PSEUDOMONAS PUTREFACIENS FROM CLINICAL MATERIAL

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INTRODUCTION

There has been growing medical interest in non-fermentative Gram-negative bacilli in recent years. This interest has been stimulated by the increasing occurrence of infections due to bacteria previously rarely encountered in human clinical sources. Reports have appeared increasingly of various infections associated with *Pseudomonas* species other than the well known species *Ps. pseudomallei* and *Ps. aeruginosa*. Concurrent with this, simplified schemes for the recognition of unusual pseudomonads have been presented in recent papers (Gilardi, 1971; Von Graevenitz, 1973). It is now possible to identify many of these bacteria in the routine diagnostic laboratory.

Ps. putrefaciens is one of the lesser known pseudomonads. It can be easily identified by its ability to produce abundant hydrogen sulphide, but may be mistaken for other H_2S producing organisms like Proteus, Salmonella and Citrobacter (Levin, 1968; Dubois *et al.*, 1975). This organism is found in soil and water and has been associated with food spoilage (Long and Hammer, 1941; Levin, 1968). It has recently been recognised in a variety of human sources and has been linked aetiologically with chronic otitis media and varicose ulcers of the leg (Von Graevenitz, 1970; Gilardi, 1972; Holmes *et al.*, 1975; Dubois *et al.*, 1975). Previous isolations of *Ps. putrefaciens* from clinical material have been reported from the United States and Europe but not elsewhere.

This paper describes 3 strains of *Ps. putrefaciens* isolated at the University Hospital, Kuala Lumpur between August 1972 and August 1975.

MATERIALS AND METHODS

The strains were recovered from clinical specimens submitted for routine bacteriological examination. Two strains were isolated in pure cultures from ear swabs and the third strain was recovered in mixed culture from a sputum specimen. The specimens had been plated routinely onto blood agar, chocolate agar and MacConkey agar (Oxoid) and incubated overnight at 37°C. Gram-negative bacilli present as pure cultures or as the predominant organism in mixed cultures were subcultured onto Kligler iron agar (Difco), peptone water sugars and other media for biochemical tests. *Ps. putrefaciens* isolates were recognised by their production of abundant H_2S (black butt in Kligler medium), failure to produce acid in 1% glucose peptone water and positive oxidase reactions. Final identification was based on the methods of Gilardi (1971) and Cowan and Steel (1965).

The isolates were tested against 18 chemotherapeutic agents by the disc-agar diffusion method of Bauer and associates (1966) using Oxoid Diagnostic sensitivity test agar.

REPORT OF CASES

Case 1 : A 22-year old Indian male machine operator was seen at the ENT Clinic for purulent discharge of the right ear. He had been having ear discharge since childhood and had not sought treatment previously. The ear discharge had been thick, yellowish-white and foul smelling for the past one week.

A swab of the ear discharge was taken for culture prior to treatment and yielded pure and profuse growth of *Ps. putrefaciens*. He was examined and found to have chronic suppurative otitis media with attic cholesteatoma and aural polyp. Radiologic examination revealed sclerosis of the mastoid region. No further cultures were done.

Case 2 : A 24-year old Chinese female student, with chronic suppurative otitis media of the right ear, was referred to the ENT Clinic by a private practitioner. Her ear discharge had not improved and the small perforation in the tympanic membrane had not been healing with treatment. *Ps. putrefaciens* was isolated in pure culture from two ear swabs taken at an interval of 7 days. She received treatment with chloramphenicol ear drops and the growth was less profuse in the second specimen. Subsequent cultures of the ear discharge yielded *Staphylococcus pyogenes* and *Ps. aeruginosa*, resistant to chloramphenicol.

Case 3 : A 58-year old Indian male with no definite occupation was seen at the University Hospital for an upper respiratory tract infection. *Ps. putrefaciens* was recovered from the mixed flora of a single sputum specimen together with *Haemophilus influenzae* and *Klebsiella ozaenae*.

RESULTS

All 3 isolates of *Ps. putrefaciens* possessed the following characteristics. They were motile, Gram-negative bacilli which grew well on nutrient agar, blood agar and MacConkey agar at room temperature and at 37°C. After overnight incubation, the colonies were about 2 mm in diameter, convex, smooth and circular with entire edges. They appeared light brown or pink initially and definitely brown on further incubation at room temperature. A green discoloration was seen under the colonies on blood agar. The organisms grew moderately well on deoxycholate citrate agar (Difco) and on nutrient agar with 7.5% sodium chloride. There was no growth at pH 5.6 on Sabouraud dextrose agar (Oxoid). Pellicle growth, turbidity and deposit were observed in nutrient broth. All the isolates grew in nutrient-broth incubated at 42°C for 18 hours but not at 4°C for one week.

