Urease Production from Clinical Isolates of Beta-Hemolytic *Escherichia coli*

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Received for publication 15 May 1978

Twenty-four lactose-fermenting, urease-producing strains of beta-hemolytic *Escherichia coli* were isolated from a variety of clinical material. All isolates were indole positive, citrate negative, and produced the characteristic green metallic sheen on eosin-methylene blue agar.

Urease production in the family *Enterobacteriaceae* is seen in *Proteus*, *Klebsiella*, *Citrobacter*, *Yersinia*, and also in species of *Serratia* and *Enterobacter* (2, 4). To the best of our knowledge, there has been only one report of urease production from clinical isolates resembling *Escherichia coli* (8).

Since December 1976, our laboratory has isolated 24 strains of lactose-fermenting, urease-producing, gram-negative bacteria resembling *E. coli*. Unlike the previous report (8), all our isolates were beta-hemolytic on 5% sheep blood agar upon initial isolation and during subsequent transfers. These isolates were not associated with nosocomial infections but were obtained from a variety of clinical specimens including: throat (15/24), urine (7/24), sputum (1/24), and wound (1/24).

Urease activity was first recognized after biochemically characterizing each of the 24 isolates by using the Analytab Products Inc. (New York, N.Y.) 20E (API 20E) System. Although the API 20E System is well evaluated (3, 6), variability within the system is also documented, especially with urease test results (1). Therefore, urease activity was also assessed by the use of: (i) Christensen urea agar (Baltimore Biological Laboratories, Cockeysville, Md.), (ii) urease test broth (BBL), and (iii) by the method of Leshner et al. (5), which uses Nessler reagent as an indicator. Also, we have verified the initial biochemical characteristics of the 24 isolates as determined with the API system, by using conventional fermentation media. All test incubations were for 24 h at 37°C.

Macrocolonies on 5% sheep blood agar grew as greyish-white, convex colonies with entire edges surrounded by a zone (2 to 3 mm) of beta-hemolysis. On eosin-methylene blue agar, all isolates had the characteristic green metallic sheen. Twenty-two isolates yielded an acid/acid triple sugar iron (TSI) agar reaction, whereas

the remaining two isolates yielded an alkaline/acid TSI reaction. These two isolates were slow lactose fermenters and did not utilize sucrose. An additional 24 h of incubation showed that the TSI reaction for these two isolates was acid/acid. All isolates produced gas but no H₂S from the TSI reaction. Results from the biochemical characterization with conventional fermentation media are shown in Table 1. Antibiotic susceptibilities, using the agar diffusion method (4), are shown in Table 2. Serological

TABLE 1. Biochemical characteristics of isolates resembling beta-hemolytic *E. coli*

Test or substrate	Positive isolates after 24 h	
	No.	% Positive
Indole	24/24	100
Citrate (Simmons)	0/24	0
β-Galactosidase	24/24	100
Methyl red	24/24	100
Voges-Proskauer	0/24	0
Hydrogen sulfide	0/24	0
KCN (growth)	0/24	0
NO ₃ -NO ₂ reduction	24/24	100
Oxidase	0/24	0
Lysine decarboxylase	24/24	100
Ornithine decarboxylase	24/24	100
Arginine dihydrolase	5/24	21
Urease		
Christensen	24/24	100
Urease test broth	24/24	100
Method of Leshner et al. (5)	24/24	100
Acid from:		
D-Glucose	24/24	100
D-Mannitol	23/24	96
D-Inositol	0/24	0
D-Sorbitol	14/17	82
Sucrose	10/24	42
Melibiose	22/24	91
L-Rhamnose	24/24	100
L-Arabinose	24/24	100
Lactose	24/24	100

TABLE 2. Antimicrobial susceptibilities of urease-producing *E. coli* isolates

Antimicrobial agent (U) ^a	No. of isolates inhibited	
	No.	% Inhibited
Ampicillin (10)	16/24	66
Sulfadiazine (0.25)	0/24	0
Gentamicin (10)	24/24	100
Nalidixic acid (30)	22/24	92
Tetracycline (30)	1/24	5
Polymyxin B (300)	22/24	92
Kanamycin (30)	22/24	92
Cephalothin (30)	21/24	88

^a All antimicrobial agents were in the form of Sensi-Discs (BBL).

characterization was not performed.

The biochemical characteristics of the 24 isolates studied clearly suggested an identification of *E. coli* (2), except for the urease activity. Although urease activity can vary within the API 20E System (1) and vary with the type of test medium used (7), all isolates were urease positive within approximately 24 h in each of the three additional test systems used (Table 1). Results obtained by using conventional fermentation media (Table 1) were comparable to the biochemical characteristics obtained from the API 20E System.

Washington and Maker (8) raised the possibility that urease production in *E. coli* isolates may be plasmid mediated. These authors also noted that three of their urease-producing *E. coli* strains were isolated from patients which had antecedent or concurrent infection with *Proteus rettgeri* or *P. mirabilis*. None of our urease-producing *E. coli* strains were isolated along with other urease-producing organisms. However, since Lewis and Rosen (Abstr. Annu. Meet. Am. Soc. Microbiol. 1973, G 218, p. 62) did show that *P. rettgeri* (lac⁺) transferred genes

for urease production and lactose fermentation to *E. coli*, further investigation concerning plasmid-mediated urease production in our beta-hemolytic *E. coli* strains is warranted.

In summary, it may be that urease production, although a rare occurrence for *E. coli*, may not be rare for isolates of beta-hemolytic *E. coli*. The possibility should at least be recognized.

ACKNOWLEDGEMENT

The technical expertise of Karl Mueller is gratefully appreciated.

LITERATURE CITED

1. de Silva, M. I., and S. J. Rubin. 1977. Multiple biotypes of *Klebsiella pneumoniae* in single clinical specimens. *J. Clin. Microbiol.* 5:62-65.
2. Edwards, R. P., and W. H. Ewing. 1972 Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
3. Hansen, S. L., and B. J. Stewart. 1976. Comparison of API and Minitest to Center for Disease Control methods for the biochemical characterization of anaerobes. *J. Clin. Microbiol.* 4:227-231.
4. Lennette, E. H., E. H. Spaulding, and J. P. Traut. 1974. Manual of Clinical Microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
5. Leshner, R. J., M. A. Gerencser, and V. F. Gerencser. 1974. Morphological, biochemical, and serological characterization of *Rothia dentocariosa*. *Int. J. Syst. Bacteriol.* 24:154-159.
6. Smith, P. B., K. M. Tomfohrde, D. L. Rhoden, and A. Balows. 1972. API system: a multitube method for identification of *Enterobacteriaceae*. *Appl. Microbiol.* 24:449-452.
7. Vuye, A., and J. Pijck. 1973. Urease activity of *Enterobacteriaceae*: which medium to choose. *Appl. Microbiol.* 26:850-854.
8. Washington, J. A., II, and M. D. Maker. 1975. Unclassified, lactose-fermenting, urease-producing member of the family *Enterobacteriaceae* resembling *Escherichia coli*. *J. Clin. Microbiol.* 2:70-71.
9. Washington, J. A., II, R. K. W. Yu, and W. J. Martin. 1971. Evaluation of accuracy of multitest micromethod system for identification of *Enterobacteriaceae*. *Appl. Microbiol.* 22:267-269.