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Recent Advances in the Study of the Taxonomy, Pathogenicity, and Infectious Syndromes Associated with the Genus *Aeromonas*

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INTRODUCTION

The past decade has witnessed an explosion of scientific interest in members of the genus *Aeromonas* as human and animal pathogens. Much of this interest is due to the association of this gram-negative bacillus with gastrointestinal disease in humans, but other areas of increasing importance are emerging, including the complicated taxonomy of the genus and virulence-associated factors potentially operative in animal and human infections. During the past 10 years, the number of medical and scientific publications on the genus has risen dramatically, with more than a fivefold increase over the number of publications produced during the 1970s and three international workshops on the genera *Aeromonas* and *Plesiomonas* having been held, the latest of which took place in Helsingor, Denmark, in September 1990. These collective works have helped to expand the medical and microbiologic interest in the genus *Aeromonas* on a global basis, and many significant advances in the study of various aspects of this genus have taken place over the past few years.

By far the greatest impact on *Aeromonas* research stems from the possible epidemiologic association of these organisms with bacterial gastroenteritis in humans. Although the role of aeromonads as gastrointestinal pathogens is still somewhat controversial, mounting evidence indicates that at least some strains are involved in diarrheal disease. Other avenues of major interest involving the genus *Aeromonas* include the identification of new aeromonad species, pathogenic groups, and virulence factors associated with defined clinical syndromes. The purpose of this article is to review and organize these advances into a suitable framework so that key areas in which future work is needed can be defined and important scientific questions can be answered. For general information on the microbiology, drug susceptibility, and ecology of aeromonads and infections produced by

TABLE 1. Current hybridization groups within the genus *Aeromonas*[a]

DNA group[b]	Genospecies	Phenospecies	Isolated from humans	Clinical frequency (relative)[c]	Unusual biochemical properties[d]
1	*A. hydrophila*	*A. hydrophila*	Yes	++++	
2	Unnamed	*A. hydrophila*	Yes	+	
3	*A. salmonicida*	*A. hydrophila*	Yes	+	Sor$^+$
4	*A. caviae*	*A. caviae*	Yes	++++	
5	*A. media*	*A. caviae*	Yes	++	
6	*A. eucrenophila*	*A. caviae*	No	−	Gfg$^+$ H_2S^+
7	*A. sobria*	*A. sobria*	No	−	
8/10	*A. veronii*	*A. sobria*	Yes	++++	
9	*A. jandaei*	*A. sobria*	Yes	++	Suc$^-$
10/8	*A. veronii*	*A. veronii*	Yes	++	ODC$^+$ ADH$^-$
11	Unnamed	*A. veronii*	Yes	+	ODC$^+$
12	*A. schubertii*	*A. schubertii*	Yes	++	Man$^-$ Ind$^-$ Gfg$^-$
13	*A. trota*	*A. sobria*	Yes	++	Cel$^+$ Amp^s

[a] Data were compiled from references 3, 6, 28, 29, 47, 56, 83, and 118.
[b] DNA hybridization groups 8 and 10 are identical, although different biotypes are known to exist.
[c] Clinical frequency: ++++, predominant clinical species; ++, 10 to 50 clinical isolates known; +, <10 clinical isolates known; −, presently not recovered from human material.
[d] Abbreviations: Sor, acid from D-sorbitol; Gfg, gas from glucose; H_2S, H_2S from cysteine-based agar; Suc, acid from sucrose; ODC, ornithine decarboxylase; ADH, arginine dihydrolase; Man, acid from mannitol; Ind, indole; Cel, cellulose; Amp^s, ampicillin susceptible.

them, the reader is advised to consult one of several recently published review articles on these topics (2, 67, 75).

TAXONOMY

Genetic Complexity

One of the most frustrating and perplexing problems concerning *Aeromonas* species has been the confusing and ever-changing picture of aeromonad taxonomy with regard to species identification at both the biochemical and the molecular levels (Table 1). From studies conducted in the mid to late 1970s, it became evident that strains originally lumped together as *Aeromonas hydrophila* were, in fact, extremely heterogeneous in biochemical, structural, and genetic properties. As early as 1981, it was recognized that although four groups could be distinguished biochemically (called phenospecies), on the basis of polynucleotide sequence relatedness, additional genetic clusters were found to exist within each of these phenospecies (108). Thus, strains phenotypically identified as *A. hydrophila*, *A. sobria*, or *A. caviae* in actuality contained five to seven distinct hybridization groups (HGs) based on DNA-DNA reassociation kinetics. Subsequent studies conducted by the Centers for Disease Control and Walter Reed Army Research Institute expanded the number of known *Aeromonas* HGs to 9 to 12 (47). Finally, Carnahan and colleagues (29) at the University of Maryland identified a new HG which has a unique profile of susceptibility to certain antimicrobial agents, including ampicillin. Despite these advances, many additional groups appear to exist, since strains that do not fall into any of the currently recognized HGs have been identified.

Genospecies Identification

Aeromonas genospecies can be defined on the basis of the reassociation kinetics between individual strains and isotopically labeled DNAs extracted from reference or type strains with standing in nomenclature. On the basis of the criteria of relative binding ratios of 70% under optimal conditions (60°C) and 55% under stringent conditions (70°C), with $\leq$5% divergence (3, 85), at least 13 published HGs have been proposed or are now known to occur. Genospecies assignment for each HG is dependent on where strains with standing in nomenclature reside (3, 48). For instance, ATCC 7966 is the type strain for *A. hydrophila*, and since this strain genetically resides in HG 1, this genospecies is so defined. In all, 11 of the 13 HGs have been named, but 2 (HG 2 and HG 11) presently are unnamed because of either an insufficient number of strains for study or the inability to find suitable markers to separate these HGs from designated genospecies. Although HGs 8 and 10 were independently established and are biochemically dissimilar (57), it has been shown recently that HG 8 is genetically identical to *A. veronii* (83). Since HG 10 (*A. veronii*) has precedence in the literature, HG 8 must be considered a biotype of *A. veronii*, even though HG 8 is much more frequently recovered from clinical material than HG 10.