Acid was not produced from glucose and other carbohydrates in peptone water or ammonium salt media, even after 3 weeks of incubation. The sugars tested included lactose, sucrose, mannitol, maltose, dulcitol, fructose, arabinose and xylose. An alkaline reaction was observed in Hugh and Leifson OF medium. Negative reactions were obtained for indole, citrate, urease, methyl red, Voges-Proskauer, malonate, phenylalanine, gluconate, aesculin, lysine decarboxylase and arginine dihydrolase tests. Nitrate was reduced to nitrite. On Seller's differential agar (Difco) only a blue slant and unchanged butt were noted. There was no fluorescence or N_2 gas production. On Kligler iron agar, the H_2S production was so marked that the stabbed butt was blackened and masked the red colour of the medium. The organisms gave positive reactions for oxidase, catalase, gelatinase (3 days), deoxyribonuclease and ornithine decarboxylase tests. The important positive characteristics are listed in Table 1.

Table 1

Important characteristics of *Pseudomonas putrefaciens*.

Gram negative rod
Motile
Brown pigment on nutrient agar (48 hours)
H_2S production in Kligler iron agar
Alkaline reaction in Hugh & Leifson OF medium
Presence of: oxidase
ornithine decarboxylase
deoxyribonuclease

All strains were sensitive to the following chemotherapeutic agents (figures indicating disc potencies): streptomycin (10μg), chloramphenicol (25μg), kanamycin (30μg), gentamicin (10μg), cotrimoxazole (25μg), carbenicillin (100μg), polymixin B (100 units), erythromycin (5μg), nalidixic acid (30μg), nitrofurantoin (200μg), neomycin (10μg) and rifamycin (30μg). They were all resistant to penicillin (4 units), cephaloridine (25μg), sulphonamides (200μg) and novobiocin (10 μg). One strain was sensitive and two were resistant to ampicillin (10μg) or tetracycline (25μg).

DISCUSSION

The morphologic, cultural and biochemical characteristics of the 3 strains of *Ps. putrefaciens* agree well with the descriptions given in previous publications (Gilardi, 1971; Riley *et al.*, 1972; Von Graevenitz, 1973; Holmes *et al.*, 1975: Rosenthal *et al.*, 1975; Dubois *et al.*, 1975). The outstanding features of this organism are its production of H_2S on Kligler iron agar, brown pigment formation on nutrient agar, non-fermentative activity on carbohydrate media, and production of ornithine decarboxylase. Identification of this pseudomonad is therefore not difficult provided that the possibility of its occurrence in human infections is borne in mind.

All 3 strains tolerated 7.5% sodium chloride, grew on deoxycholate citrate agar, grew well at 37°C, did not attack sugars and were of human clinical origin. They fit well in the second group of *Ps. putrefaciens* strains described by Riley *et al.*, (1972) and Holmes *et al.*, (1975). Both groups of investigators divided their strains (109 and 15 respectively) into 2 groups on the basis of salt tolerance, growth on Salmonella-Shigella agar (SS agar), growth at 35°C and production of acid from carbohydrates. The first group were composed of strains that could not tolerate 6 or 7% added sodium chloride. Most of them were unable to grow on SS agar or at 37°C but were able to attack a number of sugars. They were mostly from fishery rather than human sources. The second group of strains grew in the presence of added salt, grew on SS agar and at 35°C but failed to attack sugars, except for variable results with glucose and fructose, and were of human origin. Gilardi (1971) discribed 6 strains of *Ps. putrefaciens* of clinical origin which tolerated 6.5% sodium chloride, grew on deoxycholate citrate agar and SS agar and were mostly non-saccharolytic.

The antibiotic susceptibilities presented here are compatible with those reported previously (Gilardi, 1972; Holmes *et al.*, 1975; Rosenthal *et al.*, 1975; Dubois *et al.*, 1975). *Ps. putrefaciens* is one of the most sensitive pseudomonads, being susceptible to a large number of antibiotics, including erythromycin, and resistant to a few, mainly penicillin and cephaloridine.

The isolate that was recovered from Case 3 was judged to be insignificant clinically as other known pathogenic organisms were also present in the same specimen. The other two isolates were clinically significant, as they were recovered in pure cultures (one on two successive occasions). Both cases seem to have acquired their infections in the community, a point that had been stressed by Von Graevenitz (1973) and Dubois *et al.*, (1975).

All recorded infections have been noted to be generally benign and commonly limited to superficial mucosa and damaged skin (Dubois *et al.*, 1975). From published reports, only 3 strains were said to have played a pathogenic role in otitis media (Von Graevenitz and Simon, 1970; Holmes *et al.*, 1975) and one strain was clearly demonstrated as a pathogen in a deep plegmone, underlying a varicose ulcer of the leg (Dubois *et al*, 1975). Clinical

information on other reported isolates were generally poor and it is not possible, at present, to draw conclusions on the clinical significance of *Ps. putrefaciens* strains in general.

Accurate identification and speciation of unusual organisms, such as *Ps. putrefaciens* are important in determining their role in human disease. It is expected that this organism will be recognised more often in clinical material when its characteristics are more widely known.

SUMMARY

Three strains of *Pseudomonas putrefaciens* were isolated from routine clinical specimens at the University Hospital, Kuala Lumpur, Malaysia. Their cultural and biochemical characteristics, and antibiotic susceptibilities are presented. Characteristics of diagnostic value were stressed. Two isolates appeared to have played a pathogenic role in chronic otitis media.

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