III. Enteric infections

Aeromonas in enteric infections: Introductory comments

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My laboratory at Colindale has had a general interest in *Aeromonas* as a possible cause of diarrhoeal disease for many years, but a year or so ago we were rather suddenly required to provide a reference service for these organisms for the whole of England and Wales. Obviously it is impossible to become an expert in such a complex field overnight but I was driven to take a fresh look at the literature in much the same way as a complete newcomer would. My impressions and emotions at that time, and now, can be summed up in one word – confusion. Surely there cannot be any other area in the whole of medical microbiology that contains so much contradictory evidence.

I understand that an association between diarrhoea and the presence of *Aeromonas* in faeces was first reported as early as 1937 – perhaps others may know of even earlier reports. In any case this means that the role of *Aeromonas* in diarrhoeal disease has been under investigation now for 50 years or more and yet, even now, have we reached the stage where it can be unequivocally stated that *Aeromonas* causes diarrhoea?

Let us briefly look at just some of the problems. Since the early 1960's there have been numerous reports in all five continents, first (and rather rarely) of outbreaks of diarrhoea apparently associated with *Aeromonas* and, secondly, of varying frequencies of isolation of *Aeromonas* from individuals with and without diarrhoea. The reported variations resulted in part from differences in methodology but also seemed to indicate genuine differences between different populations. In most countries the faecal carriage rate in asymptomatic people was low, less than 4%, and most isolates came from diarrhoea cases. But in some countries, Thailand for example, *Aeromonas* was found with equal frequency in diarrhoeal and healthy stools – the carriage rate being about 27%.

In the 1980's the situation seemed to improve with a few studies directly comparing the isolation rates in diarrhoea and asymptomatic groups. Several of these studies, and notably those in Australia, have shown that *Aeromonas* is found 10 times more frequently in diarrhoea cases than in asymptomatic controls. However, other studies showed that patients with diarrhoea yielded an even higher frequency of isolation if another pathogen was also present at the same time – could it perhaps be that some aspect of the pathogenic process of the other pathogen was somehow stimulating the shedding in the faeces of a non-pathogenic *Aeromonas*, or might the *Aeromonas* simply be an indicator of the consumption of contaminated water that also contained the other pathogens?

In the case of other enteropathogenic organisms, especially *Escherichia coli*, the discovery of pathogenicity factors such as enterotoxin production and enteroinvasiveness led to a much better understanding of the role of the organism in diarrhoeal disease. Similar studies in *Aeromonas* seemed only to further confuse the issue. Enterotoxins, cytotoxins and haemolysins were all described – some workers were convinced that they were all different manifestations of the same toxin, while other groups thought that they were each distinct and even managed to clone the different DNA segments responsible for their production. Some workers detected invasive ability while others tried and failed to confirm it. Even human challenge experiments failed to clarify the problem since strains carrying some of these pathogenicity markers failed to cause diarrhoea when fed to human volunteers.

Even the taxonomy of the organism has increased the confusion. I won't attempt to summarise the history but the current vogue seems to be to divide the motile, mesophilic aeromonads into three species – *A. hydrophila*, *A. sobria* and *A. caviae*. This has opened up the possibility of correlating pathogenicity with species and several groups have confirmed that the accepted pathogenicity markers can be found in *A. hydrophila* and *A. sobria* but not in *A. caviae*. This must be progress because many of the strains referred to us are *A. caviae* – surely we can now tell the clinicians that these strains at least are not causes of diarrhoea. Unfortunately it appears not to be so, since some groups of workers have produced clinical and epidemiological evidence that *A. caviae* also may cause diarrhoea and they conclude that the absence of the accepted pathogenicity markers does not exclude the possibility of enteropathogenicity – a statement that sounds very familiar to those of us who were involved in the debate during the 1970's over the pathogenicity of the classical enteropathogenic *E. coli* or EPEC as they are now called.

In other organisms the use of serotyping as a very precise epidemiological marker has also helped to resolve some of the problems and we are fortunate to have Dr. Sakazaki here today to describe the first steps in this direction for *Aeromonas*. Perhaps he and the other eminent speakers here this morning can help to throw some light in our, or at least on my, confusion.

