Incidence of "Oxidase-Variable" Strains of *Aeromonas hydrophila*

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Received for publication 11 December 1978

Certain strains of *Aeromonas hydrophila* are oxidase negative when grown on gram-negative selective and differential media. Of 100 strains of *A. hydrophila* examined, 8 were found to possess this characteristic. Information is provided on how to detect these common variants of *A. hydrophila*.

The determination of oxidase activity is a key characteristic used in the identification of most gram-negative bacteria. In oxidase-positive organisms the *p*-phenylenediamine reagent reacts with oxidized cytochrome *c* complex to form reduced cytochrome *c* complex and a colored oxidation product. Organisms which do not use cytochrome *c* complex as a respiratory enzyme are oxidase negative (1).

Members of the genus *Aeromonas* are defined as being oxidase positive (3). However, an isolate of *Aeromonas hydrophila* variable in its ability to produce indophenol oxidase has recently been described (5). The isolate was oxidase negative when grown on gram-negative selective and differential media, but was oxidase positive when grown on nonselective media. It was proposed that oxidase testing be performed on colonies grown on nonselective media to assure valid results, at least until the extent and/or cause of the phenomenon has been determined. Failure to properly perform the oxidase test may lead to the misidentification of *A. hydrophila* as *Escherichia coli* or *Serratia* (3).

The strain described above was isolated in April 1976, by the Microbiology Laboratory of the University of Kentucky Medical Center, Lexington (UKMC). In March and April 1977, two additional "oxidase-variable" strains of *A. hydrophila* were isolated by the Microbiology Section, Pathology Service, Veterans Administration Medical Center, Lexington, Ky. (VAMC-LEX). The detection of these two strains prompted this study.

A review was conducted to determine the total number of *A. hydrophila* isolated by UKMC and VAMC-LEX for the 13-month period of 1 April 1976, to 30 April 1977. Also, a survey of the culture collections of Analytab Products, Div. of Ayerst Laboratories Inc., Plainview, N.Y. (API), Special Bacteriology Section, Center for Disease Control, Emory University Hospital and

Rudolf Hugh for the presence of oxidase-variable *A. hydrophila* was performed. The results of the review and the culture collection survey are described in this report.

MATERIALS AND METHODS

A. hydrophila isolates from the culture collections of API, Center for Disease Control, Emory University Hospital, and Rudolf Hugh were screened by using Kovacs (4) oxidase test on colonies grown for 24 h on MacConkey agar. Isolates found to be oxidase negative were then tested with a battery of six oxidase tests. The battery of oxidase tests were as follows: (i) Kovacs (4) method using 0.5% (wt/vol) tetramethyl-*p*-phenylenediamine dihydrochloride with a cutoff time of 10 s. (ii) Kovacs (4) method using 1% (wt/vol) dimethyl-*p*-phenylenediamine hydrochloride with a cutoff time of 10 s. (iii) Pathotec strip (General Diagnostics Div., Warner-Lambert Co., Morris Plains, N.J.) with a cutoff time of 30 s. (iv) Dropping 0.5% (wt/vol) tetramethyl-*p*-phenylenediamine dihydrochloride directly on the colonies with a cutoff time of 2 min. (v) Dropping 1.0% (wt/vol) dimethyl-*p*-phenylenediamine hydrochloride directly on the colonies with a cutoff time of 2 min. (vi) Ewing and Johnson (2) method of dropping 1% (wt/vol) alpha-naphthol and 1% (wt/vol) dimethylphenylenediamine oxalate directly on the colonies with a cutoff time of 2 min. These tests were performed on a 24-h old subculture of the oxidase-variable strains on each of the following media: sheep blood agar, brilliant green, eosin-methylene blue, MacConkey, salmonella-shigella, and xylose-lysine-deoxycholate agars. All oxidase tests were timed using a stopwatch.

All media, including Mueller-Hinton agar for susceptibility testing, were prepared by the Microbiology Section, VAMC-LEX, from commercial dehydrated bases (Difco Laboratories, Inc., Detroit, Mich.). Sheep blood agar plates were prepared by adding 5% (vol/vol) defibrinated sheep blood to tryptone blood agar base (Difco).

The identity of all oxidase-variable isolates was confirmed by using the API 20E system.

Antimicrobial susceptibility testing was performed by a standardized disk-diffusion method (6) by using

high potency antimicrobial disks (Pfizer Diagnostics Div., Pfizer Inc., Clifton, N.J.).

