In their excellent review of the classification of carbapenem-hydrolyzing metallo-β-lactamases, Rasmussen and Bush (6) proposed that such enzymes produced by Bacteroides fragilis should be assigned to a new functional subgroup, 3a. The characteristics of β-lactamases from three B. fragilis strains were cited and included those from B. fragilis QMCN3 and B. fragilis QMCN4 (7, 8). To avoid confusion in the literature, we wish to point out that these strains were first isolated at the Queen’s Medical Centre, Nottingham, United Kingdom (not Queen’s Medical College, London, United Kingdom, as stated in earlier reports by Rasmussen et al. [7, 8]) and were originally designated B. fragilis 57 and B. fragilis 97, respectively (5).

In tabulating features of these enzymes for comparison with other group 3 β-lactamases, Rasmussen and Bush indicated that data on inhibition profiles, pl values, effect of zinc ions, and the relative hydrolysis of meropenem and imipenem were incomplete. However, these and other data, which would be useful when considering the characteristics of this group of enzymes, are available.

Resistance (50% inhibitory concentration of >100 μM) of β-lactamases from these strains to six β-lactamase inhibitor compounds, including clavulanic acid and sulbactam, was reported in 1986 (5). The effect of cations on their activity has also been examined in detail (2). Zinc ions were shown to enhance activity and to be required for restoration of >90% of the activity after inhibition by EDTA. Partial restoration of activity (39 to 45%) was achieved with cobalt ions, although manganese, magnesium, and iron had no or minimal effect. The pl value of the metallo-β-lactamase of B. fragilis 97 (QMCN4) was found to be 4.6 (5) and that of B. fragilis 57 (QMCN3), initially reported as 4.2 (5), has been found to be 4.7 after repeated testing (3). Also, β-lactamase-mediated hydrolysis of meropenem has been shown to be at least as efficient as hydrolysis of imipenem (1).

Unlike Rasmussen et al. (8), we have not detected coproduction of other β-lactamases with B. fragilis 57 (QMCN3), either by isoelectric focusing following pretreatment of β-lactamase extracts with EDTA or by demonstration of residual cephalosporinase activity in the presence of the chelator (3).

Interestingly, neither B. fragilis 57 nor B. fragilis 97 exhibits high-level resistance to carbapenems. Indeed the MICs of imipenem and meropenem for B. fragilis 97 (QMCN4) are 0.5 and 2 mg/liter, respectively (1), within the normal breakpoints for susceptibility. However, the conversion of B. fragilis from low- to high-level resistance, which has been observed in a patient undergoing therapy with imipenem (9), underlines the potential clinical importance of these enzymes. Strains exhibiting low-level resistance to imipenem currently comprise about 7% of isolates of anaerobic gram-negative bacilli in Nottingham (4). They are easily overlooked by routine susceptibility testing and may pose a more significant threat than has been supposed.

REFERENCES

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Author’s Reply
We appreciate the clarification provided by Edwards and Greenwood regarding the origin of Bacteroides fragilis strains QMCN3 and QMCN4. These cultures had been deposited in the culture collection of American Cyanamid before we joined the group working with these strains. We were unaware of the original designations of these as B. fragilis 57 and B. fragilis 97 (1) and appreciate the additional information provided by the authors. This oversight underscores the need for culture collections to be designed to cross-reference new additions to their previous literature references.

Like the authors of the letter, we were unable to identify a second β-lactamase in strains QMCN3 and QMCN4 when crude extracts were examined by isoelectric focusing (4). However, we were able to demonstrate complete inhibition of the imipenem-hydrolyzing activity after treatment with 1,10-phenanthroline, a strong chelator of Zn2+, but only 60 and 12% inhibition of the nitrocefin-hydrolyzing activity in each strain, respectively (4). These results led us to believe that there were multiple β-lactamases present, as is generally found in organisms that produce carbapenem-hydrolyzing enzymes (3). These results were also substantiated by comparing the amount of cephalosporinase activity in crude extracts with that predicted from the hydrolytic activity of the purified enzymes with both imipenem and nitrocefin. In both extracts, a higher cephalosporinase activity was present in the crude extracts than that expected if only the metalloenzyme were contributing to the nitrocefin hydrolysis. In addition, p-chloromercuribenzoate, a potent inhibitor of B. fragilis CcrA metallo-β-lactamase activity at a concentration of 0.17 μM (5), was reported to be poorly active against the β-lactamase activity of B. fragilis strains 57 and 97, with 50% inhibition occurring at 100 μM (1), again indicating the presence of an additional non-metallo-β-lactamase activity in these strains.
It is possible that EDTA as used by Edwards and Greenwood was inhibitory to both the metalloenzyme and the cephalosporinase activity, a phenomenon previously reported by Yang et al. in early studies with the Sme-1 serine-based carbapenem-hydrolyzing cephalosporinase from Serratia marcescens (6). Later studies showed that the apparent inhibition of the Sme-1 enzyme by EDTA was due to pH effects in the assays (2). However, the authors are correct in their statement that there is no direct proof for the existence of a second β-lactamase in these strains.

We agree that because clinical laboratories may overlook strains that exhibit low-level expression of metallo-β-lactamases, it is important to identify B. fragilis isolates for which imipenem MICs are higher than those normally found in that laboratory. Although the strains may not exhibit clinical resistance initially, their potential for developing high-level carbapenem resistance should be recognized.

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