In addition to the problem concerning *A. veronii*, several other taxonomic problems relating to genospecies designations exist. First, although certain HGs are now taxonomically defined, they cannot in every instance be unambiguously separated from genotypically distinct groups at the biochemical level (6, 48). For example, HGs 1, 2, and 3 would in most instances be identified in the clinical laboratory as *A. hydrophila*, even though genetically speaking only HG 1 is in reality *A. hydrophila*; the other two groups, HG 2 (unnamed) and HG 3 (*A. salmonicida*), cannot at present be separated from HG 1 unless molecular methods are used. Phenotypic differences originally used to separate *A. salmonicida* from mesophilic species (nonmotile, indole negative, melaninlike pigment) now define subspecies within HG 3 rather than the genospecies. Second, there is a controversy over whether HG 4 should be named *A. caviae* or *A. punctata*, since the type strain for both species is identical (ATCC 15468). Since *A. punctata* is the older name in the scientific literature, it has precedence over *A. caviae* (48). Fortunately, one area of major interest concerns advances in genospecies identification by multilocus enzyme electrophoresis. Studies conducted by Altwegg and coworkers (4) at the Centers for Disease Control have indicated that electromorphic variations at four enzymatic loci can be used to assign 98% of the *Aeromonas* strains studied to the correct HG. Such alternative methods to DNA hybridization may

help identify other markers useful in genospecies recognition.

Phenospecies Identification

For most institutions, identification of *Aeromonas* isolates to the genospecies level is impractical because of the technical time required, reagents involved, and instrumentation costs. However, a feasible alternative to such a procedure is to identify aeromonads to the phenospecies level on the basis of a number of biochemical tests. Over the past several years, most microbiology laboratories have begun to report *Aeromonas* isolates as *A. hydrophila*, *A. sobria*, or *A. caviae* (71). This is a workable alternative to genetic methods provided the microbiologist realizes that phenospecies identification and genospecies identification do not always correlate and that the failure to use a reasonable number of biochemical tests probably increases the number of misidentifications. One interesting point is that many of the recently named HGs appear to have unique biochemical properties that are associated with specific genospecies and that are not commonly found in other members of this genus (Table 1). If these biochemical traits continue to be found unique to specific HGs assignment of aeromonads into appropriate groups should become an easier task.

New Species and Environmental Distribution

Several new *Aeromonas* species have recently been described, bringing the number of named HGs to 11. In 1988, Hickman-Brenner et al. (56) proposed the name *A. schubertii* for a group of mannitol-negative strains that originated from clinical material. This group was formerly referred to as enteric group 501 and encompassed DNA HG 12; at present, only clinical strains are known to exist. A second group biochemically similar to *A. schubertii* but with phenotypically distinct properties (indole positive, lysine decarboxylase negative) is currently referred to as *Aeromonas* group 501. This group is highly related to *A. schubertii* but shows some sequence divergence, and only a few strains have been described so far. Simultaneously with this study, Schubert and Hegazi (118) reported on a new *Aeromonas* species, named *A. eucrenophila* (DNA HG 6), which biochemically resembles an aerogenic *A. caviae*-like organism. This species has been recovered only from fish and water sources at present. Most recently, Carnahan and colleagues (28) designated DNA HG 9 as *A. jandaei*. This species, recovered from both clinical and environmental sources, is a sucrose-negative *A. sobria*-like organism. Lastly, these same workers identified an ampicillin-susceptible group of aeromonads primarily recovered from the gastrointestinal contents of humans and representing a 13th published HG (29). At the Third International Workshop on *Aeromonas* and *Plesiomonas*, Carnahan et al. (29) proposed the name *A. trota* for this newest aeromonad group.

Recently, several epidemiologic and genetic investigations have characterized the HGs for a number of human *Aeromonas* isolates primarily of fecal origin. On the basis of the cumulative results of these surveys involving 270 strains, HGs 1 (*A. hydrophila*), 4 (*A. caviae*), and 8 (genospecies *A. veronii* biotype sobria, phenospecies *A. sobria*) account for 85% of all human isolates (3, 77, 83); of the remaining 15%, two-thirds belong to *A. media* (HG 5). The relative frequencies of each of these species or HGs in clinical material are presented in Table 1.

Laboratory Identification

One of the current issues regarding *Aeromonas* species concerns how the laboratory should go about identifying aeromonads. While identification to the genus level is straightforward in virtually all cases, determining the appropriate species assignment of individual strains is controversial. One viewpoint is that *Aeromonas* strains should simply be reported as "*Aeromonas* species," "*Aeromonas hydrophila group*," or "*Aeromonas hydrophila* complex." This philosophy is based on the fact that the role of individual strains or species in gastrointestinal disease, the most common clinical presentation, has not been conclusively established. Other difficulties include an identification scheme that is both difficult and time-consuming and does not lend itself readily to smaller laboratories or to those who rely solely on automated or semiautomated systems for the identification of gram-negative bacilli. Finally, no documented reports of community or nosocomial outbreaks of *Aeromonas* disease have ever been reported.

Despite these valid assertions, an equally compelling case can be made for the identification of aeromonads to the species level. Species assignment provides definitive identification of individual strains and, in the case of the more recently described members (*A. veronii* biotype veronii, *A. schubertii*, *A. jandaei*, and *A. trota*), helps to expand our limited knowledge regarding the environmental distribution, disease spectrum, and role in pathogenesis of these bacteria. For previously established species, identification may help to shed light on new infectious syndromes, such as that recently associated with *A. hydrophila* and medicinal leech therapy (1). In the case of extraintestinal disease, several studies have shown that both *A. hydrophila* and *A. sobria* (phenospecies) appear to be more inherently invasive and pathogenic than *A. caviae* (46, 64). Since most invasive diseases involving *Aeromonas* species occur in patients with underlying malignancies or hepatic illnesses, identification of aeromonads colonizing or infecting localized sites in such individuals may provide clinicians with insight regarding the invasive potential of such strains for subsequent systemic dissemination. Finally, species identification has revealed in some instances noted differences in susceptibility profiles, principally in the cephalothin-susceptible *A. sobria* phenospecies and the proposed ampicillin-susceptible *A. trota* (29, 69, 97). While these differences in susceptibility do not always imply a therapeutic difference, they do have an impact on identification, isolation techniques, and structural and genetic differences among groups.

