IN-VITRO SENSITIVITY OF *FLAVOBACTERIUM MENINGO-SEPTICUM* TO ANTIMICROBIAL AGENTS

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FLAVOBACTERIUM MENINGOSEPTICUM was first recognised and described by King in 1959. This Gram-negative rod may cause severe meningitis in premature and newborn infants, with a very high fatality rate and serious sequelae in the surviving patients. The infection in babies usually defies all attempts at antibiotic treatment. In adults, on the other hand, the illness is a mild one, without meningitis and with rapid recovery, as shown by Olsen, Frederiksen and Siboni (1965), in a series of patients suffering from post-operative bacteraemia. The organism possesses outstanding resistance to antimicrobial agents and shows an unusual sensitivity pattern for a Gram-negative bacterium. This report deals with the in-vitro sensitivity of 11 strains to 17 antibiotics, nalidixic acid, sulphamethoxazole and trimethoprim.

MATERIAL AND METHODS

Eleven strains of *Flavobacterium meningosepticum* were tested for sensitivity. Six of them were King's serological type-strains A-F kindly supplied by Dr I. Sechter, Central Laboratories, Jerusalem, and five were isolates from CSF of infants with meningitis. Two belonged to serotype C and three to serotype E. All showed typical cultural and biochemical reactions (King).

Minimum inhibitory concentrations (MICs) of various antibiotics and nalidixic acid were determined by the tube dilution method. The medium used was Brain Heart Infusion (pH 7.2) and the inoculum contained 5×10^5 organisms. Results were read after 18 hr at 37°C. In order to estimate minimum bactericidal concentrations, 0.1 ml from all tubes without visible growth was transferred to 10 ml Brain Heart Infusion and to tryptose agar plates for the determination of the bactericidal effect. Results were recorded after 48 hr at 37°C. MICs of sulphamethoxazole and trimethoprim, alone and in combination, were determined by the plate dilution technique, using Wellcome nutrient agar which contains insignificant amounts of end-products of folate metabolism (Bushby, 1969). The inoculum was 150–200 organisms. All strains were also tested for β -lactamase production by iodo-metric assay (Perret, 1954).

RESULTS

All the strains were resistant to benzyl penicillin, methicillin, ampicillin, carbenicillin and cephaloridine, the MICs of which ranged from 50 to more than 100 μg per ml, and all produced penicillinase. All strains were resistant ($\text{MIC} > 100 \mu\text{g}$ per ml) to dihydrostreptomycin, kanamycin, gentamicin, sulphomethyl-colistin and lincomycin (table I). MICs of tetracycline, chloramphenicol, vancomycin, pristinamycin and nalidixic acid ranged from 25 to 50 μg per ml (table I). On the other hand, the organisms were found to be sensitive to erythromycin (as lactobionate), rifampicin and novobiocin. MICs of rifampicin were from 0.6 to 1.25 μg per ml and of erythromycin from 2.5 to 10 μg per ml. Minimum bactericidal concentrations ranged from 1.25 to more than 20 μg per ml for rifampicin and from 10 to more than 20 μg per ml for erythromycin (table II). Even with strains showing survival at 20 μg per ml, however, 99 per cent. of the original inoculum was killed by concentrations of 2.5–5 μg erythromycin per ml and 2.5 μg rifampicin per ml. In the case of novobiocin the MICs were between 2.5 and 10 μg per ml, with minimum bactericidal

Received 24 June 1970; accepted 25 Sept. 1970.

TABLE I

Minimum inhibitory concentration ($\mu\text{g per ml}$) of 14 antibiotics and nalidixic acid for 11 strains of *Flavobacterium meningosepticum*

Antibiotic	Number of strains inhibited by antibiotic concentration ($\mu\text{g per ml}$) of			
	25	50	100	>100
Penicillin*	0	3	8†	0
Ampicillin	0	0	6	5
Methicillin	0	0	0	11
Carbenicillin	0	0	0	11
Cephalexidine	0	0	4	7
Dihydrostreptomycin	0	0	0	11
Kanamycin	0	0	0	11
Gentamicin	0	0	4	7
Lincomycin	0	0	0	11
Sulphomethyl-colistin*	0	0	0	11
Tetracycline	2	9	...	...
Chloramphenicol	1	10	...	...
Vancomycin	8	3	...	...
Pristinamycin	9	2	...	...
Nalidixic acid	9	2	...	...

