

Susceptibility of Related Vibrios and *Vibrio fetus* to Twelve Antibiotics

J. P. BUTZLER, P. DEKEYSER, AND T. LAFONTAINE

Departments of Bacteriology, St. Pierre Hospital, Free University of Brussels, and the National Institute of Veterinary Research, Brussels, Belgium

Received for publication 20 August 1973

One hundred fourteen strains of related vibrio and seven strains of *Vibrio fetus*, all isolated from humans, were tested for susceptibility in vitro to 12 antibiotics. Mueller-Hinton agar containing 14 dilutions of the antibiotics was inoculated with undiluted overnight cultures. Gentamicin and erythromycin were the most active drugs against related vibrios and *Vibrio fetus*; lower activity was noted with chloramphenicol, streptomycin, and tetracycline; neomycin and kanamycin were even less active. The susceptibility to ampicillin was variable. More than 90% of the strains were resistant to cephalothin.

For technical reasons, related vibrio and *Vibrio fetus* infections in man have long failed to be recognized. Authors often describe (10, 17) the difficulties experienced in creating the necessary growth conditions, and this may explain the frequent failure of the organisms to grow in subculture. Furthermore, it may account for the fact that it is often impossible to carry out an antibiogram.

The purpose of this study was to determine the in vitro activity of 12 antibiotics against clinical isolates of these vibrios.

MATERIALS AND METHODS

Bacterial strains. One hundred fourteen clinical isolates of related vibrios (obtained in 1971, 1972, and 1973) and seven clinical isolates of *V. fetus* were used in the study. All of the related vibrios were isolated from stools (3, 5), except for two which were isolated from blood. The seven *V. fetus* strains were all isolated from blood (2, 20). Preservation of the vibrios was done by freeze-drying.

The frozen, dried matter was suspended in a basal medium (Albimi *Brucella* broth, Chas. Pfizer & Co., Inc.). This suspension was inoculated onto a petri dish containing fluid thioglycolate agar medium (Difco, no. 0256-01) to which were added 15% defibrinated ovine blood and, per milliliter, 25 IU of bacitracin (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.005 mg of novobiocin (The Upjohn Co., Kalamazoo, Mich.), and 0.05 mg of actidione (Upjohn Co.).

The inoculated plates were incubated for at least 3 days at 37 C in a prepared atmosphere in which two-thirds of the volume of air had been replaced by a mixture of 95% nitrogen and 5% carbon dioxide. Then the colonies were suspended in the Albimi *Brucella* broth and incubated for 24 h, again in the prepared atmosphere. The turbidity was adjusted to match a McFarland no. 3 standard.

Antibiotic susceptibility tests. A replica-inoculating apparatus (19) was used to apply the organisms to the surface of Mueller-Hinton plates containing two-fold dilutions of antibiotic. Broth cultures (24 h) were used as inoculum, and the inoculated plates were incubated for 48 h at 37 C in a prepared atmosphere in which two-thirds of the volume had been replaced by a mixture of 95% nitrogen and 5% carbon dioxide.

Antibiotics. The following antibiotics were tested: cephalothin (Lilly Laboratories, Indianapolis, Ind.), sodium penicillin and streptomycin (Specia Laboratories, Paris, France), sodium ampicillin and carbenicillin (Beecham Laboratory, Piscataway, N.J.), erythromycin (Abbott Laboratories, North Chicago, Ill.), tetracycline hydrochloride (Pfizer Inc., New York, N.Y.), chloramphenicol (Parke, Davis & Co., Detroit, Mich.), gentamicin (Schering-White Corp., Bloomfield, N.J.), neomycin sulfate (The Upjohn Co., Kalamazoo, Mich.), kanamycin sulfate (Bristol Laboratories, Syracuse, N.Y.), and colistine (Bellon Laboratories, Paris, France).

RESULTS

The minimal inhibitory concentrations (MICs) of the various antibiotics against the seven *V. fetus* strains are listed in Table 1.