Assuming that one wishes to identify aeromonads to the species level, how far does one go? Several approaches are possible. Since genetic studies conducted to date (3, 4, 6, 77, 83) indicate that ≥85% of all species identifications (phenospecies) are accurate to the genospecies level (HGs 1, 4, and 8 predominate), one alternative is to continue to biochemically identify strains of the three major phenospecies, namely, *A. hydrophila*, *A. caviae*, and *A. sobria*. Assuming that there is at present no clinical necessity for distinguishing *A. caviae* from *A. media*, phenospecies identification approaches 95% in accuracy. Strains previously reported as *A. sobria* should now be designated *A. veronii* biotype sobria to be more consistent with recent findings and current taxonomic nomenclature (3, 77). If one wishes to distinguish all *Aeromonas* species linked to human disease, then an extended biochemical scheme with at least 10 to 15 tests should be used (Table 2); some tests, such as growth in KCN and production of elastase, may not be amenable for routine

TABLE 2. Salient features in the identification of *Aeromonas* phenospecies or genospecies in the clinical laboratory

Test[a]	Result[b] for:						
	A. hydrophila[c]	*A. caviae*[d]	*A. veronii* Biotype sobria	*A. veronii* Biotype veronii	*A. schubertii*	*A. jandaei*	*A. trota*
Indole	+	+	+	+	−	+	+
LDC	+	−	+	+	+	+	+
ODC	−	−	−	+	−	−	−
Esculin	+	+	−	+	−	−	−
H_2S	+	−	+	+	NA	+	+
Acid from:							
Glucose (gas)	+	−	+	+	−	+	+
Sucrose	+	+	+	+	+	−	−
Salicin	+	+	−	+	−	−	−
Arabinose	+	+	−	−	−	−	−
Mannitol	+	+	+	+	−	+	+
Voges-Proskauer	+	−	+	+	+[e]	+	−
KCN	+	+	−	+	−	−	NA
Elastase	+	−	−	−	−	−	−
Cephalothin (30 μg)	R	R	S	S	S	R	R

[a] Abbreviations: LDC, lysine decarboxylase; ODC, ornithine decarboxylase.
[b] +, positive for 80% or more strains; −, negative for 80% or more strains; NA, data not available; S, susceptible; R, resistant.
[c] Refers to phenospecies; *A. hydrophila* (HG 2, unnamed) and *A. salmonicida* (HG 3) originating from clinical material cannot be biochemically separated at present with any degree of accuracy.
[d] Refers to both *A. caviae* (HG 4) and *A. media* (HG 5), which cannot be biochemically separated at present with any degree of accuracy.
[e] Depends on method.

laboratory use. Since two genospecies (HG 6, *A. eucrenophila*; HG 7, *A. sobria*) have not yet been recovered from clinical material, they can at present be excluded from consideration. When such an extended biotyping scheme is used, the selection of tests should not be based on the availability or ease of preparation or interpretation but on the amount of information yielded by the tests. One should also be aware that the miniaturized biochemical tests in automated or semiautomated commercial systems cannot accurately identify aeromonads to the species level. Even when differential tests are present in such systems, they often yield results discrepant from those obtained with conventional assays, and the microbiologist should be wary of drawing definitive conclusions from the results obtained with such systems.

AEROMONAS INFECTIONS

New Syndromes and Diseases

The most interesting new clinical syndrome associated with *Aeromonas* species has been serious wound infections occurring in individuals after medicinal leech therapy (1). Recently, there has been a significant increase in the use of the age-old practice of leech therapy to relieve venous congestion subsequent to microvascular or plastic surgery involving replants or skin flaps. At least eight cases of *Aeromonas* wound infections resulting from such therapy have recently been described in the literature (41, 93, 127). All infected individuals recovered after debridement, antimicrobial therapy, or both. The source of all of these illnesses appears to be the medicinal leech, *Hirudo medicinalis*, which harbors aeromonads symbiotically in its gut to promote enzymatic digestion of hematologic material ingested during engorgement.

Of the recent additions to the genus, only *A. schubertii* exhibits an unusual disease spectrum in that it has been recovered only from extraintestinal sites such as blood and wounds (56). Recently, Carnahan et al. (30) genotypically identified two additional *A. schubertii* strains, both recovered from cases of cellulitis. It may well be that this species has a propensity for causing serious extraintestinal disease, since no fecal isolates have been identified so far.

Aeromonas Gastroenteritis: an Update

Mounting clinical and epidemiologic evidence indicates that *Aeromonas* species are, indeed, enteropathogens, despite the facts that Koch's postulates remain unfulfilled (no animal model) and that no well-documented outbreak of aeromonad-associated gastroenteritis has been reported in the literature. Despite these shortcomings, some recent investigations have expanded our knowledge concerning various aspects of aeromonad-associated gastroenteritis, knowledge which should help in establishing members of this group as etiologic agents of diarrheal disease.

Genetic studies conducted on a large number of fecal isolates presumably involved in gastrointestinal disease indicate that HGs 1 (*A. hydrophila*), 4 (*A. caviae*), and 8 (*A. veronii* biotype sobria) predominate in aeromonad-associated diarrhea (3, 83). Studies conducted by San Joaquin and Pickett (115) and Kuijper et al. (82) revealed a predominant incidence of *Aeromonas*-associated diarrhea in very young children (6 months to 5 years); cytotoxigenic strains appeared to be more commonly recovered from older individuals (≥50 years). In addition to these epidemiologic surveys, increasing numbers of reports documenting the association of *Aeromonas* species with cases of chronic colitis have surfaced (42, 49, 112, 136). However, in these studies, *Aeromonas* species were recovered only from fecal material, and isolation and identification from biopsy material were not attempted; in some instances, *Aeromonas* species were recovered only in moderate amounts from stool cultures.

Probably the most striking recent findings concern immunologic evidence that patients infected with *Aeromonas* species mount specific responses against various structural

TABLE 3. Selected characteristics of structural features associated with *Aeromonas* species

Structure	Type	Diam (nm)	Molecular mass (kDa)	Chracterized by biochemical analysis	Sequence determined[a]	Gene cloned	Reference(s)
Pilus	Straight	9–10	17–18	+	(+)	No	59, 61, 117
	Flexible	5–7	4	+	+	No	
S layer	S lattice	5–8	49–58	+	+	Yes	15, 44, 45, 77, 78
Flagellum	NA[b]		36	–	–	No	117
LPS	O chain, core		46–65	+	(+)	No	35, 43, 77, 78
Protein(s)	OMP		30–59	–	–	No	5, 84

[a] Parentheses denote partial amino acid or polysaccharide sequence or structure determined.
[b] NA, not available.

or enzymatic activities of the homologous strain. In a collaborative study by Kuijper and colleagues (85), several patients with acute gastroenteritis (from four clinical groups) were found to have significant immunologic responses to *Aeromonas* cell envelopes by an enzyme-linked immunosorbent assay and were able to elicit neutralizing antibodies to the *Aeromonas*-associated cytolysin. Results of gel immunoradioassays were less convincing, since serum samples collected from infected patients reacted not only against homologous and heterologous *Aeromonas* strains but also against control *Escherichia coli* and *Vibrio cholerae* strains. The most exciting news, however, stems from the recent preliminary investigations of Jiang et al. (72), who showed that 12 of 13 patients infected with *A. hydrophila* or *A. sobria* elicited specific immunologic responses against the homologous strain by either dot blot or Western blot (immunoblot) analysis. In these individuals, a significant rise in the secretory immunoglobulin A response was detected in the 20- to 80-kDa region of lipopolysaccharide (LPS) components from infecting strains. These results, if expanded and confirmed, will go a long way in establishing *Aeromonas* species as bona fide gastrointestinal pathogens.

