CHAPTER IV

DISCUSSION

The activity of β-lactamase detected by nitrocefin solution varied among strains of the same species in B. fragilis group (Table 6). This may happen by the difference in either the ability of β-lactamase excretion or the nature of β-lactamase production individually.

Although the numbers of isolates of the species other than B. fragilis were not large, the data seemed to show that there were no differences among species of B. fragilis group in the levels of MIC to β-lactam antibiotics. The strains of B. ovatus showed a little higher level of MIC_{50} and MIC_{90} to the three antibiotics than other species (Table 8). The markedly differences in sensitivities at the MIC_{50} and the MIC_{90} for ampicillin and penicillin G may indicate the development of resistant strains.

There was not an absolute correlation between the presence of β-lactamase activity and the resistance of B. fragilis group to ampicillin and penicillin G. Since the β-lactamases of B. fragilis were predominantly active against cephalosporins but were moderately active against penicillin. Therefore the β-lactamase activity detected by chromogenic cephalosporin did not correlate to the MIC of ampicillin and penicillin. There were some strains of B.fragilis group with β-lactamase activity susceptible to ampicillin and

penicillin G and some strains resisted without β-lactamase activity (Table 9). As we know, resistance of bacteria to β-lactam antibiotics was due to a combination of many factors :- the affinity of the antibiotic for the peptidoglycan synthesising enzymes, the extent of the barrier to penetration of the antibiotic to the cell wall, and the activity of the varying amount of β-lactamase present inside the bacterial cell. The presence of a β-lactamase may not be the most important factor in the resistance of a particular species to a β-lactam compound. A compound which was hydrolyzed by a β-lactamase, may still able to kill the bacteria because it can pass freely through the outer membrane and reach inhibitory concentrations at the surface of the inner membrane [103]. Therefore, the resistance levels of bacteria to different β-lactam antibiotics may not correlate with the rates at which these antibiotics were hydrolyzed by the β-lactamase released from the bacteria because one of the resistance mechanisms to some β-lactam antibiotics was attributed to the impermeability of drugs into bacterial cells [61].

For cefoxitin, the resistance of B. fragilis group to this antibiotic was important because it was associated with high frequency of cross resistance to other β-lactam agents [104]. However, cefoxitin seemed not to be a substrate for the β-lactamase of these organisms [56,82]. Five of the 90 strains tested were highly resisted to cefoxitin. All of these highly resistance strains produced strong β-lactamase activity (Table 9). The enzymes may be able to hydrolyze cefoxitin the same as the report of other investigators [61,62], or the resistance may due to the synergistic effect of limited drug permeability and periplasmic localization of β-lactamase [64,81], or

the resistance may only be intrinsic factor [105].

From Table 11, the conjugal transfer of ampicillin resistance from B. fragilis strains to E. coli were unsuccessful. The resistance transfer may be inhibited by anaerobic environment [68,106]. Some donor strains which the MIC to ampicillin were greater than 128 µg./ml. contained some plasmids but they could not transfer the ampicillin resistance to B. vulgatus. The plasmids may lack the genetic determinants which were necessary to mediate the transfer of the R-marker. On the other hand plasmids with a molecular weight of less than 10 megadaltons were normally not self-transmissible [107], or the plasmids may not associate with ampicillin resistance. On the top of this, both E. coli and B. vulgatus No.48 may not be an appropriate recipient strains for the Bacteroides that the donors strains may not have been able to fuse with them. However, the 3 strains of B. ovatus can transfer the ampicillin resistance to B. vulgatus though the plasmid was not found in the transconjugants (Table 11). All of the transconjugants showed significant increase in β-lactamase activity (Table 12). The absence of detectable plasmid DNA (in a strain of donor and their transconjugants) suggested that the resistance marker as well as the genes responsible for conjugation could be chromosomal origin. Transferable antibiotic resistance in the absence of extrachromosomal plasmid DNA in other bacteria has been recently documented [108,109]. This may associate with conjugative transposons or the resistance genes were carried on a plasmid which can integrate into the chromosome of the transconjugants as an episome.

The increasing in the MIC for cefoxitin of the transconjugants from 8 μg./ml. to 32 μg./ml. may not be the result of the transfer of resistance marker to cefoxitin but may be the selection of mutant with defect in permeability.

The β-lactamase with pI 4.9 had been found in most strains of B. fragilis that possess β-lactamase activity. Coincidently, the value was the same as other investigators [57,82-86]. However, many reports showed different values [12,58,110]. Unfortunately, the number of tested strains were not large enough to conclude about the species specific of the enzyme. Without the evidence of transferation or the association with plasmid and with the constitutive property, β-lactamase of B. fragilis was most likely chromosomally mediated as suggested by Tally et al. [12].

Following isoelectric focusing study, some β-lactamases did not focus as a single entity but as a distinctive pattern of satellite bands (Fig. 8). The degradation, possible enzymic, of a parent β-lactamase molecule to give a family of closely related molecules was suggested [111]. The explanation was that the loss or modification of amino acid residues from the parent enzyme causing changes in the net charges of the resultant β-lactamase molecules.

The weak activity of some β-lactamases showed non-detectable band after isoelectric focusing study. The activity of these enzymes may lost during the process of study. One may concentrate the enzymes by ultrafiltration to increase the activity and render the detectable band.

From this study, the isoelectric point of β-lactamases from B. fragilis group did not fit in the classification scheme [26]. The type of β-lactamases from anaerobic bacteria may different from aerobic bacteria. Therefore, further study on the properties of these enzymes may set a new scheme for classification of β-lactamases from anaerobic bacteria.