นิพนธ์ต้นฉบับ

Evaluation of beta - lactamase - producing anaerobes from healthy and diseased subjects*

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Our study revealed that foth the healthy or diseased subjects haboured a specific-enzyme of medical importance. Moreover its presentation of Beta - lactamase is not only confined to the resistance of Beta-lactam antibiotics.

The anaerobes isolated from healthy and infectious conditions were tested for the presence of the specific Beta-lactamase. Of all the anaerobes from the 24 healthy persons, 10.86% of Beta-lactamase producing strains were from the oral cavity and 11.9% from normal vaginal-canal. From a total 195 anaerobic strains from 98 patients, 23.07% were Beta-lactamase producing and of these, 20.5% were Cl. perfringens, 43.5% B. fragilis and 50% B. melaninogenicus. None of the anaerobic cocci had the enzyme.

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ได้ตรวจหา เอ็นซัยม์ เบต้าแล็คตาเมส จากแอนแอโรบส์สายพันธุ์ต่าง ๆ จากคนปกติ 24 ราย ปรากฏว่า ในช่องปากของคนปกติ แอนแอโรบส์ที่มีเบต้าแล็คตาเมส อยู่ร้อยละ 10.86 ในช่องคลอดมีอยู่ร้อยละ 11.9 ในผู้ป่วยโรคติดเชื้อต่าง ๆ จำนวน 98 ราย ปรากฏว่า ในแอนแอโรบส์ 195 สายพันธุ์ที่แยกได้นั้น มีสายพันธุ์ที่ขับ เบต้า-แล็คตาเมส อยู่ถึงร้อยละ 23.07 แอนแอโรบิค ค็อคไค ไม่ขับเอ็นซัยม์ เบต้าแล็คตาเมสเลย ในแอนแอโรบิค บาคิลไลชนิดแกรมบวกนั้น คล็อสตริเดียม เพอร์ฟรินเจนส์ สร้างเอ็นซัยม์นี้มากถึงร้อยละ 20.4 ส่วนในบาคิลไลชนิดแกรมลบนั้น แบ็คทีรอยดีสต ฟราจิลิส และแบ็คทีรอยดีสต มีลานินโนเจ็นนิคัส สร้างเอ็นซัยม์นี้ได้ร้อยละ 43.5 และ 50 ตามลำดับ

การศึกษาชี้ให้เห็นว่า แอนแอโรบส์ ในนอร์มัล ฟลอร่าของคนปกติ มีเบต้าแล็คตาเมสแฝงอยู่ และยิ่งในสภาวะที่เป็นโรคติดเชื้อ เอ็นซัยม์นี้จะยิ่งเพิ่มมากขึ้น

อนึ่ง ได้ศึกษาความสัมพันธ์ของการดื้อเพ็นนิซิลลิน ของแอนแอโรบิค ปาโธเจนส์ สายพันธุ์ที่ขับเอ็นซัยม์ดังกล่าว ปรากฏว่า แม้ว่าแอนแอโรบิค ปาโธเจนส์นั้นจะขับเบต้าแล็คตาเมสเอ็นซัยม์ได้ก็ตาม แต่ก็ไม่ทำให้แอน-แอโรบส์นั้นดื้อต่อเพ็นนิซิลลินเต็มประสิทธิภาพ ร้อยละ 100 เสมอไป นอกจากนั้น คณะผู้วิจัยยังวิจารณ์ปัญหาในสังคม เช่นการใช้ปฏิชีวนะเบต้าแล็คแตมพร่ำเพรื่อ จุลินทรีย์แอโรบส์และแอนแอโรบส์ ย่อมจะสร้างเอ็นซัยม์นี้มากขึ้นทุกที และจะก่อปัญหาอย่างยิ่งต่อการดื้อยากลุ่มเบต้าแล็คแตม ในอนาคต

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It is well-known that aerobes and anaerobes or micro-aerophilic members dominate the indigeous, acquired and potential microflora of the skin, oral cavity, female genito-urinary-tract and penile lesions.[1,2,3] These organisms are not only abundant and highly diverse microbial flora of man but may occassionally cause primary or secondary infections. They may participate in a local or remote sepsis, often in mixed infections exhibiting pathogenic synergy and therefore pose problems in their detection, diagnosis and especially clinical management.[4]

The fact that either the beta-lactamase enzyme is detected in a specific anaerobic pathogen or its production by anaerobic bacteria can interfere with the eradication of Group A streptococci in tonsilltis treated with penicillin brought us to re-investigate our anaerobic strains sisolated from healthy and variously diseased subjects for the presence of beta-lactamase producing anaerobes.[5,6,7]

The purpose of the present article is to evaluate the roles of beta-lactamase-producing anaerobes isolated from normal subjects, and those found in common infectious diseases, between the detectable beta-lactamase enzymes and the pattern of penicillin-resistance.