VIRULENCE FACTORS

Although most of the active research on *Aeromonas* species concerns the identification of virulence factors or mechanisms potentially operative in human or animal infections, only one factor, the S layer of *A. salmonicida* (73), has been unequivocably linked to the overt pathogenicity of this species in causing serious infections in fish (salmonids). Most of the other reputed virulence factors produced by *Aeromonas* species have been linked to pathogenicity in humans by inference; that is, similar molecules have been shown to play important roles in virulence in other bacterial species, such as *E. coli*. In addition, since no animal model in which *Aeromonas*-associated gastroenteritis can be faithfully reproduced presently exists, studies of the role of products such as enterotoxins, hemolysins, and adhesins in diarrhea must await identification of a suitable model. Despite these deficiencies, substantial progress has been made in identifying a number of key structural, enzymatic, and cell-associated features that appear to play important roles in both intestinal and extraintestinal infectious processes in humans and animals.

Structural Features

Pili. While early studies had indicated that a number of *Aeromonas* strains were piliated, a detailed analysis of such structures was not undertaken until recently. Using transmission electron microscopy for ultrastructural analysis, Carrello et al. (31) observed at least two distinct morphologic types of fimbriae on *Aeromonas* strains recovered from both clinical and environmental sources. On the basis of these observations, two types of pili have been reported (Table 3); one is designated a "straight" pilus, and the other is more curvilinear and termed "flexible." These original observations have subsequently been independently confirmed in other laboratories (59, 61). The straight pilus is 0.6 to 2.0 μm in length and has a subunit molecular weight of 17,000 to 18,000; purified pili from different strains expressing such fimbriae indicate that this structure has no inherent hemagglutination capability. Sato et al. (117) have also found that the straight pilus does not appear to share immunologic cross-reactivity with *E. coli* strains expressing both colonization factor antigens I and II. Ho and colleagues (59) have sequenced this 17K molecule through the first 55 amino acid residues at the NH_2 terminus and have found remarkable homology (28 of 40 residues starting at position 7) between this molecule and *E. coli* type I and pyelonephritis-associated pili (92); the two cysteine residues also identified in this molecule allow for disulfide bridges to occur; one potential loop of 30 residues has been identified. In a similar fashion, Ho et al. (59) have sequenced the flexible pilus, which they have called a "mini pilin," because of the small molecular weight (4,000) of the protein subunit. This 46-amino-acid molecule appears to be entirely novel and shows no homology with known pilin sequences. The mini pilin is environmentally regulated and maximally expressed at 22°C, in liquid media, and under reduced iron conditions; the straight pilus appears to be constitutively expressed under all conditions. These results suggest that synthesis of the flexible mini pilin may be triggered by environmental conditions as a prerequisite for the colonization of mammalian hosts.

S layers. In the early 1980s, Kay and associates identified by transmission electron microscopy an additional layer (originally called the A layer) external to the cell wall in autoaggregating strains of *A. salmonicida* (HG 3) that were pathogenic for young Coho salmon (73); the spontaneous loss of this layer from virulent *A. salmonicida* strains resulted in significant increases in the 50% lethal doses (LD_{50}s) of these strains (1,000- to 10,000-fold) on intraperitoneal

injection into fish. Subsequent biochemical and genetic investigations indicated that this structure is a typical paracrystalline S layer composed of a single surface array protein (SAP) of 49 to 51 kDa (74). S layers extracted from different autoagglutinating *A. salmonicida* strains conferred significant surface hydrophobicity to the bacterial cell surface, and subsequent biochemical and genetic investigations indicated that these layers were the single major virulence factor of this bacterium in fish (14, 63, 133). Biochemical studies of the amino termini of a number of SAPs have indicated that the amino acid sequence is highly conserved (74), and the gene coding for a SAP has recently been cloned from one strain (15). A number of biologic or biochemical properties have been associated with *A. salmonicida* S layers in vitro and include Congo red binding, resistance to complement-mediated lysis, and enhanced association with phagocytic monocytes (63, 98, 133).

Subsequent to these studies, several laboratories have simultaneously identified S layer-positive strains of *A. hydrophila* (HG 1) and *A. veronii* biotype sobria (HG 8, phenospecies *A. sobria*) involved in a variety of animal and human infections (45, 77, 104). The human isolates have originated principally from extraintestinal infections such as bacteremia and peritonitis (70). Regardless of the anatomical source or origin (human, environmental, or animal), these strains express identical structural and genetic properties and display similar phenotypic characteristics (77, 104). In animals, S layer-containing *A. hydrophila* strains have been recovered from infected fish, birds, hamsters, cows, and pigs (45). Electrophoretic analysis of glycine-extracted SAPs indicates that these proteins are acidic molecules ranging between 52 and 58 kDa (44, 77, 78). When subjected to amino acid analysis, SAPs from *A. hydrophila* have an overall amino acid composition similar to that of SAPs from *A. salmonicida*, although these molecules share no sequence homology at their amino termini (44). Functionally, the SAPs of *A. hydrophila* and *A. veronii* biotype sobria appear to act quite differently from those of *A. salmonicida*, since the former do not confer overt surface hydrophobicity to the bacterium or preferentially absorb Congo red (104). What role the S layers of *A. hydrophila* and *A. veronii* biotype sobria play in human and animal infections remains to be determined, although one recent study suggests that their role in pathogenesis may be substantially different from the role of the S layer of *A. salmonicida* (79).

