CATEDRA DE MICROBIOLOGIA, INMUNOLOGIA Y SEROLOGIA
D. A. CONROY

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Observations on the ocurrence of pseudomonas aeruginosa in a sample of well water

From time to time, the author is called upon to effect bacteriological examinations of samples of well water in the laboratory where he is employed. These samples are brought in by members of the staff, who wish to determine the potability of the water in their wells, with particular regard to any possible contamination by 'pozos negros' or cesspools. This paper describes the results of one such examination in which *Pseudomonas aeruginosa* was encountered, and seeks to relate this occurrence to the other results obtained in tbe same examination.

Experimental methods

The methods used during the course of this work follow the scheme prescribed by Ferramola (1947), and are similar to those in use in Great Britain. Estimations of the total numbers of viable bacteria at 22°C and at 37°C were made, as also was an examination for the presence of *Clostridium perfringens*, and the enumeration of coliform bacteria. For the detection of *Cl. perfringens*, Spray's iron milk was used in preference to litmus milk, as personal experience has shown that superior results may thereby be obtained. This medium was steamed prior to inoculation in order to reduce the oxygen tension.

Following inoculation, the media were incubated at 37°C (room temperature in duplicate plates of the sample to detect the total number of viable bacteria), and incubated for 48 hours before examination.

On subsequent examination, it was noted that a greenish colour had developed on the surface of the Spray's iron milk, where this was in contact with the air. A microscopical examination showed the presence of gram negative rods in large numbers, and these were seen to be motile. Accordin-

gly, a nutrient agar plate was streaked with a loopful of this culture, and incubated overnight at 37°C. Subsequent examination of the plate showed that the surface of the medium was covered with a spreading growth, and that the medium was coloured blue-green. It was immediately suspected that the organism was *Pseudomonas aeruginosa*, and it is of interest to note that the organism was present in pure culture.

In order to determine with more accuracy whether this organism was in fact *Ps. aeruginosa*, a tube of peptone water was inoculated, from which were subsequetly inoculated peptone water sugars, Clark and Lugs' medium, nitrate broth etc., follwing 24 hours incubation at 37°C.

Results

The total viable count at 37°C was 40 organisms/ml., and at room temperature (about 21°C) there were 38 organisms/ml. None of the MacConkey broths used in the coliform count showed the presence of either acid or gas in up to 72 hours incubation. It was therefore assumed from thee results that members of the coliform group were either absent, or not present in sufficient numbers to cause acid and gas production in MacConkey broth. No typical 'stormy clot' fermentation charactehistic of *Cl. perfringens* was produced in Spray's iron milk, but the blue -green coloration described above was seen to be present to a depth of 1 cm. or so.

The organism isolated from the milk possessed the following characteristics :

i) Growth on nutrient agar was luxurious, the colonies having a dark - coloured centre, with lighter coloured borders. The whole of the medium came to be coloured blue - green, which colour increased with prolonged incubation.

ii) Growth in nutrient broth produced heavy turbidity, with both pellicle and sediment.

iii) Indole was not formed from peptone water (Kovac's method),

iv) Gelatin was rapidly liquefied,

v) No acid or gas produced from arabinose, adonitol, dulcitol, fructose, galactose, inositol, lactose, maltose, mannitol, rhamnose, saccharose, salicin, or xylose at a 1 % concentration in peptone water with phenol red indicator.

vi) Nitrates were reduced to nitrites (Griess - Ilosva method),

vii) Methyl - red and Voge - Proskauer tests were both negative,

viii) A blue - green pigment was produced which was soluble in chloroform. The pigment was tested by means of the method described by Conroy (1960), and gave reproducible results.

It was concluded that the organism isolated was a typical pyocyanogenic strain of *Pseudomonas aeruginosa*.

Discusión

Pseudomonas aeruginosa is of widespread distribution, having been isolated in many countries of the world from water, soil, faeces, plants, and foodstuffs, in addition to lesions and suppurative conditions of man and animals. A detailed study of the distribution of this organism in the waters of the Argentine Republic is given in the work of Ferramola and Monteverde (1939), which authors found it to be of extremely common occurrence.

It is of interest to consider the implications arising from the isolation of the organism in this case, in view of the fact that it was necessary to determine whether faecal contamination of water had occurred. From the results of the counts on coliforms and the total count of viable bacteria, together with the study realised to determine the presence of *Cl. perfringens.* it would be possible to classify the water as of 'Grade I'. As such it would be quite acceptable for driking purposes. However, within the course of the past fe% years, much consideration has been given to the presence of *Ps. aeruginosa* in water as being tantamount to evidence of faecal contamination. Ringen and Drake (1952) were able to isolate this bacterium in 90,40 % of the samples of sewage they examined, and concluded the organism may be found consistently occurring in sewage, and furthermore that human faeces most probably serve as the source of contamination. Curbelo and Marquez likewise feel (1954) that the finding of *Ps. aeruginosa* in water is definite evidence of the water having been contaminated from these sources.

Schiavone and Passerini (1956), give ample evidence for the utilisation of *Ps. aeruginosa* as an indicator of faecal contamination of water supplies, and consider the decisión reached at the 3rd. Interamerican Conference on Sanitary Engineering that every attention should be given to the incidence of this bacterium in waters, as much so as the universally accepted coliform index.

On the basis of the foregoing, it was suggested that the water be unsuitable for driking purposes, as there existed some doubt as to whether it may have been previously contaminated from sewage or faecal sources.

Of further interest is the finding of Reitler and Seligman (1957) that *Pseudomonas aeuriginosa* is capable of inhibiting the growth of *Escherichia coli*, sometimes to a very marked extent. These workers found that in the examination of some 1,000 samples of water, the number of coliform organisms present was related directly to the numbers of *Ps. aeruginosa* in the sample. This phenomenon was so pronounced that it may even be suggested that the numbers of coliforms is practically determined by the numbers of *Ps. aeruginosa*. It was unfortunately not possible to perform any quantitative studies during the course of his investigation. but the apparent absence of coliform types may be regarded as supporting evidence for the findings of Reitler and Seligman, in niew of the presence of the pseudomonad in almost pure culture, and the lowered number of total viable bacteria.

The conclusions drawn from this work are that the presence of *Ps. aeruginosa* in water should lead one to suspect the potability of the water

sample, even where the coliform index and other such factors may be well within the normal limits for the evidence given by many other workers, whose articles and reports contain detailed findings on this subject.

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SUMMARY

A sample of well water was examined, and it was found that the total bacterial count and the coliform count were of no significance in condemning the water as unsuitable for drinking purposes. *Pseudomonas aeruginosa* was found to be the predominating organism present, and the possible relationship between this organism and those of the coliform group is considered. Reference to recent published work is made, in an attempt to show that the sample received should rightly be regarded as having been contaminated from faecal or sewage sources.

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