A report of three strains of *Pasteurella septica* isolated in Hong Kong

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**SYNOPSIS** Three strains of *Past. septica* were isolated in Hong Kong. The clinical histories and the detailed bacteriological and serological studies are presented.

Human infection by *Pasteurella septica* is uncommon. Meyer (1958) stated that *Past. septica* was identified in 89 of 109 cases reported. The organism had been demonstrated largely in sputum and from animal bites, but it had also occasionally been isolated from cases of arthritis, conjunctivitis, septicaemia, puerperal sepsis, appendix abscess, brain abscess, meningitis, sinusitis, masotiditis, and other manifestations. Smith (1959) isolated two more strains from the throat washings of students.

All cases of *Past. septica* infection reported were from Europe and America. The occurrence of the infection in Chinese or in the East has not been found in the literature. A report is thus made on three strains of *Past. septica* isolated in Hong Kong between the period of 1958 to 1962. The first strain was isolated from the cervical swab of a woman suffering from carcinoma of the cervix, the second strain from the pus of an appendix abscess, and the third strain from the peritoneal cavity of a case of perforated duodenal ulcer.

**CASE REPORTS**

CASE 1 A Chinese woman, aged 35, was admitted to Queen Mary Hospital on 20 February 1958 with the chief complaint of foul discharge and dull, aching pain over the abdomen for three months. On examination, the external genitalia appeared normal but the cervix was replaced by cauliflower growth which infiltrated the lateral and posterior fornices. The biopsy showed anaplastic epidermoid carcinoma. The first cervical swab taken for culture one week after admission revealed a mixed growth of *Past. septica* and a non-pathogenic corynebacterium. The second swab taken on the second week after admission also yielded a pure growth of *Past. septica*. On admission, the patient had a temperature of 100°F. and blood leucocyte count 7,100 cells per c.mm. (81% polymorph neutrophils, 15% lymphocytes, 4% monocytes). The patient was treated with the insertion of cobalt, penicillin, and streptomycin for one week and the temperature became normal. The patient stayed in the hospital for one month. She was not followed up.

CASE 2 A Chinese male, 60 years old with no definite occupation, was admitted to Queen Mary Hospital on 19 February 1962 with the complaint of constant, dull, aching pain in the right lower quadrant of the abdomen for one week. The pain increased in severity three days before admission and was aggravated by flexion of the right hip. There was no history of close contact with any animal. The patient was found to be afebrile on admission with a blood leucocyte count of 5,250 cells per c.mm. A tender mass was found in the right lower quadrant of the abdomen. Operation was performed on the day of admission. A large abscess was found behind the ileocaecal area. About 150 ml. of foul-smelling, creamy pus was drained. The pus taken at the operation was found to be sterile. Two days after the operation, the patient developed a temperature of 102°F. The abdomen was rigid and tender. A second laparotomy revealed another retrocaecal abscess containing 60 ml. of foul-smelling, creamy pus. *Past. septica* was isolated in pure growth twice. Chloramphenicol and streptomycin were given to the patient and the temperature gradually returned to normal. Swabs taken from the nasal cavity, sputum, and the stool were negative for *Past. septica*. The patient continued to improve and was discharged one month later.

CASE 3 A Chinese male patient, 26 years old, was admitted to Queen Mary Hospital on 17 June 1962. He was a farmer in China and came to Hong Kong only one month before admission. He was in constant contact with cattle, poultry, dogs, and cats but had never been bitten by animals. His complaint was pain in the abdomen near the right lower quadrant associated with vomiting. No bowel movement or flatus was passed and the general condition was poor. On examination, the patient was afebrile. The blood leucocyte count was 14,000 cells per c.mm. (87% polymorph neutrophils, 8% lymphocytes, 4% monocytes, and 1% eosinophils). Radiographs of the abdomen showed a small rounded radiolucent area in the right subphrenic region suggesting free gas due to perforated...
hollow viscera. Laparotomy was done and the perforation was found at the second part of the duodenum. A peritoneal swab taken during the operation showed pure growth of *Past. septicum*. The perforation was repaired and the patient was treated with terramycin by intravenous drip and also by mouth for four days. He made satisfactory progress and was discharged nine days later. Six months later, the patient was re-admitted to the hospital with an intraperitoneal abscess. The abscess was drained and the pus revealed mixed growth of *Past. septicum* and *E. coli* on two occasions. The patient's condition improved and he was discharged 10 days later. The throat and stool swabs were negative for *Past. septicum*.

**BACTERIOLOGY**

All the swabs were cultured on human blood agar plates, nutrient agar plates, MacConkey's plates and Robertson's cooked meat media for primary isolation of pathogens. The fermentation reactions were tested over a period of two weeks at 37°C, in Durham's tubes containing 1% of the tested carbohydrate in peptone water plus Andrade's indicator. Other biochemical tests included indole production, the urea test, methyl-red and Voges-Proskauer reactions, the nitrate reduction test, and the catalase test. The motility test was done in semi-solid agar. The three strains were designated as strains I, II, and III respectively from isolation from cases 1, 2, and 3. Comparison was made with known strains of *Past. septicum* obtained from the National Collection of Type Cultures, Colindale, England: the strain numbers are NCTC 948 (mouse), 1737 (pig), 3195 (bovine), 8080 (human), 8771 (infected finger following cat bites).

