JOURNAL OF BACTERIOLOGY, Jan., 1967, p. 125-130
Vol. 93, No. 1

Antibacterial Sensitivity of *Bifidobacterium* (*Lactobacillus bifidus*)

LAWRENCE G. MILLER AND SYDNEY M. FINEGOLD
Department of Medicine, Veterans Administration Center and UCLA School of Medicine, Los Angeles, California

Received for publication 31 August 1966

ABSTRACT

The antibacterial sensitivity patterns of gram-positive, nonsporeforming, anaerobic bacilli variously classed as *Lactobacillus bifidus*, *Actinomyces bifidus*, or *Bifidobacterium* were studied by the plate dilution method. A total of 34 strains, mostly from human feces, was studied. Three species, *B. longum*, *B. adolescentis*, and *B. bifidum*, were represented with 11, 11, and 6 strains, respectively. The other six strains fell into four other species. Most strains of all types resisted 100 μg/ml or more of neomycin, polymyxin B, and nalidixic acid. They were somewhat less resistant to kanamycin and still less so to streptomycin. All strains were inhibited by less than 1 μg/ml of penicillin G and erythromycin, by 3.1 units or less per ml of bacitracin, by 3.1 μg/ml or less of chloramphenicol, and by 6.2 μg/ml or less of tetracycline and lincomycin. Most strains were inhibited by 3.1 μg/ml of vancomycin. Results were very variable with cephalothin and nitrofurantoin, with some strains quite resistant. With half of the drugs tested, there were moderate differences in sensitivity between different species. These data are discussed in relation to the effect of antimicrobial agents on bifid bacilli in the normal human fecal flora, in relation to the implications thereof, and in relation to the usefulness of several agents (particularly neomycin, nalidixic acid, and polymyxin B) in selective media for *Bifidobacterium*.

The "bifid bacteria" are anaerobic, gram-positive, nonsporeforming bacilli. These organisms are variously classified as *Bacillus bifidus*, *Bacillus bifidus communis*, *Bacteroides bifidus*, *Lactobacillus bifidus*, *Actinomyces bifidus* (15), *Bifidibacterium bifidum* (19), and *Bifidobacterium bifidum* (3). The American Society for Microbiology's subcommittee on *Lactobacilleae* has tentatively recommended removing *L. bifidus* from the genus *Lactobacillus* and placing it in a separate genus, *Bifidobacterium* Orla-Jensen 1924 (*see* ASM News, Aug. 1966, p. 29). They usually exhibit a bifurcated appearance during cultivation. *Bifidobacterium* strains may be isolated in large numbers from the feces of adults and children. They may occasionally be isolated from the vagina (19), the genitourinary tract (19), and the mouth (1) in small numbers. They have also been found on the skin and in human and cow's milk (3). Solid evidence that they are pathogenic in man or animal is lacking.

Many European authors have done morphological, serological, and biochemical studies on these organisms. Reuter (14) has suggested the recognition of eight species under the genus *Bifidobacterium*, on the basis of carbohydrate fermentation and cross-precipitin tests. A review of the current status of these organisms appeared in 1964 (3).

The antibacterial sensitivity of *Bifidobacterium* has been studied very little. Lavergne (12) tested 20 strains designated as *Bifidobacterium bifidum* against 11 antibacterial agents by the disc technique. A single strain of *B. bifidum* was reported sensitive to 3.1 μg/ml of colistin sulfate by Courtieu et al. (4). The sensitivity of 19 undifferentiated strains of *Bifidobacterium* to neomycin, kanamycin, paromomycin, and vancomycin was reported by Finegold, Miller, and Posnick (6).

Since these organisms constitute a major component of the normal bowel flora in humans, they may play an important role in certain physiological and pathophysiological processes and may help prevent colonization by pathogens such as *Staphylococcus aureus*, *Shigella*, *Salmonella*, and enteropathogenic *Escherichia coli*. It was thus deemed desirable to study in detail the antibacterial sensitivity patterns of a number of species of *Bifidobacterium* isolated from humans.