Material and Methods

A. **The subjects** : The subjects consisted of 24 normal, healthy persons, 10 male Medical Students and 14 females from the Dept. of Obstetrics and Gynecology, and 74 patients (40 females, 34 males) between 20 to 50 years of age. Thirty cases of PID,* 4 of septic-abortions, 20 of penile lesions (soft chancre-included), 2 of recurrent tonsillitis, 10 with leg-ulcers, 4 of AAC** and 4 of peritonsillar abscess participated in this study. The patients were from Chulalongkorn Hospital and Phahol-Yothin General Hospital, Bangkok, Thailand.

B. **The sampling technique** : The process of taking the appropiate clinical specimens were obtained according to Narathorn et al.[2,3]

C. **The Laboratory procedures** :

C.1 The bacterial culture. The clinical specimens were immediately inoculated on different non-selective and selective-media for anaerobes described by Narathorn et al and Holdeman et al.[2,3,8,9]

C.2 Beta lactamase assay. The isolated anaerobic strains were tested for the production of beta-lactamase by the "Well in specific media" as described by Narathorn et al and also by Chromogenic cephalosporin.*** [10,11,12]

C.3 **Antibiotic susceptibility test.** All the anaerobic pathogens that haboured beta-lactamase enzymes-were tested for the penicillin-resistant pattern by the quantitative antimicrobial susceptibility method.[13]

The anaerobic isolation, the beta-lactamase assay and susceptibility test were done at the Anaerobe Unit, Dept. of Med. Microbiology, Chulalongkorn Hosp. Med. School, This project began in February, 1982.

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* PID = Pelvic inflammatory disease.

** AAC = Antibiotic associated colitis.

*** from Glaxo, Greenford, Middlesex, England.

Result

In normal healthy subjects 10.9% of β-lactamase producing anaerobes was recovered from the oral cavity and 11.9% from vaginal flora.

In the oral cavity, 5 strains of *B. melaninogenicus* haboured beta-lactamase and in the vaginal-canal the enzyme mostly found in *B. fragilis* and Bacteroides spp. (Table 1). In the infectious conditions, *B. melaninogenicus* released highest amount (50%) of β-lactamase. (Table 2)

Out of the total 195 isolated anaerobic pathogens from common infectious diseases cases only 23.07% released β-lactamase enzymes. None of the isolated-anaerobic cocci haboured β-lactamase enzymes, while 20.4% of gram-positive Clostridium perfringens elaborated β-lactamase and 43.5% of gram-negative *B. fragilis* did (Table 2).

Table 1 Beta - lactamase - producing anaerobes from normal healthy persons.

Anaerobic genus-species	Normal subjects (24)			
	Oral flora		Vaginal flora	
	Isolated anaerobes	β-lactamase producing strains	Isolated anaerobes	β-lactamase producing strains
GRAM POSITIVE COCCI				
Peptococcus spp.	6	0	6	0
Peptostreptococcus spp.	8	0	6	0
GRAM NEGATIVE COCCI				
Veillonella spp.	4	0	6	-
GRAM POSITIVE BACILLI				
Propionibacterium spp.	4	0	4	0
Clostridium perfringens	-	-	8	1
GRAM NEGATIVE BACILLI				
Unidentified Bacteroides spp.	2	0	6	2
B. gingivitis	4	0	-	-
B. fragilis	-	-	6	2
B. melaninogenicus	12	5	-	-
Fusobacterium spp.	2	0	-	-
Fusobacterium nucleatum	4	0	-	-
Total isolated strains	46	5 (10.9%)	42	5 (11.9%)

Table 2 Beta-lactamase producing anaerobes from common infectious diseases.