**MORPHOLOGY** The three strains of *Past. septicum* on primary isolation were small Gram-negative cocco-bacilli measuring 0.5 to 1 μm in length. The organisms tended to form small groups. A proportion of them showed bipolar staining when stained by Wayson's method. A narrow capsule was demonstrable from those taken from infected animals by the modification of Muir's method.

**CULTURAL CHARACTERISTICS** Well-isolated, semi-translucent, gray colonies were observed on human blood agar after 24 hours' incubation. The surface was smooth and glistening with a diameter of 1 to 2 mm. The colonies of strain I were low convex, and were not very mucoid in character whereas those colonies of strains II and III were very mucoid, viscid, and moist in appearance. Strain I was easily emulsifiable but it was difficult to form a homogenous suspension in a saline with strains II and III. All three strains produced no haemolysis on blood agar and no growth was obtained on MacConkey's medium after one week's incubation. A very thin growth of tiny colonies was observed on the nutrient agar. In nutrient broth, an even turbidity developed in 24 hours, though some deposit was seen in cultures of strains II and III.

**FERMENTATION REACTIONS** All our strains and the N.C.T.C. strains fermented glucose, galactose, sucrose, and mannite with acid production after incubation for one day but no gas was formed. Lactose, maltose, xylose, rhamnose, salicin, dulcitol, raffinose, trehalose, and inositol were not fermented after 14 days' incubation. Sorbitol was fermented by strain I and all the N.C.T.C. strains in one day but strains II and III took nine days of incubation to show any observable fermentation reaction. On the contrary, strains II and III fermented arabinose in one day but strain I and the N.C.T.C. strains (except strain 8771) failed to ferment this sugar after incubation for 14 days. Strain N.C.T.C. 8771, however, fermented arabinose in seven days. The fermentation of glycerol was generally irregular; strains I, III, N.C.T.C. 1737, and N.C.T.C. 8771 took five days to show fermentation; strain II took nine days; whilst strains N.C.T.C. 948, N.C.T.C. 3195, and N.C.T.C. 8080 showed no visible fermentation reaction after 14 days' incubation. The results of the fermentation of sorbitol, arabinose, and glycerol are listed in Table I.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Strain No.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>N.C.T.C. Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorbitol</td>
<td>A (1)</td>
<td>A (9)</td>
<td>A (9)</td>
<td>A (1)</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>Not fermented A (1)</td>
<td>A (1)</td>
<td>Not fermented²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td>A (5)</td>
<td>A (9)</td>
<td>A (5)</td>
<td>Variable</td>
<td></td>
</tr>
</tbody>
</table>

¹Number in bracket indicates days required to show visible acid production.
²With exception of strain N.C.T.C. 8771.

All the strains tested produced indole, reduced nitrate to nitrite, gave a positive catalase test, but the urea utilization, methyl-red, and Voges-Proskauer tests were negative. The motility test was negative when the semi-solid agar medium was incubated at 37°C and at 22°C.

**SENSITIVITY TEST TO ANTIBIOTICS** The sensitivity test was done by spreading a 24-hour nutrient broth culture onto a blood agar plate. Oxford Multidisks (code nos. 30-IG and 30-IN) were placed onto the surface of the blood agar plates and the result was read 24 hours after incubation. All the strains tested were sensitive to erythromycin, novobiocin, penicillin, streptomycin, tetracycline, chloramphenicol, nitrofurantoin, and polymyxin B but resistant to sulpha furazol and bacitracin.

**ANIMAL PATHOGENICITY TEST** Only the locally isolated strains were tested for animal patho-
A report of three strains of Pasteurella septica isolated in Hong Kong

109

genicity. Four mice of the Strong A strain were inoculated intraperitoneally with 0.2 ml of 24-hour broth culture of each of the three strains. All mice died within 24 hours. Necropsy showed no gross abnormality of the internal organs except serous peritonitis. Microscopic examination of the liver and the spleen was normal. Smears taken from the peritoneal cavity and from the heart blood showed numerous Gram-negative coccobacilli. Past. septica was recovered in pure culture from the spleen, liver, heart blood, and the peritoneal cavity of these mice.

SEROLOGY Only the sera from cases 2 and 3 were available for agglutination tests against their homologous cultures. A second serum sample from case 3 was also obtained for testing six months later. The result indicated in Table II showed that the agglutinin titre in both patients' sera of cases 2 and 3 was not very high. This agreed with the result recorded by others in case of infection of Past. septica.

