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☐ Review Article ☐

Current Susceptibility Patterns of Anaerobic Bacteria

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While antibiotic resistance among anaerobes continues to increase, the frequency of antimicrobial susceptibility testing for anaerobes is declining. Because anaerobic infections are often mixed and detailed bacteriology of the organisms involved may take some time, physicians must institute empiric therapy before susceptibility testing results are available. Also, economic realities and prudent use of resources mandate that careful consideration be given to the necessity for routine susceptibility testing of anaerobic bacteria. Determination of appropriate therapy can be based on published antibiograms; however, since patterns may vary within geographic regions and even within hospitals, it is strongly recommended that each hospital center periodically test their isolates to determine local patterns and detect any pockets of resistance. As a general guide, antibiograms from the last several years of susceptibility testing at the Wadsworth Anaerobe Laboratory are reported.

Key Words: Antimicrobial susceptibility, anaerobic bacteria, resistance

The development of antibiotic resistance in anaerobic bacteria has been documented for β-lactam drugs, clindamycin and other macrolides, tetracyclines and 5-nitroimidazoles (Rasmussen *et al.* 1997). The *Bacteroides fragilis* group is one of the most antimicrobial-resistant groups of anaerobes; resistance to virtually all classes of antimicrobial agents has been reported (Rasmussen *et al.* 1997). Significant resistance is due to the production of β-lactamases from genes that can be transferred between cells (Salyers and Shoemaker, 1996), and β-lactamases capable of hydrolyzing "stable" agents have been reported (Rasmussen *et al.* 1997). Resistance to macrolides (MLS resistance) is usually due to rRNA methylases which modify the 23S component of the ribosome and has been found in both gram-negative (*Bacteroides*, *Campylobacter*, *Prevotella*) and gram-positive anaerobic rods (*Clostridium*, non-sporing rods) (Roberts, 1995).

Economic realities and prudent use of resources mandate that careful consideration be given to the necessity for routine susceptibility testing of anaerobic bacteria. Because anaerobic infections are often mixed and detailed bacteriology of the organisms involved may take some time, physicians must institute empiric therapy before susceptibility testing results are available. Determination of appropriate therapy can be based on published antibiograms; however, since patterns may vary within geographic regions and even within hospitals, it is strongly recommended that each hospital center periodically test their isolates to determine local patterns and detect any pockets of resistance. This resistance may have important implications for a clinical outcome, even though there are only limited reports which correlate susceptibility results with clinical failures. Several factors play a role in the difficulty of obtaining this kind of data: many anaerobic infections are mixed and elimination of all the organisms

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isolated may not be necessary; the effects of drainage and/or debridement will affect the outcome, even if a resistant organism is involved, and the general health of the patient, always an important factor, may be especially significant in cases in which anaerobes are involved. However, recent studies show that in serious infections, there is a correlation between susceptibility results and clinical outcomes (Rosenblatt and Brook, 1993); one example is a study in which the MIC of cefoxitin, the dose, and the duration of therapy were predictors of the outcome in a retrospective analysis of 19 patients with *B. fragilis* group infections (Snydman *et al.* 1992).

An additional cause for concern is accumulating evidence of transferable resistance among anaerobes. Such resistance transfer factors have been documented for metronidazole, cefoxitin, carbapenems, and clindamycin as well as for other compounds (Salyers, 1993; Sebald, 1994; Salyers and Shoemaker, 1996). Also, there is some evidence that exposure to the agent may induce increased resistance in isolates where the gene is present, but expressed only at low levels, which has been shown with *B. fragilis* and imipenem (Podglajen *et al.* 1994).

