APPLIED MICROBIOLOGY, July 1972, p. 143-145

Vol. 24, No. 1
Printed in U.S.A.

NOTES

Use of Acetamide Broth in the Isolation of *Pseudomonas aeruginosa* from Rectal Swabs

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Received for publication 19 April 1972

Acetamide broth, used in conjunction with ultraviolet light scanning, was superior to Pseudosel agar in the recovery of *Pseudomonas aeruginosa* from rectal swabs both in time of recognition and total positive isolations.

The recognition of *Pseudomonas aeruginosa* in the intestinal tract of hospitalized patients has been of interest for many ecological and epidemiological reasons. Media containing cetrimide as a selective agent for *P. aeruginosa* have been used frequently for isolation. Lambe and Stewart (2) recently reviewed some of the uses of cetrimide media and reported that one such medium, Pseudosel agar, was not highly selective for *P. aeruginosa*. Hedberg (1) developed acetamide agar selective for *P. aeruginosa* and found that only *Mima polymorpha* would grow in the medium as a contaminant. This report deals with the results of one aspect of studies on isolation of bacteria and yeasts from rectal swabs of burned children in which the recovery rates of *P. aeruginosa* from acetamide broth and Pseudosel agar were compared.

Rectal swabs were taken weekly on a group of acutely burned children and on a group of children with healed burns admitted for reconstructive plastic surgery. For sampling, a cotton swab was inserted into a 3-inch (ca. 7.6 cm) piece of no. 203 Latex surgical tubing (Reichhold Chemicals Inc., Cuyahoga Falls, Ohio) with one end of the tube cut to a tapered form. The swab and tubing were autoclaved in a cotton-plugged test tube. At the time of sampling, the tube was passed into the anal canal without lubrication and the swab was then pushed through the tubing into the rectum, rotated, and withdrawn again into the tubing. Swabs were rinsed in 1 ml of sterile saline, and the swab was used to streak mannitol salt agar (BBL), Littman Oxgall medium (BBL) with 80 μg of gentamicin per ml, MacConkey agar (BBL), and Pseudosel agar (BBL). One quadrant of each plate was streaked with the swab, and the remainder of each plate was streaked with a loop. The swab was then broken aseptically and placed in acetamide broth. All media were incubated aerobically at 37 C and were observed daily for 3 days. Pseudosel plates and acetamide broth were examined with a Blak-Ray model B 10-nm black light lamp (Ultra-Violet Products, Inc., San Gabriel, Calif.). Isolated colonies were streaked on MacConkey or Pseudosel agar, tested for oxidase with Kovac reagent, and tested for growth and pigment production on slants of Pseudosel agar and of F and P media (Difco). Further speciation was not routinely needed, but strains were documented as being *P. aeruginosa* by the pyocine typing method of Zabransky and Day (3). Indicator strains were kindly provided by R. J. Zabransky, Mt. Sinai Hospital, Milwaukee, Wis. Serotyping by the Verder-Evans method was done through the courtesy of J. A. Bass, North Texas State University, Denton, Texas. The results of this phase of the work are not included.

During a period of 13 consecutive weeks, 295 rectal swabs were examined from 40 acute and 47 reconstructive burned children. *P. aeruginosa* was isolated from 107 (36% positive) specimens in acetamide broth whereas 63 (21% positive) specimens yielded *P. aeruginosa* on Pseudosel agar. In no case was an isolate found

on Pseudosel agar which was not also positive in acetamide broth. Scanning of acetamide broth promoted early detection of pseudomonas, in most cases one full day before the corresponding Pseudosel agar plate (where positive) produced growth. Ultraviolet light, however, was important for use in conjunction with acetamide broth because some swab washings of rectal swabs contained feces which discolored the acetamide broth, thereby making direct detection of growth and pigment production difficult (Fig. 1). Fluorescence produced in acetamide broth was also observed without visible evidence of turbidity, but, in each case, pseudomonas was recovered. In culturing the 295 rectal swabs, five (0.01%) of the Pseudosel plates contained gross contaminants which were all strains of *Klebsiella pneumoniae*. However, as indicated from growth on MacConkey agar, various coliform bacteria were present in all of the positive specimens.

