CLINICAL MICROBIOLOGY REVIEWS, Jan. 2010, p. 35–73 Vol. 23, No. 1
0893-8512/10/$12.00 doi:10.1128/CMR.00039-09
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# The Genus *Aeromonas*: Taxonomy, Pathogenicity, and Infection

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**INTRODUCTION** ....36
**NOMENCLATURE AND TAXONOMY** ....37
  **General Principles and Practices** ....37
  ***Aeromonas* Species: Past to Present** ....37
  **Recently Described *Aeromonas* Species** ....37
    **Expansion of the genus** ....37
    **Trends in the publication of new *Aeromonas* species** ....38
    **Nomenclature and taxonomy issues** ....38
      **(i) Species status—controversial issues** ....39
      **(ii) Taxonomic descriptions in the literature** ....41
      **(iii) Other issues** ....41
  ***Aeromonas* Species—Current Status** ....41
**CLASSIFICATION** ....42
  **General Principles and Practices** ....42
  ***Aeromonas* Classification** ....42
    **The family *Aeromonadaceae*** ....42
    **Numerical taxonomy studies** ....42
    **Phylogenetic analysis of *Aeromonas* species** ....42
***AEROMONAS* AND ECOSYSTEMS** ....43
  **General Description** ....43
  ***Aeromonas* and Aquatic Environments** ....44
    **Overview** ....44
    **Fish diseases** ....44
    ***Aeromonas* and drinking water** ....45
  ***Aeromonas* and Animals** ....45
  ***Aeromonas* in Foods** ....45
**EPIDEMIOLOGY** ....45
**CLINICAL INFECTIONS AND DISEASE-ASSOCIATED SYNDROMES** ....47
  ***Aeromonas* and Gastroenteritis** ....47
    ***Aeromonas* and gastroenteritis: where are we now?** ....47
    ***Aeromonas* gastroenteritis: symptoms, peculiarities, and problems** ....48
    **Atypical *Aeromonas* gastrointestinal presentations and complications** ....50
  **Blood-Borne Infections** ....50
    ***Aeromonas* septicemia in immunocompromised persons** ....50
    ***Aeromonas* infections associated with trauma** ....52
    **Bacteremia in healthy persons** ....52
    **Sepsis and medicinal leech (*Hirudo medicinalis*) therapy** ....52
  **Skin and Soft Tissue Infections** ....52
    **Medicinal leech therapy** ....53
    **Thermal injuries** ....53
    **Zoonotic infections** ....53
  **Intra-Abdominal Infections** ....53
    **Peritonitis** ....54
    **Infections of the hepatobiliary and pancreatic systems** ....54
  **Respiratory Tract Infections** ....54
    **Pneumonia** ....55
    **Other respiratory tract infections** ....55
  **Urogenital Tract Infections** ....55
  **Eye Infections** ....55
**PATHOGENICITY** ....56
  **General Principles and Practices** ....56

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**Animal Models of Infection** ....56
**Organotrophic Disease** ....57
**Gastroenteritis** ....57
**Wound infections** ....58
**Septicemia** ....59
LABORATORY IDENTIFICATION ....60
**Isolation** ....60
**Identification** ....61
**Separation of *Aeromonas* from *Vibrio* and *Plesiomonas*** ....61
**The *A. hydrophila* complex** ....62
**The *A. caviae* complex** ....62
**Separation of *A. hydrophila* from *A. veronii* bv. sobria** ....62
**Other aeromonads isolated from clinical specimens** ....62
**Identification of aeromonads by commercial systems** ....62
**Molecular identification** ....62
**Reporting the isolation and identification of *Aeromonas*** ....64
ANTIMICROBIAL SUSCEPTIBILITY ....65
**Susceptibility Patterns and Testing Methods** ....65
**Resistance Mechanisms** ....65
**β-Lactamases and extended-spectrum β-lactamases (ESBLs)** ....65
**Quinolones** ....67
CONCLUSIONS ....67
REFERENCES ....67

## INTRODUCTION

In many ways, the history of the genus *Aeromonas* mirrors the chronicles of modern-day medical bacteriology, which spans over 100 years, from its birth as a recognized laboratory science in the late 19th and early 20th centuries through its evolution into the molecular postgenomic era. The perception of the genus *Aeromonas* by the scientific community has likewise evolved over the same interval. Initially, aeromonads were recognized only as causing systemic illnesses in poikilothermic animals. Today, the genus *Aeromonas* is regarded not only as an important disease-causing pathogen of fish and other cold-blooded species but also as the etiologic agent responsible for a variety of infectious complications in both immunocompetent and immunocompromised persons. While it is beyond the scope of this review to discuss in detail many aspects of the genus dating from the 1890s to the present, it is important to bring major historical events into perspective. Some of these benchmark achievements that have taken place over the past century are key to understanding current issues and laboratory practices regarding this group of bacteria. Seminal events in the chronology of the genus *Aeromonas* are listed in Table 1.

How much has scientific and medical interest in this genus grown? A search of PubMed using the term "*Aeromonas*" will generate approximately 663 citations covering the period 1944 thru 1980. In contrast, over the last 27 years (1981 to present) the number of research publications has grown sixfold, with the total number of entries now standing at 4,928. Similarly, in 1980, only four *Aeromonas* species had standing in nomenclature (*Aeromonas hydrophila*, *A. punctata*, *A. salmonicida*, and *A. sobria*). Today, that number is at 24, with the recent proposal of "*A. tecta*" (http://www.bacterio.cict.fr/). Finally, the first complete genome of an *Aeromonas* strain (ATCC 7966$^{T}$) has been sequenced, with 5,195 predicted protein-encoding genes identified (261). These accomplishments are a testimony not only to the molecular genomic revolution we are currently witnessing but also to how far our scientific and medical knowledge concerning this genus has evolved in 117 years.

In 2000, Joseph and Carnahan (150) authored an article in *ASM News* entitled "Update on the Genus *Aeromonas*." In that article, the authors stated that despite much progress many questions regarding this pathogen remain unanswered. Several noteworthy findings regarding the genus underscore the importance of this statement and may shed light on important global regulatory processes in bacteria of disease-causing potential. Quorum-sensing molecules have been detected in many *Aeromonas* species, including *A. hydrophila* and *A. salmonicida* (277). Although only limited data exploring the role that quorum sensing may play in this genus presently exist, the possibilities are quite extensive and include biofilm formation, control of high-cell-density populations, and regulation of virulence expression in response to environmental triggers. Graf and associates have also identified a simple two-species symbiotic model (with *Aeromonas* being one group and *Rikenella* being the other) involving the medicinal leech crop (*Hirudo verbana*) which may shed important light on which genes and regulatory factors control colonization and the establishment of permanent symbiotic relationships (249, 265). Finally, the role of aeromonads as important human pathogens in natural disasters was reinforced recently by the tsunami that struck Thailand in December 2004. In one study of 305 tsunami survivors with skin or soft tissue infections, *Aeromonas* ranked as the single most common pathogen identified, accounting for over 20% of the 641 isolates identified (62).These collective facts are a good reminder of how far we still have to go in understanding processes regulating this important human, animal, and environmental microbe.

The overall focus of this review is to provide a comprehensive update on the genus *Aeromonas*, with a particular emphasis from the clinical microbiologist's perspective, since our last review on the subject in 1998 (143). At the end of each section, a number of pertinent review articles are listed for readers desiring more in-depth information on a particular topic. For

TABLE 1. Seminal events in the history of the genus *Aeromonas*

| Date | Milestone or achievement | Comment | Reference |
|---|---|---|---|
| 1891 | Genus linked to bacteremic ("red leg") disease of frogs | No extant cultures of isolate; presumed to be *Aeromonas* | 78 |
| 1943 | Taxonomy and classification of *Aeromonas hydrophila* defined | Separation from rods with polar flagella | 275 |
| 1951 | First association of genus with human infection (fulminant metastatic myositis) | *Aeromonas* recovered from autopsy samples | 29 |
| 1968 | First major medical report describing an association of the genus *Aeromonas* with a variety of human infections | 28 cases reported; septicemia associated with liver disease (Laennec's cirrhosis) | 291 |
| 1981 | Genus contains multiple distinct species within the mesophilic group | DNA relatedness studies based upon 55 strains | 237 |
| 1986 | *Aeromonas* phylogenetically distinct from vibrios | Based upon 5S and 16S rRNA gene sequencing; proposed new family (*Aeromonadaceae*) | 49 |
| 2006 | Complete genome sequence (4.7 Mb) of *Aeromonas hydrophila* ATCC 7966$^T$ | Type strain of type species of the genus; lack of fluidity in mobile elements; clues regarding environmental metabolic repertoire | 261 |

more information on historical aspects of the genus, readers may wish to consult authoritative reviews by Ewing and colleagues (78), Altwegg and Geiss (9), and von Graevenitz (289).

## NOMENCLATURE AND TAXONOMY

### General Principles and Practices

While there are no rules governing the classification of bacteria, there are rules presiding over the nomenclature of bacteria (74). The rules forming the foundation of bacterial nomenclature are governed by the *International Code of Nomenclature of Bacteria* (*Bacteriological Code*) (269) and changes approved by the International Committee on Systematics of Prokaryotes (ICSP) (75; http://www.the-icsp.org/default.htm). The ICSP is an international committee within the International Union of Microbiological Societies that is responsible for issues arising regarding bacterial taxonomy and nomenclature. Publication of a proposal to recognize a new species, however, does not necessarily imply validity or accuracy. Publication only means that the minimum requirements have been met concerning the rules of nomenclature for describing a new taxon, such as that the species name must be described clearly, the etymology of the new name given, a description of the properties of the taxon provided, and a type strain designated (75). A standardized format for publication of names has been developed and is called the protologue (283). The rules of nomenclature apply to taxonomic categories down to the subspecies level but do not include ranks below this level, such as biovar, biogroup, biotype, and serotype (rule 5d).

### *Aeromonas* Species: Past to Present

From the creation of the genus *Aeromonas* in 1943 through the mid-1970s, aeromonads could be broken down roughly into two major groupings, based upon growth characteristics and other biochemical features (138). The mesophilic group, typified by *A. hydrophila*, consisted of motile isolates that grew well at 35 to 37°C and were associated with a variety of human infections. The second group, referred to as psychrophilic strains, caused diseases in fish, were nonmotile, and had optimal growth temperatures of 22 to 25°C. This group contained isolates that currently reside within the species *A. salmonicida*.

Beginning in the mid-1970s and continuing for almost 10 years thereafter, several groups, including the Institut Pasteur in Paris, the Centers for Disease Control and Prevention (CDC) in Atlanta, GA, and the Walter Reed Institute of Research in Washington, DC, spearheaded an effort to redefine the mesophilic group based upon DNA relatedness studies. Over that span of time, DNA hybridization investigations revealed that multiple hybridization groups (HGs) existed within each of the recognized mesophilic species (*A. hydrophila*, *A. sobria*, and *A. caviae*) (84, 237). These unnamed HGs were represented by reference strains, since in each case they could not be separated unambiguously from each other by simple biochemical means. The term "phenospecies" was coined to refer to a single heterogeneous species (such as *A. sobria*) containing multiple HGs within it. Hybridization groups were given numbers for either defined species (*A. hydrophila* = HG1) or reference strains representing unnamed species. In general, there was consensus agreement on the first 12 HGs between the Institut Pasteur and CDC. At a later time, when phenotypic markers were recognized that clearly separated these groups from one another, new species were proposed, such as *A. trota* (Voges-Proskauer [VP] negative, ampicillin susceptible), *A. schubertii* (D-mannitol negative), and *A. jandaei* (sucrose negative) (27).

### Recently Described *Aeromonas* Species

**Expansion of the genus.** Bacterial taxonomy has witnessed a logarithmic explosion in the number of proposed species over the past 2 decades. This explosion is due largely to the general availability of DNA sequencers and the relative ease with which partial or complete 16S rRNA gene sequences can be determined, as opposed to the more cumbersome and expensive gold standard, DNA-DNA hybridization. From the advent of the approved lists in 1980 to September 2007, more than a 450% increase in the number of validly published bacterial species has occurred (145). This increase has been most striking since 2000, with over 3,500 species proposed. The genus *Aeromonas* has also reflected a similar trend, with seven new

TABLE 2. Recently proposed taxa in the genus *Aeromonas*

| *Species nova* | Date | No. of strains[a] | G+C content (mol%) | DNA relatedness study | Phylogenetic analysis | Nearest neighbor[b]: DNA relatedness (%) | Nearest neighbor[b]: 16S rRNA gene sequence similarity (%) | Validity challenged | Reference |
|---|---|---|---|---|---|---|---|---|---|
| *A. tecta* | 2008 | 5 | | Yes | 16S rRNA, *gyrB*, *rpoD* | *A. eucrenophila* (40) | *A. eucrenophila* (99.5) | No | 59 |
| *A. aquariorum* | 2008 | 13 | | Yes | 16S rRNA, *gyrB*, *rpoD* | *A. eucrenophila* (55.7) | *A. trota* | No | 197 |
| *A. bivalvium* | 2007 | 2 | 62.6 | Yes | 16S rRNA | *A. caviae*, *A. media*, *A. molluscorum* (44) | *A. popoffii* (99.7) | No | 206 |
| *A. sharmana* | 2006 | 1 | 60.7 | No | 16S rRNA | | A-8[c] (99.2) | Yes | 255 |
| *A. molluscorum* | 2004 | 5 | 59.4 | Yes | 16S rRNA | *A. media* (45) | *A. encheleia* (99.7) | No | 205 |
| *A. simiae* | 2004 | 2[d] | | Yes | 16S rRNA | *A. trota* (18) | *A. schubertii* (98.3) | No | 109 |
| *A. culicicola* | 2002 | 3 | | Yes | 16S rRNA | *A. sobria* (61) | *A. jandaei* (99.9) | Yes | 234 |

[a] Number of distinct strains included in the proposal.
[b] Based upon DNA relatedness or 16S rRNA gene sequence similarity. For some studies, 16S rRNA gene sequence similarities were not reported. Values reported are minimum relatedness figures if multiple values are reported.
[c] Uncultured bacterium.
[d] May be two isolates of a single strain (253).

species described since 2002. While there are 24 validly published species names in the genus *Aeromonas* at present, the second edition of *Bergey's Manual of Systematic Bacteriology* (*Bergey's*) recognizes far fewer (192). The difference in the number of species listed in *Bergey's* and those with standing in nomenclature (Internet) is not only due to the recent description of new taxa but also because some epithets are illegitimate or heterotypic synonyms of previously published species (see below).

**Trends in the publication of new *Aeromonas* species.** Recently proposed taxa for inclusion in the genus *Aeromonas* are listed in Table 2. A number of disturbing elements can be seen in these proposals, which parallel trends noted for other genera and species. As highlighted by Frederiksen and others (97), while the number of new species/subspecies proposed in the *International Journal of Systematic and Evolutionary Microbiology* (IJSEM; formerly called the *International Journal of Systematic Bacteriology*) continues to rise, the tendency to propose new species based upon single strains (~40%) has also risen substantially. Of seven recently published *Aeromonas* species, only three of these (*A. molluscorum*, *A. aquariorum*, and *A. tecta*) were proposed based upon the analysis of more than three strains. This is in sharp contrast to well-defined species such as *A. media*, *A. veronii*, *A. schubertii*, *A. jandaei*, *A. trota*, and *A. bestiarum*, where comparable numbers are much higher (range of 7 to 15 strains, average of 10.2 strains/species). Christensen and colleagues (44) have questioned the validity of describing a new species based upon a single strain (the *Bacteriological Code* makes no recommendations) and point out potential pitfalls in doing so. They suggest that a minimum of five well-characterized strains (characterized both phenotypically and genotypically) should be the minimum standard and have proposed a revision to Recommendation 30b of the *Bacteriological Code*.

A second transparent issue concerns the range and extent of methods employed to describe new *Aeromonas* species. As outlined in a reevaluation of species definition by a prestigious ad hoc committee, certain standard methods should be included in the description of a new species, including almost complete 16S rRNA gene sequences, phenotypic properties including discriminatory markers, and mol% G+C content (274). The same committee suggested following the recommendation of Christensen et al. (44), to describe a species based upon more than one strain. As can be seen in Table 2, four studies lacked mol% G+C contents, and another proposal was based upon a single strain without DNA relatedness studies. Biochemical data available on most newly described species separated most groups from one another by three or more traits, but for some species only a single phenotype provided discrimination.

Of seven recently described species, only *A. tecta* has been recovered from clinical samples, with the remaining six species isolated from environmental sources. The ecological distribution of newly described species is also typically limited, usually to one source or site. Descriptions of new species based upon such a limited ecodistribution may significantly bias the phenotype, and even perhaps the genotype, of the species and type strain designation. In six studies where both 16S rRNA gene sequence and DNA hybridization data were available, only in the *A. tecta* study was there agreement between both methods in regards to the nearest neighbor (59). For the seventh species (*A. sharmana*), the closest neighbor by 16S rRNA gene sequencing was an uncultured bacterium (255). The validity of two of four species (*A. culicicola* and *A. sharmana*) published between 2002 and 2006 has been questioned based upon new DNA reassociation kinetic information (see below). This suggests that for the foreseeable future, DNA relatedness studies (the gold standard) still need to be included as part of a species proposal, especially if the species is defined on the basis of only a couple of strains and/or strains from a single site.

**Nomenclature and taxonomy issues.** One of the confounding problems that clinical microbiologists face regarding the role that aeromonads play in infectious diseases is how to identify them and what to call them. Part of these issues involves a twisted and convoluted legacy of species names intertwined with taxonomic history coupled to common usage, whether applied correctly or not. One of the more promising avenues for solving many *Aeromonas* nomenclature and taxonomy issues is the future use of full-genome sequencing and microarray analysis (222, 261). A recent study comparing chro-

TABLE 3. Validly published *Aeromonas* species with uncertain taxonomic status[c]

| Species | Taxonomic or nomenclature issue | Synonymous species | Method of analysis[a] | | | | Rule[b] |
|---|---|---|---|---|---|---|---|
| | | | Bio | DNA | Phy | Other | |
| *A. culicicola* | Junior subjective synonym | *A. veronii* | + | + | + | + | 24b |
| *A. sharmana* | Not an *Aeromonas* species | NA | + | − | + | − | NA |
| *A. ichthiosmia* | Junior subjective synonym | *A. veronii* | + | + | − | − | 24b |
| *A. enteropelogenes* | Senior subjective synonym | *A. trota* | + | + | − | − | 24b [principle 1 (1)] |
| *A. punctata* | Senior objective synonym | *A. caviae* | − | − | − | − | 24b [principle 1 (1)] |
| *A. allosaccharophila* | Junior heterotypic synonym | *A. veronii* | + | + | + | − | 24b |

[a] Method used in recent study of the specified taxonomic/nomenclature issue. Bio, biochemical methods; DNA, DNA-DNA hybridization; Phy, phylogenetic studies.
[b] Rules of the *Bacteriological Code* (1990 revision) that apply; others not listed may also apply. NA, not applicable.
[c] Data were obtained from references 118, 127, 128, 131, 175, 196, 226, and 253.

mosomal sequences of *A. hydrophila* ATCC 7966$^T$ to a draft sequence of *A. veronii* bv. sobria HM21 reported that >15% of genomic differences between these two strains were due to bacteriophage or hypothetical genes (261). If this pioneering study is supported by further genomic comparisons indicating significant horizontal gene transfer, microbiologists may need to reconsider how we define species based upon DNA-DNA hybridization. All of these issues cannot be summarized here; for detailed information, please refer to the chapter on *Aeromonas* coauthored by Martin-Carnahan and Joseph (192) in the latest edition of *Bergey's*. The more important and salient issues are discussed below.

**(i) Species status—controversial issues.** There are many outstanding nomenclature problems involving the genus *Aeromonas*. Some of the more prominent issues potentially relevant to clinical microbiologists are listed in Table 3. In most instances, resolution of the taxonomic/nomenclature issue requires a formal "request for an opinion" in IJSEM prior to a subsequent decision being rendered by the Judicial Commission. At the time of this writing, no formal requests for opinions have been made.

*(a)* "*Aeromonas culicicola*." In 2002, Pidiyar et al. (234) described the isolation of a new species, *A. culicicola*, from mosquitoes, utilizing DNA hybridization and 16S rRNA gene sequencing data (Table 2). Subsequent investigations by several groups do not support this proposal, based upon multiple lines of independent research. Huys and others (127) performed DNA relatedness studies and found that the type strains of *A. culicicola* (MTCC 3249$^T$) and *A. veronii* (ATCC 35624$^T$) were 79% to 88% related in reciprocal hybridization tests. These values are above the 70% relatedness threshold indicating species identity and are much higher than the 44% relatedness previously reported by Pidiyar et al., although by a different method (234). Phylogenetic studies employing housekeeping genes such as *gyrB*, *rpoD*, and *dnaJ* rather than the 16S rRNA gene have found that MTCC 3249$^T$ does not exhibit highest similarity to *A. jandaei* but, rather, clusters within the *A. veronii* group at the intraspecies level (175, 226, 253, 272). These results are also consistent with later studies conducted by Pidiyar and coinvestigators, using *gyrB* (233). Finally, there are a number of lines of phenetic data, including numerical taxonomy studies based upon API 20E and API 50CH results and fatty acid methyl ester analysis, that indicate that *A. culicicola* and *A. veronii* are biochemically indistinguishable (including utilization of D-cellobiose), except for the ornithine decarboxylase (ODC)-positive variety (*A. veronii* bv. veronii) (127). The collective result of these studies strongly suggests that "*A. culicicola*" is a later subjective synonym of *A. veronii* (127).