The NCCLS has set forth guidelines to aid microbiology laboratories in determining appropriate criteria for testing of anaerobic isolates (National Committee for Clinical Laboratory Standards. 1997). Factors which may contribute toward the development of infections involving anaerobes include various surgical procedures, trauma wounds, childbirth, aspiration pneumonia, human and animal bites, and inappropriate antimicrobial therapy. Certain physical conditions (e.g., diabetes mellitus, neutropenia, hypogammaglobulinaemia, malignancy, immunosuppression) may also predispose toward anaerobic infections. Infections in which anaerobes are commonly involved are listed in Table 1. Organisms which should be considered for testing include organisms with known variability in susceptibility patterns (the *Bacteroides fragilis* group, many other anaerobic gram-negative bacilli, and *Clostridium* species other than *C. perfringens*), organisms whose susceptibility patterns are not well known, organisms known to be especially virulent and organisms isolated in pure culture. While antibiotic resistance among anaerobes continues to increase (Cuchural *et al.* 1990; Snydman *et al.* 1996), the frequency of antimicrobial susceptibility testing for anaerobes is declining.

Table 1. Infections commonly involving anaerobes

Actinomycosis	Infected foot ulcers
Anaerobic cellulitis	Intraabdominal abscess
Appendicitis	Lung abscess
Aspiration pneumonia	Mastoiditis
Brain abscess	Neck space infection
Chronic sinusitis	Odontogenic infection
Chronic otitis media	Periodontal disease
Chronic osteomyelitis	Peritonitis
Clostridial myonecrosis	Peritonsillar abscess
Clostridium difficile-associated colitis	Pleural empyema
	Wound infections
Decubitus ulcers	Salpingitis
Endometritis	Subdural empyema
Human and animal bite infections	Tuboovarian abscess

As a guide to general descriptions of the efficacies of various agents against anaerobes, the susceptibility results from the Wadsworth Anaerobe Laboratory at the West Los Angeles Veterans Administration Medical Center are presented (Tables 2 and 3). The antibiograms presented here are summarized from the last few years of antimicrobial susceptibility tests and are composite tables of data from a large number of studies, so it is impossible to detail the number of organisms tested in each category. However, normally at least 10 – 20 strains of each species are tested, which usually results in at least 50 – 60 strains for each genus.

All bacteria were randomly selected recent clinical isolates from the Veterans Administration Wadsworth Medical Center, Los Angeles. Bacteria were identified according to established procedures (Holdeman *et al.* 1977; Summanen *et al.* 1993). Minimum inhibitory concentrations were determined by an agar dilution technique described previously (Summanen, *et al.* 1993) using an inoculum of 10^5 CFU, and Brucella base-laked blood agar. Plates were incubated in an anaerobic chamber (Anaerobe Systems, San Jose, CA.) for 48 hours at 37°C. MIC's were defined as the lowest concentration of anti-

Table 2. Susceptibility of gram-negative anaerobic bacteria

Anaerobe	% susceptible to:*						
	<50	50–69	70–84	85–95	>95		
B. fragilis	PEN†	CFP	MOX	CTT	PIP	FOX	SIT
	CIP	CTX	CRO	ZOX	AMC	BIA	LVX
	FLE	CAZ	CLR	CLI	SAM	IPM	OFX
	LOM	SPX		MIN	CPS	MEM	TVA
	AZM				TZP	CHL	MND
	ERY				TIM	CLX	
	ROX						
	TET						
Other *B. fragilis* group†	PEN	CFP	LVX	AMC	SAM	IPM	SIT
	CTX	CTT	CLR	PIP	CPS	MEM	TVA
	CAZ	MOX	CLI	FOX	TZP	CHL	MND
	CRO	OFX		ZOX	TIM	CLX	MIN
	CIP	SPX			BIA		
	FLE						
	LOM						
	AZM						
	ERY						
	ROX						
C. gracilis					PIP	MEM	TVA
					AMC	CHL	MND
					TZP	CIP	AZM
					TIM	CLX	CLI
					FOX	SIT	ERY
					ZOX	FLE	ROX
					CRO	LOM	MIN
					BIA	SPX	TET
					IPM		
Other *Bacteroides* spp.	FLE	CIP	PEN	CTT	PIP	CTX	CLX
	LOM	TET	MOX	CAZ	AMC	FOX	SIT
			OFX	CRO	SAM	ZOX	LVX
			SPX	CLR	TIM	BIA	TVA
			AZM	ERY	CFP	IPM	MND
				ROX	CPS	CHL	CLI
				MIN			
Prevotella spp.	FLE	TET	CIP	CRO	PIP	ZOX	CLX
	LOM		OFX	AZM	AMC	BIA	SIT
			SPX	CLR	SAM	IPM	TVA
			MIN	ERY	TZP	MEM	MND
				ROX	TIM	CHL	CLI
					FOX		
Porphyromonas spp.	FLE	TET		CIP	PIP	IPM	SPX
	LOM			CLR	AMC	MEM	TVA
				CLI	FOX	CHL	MND
				ERY	ZOX	CLX	AZM
				ROX	CRO	SIT	MIN
					BIA		
Sutterella wadsworthensis		CLI	MND	PIP	AMC	CRO	CIP
				TZP	TIM	IPM	FLE
				ZOX	FOX	MEM	
				TVA			

