Rapid Identification and Antimicrobial Susceptibility Profiling of Anaerobic Bacteria in Clinical Specimens

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ABSTRACT

Objective: *The aim of the current study was to evaluate the ability of API 20A, and Microscan Rapid-Anaerobe identification system to identify a spectrum of clinically significant anaerobic isolates against the gold standard conventional method. Also to determine the antimicrobial susceptibility of anaerobic isolates using different methods.* ***Materials and Methods:*** *Anaerobes were isolated from the different clinical specimens and all isolates were initially identified by conventional tests. Identification of isolates were made also by using the API 20A and Microscan systems. Antimicrobial susceptibility of all isolates were determined by disc diffusion and broth-disk method and compared to MIC determined by broth dilution method.* ***Results:*** *A total of 51 isolates were recovered from clinical specimens. API 20A and Microscan correctly identified 70.6% and 82.4% of strains to species level respectively. Eight strains were misidentified by API 20A and 9 were misidentified by Microscan. B. fragilis group isolates were the most encountered clinically significant isolates among the gram-negative anaerobes. Penicillin and ampicillin had poor activity against B. fragilis group, Prevotella spp. Eubacterium spp. and Veillonella spp. Members of the B. fragilis group were more resistant to cefoxitin, than other anaerobes (66.6%). Resistance to clindamycin varied among the species range from 11.1% to 50%. Chloramphenicol was effective against all isolates. Fusobacterium spp. were highly susceptible to penicillin and 33.3 % of Fusobacterium isolates were resistant to metronidazole. Clostridium perfringens isolates were susceptible to all agents tested. Clostridium species other than C. perfringens were more resistant than C. perfringens, with 75% of the isolates resistant to penicillin, 12.5% resistant to clindamycin, and 18.7% resistant to metronidazole. Disk diffusion method has correlated well with MIC values but give poor correlation most tested C. perfringens. The overall correlation of the broth-disk results with MIC values was 97.6% in all tests.* ***Conclusions:*** *It was concluded that Microscan RAID was easier to perform and interpret and has the potential to provide rapid identification of anaerobes with an automated reader but more expensive than API. Both system give reliable rapid identification of anaerobes. Our study indicate that there continue to be changes in susceptibility of anaerobes over time and the antimicrobial resistance patterns should be monitored regularly in order to guide empirical therapy. Attention should be paid to standardize broth-disk method as an easy, rapid and most practical to be applicable to routine use for clinical laboratories.*

INTRODUCTION

In the past few decades there have been great advances in the understanding of the role played by anaerobes in the pathogenesis of severe bacterial infections. The infectious potential of these organisms has been clarified[(1)], and the development of species- and group-specific differences in antimicrobial susceptibility patterns has been defined[(2)].

Anaerobes isolation and identification are an important part of the functions of a clinical microbiology laboratory. Conventional biochemical testing of anaerobes together with gas-liquid chromatography (GLC) are the most accurate identification method[(3)], but it has the disadvantages of being time consuming and expensive and is beyond the capabilities of many clinical laboratories.

Commercial methods which have gained acceptance for identification of anaerobes include API 20A, and rapid kits detecting preformed enzymes, such as MicroScan (American Microscan, West Sacramento, CA) have been developed and are very useful for anaerobic bacteriology. MicroScan systems mainly detect glycosidases and aminopeptidases and some other enzymes by means of chromogenic substrates. A heavy inoculum and short incubation period (4 hours in air) minimizes contamination and gives rapid results[(4)]. The microbiology laboratory without facilities for conventional anaerobe testing

requires an accurate and reliable method for anaerobe species identification.

Routine isolation, identification and susceptibility testing of anaerobic bacteria present several difficulties leading to defects in the determination of local susceptibility patterns which will guide empirical treatment protocols[5]. Over the past 10 years, there has been a significant problem with increasing resistance to antimicrobial agents among anaerobic bacteria [6,7].

Also, anaerobic bacteria are no longer entirely predictable in their susceptibility to agents that might be selected for empiric therapy[8]. Much effort has been exerted in recent years to standardize the techniques for antibiotic susceptibility testing. For the most part, the success of these efforts has been limited to the aerobic and facultative bacteria. The development of simple procedures for the anaerobes has been more difficult.