LPS. Early studies conducted by Shaw and Hodder (121) on the core region of cell wall LPS from 12 *Aeromonas* strains defined three major chemotypes that roughly correlated with existing species on the basis of the presence or absence of various hexose and heptose monosaccharides. These chemotypes (designated I to III) were subsequently classified on the basis of the presence of galactose, D-glycero-D-mannoheptose, and L-glycero-D-mannoheptose in their respective core regions. Since then, further genetic analysis of the strains used in Shaw and Hodder's study indicated that chemotype I was primarily composed of unnamed HG 2 strains (NCMB 1134, ATCC 14715, Popoff C218); chemotype III represented the *A. sobria* phenospecies (CIP 7433, HG 7; Popoff C 224, HG 8); and chemotype II was heterogeneous, containing mostly *A. caviae*-like isolates (*A. eucrenophila* NCMB 74, *A. media* Popoff C 239) but also the type strain of *A. hydrophila* (ATCC 7966, NCMB 86), which belongs to HG 1. Further studies by these workers have identified a proposed structure for each of these LPS core oligosaccharide chemotypes (11, 12, 94).

Analysis of the electrophoretic mobilities of purified or partially purified (proteinase K-treated) LPS components by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and either autoradiography or silver staining techniques indicates that at least three major resolvable fractions can be discerned by such procedures (35, 43). These fractions include slow- and faster-moving series of O side chain-core oligosaccharide bands and a fast-moving lipid A-core oligosaccharide fraction; the O side chain-core oligosaccharide region displays bands with relative mobilities of 46 to 65 kDa, compared with protein standards (35). When LPS O polysaccharide side chain profiles are electrophoretically analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, at least three major patterns are noted. The most common pattern observed among *Aeromonas* strains is a heterogeneous profile that phenotypically consists of a series of ladderlike bands usually extending throughout the length of the gel lane (43, 78). This pattern is commonly observed in many enteric bacteria, such as *E. coli* and *Salmonella* species (58). A second pattern, termed homogeneous, appears as a small number of prominent O side chain bands sometimes arranged as a doublet (43, 77, 78). This homogeneous pattern has been associated with a single *Aeromonas* serogroup and appears to play an important functional role in the anchoring of S layers to the cell surface (43, 77). Subtypes of this homogeneous pattern exist and are based on the relative distance between prominent bands (78). In two instances, namely, an S layer-positive *A. hydrophila* strain (LL1) and an *A. salmonicida* isolate (SJ-15), the proposed structure of the repeating side chain molecules has been determined (122, 123). Finally, a third pattern that is often associated with serologically rough strains exists and is characterized by the lack of detectable side chains (43, 77). In some instances, these strains appear to have some O side chain specificity despite their lack of reactivity with silver stain, since they agglutinate in specific somatic antisera and have polysaccharide side chains detectable by immunoblotting techniques (79). These strains, therefore, are probably of the SR or Ra chemotype rather than the deep-rough chemotype.

OMPs and the flagellum. There is presently very little general information on *Aeromonas* outer membrane proteins (OMPs) outside of studies conducted on specific subsets of virulent aeromonads. Aoki and Holland (5) analyzed the OMP compositions of eight *A. hydrophila* and eight *A. salmonicida* strains after Sarkosyl treatment of cell envelopes. In the case of *A. hydrophila*, the OMPs were fairly heterogeneous from one strain to another, although most strains contained a 36-kDa protein; in contrast, *A. salmonicida* strains were very similar, with a number of consistent OMPs being identified, the most abundant being a 32-kDa molecule. Under iron-restrictive conditions, both *A. hydrophila* and *A. salmonicida* were found to synthesize new iron-regulated OMPs ranging from 68 to 93 kDa. Kuijper and coinvestigators (84) studied the OMP profiles of 46 fecal *Aeromonas* strains. Major OMP bands occurred between 25 and 45 kDa, with *A. caviae* strains displaying fairly similar OMP profiles, in contrast to the greater heterogeneity observed in *A. hydrophila* and *A. veronii* biotype sobria strains.

In addition, surprisingly little information is available on the aeromonad flagellum. In a study of pili, Honma and Nakasone (61) estimated the molecular mass of the flagellum from an *A. hydrophila* strain to be 36 kDa, although no additional data were provided.

TABLE 4. Selected extracellular factors elaborated by *Aeromonas* species

Factor	Type or class	Synonym(s)	Molecular mass (kDa)	Gene cloned	Reference(s)
Hemolysin	Beta	Aerolysin; cytolysin; Asao toxin; cytolytic enterotoxin	49–54	Yes	7, 17, 20, 32, 62
	Alpha	None	65	No	91
Enterotoxin	Cytotonic	Cytotonic enterotoxin; CT-like enterotoxin	15	Yes	33, 89–91
Proteases	Thermostable	Metalloprotease	35–38	Yes	87, 111
	Thermolabile	Serine protease	68	No	87
Amylase	Alpha	None	49	Yes	53
Chitinase	None	None	96	Yes	113

Extracellular Factors

Most aeromonads elaborate a large number of extracellular enzymes that actively degrade a variety of complex protein, polysaccharide, mucopolysaccharide, and lipid-containing molecules. Although these enzymes are, in many instances, useful in identification as in the case of DNase, their roles in the physiologic functions of the bacterium or in virulence are largely unknown. To date, with minor exceptions, most extracellular factors produced by *Aeromonas* species are thought to play a role in gastrointestinal disease; this association will remain unproved until suitable models are developed for their study. Another reason for the difficulty in understanding the role that various extracellular enzymes play in pathogenesis concerns their multifunctional nature. A prime example of this latter problem is the *Aeromonas* hemolysin(s), which appears to be not only cytolytic but also enterotoxigenic. Molecules with such bifunctional properties make interpretation of data difficult. Finally, our knowledge of a number of extracellular enzymes stems from sophisticated biochemical and molecular work on only a single strain. In many instances, strains have not been sufficiently identified to the genospecies level, so that results cannot be interpreted in light of the correct taxonomic position.

Hemolysins. Probably the most striking cultural feature displayed by many *Aeromonas* strains is their ability to hemolyze erythrocytes when grown on a suitable agar-based medium (Table 4). This characteristic is principally associated with certain strains belonging to the phenospecies *A. hydrophila* and *A. sobria* and is linked to the elaboration of an extracellular hemolysin(s) (23, 71). Such a hemolysin, which typically belongs to a larger group of pore-forming bacterial cytolysins, causes leakage of the cytoplasmic contents from target cells via disruption of the normal integrity of the cell membrane (19). The end result is death, either by osmotic lysis or by a nonosmotic process.

