A bacteriological study of non-chromogenic variants of *Serratia marcescens* from human sources

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SYNOPSIS The culture and biochemical features of 10 non-chromogenic variants of *S. marcescens* isolated from clinical sources are reported. Autoagglutination titres were significantly high in sera from those patients in whom *Serratia* bacilli appeared to contribute to the illness. Since the taxonomic stature of the *Serratia* bacilli is not clear it might be better to limit the identification of this group to *Serratia* species.

Serratia bacilli are usually considered saprophytes whose natural habitat is soil, water, sewage, and food. Recently, these organisms have been described as opportunist pathogens which under certain conditions of increased susceptibility of the host can cause disease, and on rare occasions death (Wheat, Zuckerman, and Rantz, 1951; Patterson, Banister, and Knight, 1952; Haiby, McFarland, and Moore, 1961). *Serratia* strains have been invariably recognized by their pigment production, and have been classified by the colour of the pigment produced and by their growth and biochemical characteristics (Hefferan, 1903-04; Breed, Murray, and Smith, 1957; Topley and Wilson, 1955). Although the existence of non-chromogenic strains has long been known, reports of isolations from human infections are sparse in the literature. This may be due, in part, to improper identification of these organisms.

This paper reports an investigation of the cultural and biochemical characteristics of 10 non-pigmented variants of *Serratia marcescens* which we have found in clinical material from human sources.

MATERIAL AND METHODS

The source of the *Serratia* cultures investigated is given in Table I. They were isolated during a six-month period from January to August 1964. Nine strains were from adults and one strain was recovered from the blood of an infant.

MORPHOLOGY Morphological observations were made from Gram-stained smears of 18-to 24-hour cultures. Each strain was examined for capsule formation in India ink preparations. Motility was determined by a modified

TABLE I
SOURCE OF CULTURES
Clinical Diagnosis

Strain Number	Source	Clinical Diagnosis
1456	Blood	Septicaemia
2352	Blood	Septicaemia
2472	Urine	Urinary tract infection
2559	Sputum	Respiratory infection
2634	Urine	Urinary tract infection
2749	Wound	Osteomyelitis, right femur
2835	Urine	Without known clinical significance
3116	Urine	Urinary tract infection
3405	Sputum	Without known clinical significance
3441	Wound	Post-operative infection

hanging-drop technique from a six-to 18-hour tryptone broth culture (Difco).

GROWTH METHODS Primary cultures were grown on Brewer's modified thioglycollate medium¹, 4% human blood-trypticase soy agar¹, and MacConkey agar (Difco) at 37°C. The growth rate was determined at 25, 37, and 42°C. on nutrient agar slants. Four media inoculated in duplicate and incubated at 25 and 37°C. were used in surveying each strain's ability to produce pigment; potato agar¹, Gessard's glycerol peptone agar stabs (Seleen and Stark, 1943), peptone-glycerol-phosphate agar, and broth (Bunting, 1950) were observed daily for five days.

BIOCHEMICAL PROCEDURES The production of oxidase was determined by using a 1% solution of p-aminodimethylaniline monohydrochloride, while catalase activity was tested by adding 3% hydrogen peroxide to a fresh agar culture. Difco decarboxylase broth base was used to study lysine, arginine, and ornithine decarboxylase activity. Kligler iron agar containing ferrous sulphate and thiosulphate was used to demonstrate hydrogen sulphide production. Ability to produce hydrogen sulphide was further studied by inserting a lead acetate paper strip

¹Baltimore Biological Laboratory.

SEROLOGICAL STUDIES Blood serum from six patients subjected to agglutination tests with homologous organisms revealed four with agglutinin titres of 1 : 160 or greater when titres of 1 : 80 might be considered significant. Agglutinin titres of the other two patients were 1 : 10 and 1 : 40 and there was no change in titres three weeks after the first titration. It is of interest that these two patients were the same two in whom the isolation of *S. marcescens* (strain

TABLE II
BIOCHEMICAL REACTIONS OF NON-CHROMOGENIC VARIANTS OF *Serratia marcescens*