To this end, the present study aimed to evaluate the ability of API 20A, and Microscan Rapid-Anaerobe identification system to identify a spectrum of clinically significant anaerobic isolates against the gold standard conventional method. Also to determine the antimicrobial susceptibility of anaerobic isolates using different methods.

MATERIALS & METHODS

Anaerobic isolates

The anaerobes were isolated from the following clinical specimens: pus/wound/aspirates, blood, tissue and other sites. All strains were tested within approximately 1 month of isolation. All isolates were initially identified by conventional tests [3].

Isolation and identification of anaerobic bacteria by conventional biochemical tests:

Specimens were cultured for anaerobic bacteria by inoculation of different selective and non selective media. Colombia Blood agar, kanamycin-vancomycin blood agar plates supplemented with vitamin k and hemin, bacteroid bile esculin agar and neomycin Colombia blood agar were inoculated for isolation of anaerobic bacteria and incubated in anaerobic Gas-Pak jar (BBL Becton Dickinson Microbiology System, Cockeysville, MD, USA). Primary plates of various media were examined for different colonies morphologies and gram stained smears prepared from these colonies.

Identification included two levels:

Level I identification: It is a presumptive identification of many anaerobes depending on information obtained from the primary plates, i.e. gram stain, colony morphology of a pure isolate, hemolysis on BAP...etc. [9].

Level II identification: It includes group of testes for identification of anaerobic organisms to genus level and in some cases to species level using simple tests as antibiotic identification test, growth in presence of 20% oxygall, indole production, urease activity, sugar fermentation, lecithinase & lipase reaction, esculin hydrolysis, gelatin liquefaction and catalase production. Anaerobic bacteria were identified according to according to **Jousimies-Somer** *et al***.[10] and Summanen[3]**

API system

Suspensions of growth were made in Lombard-Dowell broth to a density of a McFarland standard of 3 or greater. Strips were inoculated according to manufacturer's instructions and incubated for 24 h at 37°C. Reagents were then added, and results were read according to manufacturer's instructions. Where interpretation of sugar fermentation tests was difficult, clear positive (yellow) and negative (purple) wells from the same strip were used for comparison. Sugar reactions were not compared among strips. Tests were assigned numerical values from which a profile number was generated. Identification was made by using the API Analytical Profile Index. Numbers which did not appear in the index were referred to the firm's computer facilities. For the purpose of this study, supplemental tests were not performed.

Identification of bacterial isolates using Microscan system

Microscan Rapid Anaerobe IDentification panel (RAID) (DADE BEHRING, West Sacramento, USA), is an in-vitro kit for identification of anaerobes isolated from clinical specimens. It consists of 24 dehydrated substrates. It is rehydrated and inoculated with the bacterial suspension (3-5 McFarland) and incubated aerobically for 4 hrs. Because of the Microscan RAID panel detects preformed enzymes, growth of the organism in the panel is not necessary.

Antimicrobial susceptibility testing:

Antibiotics

Metronidazole 5µg/ disc, Clindamycin 2µg/ disc, Piperacillin/Tazobactam 110µg/ disc, Ceftrixone 30 µg/ disc, Amoxicillin/Clavulanic acid 30 µg/ disc, Cefoxtine 30 µg/ disc, Penicillin 10µg/disc and Chloramphenicol 30µg/ disc (oxoid). Disks were stored refrigerated in bottles containing desiccant.

Antimicrobial susceptibility testing by disc diffusion method: Agar plate disc diffusion

technique has been used as general method**.** Columbia blood agar supplemented with vitamin K_1 and hemin was used[(11)].

Determination of MIC by the broth disk method:

Screw capped tubes, each containing 5 ml of BHI medium, were opened aseptically and the appropriate number of antibiotic disks were added aseptically from a commercial cartridge (Difco).

Disk elution:

Antibiotic disks were allowed to elute in broth for a minimum of 1 hr. Dilutions were prepared or portions were removed after this period. The final dilutions were dispensed in 0.9-ml portions into sterile glass tubes to which 0.1 ml of inoculum was added, for a total volume of 1.0 ml.

