Journal of Bangladesh College of Physicians and Surgeons
Vol. 23, No. 1, January 2005

Minimum Inhibitory Concentrations (MICs) for Imipenem of Bacteroides Fragilis - Study of 28 Strains

RS FERDOUSI[a], M HABIB[b]

Summary :

To determine the Minimum Inhibitory Concentrations (MICs) of imipenem of Bacteroides fragilis, a study was conducted in the Queens Medical Centre, University of Nottingham, England from September 1997 to August 1998. Twenty eight test strains of B. fragilis were tested for MICs of imipenem. Of them, 71.43% of B. fragilis strains showed MIC of < 8mg/L of imipenem.10.715% showed MIC of 8mg/L; 7.14% had 32mg/L and 64mg/L; 10.715% showed MIC of ≥265mg/L. Considering the break point of imipenem resistance as 8mg/L, it could be concluded from this study that 71.43% of B. fragilis strains were sensitive to imipenem.

(J Bangladesh Coll Phys Surg 2005; 23 : 9-11)

Introduction :

Bacteriodes fragilis is a well recognized anaerobic Gram negative human pathogen. It is often isolated from intra abdominal abscess, peritonitis and wound infections associated with large intestine[1]. It is implicated frequently in brain abscess secondary to otitis media[2] and is a major cause of foot ulcer in diabetic patients[3]. Bacteroides species accounts for at least a quarter of all anaerobes isolated from clinical specimens in microbiology laboratories[4].

As most infections involving B. fragilis occur in combination with aerobic pathogens, antibiotic therapy must be directed at both aerobic and anaerobic components. Metronidazole is very effective against anaerobic bacteria and resistance is so uncommon that it is considered as the drug of choice for anaerobic infections[5]. It is usually combined with gentamycin which is widely active against aerobic Gram negative rods.

Most strains of B. fragilis produce B-lactamase which hydrolyses commonly used B-lactam agents including penicillin and ampicillin. However, these antibiotics may be activated by combination with B-lactamase inhibitor B-lactam compounds e.g. clavulanic acid[5].

For monotherapy, B-lactamase stable B-lactams are available, which can be used as a therapeutic agent in the poly microbial infection in which B. fragilis species are involved[5]. These include cefoxitin and the carbapenems-imipenem and meropenem. The lowest concentration of imipenem that completely inhibit the growth of B. fragilis after 48 hours of incubation anaerobically is considered as the MIC of imipenem of B. fragilis. The aim of this study was to determine the MICs of imipenem of B. fragilis strains in the laboratory to find out its sensitivity against these strains.

Materials and Methods :

The study was conducted in the Queens Medical Centre, University of Nottingham, England from September 1997 to August 1998. Twenty nine test strains of B. fragilis were initially selected from the Nottingham Public Health Laboratory, two were originally isolated in Spain and one originated in Japan. In addition, two control strains were used, B. fragilis TAL 3636 which was cfiA (carbapenamase gene) positive and highly resistant to carbapenems[7] and B. fragilis NCTC 9344, a carbapenem sensitive strain known not to produce ß-lactamase.

Brain Heart Infusion supplemented (BHIS) with yeast extract (5g/1), haemin (5mg/L) and menadione (1 mg/L) made according to the Manufacturers instructions was used as a general growth medium. BHIS agar media was obtained by addition of 1.2% Davis agar, (Unipath). The culture plates were incubated in an anaerobic cabinet with an atmosphere of 80% nitrogen, 10% carbon dioxide and 10% hydrogen at 37^0C.

The test strains were identified by use of carbohydrate fermentation, bile tolerance and aesculin hydrolysis. The test strains were grown over night in BHIS broth at 37^0C. An inoculum of 10^6 organisms of each strain were delivered on to agar surface of carbohydrate (glucose, lactose, salicin, trehalose and mannitol) and bile/aesculin test plates with a multipoint inoculator (Denleys Instruments, Billinghurst, Sussex). The plates were examined after 48 hours of anaerobic incubation.

a. Dr. Reena Saad Ferdousi, Associate Professor, Department of Microbiology, Bangladesh Medical College
b. Dr. Mansur Habib, Associate Professor, Department of Neurology, Dhaka Medical College.
Correspondence to: Dr Reena Saad Ferdousi, Associate Professor, Department of Microbiology, Bangladesh Medical College, Dhanmondi, Dhaka.

The antibiotic used was imipenem (Merck, Sharp and Dohme, Hoddeston, Herts). Solutions of imipenem were freshly prepared by dissolving antibiotic powder in sterile distilled water.

Minimum Inhibitory Concentrations were determined by the agar incorporation method. Imipenem in suitable two fold serial concentrations were incorporated in BHIS agar. An inoculum of 10^6 were delivered onto agar surface with a multipoint inoculator. The lowest antibiotic concentration that completely inhibited growth after incubation for forty eight hours at 370C in an anaerobic cabinet was taken as the MIC.

Results :

The Bacteroides fragilis was identified by carbohydrate fermentation, bile tolerance and aesculin hydrolysis. All strains grew on BHIS agar media. There was growth on bileaesculin agar media and black zone around growth indicated aesculin hydrolysis. Four strains fermented salicin, trehalose and mannitol and were not identified as Bacteroides fragilis. So they were excluded from further study. Twenty eight strains were confirmed as B. fragilis as they fermented glucose and lactose but not salicin, trehalose or mannitol. The MIC of the 28 test strains ranged from 0.5 to >256mg/L for imipenem. The following table shows the detailed MICs of the test strains. Twenty (71.43%) B. fragilis strains were sensitive to imipenem showing MIC of <8mg/L of imipenem. Three (10.715%) strains together with the positive control showed a high level of resistance (MIC >256mg/L). It had been estimated that 10.715% of the test strains showed MIC of 8mg/L; 7.14% had 32mg/L and 64 mg/L; 10.715% showed MIC of ≥265mg/L.

