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Quantitative antimicrobial susceptibility of anaerobic bacteria from clinical specimens 1981-1983

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ได้ทดสอบ "ความไว" ของแอนแอโรบิก ปาโรเจนส์จำนวน 56 สายพันธุ์ โดยวิธี Standard dilution-technique ต่อยาด้านจุลินทรีย์ที่สำคัญทางแพทย์ 8 ชนิด ปรากฏผลว่า,

B. fragilis "ไวมาก" ต่อ คลินดามัยซิน (20 ไมโครกรัม/มล.)

Cl. perfringens "ไวมาก" ต่อ cefoxitin (0.68 ไมโครกรัม/มล.)

F. necleatum "ไวมาก" ต่อ ซอลแรมเฟนิคอลล (1.56 ไมโครกรัม/มล.)

และ *P. acnes* "ไวมาก" ต่อ เมโทรนิดาโซล (0.9 ไมโครกรัม/มล.)

แอนแอโรบิก ปาโรเจนส์ที่นำมาศึกษาทั้งหมดมีลักษณะ "คือ" ต่อแอนติไมโครเบียล เอเยนต์ประจำทุกสายพันธุ์ ยาด้านแอนแอโรบส์มีการ "คือ" สูงสุดคิดเป็น "ร้อยละ" ดังนี้, *F. necleatum* มีการ "คือ" สูงสุดต่อ ไธแอมเฟนิคอลล คิดเป็นร้อยละ 60, *B. fragilis* มีการ "คือ" สูงสุดต่อซอลแรมเฟนิคอลล คิดเป็นร้อยละ 54.55, *B. fragilis* และ *Cl. perfringens* มีการ "คือ" สูงสุดต่อ เมโทรนิดาโซล คิดเป็นร้อยละ 54.55 และ 71.43 ตามลำดับ *B. fragilis* มีการ "คือ" สูงสุดต่อ อิริย์โรมัยซิน คิดเป็นร้อยละ 54.55 แอนแอโรบส์ ที่ศึกษามีการคือต่อ เฟนิซิลลิน และ ซีฟ็อกซิทิน ปานกลาง

ปฏิชีวนะประเภท non Betalactams นั้น, คลินดามัยซิน มีค่า MIC ต่ำกว่า ส่วนปฏิชีวนะประเภท Betalactams นั้น cefoxitin นับว่ามีค่า MIC ต่ำกว่า เฟนิซิลลิน อนึ่ง ในรายงานนี้ยังวิจารณ์ปัญหาต่าง ๆ ในการทดสอบความ "ไว" ของแอนแอโรบส์ ต่อปฏิชีวนะต่าง ๆ ปัญหาสังคมไทยในการซื้อหาปฏิชีวนะโดยเสรี ตลอดจน แนะนำการพิจารณาผล antibiograms.

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A sharp rise in the frequency with which anaerobes are isolated from clinical material has occurred in recent years. This has resulted in an increased demand for routine susceptibility tests on the anaerobic isolates. Routine testing of all isolated appears to be unnecessary since many of them have predictable susceptibility patterns to commonly recommended antimicrobials. Furthermore, susceptibility test results may be misleading, since most anaerobic infections are polymicrobial, and the nature of the infectious process often changes in the interval between collection of the specimen and reporting the results. However susceptibility test are often needed for patients with serious infections such as endocarditis and brain abscess or infections requiring prolonged therapy such as osteomyelitis, or in persistent an recurrent infectious despite appropriate antimicrobial therapy. They are also needed in monitor the susceptibility of commonly isolated species so that changing patterns of resistance can be detected and the empirical basis of therapy can reflect any changes in common antibiograms. (1,1 a)

This report presents the minimal inhibitory concentrations (MIC) of 56 clinical isolates of anaerobic bacteria to 8 different antimicrobial agents in order to evaluate the significant value against anaerobic pathogens.