The tubes were simultaneously inoculated from a Pasteur pipette with one drop of a 24-h culture of the test organism in cooked meat broth. A control culture containing no antibiotics was included in each set. Incubation was at 37 C for 24 h. Susceptibility to the test antibiotic was defined as either absence of turbidity or less than 50% of the turbidity of the control culture.

MIC

The end points and corresponding MIC values were determined through visual interpretation of growth. The tests were performed in screw-capped tubes (16 by 125 mm). This media was pre-reduced by placing it in the anaerobic gas pack containing an atmosphere of 5% CO_2, 10% H_2, and 85% N_2 for 24 h. The inocula were prepared until the desired turbidity (corresponding to 10^8 colony forming units/ml) was achieved (generally overnight). The adjusted inoculum was added to each of the tubes with antibiotic-containing broth to achieve a final concentration of approximately 10^6 colony- forming units/ml (0.025 ml into 3 ml of broth). The tubes were incubated at 35 C for 24 h (or longer if growth not adequate) in the anaerobic gas pack system. The MIC was the least concentration of antibiotic that prevented visible growth. To measure the reproducibility of the test, triplicate determinations were performed at each of three testing episodes, using various lots of antibiotics, media, and anaerobic gas.

The anaerobic systems used were the GasPak (BBL). A palladium- coated aluminum catalyst (Englehard Industries, Newark) was used with each of the anaerobic systems.

Broth-Dilution Method for Determining the Antibiotic Susceptibility of Anaerobic isolates

MICs were determined by the broth-dilution technique, with BHI broths and anaerobic incubation. The same antibiotics used for disk diffusion were tested. Concentrations of antimicrobial agents tested were serial twofold dilutions ranging from 128 to 0.015 mg/ml.

Inoculum size and source

As a source of inoculum, we routinely used a 24-h culture in Cooked Meat broth because this medium gave very reproducible growth of anaerobic bacteria. CM broth is also more difficult to oxidize than other anaerobic media.

The same size and source of inocula were used for both the broth-disk and MIC methods. One drop from a Pasteur pipette of a 24-h culture of the test organism was added to each tube containing 5 ml of BHI broth to give approximately a 1:100 dilution of the culture. This was an inoculum of 10^6 to 10^7 bacteria per ml, which is 10- fold higher than the inoculum usually used for broth MIC determinations with facultative bacteria. We chose to use the higher inoculum because some anaerobic bacteria did not grow as well from smaller inocula and sometimes required more than 24 h of incubation to reach maximal turbidity in the BHI broth.

Reading results

Organisms that were resistant to an antibiotic normally grew to almost the same turbidity as the control culture, whereas susceptible organisms did not grow in the tubes containing that antibiotic. With a few organisms, the number of cells in the inoculum was high enough so that the initial generations that occurred prior to inhibition by antibiotics resulted in a light turbidity that was present with all of the antibiotics tested. In such cases, this background turbidity was disregarded, and only those tubes in which the turbidity was 50% or greater than the control culture were considered resistant.

Incubation time

We recorded both the broth-disk and MIC results after incubation for 24 h.

Correlation of the results of the broth disk, disk diffusion, and MIC methods.

To determine how well the results of the broth-disk and the disk diffusion methods correlated with MIC values, we compared the three methods using the 51 strains of anaerobic bacteria listed in Table 1.

RESULT

Identification of anaerobes:

In the present study, a total of 51 isolates were recovered from clinical specimens, identified conventionally and by using two rapid identification systems; API 20A and Microscan RAID system. Identification of isolates was shown in table (1). Identification of all anaerobic bacteria tested with the two systems is presented in Tables (2) and the major identification discrepancies are listed in table (3). API 20A correctly identified 70.6% of strains to species and 13.7% to genus only, with 15.7 incorrect. Microscan correctly identified 82.4% of strains to species, 17.6% incorrect. Eight strains were misidentified by API 20A, 9 were misidentified by Microscan.

Table (1): Anaerobes isolated in the study.