The cumulative percentage of related vibrio strains inhibited by these antibiotics is shown in Fig. 1.

Gentamicin and erythromycin were the most effective. All the related vibrio strains were inhibited by gentamicin in concentrations of less than 3.12 $\mu\text{g/ml}$; with the exception of one strain, isolated from stools (MIC 100 $\mu\text{g/ml}$), these results also apply to erythromycin. Eighty-eight percent of the strains were inhibited by concentrations of chloramphenicol and streptomycin of less than 1.56 $\mu\text{g/ml}$, whereas 79% of

TABLE 1. Antimicrobial activity of 12 antibiotics^a

Antibiotic	<i>V. fetus</i> strains						
	I	II	III	IV	V	VI	VII
Gentamicin	0.78	1.56	1.56	1.56	0.78	0.097	0.196
Erythromycin	0.39	0.048	0.39	1.56	0.78	0.078	0.39
Chloramphenicol	3.12	0.78	0.39	3.12	0.78	1.56	0.78
Streptomycin	1.56	0.196	1.56	3.12	0.196	1.56	0.196
Tetracycline	0.012	1.56	1.56	3.12	3.12	1.56	0.39
Neomycin	3.12	6.25	6.25	1.56	1.25	3.12	3.12
Kanamycin	0.012	3.12	1.56	6.25	6.25	3.12	3.12
Ampicillin	1.56	1.56	1.56	25	12.5	6.25	6.25
Penicillin	1.25	12.5	12.5	100	50	12.5	25
Colistin	100	100	100	100	50	100	100
Carbenicillin	125	250	62.5	250	250	125	125
Cephalothin	12.5	100	100	100	50	100	100

^a Values represent minimal inhibitory concentration ($\mu\text{g/ml}$).

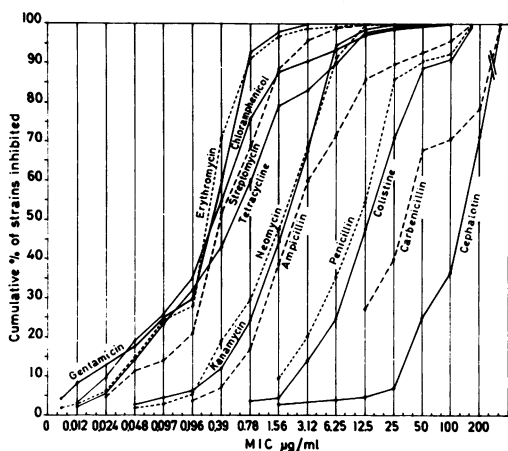


FIG. 1. Cumulative percentage of related vibrio strains inhibited by 12 antibiotics.

the strains were susceptible to this concentration of tetracycline. Related vibrios were also susceptible to low concentrations of neomycin and kanamycin. Twenty-five percent of the strains were resistant to 6.25 μg of ampicillin per ml. The MICs of penicillin, colistin, and carbenicillin were very high. Ninety-five percent of the strains were resistant to cephalothin.

DISCUSSION

For technical reasons, vibriosis in man failed to be recognized for a long time. Inadequacy of bacteriological techniques and the nutritional requirements of the causative organism made it an illness which was rarely detected. It is our opinion that *V. fetus* infections should be differentiated from related vibrio infections.

V. fetus infections in man can be found in both males and females at any age and in any profession. It appears, however, to affect primarily debilitated adults in the 40 to 70 year age group (four males to one female), infants, and pregnant women. There is no typical syndrome, but septicemic forms without septic metastases and associated with splenomegaly are seen frequently (21). Purulent meningitis and meningococcal meningitis (1, 6, 15), abortion (1, 6, 22), endocarditis (11) and pericarditis (9), cholera-like syndromes (12, 14, 23), thrombophlebitis (8, 17, 24), septic arthritis (10, 13), and jaundice with hepatomegaly (11) have been described. Isolation of the infecting organism from blood cultures has been possible in nearly all cases.