Table -I

MICs of imipenem of B. Fragilis test strains

B. fragilis strains	MIC of imipenem (mg/L)	Number (total = 28)	Percentage (N =28)
B. fragilis J8			
" " F10			
" " J1	0.5	5	17.86
" " RB11			
" " R208			
" " A1			
" " 0423			
" " Q7			
" " A3			
" " E10			
" " S1	1	10	35.71
" " R249			
" " R186			
" " 2013E			
" " R240			
" " R97			
" " R251			
" " B89	2	5	17.86
" " E9			
" " 212			
" " 119			
" " 7/5	8	3	10.715
" " T2			
" " 16/16	32		
" " 57	64	2	7.14
" " FS			
" " 288.89	256	3	10.715
" " GAI 30144	>256		

Discussion :

The majority of B. fragilis strains produce typical Bush class 2e ß-lactamase which are the primary mechanism of resistance to most ß-lactam antibiotics with the exception of ß-lactamase stable B-lactam e.g. carbapenem[8]. A minority of strains, however produce ß-lactamase that are metallo enzymes which hydrolyse nearly every class of ß-lactam antibiotic including carbapenem and are not susceptible to inhibition by classical ßlactamase inhibitors such as clavulanic acid[9,10].

In 1992, 1% of B. fragilis isolates have been shown to carry the Zinc dependant carbapenamase gene cfiA[11]. The number of B. fragilis isolates that display B-lactam antibiotic resistance is increasing and reports of carbapenamase production and carbapenem resistance are emerging[12].

The typical MIC of imipenem is 0.06mg/L, whereas the MICs of the test strains in this study ranged from 0.5 to >256 mg/L. Therefore, depending on the MICs of imipenem the isolates are categorized into four groups. Twenty of the test strains showed MIC 0.5-2mg imipenem/L. As the break point of imipenem resistance is 8mg/L, these strains were classified as sensitive, although at least a 10 fold higher antibiotic concentration was required to inhibit these strains compared to fully sensitive B. fragilis strains [13]. Three strains of B. fragilis showed MIC of 8mg/L of imipenem and they were classified as having low resistance. One strain of B. fragilis had MIC of 32mg/L and another one had 64mg/L and were classified as moderately resistant. Four strains of B. fragilis demonstrated high level of resistance with MICs of imipenem of 256mg/L or more. These values are consistent with the previous studies[14,15,16].

References :

1. Fingold S.M. Anaerobic infections in humans: an overview. Anaerobe 1995; 1:39.
2. Ingham H.R, Silcon J.B, Roxby C.M. Bacteriological study of otogenic cerebral abscesses: Chemotherapeutic role of metronidazole. BMJ 1997; 2 : 991-3.
3. Hedberg M and Nord C.E. Antimicrobial resistant anaerobic bacteria. J Chemother 1996; 8(1) : 3-16.
4. Hardic J.M and Allaker R.P. Non sporing anaerobes. Wound infections. Periodontal disease. In: Greenwood D., Slack R.C.B and Peuther J.F., eds. Medical Microbiology. 15th ed. New York: Churchill Livingstone, 1997 : 340-4.
5. Greenwood D. Inhibitors of bacterial cell wall synthesis. In: Greenwood D., ed. Antimicrobial Chemotherapy, 3`d ed. Oxford, New York, Tokyo: Oxford University Press, 1995 : 13-31.
6. Bush K. Other (3-lactams. In; O Grady F., Lambert H.P., Finch R.G. and Greenwood D, eds. Antibiotic and Chemotherapy, 7th ed. New York: Churchill Livingstone, 1997; 306-27.
7. Cuchural G.J.Jr, Tally F.P, Storey J.R Malamy M.H. Trasfer of B-lactamaseassociated cefoxitim resistance in Bacteroides fragilis. Antimicrob Agents Chemother 1986; 29 : 918-2.
8. Rasmussen B.A, Yang Y., Jacobus N., Bush K. Contribution of enzymatic properties, cell permeability and enzyme expression to microbiological activities of B-Lactam in three Bacteroides fragilis that harbour a metallo-(3 lactamase gene. Antimicrob Agents Chemother 1994; 38 :2116-20.
9. Bush K. Classification of B-Lactamases: groups 2c, 2d, 2e, 3 and 4. Antimicrob Agents Chemother 1989; 33 : 271-76.
10. Yang Y., Rasmussen B.A, Bush K. Biochemical characterization of the metallo-BLactamase CcrA from Bacteroides fragilis Tal 3636. Antimicrob Agents Chemother 1992; 36 : 1155-7.
11. Livermore D.M. Carbapenamases: the next generation of B-Lactamases? ASM News 1993; 59 : 129-35.
12. Cuchural G.J et al. Antimicrobial susceptibilities of 1292 isolates of B. fragilis group in the United States: comparision of 1981 with 1982. Antimicrob Agents Chemother 1984; 26 : 145-148.
13. Payne D. J. Metallo B-Lactamases- a new therapeutic challenge. J Med Microb 1993; 39 : 93-9.
14. Nord D.E. Hedberg M. Resistance to B-Lactam antibiotics in anaerobic bacteria. Rev Inf Dis 1990; (Supp 2) : S231-4.
15. Edwards R and Greenwood D. An investigation of B-Lactamase from clinical isolates of Bacteroides species. J Med Microb 1992; 36 : 89-95.
16. Eley A., Greenwood D. Characterisation of B-Lactamases in clinical isolates of Bacteroides. JAntimicrob Chemother 1986; 18 : 325-33.