Materials and methods

1. Materials

1.1 Pathogenic strains All anaerobic bacteria that were isolated

from the patients' clinical materials of Department of Obstetrics and Gynecology, Infectious Unit of Department of Medicine and Department of Surgery, Chulalongkorn Hospital, Bangkok, Thailand. These clinical specimens were submitted to Anaerobic Unit of Medical Microbiology Department during 12 months-period beginning February 1982. Anaerobic strains in this study were selected primarily on the basis of source and frequency of isolation from the clinical specimens. Antimicrobial susceptibility testing was performed by the method of Holdeman. (2)

1.2 Antibiotics Sources of pure antibiotics were as followed;

- 1.2.1 Thiamphenicol 996 mcg./ml, (Zambon Co.).
- 1.2.2 Chloramphenicol 10 mcg./ml. (Achadon Co.).
- 1.2.3 Cefoxitin sodium 1 gm./vial (Merck Sharp and Dome Co.).
- 1.2.4 Crystalline penicillin G sodium 400,000 Units (Glaxo Co.).
- 1.2.5 Metronidazole 950 mcg./mg. (Achadon Co.).
- 1.2.6 Tetracycline-oxytetracycline HCl 50 mg./ml. (Atlantic Co.).
- 1.2.7 Erythromycin estolate 950 mcg./mg. (Silom Medical Co.).
- 1.2.8 Clindamycin HCl 150 mg./ml. (Upjohn Company).

2. Methods

2.1 The routine isolation for anaerobic bacteria. Media for isolation of aerobic bacteria were PRAS* cooked meat glucose broth, fresh 5% sheep's blood trypticase soy agar, and specific media for *Bacteroides* sp., *Clostridial* sp.^(3,4) Anaerobic plates for strictly anaerobes were duplicated within Anaerobic chamber, Anaerobic jar with Gas generating kit (OXOID) for obligate anaerobes.

Identification of anaerobes were made on the basis of cellular morphology (Gram's stain) and colonial characters.⁽⁵⁾ Final identification was based upon the manual of the Virginia Polytechnic Institute of Anaerobes.⁽⁶⁾

All media for anaerobic culture were incubated in the Anaerobic chamber as described by Narathorn et al.⁽⁷⁾

In our laboratory we possess the full scale anaerobiosis including Anaerobic incubator, Anaerobic CO₂ cabinet, Anaerobic chamber and Anaerobic jar with Gas generator kit.** Other accessories are Gas-Liquid Chromatography, and various biochemical tests to the qualified procedures.^(8,9)

The routine standard aerobic isolation was also duplicated the anaerobic isolation.⁽⁹⁾

2.2 Media for the pathogenic strains. Anaerobic strains in this study

were selected primarily on the basis of source and frequency of isolation from the clinical specimens. Antimicrobial susceptibility testing was performed. Subcultures of anaerobic bacteria were made at the time of initial isolation and stored at 42°C according to the methods described by Holdeman.⁽²⁾ Isolated strains were incubated anaerobically for 48 hrs. in thioglycolate medium enriched with sterile sheep serum.

The broth culture was diluted to provide an inoculum of 10⁵-10⁶ C F U/ml. by Mc Farland Standard of Turbidity. The brain heart infusion broths (BHI) were prepared on the day before the test and stored overnight in the CO₂-Home Made Anaerobic Cabinet to prevent the increased oxygen absorption at room temperatures.

2.3 Antibiotic stock solutions. Antibiotic stock solutions were prepared from pure powder according to the method described by Grove and Randall.⁽¹⁰⁾ Two-fold dilutions of all antibiotic-stock solutions were incorporated in BHI so as to yield final concentrations ranging from 0.1-25 mcg./ml. as described by Narathorn et al.⁽¹¹⁾ After the specific anaerobic pathogens inoculum in the two fold antibiotic-dilutions, all tubes were incubated, including the control in the home-made anaerobic chamber.

The media preparation were stabilized at pH 7.2. The atmosphere in the anaerobic-home-made cabinet is

* Pre Reduced Anaerobically Sterilized.

** Oxoid Company, England.

also controlled with the constant amount of gases mixture. (10% H₂, 10% CO₂ and 80% N₂) They can markedly alter the antibacterial activity of some antibiotics.

The minimum inhibitory concentration, (MIC) which was determined after 48 hours of incubation, was defined as the lowest concentration permitting on growth.⁽¹²⁾

Result of Study

The anaerobic bacteria that were isolated from the patients' clinical samples were shown in Table 1. Only few anaerobic *B. melaninogenicus* V. parvula and *Peptostreptococci* species were isolated. On the contrary, many anaerobic *B. fragilis*, *Cl. perfringens*, *F. nucleatum* and *P. acnes* were from the clinical specimens.