*(b)* "*Aeromonas sharmana*." Saha and Chakrabarti (255) described this new species based upon a single environmental strain (GPTSA-6$^T$) and without DNA-DNA hybridization studies being performed. In that study, the closest 16S rRNA gene sequence match was to an uncultured bacterium, A-8 (Table 2). The closest 16S rRNA gene sequence similarity to GPTSA-6$^T$ found in a cultured organism was to *A. sobria* (95.13%) and *A. molluscorum* (95.04%), so the authors proposed the name "*A. sharmana*." However, Martínez-Murcia and collaborators (196) opined that the description of "*A. sharmana*" does not warrant inclusion within the genus *Aeromonas*. This opinion is due to a number of cardinal features associated with the genus, including the following: (i) the phylogenetic depth of the 16S rRNA gene tree for the genus *Aeromonas* is shallow, with all species exhibiting interspecies sequence similarity values of 96.7% or greater (196); (ii) all current *Aeromonas* species have been defined on the basis of interspecies 16S rRNA gene relationships of 98% or higher, with most being >99% related; (iii) two 16S rRNA gene signatures (positions 86 to 106 and 584 to 604) conserved in many strains belonging to all *Aeromonas* species are missing in *A. sharmana* (196); (iv) based upon additional phylogenetic studies involving *gyrB* and *rpoD*, *A. sharmana* is not considered to belong within the genus *Aeromonas* (253); and finally, (v) *A. sharmana* produces many biochemical reactions atypical for the genus overall, including being nitrate reductase negative, failing to produce lysine or ornithine decarboxlase or arginine dihydrolase, and lacking deoxyribonuclease activity (196, 255). Although this strain falls within the radiation of the family *Aeromonadaceae*, the long-distance arms of 16S rRNA gene branches joining *A. sharmana* to the genus do not support its inclusion within this group. Potential names for this bacterium include "*Manjusharmella aquatica*" or "*Halofoba aquatica*," neither of which has formally been proposed or validated (196). We suggest that the "List of Prokaryotic Names with Standing in Nomenclature" be amended.

*(c)* "*Aeromonas ichthiosmia*." This species was originally proposed by Schubert and coworkers in 1990 (192). Studies employing 16S rRNA gene sequencing and amplified fragment length polymorphism (AFLP) analysis have shown this species to be identical to *A. veronii* bv. sobria (192). More recently,

Huys and others (131) have shown that the type strain of *A. ichthiosmia* is 84% to 91% related at the DNA level to the type and reference strains of *A. veronii*. Phenotypically, the type strain of *A. ichthiosmia* is biochemically similar to *A. veronii* bv. sobria. *A. ichthiosmia* must be considered a later junior synonym of *A. veronii*.

*(d)* "*Aeromonas enteropelogenes*." This species was also proposed by Schubert and coworkers in 1990 (192). DNA-DNA reassociation studies performed using the type strain of *A. enteropelogenes* and type and reference strains of *A. trota* confirmed previous observations that these two species are identical (128). Testing of all type and reference strains of these two nomenspecies against a battery of 60 biochemical characters failed to detect any distinguishing characteristics. A recent PubMed search using "*A. trota*" yielded 43 citations, while a similar request using "*A. enteropelogenes*" produced 7 records. Despite these differences, *A. enteropelogenes* has priority of publication and validation in the literature (1990 versus 1991). These species are synonymous, and a decision by the Judicial Commission will eventually be required to determine which validated name is accepted (192).

*(e)* "*Aeromonas punctata*." Both *A. punctata* and *A. caviae* share the same type strain, ATCC 15468$^T$. This problem arose because the neotype strain of *A. punctata* has been reported to be NCMB 74 (equivalent to ATCC 23309) but is not on the approved lists. Furthermore, NCMB 74 is also the type strain of *A. eucrenophila*. The two species are considered to be objective synonyms of each other, although *A. punctata* predates publication and validation of the species name *A. caviae* by almost 30 years (1957 versus 1984) (192). Regarding general usage, a search of PubMed for "*Aeromonas punctata*" indicated 51 citations, while a similar search for "*A. caviae*" revealed 469 citations. Clearly, *A. caviae* is the more commonly used name, regardless of priority in the literature. To settle this taxonomic quagmire, at least two opinions will need to be rendered by the Judicial Commission regarding these issues, one involving the fact that *A. punctata* and *A. caviae* share the same type strain and one on the resulting controversy that would arise if *A. punctata* and *A. eucrenophila* eventually share the same type strain (192).

*(f)* "*Aeromonas allosaccharophila*." The validity of this species has been challenged intermittently over the last 15 years. *A. allosaccharophila* was proposed as a new *Aeromonas* species based upon the analysis of three strains, one of which was recovered from human feces (195). Issues regarding the validity of this species initially prompted further DNA relatedness studies by Esteve and collaborators, verifying their original proposal (73). However, a troubling aspect to the latter study was the lack of any homology (0% relatedness) between the type strain, CECT 4199, and the type strains of many other *Aeromonas* species, including *A. salmonicida*, *A. caviae*, *A. sobria*, *A. veronii* (including both biovars), *A. jandaei*, *A. schubertii*, and *A. trota*, given the high degree of interspecies 16S rRNA gene sequence similarity exhibited between all currently accepted species. Recent studies employing DNA hybridization assays and phylogenetic markers such as *dnaJ* strongly suggest that *A. allosaccharophila* is a later heterotypic synonym of *A. veronii* (128, 226). Huys et al. (131) provided an excellent summary of all the information for and against the validity of this species. Further DNA studies by an independent third group will be required to resolve this controversy. Regardless of the validity of this nomenspecies, as originally described this species is phenotypically heterogeneous (195), and this has been confirmed by other groups (131) as well as our own laboratory (our personal experience).

*(g) DNA hybridization group 11 and "A. encheleia."* In 1987, Hickman-Brenner and colleagues (115) at the CDC formally proposed that 8 of 10 strains previously assigned to enteric group 77 be transferred to a new ornithine decarboxylase (ODC)-positive species, *A. veronii*. The remaining two strains, CDC-1306-83 (equivalent to ATCC 35941 and LMG 13075) and CDC 715-84 (equivalent to ATCC 35942 and LMG 21755), although being ODC positive, did not warrant inclusion within this new species, based upon DNA-DNA relatedness studies. These two strains were renamed *Aeromonas* group 77 and were referred to at the time by CDC as DNA HG 11, with strain CDC 1306-83, isolated from an ankle suture, serving as the reference strain (14, 192). A third strain, CDC 3136-78 (equivalent to CCUG 30365 and LMG 13076), isolated from surface water of the Mohawk River (NY), has also been assigned to HG 11. Subsequent to these findings, ATCC 35942 was proposed as a reference strain for *A. allosaccharophila* (195) (see above).

These designations became controversial in 1996, when a phylogenetic investigation divided *A. eucrenophila* into two subgroups. While subgroup I contained the type strain of *A. eucrenophila*, subgroup II contained the type strain of *A. encheleia* plus two reference strains for HG 11, LMG 13075 and LMG 13076 (126). Although this study did not include DNA relatedness data, a subsequent study by many of the same authors, employing DNA-DNA hybridization, found both HG 11 strains to be 84% to 87% related to the type strain of *A. encheleia*, LMG 16330$^T$, further supporting synonymy between *A. encheleia* and HG 11 (129). While a 1999 phylogenetic investigation utilizing 16S rRNA gene sequence data supports the uniqueness of *A. encheleia* (193) as a species, a more recent study using *dnaJ* as a marker found *A. encheleia* and HG 11 to group together, and DNA-DNA hybridization results from the same study found HG 11 to be 85% related to *A. encheleia* GTC 2788$^T$ (226). The cumulative body of information in the published literature currently suggests that *A. encheleia* and HG 11 are equivalent; however, it is unclear how *A. eucrenophila* and *A. allosaccharophila* fit into this picture. Clearly, additional DNA work by a third independent group needs to be performed. Outcomes of such studies, at minimum, could require redefinition of each of the above-described species.

*(h) Aeromonas group 501*. Seven of eight strains referred to as enteric group 501 were found in 1988 to constitute a new species, *A. schubertii* (114). The eighth strain, CDC 2478-85 (equivalent to ATCC 43946), was only 61% related to the type strain of *A. schubertii* at 75°C, with a divergence value of 5%. The authors resolved to leave this single strain within the vernacular name, *Aeromonas* group 501, and an addendum added in proof was the description of a second strain, designated CDC 2555-87, from an open tibia fracture. Besides the genetic differences noted, both strains deviated from the idealized *A. schubertii* phenotype in being indole positive and lysine decarboxylase (LDC) negative (114). These strains clearly represented a new hybridization group and were subsequently labeled HG 13 by Martin-Carnahan and Joseph (192). By 16S

rRNA gene sequencing, *Aeromonas* group 501 is closely related to *A. schubertii*, as would be predicted from DNA relatedness studies (193). Further studies are needed.

**(ii) Taxonomic descriptions in the literature.** *(a) "A. sobria."* The species name *A. sobria* continues to be misused in publications (125, 177, 299). The species *Aeromonas sobria sensu stricto* refers to the organism originally described by Popoff and Véron in 1976 (238). Only two strains are universally recognized as belonging to this group (HG 7), namely, the type strain, CIP 7433, and CDC 9540-76, both from fish. Other strains have been described on a phylogenetic basis but have not been confirmed definitively as such by DNA hybridization (89). The issue that arises is that this species shares common phenotypes (esculin, salicin, KCN, and L-arabinose negative) with the more common biovar of *A. veronii*, which is responsible for many human infections. Barring DNA hybridization or phylogenetic studies associated with case reports or clinical studies, what authors are incorrectly reporting as "*A. sobria*" is in actuality *A. veronii* bv. sobria (192). We believe for uniformity's sake that the term "biovar sobria" should be used consistently in preference to "biotype sobria" in referring to ornithine decarboxylase-negative strains of *A. veronii*.

*(b) HG*. The term "hybridization group" (HG) is outdated with regard to the taxonomy of *Aeromonas*. Originally, the use of the term HG served a useful purpose when new *Aeromonas* species were identified at the DNA level that could not be separated phenotypically. However, with the advent of multiple phylogenetic methods and the current trend in defining a species based upon 1 or 2 strains, the use of HG(s) is irrelevant. Proposing a new species and then giving it an HG number is duplicative and adds to the general confusion in the scientific community regarding *Aeromonas* taxonomy. Furthermore, most of the recently described species are not referred to in that fashion. In practice, a more appropriate term for unnamed groups that are identified by various molecular techniques but are not going to be named at present would be "genomic species" or "genospecies," followed by a reference strain number or designation, as is often published nowadays (96).

**(iii) Other issues.** *(a) Minimal standards*. One of the obvious problems associated with the genus that is evident from the data presented in Table 2 is that there are no minimal standards for which characters should be included in a proposal to recognize a new *Aeromonas* species. As can be seen in Table 2, many of the issues we are currently facing might never have arisen if minimal standards for *Aeromonas* were available. Although the issue has been discussed repeatedly by the Subcommittee on the Taxonomy of the *Vibrionaceae*, no progress on minimal standards has been reported (221). However, the creation of a list of minimal standards may only minimize the aforementioned problems, as standards are not rules per se and genera and species names can be published outside IJSEM and then authenticated by appearing on a validation list within the journal.

*(b) Reference strains*. Principle 1 of the *Bacteriological Code* (1990 revision) is (i) to aim for stability of names, (ii) to avoid or reject names that may cause errors or confusion, and (iii) to avoid the useless creation of names (269). In order for *Aeromonas* taxonomy and nomenclature to be better in line with Principle 1 and to provide relevant biochemical characteristics that microbiologists can utilize to identify most, if not all, clinically relevant strains, it is important to create a universal collection of *Aeromonas* strains that unquestionably belong to the designated nomenspecies. This collection should contain only strains initially characterized by the gold standard, DNA-DNA hybridization, and not other methods, such as phylogenetic studies, which are subject to strain and species selection bias, method differences of phylogenetic analyses (rooted versus unrooted or neighbor joining versus maximum parsimony), and lack of a universally accepted threshold indicating species identity. Such a collection needs to have each species represented by strains of independent origins and from diverse ecohabitats, including humans, other vertebrate and invertebrate species, foods, and environmental sources, including marine and freshwater samples and soil. Finally, the collection should include at least 25 strains (if available) for each valid species, and these strains should be obtainable through a culture collection or similar vehicle at minimal cost. If such a collection were available, independent groups could more easily explore alternative molecular methods to replace DNA-DNA hybridization for species identification, more easily define discriminatory biochemical tests for species identification, and resolve taxonomic and nomenclature issues similar to those described above.

*(c) Key biochemical charts for differentiation*. When a new species is described, it is important that it can be distinguished phenotypically from its nearest neighbors, if possible. For most publications proposing new species, a biochemical chart with presumptive key tests that aid in separation of species is included. However, for genera that contain many species in addition to the *species nova*, phenotypic traits are often extracted from the original taxonomic descriptions. The problem this presents is that even though the same test (e.g., citrate utilization) may be performed in each taxonomic study, the test method, inoculation procedure, incubation period, temperature of incubation, and reading method (visual versus automated) may differ significantly. Although the chart may provide differential markers in principle, a reader trying to isolate, separate, and identify the *species nova* may have great difficulty in doing so. A concerted effort needs to be undertaken to identify key tests and test methods under standardized conditions to identify *Aeromonas* species.

## *Aeromonas* Species—Current Status

Despite all of the issues listed above, plus others not mentioned because they mostly fall outside the realm of clinical microbiology, laboratories still need a practical working knowledge of the most commonly used designations for species and groups within the genus *Aeromonas*. Some of these designations may not strictly adhere to the rules of the *Bacteriological Code*, and it may be years before a request for an opinion is offered and a judicial decision rendered on each issue. Table 4 attempts to look from a practical standpoint at *Aeromonas* taxonomy, legitimacy of proposed species, and clinical relevance.

For additional in-depth information on technical aspects of *Aeromonas* taxonomy and nomenclature, readers are encourage to consult references or reviews published by Martin-Carnahan and Joseph (192), Figueras (87), and Janda and Abbott (143).

TABLE 4. Practical view of valid and proposed species in the genus *Aeromonas*

| Species (yr[a]) | No. of subspecies[b] | Clinically significant[c] | Distinct species[d] | | Other name (yr)[e] |
|---|---|---|---|---|---|
| | | | Genetically | Biochemically | |
| *A. hydrophila* (1943) | 5 | Yes | Yes | Yes | |
| *A. salmonicida* (1953) | 5 | Yes[f] | Yes | Yes | |
| *A. sobria* (1981) | | No | Yes | No | |
| *A. media* (1983) | | Yes | Yes | Yes | |
| *A. caviae* (1984) | | Yes | Yes | Yes | *A. punctata* (1957) |
| *A. veronii* (1988) | 0[g] | Yes | Yes | Yes | *A. ichthiosmia* (1991) |
| *A. eucrenophila* (1988) | | No | Yes | No | |
| *A. schubertii* (1989) | | Yes | Yes | Yes | |
| *A. jandaei* (1992) | | Yes | Yes | Yes | |
| *A. trota* (1992) | | Yes | Yes | Yes | *A. enteropelogenes* (1991) |
| *A. encheleia* (1995) | | No | Yes | No | HG 11 |
| *A. bestiarum* (1996) | | Yes | Yes | Yes | |
| *A. popoffii* (1997) | | Yes | Yes | No | |
| *A. simiae* (2004) | | No | Yes | No | |
| *A. molluscorum* (2004) | | No | Yes | Yes | |
| *A. bivalvium* (2007) | | No | Yes | Yes | |
| *A. aquariorum* (2008) | | No | Yes | Yes | |
| *A. tecta* (2008) | | Yes | Yes | Yes | |
| *A. allosaccharophila* (1992) | | No | No | No | *A. veronii*? |
| *A. culicicola* (2002) | | No | No | No | *A. veronii* |
| *A. sharmana* (2006) | | No | Yes | No | Not an *Aeromonas* species |

[a] Year of valid proposal of species name.
[b] Based upon names with standing in the literature (http://www.bacterio.cict.fr/).
[c] Has been isolated from clinical material on more than one occasion or unequivocally linked to human disease.
[d] Based upon DNA-DNA hybridization. Biochemically distinct means that there are a number of phenotypic properties that can separate ≥90% of strains isolated in the clinical laboratory.
[e] Other names in current usage in the literature describing the same species.
[f] There is a subset of strains residing within *A. salmonicida* that grow well at 35°C to 37°C and can be isolated from clinical specimens (192). These strains are distinct from the indole-negative, nonmotile, melanin-producing isolates associated with fish diseases. See *Aeromonas* and Ecosystems, as well as Laboratory Identification.
[g] No subspecies, but two distinct biovars (veronii and sobria).

## CLASSIFICATION

### General Principles and Practices

In its simplest form, classification is the orderly arrangement of hierarchal ranks (class, order, and family) of bacteria based upon relatedness of units, as assessed by various platforms, including phenotypic and genetic methods. Relationships of species and genera within higher ranks are often visually depicted as phylogenetic trees with branches connecting relatedness groups (e.g., strains or species) into clades (taxonomic groups of common ancestry). As previously mentioned, classification of bacteria does not fall under rules that govern the nomenclature of bacteria. Rather, classification is a dynamic and constantly evolving process dependent upon genes analyzed, phylogenetic tools and methods of analysis employed, and selection of type and reference strains for analysis. For practical purposes, the legitimacy of any classification scheme is dependent upon general usage and acceptance by the scientific community.

### *Aeromonas* Classification

**The family *Aeromonadaceae*.** It has been well appreciated for over 15 years that the seminal observations of Colwell et al. (49) are correct and that the former classification system including aeromonads in the family *Vibrionaceae* is inappropriate based upon phylogenetic analyses. Multiple international studies primarily employing 16S rRNA gene sequence analysis of the genus *Aeromonas* indicate that (i) members of the genus *Aeromonas* form a distinct line within the *Gammaproteobacteria* and (ii) there is enough phylogenetic depth within the genus to warrant elevation of the genus name to the rank of family (194, 250, 301). The current edition of *Bergey's* lists three genera in the family *Aeromonadaceae*, including *Aeromonas*, *Oceanimonas*, and *Tolumonas* (*genus incertae sedis* [uncertain placement]) (192).

**Numerical taxonomy studies.** Although no longer in vogue, numerical taxonomy studies are still being performed in regards to the classification of aeromonads (285). Despite well-recognized limitations involving phenetic methods, biochemical characteristics can be extremely useful in the identification of new phenoms due to the diverse substrates attacked by members of the genus. Furthermore, phenotypic properties can be at least an indirect assessment of the diversity in a wider range of chromosomal genes than those in phylogenetic studies based on a limited number of housekeeping genes. In a 2002 investigation by Miñana-Galbis and coinvestigators (207), a collection of 202 *Aeromonas* strains primarily isolated from bivalve mollusks and water were characterized for 64 independent phenotypic traits. Two phenoms (VI and VII) were identified in this study whose strains could not be assigned to any previously recognized *Aeromonas* species. Subsequent investigations identified both groups as new species, with phenom VI being proposed as *A. molluscorum* (205) and phenom VII being proposed as *A. bivalvium* (206).

**Phylogenetic analysis of *Aeromonas* species.** A number of molecular chronometers have been used to evaluate phylogenetic relationships and relatedness among *Aeromonas* species

TABLE 5. Phylogenetic relationships within the genus *Aeromonas* deduced from analysis of selected housekeeping genes

| Study (authors [reference]) | No. of strains | Gene(s)[a] | Interspecies relatedness (%)[b] | Outliers[c] |
|---|---|---|---|---|
| Yáñez et al. (301) | 53 | *gyrB* | >3 | *A. salmonicida*/*A. bestiarum*, *A. encheleia*/HG 11 |
| Soler et al. (272) | 68 | *gyrB*, *rpoD* | >3[d] | *A. encheleia*/HG 11, *A. veronii*/*A. culicicola* |
| Küpfer et al. (175) | 28 | 16S rRNA, *gyrB*, *rpoB* | 6, 7[e] | *A. veronii*/*A. culicicola*/*A. allosaccharophila*, *A. bestiarum*/*A. salmonicida*/*A. popoffii*, *A. encheleia*/HG 11 |
| Nhung et al. (226) | 27 | *dnaJ* | >5.2 | *A. ichthiosmia*/*A. veronii*/*A. allosaccharophila*/*A. culicicola*, *A. encheleia*/HG 11 |

[a] Gene(s) analyzed in phylogenetic study.
[b] Based upon rate of nucleotide substitution determined for strains studied (most species).
[c] Pairs or groups of species and/or taxa that do not fall within expected interspecies relatedness values.
[d] For *rpoD*.
[e] For *rpoB* and *gyrB*, respectively.

and unnamed taxons, such as *Aeromonas* group 501. These evolutionary markers include the 16S rRNA, *gyrB* (B-subunit DNA gyrase), *rpoD* ($\sigma^{70}$ factor), *rpoB* ($\beta$-subunit, DNA-dependent RNA polymerase), and *dnaJ* (heat shock protein 40) genes, whose sequenced lengths of DNA typically range from 934 to 1,100 bp (175, 226, 253, 272, 301). Results from these collective studies indicate that there is less divergence in 16S rRNA gene sequences (as measured by mean sequence similarity values) than there is within the housekeeping genes described above. The greater nucleotide sequence degeneracy found in these other housekeeping genes translates into mean sequence similarity values of 89% to 92% for *gyrB*, *rpoD*, and *dnaJ*, as opposed to 98.7% for 16S rRNA (226). Consequently, the discriminatory power for the first three genes in regards to *Aeromonas* phylogeny is appreciably higher than it is for 16S rRNA. As an example, *A. trota* and *A. caviae* are distinguishable by only a single nucleotide by use of 16S rRNA as a molecular chronometer, while *gyrB* analysis reveals 57 to 69 bp differences, depending upon the particular strain sequenced (301).

Collective results from several phylogenetic investigations are shown in Table 5. For most housekeeping genes studied, intraspecies nucleotide substitution rates are <2% (different strains within the same species), while interspecies values (strains belonging to different species) are typically >3%. Using these numbers as baseline values, one can determine which groups of species do not fall within these parameters (Table 5, outlier column). Issues concerning several of these groups, most notably *A. encheleia* with HG 11 and *A. veronii* with *A. ichthiosmia*/*A. allosaccharophila*/*A. culicicola*, are discussed in Nomenclature and Taxonomy. Two studies found the interspecies nucleotide substitution rates for *A. salmonicida* and *A. bestiarum* to be considerably lower than what would be predicted for distinct *Aeromonas* species (109, 301). Such outliers may simply be a reflection of the methodology used, gene sequenced, or strains analyzed. However, these anomalies may also signal reevaluation of the legitimacy of *A. bestiarum* as a separate species. Phylogenetic studies have also found that *A. schubertii* (and *A. simiae* when included) is at the deepest branch of the genus, near its ancestral root, which is consistent with 16S rRNA trees (226, 301). Inconsistencies or anomalies associated with the phylogenetic analysis of various housekeeping genes have been detected on rare occasions. Such inconsistencies involve strains from one genomic species possessing *gyrB* or 16S rRNA gene sequence similarities closer to another species (175, 301) or intragenomic heterogeneity in the 16S rRNA gene, which can affect phylogenetic placement (213).