Table 2. Continued

Anaerobe	% susceptible to:*						
	<50	50–69	70–84	85–95	>95		
F. nucleatum	FLE			CIP	PIP	BIA	OFX
	LOM			AZM	AMC	IPM	SPX
	CLR				TZP	MEM	TVA
	ERY				TIM	CHL	CLI
	ROX				FOX	CLX	MND
					ZOX	SIT	MIN
					CRO	LVX	TET
F. mortiferum	FLE	CIP	CLI	AMC	PIP	IPM	SIT
& F. varium	LOM	SPX	TET	ZOX	TZP	MEM	TVA
	AZM	TEM		CRO	TIM	CHL	MND
	CLR				FOX	CLX	MIN
	ERY				BIA		
	ROX						
Other *Fusobacterium* spp.	FLE		CAZ	PIP	PEN	IPM	MND
	LOM		MOX	AMC	SAM	MEM	CLI
	CLR		CIP	TIM	TZP	CHL	MIN
	ERY		SPX	CPS	FOX	CLX	TET
	ROX		AZM	CTX	BIA	SIT	
				CTT			
				ZOX			
				CRO			
B. wadsworthia	AMX			CLI	PIP	ZOX	LOM
	AMP				TIC	IPM	SPX
	PEN				AMC	CHL	TVA
					SAM	CIP	MND
					CTT	SIT	MIN
					FOX	FLE	TET

*: The order of listing of drugs within percent susceptible categories is not significant. According to the NCCLS-approved breakpoints (M11-A3), using the intermediate category as susceptible. AMC, amoxicillin/clavulanate; AZM, azithromycin; BIA, biapenem; CAZ, ceftazidime; CFP, cefoperazone; CHL, chloramphenicol; CIP, ciprofloxacin; CLI, clindamycin; CLR, clarithromycin; CLX, clinafloxacin; CPS, cefoperazone/sulbactam; CRO, ceftriaxone; CTT, cefotetan; CTX, cefotaxime; ERY, erythromycin; FLE, fleroxacin; FOX, cefoxitin; IPM, imipenem; LOM, lomefloxacin; LVX, levofloxacin; MEM, meropenem; MIN, minocycline; MND, metronidazole; MOX, moxalactam; OFX, ofloxacin; PEN, penicillin; PIP, piperacillin; ROX, roxithromycin; SAM, ampicillin/sulbactam; SIT, sitafloxacin; SPX, sparfloxacin; TEM, temafloxacin; TET, tetracycline; TIM, ticarcillin/clavulanate; TVA, trovafloxacin; TZP, piperacillin/tazobactam; ZOX, ceftizoxime.

†: NCCLS approved breakpoint is 4 μg/mL. However, the breakpoint should probably be lowered to 1 μg/mL, which will considerably lower the values for % susceptible. For example, at 1 μg/mL, no strains of the *B. fragilis* group were susceptible.