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Aeromonas and *Plesiomonas* – enteric infections and fecal carriage

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Aeromonas spp. were first recognized as pathogens for cold-blooded animals. After pioneer studies by Caselitz[7] and Lautrop[18] in the sixties these members of Vibrionaceae have come to be recognized as primary human pathogens. A wide variety of clinical infections has been reported, from extraintestinal infections in immunocompromised as well as healthy persons to intestinal ones involving the small and/or the large intestine[21]. *Plesiomonas shigelloides*, also a member of the Vibrionaceae is rarely isolated from extra-intestinal infections[21]. Progress in recent years in the development of selective media to isolate and quantitate *Aeromonas* and *Plesiomonas* from water, food and human as well as animal stools[23, 24, 35] has increased our knowledge of the environmental spread of these organisms.

Both species can be isolated from fresh and salt waters ubiquitously[21]. They show a seasonal variation with higher bacterial counts during the summer[14, 16, 17] in spite of the ability of *Aero-*

monas to multiply at temperatures below 8 °C, a feature not observed in *Plesiomonas*[30]. High bacterial counts were also found in the intestine of fish and other animals in these waters[4, 7, 30, 34]. Consumption of contaminated fish was suggested as an important source of *Plesiomonas*-associated gastroenteritis in Japan, Zaire and India[4, 27, 34]. *P. shigelloides* was the probable causative agent in an oyster-associated outbreak of watery diarrhea in North Carolina[26]. Likewise, oysters associated with acute gastroenteritis in Louisiana and found negative for *Vibrio parahaemolyticus, Salmonella* and diarrhetic shellfish poison grew *A. hydrophila* at a concentration of 9.3 MPN/100 g[36]. Snake-to-human transmission of *P. shigelloides* gastrointestinal infection has been reported[8]. Both species have been isolated from the intestines of healthy and ill warm-blooded animals[4, 7, 15, 29, 34]. Studies on the survival of *Aeromonas* in foods have shown that they may represent 'new' food-borne pathogens and that food may be important in the dissemination of these organisms[15]. Finally, *A. hydrophila* may survive in chlorinated waters[20]. In view of these findings that gastrointestinal infection caused by *Aeromonas* and *Plesiomonas* can be both water- and food-borne it is certainly distressing that no clearcut descriptions of outbreaks of gastrointestinal infection with these organisms exist with the possible exception for two water-borne outbreaks with *P. shigelloides* in Japan[32] and the above-mentioned oyster-associated outbreaks.

Enteric infections

Sporadic cases of gastrointestinal infection with *A. hydrophila* and *P. shigelloides* have been reported from all parts of the world[21]. The rate of isolation of these organisms in stool cultures from symptomatic patients varies from 0.2% to 51%[10, 18] but this may in part reflect variation in culture techniques, in enrichment procedures and not the least an increased interest in these 'new enteropathogens'. In temperate climates the incidence of infection is higher during the warm season[14, 22]. In some studies the incidence was higher in children and in the elderly[2, 14]. Gastrointestinal malignancy, hepato-biliary disease, chronic inflammatory bowel disease, hospitalization and antibiotic treatment seem to be some factors shown to be associated with gastrointestinal infection with *A. hydrophila* and to some extent with *P. shigelloides*[12]. These species should also be included among causes of travellers' diarrhea[10]. The clinical picture varies. Some patients present with cholera-like, rice-water diarrhea, some with mucoid bloody stools, some with typical dysentery with fever, suggesting involvement of different levels of the intestine[21]. There are geographical differences in the symptomatology of *Aeromonas*-related infections, e.g. in North America most patients present with toxigenic diarrhea whereas in Australia as many as 20% of the patients present with colitis or dysentery[14]. Duration of diarrhea for more than ten days was a common feature in two studies[2, 12]. It has been suggested that *A. hydrophila* either causes idiopathic inflammatory bowel disease or exacerbations of it[12, 14].