## *AEROMONAS* AND ECOSYSTEMS

### General Description

Aeromonads are essentially ubiquitous in the microbial biosphere. They can be isolated from virtually every environmental niche where bacterial ecosystems exist. These include aquatic habitats, fish, foods, domesticated pets, invertebrate species, birds, ticks and insects, and natural soils, although extensive investigations on the latter subject are lacking. The vast panorama of environmental sources from which aeromonads can be encountered lends itself readily to constant exposure and interactions between the genus *Aeromonas* and humans (see Epidemiology).

The relative environmental distributions of *Aeromonas* species in selected settings, as currently known, are presented in Table 6. Several points bear mentioning. Earlier studies have indicated that three *Aeromonas* genomospecies (*A. hydrophila*, *A. caviae*, and *A. veronii* bv. sobria) are responsible for the vast majority (≥85%) of human infections and clinical isolations attributed to this genus (143). The same pattern observed clinically appears to repeat itself in most environmental samples, with *A. salmonicida* included as a predominant species in fish and water samples. In some studies, less frequently encountered species have been found to predominate in environmental samples, such as *A. schubertii* in organic vegetables (201). However, the preponderance of published data to date do not support these findings overall, and Table 6 reflects distribution patterns based upon normalized data from multiple studies. For newly described species such as *A. aquariorum* and *A. tecta*, no data exist on their relative distributions in the environment outside their initial taxonomic description, and extremely limited data are available on many other taxa described since 2004. Finally, the techniques and methods used to identify *Aeromonas* isolates to the species level vary considerably from one study to the next. The data presented in Table 6 are a compilation of the best studies on frequency distribution published to date.

TABLE 6. Minimal relative distributions of *Aeromonas* species in environmental sources

| Species | Presence of species[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Vertebrates | | Invertebrates | | | Water | | |
| | Primates | Others | Molluscs[b] | Arthropods[c] | Others[d] | Fresh | Saline[e] | Foods[f] |
| *A. allosaccharophila* | ± | ± | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. aquariorum* | 0 | ± | 0 | 0 | 0 | ± | 0 | 0 |
| *A. bestiarum* | + | ± | ± | 0 | 0 | ++ | 0 | 0 |
| *A. bivalvium* | 0 | 0 | ± | 0 | 0 | 0 | 0 | 0 |
| *A. caviae* | +++ | +++ | ++ | ++ | 0 | ++ | +++ | +++ |
| *A. encheleia* | 0 | ++ | ± | 0 | 0 | + | 0 | 0 |
| *A. eucrenophila* | ± | + | 0 | 0 | 0 | + | 0 | 0 |
| *A. hydrophila* | +++ | +++ | ± | ± | 0 | +++ | ++ | ++ |
| *A. jandaei* | + | ++ | ± | 0 | + | ± | 0 | 0 |
| *A. media* | + | 0 | 0 | 0 | 0 | + | 0 | 0 |
| *A. molluscorum* | 0 | 0 | ± | 0 | 0 | 0 | 0 | 0 |
| *A. popoffii* | ± | 0 | 0 | 0 | 0 | + | 0 | 0 |
| *A. salmonicida* | + | +++ | 0 | 0 | 0 | ++ | 0 | 0 |
| *A. schubertii* | + | ± | 0 | 0 | 0 | 0 | 0 | ± |
| *A. simiae* | ± | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. sobria* | 0 | ++ | 0 | 0 | 0 | ± | 0 | 0 |
| *A. tecta* | ± | ± | 0 | 0 | 0 | 0 | 0 | 0 |
| *A. trota* | + | 0 | 0 | 0 | 0 | 0 | 0 | ± |
| *A. veronii* | +++ | ++ | 0 | ± | ++ | ± | ++ | ++ |

[a] 0, not reported to date; ±, rare reports; +, uncommon; ++, common; +++, predominant species. Data from published studies were selected on the basis of study populations, methods of analysis and identification, and other selected factors (7, 22, 59, 129, 130, 181, 195, 197, 201, 205, 206, 207, 223, 254). Additional data were from the Microbial Diseases Laboratory, California Department of Public Health (unpublished data).
[b] Includes bivalves and snails.
[c] Insects and arachnids.
[d] Includes leeches.
[e] Estuaries.
[f] Excludes fish, shellfish, and crustaceans.

### *Aeromonas* and Aquatic Environments

**Overview.** Groundbreaking studies conducted over 30 years ago by Terry Hazen and associates identified viable *Aeromonas* in 135 of 147 (91.8%) natural aquatic habitats sampled in the United States and Puerto Rico (112). *Aeromonas* numbers were higher in lotic than in lentic systems and were higher in thermal gradients ranging from 25°C to 35°C (111, 112). *A. hydrophila* grew over a wide range of temperatures, conductivities, pHs, and turbidities, with only those habitats with extreme ranges of these parameters (extremely saline environments, thermal springs, and highly polluted waters) failing to yield aeromonads.

Today, the genus *Aeromonas* is considered to be almost synonymous with water and aquatic environments, being isolated from rivers, lakes, ponds, seawater (estuaries), drinking water, groundwater, wastewater, and sewage in various stages of treatment. Concentrations of aeromonads in these sites have been reported to vary from lows of <1 CFU/ml (groundwater, drinking water, and seawater) to highs of $10^8$ CFU/ml or more, in crude sewage or domestic sewage sludge (119). Although primarily a freshwater resident, *Aeromonas* species can be recovered from the epipelagic layer (<200 m) of the ocean (as opposed to benthic regions), most often in estuaries, existing as free-living bacteria or in association with crustaceans. Estuaries are ideally suited for aeromonads, since salinity concentrations are substantially lower there than in the deeper (benthic) regions of the ocean. One study from the Italian coast found aeromonad numbers varying from $10^2$ to $10^6$ CFU per 100 ml throughout the year (91).

**Fish diseases.** The role of aeromonads as a causative agent of fish diseases has been known for decades, longer than their comparable role in causing systemic illnesses in humans. Two major groups of fish diseases are recognized. *A. salmonicida sensu stricto* causes fish furunculosis, particularly in salmonids. The disease has several presentations, ranging from an acute form characterized by septicemia with accompanying hemorrhages at the bases of fins, inappetence, and melanosis to a subacute to chronic variety in older fish, consisting of lethargy, slight exophthalmia, and hemorrhaging in muscle and internal organs (16). Mesophilic species (*A. hydrophila* and *A. veronii*) cause a similar assortment of diseases in fish, including motile *Aeromonas* septicemia (hemorrhagic septicemia) in carp, tilapia, perch, catfish, and salmon, red sore disease in bass and carp, and ulcerative infections in catfish, cod, carp, and goby (149). Mesophilic *Aeromonas* species, most notably *A. hydrophila*, have been linked to major die-offs and fish kills around the globe over the past decade, resulting in enormous economic losses. These die-offs included over 25,000 common carp in the St. Lawrence River in 2001 (212), 820 tons of goldfish in Indonesia in 2002, resulting in a $37.5 million loss (http://www.promedmail.org/pls/otn/f?P=2400:1202:959209208141620::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,18797), and a catfish die-off in Minnesota and North Dakota in 2007 (http://www.promedmail.org/pls/otn/f?P=2400:1202:959209208141620::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,39840). In many of these instances, *Aeromonas* species were sole or copathogens causing invasive secondary

infections in immunosuppressed fish due to spawning or environmental triggers, such as high temperatures or low water levels.

***Aeromonas* and drinking water.** *Aeromonas* species can be found in various concentrations in drinking water. Although the significance of aeromonads in such samples is unknown in relationship to reputed cases of gastroenteritis, the chronic exposure of immunocompromised persons to *Aeromonas* via contaminated waters could potentially lead to invasive disease, such as septicemia (182). The World Health Organization lists *Aeromonas* in the third edition of *Guidelines for Drinking-Water Quality* (http://www.who.int/water_sanitation_health/dwq/guidelines/en/index.html). In 1998, the Environmental Protection Agency listed *A. hydrophila* on its "Drinking Water Contaminant Candidate List" (http://permanent.access.gpo.gov/lps21800/www.epa.gov/safewater/ccl/cclfs.html). Through the Consumer Confidence Report Rule, public water systems are required to report unregulated contaminants, such as *Aeromonas*, when detected (67). These reports must be filed on an annual basis (http://www.epa.gov/safewater/ucmr/data_aeromonas.html). *Aeromonas* has also been reported to enter a viable but nonculturable state, similar to other pathogens, including *Vibrio*. The significance of these observations is presently unknown (200).

### *Aeromonas* and Animals

Although not studied in nearly as intensive detail as aquatic ecosystems, aeromonads can be recovered frequently from vertebrates and other hosts, including insects. Our knowledge regarding the extent and diversity of vertebrate species harboring *Aeromonas* stems from several direct and indirect lines of evidence, namely, (i) systematic surveys of the fecal content of farm and domesticated animals; (ii) surveys of the microbial content of retail foods, including meats, poultry, and dairy products (see "*Aeromonas* in Foods"); (iii) reports describing human illnesses directly related to bites or other penetrating traumas precipitated by vertebrates such as snakes; and finally, (iv) reports of epizootic infections caused by aeromonads in susceptible species. A Turkish study recently reported that *Aeromonas* species were identified in the gastrointestinal contents of healthy sheep, cattle, and horses at frequencies ranging from 5% to 10% (32). In the disease state, aeromonads can also cause a variety of serious illnesses in both cold-blooded and warm-blooded animals. Such conditions include ulcerative stomatitis in snakes and lizards, "red leg" disease in frogs, septicemia in dogs, and septic arthritis in calves (103). *Aeromonas* spp. have also been implicated in a variety of infectious processes in seals (282) and as a cause of seminal vesiculitis in bulls (215). Together, the cumulative data strongly suggest that animals are an ever-present reservoir for the introduction and exchange of *Aeromonas* species in the environmental microbial world.

### *Aeromonas* in Foods

Transient colonization of the human gastrointestinal tract by aeromonads is most likely an indirect result of the consumption of foods and drinking water containing *Aeromonas* spp. Over the past 20 years, there have been literally dozens of studies geared toward determining both the frequency and concentration of *Aeromonas* spp. in consumable products obtained from supermarkets and retail stores (133). Although the method of analysis, use of selective and enrichment media, and types and sources of commercial products analyzed vary from study to study, the collective results from these investigations indicate that aeromonads are common inhabitants of most types of food, regardless of geographic origin. Palumbo et al. (231) found *Aeromonas* isolates universally present in all foods tested, including seafoods, raw milk, chicken, and meats such as lamb, veal, pork, and ground beef. While initial counts in these foods ranged from $<10^2$ to $>10^5$ CFU/g at 5°C, after a 7-day period at refrigeration temperatures *Aeromonas* numbers had increased 1 to 3 log in most products. Other studies have found aeromonads in dairy products (4%), vegetables (26% to 41%), and meats and poultry (3% to 70%), with the largest numbers recorded for shellfish (31%) and fish (72%) (22, 201, 225). In most of these studies, the majority of isolates were recovered after enrichment techniques rather than direct plating, indicating that *Aeromonas* concentrations were relatively low.

Further information on the genus *Aeromonas* and its association with various environmental ecosystems can be found in reviews by Edberg et al. (67), Isonhood and Drake (133), Joseph and Carnahan (149), and Kirov (164).

## EPIDEMIOLOGY

There is a frank periodicity associated with the isolation of *Aeromonas* species from the human gastrointestinal tract. Since these bacteria are not normal inhabitants of the gut (<1% of stools were positive in many reports), most studies have found the recovery of *Aeromonas* from fecal specimens to increase coincidentally with the warmer months of the year. This rise in numbers no doubt occurs because mesophilic aeromonads grow optimally at elevated water temperatures, thus leading to increased concentrations of bacteria in freshwater environments as well as in domestic water supplies (67, 159). The same seasonality noted in regards to *Aeromonas* intestinal isolates has also been observed in other extraintestinal infections, such as septicemia, where 42% to 67% of bacteremic illnesses occurred during the summer season (156, 187, 284). While the frequency of less frequently encountered extraintestinal infections caused by *Aeromonas* is more difficult to track because of their lower incidence, it is fairly safe to assume that increased concentrations of aeromonads in aquatic ecosystems during warmer months of the year translate into increased opportunities for exposure to these bacteria and thus an elevated risk of developing infection and/or colonization with these microbes.

The intimate association between aeromonads and aquatic ecosystems has led many microbiologists to almost consider the term "*Aeromonas*" to be synonymous with "water." However, in regards to the infection/colonization status of humans with aeromonads, some of these hydrophilic associations may not always be that apparent. Figure 1 depicts major and minor pathways by which humans become infected/colonized with *Aeromonas* species during the warmer seasons of the year. Most available data suggest that the majority of mesophilic isolates are acquired via contact with contaminated drinking

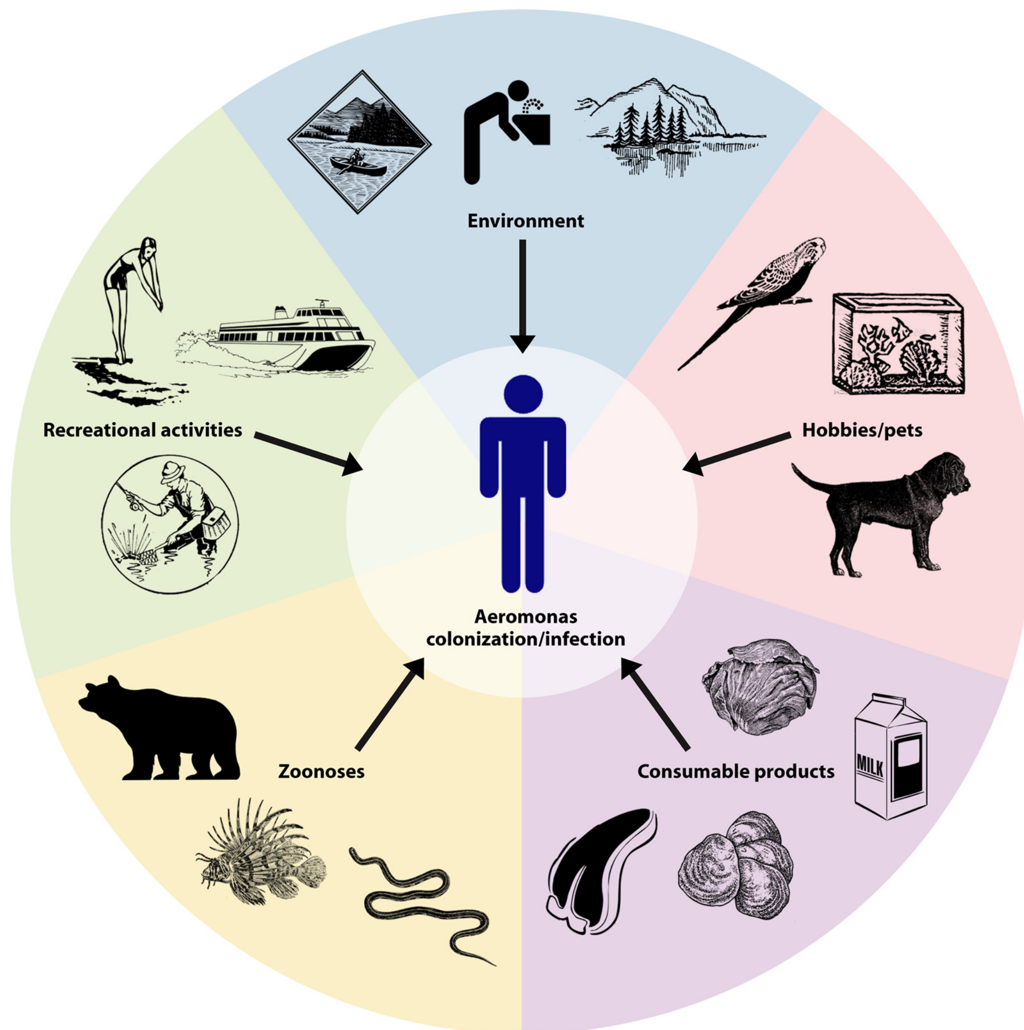


FIG. 1. Environmental sources of *Aeromonas* species potentially leading to infection or colonization in humans. Black lines indicate suspected major and minor routes of acquisition leading to colonization/infection.

water or through the ingestion of foods (produce, dairy, or meats) that are naturally exposed to aeromonads through irrigation processes or other "farm-to-table" operations. In addition to these consumable products, bivalves such as oysters and mussels are naturally bathed in estuary waters containing these organisms, and through their filter-feeding process, they actually concentrate these bacteria within their meats. In addition to these major pathways, aeromonads can also be acquired by other, less prominent routes. Recreational activities such as boating, fishing, and diving can lead to infection through major or unapparent traumas, as can near-drowning events (23, 31, 292). As urban sprawl continues to encroach upon rural environments, the potential for *Aeromonas* infections arising from zoonotic origins will increase. While infections resulting from reptile and snake bites have a long-recognized association with the genus *Aeromonas*, recent case reports have documented illnesses resulting from bites from less commonly encountered vertebrates, such as bears (10, 174).

The exact incidence of *Aeromonas* infections on a global basis is unknown. *Aeromonas* is not a reportable condition in the United States or in most other countries around the world. In 1988, California became the first state to make *Aeromonas* infections reportable. Based upon data collected from 219 patients over a 12-month period, the overall incidence of *Aeromonas* infections was 10.6 per million population, with wound infections estimated to be 0.7 per million population, with the highest incidence, 1.4 per million, recorded for persons aged 30 to 39 years (31, 163). *Aeromonas* infections, however, are no longer reportable in California. A 6-month nationwide survey of *Aeromonas* infections in France in 2006 reported 99 infections in 70 hospitals. Based upon an estimated 2006 census of 61 million, this represents a prevalence of 1.62 infections per million population, a value much lower than that reported in the California study (178). *Aeromonas* bacteremia in England and Wales is a voluntarily reportable condition, with between 47 and 116 cases tallied annually between 1990 and 2004. For 2004, the population estimate for England and Wales was 53 million, with 82 cases of *Aeromonas* bacteremia recorded. If one estimates the U.S. population in 2004 to be around 293

TABLE 7. *Aeromonas* gastroenteritis in 1988 and 2008: issues and observations

| Issue | Comment(s) | |
|---|---|---|
| | 1988 | 2008 |
| *Aeromonas*-associated outbreak | No well-circumscribed outbreak reported | No progress |
| Henle-Koch postulates | Not fulfilled | Molecular postulates also unfulfilled |
| Volunteer studies | Negative; no consistent colonization or gastroenteritis produced | No further studies attempted |
| *Aeromonas* taxonomy | Complicated; gastroenteritis perhaps linked to specific species or genotypes | Taxonomy now well defined; gastroenteritis not linked to specific genomospecies or genotypes to date |
| Virulence genes (enterotoxin) | Poorly defined | Multiple enterotoxins identified and characterized |
| Epidemiology studies | Most support a role for *Aeromonas* in gastroenteritis | No change; rare studies support opposite conclusions |

million, then the projected number of cases of *Aeromonas* septicemia in the United States for 2004 was 453, based upon the British data. This would make the incidence of *Aeromonas* septicemia in England/Wales and the United States 1.5 per million population. Clearly, both values are minimum estimates, since many cases either go undetected or are not reported. Since drinking water is thought to be one important environmental source for potential colonization/infection with aeromonads, one study reviewed the relative risk to human health posed by acquiring various pathogens in this manner. *Aeromonas* was on the very low end of the relative risk spectrum, with an estimate of 7.3 per billion (252).

## CLINICAL INFECTIONS AND DISEASE-ASSOCIATED SYNDROMES

There are few gram-negative bacteria that rival the genus *Aeromonas* in scope and breadth of human infections that they can cause. Aeromonads are literally responsible for a "cornucopia" of intestinal and extraintestinal diseases and syndromes, ranging from relatively mild illnesses such as acute gastroenteritis to life-threatening conditions, including septicemia, necrotizing fasciitis, and myonecrosis (142). The panorama of maladies linked to this genus goes far beyond those listed above and includes intra-abdominal problems, ocular disease, infections of bones and joints, and even less frequently observed conditions involving the respiratory and urogenital tracts. Based upon frequency, *Aeromonas* clinical infections fall into four broad categories, namely, (i) gastrointestinal tract syndromes, (ii) wound and soft tissue infections, (iii) blood-borne dyscrasias, and (iv) a miscellaneous "catch-all" category which includes a myriad of less frequently encountered ailments and infectious processes.

### *Aeromonas* and Gastroenteritis

Although the gastrointestinal tract is by far the most common anatomic site from which aeromonads are recovered, their role as etiologic agents of bacterial diarrhea is still problematic (144). Supporting evidence for aeromonads as intestinal pathogens stems from detailed case reports, epidemiologic case-controlled investigations on *Aeromonas*-associated diarrhea, and generally very low colonization rates in asymptomatic persons (9, 87, 117, 138, 143). Many reviews now list *Aeromonas* spp. as *bona fide* enteropathogens, yet other publications in leading peer-reviewed journals do not give even a cursory mention of this genus in regards to causes of infectious diarrhea (280). von Graevenitz (290) recently summarized the evidence in the literature both for and against the role of aeromonads in bacterial gastroenteritis and concluded that it is still controversial. He further states that even if "subsets" of aeromonads are enteropathogenic, this is of little help to the clinical microbiologist. Thus, the relative importance of this genus as a human pathogen hinges to a great extent on its proven role as a common cause of acute bacterial gastroenteritis.

