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Flavobacterium odoratum: a species resistant to a wide range of antimicrobial agents

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SUMMARY During the period 1966-77, 24 strains of *Flavobacterium odoratum* were identified from among strains of Gram-negative, non-fermentative bacteria submitted to the National Collection of Type Cultures for computer-assisted identification. The *F. odoratum* strains showed resistance to therapeutic levels of gentamicin, tobramycin, amikacin, and carbenicillin as well as to several other antimicrobial agents generally useful in the treatment of infections caused by Gram-negative, non-fermentative bacteria. Two strains isolated from amputation stumps and another three strains isolated in significant numbers from urine specimens were possibly opportunist pathogens. The biochemical characteristics of the 24 strains, the proposed neotype strain of *F. odoratum*, and three strains representative of a group, referred to at the Center for Disease Control, Atlanta as group M-4f, were compared with those of biochemically similar species which may be isolated from clinical material.

The earliest recorded isolations of the species now known as *Flavobacterium odoratum* were made from the human intestine by Stutzer (1923) and Stutzer and Kwaschnina (1929). Although the strains could not be isolated from normal faeces but only from the faeces of patients suffering from typhoid fever, acute gastroenteritis, and relapsing fever, there was no suggestion that the strains were playing a pathogenic role, and animal pathogenicity tests proved negative. Holmes *et al.* (1977) proposed a neotype strain for *F. odoratum* (NCTC 11036) and provided a revised description of the species based upon an examination of the neotype and nine field strains which had been isolated from clinical material; they could find no reports of clinical isolations of the species since it was first described. The source of the isolates and the results of biochemical and antimicrobial susceptibility tests obtained by Holmes *et al.* (1977) are, for convenience, repeated in the present paper.

The recent isolation of a further 15 strains of *F. odoratum*, 10 in the UK, suggests that the organism may be widely distributed. The present account reports the results of biochemical tests and antimicrobial susceptibility tests of 28 strains of *F. odoratum* in order that the species may be more easily recognised in the clinical laboratory.

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Material and methods

STRAINS

Twenty-eight strains of *F. odoratum* were examined. They comprised 24 field strains referred to the National Collection of Type Cultures for computer-assisted identification, the neotype strain maintained in the National Collection of Type Cultures (NCTC), Colindale (NCTC 11036), and three strains of group M-4f (Tatum *et al.*, 1974), which were found to be identical with *F. odoratum* by Holmes *et al.* (1977). Clinical details of the field strains are summarised in Table 1; fuller details on strains 1 to 9 are given by Holmes *et al.* (1977).

BACTERIOLOGICAL STUDIES

The majority of tests used (Table 2) were incubated at 37°C, and the methods employed were those used by Holmes *et al.* (1975). Susceptibility to antimicrobial agents was tested as described by Holmes *et al.* (1977).

Results

The 28 strains were Gram-negative rods and, with the exception of strain 21, all produced, on nutrient agar, bright yellow colonies, which gave off a pleasant, strong, fruity, characteristic odour. After incubation

Table 1 *Strains of* F. odoratum *examined*

Strain	*Source*	*Patient*		*Clinical condition*	*Comment*
		Age	*Sex*		
1	Urine	48	F	Cystitis	Mixed culture
2	Infected cut	34	M	Not known	No further details
3	Urine	59	F	Not known	Very scanty numbers
4	Urine	?	F	Urinary retention	Probable contaminant
5	Urine	?	?	Not known	No further details
6	Wound swab	?	?	Varicose ulcer	No further details
7	Ulcer	76	F	Thigh ulcer for 14 months following gnat bite	No further details
8	Urine	67	F	Total cystectomy, ileal loop urethrotomy	Mixed culture
9	Urine	48	M	Renal insufficiency, pyelonephritis, renal calculi	Mixed culture
10	Ear	70	M	Not known	Large numbers, pure culture
11*	Urine	54	M	Not known	Very scanty numbers
12*	Urine	87	M	Not known	Mixed culture
13*	Blood	43	M	On dialysis; pyrexia of unknown origin	Probable contaminant
14*	Urine	46	M	Not known	Mixed culture
15*	Urine	59	M	Not known	Mixed culture
16*	Urine	?	F	Not known	Scanty numbers
17	Sloughing amputation stump	58	F	Ischaemic lower limb disease	Predominant organism of mixed culture, isolated more than once
18	Urine	67	M	Multideficiency syndrome, pyelonephritis, liver disease	Mixed culture
19	Gangrenous feet	52	M	Frostbite, gangrene, cellulitis	Heavy pure culture obtained after some antibiotic treatment
20	Urine and ureter stoma	38	M	Relapsing urinary infections, bladder carcinoma	Heavy growth, sometimes pure, sometimes mixed
21	Urine	?	?	Not known	No further details
22	Urine	?	M	Syringomyelia, indwelling catheter	Heavy growth, frequent infections with different organisms
23	Urine	?	F	Relapsing urinary infection, renal insufficiency, hypertension	Heavy growth isolated on three occasions with lesser numbers of *Streptococcus faecalis*
24	Urine	19	M	Spina bifida, permanent catheter	Repeatedly isolated after a course of carfecillin and tobramycin, which cleared a *Pseudomonas aeruginosa* infection
25†	Wound swab (toe)	?	?	Not known	Group M-4f, strain B7249
26†	Urine	?	?	Not known	Group M-4f, strain B7942
27†	Sputum	?	?	Not known	Group M-4f, strain B9856
28	Unknown	?	?	Not known	Proposed neotype, NCTC 11036

