JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1999, p. 706–708 Vol. 37, No. 3
0095-1137/99/$04.00+0

Antimicrobial Susceptibility Patterns of *Aeromonas jandaei*, *A. schubertii*, *A. trota*, and *A. veronii* Biotype veronii

TIMOTHY L. OVERMAN[1,2]* AND J. MICHAEL JANDA[3]

Pathology and Laboratory Medicine Service, Veterans Affairs Medical Center, Lexington, Kentucky 40511-1093[1]; Department of Pathology and Laboratory Medicine, University of Kentucky Lexington Medical Center, Kentucky 40536-0298[2]; and Microbial Diseases Laboratory, Division of Communicable Disease Control, California Department of Health Services, Berkeley, California 94704-1101[3]

Received 22 June 1998/Returned for modification 16 October 1998/Accepted 13 November 1998

Fifty-six isolates of four *Aeromonas* species, which have been documented as causative agents of human infections or isolated from human clinical specimens, were subjected to antimicrobial susceptibility testing using a MicroScan WalkAway conventional (overnight incubation) gram-negative panel. The four species tested and the number of isolates of each were as follows: *Aeromonas jandaei*, 17; *A. schubertii*, 12; *A. trota*, 15; and *A. veronii* biotype veronii, 12. All isolates of *A. trota* were susceptible to all antimicrobial agents tested, except cefazolin (20% of isolates were resistant) and cefoxitin (13% of isolates were resistant). All isolates of *A. schubertii* and *A. veronii* biotype veronii, as well as 88% of *A. jandaei* isolates, were resistant to ampicillin. Resistance to ampicillin-sulbactam ranged from 25% of *A. schubertii* strains to 100% of *A. veronii* biotype veronii strains. Cefazolin resistance ranged from 17% of *A. veronii* biotype veronii isolates to 59% of *A. jandaei* isolates. Imipenem resistance was detected in 65% of *A. jandaei* strains and 67% of *A. veronii* biotype veronii strains. *A. jandaei* displayed resistance to piperacillin and ticarcillin in 53 and 71% of the isolates, respectively. *A. veronii* biotype veronii strains were 100% susceptible to piperacillin and 100% resistant to ticarcillin. These antibiogram data may be useful in establishing the identification of these four species when members of the genus *Aeromonas* are isolated from human clinical sources.

Since 1976 the genus *Aeromonas* has been expanded from three phenospecies to 14 nomenspecies. The majority of these newer species were originally discovered when DNA-DNA studies performed on representative strains revealed the presence of genetic heterogeneity. From these studies a number of hybridization groups (HG) were identified, and some were given species names (10). Human infections caused by *Aeromonas hydrophila* (HG 1), *A. caviae* (HG 4), and *A. veronii* biotype sobria (HG 8), formerly phenospecies *A. sobria* (HG 8), are not uncommon and have been reported in clinical microbiology and infectious disease periodicals worldwide. Susceptibility patterns of these species to various antimicrobial agents are well documented (5, 6, 12, 13, 15, 16, 18, 20), as is an apparent increase in resistance to β-lactam antibiotics (11, 14). This increased resistance is attributed to the presence of β-lactamases, including those that hydrolyze the carbapenems, in these organisms (3, 7, 17). Other species documented to cause human infections include *A. jandaei* (HG 9), *A. schubertii* (HG 12), and *A. veronii* biotype veronii (HG 10), formerly *A. veronii* (HG 10) (10). *A. trota* (HG 13) has been isolated from human clinical sources (feces and appendix) but has not been firmly established as a causative agent of human disease (10). Identification of these organisms is problematic even when widely accepted commercial identification systems, both manual and automated, are used. The identification of isolates of *A. schubertii* and *A. veronii* biotype veronii as *Vibrio damsela* and *Vibrio cholerae*, respectively, has been reported (2). The antimicrobial susceptibility patterns of these more recently recognized species of aeromonads are not well documented because of the small number of single-isolate cases reported in the scientific literature. No susceptibility studies examining reasonable numbers of these less frequently isolated *Aeromonas* species have been published to date (10). The purpose of this study was to examine such a collection of reference laboratory-identified isolates of these four species. Determination of antimicrobial susceptibility patterns may also aid in the recognition of these species in the clinical microbiology laboratory.

MATERIALS AND METHODS

Fifty-six isolates of four *Aeromonas* species, which have been documented as causative agents of human infections or isolated from clinical specimens, were subjected to antimicrobial susceptibility testing using a MicroScan WalkAway conventional (overnight incubation) gram-negative panel. The four species tested and the number of each were as follows: *A. jandaei*, 17; *A. schubertii*, 12; *A. trota*, 15; and *A. veronii* biotype veronii, 12.

