Meningitis caused by an alkali-producing pseudomonad

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SYNOPSIS The clinical and microbiological features of a case of meningitis, due to an alkali-producing pseudomonad which closely resembles *Pseudomonas pseudoalcaligenes*, are described. A respiratory infection and a course of antibiotic therapy before admission to hospital may have been predisposing factors to opportunistic infection by this normally saprophytic organism. The problems of identifying alkali-producing pseudomonads are discussed.

Meningitis in early childhood is commonly caused by *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. Infection by opportunistic bacteria, such as *Pseudomonas* spp, however, is relatively uncommon. We present this as the first isolation of *Pseudomonas pseudoalcaligenes* from cerebrospinal fluid (CSF) and as the first instance of its pathogenicity. *Ps. pseudoalcaligenes* was first described by Stanier *et al* (1966). Pedersen *et al* (1970) and Gilardi (1972) reported the recovery of this organism from many clinical specimens but in none of these instances was it considered to be pathogenic.

Case report

An 8-month-old female infant was admitted to hospital with a 24-hour history of anorexia, vomiting, drowsiness, and irritability. There had been no diarrhoea.

After contracting whooping cough at 4½ months, she developed a chronic cough. This persisted until the time of admission, despite a 14-day course of ampicillin, 125 mg four times daily, from her general practitioner, ending three days previously. Her birth history and development had been normal.

On admission her skin was ashen, with a sparse macular purpuric rash of patchy distribution, 0.5 cm in diameter, on the trunk, upper limbs, and feet. This was not typical of the maculopapular erythema found in ampicillin sensitivity. The spleen was not palpable. Her temperature was 38.6°C and pulse 180/

minute; the anterior fontanelle was tense and her throat was reddened. Occasional twitches of the limbs were noted. There was no neck stiffness.

Results of laboratory investigations were as follows: haemoglobin 10.7 g/dl; leucocytes 6500/ μ l with 60% neutrophils, 36% lymphocytes and 4% monocytes; platelet count 163 000/ μ l; ESR 36 mm fall in 1 hour (Westergren). Blood cultures were sterile after 10 days' incubation, urine was sterile, and a throat swab grew only commensal organisms. The CSF was opalescent with a leucocyte count of 400/ μ l, of which 90% were neutrophils and 10% lymphocytes. Protein concentration was 2.1 g/l and sugar 0.55 mmol/l. Numerous pleomorphic Gram-negative bacilli with distorted cell walls, indicative of antibacterial activity, were seen in a stained film from a centrifuged deposit of CSF. An organism was grown which was later shown to conform closely to *Ps. pseudoalcaligenes*. Leucocyte counts in further specimens of CSF, taken 24 and 48 hours later, were 1225 and 1200/ μ l respectively. Although stained films from both of these specimens showed scanty Gram-negative bacilli, cultures were sterile.

In view of the laboratory findings, initial antibiotic therapy was with ampicillin, 400 mg/kg per day, intravenously. After 24 hours this was changed, on the basis of the *in vitro* sensitivity of the organism recovered from the CSF, to 6 mg/kg per day of gentamicin intravenously and 2 mg daily intrathecally for seven days. Chloramphenicol, 100 mg/kg per day, was then given orally for a further seven days.

The condition of the patient was unchanged until 24 hours after starting gentamicin, but subsequently she made an uncomplicated recovery. One month later her head circumference had not increased,

hearing and vision appeared normal, and she did not seem to have suffered any regression in development.

BACTERIOLOGY

After 18 hours at 37°C, cultures yielded a moderate growth of a non-pigmented Gram-negative bacillus which grew well aerobically on blood agar and MacConkey agar, and on chocolate agar in an atmosphere of 10% CO₂.

Of the preliminary tests carried out, the following results showed that the organism was an alkali-producing pseudomonad: production of cytochrome oxidase, motile with one polar flagellum (Kirkpatrick's stain), and production of alkali in the Hugh and Leifson O-F test. The results of secondary tests (Stanier *et al*, 1966), which follow, enabled the organism to be provisionally identified as *Ps. pseudoalcaligenes*: nitrates were reduced to nitrites, growth was absent at 4°C and present at 41°C, gelatin was not liquefied, and starch was not hydrolysed.

The organism was sent to the National Collection of Type Cultures (Computer Trials Laboratory), where it was confirmed to resemble closely *Ps. pseudoalcaligenes* differing from the type strain of *Ps. pseudoalcaligenes* (NCTC 10860) by only two results of a total of 87 tests. These differences are listed as follows:

Test	NCTC 10860	Strain from CSF
Acid from glycerol (ammonium salt medium)	—	+
Growth on β -hydroxybutyrate	+	—*

*Accumulation of poly β -hydroxybutyrate inclusion granules
—positive when organism grown on nutrient agar containing β -hydroxybutyrate.