***Aeromonas* and gastroenteritis: where are we now?** One of the best approaches in trying to understand why aeromonads are not universally accepted as gastrointestinal pathogens is to compare shortcomings in the *Aeromonas* "portfolio" relative to other traditional enteropathogens (Table 7). Probably the largest single impediment to unquestionably establishing *Aeromonas* as a true gastrointestinal pathogen is the failure to identify a single clonally related outbreak of diarrhea caused by this agent. Edberg et al. (67) recently summarized the literature in regards to "suspected" food-borne disease outbreaks involving aeromonads. These outbreaks have principally involved seafoods (prawns, oysters, shrimp, and sashimi) and fish, with the number of affected persons ranging from 2 to >400. Yet the incubation periods in many of these reports were exceedingly short (<24 h), which is not suggestive of *Aeromonas*, and definitive laboratory data supporting the conclusion that aeromonads were responsible were not available (67, 172). This is surprising in light of the ubiquitous nature of these organisms in the environment and the multiple opportunities that must exist for aeromonads to cause outbreaks of diarrheal disease from contaminated foods or water. Furthermore, even more perplexing is the fact that less prominent organisms, such as *Providencia alcalifaciens*, have been established as legitimate causes of bacterial gastroenteritis by use of the same criteria. In a major outbreak of food-borne disease in Japan, a clonal strain of *P. alcalifaciens*, as determined by pulsed-field gel electrophoresis (PFGE), was recovered from multiple symptomatic patients, and subsequent studies found an immune-specific response to the bacterium in the acute- and convalescent-phase sera of 7 of 8 persons tested (219). No such comparable evidence exists for *Aeromonas*.

A second stumbling block is the inability to fulfill Henle-Koch postulates (76). Postulate 3 requires that the proposed pathogen be fully isolated from the body and grown in pure culture, and it must be shown that "it can induce the disease anew." No animal model has ever been established that can faithfully reproduce the *Aeromonas*-associated diarrheal syn-

drome, although many attempts have been made (155). Stanley Falkow proposed an addendum to Koch's postulates, in a molecular format relying on the use of genetic mutations that is more in line with today's research methodologies (82). Even with the use of this newer set of standards, molecular postulate 1 has not been fulfilled, since the phenotype is not associated exclusively with pathogenic members or strains of the genus and the same traits can be found in what are assumed to be nonpathogenic varieties (82, 290).

Other obstacles in addition to those listed above can be found. *Aeromonas* can be found in the stools of 1% to 4% of asymptomatic individuals in some studies, although the carriage rate in industrialized countries is typically <1% (117). Morgan and associates (214) challenged volunteers with high concentrations (up to $10^{10}$ CFU) of five "*A. hydrophila*" strains. Only one of five strains tested produced transient colonization (shedding) in >50% of persons tested; even more disappointing was the fact that only 2 of 57 individuals (3.5%) developed diarrhea, using ≥2 unformed stools in 24 h, with systemic or enteric symptoms, as clinical criteria. Some critics pointed to the fact that the five strains tested might not have been "hot" isolates. However, one of these strains, SSU, a CDC diarrheal isolate, is probably the most well-characterized *Aeromonas* strain at the molecular level in regards to *Aeromonas* gastroenteritis, enterotoxin genes, and potential colonization factors (70). In the study by Morgan et al. (214), orally administered challenge doses of up to $5 \times 10^{10}$ CFU of strain SSU produced no colonization or deleterious effects whatsoever in volunteers. Therefore, it is very hard to argue that good candidate strains were not selected. It is still possible that a critical virulence or colonization factor is lost upon *in vitro* passage, although current evidence to support this hypothesis is lacking.

For a protracted time, it was thought that the failure to unequivocally tie the genus *Aeromonas* to gastroenteritis was because of an extremely complicated taxonomy and the fact that a specific subset of strains existed that contained unique virulence determinants required to produce diarrhea. This no longer appears to be the case. *Aeromonas* taxonomy, once in a quagmire, has been redefined clearly over the past 20 years, based upon phylogenetic investigations, and the number of legitimate species has more than doubled (Table 4). *Aeromonas* strains implicated as causes of enteritis are not restricted to a single genomospecies or even to a particular biotype/genotype within a single taxon (6). Most *Aeromonas* species recovered from clinical samples have been implicated at least on rare occasions as a cause of diarrhea (6). While the number of *Aeromonas* enterotoxins identified has increased and these reputed virulence factors have been characterized extensively on phenotypic and molecular bases (262), these genotypes still represent only a portion of isolates implicated in causing gastroenteritis (6).

Finally, while many prior epidemiologic studies and some recent case-controlled investigations concluded that aeromonads are true enteropathogens (276), there are still a spattering of surveys that come to exactly the opposite conclusion (45). There are also a small number of cases in the literature where *Aeromonas* is unquestionably the cause of gastroenteritis and the diagnosis is based not only upon the isolation of the microorganism from feces but also on a human immune response and/or pathological evidence of infection (143). It therefore seems illogical not to conclude from the data presently available that if most fecal strains of *Aeromonas* are potentially enteropathogenic, they are so either by mechanisms not presently identified or by routes not associated with traditional enteric pathogens. The facts that a clonally defined outbreak has yet to be confirmed and that no animal model exists with which to reproduce the disease are perplexing. These anomalies suggest the possibility that comitigating factors exist in hosts that attenuate the potentiality of disease and transmissibility to others. Alternatively, many fecal isolates of *Aeromonas* may simply reflect transient colonization of the gastrointestinal tract.

***Aeromonas* gastroenteritis: symptoms, peculiarities, and problems.** The susceptible patient populations, disease presentations, and symptomatology associated with *Aeromonas* gastroenteritis have been well characterized for almost 2 decades. *Aeromonas*-associated diarrhea is a worldwide phenomenon seen in both industrialized and developing nations and spanning all age groups, and while principally observed in healthy persons, it can also be found in those suffering from underlying maladies, including immune disorders such as HIV infection (87). Holmberg and Farmer (117) described *Aeromonas* gastroenteritis as a mild, self-limiting infection. They further reviewed a number of large-scale retrospective or prospective investigations on bacterial diarrhea and found that aeromonads were present in the stools of 0.5% to 16.9% of ill persons versus 0% to 10% of controls. The higher frequencies of *Aeromonas*-associated gastroenteritis reported in this review are not substantially different from incidences reported for *Salmonella*, *Shigella*, and *Campylobacter* diarrhea in adult travelers to Sweden, Southern Europe, Africa, and Asia (276). These broad and overlapping prevalence ranges in the frequency of aeromonads in both symptomatic and asymptomatic individuals still hold true today, which is one of the confounding reasons for why *Aeromonas* gastroenteritis is still a controversial issue (see preceding section).

Since 2000, there have been relatively few new investigations into clinical and epidemiologic aspects of *Aeromonas*-associated diarrhea, and most of these studies originated in developing nations. In industrialized countries, regardless of patient populations studied, the frequency of *Aeromonas* in stool samples ranges from 2.2% to 10% (72). Similar findings have also been posted from non-European or American surveys, including a low prevalence of 0.62% in Malaysian children in an urban setting (184) and a high incidence of 13% in a Nigerian community with poor personal and environmental hygiene standards (227). There have been few prospective and no population-based studies involving *Aeromonas* gastroenteritis to date.

*Aeromonas* gastroenteritis can clinically present in five different settings, namely, as a nondescript enteritis, as a more severe form accompanied by bloody stools, as the etiologic agent of a subacute or chronic intestinal syndrome, as an extremely rare cause of cholera-like disease, or in association with episodic traveler's diarrhea (Table 8). By far the most common presentation for *Aeromonas* gastroenteritis is as secretory (watery) enteritis (87, 117, 138). In numerous retrospective studies, the secretory form has accounted for 75% to 89% of all cases of *Aeromonas* gastroenteritis where aeromonads were deemed the sole pathogen present (36, 72, 268,

TABLE 8. Gastrointestinal syndromes and secondary complications associated with *Aeromonas* infection[a]

| Category | Presentation | Description | Frequency | Reference(s) |
|---|---|---|---|---|
| Enteritis | | Inflammation of the small intestine | ++++ | |
| | Secretory GE | Watery diarrhea | ++++ | 36, 72, 108, 268, 288 |
| | Cholera-like GE | >10 liters of "rice-water" stools/day | + | 34, 106 |
| | Ileal ulceration | Associated with acute enteritis | + | 300 |
| | Intramural intestinal hemorrhage | "Stack of coins" or "picket fence" appearance of mid- to distal ileum | + | 20 |
| Colitis | | Inflammation of the colon (large intestine) | +++ | |
| | Dysentery | Diarrhea often accompanied by gripping pain and the passage of blood and mucus | +++ | 36, 41, 72, 184, 288 |
| | Segmental colitis | Severe colitis of a segment of the colon | ++ | 18, 60, 85, 186 |
| | Chronic colitis | Ulcerative colitis or proctitis with chronic inflammation of the mucosa, ulcerative lesions of the lining with bleeding | ++ | 61, 298 |
| | IIBD | Rectosigmoid spiculations and ulceration; pancolitis without skip lesions; dense chronic inflammatory process | + | 63 |
| | Ischemic colitis | Inflammation and injury of large colon due to inadequate blood supply | + | 120 |
| Complications | HUS | Hemolytic anemia, low platelet count, renal impairment | ++ | 21, 83, 88, 90 |

[a] GE, gastroenteritis; HUS, hemolytic-uremic syndrome; IIBD, idiopathic inflammatory bowel disease. ++++, predominant syndrome associated with *Aeromonas*; +++, common syndrome associated with *Aeromonas*; ++, multiple cases linked to *Aeromonas* infection; +, rarely reported.

288). Chief complaints accompanying this form of diarrhea include low-grade fevers and abdominal pain; one study noted a high frequency of vomiting (60%) in very young children with a median age of 1.2 years (72, 288). Dehydration is typically mild to moderate (36, 72). A Hong Kong study of acute bacterial gastroenteritis in adults found that the average person with *Aeromonas* enteritis had 8.6 unformed stools per day, with little or no fever, and the duration of diarrheal symptoms lasted slightly more than 3 days (36). There is some indirect evidence from one Bangladeshi investigation that children belonging to blood groups O and AB may be more susceptible to diarrheal disease (and presumably *Aeromonas*) than those belonging to groups A and B (108).

The dysenteric form of *Aeromonas* gastroenteritis is much less common, with most studies reporting frequencies of 3% to 22% (36, 72, 184, 288). Common symptoms associated with *Aeromonas* dysentery or colitis include cramping abdominal pain and mucus in stools, in addition to blood (36, 72, 138). This presentation of diarrhea often requires hospitalization (for biopsy and because of the severity of symptoms). An interesting 1987 study suggests that aeromonads may preferentially colonize the bowels of persons with hematologic malignancies such as leukemia (264). This 2-year study at Vancouver General Hospital found an 8% *Aeromonas* colonization rate in neutropenic/bone marrow transplantation patients versus a 0.24% rate in other hospitalized persons. Several of the neutropenic patients presented with bloody diarrhea and symptoms suggestive of infection. *Aeromonas* colitis has also been linked to a single case of underlying and undiagnosed colonic carcinoma (51). It may well be that persons with hematologic cancers, tumors of the gastrointestinal tract, or other underlying pathological anomalies of the alimentary canal are predisposed to colonization/infections with *Aeromonas*. Further studies are warranted in this area.

Probably one of the most underappreciated roles that *Aeromonas* plays in bacterial gastroenteritis is as a cause of subacute or chronic diarrhea. Subacute diarrhea can be defined as a diarrheal syndrome lasting from 2 weeks to 2 months, whereas chronic diarrhea lasts for >2 months (65). Both conditions are fraught with multiple clinical complications, including repeat medical visits, potentially invasive and expensive diagnostic tests, specialist consultations, laboratory testing for unusual infective agents, and whether to treat the condition or not. Individual case reports have documented *Aeromonas* gastrointestinal infections in healthy persons, lasting 17 months in one instance (243) and over 10 years in another (58). Symptoms are often nonspecific in nature and typically include multiple watery bowel movements each day, sometimes accompanied by significant weight loss over time. Some episodes are linked to foreign travel prior to the onset of disease (243, 288). One study of recent travelers to Africa, Asia, and Latin America found 50% of individuals returning with *Aeromonas*-associated diarrhea to have symptoms lasting 14 days or longer (288). The frequency of subacute or chronic diarrhea due to *Aeromonas* is presently unknown.

On extremely rare occasions, *Aeromonas* has been linked to cholera-like disease (138). The most definitive example of such infections is a case report by Champsaur et al. (34) describing cholera-like disease with "rice-water" stools in a 67-year-old Thai woman. During the first 2 days of infection, the patient lost 13 liters of "rice-water" stools and received 21 liters of intravenous fluids (saline and plasma) in an attempt to return her to normal electrolyte status. She was discharged in good condition after 7 days of hospitalization. At least four other cases of cholera-like disease linked to *Aeromonas* have been described in the literature (most prior to 1990), but in some instances the role that aeromonads played in the disease process is clouded by the coisolation of other enteric pathogens (106, 138).

Gastroenteritis is the chief health problem associated with

global travel, particularly travel to developing countries (101). The reported incubation period for *Aeromonas*-associated traveler's diarrhea is 1 to 2 days, and secretory enteritis is the most common clinical presentation, although inflammatory gastroenteritis can also occur, as well as persistent or chronic diarrhea (101). A 2003 Spanish study of 863 patients with traveler's diarrhea returning from Asia, Africa, and Latin America found that 2% of cases were caused by *Aeromonas* species (288). *A. veronii* biotype sobria and *A. caviae* were the most common species identified. The most common symptoms travelers experienced were watery diarrhea and fever with abdominal pain, in slightly over half of all patients; in 17% of cases, aeromonads were isolated along with other enteropathogens.

**Atypical *Aeromonas* gastrointestinal presentations and complications.** There are a variety of unusual presentations and complications that can result from *Aeromonas* gastroenteritis. Most of these sequelae are preceded by severe bouts of *Aeromonas* colitis or dysentery (Table 8). Individual cases of *Aeromonas* colitis have subsequently led to the development of long-term chronic conditions, such as ulcerative colitis or pancolitis, ranging in duration from months to more than a year (61, 298). In some cases, surgical resection in addition to anti-inflammatory medications is necessary in order to promote recovery (298). Aeromonads cannot be recovered from bloody stools or biopsy specimens in most instances of persons suffering from these chronic conditions. Another rare condition occasionally associated with *Aeromonas* intestinal infection is segmental colitis (18, 60, 85, 186). *Aeromonas* segmental colitis can sometimes mimic or present as ischemic colitis or Crohn's disease (18, 60, 120). While the condition can affect any portion of the colon, it most often is associated with the ascending or transverse sections. One fulminant case of *Aeromonas* segmental necrotizing gastroenteritis was associated with severe soft tissue damage, septicemia, and multiorgan failure (134). Definitive diagnosis in most instances is achieved by isolation of *Aeromonas* spp. from stool cultures or other gastrointestinal samples. Other conditions reportedly linked to *Aeromonas* enteritis/colitis include ileal ulceration (300), intramural intestinal hemorrhage with small bowel obstruction (20), and refractory inflammatory bowel disease (63).

The most serious complication potentially resulting from *Aeromonas* gastroenteritis is hemolytic-uremic syndrome (HUS). Figueras et al. (88) reviewed seven reputed cases of *Aeromonas*-associated HUS reported in the literature, including their case involving a 40-year-old woman who initially presented with a 2-day history of nonbloody watery diarrhea. However, what role aeromonads actually play in the pathogenesis of HUS in most cases is unclear, and the possibility of other, unrecognized causes of HUS, such as non-O157:H7 *Escherichia coli*, may have been overlooked or not sought. Several reports listed in this review provide only anecdotal information, and in others it is not clear whether or not the case mentioned meets the definition of HUS (e.g., the presence of schistocytes and a platelet count of $<60,000/mm^3$) (90). Furthermore, the simple de facto isolation of verocytotoxigenic aeromonads from the stools of children or adults with HUS does not imply causality, since most hemolytic strains of *Aeromonas* produce a cytolysin that is active on many eukaryotic cell lines, including Vero (83). The single best evidence for the role that aeromonads may play in the disease process comes from a case report of Bogdanović and others (21) describing the recovery of a verocytotoxigenic *A. hydrophila* strain from the feces of a 23-month-old female infant with HUS. In this case report, the authors demonstrated fourfold or greater rising neutralizing antibodies to the cytotoxin in the infant's serum, reaching a maximum titer of 1:256 at day 58 after the onset of HUS. Thus, while evidence is lacking to unequivocally link *Aeromonas* with HUS, physicians should be aware of this syndrome as a possible direct or indirect consequence of gastrointestinal infection with aeromonads.

### Blood-Borne Infections

The quintessential invasive disease associated with the genus *Aeromonas* is septicemia. In 1964, Conn described a case of "*Aeromonas liquefaciens*" septicemia and peritonitis in a 44-year-old man with Laennec's cirrhosis (50). This was soon followed by two reports describing fatal cases of *A. hydrophila* sepsis, in a 16-year-old girl with acute myelogenous leukemia (AML) (56) and in a 5-year-old girl with lymphoblastic leukemia (26). In 1968, von Graevenitz and Mensch (291) published their seminal report on *Aeromonas* infections, which included two cases of septicemia in adults with Laennec's or biliary cirrhosis. These singular observations more than 40 years ago have served as the springboard for defining patient populations most at risk of developing *Aeromonas* sepsis. Today, the medical literature is replete with publications on the topic, with over 300 citations in PubMed alone regarding *Aeromonas* septicemia at the time of writing of this review. While some epidemiologic differences in the disease spectrum of *Aeromonas* sepsis based upon geographic locales or populations studied over the years have been noted, the major parameters defining *Aeromonas* septicemia have been well established for over 20 years. Three species (*A. hydrophila sensu stricto*, *A. caviae*, and *A. veronii* bv. sobria) account for >95% of all *Aeromonas* blood-borne infections (139). Infrequently, other aeromonad species have been documented as agents of infection in culture-confirmed cases of sepsis. These species include *A. jandaei*, *A. veronii* bv. veronii, and *A. schubertii* (2, 114, 139, 203, 258). While in the past the term bacteremia defined the isolation of bacteria from blood without symptomatology, while septicemia referred to blood-borne disease with signs of infection (fever, chills), the distinction between these words has been lost in most studies involving *Aeromonas*. The two terms are used interchangeably within this review.

***Aeromonas* septicemia in immunocompromised persons.** *Aeromonas* septicemia can generally be classified into one of four categories, based upon the population affected, risk factors, precipitating events leading to disease, and modes of acquisition. These groups are listed in Table 9 in decreasing order of frequency. By far, the vast majority (>80%) of cases of *Aeromonas* septicemia are seen in persons who are severely immunocompromised (group I). Disease in this setting most often involves middle-aged males (mean age, 53 to 62 years; male/female ratio, 1.6 to 4.0:1) and is community acquired (71% to 79%) (170, 180, 187, 284). However, one recent retrospective study from Taiwan, by Tsai et al. (284), involving 45 episodes of adult bacteremia in persons with leukemia or lymphoma, found only 31% of these infections to originate in the

TABLE 9. Major categories of *Aeromonas* septicemia disease presentation

| Category | Group | Underlying risk factors | Precipitating events | Portal of entry | Mortality (%) |
|---|---|---|---|---|---|
| I | Immunocompromised persons | Hepatobiliary disease, malignancy | Recent antineoplastic chemotherapy, neutropenia | Gastrointestinal tract, soft tissue, intra-abdominal route, contaminated indwelling devices | 32–45 |
| II | Trauma patients | Can vary from none to multiple conditions, including diabetes | Crush injury, penetrating injuries, near-drowning events, burns | Cutaneous-subcutaneous tissues, respiratory tract | 60 |
| III | Healthy persons | None apparent at time of presentation | None noted | Unknown | <20 |
| IV | Reconstructive surgery patients | Malignancy, traumatic injury resulting in amputation | Medicinal leech therapy | Tissue flap | <5 |

community. The exact reason for such a high frequency of health care-associated *Aeromonas* septicemias in that publication is not clear. *Aeromonas* septicemia occurs throughout the year, but a higher frequency of cases is typically observed during the summer or warmer months of the year (187, 284).

Immunocompromised persons at greatest risk of developing *Aeromonas* septicemia are those with myeloproliferative disorders or chronic liver disease (e.g., Laennec's cirrhosis or viral hepatitis). Ko and others (170) studied the largest single group of reported episodes of *Aeromonas* bacteremia ($n$ = 143) to date, spanning a 10-year period. In that study, they found the major underlying illnesses associated with systemic infection to be hepatic cirrhosis (54%) and malignancy (21%). Other recent studies have reported similar findings regarding predisposing conditions for sepsis, with chronic liver disease (26% to 36%), neoplasia (33%), and biliary disease (24%) as the three leading conditions (187, 284). Among blood dyscrasias, Tsai et al. (284) found AML to predominate, followed by myelodysplastic syndromes, non-Hodgkin's lymphoma, and acute lymphocytic leukemia. Many other underlying conditions or complications have been associated with *Aeromonas* septicemia, and these include diabetes mellitus, renal problems, cardiac anomalies, and various other hematologic conditions, including aplastic anemia, thalassemia, multiple myeloma, and Waldenstrom's macroglobulinemia (46, 142, 203, 258, 281).

Unfortunately, there are no clinical features distinguishing *Aeromonas* septicemia from those caused by other gram-negative bacteria. The most common symptoms associated with *Aeromonas* bacteremia include fever (74% to 89%), jaundice (57%), abdominal pain (16% to 45%), septic shock (40% to 45%), and dyspnea (12% to 24%) (180, 284). Diarrhea immediately preceding or concurrent with the onset of bacteremia occurs in a very small percentage of cases (9% to 14%). Most infections are monomicrobic, accounting for between 60% and 76% of all reported illnesses (170, 180, 187, 284). When polymicrobic septicemia occurs, *Aeromonas* infections are most often found in association with *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (180, 284). Llopis et al. (187) found that in 23% of cases of *Aeromonas* septicemia, a second anatomic site was also positive for these organisms, with this site most often being ascitic fluid, bile, wounds, or urine. On rare occasions, a patient with *Aeromonas* bacteremia may relapse or experience a second episode of aeromonad sepsis, separated by two or more months. The frequency of repeat *Aeromonas* infections ranged from 1.4% to 9.8% in several investigations (170, 187, 284). In these instances, it was not always clear whether reinfection occurred from a protected nidus or a clonally distinct strain caused the second bacteremia.

The frequency of primary bacteremia due to *Aeromonas* in this population has been estimated to range from 40% to 57% (170, 187), with secondary cases often seeding from endogenous foci, including peritonitis, soft tissue infections, or biliary disease. One study has suggested that a common dietary staple, seafood, may be heavily contaminated with aeromonads in Southeast Asia and may serve as a vehicle for constant gastrointestinal colonization/infection with these organisms (284). In individuals in this region with hematologic malignancies, antineoplastic medications may cause disintegration of the gastrointestinal mucosa and allow transmigration of seafood-derived aeromonads from the bowel into the circulatory system (284). Contaminated lines, such as catheters and transhepatic drainage devices, can also serve as portals of entry to seed bloodborne infections from internal or external sources (25, 64, 122).