Table 1 The result of mean MIC value (mcg./ml.) of the current antimicrobial agents against some anaerobic pathogens of medical importance

	<i>B. fragilis</i> (22) ⁺	<i>Cl. perfringens</i> (14)	<i>F. nucleatum</i> (10)	<i>P. acnes</i> (10)
a. Thiamphenicol	14.28*	<u>2.12</u> **	2.42	2.27
b. Chloramphenicol	18.11	1.28	<u>1.56</u>	1.40
c. Cefoxitin	3.69	<u>0.68</u>	2.07	1.09
d. Penicillin	14.35	<u>1.04</u>	7.42	1.09
e. Metronidazole	9.16	1.51	3.52	<u>0.90</u>
f. Tetracycline	13.55	1.73	7.35	<u>1.41</u>
g. Erythromycin	8.43	1.88	2.15	<u>0.97</u>
h. Clindamycin	2.0	0.98	1.84	<u>0.96</u>

* Mean MIC values in mcg./ml.

** The underline-figures indicate the lowest MIC of the current antibiotic against the given anaerobes.

()⁺ in parenthesis were the number of isolated strains.

Table 2 The percentage of drugs resistance strains were distributed.

	<i>B. fragilis</i> (22) ⁺	<i>Cl. perfringens</i> (14)	<i>F. necleatum</i> (10)	<i>P. acnes</i> (10)
a. Thiamphenicol	36.36*	42.86	<u>60</u>	50
b. Chloramphenicol	54.55**	42.86	20	<u>80</u>
c. Cefoxitin	36.36	<u>64.29</u>	50	50
d. Penicilin	36.36	<u>50.0</u>	40	<u>50</u>
e. Metronidazole	54.55	<u>71.43</u>	40	30
f. Tetracycline	36.36	35.71	40	<u>60</u>
g. Erythromycin	<u>54.55</u>	42.86	50	50
h. Clindamycin	<u>45.45</u>	42.86	40	40

* Percentage of resistant strains.

** The underline figures indicate the high percentage of resistant strains.

()⁺ in parenthesis were the number of isolated strains.

Table 3 Incidence of isolated anaerobic pathogens from corresponding clinical specimens of various clinical conditions. (1981-1982)

Clinical- diagnosis (infection)	No. of patients*	Clinical specimens	Isolated bacteria (strains)													
			Anaerobes						Aerobes							
			<i>B. fragilis</i>	<i>B. melaninogenicus</i>	<i>Cl. perfringens</i>	<i>F. nucleatum</i>	<i>P. acnes</i>	<i>V. parvula</i>	Peptostreptococcus sp.	Diphtheroids	<i>Strept. pneumoniae</i>	Alpha streptococci	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Pseudomonas</i> sp.	<i>Streptococcus fecalis</i>
1. Post-operative wound sepsis after appendectomy	6	P	3	-	2	-	1	1	-	-	-	3	-	-	-	
2. Cholecystitis after cholecystectomy	4	B	4	-	1	-	-	-	-	-	-	4	-	-	1	
3. Pulmonary abscess	2	P	-	-	1	2	-	-	-	-	1	-	-	-	-	
4. Pelvic peritonitis and pyometritis (Cesarian section)	2	E	2	-	-	-	-	-	-	-	-	2	1	-	-	
5. Tubo ovarian abscess	3	E	2	-	-	-	-	-	-	1	-	2	-	-	-	
6. Criminal abortion	4	C	3	-	3	-	1	-	-	-	-	1	1	-	1	
7. Brain abscess	3	P	1	-	1	-	-	-	-	-	1	-	-	-	1	
8. Peritonsillar abscess	6	P	-	-	-	6	-	-	-	1	-	-	-	-	-	
9. Post operative cheek cyst.	4	P	-	-	-	-	4	-	-	2	-	-	-	-	-	
10. Liver abscess	4	P	2	-	2	-	-	-	-	-	-	-	-	-	-	
11. Perianal abscess for cancer in rectum	4	P	2	-	2	-	-	-	-	-	-	1	1	1	2	
12. Cholangitis (hepatobiliary calculus)	3	B	2	-	2	-	-	-	-	-	-	-	1	1	-	
13. Acnes pustule	3	P	-	-	-	-	4	-	-	3	1	-	-	-	-	
14. Cholangitis (cancer of common bile duct)	1	B	1	-	-	-	-	-	-	-	-	2	1	-	-	
15. Subdural empyema	2	E	-	1	-	2	-	-	-	-	-	1	1	-	-	
16. Non specific urethritis	4	E	-	1	-	-	-	1	1	1	2	-	-	-	-	
Total			22	2	14	10	10	2	1	7	6	1	15	5	2	5

* N.B. : B = bile; C = cervical swab; E = exudate; P = pus.