†: Excluding *B. fragilis.*

microbial resulting in no growth, a haze, one discrete colony or multiple tiny colonies, or a marked change in the appearance of growth as compared to the control plate (in the case of persistent light [slight] growth) (National Committee for Clinical Laboratory Standards. 1993). Reference strains of *Bacteroides*

Table 3. Susceptibility of gram-positive anaerobic bacteria

Anaerobe	% susceptible to:*						
	<50	50–69	70–84	85–95	>95		
Peptostreptococcus spp.	LOM	FLE TET ROX	CIP OFX AZM CLR ERY	LVX CLI MIN	PEN PIP AMC SAM TZP TIM CFP CPS	CTT FOX CAZ ZOX CRO BIA IPM	MEM CHL CLX SIT SPX TVA MND
C. difficile†	FOX ZOX CIP FLE LOM SPX	CLI MIN TET AZM CLR ERY ROX		CRO BIA CHL	AMP PIP TIC AMC SAM	TZP TIM CTT IPM MEM	CLX SIT TVA MND
C. ramosum	CIP FLE LOM AZM CLR ERY ROX	SPX MIN TET	FOX	AMP PIP SAM CHL TVA CLI	AMC TZP TIM	ZOX IPM CLX	SIT MND
C. perfringens		TET	MIN	LOM CLI	AMP PIP TIC SAM AMC TZP TIM CTT	ZOX BIA IPM CHL CIP CLX SIT FLE	SPX TVA MND AZM CLR ERY ROX
Other *Clostridium* spp.	CAZ FLE LOM	CFP CTX FOX ZOX CRO CIP AZM CLR ERY ROX	LVX OFX SPX CLI TET	MOX	AMX AMP CAR PEN PIP	TIC SAM AMC BIA IPM CHL	CLX SIT TVA MND MIN
Nonspore-forming gram-positive rod	FLE LOM	CIP OFX MND	CFP MOX SPX TET	CTT FOX CRO CPS TVA AZM CLR ERY ROX	PEN PIP AMC SAM TZP TIM	FTX ZOX BIA IPM MEM CHL	CLI CLX SIT LVX MIN

*: The order of listing of drugs within percent susceptible categories is not significant. According to the NCCLS-approved breakpoints (M11-A3), using the intermediate category as susceptible. AMP, ampicillin; AMX, amoxicillin; TIC, ticarcillin. See Table 2 footnote for other antimicrobial agents.

†: Breakpoint is used only as a reference point. *C. difficile* is primarily of interest in relation to antimicrobial induced pseudomembranous colitis. These data must be interpreted in the context of level of drug achieved in the colon and impact of agent on indigenous colonic flora.

fragilis (ATCC 25285) and *B. thetaiotaomicron* (ATCC 29741) were used as controls in each test. Antimicrobial agents were obtained as powders from pharmaceutical companies.

The most active agents against the *Bacteroides fragilis* group were the β-lactam/β-lactamase inhibitor combinations, the carbapenems (imipenem, meropenem), the newer fluoroquinolones (trovafloxacin, moxifloxacin, clinafloxacin, sitafloxacin), and metronidazole. Cefoxitin was active against most strains of *B. fragilis* but less active against other members of the group. Both *Prevotella* and *Porphyromonas* species exhibited some resistance to the macrolides, and were very resistant to tetracycline. Most of the anaerobes tested were highly resistant to fleroxacin and lomefloxacin. Among the gram-positive anaerobes, clostridia other than *C. perfringens* showed considerable resistance to the macrolides and to many of the cephalosporin compounds. Agents with excellent activity included β-lactam/β-lactamase inhibitor combinations, carbapenems, trovafloxacin and metronidazole. *Peptostreptococcus* species differed among their resistance patterns (Table 4). *P. anaerobius* and *P. asaccharolytica* were more resistant to several agents (e.g., amoxicillin/clavulanate, clindamycin, penicillin G) than were the