Isolates	*No.*	*%*
Bacteroides spp. (9)		
Bacteroides ovatus	5	9.8
B. distasoni	3	5.6
B. uniformis	1	2.0
Clostridium spp. (22)		
C. histolyticum	4	7.8
C. sporogenes	4	7.8
C. diffcile	4	7.8
Clostridium perfringenes	6	11.8
Clostridium bifermentans	3	5.6
Clostridium sordellii	1	2.0
***Eubacterium spp.* (7)**		
Eubacterium lentum	5	9.8
Eubacterium limosum	2	3.9
***Fusobacterium spp.* (6)**		
F. mortiferum	2	3.9
F. nicrophorum	4	7.8
Vielonella parvula	4	7.8
Prevotella spp.	3	5.6
Total	51	

Table (2): Identification of anaerobic isolates by API and Microscan.

Anaerobes (No. of strains tested)	*No. of correct identification to Species*		*Genus*	*No. of incorrect Identification*	
	API	Microscan	API	API	Microscan
***Clostridium* spp. (22)**	17	17	4	1	5
***Eubacterium* spp. (7)**	3	7	0	4	0
***Fusobacterium* spp. (6)**	2	6	1	3	0
***Bacteroides* spp. (9)**	7	6	2	0	3
***Vielonella* spp. (4)**	4	4	0	0	0
***Prevotella* spp. (3)**	3	2	0	0	1
Total (51)	36	42	7	8	9
% of total	70.6	82.4	13.7	15.7	17.6

Table (3): Identification discrepancies with API and Microscan.

Isolates	***Identification***	
	API	**Microscan**
F. mortiforum	***B. distasoni, B. ovatus***	***F. motiforum***
F. nicrophorum	***F. nicrophorum, Porphyromonas assacharolyticus, B. uniformis***	***F. nicrophorum***
B. distasoni	***B. distasoni, B. ovatus***	***B. distasoni, F. nicrophorum***
C. histolyticum	***C. spp, C. sporogenes, C. botulinum***	***E. lentum***
C. sporogenes	***C. sporogenes, C. spp., C. botulinum***	***E. lentum, C. sordellii***
C. diffcile	***C. spp., C. butyricum, B. ovatus***	***C. difficile***
Eubacterium lentum	***C. butyricum, C. spp.***	***Eubacterium lentum***
Eubacterium limosum	***C. butyricum, C. spp.***	***Eubacterium limosum***

Antimicrobial susceptibility of isolated anaerobes:

A total of 51 anaerobes were tested; percentages of susceptible, intermediate and resistant organisms; MIC ranges for the anaerobic Gram-negative organisms and the anaerobic Gram-positive organisms are shown in Table 4. The rates of susceptible, intermediate, and resistant isolates were determined by using the NCCLS breakpoints. *B. fragilis* group isolates were the most encountered clinically significant isolates among the gram-negative anaerobes. Penicillin and ampicillin had poor activity against *B. fragilis* group isolates and *Prevotella* spp. Penicillin resistance also was found in the majority of *Eubacterium* spp. (71.4%) and Veillonella spp. (75%). Members of the *B. fragilis* group were more resistant to cefoxitin, with resistance rates of 66.6%. Resistance to clindamycin varied among the species range from 11.1% to 50%. Chloramphenicol was effective against all isolates. *Fusobacterium* spp. isolates were all penicillin susceptible, but one *F. necrophorum* (16.7%) isolate was resistant to penicillin and 33.3 % of *Fusobacterium* spp. isolates were resistant to metronidazole. Of the gram-positive isolates, the *Clostridium perfringens* isolates were susceptible to all of the agents tested. *Clostridium* spp. other than *C. perfringens* were more resistant than *C. perfringens*, with 75% of the isolates resistant to penicillin, 12.5% resistant to clindamycin, and 18.7% resistant to metronidazole. Cefoxitin was less active against species of the *B. fragilis* group than other tested anaerobes.

The susceptibility of each of the 51 isolates to the seven antibiotics was determined by the broth-disk, disc diffusion and MIC broth dilution methods. Listed in table 5 was the percentage of tests in which the broth-disk results did correlate with the MIC data with each of the species tested. The overall correlation of the broth-disk results with MIC values was 97.6% in all tests.