Discussion

One of the main reasons why our knowledge about the anaerobic bacteria, especially in human pathology, is not on the same plane with the knowledge of the living habits of oxygen tolerant bacteria comes from the technical difficulties to obtain pure culture for study the living habits of these bacteria. However, most anaerobes can be cultivated with methods currently available if one carefully controls the factors that affect the growth of the anaerobic bacteria. In view of this situation, in this study only 56 isolated anaerobic pathogens were studied. Moreover, in the three decades since, some of the methods have been refined, but little that is basically new has been proposed.⁽¹³⁾

In contrast to the voluminous literatures on the in vitro susceptibility testing of clinically significant aerobic and facultatively anaerobic bacteria, only a few references exist with regard to the anaerobes, and most of these are concerned with nonsporeforming gram negative bacilli.^(14,15,16) Serial dilution susceptibility test of local anaerobic *B. melaninogenicus*, *V. parvula* and *Peptostreptococci* species were not shown. Normally these anaerobes are not the medical problems because they seldom develop resistant mutants.⁽¹⁶⁾ *B. fragilis*, *F. nucleatum*, *Cl. perfringens* and *P. acnes* are the anaerobes of medical importance as they are isolated from the patients' infectious clinical specimens. In these

circumstance, these anaerobes are infectious, strains.⁽¹⁷⁾ It is worth to notice that the clinical specimens harbour both the aerobic and anaerobic pathogens. Therefore mixed culture are the important role of infectious diseases. Moreover, in our study one cannot explain why aerobic *Pseudomonas* species and *Staphylococcus* species are seldom isolated in the pus or exudate. (Table 3)

Bacteroides fragilis has been found frequency in the intra-abdominal infections and in the pelvic inflammatory diseases. These anaerobes harbour the Betalactamase enzymes. Between the Betalactams in this study, cefoxitin is the most sensitive drug as compare to penicillin. (Table 2)

Many anaerobic infections are mixed, involving facultative and aerobic forms also. In view of this situation, one can finally realize that serous mixed infection, cefoxitin should be used with aminoglycosides. On the contrary, clindamycin, metronidazole or thiamphenicol should be considered in Betalactamase producing anaerobic infection.

Cefoxitin can also administer alone against the serous mixed infection but one must be careful about cefoxitin-drug-adverse reaction. (skin rash or plebitis)

Among the non-Beta-lactam antibiotics, clindamycin has the lowest mean MIC against many anaerobes (Table 2).

Clindamycin has been recognized in the U.S.A. as agent of choice in the treatment of anaerobic infection but this drug is primarily known to induce

pseudomembranous colitis.⁽¹⁸⁾ Although many studies have been compared metronidazole and clindamycin for the treatment of intra-abdominal and pelvic infections and finally found that these drugs are equivalent in safety and efficacy treatment.⁽¹⁹⁾ However the mean MIC of clindamycin (Table 2) demonstrates more activity and also more expensive than metronidazole. In this circumstance, metronidazole should be considered. However the fact that; metronidazole crosses the placental barrier and enters the fetal circulation rapidly and because its effects on fetal development are not definitely known, the Food and Drug Administration, U.S.A. has held that the drug is contraindicated during the first trimester of pregnancy. Moreover the drug may have a disulfiram-like-effect. (Antabuse effect) One should therefore administer these 2 drugs carefully.

Actually *Cl. perfringens* are the normal microbial intestinal flora of normal healthy person. These anaerobes cause serious infection when they are induced in the accidental wound. The victim's status is fatal if *Cl. perfringens*' alpha toxin was released.⁽²⁰⁾ Normally these anaerobes are sensitive to penicillin. In this study, the infectious strains are more sensitive to cefoxitin than penicillin. (Table 2) *Cl. perfringens* is also very sensitive to erythromycin. (Table 2)

Among the non Beta-lactam drugs, chloramphenicol is more sensitive to *Cl. perfringens* than others, calculated on

the ground of the mean MIC. (Table 2) Chloramphenicol is a potent inhibitor of protein synthesis in microorganisms. It blocks the attachment of amino-acids to the nascent peptide on the 50-S unit of ribosomes by interfering with the action of peptidyl transferase.⁽²¹⁾ Thiamphenicol, the analog of chloramphenicol with substitution of the p-nitro group with methylsulfonyl moiety, has probably the same mechanism of action. However, from Tables 2-3, thiamphenicol is less sensitive than chloramphenicol. (in vitro).