* For penicillin and sulphomethyl-colistin, concentrations are given in IU per ml.

† Number of strains given as sensitive at a stated antibiotic concentration are those not sensitive at the next lower level. ... = Test not done.

TABLE II

Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) in $\mu\text{g per ml}$ of 3 antibiotics for 11 strains of *Flavobacterium meningosepticum*

Antibiotic	Estimation	Number of strains inhibited or killed by antibiotic concentration ($\mu\text{g per ml}$) of						
		0.6	1.25	2.5	5	10	20	>20
Erythromycin	MIC	0	0	3	7*	1	0	0
	MBC	0	0	0	0	5	3	3
Rifampicin	MIC	7	4	0	0	0	0	0
	MBC	0	1	3	3	0	0	4
Novobiocin	MIC	0	0	3	6	2	0	0
	MBC	0	0	0	0	0	1	10

* Number of strains given as sensitive at a stated antibiotic concentration are those not sensitive at the next lower level.

concentrations higher than 40 μg per ml (except for one strain), although 40 μg per ml killed 99 per cent. of the original inoculum of all strains.

Nine strains were inhibited by 20 μg sulphamethoxazole per ml and two by 40 μg per ml. Ten were inhibited by 10 μg trimethoprim per ml and one by 20 μg per ml. A combination of these two agents (1 part trimethoprim and 10 parts sulphamethoxazole) proved to be more bacteriostatic than either alone and inhibited growth of all strains at 5 μg per ml.

DISCUSSION

No antibiotic has so far been found to be really effective in the treatment of neonatal meningitis due to *Flavobacterium meningosepticum*. King in 1959 and later George, Cochran and Wheeler (1961), Sugathadasa and Arseculeratne (1963), Vandepitte, Eeckels and Seynhaeve (1965) and Watson, Krogh and Jones (1966) tested the sensitivity of this bacterium by the disk-diffusion method. Most of these investigators found sensitivity to erythromycin and resistance to penicillin and to the polymyxins. Varying results were obtained with streptomycin, tetracycline and chloramphenicol. George *et al.* reported also sensitivity to novobiocin, oleandomycin, vancomycin and ristocetin. Olsen (1967*a*) who used the 50 per cent. inhibitory concentration method found sensitivity to erythromycin, novobiocin, fucidin and to a certain degree to penicillin. Olsen (1967*b*) also noted an increased effect of these agents (except penicillin) at temperatures above 37°C.

All strains we tested by the tube dilution method of estimating minimum inhibitory concentrations at 37°C were highly resistant to the penicillins, cephaloridine, the aminoglycosides, colistin and lincomycin, and resistant to tetracycline, chloramphenicol, vancomycin, pristinamycin and nalidixic acid. The pronounced resistance of a Gram-negative rod to colistin and to gentamicin seems especially noteworthy. The organism was sensitive to erythromycin, rifampicin and novobiocin. All three agents also showed bactericidal activity, although in concentrations that are attainable in the cerebrospinal fluid only by intrathecal injection. Rifampicin was slightly more efficient than either novobiocin or erythromycin. The latter has in fact been used in the treatment of this infection, though with little effect (George *et al.*). Relative sensitivity was found to trimethoprim and to a combination of trimethoprim and sulphamethoxazole. Although sulphonamides are generally contra-indicated in the first weeks of life, Morzaria, Walton and Pickering (1969) reported successful treatment of neonatal meningitis with this combination, after other drugs had failed.

Advantage may be taken of the outstanding resistance to antibiotics of *Flavo. meningosepticum* in designing selective media. Media containing gentamicin alone or in combination with other antibiotics may be very useful for the isolation of the organism from mixed bacterial populations in water, stools, and throat swabs, or from instruments and general hospital surroundings during a search for the source of hospital infections due to this organism.

SUMMARY

The in-vitro sensitivity of 11 strains of *Flavobacterium meningosepticum* to 20 antimicrobial agents was studied. They were resistant to 14 antibiotics at present in clinical use, as well as to nalidixic acid. On the other hand, they were sensitive to erythromycin, novobiocin and rifampicin. Sensitivity was also found to sulphamethoxazole and trimethoprim and to a combination of these two drugs, which acted in synergy.

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