The results of the disk diffusion and MIC methods were compared. Disk diffusion method correlated with MIC values as shown in table 4. However, the disk diffusion method give poor correlation most tested *Clostridium perfringens*. For this organism, the strains appear resistant by disk diffusion; while the MIC results indicated that the strains were susceptible to the tested antibiotics.

Table (4): MICs, MICs range, MICs average (µg/ml) and susceptibility of the selected antibiotics against anaerobic bacteria by broth-dilution and disc diffusion methods.

Species tested	S	I	R	MIC range µg/ml	MIC average µg/ml	Species tested	S	I	R	MIC range µg/ml	MIC average µg/ml
Bacteroides Fragilis group (9)						***Clostridium perfringenes***					
Penicillin	-	-	100	**2-256**	>128	Penicillin	100	-	-	0.06-0.5	0.25
Ampicillin	-	-	100	32-256	128	Ampicillin	100	-	-	0.06-0.12	0.12
Amox/clav	44.4	22.2	33.4	0.25-64	16	Amox/clav	100	-	-	0.06-0.12	0.12
Cefoxitin	33.4		66.6	1-128	32	Cefoxitin	100	-	-	0.5-2	2
Clindamycin	77.8	11.1	11.1	0.12->128	8	Clindamycin	100	-	-	0.06-2	2
Chloramephinecol	100	-	-	2-32	4	Chloramephinecol	100	-	-	0.5-2	2
Metronidazol	100	-	-	0.5-16	*2*	*Metronidazol*	100	-	-	0.5-2	2
Pipracillin/tazo	88.9	-	11.1	0.25-64	*32*	*Pipracillin/tazo*	100	-	-	0.12-0.5	0.5
Clostridium spp.(16)						***Eubacterium spp.(7)***					
Penicillin	25	-	75	0.06-32	16	Penicillin	14	28.6	71.4	0.06-4	4
Ampicillin	68.8	-		31.8	16	Ampicillin	28.6	14	71.4	0.06-4	4
Amox/clav	87.5	-	12.5	0.06-16	16	Amox/clav	100	-	-	0.06-1	1
Cefoxitin	100	-		0.06-16	16	Cefoxitin	100	-	-	0.5-8	8
Clindamycin	87.5	-	12.5	0.06-4	4	Clindamycin	100	-	-	0.06-1	0.5
Chloramephinecol	100	-		0.5-4	4	Chloramephinecol	100	-	-	0.5-1	1
Metronidazol	68.8	12.5	18.7	0.12-128	128	*Metronidazol*	100	-	-	0.12-1	0.5
Pipracillin/tazo	100	-	-	0.03-8	4	*Pipracillin/tazo*	100	-	-	0.12-32	32
Fusobacterium spp(6)						***Vielonella spp.(4)***					
Penicillin	83.3		16.7	0.06-16	0.12	Penicillin	25	-	75	1-16	8
Ampicillin	100		-	0.06-16	0.12	Ampicillin	25	-	75	0.25-128	8
Amox/clav	100	-	-	0.06-4	1	Amox/clav	100	-	-	0.12-4	4
Cefoxitin	100	-	-	0.12-8	2	Cefoxitin	100	-	-	0.12-8	8
Clindamycin	100	-	-	0.06-0.5	0.12	Clindamycin	50	-	50	0.06-0.25	0.12
Chloramephinecol	100	-	-	0.5-16	4	Chloramephinecol	100	-	-	0.5-8	4
Metronidazol	66.7	-	33.3	0.06-2	0.25	*Metronidazol*	50	-	50	0.12-2	2
Pipracillin/tazo	100	-	-	0.03-16	0.25	*Pipracillin/tazo*	100	-	-	0.12-16	16
prevotella spp(3)											
Penicillin	33.3	-	66.7	0.06-16	16						
Ampicillin	33.3	-	66.6	0.12-64	16						
Amox/clav	100	-	-	0.06-8	4	**Amox/clav = Amoxicillin / Clavulinic acid**					
Cefoxitin	100	-	-	0.12-8	4	***Pepracillin / tazo = Pepracillin / Tazobactam***					
Clindamycin	100	-	-	0.06-128	0.25						
Chloramephinecol	100	-	-	1-4	4						
Metronidazol	66.7	-	33.3	0.12-128	32						
Pipracillin/tazo	100	-	-	0.12-32	32						

Table (5): Correlation of the broth disk diffusion tests with MIC results.