At least two major classes of hemolysins expressed by *Aeromonas* strains have been reported. One class, originally termed "aerolysins" by Bernheimer and Avigad (17), comprises typical beta-hemolysins that produce clear zones of hemolysis on blood agar. The *Aeromonas* beta-hemolysins are heat labile (56°C, 5 min) proteins with molecular weights of 49,000 to 53,000 (7, 17, 20, 32). The aerolysin is synthesized in a precursor form, from which the signal sequence is removed prior to export across the bacterial outer membrane (62). Studies of these enzymes have suggested that their activity is not enhanced in the presence of divalent cations (17), while investigations of the effect of various proteases on crude, partially purified, or purified hemolysin preparations have produced conflicting results (17, 20, 91). At least two different types of beta-hemolysin are known to be produced by aeromonads, as determined by biochemical and immunologic studies (7, 8, 80). Both proteins have molecular weights of ca. 50,000, although their isoelectric points differ significantly (5.3 to 5.4 versus 7.6). Results of immunodiffusion assays indicate that the hemolysins (designated AH-1 and CA-11) are immunologically related and share some epitopes in common (8). A second class of hemolysins, termed alpha-hemolysins, has been primarily studied by a number of Swedish investigators (91, 130). The alpha-hemolysin is elaborated during the stationary phase and is not expressed when temperatures exceed 30°C. When observed on blood agar, this hemolysin produces an opaque, incomplete type of hemolysis that is often seen as the inner hemolytic zone of a strain producing "double-zone" hemolysis. The alpha-hemolysin appears to be a 65,000-molecular-weight protein with a pI of 4.8 (91). Both alpha- and beta-hemolysins have observable but different effects on cell culture lines, although the effect of the beta-hemolysin appears irreversible (130).

In addition to the above-described cytotoxic properties for mammalian cells, an additional in vivo activity has been ascribed to the beta-hemolysin. In pioneering studies by Burke and colleagues (25, 26), a close association between the expression of a cell-free hemolysin by aeromonads and enterotoxigenic activity in suckling mice was noted, although evidence directly linking these two biological activities was lacking. Subsequent studies by Asao et al. (7, 8) and Bloch and Monteil (20) with purified hemolysin preparations unequivocably demonstrated this protein to be enterotoxigenic in rabbit ileal loops and in suckling mice; antibodies raised against this purified hemolysin completely neutralized both the cytotoxic and the enterotoxic properties (81, 128). Besides this potential role in gastrointestinal disease, the beta-hemolysin has also been implicated in systemic disease, as isogenic mutants from which the aerolysin gene has been specifically deleted show reduced lethal capabilities in mice, as assessed by LD_{50}s (32). Purified beta-hemolysin has a minimum lethal dose upon intraveneous injection into mice of 0.06 μg (7, 8). These collective studies indicate that the beta-hemolysin is an enterotoxigenic cytolysin; similar functions for the alpha-hemolysin of *Aeromonas* species have not been described to date.

Enterotoxin. In addition to the cytolytic enterotoxin (beta-hemolysin) produced by many *Aeromonas* strains, at least one other enterotoxigenic factor has been described. In the

early 1980s, Ljungh and associates reported on the partial separation and purification of an enterotoxin that was distinct from the alpha- and beta-hemolysins previously described (89, 90). This enterotoxin caused fluid accumulation in rabbit ileal loops and gave a positive response in the rabbit skin test. When Y1 adrenal cells were exposed to this enterotoxin, rounding, concomitant steroid secretion, and increased intracellular levels of cyclic AMP were observed. These collective results suggested that this molecule was a cytotonic enterotoxin.

While early studies (33, 89, 90) indicated no serologic cross-reactivity or neutralization of the *Aeromonas* cytotonic enterotoxin by cholera toxin antibodies or antisera raised against the heat-labile and heat-stable enterotoxins of *E. coli*, subsequent investigations indicated that 5 to 20% of the *Aeromonas* strains screened in reverse passive latex agglutination or enzyme-linked immunosorbent assays produced a cholera toxin cross-reactive factor that was enterotoxigenic (27, 37, 60, 109, 124). This enterotoxin caused fluid accumulation in suckling mice and elongation of CHO cells in vitro. *Aeromonas* strains probed for cholera toxin sequences in colony hybridization assays were found to sporadically react against the A2- and B-subunit probes (119). Although not firmly established, it appears that the cytotonic and cholera toxin cross-reactive factor-like enterotoxins are very similar or identical in most instances, although physiochemical characterization of these molecules is still lacking. The cytotonic enterotoxin from one *Aeromonas* strain (AH2) has recently been successfully cloned (33) into *E. coli* LE392(pAH195). Whether certain cytotoxic enterotoxins (hemolysins) also contain epitopes cross-reactive with cholera toxin antibodies is presently unknown.

Proteases. Proteases are enzymes that are capable of cleaving peptide bonds. A number of extracellular proteases produced by gram-negative bacteria are thought to play important roles in pathogenesis and virulence. Included in this group are the 33-kDa elastase of *Pseudomonas aeruginosa* and the 50- and 56-kDa proteases of *Serratia marcescens* (135). A number of these extracellular bacterial proteases have been implicated in specific illnesses, including wound infections and pulmonary disease.

Aeromonas isolates secrete at least four or five different proteases, as determined of pH optima and substrate specificities (102). Two major proteases produced by *A. hydrophila* have been described by Leung and Stevenson (87). One enzyme is a heat-stable protease that is inactivated by EDTA and appears to belong to the general class of thermostable metalloproteases; the other protease is heat labile (56°C, 30 min) and belongs to the thermolabile serine protease family. Most *Aeromonas* strains analyzed to date by immunologic methods secrete both proteases extracellularly, although some elaborate only one or the other. Recently, a thermostable protease with a molecular weight of 38,000 was cloned and expressed in *E. coli* (111). Although it is similar to the thermostable proteases described by Leung and Stevenson (87), it differs in some aspects, such as temperature inactivation and inhibition by other chemical agents. These differences suggest that some differences exist in proteases of the same class produced by different *Aeromonas* strains. Although the role of proteases in *Aeromonas* pathogenicity is unclear, the LD_{50} of a mutagenized strain of *A. salmonicida* deficient in protease activity was 1,000- to 10,000-fold higher than that of the parent strain when injected intraperitoneally into either salmon or rainbow trout (114). Partially purified heat-labile and heat-stable protease fractions obtained from *A. hydrophila* had LD_{50}s of 18.0 and 3.0 μg of protein per g of catfish, respectively, when injected intraperitoneally (131).

Siderophores. Siderophores are low-molecular-weight compounds with high affinities (binding capacities) for various organic and inorganic forms of iron, particularly under iron-limiting conditions (38). These compounds are thought in many instances to play important roles in the establishment of infection, and the hydroxymate class of siderophores has been associated with gram-negative bacteria, such as *E. coli*, producing invasive disease (105).