There are only a couple of clues which, if present, may aid the clinician in suspecting *Aeromonas* sepsis as opposed to the multitude of more commonly encountered cases due to gram-negative bacilli. A patient with a history of contact with estuarine or freshwater habitats or an occupation associated with these environs may suggest *Aeromonas*. Reports of *Aeromonas* septicemia in immunocompromised persons linked to occupations such as fishing or boating (217, 281) or in aquarium hobbyists (25) have been made. The second potential indicator of *Aeromonas* infection is the presence of ecthyma gangrenosum-like lesions in the form of petechiae or bullae as a consequence of bacteremia. While ecthymotic lesions are more traditionally associated with *Pseudomonas aeruginosa* infections, these cutaneous manifestations are also associated with aeromonads. Various retrospective studies place the frequency of ecthyma gangrenosum lesions between 2% and 4% in cases of *Aeromonas* septicemia (187, 284).The attributable or direct mortality rate for *Aeromonas* septicemia is 33% or higher in most recent studies, although the methods of calculation of this value vary significantly from one study to another (170, 180,

187, 284). Multiple independent or linked variables have been associated with a poor prognostic outcome. These variables include altered consciousness, septic shock, liver cirrhosis or cancer as an underlying condition, >1 set of blood cultures positive for *Aeromonas*, a community-acquired focus, or secondary bacteremia (170, 180, 284).

***Aeromonas* infections associated with trauma.** A common but less frequently encountered subset of cases of *Aeromonas* septicemia involves persons with a history of a major traumatic event immediately preceding their septic episode. Unlike group I cases, group II patients often do not have preexisting conditions that predisposed them to invasive aeromonad infection. Rather, these traumatic events are almost always community associated and can result from various insults, only the most common of which are mentioned here. Necrotizing fasciitis, myonecrosis, or cellulitis as a result of a crush injury such as a car accident or a penetrating trauma (e.g., prick injury while deboning fish or a snake bite) can lead to severe disease requiring amputation of a limb to fulminant cases of aeromonad sepsis (4, 211). Lee and colleagues (183) found *Aeromonas* to cause 14% of cases of necrotizing fasciitis in persons with liver cirrhosis in a 12-year study. While the initial traumatic event tied to these infections was not described in the study, these patients did have serious underlying conditions associated with their illness, which probably contributed significantly to the overall observed mortality rate of 67%.

Traumas received from burns caused by oil rig or gas tank explosions (40, 161), electrical arcs (40), attempted suicides (177), or other events can result in sepsis. Fires are often initially neutralized with local water supplies, which may seed aeromonads into traumatized or devitalized tissues (161). Near-drowning events in irrigation ditch water (209) or seawater (217) can also lead to *Aeromonas* pneumonia and septicemia. In some fatal cases of sepsis, the suspected precipitating events leading to infection and the patient's demise (consumption of nonpotable water at the beach, bathing legs in a bucket of water) may not even be viewed as significant at the time of their occurrence (105, 246). The observed mortality rate for recent reported infections in this grouping approaches 60%. In many instances, the high mortality rate associated with group II infections is as much related to the trauma itself as to the infecting agent.

**Bacteremia in healthy persons.** A small but increasing group of patients apparently present with *Aeromonas* bacteremia (i) without recognized risk factors for infection (group I) and (ii) with no major trauma or precipitating event recognized that would introduce these organisms into the circulatory system (group II). Kao and coinvestigators (153) described a fatal case of *A. hydrophila* septicemia in a 5-year-old girl with high fever, lethargy, a poor appetite, and blood-tinged sputum. She had previously been healthy and had no recent recreational aquatic activities (swimming) or airway aspirations. She rapidly developed septic shock and died 4 hours after admission. Roberts et al. (244) reported a case of *A. veronii* bv. sobria bacteremia and septic arthritis in an elderly male with a 1-week history of right shoulder pain. Again, this patient had no history of travel, water contact, or trauma. He was treated with a fluoroquinolone and responded favorably, although he died 2 months later because of multiorgan failure secondary to a gastrointestinal bleed. Probably the most unusual case reported concerns a healthy 42-year-old male with dysuria, left flank pain, chilliness, and a diagnosis of right epididymitis and left pyelonephritis (19). Blood cultures grew *A. hydrophila*. On further questioning, the patient indicated that he had had recreational sex with his wife in his swimming pool 24 h prior to the onset of symptoms.

In addition to these cases, a number of other case reports have described *Aeromonas* sepsis with various sequelae in apparently healthy adults, including one recent French study which reported that 30% of patients presenting with bacteremia had no underlying health disorders (125, 151, 178, 260). However, it should be mentioned that many of these individuals were elderly (151, 244, 260), were involved in heavy alcohol consumption (125), or had professions compatible with aquatic exposures (151). While it is difficult to estimate the mortality rate in this group, it appears to be considerably lower than that observed in group I and II infections, probably due to the better immune status of affected individuals.

**Sepsis and medicinal leech (*Hirudo medicinalis*) therapy.** Medicinal leeches are often applied to tissue flaps or replantation areas as a result of plastic or reconstructive surgery to relieve venous congestion. Since leeches harbor aeromonads symbiotically, there is a risk of infection associated with such procedures. Under normal circumstances, resultant infections are normally localized (cellulitis). However, in a few instances, invasive disease has been reported. *Aeromonas* septicemia has been reported as a consequence of leech therapy in males suffering from crush injuries, accidental amputations, or plastic surgery related to malignancies (77, 86, 110) and has also seeded secondary infections of the central nervous system (CNS), such as meningitis (229). Most of the aeromonads recovered from such illnesses have been identified as *A. veronii* bv. sobria ("*A. sobria*"). All patients to date have had favorable outcomes from their resulting infections.

### Skin and Soft Tissue Infections

The second most common anatomic site from which aeromonads are recovered is the integument and deeper soft tissues underlying the epidermis. *Aeromonas* species can be associated with a variety of skin and soft tissue infections (SSTIs), ranging from mild topical problems such as pustular lesions to serious or life-threatening infections. The latter manifestations can range from infections of subcutaneous tissues (cellulitis) to processes involving the deeper layers of skin and subcutaneous tissues while spreading along fascial planes (necrotizing fasciitis) with the potential to cause severe damage to muscle tissue (myonecrosis). Necrotizing fasciitis or myonecrosis is most often seen in persons with liver disease or malignancy (55, 183). Such devastating disease can be associated with high mortality rates approaching 60% to 75% (183); a favorable outcome is inherently dependent upon early recognition of the condition, with appropriate therapeutic intervention (debridement, irrigation, and/or antimicrobial therapy). However, scarce cases have been described for children and adults without underlying systemic illness or immune dysfunction (208). Other secondary sequelae can also result from serious wound infections, including inflammation of joints and bone (septic arthritis) and disseminated invasive disease (septic shock) (68, 176).

More than 90% of *Aeromonas* wound infections are com-

munity acquired and occur in persons of ≥10 years of age (142, 178). Such illnesses are often (>70%) a direct consequence of traumatic occupational injuries or unexpected exposures via recreational sporting activities (such as swimming, fishing, and football) (142, 178). In such circumstances, the body sites most often affected include the hands, feet, arms, and legs. Some medical procedures, including medicinal leech therapy and elective surgery, can also predispose persons to developing *Aeromonas* wound infections (17, 210). Surgical site infections (SSIs) caused by *Aeromonas* are an extremely rare event but have been reported subsequent to medical procedures, including appendectomies, cholecystectomy, and colectomy (279). Virtually all reported SSIs have developed in persons with preexisting gastrointestinal or biliary disease; over three-fourths of these infections are polymicrobic. The gross mortality rate is <5%.

Unapparent or obvious traumatic events can result in various types of wound infections. Simple abrasions or lacerations can lead to significant disease if abraded areas come into direct contact with contaminated aquatic environments, including mud, streams, and lakes (68, 176, 286). More pronounced tissue damage can result from penetrating traumas, such as animal bites or the introduction of foreign bodies (soil, wood, or metal) containing aeromonads into deeper tissues via road accidents (4, 178, 179). Finally, major traumatic events, such as car or motorcycle accidents that produce open fractures with severe tissue and muscle damage, provide fertile ground for *Aeromonas* infections (211). The greater the initial insult, the more likely it is that serious life-threatening *Aeromonas* disease will result from infection.

A number of new syndromes or disease associations have been linked to *Aeromonas* wound infections. *Aeromonas* species may be important pathogens in natural disaster situations, such as hurricanes and typhoons. Water samples taken from the New Orleans Superdome and Charity Hospital post-Hurricane Katrina detected *Aeromonas* at concentrations of $10^6$ to $10^7$ CFU/ml (239). The tsunami that struck Thailand in December 2004 resulted in many SSTIs resulting from the most common problem, foreign bodies which included seawater, sand, coral, and vegetation (116, 190). *Aeromonas* was the most common pathogen identified, accounting for 22.6% of all isolates recovered from 396 persons with SSTIs (116). Though not of the same magnitude, *Aeromonas* was still recorded as the 9th most common bacterial wound pathogen in Wenchuan survivors subsequent to the 8.0 earthquake that devastated Sichuan, China, on 12 May 2008 (278). The first outbreak of wound infections associated with "mud football" was recently reported from Australia (286). Twenty-six persons participating in this charity event presented to emergency rooms with scratches or pustules (20 to 30 lesions/person) on their trunk and limbs. Three swab samples (from pustules and debridement) yielded *A. hydrophila*. All 26 players were exposed to mud, river water, or both before, during, or after the game. *A. hydrophila* was also recovered from river water, but DNA fingerprints did not match those of human isolates. Most recently, the first case of *Aeromonas* folliculitis associated with a home spa bath was published (218). Skin eruptions consisted of nodular, painful lesions in the genital area, initially resembling herpes simplex virus (HSV) folliculitis. Culture of pustular material grew *A. hydrophila*, and treatment with ciprofloxacin (6 weeks) resolved the infection. The spa was noted to be unhygienic, with earthworms living in the pipes and filter.

**Medicinal leech therapy.** *Aeromonas* infection is a recognized risk factor associated with the use of medicinal leeches to relieve postsurgical venous congestion. A 2-year retrospective study from Belgium found 4 of 47 patients (8.5%) infected, coinfected, or colonized with aeromonads (17). In another, 5-year retrospective study from France, it was estimated that the *Aeromonas* infection rate in over 200 patients treated with leeches varied from 2.4% to 4.1% (259). One source for some of these infections was an aquarium used to house leeches in a hand surgery unit that was filled with tap water contaminated with aeromonads. Cellulitis is the most common symptom associated with *Aeromonas* infections in this setting (86, 259), although bacteremia has occasionally been reported (see "Blood-Borne Infections"). However, many other consequences related to leech-associated aeromonad infections can occur, including partial necrosis or loss of flaps or grafts and amputations. Wound infections caused by *Aeromonas* can even occur several days after the cessation of leech therapy, as reported for a 47-year-old female undergoing reconstructive breast surgery (13).

**Thermal injuries.** Flame-induced or electrical burns involving major surface areas of the body are often initially controlled or extinguished by partial or total immersion of injured tissues in aeromonad-contaminated waters from taps, drains, or creeks (161). Such acts may result in *Aeromonas* colonization or infection of devitalized tissues, ranging from cellulitis to septicemia (40, 161, 177, 297). In most instances, infections are due to a single strain, although Lai et al. (177) described a case of *Aeromonas* sepsis, in an 80-year-old man who suffered a burn to 40% of his body, caused by two separate species, *A. hydrophila* and *A. veronii* bv. sobria. Mortality rates in recently reported series of *Aeromonas* infections in burn patients approximate 20%.

**Zoonotic infections.** Probably one of the more underappreciated routes by which *Aeromonas* wound infections can result is via bites of various animal species. The oropharyngeal flora of reptiles, and snakes in particular, often harbors aeromonads. Wound infections from cellulitis to necrotizing fasciitis have resulted from water moccasin, cobra, and viper snake bites (10, 147, 217). A stingray was found to cause a laceration in the dorsal aspect of the left foot of an 11-year-old boy swimming in a river in Argentina (236). The resultant wound infection was edematous and tender with mild crepetation. The necrotic border of the lesion drained purulent fluid with a "fishy" smell. Wild animals such as grizzly bears (174) or tigers (66) have been documented to produce traumatic bites on the scalp or shoulders, resulting in significant wounds containing aeromonads as part of mixed microbial populations. It is not clear in each of these instances what role, if any, aeromonads played in either the disease process or wound healing.

### Intra-Abdominal Infections

The term "intra-abdominal infection" is often viewed as being synonymous with peritonitis. In reality, intra-abdominal infections refer to infections that spread beyond the hollow viscus of origin into the peritoneal space. Such infections include pancreatitis, acute cholangitis, and hepatic abscesses as

well as peritonitis. Intra-abdominal infections are important medical problems in Southeast Asia, where the frequency of *Aeromonas*-associated peritonitis is much higher than that observed in the United States or Europe. As with the case of *Aeromonas* septicemia, most intra-abdominal infections are community acquired and are found in middle-aged males with one or more underlying diseases.

**Peritonitis.** A number of serious infectious complications are found in cirrhotic patients (24). Peritonitis is an inflammation of the peritoneum, the serous membrane lining the abdominal cavity. It can be found in three clinical settings, namely, spontaneous bacterial peritonitis (SBP), chronic ambulatory peritoneal dialysis, or direct extension from the gut (intestinal perforation) (299). Cases of peritonitis can basically be categorized into two groups, primary and secondary. The rarer form is primary peritonitis, which results from spread of an infection from the blood or lymph into the peritoneum. Secondary peritonitis, which is the more common form, most often results from extension of infections from the biliary or gastrointestinal tract. Huang et al. (124) retrospectively reviewed 49 cases of primary or secondary *Aeromonas* peritonitis that occurred in Taiwan between 1994 and 2003. Several differences were found between the two groups. Primary *Aeromonas* peritonitis was most often detected in persons with liver disease (97%) and accompanied by bacteremia (50%). Infections were community acquired in 73% of cases, and 100% of ascitic cultures were monomicrobic; two patients were infected with the same strain in the urinary tract prior to the onset of peritonitis. In contrast, 44% of secondary peritonitis cases were health care-associated illnesses, and 85% of peritoneal cultures were polymicrobic in nature, typically involving other gram-negative rods, such as *E. coli* and *K. pneumoniae*. Only 7% of these illnesses were seen in persons with liver disease or concomitant bacteremia. Attributable mortality rates in primary and secondary cases were 23% and 15%, respectively.

SBP is an infection of ascitic fluid normally seen in those with severe underlying liver disease. *Aeromonas* is the third most common gram-negative cause of SBP in Korea and Taiwan. Choi and coinvestigators (41) retrospectively reviewed 43 definite and probable cases of SBP due to *Aeromonas* in cirrhotic patients over a 10-year period and matched these cases by sex/age to control subjects with SBP caused by other bacteria. Overall, the *Aeromonas* SBP group differed from the control group in two aspects, namely, most infections were observed during the summer months and 25% of cases were preceded by diarrhea prior to the onset of SBP. The mortality rate was 23% in this series, and septic shock was found to be a poor prognostic indicator. A later, 16-year retrospective Taiwanese study evaluated 31 cases of *Aeromonas* SBP in patients with advanced liver disease (299). All cases of SBP were caused by either *A. hydrophila* or *A. veronii*. The gross mortality rate was 56%.

*Aeromonas* peritonitis can also present as a consequence of continuous ambulatory peritoneal dialysis (37, 302). In many instances, these patients have underlying liver disease (e.g., adenocarcinoma or hepatitis) that may or may not be recognized at the time of infection (37, 302). *A. hydrophila* is by far the most common species associated with bacterial peritonitis in Southeast Asia, accounting for 95% or more of all reported cases, although other species, including *A. veronii* bv. sobria, may be involved (41, 124, 202). In most cases, it is unclear where the source of infection originates. Yang et al. reported a case of peritonitis in a 68-year-old female who had consumed freshwater fish both at a restaurant and at home multiple times a week prior to the onset of her symptoms (302). In another instance, the coisolation of *Shewanella putrefaciens* from dialysis fluid suggested a marine focus for this illness (37). In several large series of cases, only a few medical histories had frequent water exposure suggesting an environmental origin (124).

**Infections of the hepatobiliary and pancreatic systems.** Acute suppurative cholangitis is one of the most common medical complications of the hepatobiliary tree associated with aeromonads. Two recent studies, one from Hong Kong and another from Michigan, place the frequency of cases of cholangitis due to *Aeromonas* between 1.3% and 2.9% (35, 47). As opposed to other disease syndromes, most cases of *Aeromonas* cholangitis are mixed infections (>85%), typically associated with members of the *Enterobacteriaceae*, *Enterococcus*, or *P. aeruginosa*. This fact suggests a gastrointestinal origin for these illnesses. Many persons developing *Aeromonas* cholangitis have had previous attacks of the condition (35). Rare cases of recurrent *Aeromonas* cholangitis, separated by 12 and 22 months, have been described (47) in the literature, although it is not clear whether or not the same strain was involved.

Virtually all patients presenting with *Aeromonas*-associated cholangitis have one of several underlying conditions: cholelithiasis or choledocholithiasis, cholangiocarcinoma, pancreatic carcinoma, or nonmalignant biliary strictures (35, 47). Clark and Chenoweth (47) found liver transplantation as a comorbid condition in 4 of 15 patients with cholangitis. They also reviewed the medical literature on the subject and found the most common underlying conditions and comorbidities for 39 patients with *Aeromonas* hepatobiliary or pancreatic infection to be cholelithiasis (33%), malignancy (33%), other immunocompromised conditions (28%), and recent surgical procedures (21%). In the Hong Kong study, the gross mortality rate was 10% and the mortality rate attributed to *Aeromonas* was 0% (35). Comparable numbers in the Michigan study were 29% and 11.8%, respectively (47).

De Gascun and others (57) recently published a case report describing a fatal case of *Aeromonas* sepsis linked to a pancreatic abscess. The patient, a 50-year-old man with alcohol-related liver disease and chronic pancreatitis, presented with abdominal pain, hematemesis, and weight loss. He died 6 days after admission, and postmortem cultures of pus from a fibrocytic pancreas yielded *A. hydrophila* and an unidentified anaerobe. Death was attributed to secondary sepsis resulting from the infected abscess.

### Respiratory Tract Infections

*Aeromonas* species are occasionally encountered in sputum or other respiratory tract secretions from a variety of hospitalized patients. In the past, in the vast majority of cases, these isolates have been regarded to represent transient colonization only (143). Even recent reports describing the isolation of mucoid and nonmucoid variants of *A. hydrophila* from cough swabs of an 11-month-old infant with cystic fibrosis have been thought to reflect brief asymptomatic colonization rather than

infection and to have resulted from home aerosolization of bacteria from one of several tropical aquaria (54).

Our concepts regarding the genus *Aeromonas* and respiratory disease may need to change, though. A decade ago, only a few legitimate cases of respiratory tract disease due to *Aeromonas* were available. These infections ranged from epiglottitis to empyema, lung abscesses, and pneumonia in those with no comorbid conditions or in individuals with traditional immunocompromised states associated with the genus. Today, while not large in number, an increasing body of cases document aeromonads causing serious respiratory tract infections. Such illnesses are often difficult to diagnose and present a diagnostic challenge to the clinician and microbiologist alike.

**Pneumonia.** By far the most frequent respiratory complication associated with the genus *Aeromonas* is pneumonia. Cases of bacterial pneumonia are typically found in two distinct populations. The first group involves major trauma, the most common of which is near-drowning events, of which there are an estimated 16,000 to 160,000 instances in the United States annually (69). Recent cases of *Aeromonas* pneumonia accompanying septicemia have been linked to near-drowning events involving seawater (217), a shallow irrigation ditch, and other massive aquatic exposures (178, 209). The rapid demise of patients in this setting can be as quick as 9 h from the initial insult to time of death (209). In a second scenario, there is no obvious event leading to respiratory disease or, in most cases, a defined vehicle of infection. Patients often present with high fever, a productive cough (hemoptysis), vomiting, chest pain, and/or respiratory failure (153, 216, 220, 246). While many adults with *Aeromonas* pneumonia have preexisting underlying conditions, such as liver cirrhosis, renal disease, or multiple sclerosis, children often do not. Aspiration pneumonia is suspected in some of these cases (216), while polluted water is thought to have been the vehicle of infection in one instance (246). Blood cultures are the most common specimen found positive for aeromonads, but others include endotracheal samples, bronchoalveolar lavage or secretions, and postmortem samples, such as lung and pleural effusions. As with the first group, these infections often have a very rapid downhill course, with the time between hospital admission and death ranging from 4 to 48 h. In one fatal case, two distinct colony types of *A. hydrophila* were detected, one of which was resistant to multiple antimicrobial agents, including piperacillin and imipenem, which may have contributed to the negative outcome (220). The mortality rate associated with *Aeromonas* pneumonia from recent case reports is approximately 50%.

**Other respiratory tract infections.** Several cases of spontaneous bacterial empyema caused by *A. hydrophila* or *A. veronii* bv. sobria have been reported from Southeast Asia for males with underlying cirrhosis due to hepatitis B virus (39, 162, 295). All three men, whose ages ranged from 27 to 54 years, presented with dyspnea; two had high fevers, and one had pleuritic pain. Pleural fluid obtained from each patient had leukocyte counts varying from 14,900 to 44,800/mm$^3$, with 80% to 95% neutrophils. Blood cultures were negative in two of the three cases, and there was no evidence of pneumonia by imaging. Presumed sources of infection included ascites or transient hematogenous seeding. All three patients recovered from their empyema episodes.

Bossi-Küpfer and colleagues reported a case of tracheobronchitis in a healthy 19-year-old man who suffered a near-drowning event when he was submerged in a river in Switzerland for several minutes (23). Upon bronchoscopy, *Aeromonas* was recovered as the predominant microorganism. Although his respiratory condition improved, he subsequently succumbed due to severe neurologic impairment. A severe case of *Aeromonas* epiglottitis progressing to necrotizing fasciitis was also reported for a 61-year-old man with cirrhosis (12). *A. hydrophila* was recovered from his blood, epiglottitis specimens, soft tissue of the neck and fascia, and a rectal swab. He underwent surgery and postoperatively received ceftriaxone, to which he responded favorably.