Number of Strains	Sucrose Maltose Mannitol Salicin Inositol Galactose Glycerol Trehalose	Xylose Dulcitol Arabinose Raffinose Rhamnose Malonate	Glucose	Lactose	Adonitol	Sorbitol	Citrate Voges-Proskauer Catalase Nitrites Motility Gelatin Serum Liquefaction	Indole Methyl Red Oxidase Phenyl- pyruvic Acid	H ₂ S	Urea	Litmus Milk	Decarboxylase		
												Lysine	Arginine	Ornithine
1	A	O	A _b	O	A _s	A	+	—	+w	+w	A.C.D.	+	—	+
2 ¹	A	O	A	O	A _s	A	+	—	+w	—	A.C.D.	+	—	+
2	A	O	A	O	O	A	+	—	+w	+w	A.C.D.	+	—	+
1	A	O	A _b	A _s	A _s	A	+	—	+w	+w	A.C.D.	+	—	+
2	A	O	A	A _s	A _s	A	+	—	+w	+w	A.C.D.	+	—	+
1	A	O	A	A _s	O	A	+	—	+w	+w	A.C.D.	+	—	+
1	A	O	A	O	O	O	+	—	+w	+w	A.C.D.	+	—	+

¹One of these two strains produced a faint pigment on subculture.

A = Acid without gas. A_b = acid with bubble of gas; A_s = acid produced slowly; +w = weakly positive; A.C.D. = acid, coagulated, digested.

nos. 2835 and 3405 of Table I) was thought to be of no clinical significance.

ANTIBIOTIC SENSITIVITY STUDIES All 10 strains were highly sensitive to kanamycin and neomycin, while nine strains showed some degree of susceptibility to chloramphenicol. Of the semisynthetic penicillins studied, only ampicillin was slightly effective. All 10 organisms were uniformly resistant to penicillin, methicillin, oxacillin, erythromycin, novobiocin, tetracycline, nitrofurantoin, and all of the sulphonamides tested. Results of these studies are listed in Table III.

TABLE III

DRUG SUSCEPTIBILITY TESTS OF 10 NON-CHROMOGENIC VARIANTS OF *Serratia marcescens*

Antimicrobial Drug and Disc Concentrations	Sensitive ¹	Moderately Sensitive ²	Resistant ³
Penicillin (2, 10 units)	0	0	10
Methicillin (5 µg.)	0	0	10
Oxacillin (1 µg.)	0	0	10
Ampicillin (2, 10 µg.)	0	3	7
Streptomycin (2, 10 µg.)	0	1	9
Tetracycline (5, 30 µg.)	0	0	10
Chloramphenicol (5, 30 µg.)	3	6	1
Erythromycin (2, 15 µg.)	0	0	10
Novobiocin (5, 30 µg.)	0	0	10
Kanamycin (5, 30 µg.)	10	0	0
Neomycin (5, 30 µg.)	10	0	0
Colistin (2, 10 µg.)	1	2	7
Polymyxin (50, 300 units)	0	2	8
Nitrofurantoin (100 µg.)	0	0	10
Gantrisin (0.25, 1.0 mg.)	0	0	10
Sulphadiazine (0.25, 1.0 mg.)	0	0	10
Sulphathiazole (0.25, 1.0 mg.)	0	0	10

¹Sensitive = growth inhibited by both concentrations

²Moderately sensitive = growth inhibited by higher concentration

³Resistant = growth not inhibited by either concentration

RESULTS OF ANIMAL VIRULENCE TESTS The *Serratia* bacilli were lethal to white mice. Necropsy revealed no gross abnormalities of the internal organs except serous peritonitis. On microscopy the liver, spleen,

kidneys, and adrenals were normal. Smears taken from the heart blood and peritoneal exudate showed many Gram-negative bacilli. Organisms recovered in pure culture from the heart blood, peritoneal fluid, liver, spleen, and kidneys were identified culturally and by slide agglutination tests as *S. marcescens*. The rabbits showed no signs of illness during a period of observation of one month.

DISCUSSION

Non-chromogenic variants of *S. marcescens* have generally not been recognized in culture studies until the last few years. This may be due to the emphasis that has been placed on the association of pigmentation with *Serratia* colonies. The increased isolation of these bacilli in our laboratory is, at least in part, related to the increased use of definitive tests to identify Gram-negative bacilli.