MATERIALS AND METHODS

Thirty-four strains of *Bifidobacterium* were studied. The source and species designation, as determined by Reuter (14), of these organisms is listed in Table 1. Of the strains used in this study, 21 were isolated in our laboratory. The two strains isolated from abscesses were part of a mixed flora; neither strain is considered to have contributed to the pathological process. The remaining 13 organisms were kindly sent to us by G. Reuter.

All of the organsisms were gram-positive, nonspore-forming, anaerobic bacilli. They fermented glucose without producing gas and yielded acetic, lactic, and formic acids. They were all catalase-negative; they did not produce indole or hydrogen sulfide, nor did they reduce nitrate. Gelatin was not liquefied. Milk was coagulated in 24 to 48 hr, and the final *p*H was 4.0 to 4.8. They were nonmotile. Under some conditions, they exhibited true branching and a degree of pleomorphism.

All of the media used were supplemented with 0.2% yeast extract and 5 μg/ml of hemin. The fluid medium used was Trypticase Soy Broth (BBL) with 0.7 μg/ml of L-cystine added. The solid medium used was Eugonagar (BBL).

Sensitivity testing was done by plate dilution technique with a Steers replica inoculating apparatus (18). The organisms were grown for 48 hr in supplemented Trypticase Soy Broth. The growth was diluted so as to obtain 10^5 to 10^6 organisms per milliliter for use as inoculum.

Laboratory standards of 14 antibacterial agents were weighed, dissolved, and diluted in appropriate diluents prior to addition to the base medium, Eugonager. The antibacterial agents tested and the concentrations used are listed in the remaining tables. Appropriate control plates were set up. After inoculation, the plates were incubated at 37 C in a National Anaerobic Incubator containing an atmosphere of 90% nitrogen and 10% carbon dioxide obtained after six flushings with nitrogen. The tests were read after 5 days.

TABLE 1. *Source and species differentiation of bifidobacterium strains*

Species of *Bifidobacterium*	Source of Strain		ATCC no.
	Author's isolates[a]	Reuter's strains[b]	
B. adolescentis	8	3	15703, 15705, 15706
B. bifidum	4	2	15696
B. breve	—	2	15700, 15701
B. infantis	—	1	15697
B. liberorum	—	1	15702
B. longum	9	2	15707, 15708
B. parvulorum	—	2	15698

[a] All of these strains were isolated from feces, except for two strains of *B. adolescentis* which were isolated from abscesses.

[b] Eleven of these strains have been deposited in the American Type Culture Collection under the numbers noted (2nd Supplement to Seventh Edition, ATCC Catalogue). All 11 strains were isolated from the intestine of human adults or infants.

RESULTS

The results of sensitivity of *Bifidobacterium* to two penicillin-like antibiotics (penicillin G and cephalothin) are shown in Table 2. Over one-half of the strains were sensitive to less than 0.2 μg/ml of penicillin G. *Bifidobacterium* strains were distinctly more resistant to cephalothin; this was especially true of *B. longum*.

The strains studied were quite resistant to the streptomycin-like antibiotics (Table 3). Most of the strains were resistant to 100 μg/ml of neomycin. They were less resistant to kanamycin and even less so to streptomycin. Kanamycin appears to be somewhat less active against *B. adolescentis* than neomycin is.

Table 4 shows the sensitivity of the strains to antibiotics with a primarily gram-positive spectrum, bacitracin, erythromycin, lincomycin, and vancomycin. The strains studied were most sensitive to erythromycin and were quite sensitive to the other three agents as well.

The broad-spectrum antibacterial agents, chloramphenicol, tetracycline, and nitrofurantoin, were all effective against bifid bacilli, with chloramphenicol the most effective (Table 5). Five strains of *B. adolescentis* showed resistance to 25 μg/ml of nitrofurantoin.

Bifidobacterium strains were very resistant to agents with a gram-negative spectrum (Table 6). All of the strains were resistant to at least 100 μg/ml of nalidixic acid, and all but five were resistant to 100 μg/ml or more of polymyxin B.