## Urogenital Tract Infections

*Aeromonas* species are occasionally implicated in infections of the urogenital tract, although such disease has received little attention from the scientific and medical communities. It is also not clear how common or infrequent such urogenital tract infections (UTIs) are, since they often receive only cursory mention in published studies (124). Hsueh et al. (122) described a UTI with bacteremia caused by *A. veronii* bv. sobria in a 69-year-old male with diabetes mellitus and chronic hepatitis. He was treated successfully with ceftriaxone but subsequently developed necrotizing fasciitis caused by the same organism. *A. popoffii*, a rare human pathogen, was the cause of a UTI in a 13-year-old boy with congenital spina bifida and myelomeningocele (123). Introduction of the infection appeared related to the replacement of a urinary catheter. Urine cultures yielded pure growth of *A. popoffii*, which was identified by 16S rRNA gene sequencing. A 39-year-old man with a 2-month history of increased urination, dysuria, and hematuria developed cystitis due to *A. caviae* (5). He was apparently healthy otherwise, and no potential source for his infection was found.

An unusual case of *Aeromonas* prostatitis was reported for a 39-year-old male with chronic alcohol consumption (125). Computed tomography showed a fatty liver and prostatitis. Blood and urine cultures grew *A. veronii* bv. sobria ("*A. sobria*"). No source for his infection was discovered, but the authors speculated that his lower socioeconomic status may have increased the likelihood of exposure to soil or water containing *Aeromonas* spp.

## Eye Infections

*Aeromonas* species can cause ocular disease ranging from endophthalmitis to keratitis and corneal ulceration (158, 235, 240, 270). In many instances, there is no known preceding trauma or exposure to environmental sources potentially containing aeromonads. However, soft contact lenses have been found to contain *Aeromonas* on occasion, among other microbes (121). Pinna and others (235) described a case of *A. caviae* keratitis in a 35-year-old man that was associated with contact lens wear. The infection was associated with replaceable soft lenses that were kept in a lens case which was never replaced or cleaned. Furthermore, the patient occasionally rinsed his lenses in tap water.

For additional information regarding the role of *Aeromonas* species in human infections, the reader is advised to consult

the reviews of Altwegg and Geiss (9), Figueras (87), Janda and Abbott (142, 143), Khardori and Fainstein (159), and Zhiyong et al. (305).

## PATHOGENICITY

### General Principles and Practices

Pathogenicity can be defined in its most recognizable form as the capacity of microbial agents to cause disease in a particular host and, in regards to this review, in humans (28, 80). The definition of microbial pathogenicity is by its very nature a constantly evolving one, and recent proposals suggest a redefining of pathogenicity as simply the ability to cause damage in a host (28). Such definitions are problematic in light of today's health care-associated infections and the large number of persons living in communities with a variety of preexisting medical complications, including immunocompromised states and diabetes. Several cardinal features help to define infections and pathogenicity, including the inoculum and route of infection, host susceptibility, and virulence characteristics of a given strain (80). A second cardinal feature innately associated with pathogenicity is virulence. Virulence can be defined roughly as the ability of a particular strain to incite disease at a specified end point. For intact bacteria, this can be measured in multiple ways, such as lethality studies (50% lethal dose [$LD_{50}$]), strength of pathogenicity (degree of invasiveness or production of toxins), or other attributes (28). Classic definitions of pathogenicity link virulence to specific toxins or to cell-associated characteristics, such as capsule production. Pathogens and virulence factors were originally defined on the basis of Koch's postulates, modified to fit the molecular era (82). Yet even these revised postulates do not fit all current situations.

What has changed? With the advent of molecular genomics and whole-genome sequencing (261), an endless array of genes and potential virulence factors can be identified on the basis of homologs in other species. Yet this is guilt by association only and does not necessarily imply causality. Virtually all genes identified nowadays are listed as virulence genes in one format or another, yet few, if any, clearly fulfill this definition by directly being responsible for causing pathological damage in the host. Proposals have been made for changing and redefining the concept of what constitutes a virulence gene (296). It is also evident that strain virulence for most nonclassical species is polygenic in nature, requiring two or more genes to act in concert in ways that are poorly understood for most species at present.

Problems regarding microbial pathogenicity are even more confounding in regards to *Aeromonas* species. Only two *Aeromonas* infections in humans (gastroenteritis and wound infections) clearly predominate in healthy people, as opposed to those with underlying illnesses. In the former instance, it is presently unknown if all or most aeromonads recovered from stools cause intestinal symptoms, which microbial factors are critical in this infectious process, and why these factors are not exclusively associated with a subset of "diarrheagenic strains." The inability to clearly distinguish "infecting" from "colonizing" strains in the gastrointestinal tract makes it unfeasible presently to establish collections of enteropathogenic and nonenteropathogenic strains. Additional problems include no well-circumscribed outbreaks of *Aeromonas* gastroenteritis and the lack of an animal model to reproduce such a syndrome. Such factors make it impossible to determine which genetic characteristics might be important in *Aeromonas* gastrointestinal colonization and infection (see "*Aeromonas* and gastroenteritis: where are we now?"). In comparison, little attention has been paid to wound models of infection, probably because of their lower frequency of occurrence in clinical infections.

Yet we know that *Aeromonas* pathogenicity is not simply a random event. Only 3 of more than 15 recognized *Aeromonas* species (*A. hydrophila sensu stricto*, *A. caviae*, and *A. veronii* bv. sobria) produce the vast majority of systemic infections in humans (139, 143). Animal studies, largely now a thing of the past, generally but not universally support the enhanced pathogenicity of these species, as assessed through $LD_{50}$ studies, with a 4-$log_{10}$ difference noted in pathogenicity between the most virulent and least virulent strains (140). Environmental studies indicate that while these species may be relatively common in some ecologic niches, they are not for the most part the predominant species in drinking and surface water samples and in foods, suggesting that the overall process of disease production in a susceptible host involves, at least in part, "selection" of strains with certain characteristics that favor infection (22).

It is beyond the scope of this review to provide a definitive analysis or exhaustive compilation of all virulence factors, markers, or pathogenicity studies published concerning the genus *Aeromonas*. Rather, an attempt is made to provide an overview of our present knowledge on the subject, focusing on the most recently published data on the subject as much as is possible.

### Animal Models of Infection

The animal model of infection that everyone needs (gastroenteritis) is still lacking. Repeated attempts to develop such a standard, although without success, continue to be made, such as through the use of clindamycin-pretreated rats (155). However, several real or potential models for studying *Aeromonas* pathogenicity do exist and offer some opportunities to understand the genetic basis for how aeromonads interact with susceptible hosts (Table 10).

By far the most novel and attractive system developed recently is the medicinal leech model of Graf and collaborators (104, 265). The microbiota of the leech's crop is normally populated by only two resident symbiotic microbial species, one of which (*Aeromonas*) can be grown *in vitro* and is amenable to genetic manipulation. Silver et al. (266) have identified several classes of genes that play important roles in colonization of the leech digestive tract, including bacterial cell surface modifications, regulatory factors, nutritional elements (amino acid and phosphate transporters), and genes involved in type three secretion systems (TTSS). Mutants in specific genes, such as that encoding Braun's major outer membrane lipoprotein or a gene encoding a cytoplasmic membrane component of a TTSS, compete 10,000- to 25,000-fold less efficiently against wild-type or competitor strains in colonization of the leech's crop (265, 266). Even more interesting is the fact that Graf has identified seven or more genes in *A. veronii* that are colonization mutants and whose function is

TABLE 10. Current and prospective models of *Aeromonas* infection

| Model | Species | Process studied | *Aeromonas* sp. | Implicated genes[a] | Reference(s) |
|---|---|---|---|---|---|
| Medicinal leech | *Hirudo medicinalis*, *H. orientalis*, *H. verbana*, *Macrobdella decora* | Symbiosis; digestive tract colonization and associations | *A. veronii* bv. sobria, *A. jandaei* | *lpp*, *ascU* | 104, 265, 266 |
| Blue gourami | *Trichogaster tricopterus* | Septicemia; bacterial phagocytosis; immune responses | *A. hydrophila* | *aopB*, *aopD*, *ascN* | 92, 303, 304 |
| Zebrafish | *Danio rerio* | Immune responses | *A. hydrophila* | | 247 |
| Slime mold (amoebae) | *Dictyostelium discoideum* | Overt pathogenicity | *A. salmonicida*, *A. hydrophila* | | 98 |
| Nematode | *Caenorhabditis elegans* | Toxicity to worm; survivability | *A. hydrophila* | | 52 |

[a] *aopB*, *Aeromonas* outer protein B gene; *aopD*, *Aeromonas* outer protein D gene; *ascN*, *Aeromonas* secretion protein N gene; *ascU*, *Aeromonas* secretion protein U gene; *lpp*, lipoprotein gene.

presently unknown (266). Such a model offers exciting opportunities to potentially discover important genes and gene products critical to colonization of digestive tracts in susceptible hosts.

A number of other systems are not nearly as well developed as the medicinal leech model but merit mentioning. Several of these involve tropical aquarium fish. The blue gourami model of septicemic disease has been used extensively by Leung and partners to study the pathogenicity of several microbial species, including *Edwardsiella tarda* and *A. hydrophila* (273). Genome walking experiments have identified a series of open reading frames (ORFs), including two (*apoB* and *apoD*) involved in TTSS, that have reduced virulence in the blue gourami and are more easily phagocytized by gourami phagocytes (303). Further studies employing genomic subtraction experiments identified 19 putative virulence factors and 7 ORFs in *A. hydrophila* PPD134/91 (304). However, the only mutant in this study with an appreciable difference in virulence in the blue gourami was another homologue (AscN) of a *Yersinia* protein involved in TTSS (304). In both studies, the best mutants altered $LD_{50}$ values in fish only 1 log (10-fold), far below the threshold value of a 2-log (100-fold) difference that most researchers would like to achieve. Both the blue gourami and zebrafish have also been used to study host immune responses to challenge with *A. hydrophila* (92, 247). Challenge studies in zebrafish with viable, heat-killed, or extracellular products of *A. hydrophila* elevated expression of tumor necrosis factor, interleukins, and interferon in pathologically damaged organs, such as the kidney (247).

Several other interesting models of infection have also been proposed. Use of the unicellular amoeba *Dictyostelium* to assess virulence by the growth of this organism on lawns of bacteria has been suggested (98). Virulent strains of *A. salmonicida* and *A. hydrophila* are nonpermissive (no plaques), while an avirulent mutant strain of *A. hydrophila* with a TTSS defect was found to be permissive (plaques). Finally, several strains of *A. hydrophila* have been shown to produce rapid toxic (death) effects in *Caenorhabditis*, although avirulent strains were not tested (52). It may well be that this worm could serve in a similar fashion to *Dictyostelium* as a quick measure of overt pathogenicity or to assess virulence in genetic mutants.

## Organotrophic Disease

At present, there are no suitable models developed to study the vast majority of diseases potentially caused by aeromonads. This includes the most frequently encountered syndromes, such as gastroenteritis and wound infections. While $LD_{50}$ studies with either immunocompetent (140) or immunocompromised (188) mice may give a fair approximation of the overt virulence of *Aeromonas* species or strains in a septicemic model, the common route of inoculation used (intraperitoneal) is atypical of the *in vivo* situation, that is, translocation of bacteria from the gastrointestinal lumen into the circulatory system. Probably the best way of looking at *Aeromonas* pathogenicity at present is by the type of infections caused, general knowledge regarding the disease process in other traditional gram-negative enteropathogens, and drawing rough conclusions concerning types of virulence factors associated with these illnesses (81).

**Gastroenteritis.** To be a successful enteropathogen, a bacterium must gain entry into a host, bypass normal physiologic barriers, find a particular niche, avoid host defense mechanisms, and produce disease (80). In the case of *Aeromonas* gastroenteritis, the presumed route of infection is via oral ingestion of contaminated foods or water. Ingested bacteria must then bypass the deleterious effects of gastric acidity and take up residence in the small or large intestine, competing successfully against autochthonous microorganisms.

While many bacterial genes and potential virulence factors must be involved in this complicated process, only a few have been studied in any great detail. One potential pathway by which *Aeromonas* could theoretically circumvent the harmful effects of low acid pH in the stomach is by an acid tolerance response similar to that described for a number of enteric pathogens, including *Salmonella enterica* serovar Typhimurium and *E. coli* (15). Karem et al. (154) have adapted a strain of *A. hydrophila* to withstand pH 3.5 through a process similar to that recorded for salmonellae. Adaptive acid tolerance required protein synthesis but was independent of the iron concentration. Such a process, if consistently found in many other *Aeromonas* strains, would help to facilitate subsequent colonization of the gastrointestinal tract.

Once aeromonads enter the gastrointestinal tract, a series of events must unfold in which they compete successfully against

normal flora with their elaboration of by-products of metabolism and bacteriocin-like compounds, to attach and colonize the lumen of the intestine or bowel. One can think of this process potentially involving a series of interrelated steps, including directed locomotion→attachment to gastrointestinal epithelium→biofilm formation→colonization→elaboration of virulence factors→infection. Two factors thought to play intimate roles in these processes are bacterial flagella and pili. *Aeromonas* produces two types of flagella, a constitutively expressed polar flagellum (Pof) and multiple inducible lateral flagella (Laf) (192). Pof produces swimmer cells in liquid environments, while Laf induces swarming motility on solid medium surfaces (167). One can envision Pof playing an important role in the initial attachment of bacteria to the gastrointestinal epithelium, while Laf could play an important role in subsequent processes, including increased cell adherence, biofilm formation, and long-term colonization. Studies conducted with HEp-2 cells showed that reintroduction of *laf* genes into *laf*-negative mesophilic isolates increased adhesion and invasion of epithelial cells as well as promoting biofilm formation (102). Similarly, two morphologically distinct types of pili exist in *Aeromonas*, consisting of short and rigid (common) ones that are related to type I and Pap pili of *E. coli* and long, wavy, type IV pili (192). Furthermore, two families of type IV pili have been found, namely, those related to bundle-forming pili (Bfp), which appear to mediate adherence to enterocytes (Henle 407 and Caco-2 cells), and a second family, called type IV *Aeromonas* pilus (Tap), encoded by a gene cluster designated *tapABCD* (137, 166, 192). While there is considerable evidence that Bfp pili are significant factors in intestinal colonization, there currently are no credible data to suggest an identical function for Tap (165).

Biofilm development may also be regulated by quorum sensing in *Aeromonas* (189). Most, if not all, *Aeromonas* species contain *luxRI* homologs, encoding an acyl-homoserine lactone (acyl-HSL)-dependent transcriptional activator (146). Mutation in the *luxS* gene in one clinical isolate of *A. hydrophila*, strain SSU, significantly altered biofilm development and enhanced virulence in the septicemic mouse model but did not appreciably affect cytotoxic or hemolytic production or TTSS activity (171). Quorum sensing and lactone production also appear to act in concert with TTSS to regulate the expression of at least one *Aeromonas* enterotoxin in the diarrheal isolate SSU, as enterotoxin production increased as bacterial cell density increased (263).

Once established in the gastrointestinal tract, aeromonads can apparently produce diarrhea by elaboration of enterotoxigenic molecules, causing enteritis, or by invasion of the gastrointestinal epithelium, producing dysentery or colitis. Conceivably, simultaneous expression of both enterotoxins and invasins is also possible. One of the problematic issues in this area concerns the plethora of enterotoxigenic factors described and the lack of consensus on standardization of terminology regarding these factors between different research groups (42). These molecules fall into several broad categories, including cytolytic toxins with hemolytic activity and cytotonic enterotoxins. Probably the best known and well characterized of these toxins is the β-hemolysin of *A. hydrophila*, often referred to as Bernheimer's aerolysin. This pore-forming toxin is found in 75% or more of *A. hydrophila* strains, as well as in many other species, including *A. veronii* ("*A. sobria*"), *A. caviae*, and *A. trota* (99, 113). A second family of β-hemolysins exhibits significant amino acid sequence homology to the HlyA hemolysin of *Vibrio cholerae* (137) and is also referred to as AHH1 in the literature (113). HlyA is widely dispersed in *Aeromonas* species and is virtually ubiquitous in *A. hydrophila*; it is also found in *A. caviae* (35%), *A. veronii* (12%), *A. trota*, and *A. jandaei* (113, 294). A third *Aeromonas* cytotoxic enterotoxin, Act, is a type II secreted pore-forming toxin with hemolytic activity (262). Act induces fluid accumulation in ligated intestinal loops and stimulates proinflammatory responses by increased cytokine production through elevated tumor necrosis factor, IL-1β, and IL-6 levels (43).

Many other toxins or factors have been described that may play roles in *Aeromonas*-induced gastrointestinal disease pathology. At least two cytotonic toxins have been identified, i.e., an *Aeromonas* heat-labile cytotonic enterotoxin designated Alt and a heat-stable cytotonic enterotoxin named Ast (262). A vacuolating toxin has also been found in certain strains of *A. veronii* bv. sobria. The toxin was recently partially purified and appears to be a 60-kDa nonhemolytic enterotoxin that acts as a serine protease and causes apoptosis in Vero cells (199). It can be neutralized partially by antibodies produced against aerolysin. Invasins have also been reported, but they are often difficult to detect *in vitro*, as cytolytic toxins often mask the potential invasive capabilities of strains entering human epithelial cells, such as HEp-2 or HeLa cells, or enterocytes. Limited studies suggest that only a fraction of *Aeromonas* strains are invasive (53), and the relative degree of invasion is considerably less than that observed for classic enteropathogens, such as enteroinvasive *E. coli*, *Shigella*, or *Yersinia enterocolitica* (102).

Presently, there are a multitude of unresolved questions and issues regarding the role that each of these factors plays in *Aeromonas*-associated gastroenteritis. For example, the prototypical diarrheal isolate SSU contains at least four distinct factors with enterotoxigenic capabilities *in vitro*, namely, Hly, Act, Alt, and Ast (71). What role does each or any of these play in diarrhea, and is this gene assortment representative of other fecal isolates representing diverse species associated with gastroenteritis? Some factors, such as Act, are also found in species infrequently associated with human disease, such as *A. trota* or *A. bestiarum* (192). Although differences in restriction maps and flanking sequences of these genes occur in different strains and species, it is hard to imagine how Act could play an important role in diarrhea, given its widespread distribution in species and in the environment. Other important factors must also be operative. Finally, sophisticated studies by Chopra and others (79), using microarray analysis, indicated that the expression of 221 genes was altered when wild-type and mutagenized SSU strains were used to infect mice. This clearly demonstrates the enormity of the situation involving polygenic expression in both the pathogen and the host.

**Wound infections.** There is a paucity of information in the literature on experimental studies conducted on the pathogenicity of *Aeromonas* in wound infections. Despite these shortcomings, it is likely that pathogenicity in aeromonads involves similar steps and virulence factors to those described for another gram-negative wound pathogen, *P. aeruginosa*. Figure 2 depicts a hypothetical model regarding

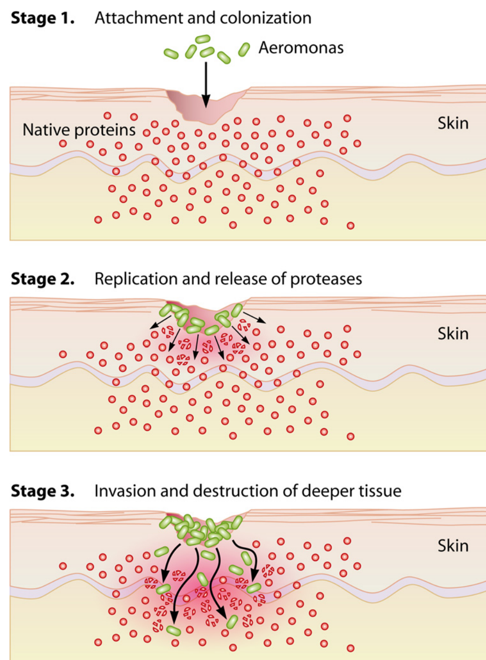


FIG. 2. Hypothetical model of *Aeromonas* wound infection. The process involves three major stages. (1) Attachment and initial colonization of wound site; (2) elaboration of proteases and degradation of proteinaceous material as an energy source, leading to multiplication of bacilli; (3) migration of aeromonads into deeper tissues due to a gradient effect (higher concentration of proteins) via chemotactic motility.

how *Aeromonas* might cause superficial or deep-seated wound infections with possible systemic extension. Infection requires attachment at the local site, degradation of biological molecules (proteins) as an energy source for replication, and then invasion of deeper tissues in response to a chemotactic protein gradient.

Several factors probably play important roles in this process, in addition to adhesive factors needed for step 1 that are listed under gastroenteritis. *Aeromonas* species elaborate a wide range of microbial proteases (metalloproteases, serine proteases, and aminopeptidases) capable of degrading complex biologic proteins present in serum and connective tissue, including albumin, fibrinogen, elastin, and collagen (107, 132, 136, 137). Degradation of such tissues and proteins can serve as an energy source for subsequent multiplication. When nutrient sources become depleted, a chemotactic gradient then develops, with higher protein concentrations in deeper tissues and lower protein concentrations in superficial areas already colonized by aeromonads. Most aeromonads (80% to 95%) exhibit chemotactic motility in response to amino acids, proteins, or mucins (136). Such directed chemotactic responses should trigger rapid migration of *Aeromonas* into subcutaneous tissues via motility, leading to colonization of environments with enriched nutrients. Many other factors also probably play important roles in wound infections, including quorum sensing and TTSS.

**Septicemia.** Most cases of primary *Aeromonas* septicemia apparently arise through endogenous translocation of bacteria from the gastrointestinal tract into the circulatory system. Secondary cases often involve seeding of aeromonads into the bloodstream from infected wounds, peritonitis, or biliary disease. Models to study the progression of such diseases are presently unavailable, although intraperitoneal inoculation of bacilli into normal or immunocompromised mice is probably a reasonable simulation of secondary *Aeromonas* bacteremia associated with peritonitis.