Almost all strains of *A. salmonicida*, *A hydrophila*, *A. sobria*, and *A. caviae* studied elaborate one or more types of siderophores (13, 36). In *A. salmonicida*, there are at least two difference mechanisms for iron acquisition; one involves an inducible low-molecular-weight siderophore (36). In the case of mesophilic aeromonads, most *A. hydrophila* and *A. caviae* strains and some *A. sobria* strains produce a novel siderophore termed amonabactin (13). Two forms of this catecholate siderophore exist and appear to function by enhancing the growth of the bacteria in iron-deficient media. Other strains of *A. sobria* which do not produce amonabactin appear to synthesize other types of catecholate siderophores.

Other extracellular factors. *Aeromonas* spp. secrete a wide array of extracellular substances, yet few of these factors have been studied with any degree of detail. The amylase and chitinase genes from one strain of *A. hydrophila* (JMP 636) have been cloned into *E. coli* (53, 113). The amylase gene has also been sequenced and shows relatedness at three apparently functional regions to amylase genes from other bacterial and fungal species.

Distinctions between true cytolysins and cytotoxins are somewhat dubious, but a true cytotoxin, which kills cells without causing overt lysis, has never been convincingly demonstrated for *Aeromonas* species. Recently, cytotoxic activity in *A. caviae* against HEp-2 cells was reported by Namdari and Bottone (100). This cytotoxic activity is present in cell-free supernatants only at a low level and under unusual growth conditions; if substantiated, however, it may provide evidence linking specific virulence-associated factors with the pathogenicity of this phenospecies. Surveys of the frequency of Vero- or Shiga-like toxins in various microbial species have failed to identify such activities in *Aeromonas* species by genetic (probe; polymerase chain reaction amplification), immunologic, and cytologic assays (18, 52, 107).

Cell-Associated Factors

Many important virulence determinants involved in microbial pathogenicity are not elaborated extracellularly per se but instead are associated with the bacterial cell surface or are located internally in the cytosol. Examples of such factors include invasin determinants, serum resistance, iron-regulated OMPs, and the ability to avoid immune surveillance systems through antigenic variation (51, 126). While many of these factors have only recently been addressed in *Aeromonas* species, initial findings seem to indicate that further studies of their potential role in the pathogenic process are warranted.

Invasins. Although gastrointestinal syndromes (dysentery, colitis) associated with some cases of *Aeromonas*-linked diarrhea are compatible with an invasive mechanism, there is surprisingly little experimental evidence to support this hypothesis. One major obstacle in this area concerns the potent cytolysin or hemolysin that is expressed by many *A.*

TABLE 5. Pathogenicity of *Aeromonas* strains in various animal models

Animal	Strain or species	No. tested	Route[a]	Range (CFU)	Virulence criterion[b]	Reference
Mouse	NMRI[c]	24	i.p.	10^3–10^8	$<10^7$	39
	B10.T6R	3	i.m.	10^7–10^8	ND	21
	Swiss-Webster	80	i.p.	10^5–10^9	$\leq 10^{7.7}$	68
Fish	Rainbow trout	25	i.m.	10^4–$>10^7$	$\leq 10^{5.5}$	95
	Goldfish	3	i.m.	10^5–10^6	ND	21
	Rainbow trout	56	i.p.	10^4–$>10^7$	$<10^6$	116
	Channel catfish	9	i.p.	10^4–$>10^7$	ND	40

[a] i.p., intraperitoneal; i.m., intramuscular.
[b] LD_{50} observed in individual studies as the cutoff for virulent or highly pathogenic strains. ND, not determined.
[c] NMRI, Naval Medical Research Institute.

hydrophila and *A. sobria* strains and that interferes with invasion assays by producing irreversible, deleterious effects on cultured epithelial cell lines. Despite these technical difficulties, three reports supporting such a mechanism and concept have surfaced. In 1985, Lawson et al. (86) reported that 6 of 10 *A. hydrophila* strains studied were invasive in HEp-2 cells by both visual and quantitative criteria; the plasmid compositions of these invasive strains suggested that invasin determinants were probably chromosomally located. Several years later, in a cohort study from Chile, five *A. sobria* strains and one *A. hydrophila* strain were also found to be invasive in HEp-2 cell monolayers, although definitive evidence (photomicrographs, electron micrographs, or quantitative assays) was not provided (50). Gray et al. (54) recently showed that between 14 and 36% of *Aeromonas* strains recovered from animals or environmental sources were invasive in HEp-2 cells by their invasion criteria; *A. sobria* was the most invasive phenospecies noted in this study. Genetic investigations with a variety of probes of the *ipa* (invasion plasmid antigen) gene of *Shigella flexneri* have failed to detect homologous sequences in a limited number of aeromonads studied by hybridization techniques (134).

Serum resistance. Many psychrophilic and mesophilic aeromonads are resistant to the complement-mediated lysis of fresh pooled human, rabbit, or fish serum (65, 98). Studies of mesophilic phenospecies indicate that the majority of *A. hydrophila* and *A. sobria* strains are more refractive to the bactericidal activity of high concentrations of pooled serum than is *A. caviae*; serum-susceptible strains appear to activate complement by one or both pathways (classical and alternative), leading to rapid lysis (22). In one study by Mittal et al. (95), serum resistance was directly linked to the *Aeromonas* group most virulent for trout. The *traT* gene, which codes for a major OMP in *E. coli* that is responsible for serum resistance and is associated with invasive infections, was not detected in 66 *Aeromonas* strains screened in colony hybridization assays with a 700-bp probe (96).

Plasmids. Between 20 and 100% of the *A. salmonicida*, *A. hydrophila*, and *A. sobria* strains surveyed harbor one or more extrachromosomal elements with molecular masses ranging from 2 to 100 MDa (16, 34, 132). Most typical strains of *A. salmonicida* contain plasmids, and the plasmid profiles from strain to strain appear fairly homogenous (34, 132); individual strains have been noted to contain up to nine separate plasmids simultaneously (34). In *A. hydrophila* and *A. sobria*, the frequency of plasmid carriage is lower (20 to 58%) and more strain-to-strain variation in profiles is observed (34, 132). Many of these aeromonad plasmids are R factors of incompatibility groups A to C (34, 55, 132); resistance markers harbored on such elements include those for tetracycline, ampicillin, tobramycin, and kanamycin. In one report (132), a plasmid-cured derivative of a wild-type *A. hydrophila* strain displayed a number of differences from the parental strain, including indole and gelatinase production and alterations in surface properties in addition to tetracycline resistance.