*Strains 11-16 were isolated in the same hospital over a period of more than two years, at intervals of at least two months.
†Strains 25-27 are isolates of group M-4f (Tatum *et al.*, 1974) sent to us by R. E. Weaver, Atlanta, Georgia, USA.

for 24 hours on nutrient agar four colonial types were observed among the strains. Strains 4, 5, 6, 7, 8, 15, 21, and 22 produced effuse, spreading colonies of 3 to 4 mm in diameter with raised, shiny centres and dull, matt, spreading edges. On further incubation the whole colony became smooth and shiny (colonial type 1 of Holmes *et al.* (1977)). Colonies of strains 1, 3, 11, 12, 13, 18, and 28 (NCTC 11036) showed the same appearance after 24 hours as those of type 1, but they were smaller, about 1·0 to 1·5 mm in diameter (colonial type 2 of Holmes *et al.* (1977)). Colonies of strains 2, 9, 10, 14, 16, 17, 19, 23, 24, 25, 26, and 27 were smooth, shiny, and convex with no spreading edge and with a diameter of 0·5 to 1·0 mm after 24 hours; however, after further incubation, the colonies took the same appearance as those of type 1 (colonial type 3 of Holmes *et al.* (1977)).The colonies of strain 20 were mucoid and thus constituted a fourth colonial type not observed by Holmes *et al.* (1977) in their series.

The biochemical test results for the strains are given in Table 2 and are arranged by the tests in which all strains gave a positive or negative result and tests in which the strains differed. The strains were very homogeneous with respect to their biochemical characteristics, showing differences between the strains in only eight of the 65 tests carried out. Despite giving negative results for most of the biochemical characters tested (Table 2), the strains had several distinguishing characteristics by which they may be recognised. All but one of the isolates produced a bright yellow pigment on nutrient agar and gave off a characteristic fruity odour. All of the isolates were non-motile and non-saccharolytic, and produced an alkaline reaction in glucose Hugh and Leifson O-F medium. All of the isolates also digested casein and produced both extracellular deoxyribonuclease and gelatinase. Urease was produced, and a particular characteristic of the strains was their ability to reduce nitrite but not nitrate.

Results of susceptibility tests to antimicrobial

Table 2 *Biochemical characters of* F. odoratum

All strains positive in:

Alkaline reaction in O-F test	Cytochrome-oxidase production	Growth at 37°C	Nitrite reduction
Casein digestion	Deoxyribonuclease production	Growth at room temperature†	Tween 20 hydrolysis
Catalase production	Gelatinase production*	Growth on MacConkey's agar	Urease production

All strains negative in:

Acid from following ammonium salt sugars:			
Adonitol	Raffinose	Arginine desimidase	Motility§
Arabinose	Rhamnose	Arginine dihydrolase	Nitrate reduction
Cellobiose	Salicin	Fluorescence on King's medium B	Opalescence on lecithovitellin agar
Dulcitol	Sorbitol	Gas from PWS glucose	Ornithine decarboxylase
Ethanol	Sucrose	Gluconate oxidation	Phenylalanine deamination
Fructose	Trehalose	Growth at 42°C	Poly-β-hydroxybutyrate inclusion granules
Glucose	Xylose	Growth on cetrimide	Reduction of selenite 0·4 g/100 ml
Glycerol	Acid from PWS glucose‡	Growth on Simmons' citrate	Starch hydrolysis
Inositol	Acid from glucose 10 g/100 ml	Hydrogen-sulphide production‖	β-Galactosidase production (ONPG)
Lactose	Acid from lactose 10 g/100 ml	Indole production	3-Ketolactose production
Maltose	Aesculin hydrolysis	Lysine decarboxylase	
Mannitol	Alkali production on Christensen's citrate	Malonate utilisation	