Table 7 lists the results of the sensitivity tests (with all agents) on the species represented by fewer than three strains.

On the whole, there were very few differences among species with regard to sensitivity to antibacterial agents. However, *B. adolescentis* was more sensitive to neomycin and polymyxin B and more resistant to nitrofurantoin than the other two groups studied. *B. bifidum* was relatively resistant to bacitracin, vancomycin, and polymyxin B; *B. longum* was relatively resistant to penicillin G, cephalothin, erythromycin, and lincomycin.

DISCUSSION

The majority of strains used in this study are the strains which are most commonly isolated from feces in adults; *B. adolescentis*, *B. longum*, and *B. bifidum* (15).

Lavergne et al. (12), in studying the effect of 11 antibiotics on 20 strains of *B. bifidum* isolated from infants' stools, utilized the disc technique.

TABLE 2. *Sensitivity of Bifidobacterium to penicillin-like antibiotics*

Antibiotic	Species	Total no. of strains	Minimal inhibitory concn (μg/ml)									
			<0.2	0.2	0.39	0.78	1.56	3.12	6.2	12.5	25.0	50.0
Penicillin G	*B. adolescentis*	11	9[a]		1	1						
	B. bifidum	6	5		1							
	B. longum	11	2		5	4						
	Other species	6	2		4							
Cephalothin	*B. adolescentis*	11				2	3	2	1	2	1	
	B. bifidum	5					1	3	1			
	B. longum	11							1	1	8	1
	Other species	5							2	3		

[a] Number of strains sensitive to the various minimal inhibitory concentrations.

TABLE 3. *Sensitivity of Bifidobacterium to streptomycin-type antibiotics*

Antibiotic	Species	Total no. of strains	Minimal inhibitory concn (μg/ml)									
			<12.5	12.5	25	50	100	200	400	>400	800	1,600
Kanamycin	*B. adolescentis*	11					6[a]	2	3			
	B. bifidum	5						4	1			
	B. longum	11					3	1	4		2	1
	Other species	5				1	3		1			
Neomycin	*B. adolescentis*	11		1	2		1	4	3			
	B. bifidum	5							3		1	1
	B. longum	11						3	5		2	1
	Other species	6						4			1	1
Streptomycin	*B. adolescentis*	11	1	1	1	6	1			1		
	B. bifidum	6			3	2				1		
	B. longum	11		2	3	2	2	1		1		
	Other species	6		1	1	1				3		

[a] Number of strains sensitive to the various minimal inhibitory concentrations.

TABLE 4. *Sensitivity of Bifidobacterium to antibiotics with gram-positive spectrum*

Antibiotic	Species	Total no. of strains	Minimal inhibitory concn (units/ml for bacitracin; μg/ml for others)							
			<0.2	0.2	0.39	0.78	1.56	3.1	6.2	25
Bacitracin	*B. adolescentis*	11	3[a]	1	5	1		1		
	B. bifidum	5					4	1		
	B. longum	11	2	3	5		1			
	Other species	6			3	2	1			
Erythromycin	*B. adolescentis*	11	10	1						
	B. bifidum	6	5	1						
	B. longum	11	2	3	5	1				
	Other species	6	3	3						
Lincomycin	*B. adolescentis*	11	4	2	3	1	1			
	B. bifidum	6		1	2	2	1			
	B. longum	11				2	7		2	
	Other species	5		1	2		2			
Vancomycin	*B. adolescentis*	11		1	4	5				1
	B. bifidum	6					6			
	B. longum	11			4	7				
	Other species	5			1	3	1			

[a] Number of strains sensitive to the various minimal inhibitory concentrations.