Antibiotic sensitivity tests were carried out in our laboratory by the disc diffusion method on Oxoid Diagnostic Sensitivity Test agar with the addition of 5% lysed horse blood. The pseudomonad was found to be sensitive to the following antibiotics (with the antibiotic content of each disc in parenthesis): tetracycline (10 μ g), chloramphenicol (50 μ g), carbenicillin (100 μ g), cephaloridine (30 μ g), cotrimoxazole (25 μ g), colistin (10 μ g), gentamicin (10 μ g), neomycin (30 μ g), sulphafurazole (100 μ g), and streptomycin (25 μ g); and resistant to ampicillin (25 μ g) and cephalixin (30 μ g). The minimum inhibitory concentration of ampicillin, determined by the plate dilution method, was 60 μ g/ml using an inoculum of 10⁴ organisms/ml.

Discussion

Stanier *et al* (1966) examined several pseudomonads which they recognized as strains of a new species, *Ps. pseudoalcaligenes*. Among these was an isolate from a sinus discharge (ATCC 17440, NCTC 10860). Of 54 isolations of *Ps. pseudoalcaligenes* reported by Pedersen *et al* (1970), 32 were from sputum, four from urine, and the remainder from the hospital environment. Gilardi (1972) reported one isolation from a postoperative infection of the knee and another from the blood and sputum of a patient with pneumonia. In none of these instances was *Ps. pseudoalcaligenes* considered to be pathogenic.

The alkali-producing pseudomonads are difficult to recognize and in a routine laboratory oxidase-positive, Gram-negative bacilli which produce alkali in the Hugh and Leifson O-F test are often identified as *Alcaligenes* spp. Indeed Gilardi (1972) and von Graevenitz (1973) consider that previously reported pathogenic strains of *Alcaligenes* spp. may have been alkali-producing pseudomonads.

Although Snell (1973) does not include flagellar arrangement in his identification scheme, flagellar stains are of value in separating *Alcaligenes* spp from pseudomonads (Sutter, 1968; von Graevenitz, 1973); *Alcaligenes* spp have peritrichous flagella whereas those of pseudomonads are polar.

Separation into species within the group of alkali-producing pseudomonads presents problems to taxonomists, as this group is biochemically unreactive in conventional tests. The five members in the group, *Ps. acidovorans* (den Dooren de Jong, 1926), *Ps. alcaligenes* (Monias, 1928), *Ps. diminuta* (Leifson and Hugh, 1954), *Ps. testosteroni* (Marcus and Talalay, 1956), and *Ps. pseudoalcaligenes* (Stanier *et al*, 1966), were first characterized by their utilization of a wide range of carbon compounds as sole energy source. Identification schemes have been devised by Stanier *et al* (1966), Pedersen *et al* (1970), and Snell (1973) but carbon source utilization tests are difficult to carry out in a routine laboratory. Until more conventional tests can be used the identification of the alkali-producing pseudomonads will remain a problem.

The antibiotic sensitivities of previous isolates of *Ps. pseudoalcaligenes* have been discussed by Pedersen *et al* (1970), who examined 28 strains, all of which were sensitive to chloramphenicol, 96% sensitive to erythromycin and tetracycline, and 50% sensitive to ampicillin. Of 16 strains examined by Gilardi (1972), most were found to be sensitive to tetracycline, neomycin, kanamycin, polymyxin, and gentamicin; only 6% were reported to be sensitive to ampicillin.

In this case the organism was recovered only from

the initial CSF, but Gram-negative bacilli were seen in stained films in specimens taken 24 and 48 hours later. High dosage may have caused an ampicillin level sufficient to sterilize the CSF in 24 hours, despite the relative insensitivity of the bacilli. McCracken (1972) reported an ampicillin level of 59 µg/ml in the CSF of a neonate two and three-quarter hours after receiving a daily intravenous dose of 250 mg/kg.

Although *Ps. pseudoalcaligenes* has not previously been reported as causing meningitis, Patrick *et al* (1975) reported a case of meningitis caused by *Ps. maltophilia* in a 70-year-old man with no history of recent antibiotic therapy and no previous debility apart from chronic bronchitis. The respiratory tract was considered to be the most likely route of infection but there was no supporting clinical and laboratory evidence. The present case differs in that an antibiotic had been given in the previous fortnight, specifically for a chest infection. The patient was therefore a candidate for opportunistic infection by reason of her age, repeated chest infections following whooping cough, and a recent course of a broad-spectrum antibiotic. We consider that the respiratory tract was the most likely route of infection in this case.

The initial diagnosis was complicated by a purpuric rash, suggesting meningococcal infection, and by the presence of pleomorphic Gram-negative rods resembling *Haemophilus influenzae*. Ampicillin was given until *in vitro* antibiotic sensitivity tests showed the organism, later identified as an unusual pseudomonad, to be resistant.

This stresses the importance of isolating the causative organism in meningitis, and the possibility of this being an opportunistic pathogen should be borne in mind if predisposing factors exist.

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