ceftriaxone did not appear to be related to the production of a cephalosporinase).

**MBCs.** With regard to the determinations of individual MBCs, all 4 strains of *Bacteroides* were resistant to the commonly used β-lactam antibiotic ceftriaxone, while the *E. coli* strain used in these experiments displayed good susceptibility to ceftriaxone, with MBCs in the <0.25-mg/liter range (Fig. 2A).

**Mixing study results.** Results of the mixing studies demonstrated that, in repeated broth microdilution MBC testing by standard methods, the geometric mean MBC of ceftriaxone for *E. coli* was raised when it was determined in the presence of *ccrA*-positive *Bacteroides* strains expressing rapid hydrolytic activity against nitrocefin but not when determined in the presence of cephalosporin-resistant but *ccrA*-negative *Bacteroides* strains displaying poor nitrocefin hydrolysis (Fig. 2B). Incubation with *Bacteroides* strains 1 or 3 raised the MBC of ceftriaxone required to kill a susceptible *E. coli* strain from <0.25 mg/liter to a geometric mean

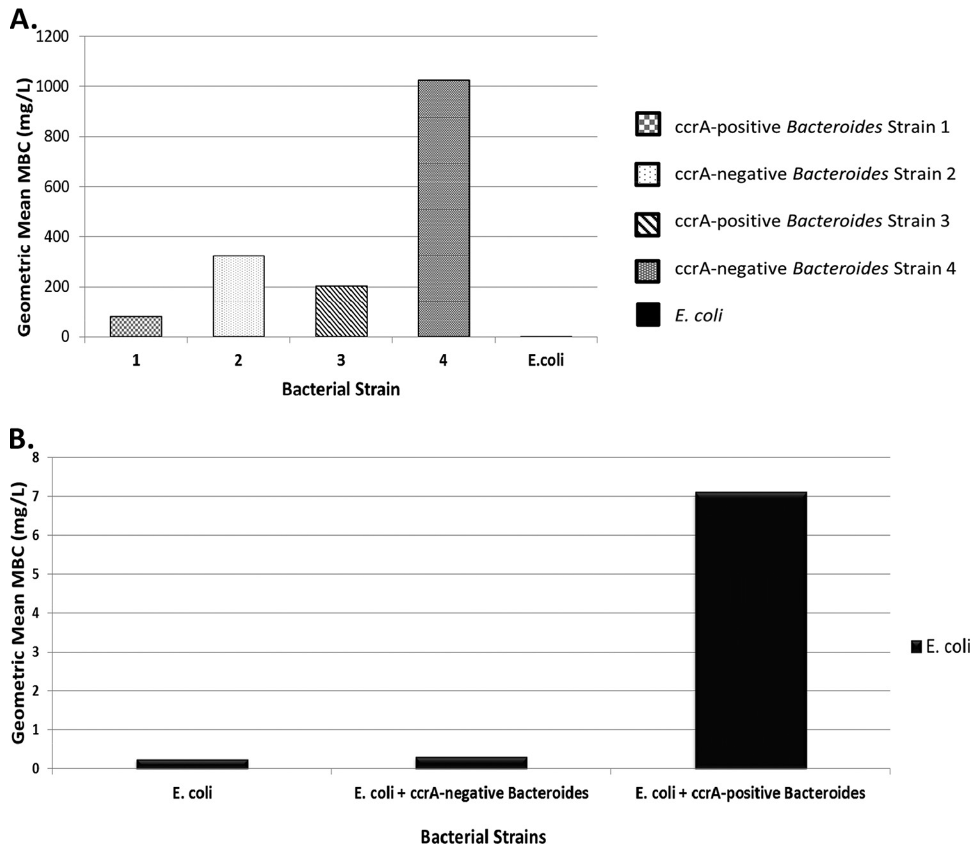


**FIG 2** MBC determinations. (A) Geometric mean MBCs of ceftriaxone for each of the five bacterial species. All 4 strains of *Bacteroides* were resistant to the commonly used β-lactam antibiotic ceftriaxone. The *E. coli* strain used in these experiments, however, displayed good susceptibility to ceftriaxone, with MBCs in the <0.25-mg/liter range. (B) Geometric mean MBCs of ceftriaxone for the *E. coli* strain when it was grown alone, in the presence of *ccrA*-negative non-β-lactamase-producing *Bacteroides* spp., or in the presence of *ccrA*-positive β-lactamase-producing *Bacteroides* spp. All MBC determinations were made at least in triplicate.

value of 7.12 mg/liter or a pooled average MBC of 29 mg/liter ($P = 0.009$), whereas incubation with *Bacteroides* strains 2 or 4 had no significant effects (geometric mean MBC change from <0.25 mg/liter to 0.31 mg/liter or pooled average MBC change to 0.33 mg/liter; $P = 0.17$). Survival of the *E. coli* isolates in the presence of *Bacteroides* strains 1 and 3 was not secondary to the development of resistance, as repeat MBC testing on recovered *E. coli* isolates from the mixing studies with *Bacteroides* still showed it to be susceptible to ceftriaxone (MBC, <0.25 mg/liter).

In a previous series of animal experiments, we showed that β-lactamase production by a *B. thetaiotaomicron* isolate could protect susceptible organisms within the same microbiota from β-lactam antibiotics (8). In the present study, we demonstrated that the magnitude of this effect is correlated with the genetic and phenotypic characteristics of the β-lactamase background in the protecting anaerobes. Cephalosporinase-producing anaerobes that possess a broad-spectrum β-lactamase gene allowed survival of a susceptible *E. coli* isolate despite high concentrations of ceftriaxone, while cephalosporin-resistant anaerobes without cephalosporinase activity did not.

These results highlight a potential mechanism by which antimicrobial resistance in bacteria—usually an undesirable phenomenon—may one day be leveraged to benefit hospitalized patients. According to recent estimates, *Bacteroides* group organisms (*B. fragilis*, *B. distasonis*, *Bacteroides ovatus*, *B. thetaiotaomicron*, and *Bacteroides vulgatus*) may comprise up to one-fourth of the indigenous intestinal microbiota in humans (19); our results suggest that β-lactam resistance in these microorganisms may not always be a bad thing. From an ecological perspective, such findings may also represent part of the explanation for why only some β-lactam-treated patients conserve their microbiome in the hospital setting and resist colonization by nosocomial pathogens.

Our study has some limitations. It is an *in vitro* examination of microbial interactions that usually take place within a complex ecosystem, and as such, it serves as a model. Second, it would be of interest to characterize the mechanism of resistance to β-lactam antibiotics in the non-β-lactamase-producing *Bacteroides* isolates, and it may have been of interest to measure the amount of ceftriaxone present in the wells by high-pressure liquid chromatography (HPLC) or other methods after the mixed incubation of multiple organisms. Third, many hospitalized patients receive more than one class of antibiotic. Any pharmaceutical, probiotic, or ecologic strategy attempting to capitalize on these findings would have to be accompanied by effective antimicrobial stewardship, as β-lactamases produced by gut anaerobes would not protect against non-β-lactam classes of antibiotics that are secreted into the intestinal tract.

In summary, we have shown that the degree of protection from

β-lactams conferred by common anaerobes on other members of the gut microbiome *in vitro* appears to be associated with their *bla* genetic and phenotypic profiles and not simply with the presence of β-lactam resistance in the protecting anaerobes. Further investigations to determine the importance of this mechanism of protection in hospitalized patients are indicated, as these results may inspire novel approaches to prevent pathogen colonization.

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