

Pasteurella Haemolytica Var. Ureae

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SUMMARY

The eleven strains of *Pasteurella haemolytica* var. *ureae* were isolated from discharges of respiratory tract and the eyes.

Three strains were isolated in pure cultures from the diseased conditions (chronic dacryocystitis), but further clarifications for their pathogenicities to man were urged.

INTRODUCTION

There is little doubt many cultures, which are discarded without interest in the daily routine work, may contain *Pasteurella* species, which are mistaken for *Haemophilus*, *Neisseria* or some other normal flora.

During six months, April to September 1964, eleven strains of *Pasteurella hemolytica* var. *ureae* were isolated. Seven strains were isolated from the sputum, three strains from the eye discharges and one strain from the gastric lavage. Henriksen (1960, 1961 a) isolated several strains of *Pasteurella* species from human respiratory discharges and reported eight strains of *pasteurella haemolytica* var. *ureae*. (Henriksen 1961 b, Omland and Henriksen 1961).

MATERIALS AND METHODS

Organisms:

Strain 1819 was isolated in pure culture from the eye discharge of 16 years old male who suffered from chronic dacryocystitis.

Strain 1926 was isolated from the sputum of 31 years old male who suffered from the upper respiratory infection. *Diplococcus pneumoniae* was also isolated from the same specimen.

Strain 2316 was isolated from the sputum of 32 years old female who was suffering from pulmonary infiltration. *Haemophilus haemolyticus* was also found in cultures from the same specimen.

Strain 3014 was isolated from the sputum of 19 years old male who suffered from pulmonary tuberculosis. *Diplococcus pneumoniae* was also present.

Strain 3127 was isolated from the sputum of 20 years old male who suffered from bronchiectasis, together with *Haemophilus influenzae* and *Diplococcus pneumoniae*.

Strain 4565 was isolated in pure culture from the eye discharge of 31 years old female who suffered from chronic dacryocystitis.

Strain 4735 was isolated in pure culture from the eye discharge of 41 years old female who suffered from chronic dacryocystitis.

Strain 4741 was isolated from the gastric lavage material of 6 years old boy who suffered from pleurisy together with *Diplococcus pneumoniae*.

Strain 5130 was isolated from the sputum of 14 years old male who suffered from lung abscess.

Strain RH1 and RH2 were isolated from the sputum of patients respectively, of whom no clinical informations were available.

Sugar fermentation:

Bacto phenol red broth was used as a basal medium. In case of uncertain results, the test was repeated with the same medium after adding 10 per cent rabbit serum.

Indol test:

One per cent Tryptone medium was used and tested by Ehrlich's reagent.

methyl red and voges-Proskauer reactions.

Two days old cultures grown in 0.7 per cent peptone water which added with 0.5 per cent glucose and 0.5% disodium phosphate were used. The reagents were added to the cultures respectively for these tests and read.

Citrate utilization:

Simmons medium was used and observed after 2 days of culture.

Urease reaction:

Christensen's urea agar was used and read 4 times after 30 minutes, 1 hour, 1 and 2 days.

Oxidase reaction:

Aqueous solution of 0.5 per cent tetramethyl-p-phenylenediamine hydrochloride were prepared immediately before use.

Sensitivity tests to antibiotics:

The procedure of Ericsson (1960) was used.

RESULTS

Morphology:

Gram-stained film of each strain, prepared from a 18-24 hours blood agar culture, was examined. The findings were rather pleomorphic rods, Ca. 0.5 to 0.7 micron wide, with rounded ends, varying from short oval cells to short and often curved filaments.

Many short rods showed distinct bipolar staining. Some long rods were observed with one or more unstained "vacuoles".

Colonies:

Blood agar plate was prepared from a commercial dehydrated blood agar base by addition of human blood containing a citrate-anticoagulant solution.

All strains regularly produced a green discoloration zone with a more or less marked, but usually faint partial haemolysis.

Colonies revealed a slightly translucent and mucoid appearance, the diameter being about 2mm after 24 hours of incubation.

Growth:

The strains were fastidious in their nutritional requirements showing comparatively short period of viability on blood agar when observed by subculturing or deep-freezing in glycerol broth stock

Biochemical reactions:

Our strains revealed negative result for lactose, but positive for mannitol, maltose and sucrose. Indol and M.R. tests were negative.

The urease reaction was extremely rapid and strong (starting after few minutes and completed after 2 hours).

The oxidase reaction showed distinctly positive.

Sensitivity to antibiotics:

Four strains (1819, 1926, 4565 and 5130) were examined the sensitivities to six antibiotics. All strains showed sensitive to penicillin, streptomycin, chloramphenicol and tetracycline. Three strains were sensitive to sulfonamide but one strain (1819) was resistant to it.

Three strains were sensitive to erythromycin but one strain (4565) was moderately resistant to it.

DISCUSSION

P. multocida (*P. septica*) is a general name of species characterized by producing haemorrhagic septicemia in various animals and infections in man in close contact among them.

There is now a tendency to classify the species into *P. pneumotropica* (Henriksen 1962, Jawetz 1950), *P. haemolytica* (Biberstein et al. 1960, Newsom and Cross 1932, Smith 1961) and its variety to urea (Henriksen and Jyssum 1960, Jones 1962).

Among Pasteurella species, biochemical differentiations were shown as following table 1. van Loghem (1944-5) proposed to include the two species of *P. pestis* and *P. pseudotuberculosis* into the genus *Yersinia*.