Anaerobic genus-species	Isolated anaerobes from infectious diseases							Total isolated anaerobic pathogens	β-lactamase producing strains
	PID (30)*	Septic abortion (4)	Sexually transmitted lesion (20)	Peritonsilitar abscess (4)	Recurrent tonsillitis (2)	Leg ulcers (10)	A A C (4)		
GRAM POSITIVE COCCI									
Peptococcus spp.	2	-	4	-	2	2	-	10	0
Peptostreptococcus spp.	10	2	4	2	2	3	1	24	0
GRAM NEGATIVE COCCI									
Veillonella spp.	8	2	2	2	2	-	-	16	0
GRAM POSITIVE BACILLI									
Lactobacilus spp.	3	-	-	-	-	-	-	3	0
Propionibacterium spp.	2	-	4	2	-	2	-	10	2(20%)
Clostridium difficile	-	-	-	-	-	-	2	2	0
Clostridium perfringens	28	3	4	-	-	14**	-	49	10(20.4%)
Clostridium novyi	4	2	2	-	-	4	-	12	4(33.3%)
GRAM NEGATIVE BACILLI									
Unidentified Bacteroides spp.	6	2	2	-	-	-	-	10	4(40%)
B. gingivitis	-	-	2	2	2	-	-	6	2(33.35%)
B. fragilis	15	5**	1	1	1	-	-	23	10(43.5%)
B. melaninogenicus	10	-	2	2	2	4	-	20	10(50%)
Fusobacterium spp.	-	-	-	-	2	-	-	2	1(50%)
Fusobacterium nucleatum	-	-	6	-	2	-	-	8	2(25%)
Total isolated strains	88	16	33	11	15	29	3	195	45(23.07%)

* In parenthesis were the number of cases in each infectious disease.

** The samples haboured more than one strain of the anaerobes.

The Laboratory data from Table 4 revealed the probable degree of β-lactamase activity and the significant role of penicillin resistance.

Discussion

Bacteria can become resistant to drugs by the following mechanisms. Ordinary Mutations may occur in genes on the bacterial chromosome which once arisen in a bacterial population, may be transfered to others by transformation, transduction or conjugation depending on which mechanism the species in question is capable of performing.[14]

In aerobic pathogens, 60-90 per cent of resistance genes are carried on plasmids. Since many of these are self transferable and can mobilize others which are not, drug resistance can spread through a population of sensitive bacteria in epidemic fashion.

However resistance to penicillin or other beta lactam antibiotics has been found

in virulent anaerobic bacteria to be plasmid determined. Whether the plasmids can undergo conjugal transfer is doubtful. The specific plasmids can be carried from cell to cell and the majority of them now carry plasmids,[14,15] the acquisition of plasmids accounts the majority of the failures of beta lactam antibiotic therapy.[14]

In Thailand, the bacterial beta-lactamase or formerly penicillinase enzyme was qualitatively detected since 1972.[10] Since then many new promising methods have been recommended.[11]

Beta-lactamase-enzyme-producing anaerobes in the normal healthy people demonstrate the probable role of latent beta-lactamase plasmids in the community. As Thai people can buy antibiotics freely from any drug-store without a physician's prescription, they often receive subtherapeutic doses of beta-lactam antibiotics. (Table 3).

Table 3 Age and sex - distribution of the normal and infectious entities.

Subjects or Patients	Age (years)				Sexes		Total
	20-30	31-40	41-50	over 50	Male	Female	
Normal healthy	3	8	13	-	10	14	24
PID	5	10	15	-	-	30	30
Septic abortion	-	4	-	-	-	4	4
Sexually transmitted lesions	5	10	5	-	20	-	20
Peritonsillar abscesses	2	2	-	-	2	2	4
Recurrent tonsillitis	-	1	1	-	2	-	2
Leg ulcers	2	2	6	-	8	2	10
AAC	-	2	2	-	2	2	4
total	17 (17.3%)	39 (39.8%)	42 (42.8%)	- -	44 (44.9%)	54 (55.1%)	98

Beta-lactamase-plasmids are latent in the anaerobes of the oral cavity and vaginal microbial flora (Table 3). However, beta-lactamase plasmids may be transfered from elsewhere especially from the aerobic flora.

In infectious conditions, beta-lactamase are undetectable in anaerobic-gram-positive and gram negative cocci. Resistance to penicillin may therefore be from other mechanisms as mentioned above. (Table 2). Many anaerobic gram-positive and gram-negative bacilli produce the specific enzymes. All the anaerobic pathogens are isolated from patients between 20 to 50 years of age without any underlying-diseases or nosocomial infections (Table 3).

