Further isolations of non-flagellate *Pseudomonas aeruginosa*

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SYNOPSIS Three strains of non-flagellate *Ps. aeruginosa* isolated from pathological specimens have already been described. The purpose of this paper is to report further isolates. All strains have been shown to be different types isolated from a variety of infections.

Since the isolation of three non-flagellate strains of *Ps. aeruginosa* reported recently (Lee, 1973), I have during the course of routine microbiological investigations isolated further distinct strains from typical *Pseudomonas* infections. Despite exhaustive attempts to induce flagellation and motility, all six strains have remained unchanged. Other isolates were not motile on primary isolation but motility was induced by cultural methods.

Isolations

Non-flagellate *Ps. aeruginosa* was isolated from an infected ear, a urinary tract infection, and an operation wound, each isolate from a different patient. The strains are referred to as strains 4, 5, and 6 respectively.

MATERIALS AND METHODS

Primary isolation was made by inoculating the specimens directly onto nutrient blood agar and MacConkey agar and the organisms were identified using the biochemical and typing methods previously employed.

INVESTIGATIONS FOR MOTILITY

Since the original work on non-motile strains was initiated a standard procedure has been adopted. Twenty-four-hour peptone water cultures incubated at both 37 and 24°C were examined by the hanging-drop technique. Any motile isolates were discarded. Those showing no signs of motility were examined further. The centre tube of a bottle of Craigie's medium was inoculated using a straight wire and incubated at 37°C for four to six days. At the same time agar plates of motility test medium

EE (Difco) were seeded by multiple stab culture. These were examined after 18 and 36 hr for hazy zones around the stab line denoting motility.

Control motile strains were examined with all tests. The growth from Craigie tubes was examined microscopically for motility; if it was not motile it was inoculated into motility test medium and a further Craigie tube.

Results

The strains 4, 5, and 6 were shown by biochemical tests, phage, pyocine, and serological typing methods to be *Ps. aeruginosa*. Electron microscopy was employed to study bacterial morphology. The three strains described here were non-flagellate whereas a control motile strain showed typical polar flagella. Other results are presented in tables I and II. During the examination of *Ps. aeruginosa* strains isolated from routine microbiology of hospital, general practice, and other sources of pathological speci-

Cultural and Biochemical Characteristics	Strain Number		
	4	5	6
Anaerobic growth	-	-	-
Green pigment on nutrient agar	+	+	+
Trimethylamine odour	+	+	+
Growth at 42°C	+	+	+
Growth on cetrimide agar	+	+	+
Oxidase (Kovacs)	+	+	+
Catalase	+	+	+
Nitrate reduction to gaseous nitrogen	+	+	+
Arginine dihydrolase	+	+	+
Gluconate oxidation	-	-	-
Gelatin liquefaction	+	+	+
Colony	Rough	Mucoid	Rough

Table I Cultural and biochemical properties of the non-flagellate *Ps. aeruginosa* strains

Typing Method	Strain Number		
	4	5	6
Pyocine	3	1d	3
Phage	Lysis +	No lysis	Lysis +
Serological	6	Not typable	7

Table II Results of typing methods

mens, most were motile after incubation in peptone water at 37°C. Occasional strains were isolated, however, that were not motile or only sluggishly motile on primary isolation. Active motility was induced by passage through Craigie's medium. Motility test medium EE (Difco) was found to be invaluable in these tests, a motile strain having a large hazy growth around the stab inoculum, a non-motile strain no such growth. Some of the feebly motile strains produced very small zones but these were readily seen when incubation was repeated. All the feebly motile forms were shown to be actively motile after passage through Craigie tubes. Despite repeated passage through Craigie tubes and repeated examinations by hanging drop methods and multiple stab inocula into motility

medium, the phenomenon of motility was not induced by strains 4, 5, and 6.

Discussion

Six distinct strains of non-flagellate *Ps. aeruginosa* have been isolated as prime pathogens as a result of routine work in a small hospital over a period of one year. The fact that the strains are different and that they are being isolated from more than one source and from a variety of infections raises the question of how common non-flagellate *Ps. aeruginosa* is in pathological sites. Approximately 60 actively motile strains were also isolated during this study. Many laboratories do not examine all isolates of *Pseudomonas* for motility and flagellation. It seems to me that the categorical definition of *Ps. aeruginosa* as motile rods is suspect, especially if these findings are corroborated elsewhere.

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Reference

- Lee, F. W. (1973). Non-flagellate *Pseudomonas aeruginosa* in pathological material. *J. clin. Path.*, 26, 826-827.