### TABLE II

**AGGLUTININ TITRES OF SERA OF CASES 2 AND 3**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Cultures</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>N.C.T.C. 8080</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 2</td>
<td>Not tested</td>
<td>1 : 20</td>
<td>Not tested</td>
<td>Not tested</td>
<td>1737</td>
</tr>
<tr>
<td>Case 3 (1st sample)</td>
<td>1 : 20</td>
<td>1 : 20</td>
<td>1 : 80</td>
<td>1 : 40</td>
<td></td>
</tr>
<tr>
<td>Case 3 (2nd sample)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

Agglutination tests were also performed with immune sera prepared by inoculation of the three Hong Kong strains, strain N.C.T.C. 1737, and strain N.C.T.C. 8080 into rabbits. Strain N.C.T.C. 8080 was chosen because it was a human strain and strain N.C.T.C. 1737 was serologically related to a human strain (Bartley and Hunter, 1947; Bartley, 1960). The procedure of immunization consisted of six subcutaneous injections of boiled cultures (100°C. for one hour) during the first two weeks; six subcutaneous injections of heat-inactivated cultures (56°C. for half hour) for the following two weeks; finally six injections of 0.2% formaldehyde inactivated cultures for another two weeks. Living cultures were not used because rabbits succumbed to them even though they had previously received killed cultures.

Animals were bled one month after the last injection and sera were tested. Two-fold dilutions of the sera were made and the antigens were prepared from heat-killed cultures. The results of agglutination reactions are presented in Table III.

### TABLE III

**CROSS-AGGLUTINATION TEST USING IMMUNE RABBIT SERA**

<table>
<thead>
<tr>
<th>Serum</th>
<th>Cultures</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>N.C.T.C. 8080</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1 : 640</td>
<td>0</td>
<td>0</td>
<td>1 : 80</td>
<td>1 : 160</td>
</tr>
<tr>
<td>II</td>
<td>1 : 40</td>
<td>1 : 40</td>
<td>0</td>
<td>0</td>
<td>1 : 40</td>
</tr>
<tr>
<td>III</td>
<td>1 : 80</td>
<td>0</td>
<td>1 : 20</td>
<td>0</td>
<td>1 : 20</td>
</tr>
<tr>
<td>N.C.T.C. 1737</td>
<td>1 : 320</td>
<td>1 : 40</td>
<td>0</td>
<td>1 : 1280</td>
<td>0</td>
</tr>
<tr>
<td>N.C.T.C. 8080</td>
<td>1 : 160</td>
<td>1 : 20</td>
<td>0</td>
<td>1 : 40</td>
<td>1 : 5120</td>
</tr>
</tbody>
</table>

**DISCUSSION**

According to Meyer (1958) immunologically related strains of Past. septica from different countries might have characteristic carbohydrate fermentation patterns. However, our strains did not fall into any of the groups of Rosenbusch and Merchant (1939) who used arabinose, xylose, and dulcitol for grouping. In fermentation reactions of sorbitol and arabinose, strains II and III isolated in 1962 were distinctly different from strain I isolated in 1958 and the N.C.T.C. strains.

The relationship of Past. septica infection to animal bites is well established but the cases reported in the present series did not reveal such a linkage. Case 3 gave a history of close contact with animals but no history of being bitten by any of them. It appears, therefore, that a patient may have a primary focus such as carcinoma, appendix abscess, or perforated duodenal ulcer and that Past. septica acts as secondary invader. Nevertheless, the finding of Pasteurella species from animal origin in human diseases has definite clinical significance. This fact was illustrated in case 3. The re-admission of the patient suffering from an intraperitoneal abscess was definitely due to Past. septica.

All our strains and the N.C.T.C. strains were sensitive to the common antibiotics. Immediate subsidence of fever was noticed in all the patients after antibiotic therapy and this is further evidence that the infection with Past. septica was of clinical significance.

Pizey (1953) reported a case of Past. septica arthritis involving several joints in a baby aged 3
weeks, the youngest age incidence on record. There was no history of trauma or of contact with animals and the mode of infection was unknown. In case 1 of our series, *Past. septica* was isolated from the cervix. If the organism is present in the female genital tract, it is also possible that it may gain entrance to the uterus during labour and be transmitted to the foetus, though the exact role of *Past. septica* infection has yet to be determined.

The agglutinin titres in sera of our cases were low. This fact was reflected by the low agglutinin titre in the immune rabbit sera. This finding agrees with those of Bartley and Hunter (1947) and of Bartley (1960) who showed that a patient suffering from *Past. septica* nasal sinusitis had no agglutinin titre in his serum in 1947 and that the titre was only 1:40 when he was re-tested in 1959. No demonstrable agglutinin was found in case 3 of our series when he was re-admitted to hospital.

In our results, strains II and III were very poorly antigenic. It may be due to the fact that the strains were mucoid as shown by their colonial appearances and that the presence of the M substance may make the strain poorly antigenic (Meyer 1958). It is also possible that the antigenic substance closely bound to the living organism is destroyed by heat during the preparation of the antigen. In view of the low agglutinin titre in the immune sera, an agglutinin-absorption test was not done.

We wish to thank Professor Daphne Chun, Professor F. E. Stock, and Mr. J. Chen for the clinical histories of cases 1, 2, and 3, Dr. G. C. Turner for the isolation of strain 1, and Dr. C. T. Huang for advice and encouragement.

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