Various antibiotics, alone or in association, have been used in the treatment of *V. fetus* infections. Treatment with penicillin alone has frequently been unsuccessful (4, 18, 22), but chloramphenicol (13), chlortetracycline (19), and tetracycline (4, 14) have produced good results, and combinations of penicillin and streptomycin (16), penicillin and chlortetracycline (13), penicillin and oxytetracycline, and dihydrostreptomycin and tetracycline have been curative. Combinations of penicillin or streptomycin with a broad spectrum antibiotic have also been used with good results.

Strains tested *in vitro* by the disk-plate method have been found to be resistant to nalidixic acid, oxacillin, novobiocin, bacitracin and, frequently also, to penicillin. They were always susceptible to erythromycin, chloramphenicol, tetracycline and streptomycin.

Over a period of 5 years, we have observed seven patients with *V. fetus* septicemia. Six of the seven patients were debilitated. Four of

them were treated successfully with tetracycline (250 mg three times daily intravenously [i.v.]). One patient recovered rapidly on ampicillin (8 g i.v. per day). One other patient, who was not debilitated, was hospitalized with septic shock and cholecystitis; upon administration of gentamicin (80 mg three times per day i.v.), symptoms disappeared within 48 h. Finally, one patient, who was cirrhotic, developed a *V. fetus* septicemia and died during treatment with cephalothin (12 g i.v.).

The seven strains were susceptible to gentamicin, erythromycin, chloramphenicol, streptomycin, tetracycline, neomycin and kanamycin. Three of the seven were susceptible to ampicillin. All were resistant to penicillin, colistin, carbenicillin, and cephalothin.

Related vibrio infections in man have generally been found in young children and infants. Up to now, 58 cases have been reported. The bacteriological diagnosis of the 13 cases first described was made by blood culture. In other instances, the organism was isolated from feces. Enteritis due to strains of related vibrio has been treated successfully with various antibiotics such as neomycin, tetracycline, penicillin, and chloramphenicol given orally.

During the past three years, with a new filtration technique (3, 5), we have isolated related vibrios in 5% of the cases of enteritis in infants and children. These infections have been successfully treated with tetracycline (50 mg per kg per day), erythromycin (50 mg per kg per day), furazolidon (5 mg per kg per day), and neomycin (50 mg per kg per day) administered orally. There were a few failures with oral ampicillin (50 mg per kg per day). Finally, in several instances treatment with colistine (150,000 U per kg per day) was unsuccessful.

Three patients with related vibrio septicemia were treated successfully, one with 2 g of chloramphenicol per day orally, another with erythromycin orally (50 mg per kg per day), and the third with ampicillin (100 mg per kg per day orally).

By using the disk-plate method, we have tested susceptibility of related vibrios and *V. fetus* to lincomycin, vancomycin, rifampin, oxacillin, and furazolidon. All vibrios were susceptible to furazolidon and resistant to the other four antibiotics.

Plastridge et al. have tested the susceptibility of 149 strains to 10 antibiotics; 31 of the strains were of human origin. The 28 tested strains of *V. fetus* appeared to be more resistant to novobiocin and tetracycline than were the bovine and ovine strains. The three related vibrio strains

were more resistant to penicillin than the others.

It is our opinion that no major differences exist between related vibrio strains and *V. fetus* in their susceptibility to antibiotics. In patients with related vibrio and *V. fetus* septicemia, gentamicin is the treatment of choice. In cases of enteritis, we have had excellent results with furazolidon, erythromycin, and tetracycline.