All isolates from acetamide broth grew on Pseudosel agar as a subculture medium. Isolates were tested on Pseudosel, F agar and P agar slants, and on acetamide broth. Fluorescence was produced in all media, but, because acetamide broth was colorless and had no natural fluorescence, pigment production in acetamide broth was more brilliant (Fig. 2). Other species of the genus *Pseudomonas* were not

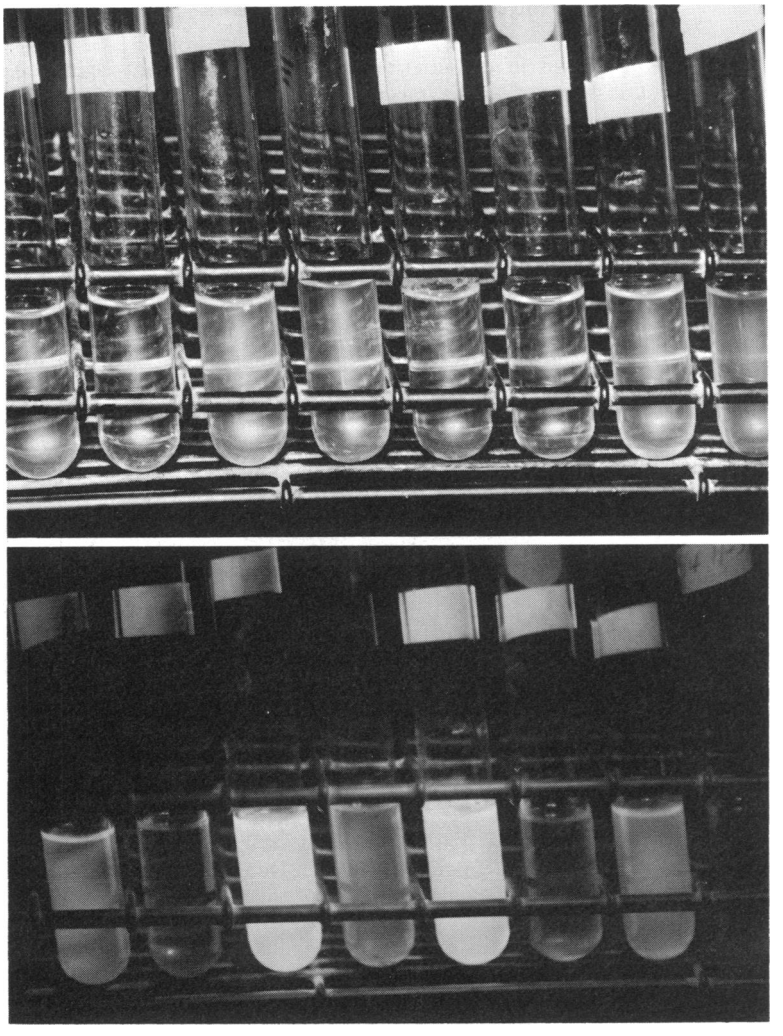

FIG. 1. *Rack containing acetamide broth showing appearance of tubes in regular light (top) and with ultraviolet light (bottom). Swabs were removed for photograph. Bottom figure, third and fifth tubes from left are positive for pseudomonas.*

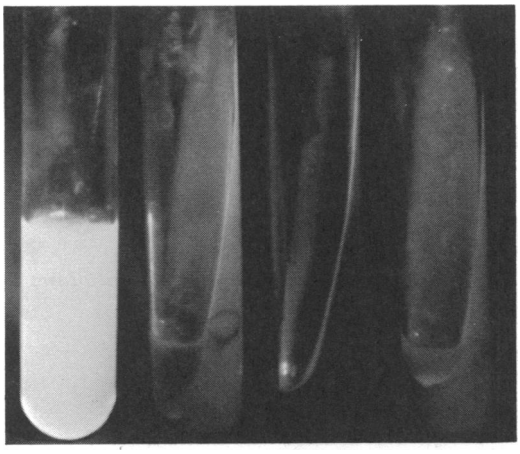

FIG. 2. *Culture of Pseudomonas aeruginosa in ultraviolet light. From left to right, acetamide broth, Pseudosel, F, and P slants incubated for 24 hr.*

encountered in the study and have been too rare to collect and examine for growth in acetamide broth.

Considering the swab used for the rectal sample was used to streak four plates before being placed in acetamide broth, the results of this study indicate that acetamide broth, as originally described by Hedberg (1), is superior to Pseudosel agar as a single isolation medium for *P. aeruginosa*, and ultraviolet light contributes significantly to its use. The broth has also been valuable for large-scale environmental studies where numerous swabbings of sinks and other areas were cultured, and whole racks of tubes could be scanned with ultraviolet light.

This investigation was supported by funds from the Shriners of North America.

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