Table 4. Antibiograms of *Peptostreptococcus* species

Species	AMC	AMP	SAM	CFP	CTT	FOX	ZOX	CHL	CIP	CLI	IPM	MND	PEN	PIP	TIC	TIM	TVA
P. anaerobius																	
GM MIC	0.4	1.8	4.0	0.9	6.1	2.1	1.3	2.4	1.2	0.4	0.1	0.6	0.7	0.8	25.4	1.1	0.1
No. resistant	3	4	0	0	3	0	0	1	1	3	0	1	2	0	1	2	0
No. tested	21	9	1	6	21	43	43	35	24	47	43	52	6	7	3	18	9
P. asaccharolytica																	
GM MIC	0.4	0.2	0.4	0.1	1.8	0.7	1.1	2.0	2.7	0.6	0.1	0.9	0.7	0.2	0.9	0.2	0.4
No. resistant	1	0	0	0	0	0	0	0	13	11	0	1	3	0	0	0	0
No. tested	18	5	2	7	23	44	39	27	25	52	44	57	7	8	10	20	6
P. magnus																	
GM MIC	0.2	0.8	0.2	0.1	1.9	0.9	4.4	3.5	0.6	1.1	0.1	0.6	0.2	0.3	1.3	0.7	0.2
No. resistant	0	2	0	0	0	0	0	0	1	12	0	1	0	0	0	0	0
No. tested	35	7	6	8	38	69	60	39	33	83	77	83	8	12	12	25	6
P. micros																	
GM MIC	0.1	0.2	0.2		0.9	0.8	1.0	2.0	0.8	0.2	0.1	0.3		0.3	0.6	0.1	0.2
No. resistant	1	0	0		1	0	0	0	0	0	0	0		0	0	0	0
No. tested	63	5	12	0	35	62	63	44	43	96	87	98	0	18	20	36	6
P. prevotii																	
GM MIC	0.1	0.3	0.2	0.1	1.4	0.5	0.6	2.3	2.8	0.6	0.1	0.6	0.1	0.4	0.4	0.2	0.6
No. resistant	0	0	0	0	0	0	0	0	10	6	0	0	0	0	0	0	0
No. tested	8	6	3	4	24	44	35	19	20	45	42	45	4	5	7	16	6
P. productus																	
GM MIC	2.8				2.0	8.0	8.0	8.0	0.5	0.1	0.4	0.4		32.0		0.5	
No. resistant	0				0	0	0	0	0	0	0	0		0		0	
No. tested	2	0	0	0	2	2	2	1	1	2	2	3	0	1	0	1	0
Peptostreptococcus sp.																	
GM MIC	1.0		1.0		0.5	1.0	0.5	1.0	1.0	0.1	1.0	0.5		1.0	1.0	0.1	1.0
No. resistant	0		0		0	0	0	0	4	0	0	0		0	0	0	0
No. tested	9	0	1	0	8	11	11	6	9	12	13	17	0	5	1	6	5
P. tetradius																	
GM MIC	0.04				0.9	0.2	1.0	1.0	5.3	0.3	0.1	1.1		0.1		0.1	
No. resistant	0				0	0	0	0	4	1	0	0		0		0	
No. tested	7	0	0	0	12	15	19	3	5	13	13	7	0	5	0	8	0

Abbreviations: see Tables 2 and 3 footnote.
GM MIC: Geometric mean MIC (μg/mL)
Note: Shaded areas indicate species with higher percentages of resistant strains.

other species tested.

We have emphasized in several publications that many of the isolates tested cluster around breakpoint values for many antimicrobial agents (β-lactam drugs and clindamycin, especially). Also, the accuracy of most of the susceptibility techniques used (agar dilution and broth microdilution) is plus/minus one two-fold dilution. The combination of these two factors means that the percent organisms that are reported as susceptible at a given breakpoint may vary widely, and this variation may have little or no significance. For some time we have reported the percentages susceptible at a range of dilutions bracketing the breakpoint concentration. In this report, the data is presented as groups of agents having a percentage range of activity against specific anaerobic groups or species. Again, at times an agent was on the borderline between two groups and an arbitrary decision was made as to placement (generally based on the most recent tests performed).

In summary, it is important for clinical laboratories to recognize those situations where susceptibility testing of anaerobes may be an important factor in clinical management. Published reports by large research centers may be used as general guides in determining therapy, but individual hospitals are strongly encouraged to test batches of their isolates periodically to determine if there is emerging resistance. Reports of resistance to agents previously highly active against anaerobes (β-lactam/β-lactamase inhibitor combinations, carbapenems and metronidazole) are of particular concern. The ability of organisms to transfer their resistance genes mandates that global resistance patterns should be carefully and continuously monitored.

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