Species tested	No. of tests	% of correct
Bacteroides ovatus	35	97
B. distasoni	21	100
B. uniformis	7	100
C. histolyticum	28	93
C. sporogenes	28	96
C. diffcile	28	100
Clostridium perfringenes	42	100
Clostridium bifermentans	21	95
Clostridium sordellii	7	100
Eubacterium lentum	35	97
Eubacterium limosum	14	93
F. mortiferum	14	100
F. nicrophorum	28	100
Vielonella parvula	28	96
Prevotella spp.	21	100
Total	357	97.6

DISCUSSION

Anaerobic bacteria have been implicated as the causative agents in several types of tissue infections, and clinical laboratories are often requested to process samples for anaerobes[12,13].

This study evaluated the Microscan RAID, and API 20A systems for the identification of anaerobic bacteria. A total of 51 isolates from clinical specimens were examined and included 22 *Clostridium* spp., 9 *Bacteriodes* spp., 6 *Eubacterium* spp., 5 *Fusobacterium* spp., and 3 *Prevotella* spp. and 4 *Veillonella* spp. All isolates were initially identified by the procedures outlined by Summanen[3], identifications from the RAID and API 20A systems were compared with these initial identifications.

In general, the total technical time required to set up and read each of the two identification system was comparable. API is nearly more cost effective than Microscan panel. At the recommendation of manufacturers, API and Microscan were read after 24 hrs. Interpretation of API sugar reactions required more technical expertise than is the case with most other Analytab products. Sugar fermentation reactions, especially cellobiose, were difficult to read. Indole reactions were generally easily interpreted, but had to be read within 5 min after addition of Ehrlich's reagent to avoid false-positive reactions. Microscan reactions were read automatically so overcome error encountered with API interpretation. Also additional tests most commonly required for accurate species identification of organisms with API, which include; black colonial pigmentation, Gram stain, lecithinase, lipase, and motility were not required by Microscan.

RAID identified 82.3% correctly to the species level and 17.6% were misidentified. API 20A identified 68.6% correct to the species level, 11.7% to genus only and 15.6% incorrect. Gulletta *et al.,* 1985 reported higher identification percentage of strains by the API 20A to species level (85.2%) but same incorrect identification rate. Stoakes *et al*., 1990[4] was reported a lower accuracy for Microscan (70%) in identifying anaerobes than our finding. This could be attributed to the increased experience in dealing with the system.

It should be noted that, because of the small sample size for some species, a high percentage of incorrect identification does not necessarily imply that the systems could never identify that organism.

API 20A was found to have the lowest sensitivity in identifying *C. difficile* in the present study. Head and Ratnam[14] was found high sensitivity of API in identifying *C. difficile* (95.6%). Although API 20A is a more established system for identification of anaerobic bacteria and also more cost effective[15], Microscan RAID appear to be more promising for the identification of anaerobicrtde bacteria.

MicroScan system does not prompt the user to try specific supplementary tests. However, a number of rapid, simple tests, such as bile tolerance, gas chromatography, Gram staining, pitting of media, production of catalase, indole, lecithinase, lipase, fluorescence, and pigment, and esculin hydrolysis, can help obtain higher level of correct identification. The potential of the Microscan system to provide rapid identification of anaerobes with an automated reader is of great interest to diagnostic laboratories.

Clinical microbiology laboratories are faced with the challenge of accurately detecting emerging antibiotic resistance among a number of bacterial pathogens. Anaerobic bacteria are no longer entirely predictable in their susceptibility to agents that might be selected for empiric therapy[16]. The increasing resistance to antimicrobial agents among anaerobic pathogens has been a global problem in the past two decades[17,18,19]. The rates of resistance show clinically important variations among geographic areas and between countries[8].

Therefore, clinical microbiology laboratories may not be able to rely on a single susceptibility testing method or system to detect all those emerging resistant or fastidious organisms. For reliable detection, laboratories may need to employ conventional, quantitative susceptibility testing methods or use specially developed, single concentration screening tests for some resistant species [(16)].