Adherence. Aeromonads have been shown to bind to erythrocytes, yeast cells, buccal epithelial cells, and rabbit brush borders via adhesive mechanisms involving afimbrial proteins exposed on the bacterial cell surface (10, 88). In one instance, a reputed 43-kDa afimbrial OMP involved in hemagglutination was identified in an *Aeromonas* strain by cell fractionation and immunochemical techniques (9).

PATHOGENICITY

One of the major drawbacks in studying virulence determinants related to *Aeromonas* pathogenicity has been the inability to establish appropriate organ or animal models that faithfully reproduce the specified disease observed in vivo. This situation is particularly acute in the case of *Aeromonas*-associated gastroenteritis. Establishing such models is critical for identifying strains of high- and low-virulence potential and for comparing extracellular and cell-associated factors associated with these strains which eventually lead to the recognition of determinants and gene products involved in microbial pathogenicity (125). Once virulence determinants are identified, phenotypic markers that allow accurate and rapid identification of pathogenic strains and the epidemiology associated with such infections can be sought.

Animal Studies

Most investigations determining the relative pathogenicities of individual *Aeromonas* strains have used either mouse or fish models (21, 39, 40, 68, 95, 116) for assessing relative virulence, although limited studies have also been performed with chicks and turkey poults (120). Values obtained from such investigations indicate that between 10- and 1,000-fold fewer bacteria are required to produce mortality in susceptible fish than in susceptible mice (Table 5). On the basis of the cumulative results of these studies, a number of general conclusions can be drawn: (i) the phenospecies *A. hydrophila* and *A. sobria* are inherently more virulent than the phenospecies *A. caviae* (66, 68, 103, 116), (ii) within phenospecies (and probably genospecies) significant variations in strain-to-strain virulence potential exist (39, 40, 68, 116), and

(iii) these variations in virulence potential cannot be ascribed to a single extracellular or cell-associated product, with the possible exception of the previously noted S layer and *A. salmonicida*.

Few attempts to manipulate the virulence potential of individual isolates have been reported. Brenden and Huizinga (21) showed that X-irradiated goldfish and mice were more susceptible to infection by two of three *A. hydrophila* strains tested; one avirulent strain showed no appreciable difference under either condition. Similarly, strains of *A. hydrophila* and *A. sobria* studied in BALB/c mice showed no difference in virulence when injected alone, when supplemented with iron-dextran or lysed sheep blood, or when mice were immunosuppressed via cadmium chloride (129); the results of the latter study are somewhat clouded by the fact that exact LD_{50}s were not determined for each strain.

Wound Models

Very limited information on models that could be used to study *Aeromonas* wound infections is presently available. Brook et al. (24) evaluated the ability of 24 *Aeromonas* strains to produce abscesses upon subcutaneous inoculation into mice. Responses in mice were strain dependent and were characterized by epidermal sloughing, alopecia, and/or abscess formation; three avirulent strains produced no observable effect. A potential model for studying *Aeromonas* wound infections induced by medicinal leech therapy has also been reported (110). This model involves the creation of two abdominal flaps in rabbits by aseptic techniques prior to the application of leeches.

Gastrointestinal Models

Individual *Aeromonas* strains can be assayed for enterotoxigenic capabilities in a variety of systems, including suckling mice and rabbit ileal loops (8, 20, 26, 32, 91, 128), but no model that faithfully reproduces the diarrheal disease syndrome associated with *Aeromonas* infection has ever been convincingly demonstrated. Recent studies by Pazzaglia et al. (106), who used the removable intestinal tie adult rabbit model, revealed histologic changes consistent with colonization and infection of the ileum by *Aeromonas* species. Gastrointestinal lesions varied in severity from mild enteritis to hemorrhagic necrosis of the ileum. Although this model does not reproduce diarrheal disease produced by aeromonads, it may serve as a useful prototype for studying the initial steps of gastrointestinal infection (colonization, invasion).

Virulence Markers

While inherent pathogenic differences do exist among aeromonads, few virulence markers reported in the literature go beyond the definition of pathogenicity at the phenospecies level. One group of major interest, however, is *Aeromonas* serogroup O:11 strains, which are primarily associated with severe invasive disease in both humans and animals (70, 104). These strains are characterized by their autoagglutination or aggregation in broth, the presence of an unusual LPS side chain architecture, and the possession of a SAP in the form of an S layer (64, 77, 78). Such strains, predominantly found in the *A. hydrophila* and *A. sobria* phenospecies, can be recognized in the clinical laboratory by phenotypic and serologic tests (76).

Namdari and Bottone (99) have reported that the loss of viability of *Aeromonas* organisms grown in broth containing 0.5% glucose is associated with enteropathogenicity and virulence in mesophilic aeromonads. This reaction, termed the suicide phenomenon, appears to involve glucose catabolite repression of the tricarboxylic acid cycle, leading to acetic acid accumulation (101). This marker, apparently linked to species- and strain-specific virulence and enteropathogenicity, has not been confirmed by other investigators to date.

CONCLUSIONS

The past decade has witnessed a remarkable change in our understanding of the role that aeromonads play in animal and human diseases. Major recent advances include the definition of new species, the identification of novel or new structural components (mini pilin, S layers), and the genetic characterization of reputed virulence factors (hemolysins, proteases). The recent identification within species of specific virulent subgroups that cause a wide array of infections throughout the animal kingdom is also a significant finding (77).

Despite these accomplishments, many important questions remain unanswered. Of cardinal importance is the need to develop an animal model, if possible, that reproduces *Aeromonas*-associated diarrhea, thereby unequivocably establishing the enteropathogenic role of aeromonads in gastroenteritis. Genetically altered strains need to be phenotypically typed in light of the new and expanded taxonomy, so that results obtained with one strain can be accurately translated into reasonable expectations for the genospecies as a whole. Studies characterizing detailed virulence products in the anaerogenic aeromonads needs to be performed so that the role of these species in various disease processes can be clarified. Furthermore, studies based on sound models need to be published so that extracellular and cell-associated products detected both qualitatively and quantitatively can be compared among pathogenic and nonpathogenic strains. The frequency of HGs in the environment needs to be addressed to more fully understand the epidemiology involved in *Aeromonas*-associated infections.

It is likely, on the basis of our current knowledge of microbial pathogenesis in gram-negative bacteria, that virulence in *Aeromonas* species is polygenic in nature, involving a complex interaction of bacterial and host factors. It is hoped that, in the next few years, a number of the key questions regarding this genus will be addressed, thus leading to an understanding of the significance of this genus in clinical microbiology.

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