

Vancomycin as a Selective Agent for Isolation of *Bacteroides* Species

A. J. VAN WINKELHOFF AND J. DE GRAAFF

Research Group of Commensal Infections, Department of Medical and Oral Microbiology, Vrije Universiteit, 1007 MC Amsterdam, The Netherlands

Received 29 June 1983/Accepted 22 August 1983

Thirty saccharolytic and asaccharolytic black-pigmented *Bacteroides* strains were tested for their susceptibility to vancomycin. All asaccharolytic strains appeared to be partly or completely inhibited at a concentration of 7.5 µg/ml, whereas most saccharolytic strains were resistant to this concentration. The use of vancomycin in *Bacteroides* selective media is discussed.

The genus *Bacteroides* contains about 25 species of obligately anaerobic non-spore-forming gram-negative bacilli. Members of this genus have been found in association with many diseases in humans, ranging from relatively benign to severe infections. These *Bacteroides* species form part of the anaerobic flora present on various mucous membranes of the body. Commonly, they are found in mixed cultures, and they probably play a key role in synergistic infections in humans and animals, including the various types of periodontal diseases.

Many investigators have reported the use of selective media for the isolation of *Bacteroides* species from clinical specimens, i.e., nonoral sites (1, 3, 5). These media contain a combination of kanamycin and vancomycin (respectively, 10 to 1,000 and 7.5 µg/ml) in Brucella agar, blood agar base, or Trypticase (BBL Microbiology Systems) soy agar, all supplemented with hemin (5 µg/ml) and menadione (10 µg/ml). In our study on the occurrence of *Bacteroides* species in periodontal patients, we have been investigating the usefulness of antibiotics as a selective agent in various media. We focused our work on kanamycin and vancomycin. Using the disk diffusion method, we tested nine strains of *Bacteroides gingivalis* for susceptibility to these antibiotics. These strains appeared to be susceptible to vancomycin and resistant to kanamycin. In a following experiment, we investigated the response to vancomycin with the agar dilution method.

The medium used in these experiments consisted of BM agar (13), supplemented with laked rabbit blood, hemin (5 µg/ml), and menadione (10 µg/ml). Vancomycin (Sigma Chemical Co.) was added to the medium in concentrations of 3.5, 5.0, and 7.5 µg/ml. After inoculation, the plates were incubated under 80% N₂, 10% H₂, and 10% CO₂ at 37°C. Examination took place

after 7, 14, and 21 days. This experiment was carried out three times with the same medium without vancomycin (control). Strains used, their sources, sites of isolation, and the results of the susceptibility tests are shown in Table 1.

At a concentration of 3.5 µg/ml, vancomycin appeared to be inhibitory to all *B. asaccharolyticus* strains; only a few small colonies developed. At a higher concentration (5.0 µg/ml) other important differences were observed. Some strains were partly inhibited: *B. levii* (HG67), several *B. gingivalis* strains (HG91, HG94, HG185, HG371, HG378, and HG379) and *B. capillosus* (HG99).

Complete inhibition was found for all the *B. asaccharolyticus* and two black-pigmented *Bacteroides* strains (HG181 and HG182) isolated from human dental root canals and not belonging to either *B. gingivalis* or *B. asaccharolyticus* (13). Strain HG370 showed DNA homology with HG181 and HG182 and belongs, therefore, to the same species (unpublished data). HG370, however, showed minimal growth at a concentration of 5.0 µg of vancomycin per ml.

At a concentration of 7.5 µg of vancomycin per ml only *B. loeschii* (HG64), the *B. intermedius*, the *B. melaninogenicus*, *B. corporis* (HG119), *B. oralis* (HG183), and *B. corrodens* (HG324) were resistant and grew without any inhibition. All other representatives were partly or completely inhibited at this concentration.

Shah et al. (8) reported major differences in susceptibility to vancomycin between saccharolytic and asaccharolytic *Bacteroides* strains at a concentration of 10.0 µg/ml. They also found variation within the *B. intermedius* and *B. melaninogenicus* groups.

Our results agree with a study of Sasaki and Takazoe (7), who found a strain of *B. gingivalis* (NA88) to be susceptible to vancomycin at a concentration of 7.5 µg/ml in blood-agar medi-