Non-pigmented strains of *S. marcescens* may be confused with several members of the *Enterobacteriaceae* family. Only by routine use of a battery of bacteriological tests can these bacilli be separated from other enteric bacteria and properly identified. Colonial morphology on blood agar is quite similar to other enteric organisms, while growth on MacConkey agar resembles that of the non-lactose fermenters. It is probable that cultures of non-chromogenic *S. marcescens* have been mistaken for anaerogenic *Paracolobactrum* species (especially the *Hafnia* group) or for *Aerobacter cloacae*, *Aerobacter* subgroup C (*liquefaciens*), and, to a lesser extent, for other members of the *Klebsiella-aerogenes* group. Non-chromogenic *S. marcescens* strains differ from the paracolon group in failing to ferment xylose, arabinose, or rhamnose, and by the rapid liquefaction of gelatin in 24 hours. In addition, cultures of the *Hafnia* group ferment malonate, and fail to ferment inositol or sorbitol. Some of the paracolon

bacilli give variable indole, methyl red, Voges-Proskauer, and citrate reactions whereas *S. marcescens* almost always give uniform results (— — + +). *Aerobacter cloacae* is similar in IMVic (indole, methyl red, Voges-Proskauer, citrate) and gelatin reactions even though the latter reaction is slower. Also, *A. cloacae* ferments rhamnose, while *S. marcescens* does not. *Aerobacter* subgroup C organisms may be distinguished from *Serratia* bacilli mainly through their ability to ferment arabinose and raffinose. Some *Aerobacter* strains, such as *Aerobacter* subgroup C, produce similar decarboxylase activity. The key features which appear to differentiate *S. marcescens* from these closely related *Aerobacter* strains are their anaerogenic nature and failure to produce acid from xylose, arabinose, or raffinose.

In contrast to the findings of Fulton, Forney, and Leifson (1959), we were unable to demonstrate non-motile, gelatin-negative, and arabinose-positive strains. On the contrary, we found distinct motility on primary isolation and rapid liquefaction of gelatin within 24 hours as key identifying characters. With the exception of urease production by some of our strains, other biochemical characteristics are in agreement with those noted by Fulton *et al.* (1959; Bövre and Tönjum, 1963). Some of the strains that produced weak urease reactions on initial isolation in the weakly buffered urea agar tend to lose urease activity on subculture. Weak acid reactions may occur in fermentation media which may result in a reversion of the pH to the alkaline side. This was particularly true with salicin and adonitol. Non-chromogenic variants of *S. marcescens* may be best characterized by their anaerogenic nature, motility, rapid liquefaction of gelatin, ability to produce small amounts of H₂S on lead acetate paper, production of acid from glucose, sucrose, maltose, mannitol, salicin, and absent or delayed acid from lactose, and lack of fermentative action on xylose, arabinose, rhamnose, raffinose, and malonate.

Most reports of sensitivity studies *in vitro* attest to the high degree of susceptibility of *Serratia* to kanamycin and neomycin, but beyond this there are

many differences. Because of variable resistance to most antimicrobial agents it seems imperative that sensitivity studies be undertaken for each individual strain.

The role that *Serratia* may have in producing human infections has not been completely resolved. *Serratia* has been associated with meningitis, septicemia, endocarditis, urinary and respiratory tract infections, bronchiectasis, otitis media, sinusitis, skin and wound infections. Eight of the strains reported here were thought to have contributed to the patient's illness. Further clinical evaluation may rest with the recognition of non-chromogenic *Serratia* strains on culture.

The taxonomic stature of *Serratia* is not clear as evidenced by the differences in present day classification schemes (see Breed *et al.*, 1957; Topley and Wilson, 1955). Pigmentation can no longer be regarded as a prime requisite for classification. Antigenic studies, such as those in progress at the Communicable Disease Centre (Ewing, Davis, and Reavis, 1959), may give a clearer picture of the taxonomic position of these organisms. It might be worthwhile at the present time to limit the identification of these bacteria to *Serratia* species.

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