About the disadvantages of chloramphenicol, although it infrequently causes gastrointestinal upsets. However prolonged administration of more than 3 gm. daily to adults regularly results in abnormalities of early forms of red blood cells, elevation of serum iron and anemia. These changes are reversible upon discontinuance of the drug. Very rare individuals exhibit an apparent idiosyncrasy to chloramphenicol and develop severe or fatal depression of bone marrow function. The mechanism of this aplastic anemia is not understood but it is distinct from the dose-related reversible effect described above.⁽²¹⁾

In premature and newborn infants, Chloramphenicol can induce collapse (Gray syndrome) because the normal mechanism of detoxification (glucuronide conjugation in the liver) is not yet developed. In view of clinical use, the mentioned side effects are not seen in thiamphenicol.^(22,23) Therefore one should consider carefully about these two drugs.

Propionibacterium acnes is one of the comedonal bacteria and is the skin normal flora and the area of sebaceous glands.⁽¹⁶⁾ From Table 2, anaerobic propionibacterium acnes is more sensitive to tetracycline and erythromycin than others. Tetracycline, formerly the drug of choice for *B. fragilis* infections and very useful in anaerobic infections, is now relegated to a much lower position because of the development of resistance. Wide-spread tetracycline resistance in anaerobic bacteria has been prompted studies to elucidate its mechanism. Fayolle has demonstrated that the uptake of tetracycline by *B. fragilis* is similar to *E. coli*.⁽²⁴⁾ However it is not known whether tetracycline resistant bacteroides possess a tetracycline exit mechanism which pumps tetracycline out of cells, as recently demonstrated in *E. coli*.⁽²⁵⁾ Therefore tetracycline, once a standard treatment for anaerobic infection now exhibits high resistance with the exception of *P. acnes*. (Table 2)

Erythromycin, the macrolides inhibits *P. acnes* and *Cl. perfringens* better than others (Table 8). This drug is therefore chiefly useful in many anaerobic infection.

Fusobacterium nucleatum, the normal inhabitant of throat and buccal cavity, may become potential pathogens in mixed infection of oral cavity, and respiratory tract.⁽¹⁶⁾ In this study, chloramphenicol is still the drug of choice, white clindamycin, cefoxitin,

thiamphenicol and erythromycin are the alternative drugs. *F. nucleatum* resists to tetracycline. (Table 2)

In fact, the method is sensitive directly in mcg./ml. but this method is time-consuming and is not suitable for daily routine use. The improved micro-dilution method should be considered for anaerobic susceptibility testing of the daily isolated-pathogens.⁽²⁶⁾

In general, antimicrobial therapy for anaerobic infection requires high dosage and prolonged treatment because of the tissue necrosis and tendency to relapse.⁽¹⁶⁾

However, in our series using the local isolated anaerobic pathogens from the patients' clinical specimens, they exhibit MIC values higher than others.⁽²⁷⁾ Among the substantial problems, one possibility-factor concerns the inadequate use of antibiotics. In Thailand, most of the citizens have already received "pretreatment" with potentially obscuring therapy. Owing to the fact that Thai people widely used antibiotics more freely as one can obtain the Beta lactam antibiotics from any drug store. Many anaerobic bacteria contain at least one plasmid that carries the gene for Beta lactamase production and then becomes resistant to Beta lactam antibiotic.⁽²⁴⁾ Moreover the Beta-lactam-plasmids are transmissible among the anaerobic pathogens and they may have acquired from other organisms also, these events are responsible for the higher MIC than other.⁽²⁷⁾

Some antibiotics used in this study are supplied from the local pharmaceutical companies. However all of them are exactly the pure chemical substances and are original imported for repacking in Thailand. In these circumstance, the anti-microbial potency are reliable within the expiratory date.

All antimicrobial-agents demonstrate the highest percentage of resistance ;

tiamphenicol posses the 60% resistance to *F. nucleatum*, chloramphenicol posses the 54.55% resistance to *B. fragilis*, metronidazole exhibit the 54.55% and 71.43% of resistant *B. fragilis* and *Cl. perfringens* respectively, erythromycin has 54.55% of resistant to *B. fragilis*.

The percentage of resistance to tetracycline, cefoxitin and penicillin habour fairly resistant anaerobes.

These circumstance expresses that the anaerobic pathogens always habour the significant percentage of resistance to many current antimicrobial agents.

Physicians should therefore consider the "antibiogram" carefully to avoid misleading of antibiotic therapy. Only infectious strains of anaerobic bacteria from the serious infectious victims or prolonged infectious diseases are very valuable for susceptibility test.

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