While many isogenic mutants show a loss of virulence ($LD_{50}$ values) in the mouse septicemic model compared to wild-type strains, it is unlikely that factors such as enterotoxins or global regulatory systems such as TTSS or quorum sensing in and of themselves are overt virulence factors specifically associated with bacteremia. Rather, bacteria are exposed to a number of host defense mechanisms that pathogens must overcome in order to proliferate in extraintestinal spaces. It is well recognized that *Aeromonas* strains are not randomly associated with septicemia, but rather most infections (90%) are caused by a very limited number of genomospecies (139). Within these septicemia-producing species, specific subsets of strains with certain markers or attributes are likely responsible for most blood-borne disease. Studies demonstrate that aeromonads belonging to serogroups O:11, O:16, O:18, and O:34 (Sakazaki and Shimada scheme) are associated with most cases of bacteremia, implying that lipopolysaccharide (LPS) antigens and architecture are important in systemic disease pathogenesis (139). Because of their LPS or the possession of S layers, most bacteremic *Aeromonas* isolates are resistant to the lytic effects of the classical complement pathway (139, 141, 204). Resistance is linked to the rapid degradation of C3b and the failure of terminal components of the pathway to bind and form the lytic membrane complex (204).

Krzymïnska et al. (173) used the J774 macrophage cell line to study phagocytosis of 26 strains of *Aeromonas*. Most *Aeromonas* strains were poorly phagocytized by J774 cells, regardless of species designation. However, the uptake of strains of *A. veronii* bv. veronii and *A. hydrophila* was less efficient than that of *A. caviae* in this model. Internalized bacteria continued to replicate in J774 cells for 3 h postinfection in 31% of strains studied, suggesting that aeromonads have an avoidance mechanism to counteract intracellular killing (173). A prototype bacteremic strain of *A. hydrophila* has also been studied in regards to comparative pathogenicity with *K. pneumoniae* and a control strain of *E. coli*. *A. hydrophila* was more virulent in BALB/c mice and caused higher levels of tumor necrosis factor, IL-1β, and IL-6 in human whole blood than did a blood isolate of *K. pneumoniae* (169). In intramuscular inoculation studies, the *Aeromonas* blood isolate produced a more intense inflammatory response in infected mice than did *K. pneumoniae* and was the only strain to cause myonecrosis (169). This suggests that *Aeromonas* and some of its biologic products are important activators of cytokine induction and inflammatory responses.

There are literally dozens of additional extracellular or cell-associated factors that may play roles in *Aeromonas* pathogenicity that are beyond the scope of this review. For further information on these topics, the reader is invited to consult reviews by Chang and Janda (38), Martin-Carnahan and Joseph (192), Chopra and Houston (42), and Janda (137).

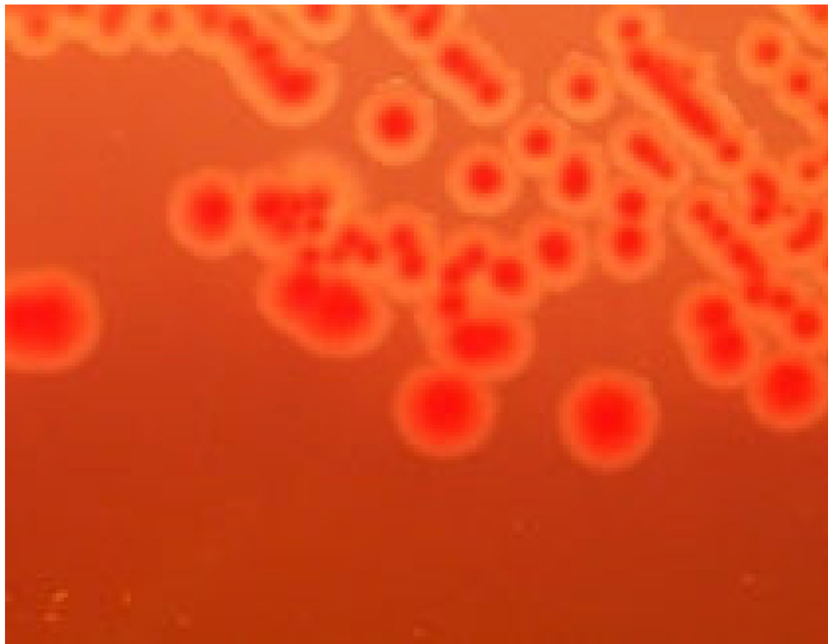

FIG. 3. Bull's-eye-like colonies of *A. caviae* on CIN agar at 48 h.

## LABORATORY IDENTIFICATION

### Isolation

Transport of specimens, particularly stool, to the laboratory can be achieved in a variety of transport media (Cary-Blair, Amies, or modified Stuart's medium, buffered glycerol in saline), although it is generally agreed that Cary-Blair medium is the most suitable (192). Transport at room temperature yields the greatest recovery. When specimens are transported at 4°C for 24 h, colony counts decline and may only rebound, if at all, after being held for several days at that temperature.

Isolation of members of the *Aeromonadaceae* from clinical sources is relatively simple. Aeromonads of clinical significance grow well on noninhibitory laboratory media used for culture of bacteria from sterile sites as well as on most enteric isolation media, with the exception of thiosulfate-citrate-bile salts-sucrose (TCBS) agar. Although growth is not a problem on routine enteric isolation media (MacConkey, XLD, HE, SS, and DC media), lactose-negative isolates must be differentiated from commonly isolated pathogens such as *Salmonella* and *Shigella*, or if the organism ferments lactose or sucrose, it may be assumed to be normal flora and be overlooked. However, cefsulodin-irgasan-novobiocin (CIN) agar, used for the isolation of *Yersinia*, has been found to support the growth of *Aeromonas* as well as *Plesiomonas shigelloides* (personal observation), making this agar multifunctional and hence increasing its cost-effectiveness. Like *Yersinia*, *Aeromonas* forms a bull's-eye-like colony due to fermentation of D-mannitol (Fig. 3), while *P. shigelloides*, which does not ferment D-mannitol, produces colorless colonies. Usually, *Citrobacter* spp. are the only normal fecal flora that grow on CIN with any frequency, and regrettably, their colony morphology is similar to that of *Yersinia* and *Aeromonas*. Because of false-negative reactions due to acid produced by fermentation of D-mannitol, an oxidase test which readily separates *Aeromonas* from *Yersinia* and citrobacters cannot be performed directly from CIN agar.

In a study comparing CIN with ampicillin blood agar (ABA; blood agar with 20 μg/ml of ampicillin), *Aeromonas* was recovered from 22 (51%) and 36 (84%) of 43 stools, respectively, although 7 (16%) strains were isolated only from CIN (157). ABA also has the advantage over CIN agar in that hemolytic colonies can readily be tested for oxidase, which dramatically reduces screening. On the other hand, ABA is useful only for the recovery of *Aeromonas*, and if screening is based on hemolysis, approximately 10% of *Aeromonas* isolates will be missed because they are nonhemolytic (laboratories using BA plates for isolation will also miss these isolates). Also, on ABA, all ampicillin-sensitive isolates, including almost all strains of *A. trota*, which is an ampicillin-susceptible species, would be inhibited. An alternative medium, produced by Lab-M, is *Aeromonas* agar (AA), which also appears to be superior to CIN agar for the isolation of aeromonads (11). This highly selective medium, like CIN, contains irgasan, but it uses D-xylose (which aeromonads do not ferment) as a differential characteristic. In one study, the numbers of aeromonads recovered from stool doubled when AA was added to the testing regimen (11). Oxidase testing can be performed directly from the medium for colonies in areas where there is no acid produced from fermentation of D-xylose by fecal flora. Pseudomonads, which are indistinguishable from aeromonads on AA (oxidase-positive, translucent pink colonies), can be separated by their oxidative metabolism. Finally, xylose-galactosidase agar (XGA) is a medium designed for recovery of aeromonads, salmonellae, shigellae, and yersiniae (100). In comparing XGA to CIN, the authors who designed XGA actually isolated more aeromonads from CIN but found fewer false-positive colonies on their medium (11% versus 60%). Regrettably, a later 2004

TABLE 11. Differentiation of *Aeromonas* from *Vibrio* and *Plesiomonas*[a]

| Organism | Growth on substrate | | | Presence of enzyme | | | Fermentation of *myo*-inositol |
|---|---|---|---|---|---|---|---|
| | Growth on 0% NaCl[b] | O129 | Growth on TCBS | LDC | ADH | ODC | |
| *Aeromonas* species | Gr | R | NGr | + | + | − | − |
| | | | | − | + | − | |
| | | | | + | − | + | |
| *Vibrio cholerae*/*V. mimicus* | Gr | S[c] | Yellow colony/green colony | + | − | + | − |
| Other vibrios | NGr | PS | Yellow or green colony | + | − | + | − |
| | | | | − | + | − | |
| *Plesiomonas shigelloides* | Gr | R/S | NGr | + | + | + | + |

[a] Abbreviations: O129, vibriostatic agent 2,4-diamino-6,7-diisopropylpteridine; LDC, lysine decarboxylase; ADH, arginine dihydrolase; ODC, ornithine decarboxylase; Gr, growth; NGr, no growth; R, resistant; S, sensitive; PS partially sensitive.

[b] Difco nutrient broth, contains no trace of NaCl.

[c] Infrequent strains of *V. cholerae* serogroup O1 and all strains of serogroup O139 are resistant.

study found that XGA was not an acceptable alternative for use as a routine isolation medium because the sensitivity and specificity for the detection of salmonellae were unacceptable (251).

Other techniques generally used for retrieval of fecal pathogens are of little or no utility for aeromonads. Enrichment broths are not recommended because most strains recovered by enrichment procedures, even when enterotoxigenic, are not associated with diarrhea (245). Many of the DNA probes developed for *Aeromonas* have a very narrow spectrum, as they are often developed for a specific species, which limits their usefulness for routine clinical specimens (137). However, for studies aimed at determining the prevalence and species distribution of aeromonads in certain clinical settings (e.g., gastrointestinal) or in environmental samples (food and water), molecular probes may be useful. In these settings, particularly when aeromonads are present in small numbers compared to other bacteria present, they are more efficient than protocols using selective media, which require enrichment with alkaline peptone water when samples are negative. A number of species-specific probes have been developed over the past 20 years for some genomic groups, including *A. hydrophila*, *A. trota*, *A. schubertii*, and *A. jandaei* (137). Two probes, one designed to detect glycerophospholipid-cholesterol acyltransferase and the other directed at an outer membrane protein, do detect all members of the genus (33, 160). The digoxigenin-labeled genus-specific DNA probe reported by Chacón and others (33) appears to pick up >98% of aeromonads and is nonreactive in colony hybridization assays against phenotypically similar bacteria, such as *Vibrio* species and *P. shigelloides*. A digoxigenin-labeled DNA probe directed against an OmpA homologue produced a positive reaction in colony hybridization assays against all 40 *Aeromonas* isolates, while the probe remained unreactive against several other gram-negative pathogens, including *Vibrio* species (160). Neither probe is commercially available.

For retrieval of aeromonads from nonhuman sources, there are a number of media that have been developed depending on whether the specimen is from water, food, or the environment. The review by Martin-Carnahan and Joseph (192) provides a brief recap of the media used for these purposes.

## Identification

*Aeromonas* spp. are oxidase-positive, facultatively anaerobic, gram-negative rods that grow readily on basic laboratory media such as heart infusion agar. Among species isolated from humans, >90% of strains produce β-hemolysis on sheep blood agar, with the exception of *A. popoffii* and *A. trota* (0% and 50%, respectively). Species of this genus (with the exception of *A. schubertii*) are considered to be indole positive, but we have strains in our collection, particularly *A. caviae* strains, that remain negative after 7 days even when extracted with xylene. Likewise, rare strains of *A. caviae* hydrolyze urea, a characteristic presumed to be negative in aeromonads. Identification of *Aeromonas* to the species level can be very challenging, and identification of strains from nonsterile sites may not be practical. Very few clinical laboratories will be able to identify the clinically significant species of this genus beyond complexes or groups (i.e., *A. hydrophila* complex or *A. caviae* complex), and for practical purposes, it is not necessary at this time. Likewise, it can be difficult to separate *A. veronii* bv. sobria from *A. hydrophila* by using conventional biochemical tests. For the most part, the only other genera that they may be confused with are *Vibrio* and *Plesiomonas*.

**Separation of *Aeromonas* from *Vibrio* and *Plesiomonas*.** *Aeromonas* spp. can be separated from vibrios by their ability to grow in nutrient broth (Difco formulation; no salt) without NaCl supplementation, their inability to grow on TCBS agar, and their resistance to the vibriostatic agent 2,4-diamino-6,7-diisopropyl-pteridine (O129) (Table 11). Some strains of *V. cholerae* O1 and all strains of *V. cholerae* O139 are now resistant to O129, but their decarboxylase pattern is very different from that of aeromonads, except for *A. veronii* bv. veronii. A positive reaction in esculin or salicin and production of gas from glucose will identify the strain as *A. veronii* bv. veronii. The ability of aeromonads to grow on TCBS agar can vary depending on the manufacturer, but if growth is present, *Aeromonas* colonies usually range in size from very small (~1 mm) to pinpoint colonies. Since the fermentable substrate in TCBS is sucrose and *Aeromonas* spp. are variable sucrose fermenters, colonies can appear either green or yellow, depending on the species. It also can be very difficult to differentiate *A. caviae* from some strains of *Vibrio fluvialis*. The latter agent, although usually requiring salt, can on occasion grow in a variety of

media without NaCl supplementation, and the zone around the O129 disk can be very small, approaching 6 mm. Fermentation of cellobiose but not D-arabitol will set *A. caviae* strains apart. *P. shigelloides* is positive for lysine and ornithine decarboxylases and for arginine dihydrolase and ferments *myo*-inositol, characteristics that are not found in any aeromonads.

**The *A. hydrophila* complex.** There are three species in the *A. hydrophila* complex, namely, *A. hydrophila sensu stricto*, *A. bestiarum*, and *A. salmonicida*; the last two species are only rarely seen in human specimens (feces), and the clinical microbiologist will seldom encounter them. *A. bestiarum* is the more difficult species to separate from *A. hydrophila*; it is less likely to decarboxylate lysine (50%), and it utilizes urocanic acid (94%) but not DL-lactate (0%) (values are versus 100%, 12%, and 80%, respectively, for *A. hydrophila*). Nonhuman isolates of *A. salmonicida* grow optimally at 22 to 25°C and are mostly nonmotile, and some subspecies produce a diffusible brown pigment. Human isolates of *A. salmonicida*, which do not belong to any of the five known subspecies, are motile, grow at 35°C, and can primarily be differentiated from the other members of this complex by fermentation of D-sorbitol and lactose. *A. hydrophila sensu stricto* can generally be separated from other species isolated from humans by a combination of biochemical tests, many of which are found in both conventional biochemical panels and commercial systems (Table 12).

**The *A. caviae* complex.** Members of the *A. caviae* complex include *A. caviae sensu stricto*, *A. media*, and *A. eucrenophila*. Although reports of human isolates of *A. media* in the literature are rare, we have received five clinical isolates (feces [$n$ = 2], bile [$n$ = 1], and wound [$n$ = 2; finger and knee] isolates) since 2007. Likewise, there are no published reports of *A. eucrenophila* in humans, but we have a fecal isolate and a knee wound isolate, from 2006 and 2008, respectively. Separation of these species by typical biochemical tests in conventional panels or commercial systems is not possible, although a positive citrate reaction at 24 h would indicate that the strain is *A. caviae* and gas from glucose would indicate that the strain is *A. eucrenophila*. Glucose-1-phosphate is the most helpful biochemical in distinguishing *A. caviae* (0% positive for >180 strains) from *A. media* and *A. eucrenophila* (both 100% positive [$n$ = 16 and $n$ = 9 strains, respectively]), including all clinical strains. To date, our human strains of *A. eucrenophila* and *A. media* are all positive on GCF (gelatin-cysteine-thiosulfate-ferric agar), while *A. caviae* strains (>180 strains) are uniformly negative. Table 12 lists other reactions helpful in separating *A. media* and *A. eucrenophila*.

**Separation of *A. hydrophila* from *A. veronii* bv. sobria.** Among tests available in commercial systems, fermentation of L-arabinose and hydrolysis of esculin are the two most helpful in differentiating *A. hydrophila* from *A. veronii* bv. sobria (Table 12). Production of elastase and hydrolysis of arbutin by *A. hydrophila* (74% and 76%, respectively, versus 0% for both for *A. veronii* bv. sobria) are also useful reactions.

**Other aeromonads isolated from clinical specimens.** Table 12 gives a list of reactions that are of assistance in separation of other aeromonads. Notably, *A. veronii* bv. veronii strains are ornithine decarboxylase positive, *A. jandaei*, *A. schubertii*, *A. popoffii*, and most strains of *A. trota* do not ferment sucrose, *A. schubertii* does not produce gas from glucose, and *A. trota* strains are susceptible to ampicillin.

**Identification of aeromonads by commercial systems.** As noted in a number of publications and summarized in Table 13, commercial systems incorrectly identify members of this genus frequently, which may result in major or very major errors (2, 143, 228, 232, 271). The problem with these misidentifications is essentially twofold. The first problem involves strains identified correctly to the genus level (*Aeromonas*) but incorrectly to the species level, as well as strains that are misidentified as *Vibrio* spp. At issue are the high mortality rates associated with *A. hydrophila* and *A. veronii* bv. sobria isolated from sepsis and necrotic wound infections and the more aggressive treatment necessary for these agents than for an organism such as *Vibrio alginolyticus* (Table 13). The second problem is that strains identified as *V. cholerae* set in motion a number of public health responses designed to prevent potential outbreaks, which are costly to governmental agencies and can have significant impact on individuals (2).

Commercial systems should be capable of correctly identifying *A. hydrophila*, *A. veronii* bv. sobria, and *A. caviae*, using reactions for lysine and ornithine decarboxylases, arginine dihydrolase, Voges-Proskauer fermentaiton, esculin/salicin, and L-arabinose, along with supplemental tests for oxidase and gas production. The reason for their continuing poor performance in identifying *Aeromonas* is unclear. Performance of adjunct tests for salt requirement for growth and O129 susceptibility prior to inoculation into commercial systems would help to alleviate confusion with *Vibrio* spp. Unfortunately, these are not tests routinely available in clinical laboratories.

**Molecular identification.** Molecular identification, albeit currently in vogue as a means of bacterial identification, has limited applications in the microbiology laboratory with regards to *Aeromonas*. This is principally due to the low frequencies of human *Aeromonas* infections reported in the United States and other industrialized nations, such as France (178), limited data suggesting a need for definitive identification past the complex level (see above), and no significant correlation between species and concentration in the gastrointestinal tract and the disease state. Molecular identifications, however, are still useful on a nonresearch basis under certain circumstances. These circumstances include definitive identification of isolates with aberrant biochemical properties (≥2 tests), for cases of recurrent disease (e.g., biliary), in the description of new disease settings or resistance patterns associated with aeromonads, for public health surveillance activities, and for publication purposes.

The most commonly utilized molecular technique in the clinical laboratory for genus and species identification of bacteria is 16S rRNA gene (SSU) sequencing (145). In the case of *Aeromonas*, this molecular technique is problematic. While some case reports have found SSU sequencing to be particularly useful in definitive species identification (123), others have not (5). The reasons for these discrepancies revolve around the apparent mosaic evolution of *Aeromonas rrn* operons (213). Intragenomic heterogeneity manifested by *rrn* nucleotide polymorphisms has been detected in most *Aeromonas* species, ranging from a low of 0.06% to a high of 1.5% (8, 213). At one extreme is *A. veronii*, which contains 6 copies of SSU, which may differ from one another by up to 1.5%. Such large sequence divergence values preclude its use for definitive *Aeromonas* species identification. One very-large-scale study of 999

TABLE 12. Differentiation of *Aeromonas* species[a]

| Species | Frequency (%) of characteristic in clinical specimens | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | VP | LDC | ADH | ODC | Gas/ glucose | Fermentation of: | | | | | | Esculin | GCF | Elastin | PZA | DL-Lactate | Urocanic acid | Arbutin | G-1-P | Amp[r] |
| | | | | | | L-Arabinose | D-Rhamnose | Lactose | Sucrose | Mannitol | D-Sorbitol | | | | | | | | | |
| Common species | | | | | | | | | | | | | | | | | | | | |
| *A. hydrophila*[b] | 95 | 98 | 100 | 0 | 95 | 58 | 7 | 27 | 97 | 98 | 0 | 99 | 100 | 74 | 22 | 71 | 26 | 76 | NT | 100 |
| *A. veronii* bv. sobria | 94 | 98 | 100 | 0 | 89 | 91 | 0 | 22 | 100 | 99 | 0 | 7 | 96 | 0 | 56 | 0 | 1 | 0 | NT | 100 |
| *A. caviae*[c] | 0 | 0 | 94 | 0 | 0 | 100 | 1 | 79 | 100 | 100 | 0 | 97 | 0 | 0 | 84 | 95 | 96 | 2 | 1 | 100 |
| Infrequent species | | | | | | | | | | | | | | | | | | | | |
| *A. veronii* bv. veronii | 83 | 100 | 0 | 100 | 92 | 8 | 0 | 83 | 100 | 100 | 0 | 92 | 100 | 0 | 0 | 0 | 0 | NT | NT | 100 |
| *A. jandaei* | 88 | 100 | 100 | 0 | 100 | 0 | 0 | 18 | 0 | 100 | 0 | 0 | 88 | 0 | 0 | 7 | 7 | NT | NT | 100 |
| *A. trota* | 6 | 100 | 100 | 0 | 71 | 0 | 0 | 12 | 24 | 71 | 0 | 12 | 65 | 0 | 13 | 94 | 69 | NT | NT | 6 |
| *A. schubertii* | 17 | 83 | 92 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 67 | 0 | NT | NT | 92 |
| *A. popoffii* | 100 | 0 | 100 | 0 | 100 | 57 | 0 | 0 | 0 | 100 | 0 | 0 | 100 | 0 | 0 | 86 | 71 | NT | NT | 100 |
| Rare species | | | | | | | | | | | | | | | | | | | | |
| *A. bestiarum*[b] | 63 | 50 | 88 | 0 | 75 | 100 | 69 | 13 | 100 | 100 | 0 | 94 | 81 | 13 | 50 | 0 | 94 | NT | NT | 94 |
| *A. salmonicida*[b] | 64 | 50 | 71 | 0 | 71 | 93 | 0 | 86 | 100 | 93 | 79 | 93 | 71 | 43 | 29 | 0 | 100 | NT | NT | 93 |
| *A. media*[c] | 0 | 0 | 94 | 0 | 0 | 100 | 0 | 94 | 100 | 100 | 0 | 88 | 0 | 0 | 31 | 38 | 100 | 80 | 100 | 91 |
| *A. eucrenophila*[c] | 0 | 0 | 82 | 0 | 73 | 82 | 20 | 55 | 64 | 100 | 0 | 82 | 82 | 0 | 100 | 0 | 9 | NT | 100 | 100 |

[a] Data from reference 1 and MDL data from 2003 to 2009, except for *A. hydrophila*, *A. veronii* bv. sobria, and *A. caviae* data, which are from 2006 to 2008. Abbreviations: VP, Voges-Proskauer; LDC, lysine decarboxylase; ADH, arginine dihydrolase; ODC, ornithine decarboxylase; GCF, gelatin cysteine thiosulfate ferric agar; PZA, pyrazinamidase; G-1-P, glucose-1-phosphate; NT, not tested.