TABLE 5. *Sensitivity of Bifidobacterium to broad-spectrum antibacterial agents*

Antibiotic	Species	Total no. of strains	Minimal inhibitory concn (μg/ml)							
			0.78	1.56	3.1	6.2	12.5	25	50	100
Chloramphenicol	*B. adolescentis*	11		6[a]	5					
	B. bifidum	6		5	1					
	B. longum	11		5	6					
	Other species	6	1	3	2					
Nitrofurantoin	*B. adolescentis*	11		1	3	1	1		4	1
	B. bifidum	6		1	2	3				
	B. longum	11		4	4	1	2			
	Other species	6		1	1		2	2		
Tetracycline	*B. adolescentis*	11	1	3	5	2				
	B. bifidum	5		1	4					
	B. longum	11		2	6	3				
	Other species	5		2	3					

[a] Number of strains sensitive to the various minimal inhibitory concentrations.

TABLE 6. *Sensitivity of Bifidobacterium to antibacterial agents with gram-negative spectrum*

Antibiotic	Species	Total no. of strains	Minimal inhibitory concn (μg/ml)								
			<25	25	50	100	200	400	800	1,600	>1,600
Nalidixic acid	*B. adolescentis*	11					1[a]	8	2		
	B. bifidum	6						2	4		
	B. longum	11						5	5		1
	Other species	6					1	3	2		
Polymyxin B sulfate	*B. adolescentis*	11	1		1	2	6		1		
	B. bifidum	6							4	1	1
	B. longum	11				1	2	4	3	1	
	Other species	6						4		2	

[a] Number of strains sensitive to the various minimal inhibitory concentrations.

TABLE 7. *Sensitivity[a] of other species[b] to antibacterial agents*

Antibiotic	*B. breve* (a)[c]	*B. breve* (b)[c]	*B. infantis*	*B. liberorum*	*B. parvulorum* (a)[c]	*B. parvulorum* (b)[c]
Bacitracin	0.39	0.78	0.78	1.56	0.39	0.39
Cephalothin	12.5	12.5	12.5	6.2	6.2	—
Chloramphenicol	1.56	3.1	3.1	1.56	0.78	1.56
Erythromycin	0.2	0.2	0.2	<0.1	<0.1	0.2
Kanamycin	50	100	400	100	100	—
Lincomycin	0.39	1.56	1.56	0.39	0.2	—
Nalidixic acid	800	400	800	400	400	200
Nitrofurantoin	1.56	12.5	12.5	25	3.1	25
Neomycin	200	1,600	800	200	200	200
Penicillin G	<0.1	0.39	0.39	0.39	0.39	<0.1
Polymyxin B	400	1,600	1,600	400	400	400
Streptomycin	12.5	50	>400	>400	25	>400
Tetracycline	1.56	3.1	3.1	1.56	3.1	—
Vancomycin	0.78	0.78	0.78	1.56	0.39	—

[a] Minimal inhibitory concentration is measured in units per ml for bacitracin, μg/ml for other agents.
[b] One strain of each species or variant was studied.
[c] Variant according to Reuter.

They did not biochemically characterize their strains, and it is not certain whether they consider *B. bifidum* a specific species, or whether they used this as a general term for *Bifidobacterium* (there are seven different species found normally in infants' stools). Our results generally agree with their results for those antibiotics studied in common (penicillin G, chloramphenicol, erythromycin, streptomycin, and neomycin). However, their strains were relatively resistant to chlor- and oxytetracycline, whereas our strains were sensitive to tetracycline. These authors also found their strains to be generally sensitive to spiramycin and oleandomycin, drugs closely related to erythromycin. Framycetin, related to neomycin and streptomycin, was relatively inactive against their bifids. Finally, they found their strains to be sensitive to novobiocin.

Earlier limited studies on unspeciated *Bifidobacterium* strains with kanamycin, paromomycin, neomycin, and vancomycin (6), and lincomycin (5) gave results similar to those obtained in the present study for the four antibacterial agents used in common (paromomycin was not used in the present study).