Strains differ in:	*Positive*	*Negative*	*Strains giving the less common result*
Gelatin stab liquefaction	27	1	7
Growth on β-hydroxybutyrate	27	1	17
Production of yellow pigment	27	1	21
Tween 80 hydrolysis	25	3	1, 9 and NCTC 11036
Tyrosine hydrolysis	13	15	1, 2, 3, 4, 5, 7, 8, 9, 12, 16, 17, 26 and NCTC 11036
Pigment production on tyrosine agar	3	25	4, 6, 8
Growth at 5°C	1	27	22
KCN tolerance	1	27	24

*By plate method.
†18-22°C.
‡PWS = peptone water sugar.
‖By both lead acetate paper and triple sugar iron agar methods.
§At both 37°C and room temperature.

agents are given in Table 3. The strains were fully resistant to clinically obtainable levels of streptomycin, kanamycin, gentamicin, tobramycin, amikacin, ampicillin, carbenicillin, chloramphenicol, tetracycline, polymyxin B, and erythromycin; and resistant or moderately sensitive to sulphamethoxazole, co-trimoxazole, cephaloridine, and nalidixic acid.

Table 3 *Susceptibility of* F. odoratum *to antimicrobial agents**

Antimicrobial agent	*MIC (μg/ml)*									
	<1	*1*	*2*	*4*	*8*	*16*	*32*	*64*	*128*	*>128*
Sulphamethoxazole			1	2	2		2		4	15
Co-trimoxazole				4	1	2	3	4	10	2
Streptomycin								1	2	23
Kanamycin										26
Gentamicin								1	1	24
Tobramycin										17
Amikacin								1	1	15
Ampicillin				1		6	7	3	5	4
Carbenicillin							2	2	12	10
Cephaloridine		1	1	4	7	9	3		1	
Erythromycin	2	9	10	2				2		1
Chloramphenicol				2	4	8	10		1	1
Tetracycline				1		1	3	14	7	
Polymyxin B								1		25
Nalidixic acid				8	5	12	1			

*26 strains tested except for tobramycin and amikacin, for which 17 strains were tested.

Discussion

Three organisms occasionally isolated from clinical material may be confused with *F. odoratum: Alcaligenes odorans, Flavobacterium breve*, and the group IIf of Tatum *et al.* (1974). Like *F. odoratum* both *A. odorans* and group IIf produce an alkaline reaction in glucose Hugh and Leifson O-F medium and are non-saccharolytic in our ammonium salt sugar medium (except for acid production from ethanol by *A. odorans*). Strains of *A. odorans* also produce a fruity odour and reduce nitrite but not nitrate. Strains of group IIf, which show affinities to both the genus *Flavobacterium* and the genus *Moraxella* (Owen and Snell, 1973), differ from *F. odoratum* in producing indole (however, a sensitive method, extraction with xylene followed by the addition of Ehrlich's reagent, is necessary to demonstrate the

indole production). Holmes *et al.* (1978) have proposed a neotype strain for *F. breve* (NCTC 11099) and have provided a revised description of the species based upon an examination of six clinical isolates (two from urine specimens) and a strain maintained in a culture collection (ATCC 14234). Like *F. odoratum*, *F. breve* is yellow-pigmented and is also resistant to therapeutic levels of antimicrobial agents such as gentamicin and carbenicillin, which are generally useful in the treatment of infections caused by Gram-negative, non-fermentative bacteria. However, when incubated at 30°C, unlike *F. odoratum*, strains of *F. breve* generally produce acid from glucose and maltose in ammonium salt sugar medium. Strains of *F. breve* further differ from *F. odoratum* in failing to reduce nitrite or to produce urease and in producing indole (using Ehrlich's reagent). Characters by which *F. odoratum* may be distinguished from *A. odorans*, *F. breve*, and group IIf are given in Table 4.