RESULTS

After the first oxidase-variable strain of *A. hydrophila* was isolated from bile at UKMC in April 1976 (5), the microbiology personnel at UKMC and VAMCLEX were instructed to retain any additional isolates for further study. Two additional strains were isolated at VAMCLEX in March and April 1977. The strains were isolated from patients who were in geographically separate divisions of the hospital, and differed biochemically, with respect to the Voges-Proskauer test. The isolation of three such strains in a 13-month period prompted a review of the *A. hydrophila* isolates at UKMC and VAMCLEX during the period 1 April 1976 to 30 April 1977, as well as a search of various culture collections for the existence of other oxidase-variable strains.

The review of clinical isolates revealed that UKMC had isolated *A. hydrophila* 27 times from 20 different patients. Only one of these isolates was oxidase variable. *A. hydrophila* was isolated nine times from five different patients by VAMCLEX during the same period. Two of those isolates were oxidase variable. After eliminating multiple isolates from the same source, *A. hydrophila* had been isolated from 25 patients, and three of the isolates were oxidase variable.

Five isolates of *A. hydrophila* were sent from Emory University Hospital to VAMCLEX for evaluation. One of the five, isolated from feces in 1970 and used as a quality control strain, was found to be oxidase variable.

The aerobic laboratory of API screened isolates of *A. hydrophila* from the API, Center for Disease Control, and Rudolf Hugh culture collections by using the Kovacs (4) oxidase procedure. Four oxidase-variable strains were found among the 70 isolates screened. These isolates were then sent to VAMCLEX for further evaluation.

All eight isolates of oxidase-variable *A. hydrophila* were subjected to a battery of six different oxidase test procedures. These tests were performed on colonies growing on six different media for a total of 36 individual oxidase tests per strain. *Pseudomonas aeruginosa* (ATCC 27853) was tested in the same manner as the *A. hydrophila* strains and served as the positive control for all test procedures. Tables 1 and 2 list the average values for the oxidase tests for *P. aeruginosa* (ATCC 27853) and for the eight oxidase-variable strains of *A. hydrophila*, respectively. The data show that in all cases *P. aeruginosa* was oxidase positive, while the *A.*

TABLE 1. Oxidase reactions of *P. aeruginosa* (ATCC 27853) on various media

Medium ^a	Oxidase Method ^b :					
	TPD-K (10 s) ^c	DPD-K (10 s)	PTS (30 s)	TPD-D (120 s)	DPD-D (120 s)	EJR (120 s)
SBA	2 ^d	3	8	36	35	34
BG	4	4	14	44	38	46
EMB	5	6	14	39	40	22
MC	3	5	12	24	29	29
SS	1	2	3	24	24	24
XLD	5	6	9	42	46	29

^a SBA, 5% sheep blood agar; BG, brilliant green agar; EMB, eosin-methylene blue agar; MC, MacConkey agar; SS, salmonella-shigella agar; XLD, xylose-lysine-deoxycholate agar.

^b TPD-K, tetramethyl-*p*-phenylenediamine-Kovacs; DPD-K, dimethyl-*p*-phenylenediamine-Kovacs; PTS, PathoTec strip; TPD-D, tetramethyl-*p*-phenylenediamine-drop; DPD-D, dimethyl-*p*-phenylenediamine-drop; EJ, Ewing and Johnson reagents.

^c Cutoff time.

^d Time in seconds for positive reaction.

TABLE 2. Oxidase reactions of eight strains of *A. hydrophila* on various media

Medium ^a	Oxidase Method ^b :					
	TPD-K (10 s) ^c	DPD-K (10 s)	PTS (30 s)	TPD-D (120 s)	DPD-D (120 s)	EJR (120 s)
SBA	1 ^d	1	4	29	43	20
BG	—	—	—	—	—	—
EMB	—	—	—	—	—	—
MC	—	—	—	—	—	—
SS	—	—	—	66 (4) ^e	—	—
XLD	—	—	—	50 (1)	—	—

^a See footnote *a* of Table 1 for abbreviations of media.

^b See footnote *b* of Table 1 for abbreviations of oxidase methods.

^c Cutoff time.

^d Time in seconds for positive reaction.

^e Number of strains positive.

hydrophila strains were oxidase positive only on sheep blood agar, with the exception of four strains which were positive with tetramethyl-*p*-phenylenediamine dihydrochloride on salmonella-shigella agar. One of those strains was also positive with the same procedure on xylose-lysine-deoxycholate agar.

Oxidase-variable strains of *A. hydrophila* were isolated from a variety of sources. One strain was isolated from each of the following: bile, blood, finger wound, great toe ulcer, stool, and throat. The sources of the two remaining isolates are unknown.