Information on the effect of antibacterial agents on *Bifidobacterium* in vivo has been collected during studies of the effect of such agents on the normal fecal flora of humans (5, 7–11, 16, 17). Parenteral streptomycin effected a distinct reduction in bifid counts in a limited study (16). Seeliger (17) noted little effect of erythromycin on the bifid flora. Ampicillin when given orally in doses of 1 to 3 g per day reduced the bifidus counts (11). One patient given 2 g per day of ampicillin showed only a slight decrease in bifids (7). Oral paromomycin, when used in doses of 40 mg/kg of body weight, reduced bifids to a level at which they were no longer detectable in six of eight patients (10). Tetracycline and oxytetracycline effected elimination or a profound reduction of bifids in 14 patients studied by Haenel (9). Similar results were obtained by Gross (8). Another study, with four patients (7), showed a lesser but definite reduction in bifids. Other studies by our group (5, 7) evaluated the effect of several other agents on the bifid population. In these studies, we found that oral neomycin eliminated or reduced *Bifidobacterium* counts in five of six cases; oral kanamycin effected elimination or reduction in four of eight cases; novobiocin markedly reduced bifids in two patients; sulfadimethoxine produced no change in bifid flora in two patients; oral lincomycin eliminated them in five patients; intramuscular lincomycin eliminated bifids in two patients; and oral colistin sulfate effected no change in three patients. The lack of effect of oral colistin on bifids in the fecal flora was unexpected in terms of the sensitivity reported by Courtieu et al. (4) on a single strain, but was consistent with the resistance of bifids to polymyxin B (a closely related drug) shown in our study. Nalidixic acid had little effect on *Bifidobacterium* counts in two patients (Finegold et al., *unpublished data*).

Clearly, then, many antibacterial agents in common use change significantly the bifid content of the gut. Changes of this type may be of the greatest importance, since *Bifidobacterium* is among the most prevalent of the normal flora, typically being present in counts of 10^9 to 10^{10} per gram of feces and outnumbering *E. coli* and other aerobes 100 to 1 (7, 10, 13). The significance of the effect of antibiotics on fecal bifids is uncertain, but elimination of one of the major components of the normal flora would theoretically offer an opportunity for pathogens such as *S. aureus*, *Salmonella*, *Shigella*, and enteropathogenic *E. coli* to implant themselves in the intestinal tract much more readily than would be true normally. In this connection, it has been shown that *Salmonella* causes disease in mice much more readily when these mice have been pretreated with oral streptomycin, and that this effect is related to elimination of *Bacteroides* from the normal flora by the drug (2). We are unaware of any studies quantitating various components of the normal flora in patients with active enteritis due to enteric pathogens. This type of study is crucial to a better understanding of the potential protective role of bifids or other elements of the normal flora.

The role of bifid bacilli in the intestine in various physiological and pathophysiological processes affecting the host is unknown, but any such role might be modified during administration of certain antimicrobial agents.

Finally, our data suggest that several antibacterial agents might be useful in selective media for bifid bacilli. Since bifid bacilli are typically found in mixtures with many other anaerobes and aerobes, selective media should greatly facilitate their recovery. This would be particularly true where other organisms were much more prevalent (stools of patients receiving certain antibiotics). Mitsuoka et al. (13) have already utilized neomycin (200 μg/ml) plus paromomycin (20 and 50 μg/ml) in selective media; however, these authors noted that the media were not fully selective and in some cases failed to promote growth of *Bifidobacterium*. In addition, our currently reported studies indicate that the use of 200 μg/ml of neomycin would prevent growth of almost half of the bifid strains. The present study also indicates that nalidixic acid and polymyxin B might prove useful in selective media for bifid bacilli.

ACKNOWLEDGMENTS

This investigation was supported by grants from The Upjohn Co. (Kalamazoo, Mich.), Eli Lilly & Co. (Indianapolis, Ind.), Winthrop Laboratories (New York, N.Y.), and by Public Health Service Training Grant 1-T1-AI-309.