The Enteric Unit of the Microbial Diseases Laboratory, California Department of Health Services, Berkeley, Calif., identified all of the isolates by a battery of 65 biochemical tests (1, 9). The Clinical Microbiology Laboratory of the Department of Veterans Affairs Medical Center, Lexington, Kentucky, performed all of the antimicrobial susceptibility tests.

Isolates were shipped in vials containing 2.5 ml of motility medium containing 0.3% agar. Upon receipt, the vials were subcultured to sheep blood agar plates (SBAP) (Becton Dickinson Microbiology Systems, Loveton, Md.) and incubated in an ambient air incubator at 35°C for 18 to 24 h. The SBAP were examined for culture purity, and a Kovács oxidase test was performed with an isolated colony. A second colony isolated from each SBAP was subcultured to a second SBAP and incubated as described above for an additional 18 to 24 h. Colonies from the second subculture were subjected to antimicrobial susceptibility testing using the MicroScan Walkaway 40 system (Dade-Behring, West Sacramento, Calif.). Five well-isolated colonies of each strain were picked by using the MicroScan Prompt Inoculation System-D. The standardized inoculum was added to MicroScan Negative Combo Panel Type 16 microwell trays by using the MicroScan Renok device. Panels were placed in the MicroScan Walkaway 40 for overnight incubation and were read automatically by the instrument.

RESULTS

The antibiograms of *A. jandaei* (HG 9), *A. schubertii* (HG 12), *A. trota* (HG 13), and *A. veronii* biotype veronii (HG 10)

* Corresponding author. Mailing address: Pathology and Laboratory Medicine (113CDD), VA Medical Center, Lexington, KY 40511-1093. Phone: (606) 233-4511. Fax: (606) 281-4970. E-mail: toverma@pop.uky.edu.

TABLE 1. Antibiograms for *Aeromonas* species used in this study

Organism (*n*)[a]	% Susceptible to:																		
	Ampicillin	Ampicillin-sulbactam	Piperacillin	Ticarcillin	Cefazolin	Cefotaxime	Cefoxitin	Ceftazidime	Ceftriaxone	Cefuroxime	Amikacin	Gentamicin	Tobramycin	Imipenem	Aztreonam	Ciprofloxacin	Ofloxacin	Trimethoprim-sulfamethoxazole
A. jandaei (17)	12	12	47	29	41	100	94	100	100	100	100	100	94	35	100	100	100	100
A. schubertii (12)	0	75	92	83	67	100	83	100	100	92	92	92	58	100	100	100	100	100
A. trota (15)	100	100	100	100	80	100	87	100	100	100	100	100	100	100	100	100	100	100
A. veronii[b] (12)	0	0	100	0	83	100	100	100	100	100	100	100	83	33	100	100	100	100

[a] *n*, number of strains tested.
[b] *A. veronii* biotype veronii.

are shown in Table 1. The MICs at which 50% of isolates were inhibited (MIC_{50}s) and MIC_{90}s of *A. jandaei* (HG 9), *A. schubertii* (HG 12), *A. trota* (HG 13), and *A. veronii* biotype veronii (HG 10) are shown in Table 2.

DISCUSSION

Aeromonas species have been the subject of a number of antimicrobial susceptibility studies over the last 30 years. Most of these studies have usually involved the three readily recognized phenospecies, *A. hydrophila*, *A. caviae*, and *A. veronii* biotype sobria (5, 6, 12, 13, 15, 16, 18, 20). In the last decade, increased resistance of these organisms to β-lactam antibiotics has been described in Japan (14) and Taiwan (11).

Until now, susceptibility data for these less frequently isolated *Aeromonas* species have been lacking (10). The results of this study indicated that these species of the genus *Aeromonas* were more susceptible to narrow-spectrum cephalosporins than the more frequently isolated species. However, resistance also occurred in these less frequently isolated species.

A. jandaei was the most resistant of the four species. Most isolates were resistant to penicillins, including both piperacillin and ticarcillin. Sixty-five percent of the *A. jandaei* strains were also resistant to imipenem. Previously reported *Aeromonas* resistance to imipenem varies from 3% (14) to 14% (11) for *A. veronii* biotype sobria and is 8% for *A. hydrophila* (11, 14). Recognition of *A. jandaei* isolates may be enhanced by the increased resistance to penicillins, including piperacillin and ticarcillin, and to imipenem. It is notable that this species which was the most resistant is, of the four species in this study, the one most frequently isolated from blood in clinical infections (10).

