

CHAPTER III

RESULTS

1. β -lactamase Production

The four species of 90 strains of Bacteroides fragilis group obtained from various clinical specimens were studied (Table 5). Their β -lactamases activity were assayed with nitrocefin. As shown in Table 6, 85 of 90 Bacteroides strains produced β -lactamase. These included 96 percent of B. fragilis, 57 percent of B. vulgatus and 100 percent of B. ovatus and B. distasonis. Only 5 of 90 strains included two strains of B. fragilis and three strains of B. vulgatus did not produce β -lactamase. Most Bacteroides strains produced moderate to small amount of β -lactamase.

2. Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of these organisms to three β -lactam antibiotics were shown in Table 7. The MIC₅₀, MIC₉₀ and range of the MIC of the isolates were demonstrated in Table 8. Most Bacteroides strains had the MIC of ampicillin and penicillin between 16 and 32 $\mu\text{g./ml.}$ On the contrary, they were more susceptible to cefoxitin than ampicillin and penicillin. The correlation of β -lactamase activity and the level of MIC were shown in Table 9 and Table 10. Many β -lactamase producing strains resisted to ampicillin and penicillin.

3. Transferability of β -lactamase Production

As shown in Table 11, the transferability of ampicillin resistance from 30 isolates Bacteroides were tried in E. coli K12 J53 or E. coli C600 by both broth mating and filter mating without success included studies which the donor cell was enhanced for increase plasmid copies by pretreated with 64 $\mu\text{g./ml.}$ of ampicillin before filter mating. The total of 24 strains of β -lactamase producing strains of B. fragilis, B. ovatus, and B. distasonis were further mated with a strain of the non- β -lactamase producing B. vulgatus. Only three strains of B. ovatus were able to transfer the antibiotic resistance to the recipient (Table 11). The MIC for the donors, the recipient and the transconjugants to ampicillin, penicillin G and cefoxitin, the β -lactamase activity and the pI of the β -lactamases of these three strains were shown in Table 12. The transconjugants had β -lactamase activity, the MIC to ampicillin and penicillin G similar to the donor. The MIC for the transconjugants to cefoxitin were however slightly lowered than the donors except the strain T No. 30.

4. The Study on Plasmid Extraction

As shown in Fig. 7, the study of plasmid DNA by alkaline extraction of the three strains of B. ovatus which were transferable showed that plasmid of M.W. 2.2 kb. was demonstrated only in the donors but not in the transconjugants.



5. Isoelectric Focusing Study

As shown in Table 13 and Fig. 8, the isoelectric point of β -lactamases from B. fragilis group in this study was 4.9 (30 of 35 strains) and 4.9, 5.1 (5 of 35 strains) which was similar to various investigators [9,82,83,85,88]. The 9 strains of B. ovatus had pI value of 4.3 which was similar to the report of Dornbusch et al. [82]. The 5 strains of B. distasonis focused the pI value at 4.8 as reported by Tally et al. [12]. Last of all 2 strains of B. vulgatus produced β -lactamase with the pI value of 4.5 similar to the report of Maskell et al. [9]. Some strains of B. ovatus and B. distasonis also had another pI minor band at pH 4.6 and 4.9 respectively.

The isoelectric point of β -lactamases from transconjugants and donors had major bands at pH 4.3.

In few strains which have weak activity of β -lactamase, it is not possible to visualize the bands on isoelectric focusing no matter the induction of β -lactamase was introduced with ampicillin 32 $\mu\text{g./ml.}$