For *Yersinia* group, minidefinition made by Cowan (1965) are as follows:

Gram-negative rods; motile or non-motile. Catalase positive, oxidase-negative. Facultatively anaerobic. Attack sugars fermentatively without gas production; do not attack sucrose. Grow on MacConkey agar. The minidefinition (Cowan 1965) for *P. multocida* group was mentioned as the followings:

Gram-negative rods; non-motile. Catalase-

Table 1.

Biochemical reactions of the *Pasteurella* species

| | Lac-tose | Man-nitol | Mal-tose | Sacc-harose | Sor-bitol | Xyl-ose | Indol | M. R. | H ₂ S | Ure-ase | Oxi-dase |
|---|----------|-----------|----------|-------------|-----------|---------|-------|-------|------------------|---------|----------|
| <i>P. multocida</i> | -/+ | + | - | + | + | +/- | + | - | + | - | + |
| <i>P. pneumotropica</i> | -/+ | - | + | + | - | + | + | - | + | + | + |
| <i>P. pseudotuberculosis</i> | - | + | + | - | - | + | - | + | -/+ | + | - |
| <i>P. pestis</i> | - | + | + | - | - | + | - | + | w+ | - | - |
| <i>P. haemolytica</i> | +/- | + | + | + | + | + | - | - | +/- | - | + |
| <i>P. haemolytica</i> var. <i>ureae</i> | - | + | + | + | +/- | +/- | - | - | - | ++ | + |

+ : Positive reaction

- : Negative reaction

W+ : Weak positive reaction

+/- : Mostly positive and sometimes negative reaction

-/+ : Mostly negative and sometimes positive reaction

positive; Oxidase-positive or negative. Facultatively anaerobic. Attack sugars fermentatively without gas production; ferment sucrose. Do not grow on MacConkey agar (few exceptions).

Concerning the haemolysis on the blood agar plate, Henriksen (1961 b) pointed out that *P. haemolytica* var. *ureae* produces only the characteristic green discoloration and partial haemolysis when the pH of the blood agar is on the acid side, while entirely inactive on the alkaline side.

Whereas, the beta-haemolysis produced by the strains of *P. haemolytica* var. *haemolytica* also depends upon the pH but tends to disappear on the acid side of the media.

It is somewhat interesting that most of Gram negative rods show resistant to penicillin, but all these isolated strains of *Pasteurella haemolytica* var. *ureae* revealed sensitive to penicillin.

Fastidiousness in their nutritional requirements is regarded as somewhat similar character of strains of *Haemophilus influenzae*.

The strains of *Pasteurella haemolytica* var. *ureae* possessed a very low degree of pathogenicity to mouse and guinea pig, but one of the strains was isolated in pure culture from a diseased condition (sinusitis; Omland and Henriksen 1961).

In cases of three chronic dacryocystitis, all showed pure culture of *Pasteurella haemolytica* var. *ureae*.

The question of its pathogenicity to man remains to be clarified.

REFERENCE

- 1) Biberstein, E. L., Gills, M. and Knight, H. 1960: *Serological types of Pasteurella haemolytica*. *Cornell Vet.* 50:283
- 2) Cowan, S. T. 1965: *Manual for the identification of medical bacteria*. Cambridge University Press, Great Britain.
- 3) Ericsson, H. 1960: *Rational Use of Antibiotics in Hospitals*. *J. Clin. Lab. Invest.*, 12, Suppl. 50,
- 4) Henriksen, S. D. and Jyssum, K. 1960: *A New variety of Pasteurella haemolytica from the human respiratory tract*. *Acta path. et microbiol. scandinav.*, 50:443,
- 5) Henriksen, S. D. and Jyssum, K. 1961: *A study of some Pasteurella strains from the human respiratory tract*. *Acta path. et microbiol. scandinav.*, 51:354,
- 6) Henriksen, S. D. 1961: *Pasteurella haemolytica* var. *ureae*. *Action on blood agar and serological reactions*. *Acta path. et microbiol. scandinav.*, 53:425.
- 7) Henriksen, S. D. 1962: *Some Pasteurella strains from the human respiratory tract. correction and supplement* *Acta path. et microbiol. scandinav.*, 55:355,
- 8) Jawetz, E. 1950: *A pneumotropic pasteurilla of laboratory animals*. 1. *Bacteriological and Serological characteristics fo the organism*. *J. infect. Dis.* 86:172.
- 9) Jones, D. M. 1962: *A pasteurilla-like organism from the human respiratory tract*. *J. Path. Bact.* 83:143.
- 10) van Loghem, J. J. 1944-5: *The classification of the plague bacillus*. *Antonie van Leeuwenhoek I.*

Microbiol. Serol. 10:15, 1944-5.

- 1) Newsom, I. E. and Cross, F. 1932: *Some bipolar organisms found in pneumonia in sheep.* *J. Amer. vet. med. Ass.*, 80:715.
- 12) Omland, T. and Henriksen, S. D. 1961: *Two new strains of Pasteurella haemolytica var. ureae*

isolated from the respiratory tract. *Acta path. et microbiol. scandinav.*, 53:117.

- 13) Smith, G. R. 1961: *The characters of two types of Pasteurella haemolytica associated with different pathological conditions in sheep.* *J. Path. Bact.* 81:431.