In our series, there were 43.47 per cent beta-lactamase positive *B. fragilis* and 50 per cent from B.melaninogenicus. The percentage of positive findings was lower compared to other studies[16] (Table 2) This may have been due to the naure of the plasmids and the local community. As a matter of fact in a developing country like Thailand beta-lactamase-producing anaerobes ought to have been found more frequently than mentioned in Table 2-4

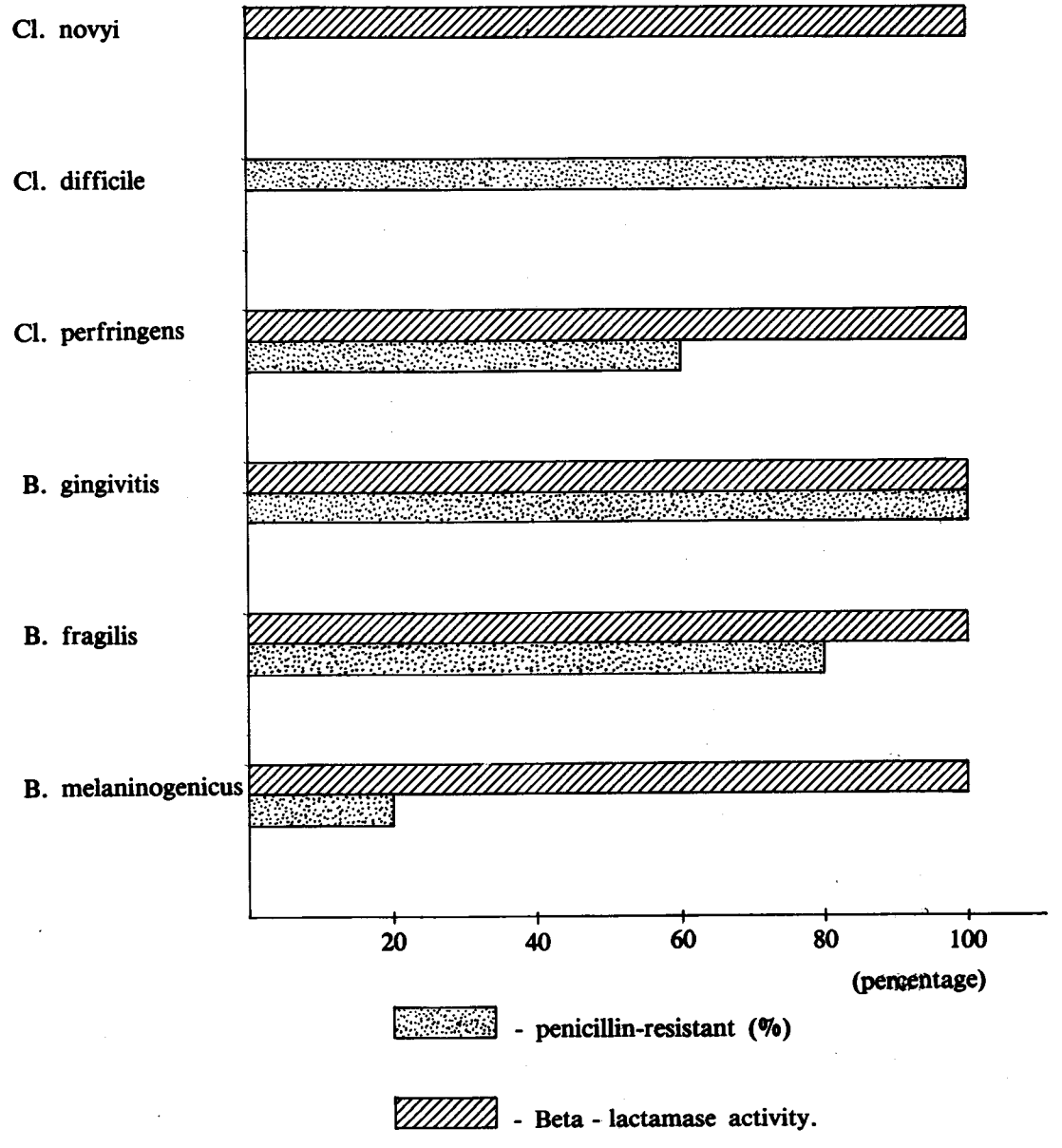
Table 4 Distribution of Beta lactamase producing anaerobes in corresponding with the infectious entities and the significant-role of penicillin-resistance pattern in Beta-lactamase producing anaerobic pathogens.