Identification of these organisms is not difficult, provided one is aware of their existence. The biochemical characteristics of these eight isolates are similar to those previously published

for an oxidase-variable strain of *A. hydrophila* (5). Some variations were observed with the Voges-Proskauer, gelatin liquification, and sorbitol and amygdalin fermentation reactions. These variations resulted in the five biotypes or API profiles listed in Table 3. These organisms could be misidentified as *E. coli* if oxidase activity was tested from a gram-negative selective and differential medium or if oxidase activity was not tested. This is particularly true if only a small number of biochemical tests were used in the identification process.

One other key characteristic is that all eight of these isolates were lactose positive on the differential media used in this study. No lactose-negative strains of *A. hydrophila* have been found to be oxidase variable. However, not all strains of lactose-positive *A. hydrophila* were oxidase variable.

Table 3 also lists the identifications of the eight isolates for both the "oxidase-negative" and oxidase-positive variations based on the API seven-digit profile.

Antibiograms were determined for eight oxidase-variable and 15 "oxidase-constant" strains of *A. hydrophila*. All eight oxidase-variable strains were resistant to ampicillin, carbenicillin, and cephalothin and were susceptible to chloramphenicol, gentamicin, kanamycin, nitrofurantoin, polymyxin B, tetracycline, and trimethoprim-sulfamethoxazole. Seven of the eight were susceptible to sulfonamides. Of the 15 oxidase-constant strains, one was susceptible to ampicillin and carbenicillin. Four were susceptible and three were intermediate to cephalothin. However, none of these seven strains were susceptible to all three beta-lactam antibiotics. One strain was resistant to sulfonamides. All 15 strains were susceptible to chloramphenicol, gentamicin, kanamycin, nitrofurantoin, polymyxin B, tetracycline, and trimethoprim-sulfamethoxazole.

TABLE 3. API profiles of eight oxidase-variable strains of *A. hydrophila*

No. of strains	Oxidase negative		Oxidase positive	
	Profile	Identification	Profile	Identification
1	3044122	None	3044126	<i>A. hydrophila</i> (1/1,823) ^a
1	3046122	None	3046126	<i>A. hydrophila</i> (1/107)
4	3046123	None	3046127	<i>A. hydrophila</i> (1/68)
1	3047123	None	3047127	<i>A. hydrophila</i> (1/34)
1	3047522	None	3047526	<i>A. hydrophila</i> (1/751)

^a API Profile index probability.

DISCUSSION

A total of 100 strains of *A. hydrophila* were studied to determine the incidence of oxidase variability. Twenty-five of the isolates were reviewed retrospectively, and 75 were characterized prospectively. Eight of the 100 strains were found to be oxidase variable. The 8% incidence indicates that these strains are common variants of *A. hydrophila*. Further evidence to support this statement comes from the fact that five additional oxidase-variable strains have been isolated but were not included in this study. Three were isolated in Lexington, Ky. (two at UKMC, one at VAMCLEX), and two were isolated in Detroit Mich. (personal communication from William J. Brown).

The oxidase-variable and oxidase-constant strains of *A. hydrophila* do not have significantly different antibiograms, except that the oxidase-constant strains appear to be somewhat more susceptible to cephalothin than are the oxidase-variable strains.

Laboratories using minimal biochemical sets to identify *Enterobacteriaceae* might identify the oxidase-negative variation of these *A. hydrophila* strains as *E. coli*. Resistance to ampicillin, carbenicillin, and cephalothin is not usual for *E. coli*, but would not necessarily alert the laboratory that it may be dealing with an organism other than *E. coli*.

For the biotypes studied to date, the API profiles for the oxidase-negative variations listed in Table 3 are not in the profile index, but fall between valid profiles for *A. hydrophila*. The API profiles for the oxidase-positive variations listed in Table 3 are considered to be excellent identifications for *A. hydrophila*. Thus, the finding of an oxidase-negative organism whose profile index number is not listed, yet is between two valid profiles for *A. hydrophila*, should lead to retesting the oxidase activity using colonies grown on blood agar.

Another fact that may be useful in recognizing oxidase-variable strains of *A. hydrophila* is that they ferment lactose on the differential media employed in this study. Oxidase-constant strains may or may not ferment lactose. At this time, no oxidase-variable strains have been isolated which fail to ferment lactose.

In order to facilitate the proper identification of oxidase-variable strains of *A. hydrophila*, it is recommended that the oxidase test be performed only from a nonselective medium such as blood agar. If there is need to test an organism growing on a gram-negative selective and differential medium for oxidase activity, such testing should be limited to non-lactose-fermenting colonies.

ACKNOWLEDGMENTS

We thank Edward O. Hill, Rudolf Hugh, and Robert E. Weaver for kindly providing many of the strains used in this study.

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