The approach of many investigators has been to use conventional techniques for aerobic and facultative organisms to test the drug susceptibility of anaerobes in oxygen less environment. Therefore, antimicrobial susceptibilities have been determined by disk-diffusion, agar dilution, and broth-dilution techniques. Although there are several common sources of variation for these techniques, e.g., inoculum concentrations, pH of the medium, and components of the medium, one major disadvantage of the disk-diffusion and agar-dilution techniques for testing anaerobic bacteria in comparison with the broth-dilution technique is that the organisms are much more likely to be subjected to lethal doses of oxygen when they are spread onto the surface of agar. Furthermore, the addition of agar to a broth medium adds to the complexity of the medium and to the possibility of some effects on the susceptibility test. For these reasons we preferred to use a broth-dilution technique as our reference method for determining minimal inhibitory concentrations (MICs) of several antimicrobial agents for various anaerobic bacteria.

Schneierson[(20)] devised a simple method for MIC determination by using standard commercial disks as the source of antibiotic and eluting the disks in broth. Modifications of his method were used for susceptibility testing of anaerobic bacteria. We reexamined the method of Schneierson[(20)] with modification of pre-reducing the media and using a control. In the present study there was a good correlation between MIC results obtained by the broth-disk method and those obtained with broth-dilution method. Both methods allowed us to determine antibiotic susceptibility in a completely anaerobic environment. Also, this method takes the advantages of broth-dilution technique that it can be used for simultaneous determination of minimal inhibitory and bactericidal concentrations; it can be used to test several antibiotics in synergism studies.

The overall correlation of the broth-disk results with MIC values was 97.6% in all tests. Wilson *et al*., 1990 reported reproducibility of 96.7% between the two methods which is in agreement with the present study. Also, Jorgensen *et al.*[(21)] found good correlation between broth disk methods and report that the majority of errors with all of the disk elution methods occurred with isolates most notably members of the *Bacteroides fragilis* group.

We think the source of error in the broth-disk method is the actual concentration of antibiotic contained in commercial antibiotic disks. This method simply designates whether the MIC is above or below concentrations normally attained in the blood. The broth-disk method would seem particularly useful for clinical laboratories which currently identify anaerobes, since susceptibility tests could be performed at the same time as the biochemical tests by the same technician. Broth disk method needs to be adequately standardized, but it can be useful for rapid comparisons of the activity of various drugs and for surveillance of resistance patterns.

In the present study disk diffusion method correlated well with MIC values as shown in table 4. Overall, full agreement (84.5% to 94.6%) between the reference broth dilution MICs and the disk diffusion method was also reported by Jorgensen *et al.*[(21)]. Shungu *et al*., 1985 found also an excellent correlation between the reference agar dilution method and broth disk elution procedures for imipenem (100% agreement). The number of incorrect results was attributed to growth failures with some isolates and difficulties in interpreting results.

Susceptibility testing of obligate anaerobic bacteria is not a standard procedure in many clinical microbiology laboratories at this time. This is due in part to the success of some empirical antibiotic regimens recommended for treating anaerobic infections and also to the technical difficulty of performing anaerobic testing[(5)]. In addition, the CLSI reference MIC method for anaerobes is an agar dilution procedure that requires preparation of antibiotic dilutions and special media at the site of testing[(22)].

Some laboratories may choose to perform periodic surveillance testing of stored clinical isolates in order to generate local data to guide clinicians with empirical therapy. However, the need for some form of routine testing may become more important, since resistant anaerobes have been found at some centers[(16,2)].

The study shows changes in susceptibility patterns. Our results were consistent with results of previous studies[(23,24,2)] in which the level of chloramphenicol susceptibility remained unchanged. Metronidazole and

chloramephenicol remain the most effective agent; but with little increase in metronidazole resistant strains. Resistance was more common among the β-lactam agents than other tested antibiotics. *Prevotella* spp. were overall more susceptible than the *B. fragilis* group but poorly susceptible to penicillin. With the exception of one isolate of *F. necrophorum*, all other *Fusobacterium* spp. tested were susceptible to all agents. The high susceptibility of *Fusobacterium* spp. was also reported by **Teng** *et al*.[25], Litterio *et al*. and Liu *et al*.[8]. *C. perfringens* isolates were susceptible to all agents tested. Other *Clostridium* spp. were more resistant to penicillin. *Eubacterium* spp. were generally considered to be susceptible to penicillin.