Anaerobic genus-species	Total isolated strains	β-lactamase producing strains (%)	Isolated strains from infectious diseases							Total β-lactamase producing strains	No. of penicillin resistant strains (%)	Probable degree of β-lactamase activity
			PID (30)*	Septic abortion (4)	Sexually transmitted lesion (20)	Peritonsillar abscess (4)	Recurrent tonsillitis (2)	Leg ulcers (10)	A A C (4)			
GRAM POSITIVE BACILLI												
Lactobacillus spp.	3	-	-	-	-	-	-	-	-	0	3	0
Propionibacterium spp.	10	2(20)	-	-	-	2	-	-	-	2	0	0
Clostridium difficile	2	0	-	-	-	-	-	-	0	0	2	0
Clostridium perfringens	49	10(20.4)	4	2	2	-	-	1	1	10	6(60)	+3
Clostridium novyi	12	4(33.3)	-	2	-	-	-	2	-	4	0	0
GRAM NEGATIVE BACILLI												
Unidentified Bacteroides spp.	10	4(40)	2	2	-	-	-	-	-	4	4(100)	+4
B. gingivitis	6	2(33.3)	-	-	-	-	2	-	-	2	2(100)	+4
B. fragilis	23	10(43.4)	8	2	-	-	-	-	-	10	8(80)	+3
B. melaninogenicus	20	10(50)	5	1	-	2	2	-	-	10	2(20)	+1
Fusobacterium spp.	2	1(50)	-	-	-	-	1	-	-	1	1(100)	+4
Fusobacterium nucleatum	8	2(25)	-	1	-	1	-	-	-	2	0	0
Total strains	145	45	19	10	2	5	5	3	1	45	28	

* In parenthesis were the number of cases in each infectious disease.

From Table 4, one can tabulate the significance of β-lactamase in relationship to penicillin resistance, as follow : (Figure 1)

Figure 1 The relationship between the beta-lactamase producing activity and the penicillin-resistant pattern in anaerobic strains.



a **The probable 100 per cent beta-lactamase activity** : The unidentified Bacteroides spp., *B. gingivitis,* and Fusobacterium spp. demonstrate the important role of beta-lactamase in destroying penicillin potency and therefore showing 100 per cent resistance to penicillin. (Table 5)

Table 5 Probable role of β-lactamase activity and penicillin resistant pattern of β-lactamase producing anaerobes in vitro.

In vitro percentage of		Interpretation (degree of β-lactamase activity)
β-lactamase producing anaerobes	Penicillin-resistant strains	
100%	100%	+ 4
100%	> 50%	+ 3
100%	50%	+ 2
100%	< 50%	+ 1
100%	0	0 or doubtful

b **The over 50 per cent beta-lactamase activity** : Although beta-lactamase was seen in *Cl. perfringens,* and *B. fragilis* only penicillin was mostly destroyed. The activity of bacterial beta-lactamase enzyme in this situation was partial. (Table 5)

c. **The 50 per cent beta-lactamase activity** : Beta-lactamase activity producing exactly 50 per cent was not seen in our series.

d. **The less than 50 per cent beta-lactamase activity**: This can seen in *B. melaninogenicus* that partially destroyed penicillin. (Table 5)

e. **The probably non benefit beta-lactamase activity** : These strains habour specific enzymes which can-not destroy the beta-lactam antibiotics. The anaerobic pathogens were therefore sensitive to penicillin. (Table 5)

Also from Table 4, *Cl. difficile* had no beta-lactamase activity yet, these anaerobes showed 100 per cent resistance to penicillin.

From our study, beta-lactamase was the potent inhibitor of beta-lactam antibiotics in vitro. In vivo many factors may enhance the resistance-pattern penicillin has a high protein-binding in serum, or some times is unable to attach and be absorbed into the bacterial cell-wall of anaerobes. However penicillin is still used in clinical practice due to its in expensiveness for most anaerobic and aerobic pathogens.

The percentage of penicillin resistance will increase in the future owing to the subtherapeutic usage by druggists, and the latent beta-lactamase enzyme in the normal flora of the community. and will brings about a substantial change in the use of beta-lactam antibiotics in serious infections

The "well in media" or the chromogenic cephalosporin method was equally sensitive, although the latter was more

practical and less time consumming. The "well in media" was complicated, time-consuming and required special, experienced operators.

One notice that anaerobic *B. melaninogenicus* played a significant role in the oral cavity (Table 2.), while *B. fragilis* and other Bacteroides spp. were potent penicillin inhibition in infectious diseases (Table 2,4).

Finally, the anaerobes play an important role in the normal flora of human-beings. However, they may turn harmful "Oppor tunistic" if they incorporate β-lactamase plasmids as shown in this article.

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