TABLE 1. Strains used, sources, and susceptibility to vancomycin

Biotype	Collection no.	Source	Site of isolation	Susceptibility to vancomycin ($\mu\text{g/ml}$) ^a		
				3.5	5.0	7.5
<i>B. loeschii</i>	HG64	H. N. Shah, ATCC 15930	Gingival crevice	++	++	++
<i>B. levii</i>	HG67	H. N. Shah, LEV. 1	Bovine rumen	++	±	-
<i>B. corporis</i>	HG119	H. N. Shah, 532-70A	Clinical specimen	++	++	++
<i>B. oralis</i>	HG183	L. V. Holdeman, D27B-24	Dental plaque	++	++	++
<i>B. capillosum</i>	HG99	S. S. Socransky	Gingival crevice	++	+	±
<i>B. ureolyticus</i>	HG324	Own isolate	Dental root canal	++	++	++
<i>B. macacae</i>	HG317	ATCC 33141	Monkey dental plaque	++	++	+
<i>B. disiens</i>	HG129	H. N. Shah, 7880	Clinical specimen	++	++	++
<i>B. asaccharolyticus</i>	HG71	H. N. Shah, NCTC 9337	Infected hemorrhoids	±	-	-
	HG79	H. N. Shah, VPI 4199	Human feces	±	-	-
	HG107	H. N. Shah, ATCC 25260	Empyema	±	-	-
	HG111	ATCC 27067	Human leg wound	±	-	-
	HG364	D. Mayrand, BM 4	Dental plaque	±	-	-
<i>B. gingivalis</i>	HG66	H. N. Shah, W 83	Clinical specimen	++	++	+
	HG76	H. N. Shah, W 50	Clinical specimen	++	++	+
	HG91	S. S. Socransky, 381	Oral specimen	++	+	±
	HG94	S. S. Socransky, 376	Oral specimen	++	+	-
	HG185	Own isolate	Oral specimen	++	+	±
	HG371	H. N. Shah, P 210	Dental plaque	++	+	±
	HG378	Own isolate	Gingival crevice	++	+	-
	HG379	Own isolate	Gingival crevice	++	+	-
<i>Bacteroides</i> sp.	HG181	Sundqvist, H11a-e	Dental root canal	±	-	-
	HG182	Sundqvist, BN11a-f	Dental root canal	±	-	-
	HG370	Own isolate	Dental root canal	+	±	-
<i>B. intermedius</i>	HG65	H. N. Shah, T588	Dental plaque	++	++	++
	HG110	ATCC 25611	Empyema	++	++	++
<i>B. melaninogenicus</i>	HG73	H. N. Shah, VPI 9343	Great toe	++	++	++
	HG118	H. N. Shah, N 334-71H	Oral specimen	++	++	++

^a Symbols: -, complete inhibition of growth; ±, growth of just a few colonies; +, growth with small colonies; and ++, no growth inhibition.

um. *B. intermedius* and *B. melaninogenicus* were partly inhibited and grew with small-colony morphology. Duerden (4) used vancomycin as a selective agent for the isolation of *Bacteroides* species from the gingival crevice at a concentration of 2.5 $\mu\text{g/ml}$. This concentration may be considered below the minimal inhibition concentration for many *Bacteroides* species. In a recent study on the antimicrobial susceptibility of various bacterial species from periodontal sites, Sutter et al. (11) found a few asaccharolytic black-pigmented *Bacteroides* strains to be susceptible to vancomycin at concentrations of 4.0 and 8.0 $\mu\text{g/ml}$.

It is known that patients with adult periodontitis can harbor high numbers of *B. gingivalis* which can comprise more than 40% of the cultivable subgingival plaque flora (6). Particularly *B. gingivalis* is often found at the most diseased sites (10, 12, 14) and this species is generally thought to be very important in the etiology of adult periodontitis.

However, Singletary et al. (9) recently used a selective medium for the isolation of *Bacteroides* species from subgingival plaque containing 7.5 μg of vancomycin per ml in a blood-agar

medium. The mean age of the patients in their experiments was 43.5. They found that the number of colonies on the *Bacteroides* selective medium dropped markedly after initial periodontal therapy.

Our results and the findings of other investigators concerning the susceptibility of *Bacteroides* spp. to vancomycin suggest that the results and conclusions of Singletary et al. (9) concerning colony counts should be rejected. They probably excluded *B. gingivalis* from their experiments and isolated only the black-pigmented *Bacteroides* resistant to vancomycin at a concentration of 7.5 $\mu\text{g/ml}$, i.e., mainly *B. intermedius* and *B. melaninogenicus*. In our opinion investigators concerned with oral bacteriology should be reluctant to use materials and methods from nonoral bacteriology studies until it is verified that the method is useful for oral microorganisms. Major differences may exist between oral and nonoral specimens; in fact, frequently species may be involved which are not closely related, despite similar generic names. Therefore, it does not make sense to us to speak generally of selective media for *Bacteroides* (2).

Even within nonoral bacteriology, one should

be careful in using selective media with vancomycin for the isolation of black-pigmented asaccharolytic *Bacteroides*. Our results clearly show the susceptibility of *B. asaccharolyticus* to vancomycin at a concentration much lower (3.5 µg/ml) than the concentration used in most recommended media described by Dowell and Lombard (7.5 µg/ml) (2).

Therefore, we recommend that for the isolation of black-pigmented asaccharolytic *Bacteroides*, one should not use vancomycin at all, not even at a concentration of 3.5 µg/ml.

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