In agreement with other reports, the susceptibility results varied among genera and species[8]. The increased resistance of the *B. fragilis* group to cefoxitin and clindamycin is noted which in consistent with those reported by Naidoo *et al*.[2]. In contrast, a previous study in Taiwan[25] showed high susceptibility to cefoxitin. *Fusobacterium* isolates remained susceptible to cefoxitin. The high rates of resistance to cefoxitin in *B. fragilis group* species were unusual.

As expected, the B-lactams were more active in gram-positive than in gram-negative anaerobes. According to CLSI[22] guidelines, members of the *B. fragilis* group are presumed to be resistant to ampicillin. The rate of resistance to amoxacillin-clavulinic in *B. fragilis* group isolates increased from those reported in previous studies[25,26]. The resistance is comparable to those reported by Marina *et al*.,[27]. On the other hand resistance to piperacillin-tazobactam was low (11.1%). In contrast, Fernández-Canigia *et al*.[26] found that members of *B. fragilis* group were the most resistant isolates to ampicillin-sulbactam and piperacillin-tazobactam.

Rates of resistance to clindamycin increased in the present study. Clindamycin has long been considered the drug of choice for treatment of anaerobes. However, over the past 20 years, there has been a significant increase in the rate of resistance to clindamycin among isolates of the *B. fragilis* group in many areas[8,28]. In our study, the overall activities of clindamycin against the *B. fragilis* group were poor. It was also reported that clindamycin resistance is associated with hospital-acquired infections[25]. In the present study, rates of resistance to clindamycin were found also for *Veillonella* spp. (50%), and *Clostridium* spp. (31%).

The rates of resistance to metronidazole for several gram positive anaerobes, other than *C. perfringens*, were higher than those for gram-negative anaerobes. Among gram-positive anaerobes, *C. perfringens* was the most susceptible. Other *Clostridium* species were less susceptible to penicillin, ampicillin and metronidazole but unchanged susceptibility to piperacillin-tazobactam. Jamal *et al*.,[7] found that Piperacillin-tazobactam was the only antimicrobial agent to which all the isolates were uniformly susceptible. Bartlett[29] and Liu *et al*.[8] reported higher resistance of *Clostridium* species to metronidazole (36%). Based on our findings metronidazole, cefoxitin, piperacillin/ tazobactam and amoxicillin/clavulanate remain good empirical choices when anaerobes are expected.

The icreased rates of nonsusceptibility to commonly used antianaerobic agents mandate our attention, and periodic monitoring of the trend of the resistance is crucial. The antimicrobial resistance of anaerobic pathogens adversely affects clinical outcome, resulting in treatment failure and mortality

These data indicate that there continue to be changes in susceptibility. The development of antibiotic resistance in anaerobic bacteria has a tremendous impact on the selection of antimicrobial agents for empirical therapy. Some laboratories may choose to perform periodic surveillance testing of stored clinical isolates in order to generate local data to guide clinicians with empirical therapy[16]. However, the need for some form of routine testing may become more important, since resistant anaerobes have been isolated.

CONCLUSIONS

It was concluded that Microscan RAID was easier to perform and interpret but more expensive than API. Microscan has the potential to provide rapid identification of anaerobes with an automated reader. Both system give reliable rapid identification of anaerobes.

Our results lead us to believe that broth-disk method can be used routinely in the clinical bacteriology laboratory to test the antimicrobial susceptibility of anaerobic bacteria. Although broth disk elution test is not the standard method for anaerobes susceptibility, attention should be paid to standardize such easy and rapid method to be applicable to routine use, because agar dilution is not a practical method for most clinical laboratories. The procedures represent one of the most practical means for clinical laboratories to perform routine

antibiotic susceptibility tests on anaerobic bacteria. Because of the occurrence of resistance to most classes of current anti-anaerobic antibiotics, it is recommended that the antimicrobial resistance patterns be monitored regularly in order to guide empirical therapy.

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