A. schubertii isolates had a susceptibility pattern very similar to those that have been reported for *A. hydrophila* and *A. caviae* (12).

A. trota was the most susceptible of the four species, with all

TABLE 2. MICs for *Aeromonas* species in this study[a]

Antimicrobial agent	*A. jandaei* (17)		*A. schubertii* (12)		*A. trota* (15)		*A. veronii*[b] (12)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Ampicillin	—[c]	—	—	—	4	8	—	—
Ampicillin-sulbactam	—	—	8	16	8	8	—	—
Piperacillin	32	—	8	16	8	8	8	16
Ticarcillin	64	—	8	32	8	8	32	64
Cefazolin	16	—	4	—	4	16	4	—
Cefotaxime	4	4	4	4	4	4	4	4
Cefoxitin	2	8	2	—	8	16	2	4
Ceftazidime	2	2	2	2	2	2	2	2
Ceftriaxone	4	4	4	4	4	4	4	4
Cefuroxime	2	2	2	8	2	4	2	2
Amikacin	4	8	4	16	4	8	8	16
Gentamicin	2	4	2	4	2	2	2	4
Tobramycin	4	4	4	—	2	2	4	6
Imipenem	8	—	4	4	4	4	8	—
Aztreonam	8	8	8	8	8	8	8	8
Ciprofloxacin	1	1	1	1	1	1	1	1
Ofloxacin	2	2	2	2	2	2	2	2
Trimethoprim-sulfamethoxazole	0.5	0.5	0.5	0.5	0.5	2	0.5	0.5

[a] MIC_{50}s and MIC_{90}s are given in micrograms per milliliter. Parenthetical values after strains are numbers of strains tested.
[b] *A. veronii* biotype veronii.
[c] —, no MIC_{50} or MIC_{90}.

isolates susceptible to ampicillin. Susceptibility to ampicillin is a characteristic of *A. trota* (4) and appears to be unique to this species because the more frequently isolated species of *Aeromonas* are usually resistant to ampicillin (11, 12, 14). Eighty percent of the *A. trota* strains were also susceptible to cefazolin. With the exception of *A. veronii* biotype sobria (8), the other species of the genus *Aeromonas* are resistant to narrow-spectrum cephalosporins (5, 6, 12, 13, 15, 16, 18, 20). Recognition of *A. trota* isolates may be enhanced by the increased susceptibility to narrow-spectrum cephalosporins and to penicillins, especially ampicillin.

A. veronii biotype veronii isolates displayed 100% susceptibility to piperacillin and 100% resistance to ticarcillin. Sixty-seven percent of the *A. veronii* biotype veronii strains were also resistant to imipenem. *A. veronii* biotype veronii was the only species to exhibit any significant aminoglycoside resistance, with 42% of the isolates being resistant to tobramycin. Previously reported values for *Aeromonas* resistance to tobramycin are 23% for *A. veronii* biotype sobria and 25% for *A. caviae* and *A. hydrophila* (11). Eighty-three percent of the *A. veronii* biotype veronii isolates were susceptible to cefazolin. Recognition of *A. veronii* biotype veronii isolates may be enhanced by the susceptibility to piperacillin coupled with resistance to ticarcillin, resistance to imipenem, and susceptibility to narrow-spectrum cephalosporins.

The recommended therapy for infections caused by members of the genus *Aeromonas* is the use of fluoroquinolones. However, fluoroquinolones should not be used in treating pediatric patients. Alternative therapies include trimethoprim-sulfamethoxazole, aminoglycosides, imipenem, meropenen, parenteral cephalosporins (expanded spectrum and broad spectrum), and tetracyclines (19). The data from the present study indicated the following. (i) *A. veronii* biotype veronii and *A. schubertii* had markedly increased resistance to tobramycin. (ii) *A. veronii* biotype veronii and *A. jandaei* were generally resistant to imipenem. (iii) *A. schubertii* and *A. trota* were less susceptible to cefoxitin, an expanded-spectrum cephalosporin, than to broad spectrum cephalosporins. Perhaps tobramycin, imipenem, and cefoxitin should be removed from the list of alternative therapies.