LITERATURE CITED

- Burgert, W., Jr., and K. W. C. Hagstrom. 1964. *Vibrio fetus* meningoencephalitis. *Arch. Neurol.* **10**:196-199.
- Butzler, J. P., H. Bleiberg, P. Dekeyser, M. Detrain, L. Van Cauwenberghe, and P. Petit. 1972. Un cas mortel de septicémie à *Campylobacter fetus* intestinalis. *Med. Mal. Infect.* **4**:157-160.
- Butzler, J. P., P. Dekeyser, M. Detrain, and F. De Haen. 1973. Related vibrio in stools. *J. Pediat.* **82**:493-495.
- Collins, H. S., A. Blevins, and E. Benter. 1964. Protracted bacteremia and meningitis due to *Vibrio fetus*. A case report. *Arch. Intern. Med.* **113**:361-364.
- Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon. 1972. Acute enteritis due to related vibrio: first positive stool cultures. *J. Infect. Dis.* **125**:390-392.
- Eden, A. N. 1966. Perinatal mortality caused by *Vibrio fetus*. *J. Pediat.* **68**:297-304.
- Jackson, J. F., P. Hinton, F. Allison, Jr. 1960. Human vibriosis. *Amer. J. Med.* **28**:986-989.
- Kahler, R. L., and J. Sheldon. 1960. *Vibrio fetus* infection in man. *N. Engl. J. Med.* **262**:1218-1222.
- Killam, H. A. W., J. G. Crowder, A. C. White, and J. H. Edmonds. 1966. Pericarditis due to *Vibrio fetus*. *Amer. J. Cardiol.* **17**:723-728.
- Kilo, C., P. O. Hageman, and J. Marzi. 1965. Septic arthritis and bacteremia due to *Vibrio fetus*. *Amer. J. Med.* **38**:962-971.
- King, E. O. 1957. Human infections with *Vibrio fetus* and a closely related vibrio. *J. Infect. Dis.* **101**:109-128.
- King, E. O. 1962. The laboratory recognition of *Vibrio fetus* and vibriosis. *Ann. N.Y. Acad. Sci.* **98**:700-711.
- King, S., and D. Bronsky. 1961. *Vibrio fetus* isolated from a patient with localised septic arthritis. *J. Amer. Med. Ass.* **175**:1045-1048.
- Mandel, A. D., and R. C. Ellison. 1963. Acute dysentery syndrome caused by *Vibrio fetus*. Report of a case. *J. Amer. Med. Ass.* **185**:536-538.
- Reyman, T. A., and B. Silberberg. 1969. *Vibrio fetus* septicemia. *Amer. J. Clin. Pathol.* **51**:578-583.
- Robin, L. A., G. Duprey, J. F. Juannot, P. Paris, H. Magard, J. Mignard, and P. Berteau. 1962. A propos de trois cas de vibriose humaine (*Vibrio fetus*) dont une méningite. *Presse Méd.* **70**:321-323.
- Ruben, F. L., and E. Wolinsky. 1968. Human infection with *Vibrio fetus*. *Antimicrob. Ag. Chemother.* **1967**, p. 143-149.
- Spink, W. W. 1957. Human vibriosis caused by *Vibrio fetus*. *J. Amer. Med. Ass.* **164**:180-182.
- Steers, E., E. L. Foltz, and B. S. Graves. 1959. Inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother.* **9**:307-311.
- Sternon, J., J. P. Butzler, M. Detrain, and P. Dekeyser. 1971. Enterites aiguës et septicémies à *Vibrio fetus*. *Acta Gastro-enterolog. Belg.* **34**:550-561.
- Stille, W., and E. B. Helm. 1969. Sepsis und Meningitis

- durch *Vibrio fetus*. Deut. Med. Wochensch. **48**:2484-2488.
22. Vinzent, R., J. Dumas, and N. Picard. 1947. Septicémie grave au cours de la grossesse due à un *Vibrio* avortement consécutif. Bull. Acad. Nat. Méd. Paris **131**:90-92.
23. Wheeler, W. E., and J. Borchers. 1961. Vibrionic enteritis in infants. Amer. J. Dis. Child. **101**:60-66.
24. White, W. D. 1967. Human vibriosis: indigenous cases in England. Brit. Med. J. **2**:283-287.