[b] Members of the *A. hydrophila* complex.

[c] Members of the *A. caviae* complex.

TABLE 13. Identification of *Aeromonas* by commercial systems[a]

| System | Panel or kit | Species identification | | Comment |
|---|---|---|---|---|
| | | Commercial | Reference | |
| Phoenix 100 ID/AST | NID | *A. caviae* | *A. sobria* | Acceptable ID |
| | NID | Three species | *A. hydrophila* | |
| | NID | *A. caviae* | *A. hydrophila* | Major error |
| | NID | *A. veronii* | *A. hydrophila* | Major error |
| | NID | *A. caviae* | *V. alginolyticus* | Very major error |
| BBL Crystal | E/NF | *A. hydrophila* | *A. bestiarum* | Acceptable ID |
| | E/NF | *A. hydrophila* | *A. caviae* | Major error |
| | E/NF | *A. hydrophila* | *A. media* | Acceptable ID |
| | E/NF | *A. hydrophila* | *A. eucrenophila* | Acceptable ID |
| | E/NF | *A. veronii* | *A. sobria* | Acceptable ID |
| | E/NF | *A. hydrophila* | *A. jandaei* | Error |
| | E/NF | *A. hydrophila* | *A. veronii* bv. veronii | Major error |
| | E/NF | *A. hydrophila* | *A. schubertii* | Error |
| | E/NF | *A. hydrophila* | *A. trota* | Error |
| MicroScan Walk/Away | Combo Neg 1S | *A. hydrophila* group | *A. bestiarum* | Acceptable ID |
| | Combo Neg 1S | *A. hydrophila* group | *A. caviae* | Major error |
| | Combo Neg 1S | *A. hydrophila* group | *A. media* | Acceptable ID |
| | Combo Neg 1S | *V. fluvialis* | *A. eucrenophila* | Very major error |
| | Combo Neg 1S | *P. multocida* | *A. sobria* | Very major error |
| | Combo Neg 1S | *A. hydrophila* | *A. veronii* bv. veronii | Major error |
| | Combo Neg 1S | *A. hydrophila* group | *A. schubertii* | Error |
| | Combo Neg 1S | *A. hydrophila* group | *A. trota* | Error |
| Vitek | GNI+ | *V. alginolyticus* 1 | *A. veronii* bv. sobria | Very major error |
| | GNI+ | *V. alginolyticus* 2 | *A. veronii* bv. sobria | Very major error |
| | NS | *V. damsela* | *A. schubertii* | Very major error |
| API | 20E | *V. cholerae* | *A. veronii* bv. veronii | Very major error |

[a] Data are from references 2, 228, 232, and 271. Error, misidentification of an uncommon *Aeromonas* species as a common one; major error, misidentification of a common species or complex as another common species or complex; very major error, misidentification of another genus and species as *Aeromonas* or vice versa. NS, not stated.

*Aeromonas* strains found that 8.1% of isolates could not be assigned to a specific species based upon 16S rRNA gene restriction fragment length polymorphism (RFLP) (8). Furthermore, DNA-DNA hybridization values, long the gold standard in the description and validation of bacterial species, may not correlate well at all with SSU gene sequence similarities. For instance, although *A. caviae* and *A. trota* exhibit only 30% relatedness at the DNA level, their 16S rRNA sequences differ by only 3 nucleotides or less (213). *A. sobria* and *A. veronii* are 60 to 65% related in DNA pairing studies, yet they differ by 14 nucleotides in their 16S rRNA sequences (194). Other studies have found that *A. salmonicida* and *A. bestiarum* differ by only 2 nucleotides and cannot be distinguished by 16S rRNA gene sequencing (198). It is therefore apparent that 16S rRNA gene sequencing is not a useful technique for *Aeromonas* species identification. Housekeeping genes that show much more promise in this area include *gyrB* and *rpoD* (5, 8, 213). However, neither of these genes is linked to an off-the-shelf product such as MicroSeq, meaning that extensive validation of an in-house test would be required to meet Clinical Laboratory Improvement Amendments (CLIA) standards.

There may be some occasions where molecular fingerprinting of *Aeromonas* isolates is required to determine strain relatedness. These could include recurrent infections, temporal clusters of isolates in a medical unit, pseudo-outbreaks of disease, or linking an individual infection to an environmental source or inanimate object. A good first approximation is always to determine the phenospecies or complex of the strain in question. Since this genus is so phenotypically diverse (in carbohydrate metabolism), these characteristics are often useful even if all isolates belong to the same group (e.g., *A. hydrophila*). When a molecular fingerprinting technique is needed, RFLP, random amplified polymorphic DNA (RAPD), and enterobacterial repetitive intergenic consensus (ERIC) sequences have been found to be satisfactory under most circumstances (38). Pulsed-field gel electrophoresis (PFGE) employing restriction endonucleases XbaI, SpeI, and SwaI has also been used to fingerprint strains in several surveys (38). One powerful tool pioneered by Huys and colllaborators (126, 130, 131) is amplified fragment length polymorphism (AFLP) analysis. AFLP analysis has repeatedly been demonstrated to be an extremely useful tool in the classification and subtyping of aeromonads.

**Reporting the isolation and identification of *Aeromonas*.** Reporting the isolation of aeromonads from feces raises a number of concerns given that their involvement in gastroenteritis remains uncertain. However, there are a number of situations where reporting of these agents may be significant. The presence of *Aeromonas* in bloody stool has masked serious conditions such as colonic carcinoma and inflammatory bowel disease, the latter of which appeared refractory to treatment because of the *Aeromonas* (51, 63). Conversely, reporting the

presence of an agent such as *A. hydrophila* can eliminate a presumptive diagnosis of chronic inflammatory disease (63). Similarly, physicians need to be informed of the presence of *A. hydrophila* and *A. veronii* bv. sobria in the stools of immunocompromised patients, even if they are only colonizers, since these species are inherently invasive and the risk of disseminated disease is high for these patients (143). It is our practice to notify physicians of *Aeromonas* in stool if the organism is isolated in pure culture or in significant numbers. If another pathogen is present, which is often the case, *Aeromonas* may still be reported depending upon the number of organisms present; the presence of the aeromonads may explain continuing symptoms following appropriate therapy for the first agent. Laboratories can add comments regarding the unknown significance of these strains when isolated from stool, but the physician cannot make an informed clinical decision without the data that only the laboratory can provide.

It is important that *A. hydrophila* and *A. veronii* bv. sobria be identified or separated from other aeromonads and less serious *Vibrio* species (*V. alginolyticus* and *Vibrio parahaemolyticus*) because of the aggressive nature of their infections. However, the ability to differentiate these two species can be a challenge given the few phenotypic tests available and the fact that several of the most useful assays are not accessible in most clinical laboratories. In these cases, a report of "*A. hydrophila/A. veronii* bv. sobria, unable to differentiate" would be reasonable. Strains of *A. hydrophila* and *A. caviae* rarely are separable from other members of their respective complexes without extensive testing, and they should be reported as "*A. hydrophila* complex" or "*A. caviae* complex." When other strains are encountered that cannot be identified to the species level, they may be reported as "*Aeromonas* species not *A. hydrophila/A. veronii* bv. sobria," and if required for actual species identification, such as in cases of recurrent disease, they may be submitted to a reference laboratory.

For more in-depth information on the isolation and identification of aeromonads, the reader is invited to read the reviews of Altwegg (9), Chang and Janda (38), Edberg et al. (67), and Joseph and Carnahan (149).

## ANTIMICROBIAL SUSCEPTIBILITY

### Susceptibility Patterns and Testing Methods

In our 1998 review on the genus *Aeromonas*, we stated that "One key area that has received little attention has been the *in vitro* susceptibility of *Aeromonas* species to chemotherapeutic agents" (143). Surprisingly, very little has changed in this regard over the intervening years. Only three major studies dealing with the general susceptibility of aeromonads to various classes and combinations of antimicrobial agents have been published since 1998, and in only two of these investigations have susceptibility data been reported for *Aeromonas* species other than *A. hydrophila*, *A. caviae*, and *A. veronii* bv. sobria (152, 230). Much of the susceptibility information we have on this genus is based solely upon these three major species associated with human disease, and it is not entirely clear whether those patterns can be extrapolated to other less frequently encountered taxa causing illness.

The overall susceptibility profile for the genus *Aeromonas* does not appear to have changed appreciably from what was recorded in studies conducted between the mid-1980s and mid-1990s. Inducible chromosomal β-lactamases are still the major resistance mechanism for most aeromonads, although expression of metallo-β-lactamases active against carbapenems is also a concern (137, 305). Although long recognized as a rapid grower, consensus guidelines for the testing of infrequently encountered pathogens, including *Aeromonas* and *Plesiomonas*, have just been published by the Clinical and Laboratory Standards Institute (CLSI) (148). CLSI recommends the use of cation-adjusted Mueller-Hinton broth for MIC microdilution testing, while Mueller-Hinton agar is recommended for disk diffusion testing (148). CLSI document M-45A provides interpretive criteria for disk diffusion and MIC testing for the three primary species plus *A. jandaei* and *A. schubertii* (48). However, this guideline cautions that most currently available susceptibility data are based upon studies performed on the three predominant species only.

Several other general conclusions can be drawn regarding the susceptibility patterns of *Aeromonas* species. The use of different methods to assess MICs for aeromonads does not appear to influence interpretation of susceptibility, for the most part (152). The singular exception to this rule may be in the interpretation of susceptibility status in regards to antifolates (trimethoprim, sulfonamides, trimethoprim-sulfonamide combinations) or certain β-lactamase–inhibitor combinations, including amoxicillin-clavulanic acid (305). The susceptibility status of *Aeromonas* isolates for therapeutically active drugs also appears to be independent of species designation. Such a conclusion takes into consideration that most *A. trota* strains are susceptible to ampicillin yet use of this β-lactam is contraindicated in regards to treatment of *Aeromonas* infections. While some species-specific susceptibility differences have been found in select studies, these results should be considered preliminary at present (152, 230). There also do not appear to be any significant differences in the susceptibilities of aeromonads to antimicrobial agents based upon origin of isolation (clinical versus environmental), although certainly more studies need to be performed in this area (152). The general susceptibility profile of the genus *Aeromonas* for class-specific antibiotics is depicted in Table 14. However, this table should be viewed only as a general baseline for the genus, given that percentages of drug resistance may vary significantly due to individual species, geographic locales, or environmental selection pressures.

### Resistance Mechanisms

**β-Lactamases and extended-spectrum β-lactamases (ESBLs).** The single most problematic area concerning *Aeromonas* species and antimicrobial susceptibility testing is the expression by aeromonads of one or more unrelated inducible β-lactamases with activity against a wide variety of β-lactam antibiotics, including penicillins, cephalosporins, and extended-spectrum cephalosporins. Three principal classes of β-lactamases are recognized in *Aeromonas* species, namely, a class C cephalosporinase, a class D penicillinase, and a class B metallo-β-lactamase (MBL) (Table 15) (185). Fosse et al. (93) characterized strains producing these β-lactamases into five major patterns, including (i) *A. hydrophila* complex strains expressing class B, C, and D β-lactamases; (ii) *A. caviae* strains expressing class C and D β-lactamases; (iii) *A. veronii* group strains con-

TABLE 14. General susceptibility profiles for most clinically relevant *Aeromonas* isolates

| Susceptibility profile (% of isolates)[j] | Antibiotic family |
|---|---|
| Susceptible (90–100) | Aminoglycosides |
| | Carbapenems |
| | Cephalosporins (extended spectrum) |
| | Cephalosporins ("fourth generation") |
| | Macrolides[g] |
| | Monobactams |
| | Nitrofurans |
| | Penicillins[i] (extended spectrum) |
| | Phenicols |
| | Quinolones |
| | Tetracyclines |
| Variable (70–90) | Aminoglycosides[a] |
| | Antifolates[c] |
| | Cephalosporins[d] (expanded spectrum) |
| Resistant (<70) | Antifolates[b] |
| | Cephalosporins (narrow spectrum) |
| | Penicillins[e] (extended spectrum) |
| | Macrolides[f] |
| | Penicillins[h] (narrow spectrum) |

[a] Tobramycin.
[b] Sulfamethoxazole.
[c] Trimethoprim-sulfamethoxazole.
[d] Cefoxitin.
[e] Amoxicillin, ampicillin, ampicillin-sulbactam, ticarcillin.
[f] Clarithromycin.
[g] Azithromycin.
[h] Oxacillin, penicillin.
[i] Azlocillin, piperacillin, piperacillin-tazobactam.
[j] Percentages of susceptible isolates were derived from references 137, 152, 230, 287, and 305.

taining class B and D lactamases; (iv) *A. schubertii* strains harboring class D lactamases; and (v) *A. trota* strains with class C β-lactamases. It also appears that many *A. veronii* bv. sobria isolates also produce a class C cephalosporinase (293). Individual strains can harbor up to three different β-lactamases which are under a single mechanism of coordinate expression (293).

Class C cephalosporinases belonging to the AmpC family are typically resistant to cephamycins (e.g., cefoxitin and cefotetan) and extended-spectrum cephalosporins. They are also resistant to the effects of β-lactamase inhibitor compounds such as clavulanic acid, tazobactam, and sulbactam (94). Class D penicillinases often exhibit sequence similarity to the OXA family of enzymes and show higher rates of hydrolysis for cloxacillin and carbenicillin than for benzylpenicillin (242). Much less frequently, sporadic cases of infection involving aeromonads have been published where the infecting strain possessed a class A β-lactamase belonging to the TEM family of ESBLs, a trait typically associated with the family *Enterobacteriaceae*. Most of these ceftazidime-resistant infections have been reported from France, where an outbreak clone of *Enterobacter aerogenes* possessing TEM-24 may have horizontally transferred this 180-kb plasmid to different *Aeromonas* species (95, 191).

*Aeromonas* isolates containing class B MBLs are extremely problematic, as they cannot routinely be detected using commercial products such as Etest for ESBLs (AB Biodisk, Solna, Sweden). Rather, MBLs must be detected using a double-disk method employing either ceftazidime or imipenem, with a second disk containing 500 mM EDTA with or without β-mercaptoethanol (185, 224). The most common MBL produced by *Aeromonas* species is of the "CphA" type, whose sequences appear to be widely distributed in *A. hydrophila* and *A. veronii* isolates (293). Recently, two other MBLs (VIM and IMP) have been detected in strains of *A. hydrophila* and *A. caviae*, encoded on an integron and a plasmid, respectively (185, 224). In both instances, these MBL-producing strains were resistant to most β-lactams, including ceftazidime, cefepime, imipenem, and piperacillin-tazobactam; both strains were susceptible to aztreonam *in vitro*. *De novo* resistance to imipenem has been reported for an 88-year-old woman with cholangitis who was initially treated with multiple antibiotics, including ciprofloxacin and ampicillin-clavulanate, for a severe UTI (257). She was readmitted approximately 2 weeks after this UTI episode for treatment of choledocolithiasis. Two bile samples yielded nine phenotypically and morphologically distinct variants of a single clonal strain of *A. veronii* bv. sobria. Of these nine isolates,

TABLE 15. Selected β-lactamases, ESBLs, and carbapenemases produced by *Aeromonas* species[a]

| Group | Ambler class | Family | Name | Location | Species |
|---|---|---|---|---|---|
| Serine β-lactamases | C | AmpC | AsbA1 | Chromosomal | *A. jandaei* |
| | D | OXA | AsbB1 | Chromosomal | *A. jandaei* |
| | D | Penicillinase | AmpH, AmpS | Chromosomal | *A. caviae*, *A. veronii* bv. sobria, *A. hydrophila* |
| | C | AmpC (FOX-1) | CAV1 | Chromosomal | *A. caviae* |
| | C | AmpC | CepS, CepH | Chromosomal | *A. caviae*, *A. veronii* bv. sobria, *A. hydrophila* |
| | A | TEM | TEM-1-like, TEM-24 | Plasmid | *A. hydrophila*, *A. caviae* |
| Metallo-β-lactamases | B | Carbepenamases | AsbM1 | Chromosomal | *A. jandaei* |
| | B | Carbepenamases | CphA | Chromosomal | *A. hydrophila*, *A. veronii* bv. sobria, *A. veronii* bv. veronii, *A. jandaei* |
| | B | Carbepenamases | ImiS | Chromosomal | *A. veronii* bv. sobria |
| | B | IMP | IMP-19 | Plasmid | *A. caviae* |
| | B | VIM | VIM | Integron | *A. hydrophila* |

[a] Data are from references 94, 95, 185, 191, 224, 241, 248, 257, and 293; most β-lactamase abbreviations are found in the work of Jacoby (135). Abbreviations: Asb, *Aeromonas sobria* β-lactamase; CAV, found in *A. caviae*; Cep, chromosomal cephalosporinase; CphA, carbapenem hydrolyzing *A. hydrophila*; ImiS, imipenemase from *A. veronii* bv. sobria; IMP, active on imipenem; TEM, named for patient Temoneira; VIM, verona integron-encoded metallo-β-lactamase.

seven were resistant to imipenem, with a MIC of 32 μg/ml. Reverse transcription-PCR of one susceptible and one resistant isolate indicated overproduction of ImiS expression in the imipenem-resistant variety. It appears that this variant was selected during treatment.

**Quinolones.** *Aeromonas* strains are almost universally susceptible to fluoroquinolones. A 2003 investigation looking at the susceptibility of 64 clinical isolates of *A. hydrophila* to various fluoroquinolones found the best *in vitro* activity associated with levofloxacin (0.25 μg/ml), gatifloxacin and ciprofloxacin (0.5 μg/ml), and moxifloxacin (1 μg/ml), based upon $MIC_{90}$s (168). Resistance, while rare, has been reported. Sinha and colleagues detected high-level chromosomal resistance to nalidixic acid, ciprofloxacin, and norfloxacin in several *A. caviae* strains (267). In four strains, double mutations were detected in the *gyrA* gene of the DNA gyrase, while a single mutation was also detected in the *parC* gene of topoisomerase IV. Quinolone resistance has also been associated with the plasmid-mediated 218-amino-acid QnrA protein. Two reports have detected QnrS determinants (41% to 60% amino acid identity with QnrA) in two environmental isolates of *A. media* and *A. caviae* and in one clinical isolate of *A. veronii* (30, 256). In the latter instance, the *A. veronii* strain was resistant not only to nalidixic acid but also to ciprofloxacin and levofloxacin.

## CONCLUSIONS

During the past decade, we have witnessed an explosion in research studies tailored to understanding the molecular biology of the genus *Aeromonas*, culminating with the sequencing of the genome of *A. hydrophila* ATCC 7966$^T$ (261). Polyphasic taxonomic studies involving the sequencing of housekeeping genes coupled to traditional phenetic approaches and gold standard assays, such as DNA-DNA hybridization, have continued to identify new *Aeromonas* species, thus expanding the phylogenetic breadth, depth, and diversity of these environmental microorganisms. DNA sequencing has also led to the identification of potential genes with significant homologies to virulence determinants in other pathogenic species. New models of *Aeromonas* infection, such as the medicinal leech, blue gourami, and zebrafish models, show promise for shedding new light on microbial gene regulation, control, expression, and pathogenicity.

Yet despite all of these accomplishments, in many ways we are no closer to unraveling many of the mysteries surrounding these microbes that are important to clinical microbiologists. If aeromonads are indeed truly enteropathogenic, why have there been no recognized outbreaks of diarrheal disease? Why have we not been able to find an animal model with which to faithfully reproduce Koch's postulates? While the medicinal leech model of Graf (104, 181, 265) shows promise, the microbial flora of the leech's digestive tract is simplistic in comparison to the complex bacterial ecoflora that *Aeromonas* encounters in the small and large intestines of humans. Furthermore, while "virulence homologs" have been identified in many *Aeromonas* species, this is at best only an indirect association with pathogenicity that can be established conclusively only by using correct organotrophic models (e.g., enterotoxins in a diarrheal model). Perhaps microarray-based comparative genomic studies of clinical isolates conducted in a fashion similar to those performed with the fish pathogen *A. salmonicida* will uncover important underlying universal themes governing persistence, infectivity, and disease-causing capabilities (222).

While many questions remain unanswered, there are still a number of things that can be accomplished immediately. At present, it is unreasonable to expect that clinical microbiologists will routinely identify aeromonads by any mechanism other than phenotype, given their infrequent occurrence. A collection of reference strains representing all known clinically relevant *Aeromonas* species, with defined genotypes (DNA-DNA hybridization) and phenotypes, should be established to aid researchers in developing better commercial products with which to identify this group of organisms to the genus and species levels. A companion set of strains of known pathogenicity in different animal models should also be made available for researchers studying pathogenicity. Both sets of strains should be made available to the scientific community at large for a nominal fee. In this way, long-standing issues or problems with studies related to the use of strains of undefined genotype, questionable taxonomic position, clinical significance, pathogenicity, etc., can be put to rest. It is probably a good idea to also develop a collection of strains with unusual resistance mechanisms, including those encoding metallo-β-lactamases.

This genus continues to surprise us. We have discovered new disease associations involving serious infections linked to natural disasters and found in new settings (prostatitis). This trend is likely to continue for some time, as more clinicians become familiar with these bacteria. Hopefully, during the next decade, many of the important mysteries surrounding this genus will be solved by the next generation of microbiological sleuths.

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