LITERATURE CITED

1. BEERENS, H., A. GÉRARD, AND J. GUILLAUME. 1957. Etude de 30 souches de *Bifidibacterium bifidum* (*Lactobacillus bifidus*). Caracterisation d'une variété buccale. Comparaison avec les souches d'origine fécale. Ann. Inst. Pasteur Lille **9**:77–85.
2. BOHNHOFF, M., C. P. MILLER, AND W. R. MARTIN. 1964. Resistance of the mouse's intestinal tract to experimental Salmonella infection. I. Factors which interfere with the initiation of infection by oral inoculation. J. Exptl. Med. **120**: 805–816.
3. BRAUN, O. H., J. DEHNERT, K. HOFFMANN, M. KIENITZ, J. B. MAYER, H. REPLOH, G. REUTER, H. P. R. SEELIGER, AND H. WERNER. 1964. Die Bifiduskeime des Menschen. Deut. Med. Wochschr. **89**:1647–1652.
4. COURTIEU, A. L., J. J. MONNIER, P. DE LAJUDIE. AND F. N. GUILLERMET. 1960. Spectre antibactérien de la colistine vis-à-vis de 1200 souches. Ann. Inst. Pasteur Suppl. **100**:14–31.
5. FINEGOLD, S. M., N. E. HARADA, AND L. G. MILLER. 1966. Lincomycin: activity against anaerobes and effect on normal human fecal flora. Antimicrobial Agents and Chemotherapy—1965, p. 659–667.
6. FINEGOLD, S. M., A. B. MILLER, AND D. J. POSNICK. 1965. Further studies on selective media for Bacteroides and other anaerobes. Ernaehrungsforschung **10**:517–528.
7. FINEGOLD, S. M., D. J. POSNICK, L. G. MILLER, AND W. L. HEWITT. 1965. The effect of various antibacterial compounds on the normal human fecal flora. Ernaehrungsforschung **10**:316–341.
8. GROSS, H. 1956. Beeinflussung der Darmflora durch Breitband-Antibiotica. Neue Osterr. Z. Kinderheilk. **1**:556–564.
9. HAENEL, H., F. KÖHLER, H. MERTSCH, AND C. PARDEMANN. 1960. Uber den Einfluss von Tetracyclinen auf die faekale Microbiozonose von TPE Dauerausscheidern. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **177**:41–61.
10. HAENEL, H., H. MERTSCH, F. KÖHLER, AND W. MÜLLER-BEUTHOW. 1965. Veranderungen der faekalen Mikroflora bei Jugendlichen durch Paromomycin. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **195**:385–392.
11. KNOTHE, H., AND B. WIEDEMANN. 1965. Die Wirkung von Ampicillin auf die Darmflora des gesunden Menschen. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **197**:234–243.
12. LAVERGNE, E. DE, J. C. BURDIN, J. SCHMITT, AND M. MANCIAUX. 1959. Sensibilité de Bifidibacterium bifidum à onze antibiotiques. Ann. Inst. Pasteur **97**:104–107.
13. MITSUOKA, T., T. SEGA, AND S. YAMAMOTO. 1965. Eine verbesserte Methodik der qualitativen und quantitativen Analyse der Darmflora von Menschen und Tieren. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **195**:455–469.
14. REUTER, G. 1963. Vergleichende Untersuchungen über die Bifidus-Flora im Säuglings- und Erwachsenenstuhl. Zentr. Bakteriol. Parasitenk. Abt. I Orig. **191**:486–507.
15. ROSEBURY, T. 1962. Microorganisms indigenous to man. McGraw-Hill Book Co., Inc., New York.
16. SCHABINSKI, G., AND R. ALTHAMMER. 1958. Untersuchungen über die Darmwirksamkeit von Streptomycin. Klin. Wochschr. **36**:1013–1017.
17. SEELIGER, H. P. R. 1957. Die Wirkung von Erythromycin auf die Darm-Flora des gesunden Menschen. Arzneimittel-Forsch. **7**:629–633.
18. STEERS, E., E. L. FOLTZ, AND B. S. GRAVES. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemotherapy **9**:307–311.
19. WEINBERG, M., R. NATIVELLE, AND A. R. PRÉVOT. 1937. Les microbes anaerobies. Masson et Cie, Paris.