While there is no definite evidence that any of our strains were acting as pathogens it is possible that five were doing so. Strain 17, although isolated in mixed culture from an amputation stump, was the predominant organism and was isolated on more than one occasion. Strain 19 was the only organism isolated, and as a heavy growth, from gangrenous feet. Strains 20, 22, and 23, like many of our isolates, came from urine specimens, but all three of these strains were isolated in significant numbers (> 100 000 bacterial cells/ml). Stutzer (1923) considered that his strains of *Bacterium faecale aromaticum* (as *F. odoratum* was then called) played only a saprophytic role in the human intestine because he could not demonstrate pathogenicity of his strains in experimental animals. Intradermal, intraperitoneal, and intravenous injections of cultures of *B. faecale aromaticum* into mice, guinea-pigs, and rabbits produced no demonstrable response (Stutzer, 1923). Stutzer and Kwaschnina (1929) could not recover *F. odoratum* from normal faeces, and they isolated strains of this species only from the faeces of patients with typhoid fever, acute gastroenteritis, and relapsing fever. They did not suggest that it followed that *F. odoratum* was playing a pathogenic role in these patients but they did consider that isolation of this species was an indication of pathological processes in the intestine. It is possible, from the clinical circumstances in which our strains were isolated, that *F. odoratum* may be a low-grade opportunist pathogen. The resistance of *F. odoratum* to a wide range of antimicrobial agents (including gentamicin, tobramycin, amikacin, and carbenicillin) to which Gram-negative, non-fermentative bacteria might be expected to be susceptible suggests that any infections due to this species would prove difficult to treat, especially in the case of systemic infections. Drasar *et al.* (1976) reported minimum inhibitory concentrations (MICs) for seven *Flavobacterium* strains they examined. They did not, unfortunately, identify their strains to species level nor report their biochemical characteristics but,

Table 4 *Characteristics for differentiation of* F. odoratum *and phenetically similar bacteria**

Test	Flavobacterium odoratum	Alcaligenes odorans	Flavobacterium breve	*Group IIf*
Acid from ASS† glucose	−	−	6/7‡	−
Acid from ASS ethanol	−	+	−	−
Acid from ASS maltose	−	−	6/7	−
Alkali production on Christensen's citrate	−	+	−	−
Alkali production in glucose Hugh and Leifson O-F medium	+	+	−‖	+
Casein digestion	+	−	+	+
Deoxyribonuclease production	+	−	6/7	11/16
Gelatinase production	+	−	+	+
Growth at 42°C	−	+	−	+
Growth on cetrimide	−	+	−	−
Growth on Simmons' citrate	−	+	−	−
Motility	−	+	−	−
Nitrite reduction	+	+	−	−
Production of yellow pigment	27/28	−	+	−
Tween 20 hydrolysis	+	−	+	+
Tween 80 hydrolysis	25/28	−	3/7	6/16
Tyrosine hydrolysis	13/28	+	−	−
Urease production	+	−	−	−

*Symbols: +, all strains tested positive; −, all strains tested negative.
The phenotypic results for *F. odoratum* were derived from this study, those for *F. breve* were derived from Holmes *et al.* (1978), while those for *A. odorans* and group IIf are from NCTC unpublished data.
†ASS, ammonium salt sugar medium. *F. breve* produces acid only at 30°C and may require incubation for more than five days before a positive result is obtained.
‡Number of strains showing characters/number of strains tested.
‖Of seven strains examined, three gave an oxidative reaction, and in four no change in the medium was observed.

like our strains, their seven had MICs for gentamicin of $\geqslant$ 128 μg/ml, for tobramycin of $>$ 256 μg/ml, and for amikacin of $\geqslant$ 64 μg/ml. They further stated that 'the problem of treating infections caused by gentamicin-resistant *Flavobacterium* spp . . . remains unsolved'.

We are grateful to all those who sent us strains of *F. odoratum* for identification, especially those who also kindly provided additional clinical details relating to the isolation of their strains. In particular, we thank the following: G. Fenwick for sending us the series of strains 11 to 16; A. A. B. Mitchell for supplying extensive clinical information relating to the isolation of strain 19; R. E. Weaver for supplying us with a culture of strain 22 and three strains of group M-4f, and for putting us in contact with J. Vandepitte; and J. Vandepitte for supplying us with cultures of strains 20, 21, and 23 and for passing on our requests for further clinical details to those who originally isolated strains 20, 21, 22, and 23.

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