REFERENCES

1. **Abbott, S. L., W. K. W. Cheung, S. Kroske-Bystrom, T. Malekzadeh, and J. M. Janda.** 1992. Identification of *Aeromonas* strains to genospecies level in the clinical laboratory. J. Clin. Microbiol. **30:**1262–1266.
2. **Abbott, S. L., L. S. Seli, M. Catino, Jr., M. A. Hartley, and J. M. Janda.** 1998. Misidentification of unusual *Aeromonas* species as members of the genus *Vibrio*: a continuing problem. J. Clin. Microbiol. **36:**1103–1104.
3. **Bakken, J. S., C. C. Sanders, R. B. Clark, and M. Hori.** 1988. β-Lactam resistance in *Aeromonas* spp. caused by inducible β-lactamases active against penicillins, cephalosporins, and carbapenems. Antimicrob. Agents Chemother. **32:**1314–1319.
4. **Carnahan, A. M., T. Chakraborty, G. R. Fanning, D. Verma, A. Ali, J. M. Janda, and S. W. Joseph.** 1991. *Aeromonas trota* sp. nov., an ampicillin-susceptible species isolated from clinical specimens. J. Clin. Microbiol. **29:** 1206–1210.
5. **Fainstein, V., S. Weaver, and G. P. Bodey.** 1982. In vitro susceptibilities of *Aeromonas hydrophila* against new antibiotics. Antimicrob. Agents Chemother. **22:**513–514.
6. **Fass, R. J., and J. Barnishan.** 1981. In vitro susceptibilities of *Aeromonas hydrophila* to 32 antimicrobial agents. Antimicrob. Agents Chemother. **19:** 357–358.
7. **Iaconis, J. P., and C. C. Sanders.** 1990. Purification and characterization of inducible β-lactamases in *Aeromonas* spp. Antimicrob. Agents Chemother. **34:**44–51.
8. **Janda, J. M., and M. R. Motyl.** 1985. Cephalothin susceptibility as a potential marker for the *Aeromonas sobria* group. J. Clin. Microbiol. **22:**854–855.
9. **Janda, J. M., S. L. Abbott, S. Khashe, G. H. Kellogg, and G. H. Shimada.** 1996. Further studies on biochemical characteristics and serologic properties of the genus *Aeromonas*. J. Clin. Microbiol. **34:**1930–1933.
10. **Janda, J. M., and S. L. Abbott.** 1998. Evolving concepts regarding the genus *Aeromonas*: an expanding panorama of species, disease presentations, and unanswered questions. Clin. Infect. Dis. **27:**332–344.
11. **Ko, W. C., K. W. Yu, C. Y. Liu, C. T. Huang, H. S. Leu, and Y. C. Chuang.** 1996. Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. Antimicrob. Agents Chemother. **40:**1260–1262.
12. **Koehler, J. M., and L. R. Ashdown.** 1993. In vitro susceptibilities of tropical strains of *Aeromonas* species from Queensland, Australia, to 22 antimicrobial agents. Antimicrob. Agents Chemother. **37:**905–907.
13. **Kuijper, E. J., M. F. Peeters, B. S. C. Schoenmakers, and H. C. Zanen.** 1989. Antimicrobial susceptibility of sixty human fecal isolates of *Aeromonas* species. Eur. J. Clin. Microbiol. Infect. Dis. **8:**248–250.
14. **Morita, K., N. Watanabe, S. Kurata, and M. Kanamori.** 1994. β-Lactam resistance of motile *Aeromonas* isolates from clinical and environmental sources. Antimicrob. Agents Chemother. **38:**353–355.
15. **Motyl, M. R., G. McKinley, and J. M. Janda.** 1985. In vitro susceptibilities of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* to 22 antimicrobial agents. Antimicrob. Agents Chemother. **28:**151–153.
16. **Overman, T. L.** 1980. Antimicrobial susceptibility of *Aeromonas hydrophila*. Antimicrob. Agents Chemother. **17:**612–614.
17. **Rasmussen, B. A., and K. Bush.** 1997. Carbapenem-hydrolyzing β-lactamases. Antimicrob. Agents Chemother. **41:**223–232.
18. **Reinhardt, J. F., and W. L. George.** 1985. Comparative in vitro activities of selected antimicrobial agents against *Aeromonas* species and *Plesiomonas shigelloides*. Antimicrob. Agents Chemother. **27:**643–645.
19. **Sanford, J. P., D. N. Gilbert, R. C. Moellering, Jr., and M. A. Sande.** 1997. The sanford guide to antimicrobial therapy, 27th ed. Antimicrobial Therapy, Inc., Vienna, Va.
20. **San Joaquin, V. H., R. K. Scribner, D. A. Pickett, and D. F. Welch.** 1986. Antimicrobial susceptibility of *Aeromonas* species isolated from patients with diarrhea. Antimicrob. Agents Chemother. **30:**794–795.