In healthy patients the infection is usually self-limiting although some patients have been hospitalized and treated with antibiotics (beside rehydration). Most patients who develop septicemia after enteric *Aeromonas* infection are immunosuppressed. In some of these patients skin involvement as in *Pseudomonas* infection, Ecthyma gangrenosum, is seen. Sepsis, skin involvement and toxemia has been described also in previously healthy patients in relation to *Aeromonas*[9]. Data on infective dose and incubation time with *Aeromonas*- and *Plesiomonas*-related enteric infections are lacking.

Fecal carriage

The incidence of asymptomatic carriage of *Aeromonas* varies in different geographical areas. It is generally higher in areas where the bacterial counts in fresh or salt waters are high like in Thailand where 27% of the Thai population harbored *Aeromonas* in one study[25]. *Aeromonas* was recovered from all drinking jars and from 74% of the canals in the study area. In other countries like Denmark and Australia only one asymptomatic carrier was found among 4500 and 1250 persons studied, respectively[13, 18]. The incidence of asymptomatic fecal carriage of *P. shigelloides* is even lower, e.g. 0.003% in Japan[4]. However, in Thailand, *P. shigelloides* was isolated from patients and controls with the same frequency[25].

Discussion

At present there seem to be geographical differences in the expression of virulence properties of *Aeromonas*. Enteroinvasive strains were detected in Australia but not in France[19, 22]. Cholera toxin-crossreacting enterotoxin was detected in strains from Australia, Texas and Japan[13, 16, 31] but not from South Africa, Thailand or Ethiopia[11, 21, 33]. Only 13% of hemolytic strains were suckling mouse test positive in Thailand versus > 90% in Australia[11, 13], and strains from Australia and India expressed different hemagglutination patterns[11, 28]. Furthermore, *A. caviae* was not correlated with diarrheal disease in Australia and USA[2, 13] whereas it was the most common *Aeromonas* species isolated in Switzerland and France[3, 22].

Our knowledge today of phenotypic characteristics of *Aeromonas* and *P. shigelloides* is not sufficient to discriminate enteropathogenic strains from strains lacking virulence markers. It seems unlikely that certain biochemical markers can be used to separate enterovirulent strains from nonvirulent ones in stool cultures since the pathogenesis of *Aeromonas* and *P. shigelloides* enteric infection is complex. *Aeromonas* isolated from patients with enteric infection may produce a battery of virulence factors which may have to be produced in certain combinations to render the organism enterovirulent and produce a cholera-like or Shigella-like disease (like *E. coli* enterotoxigenic or enteroinvasive strains). Furthermore, expression of different surface adhesins may determine the level of adhesion in the gut. It seems most likely that many isolates from water and aquatic organisms do not possess these adhesins and virulence factors and hence are unable to colonize the human intestine.

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Epidemiological studies of *Aeromonas*-related diarrheal diseases

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Published studies of the epidemiology of *Aeromonas*-associated diarrheas are relatively few. Some are based on studying matched cases and controls from hospital or clinic populations, and others on prospectively monitored groups, such as U.S. Peace Corps volunteers, entering a highly endemic area of diarrheal diseases. No data are yet published from prospective community-based studies.

Perhaps the major reasons for this relative lack of information are 1) a relatively low isolation rate when standard enteric media are used, and 2) lack of clear evidence that the organism is an enteropathogen. Most comprehensive studies of community or hospital diarrheal diseases fail to even include the organism in the list of etiologies.

By using special enrichment and selective media, however, *Aeromonas* isolation rates from stool can be improved dramatically[1,5], and in our studies in Peru, this organism is now one of the most frequently isolated from stool specimens.

Review of published literature

The epidemiologic data that are available appear to be conflicting. Studies from Thailand demonstrated a relatively high rate (9–30%) of *Aeromonas* stool isolation in the indigenous population presenting to a treatment facility regardless of whether or not diarrhea was present[7]. In persons newly arrived in this highly endemic area (U.S. Peace Corp volunteers), however, isolation rates were significantly higher among the volunteers when they had diarrhea (31–48%) than when they did not (9-15%) ($p < 0.001$)[2,3].

Studies from a treatment center in Perth, Western Australia demonstrated a significantly higher rate of *Aeromonas* isolation in children seen with diarrhea (10.8%) than with matched controls (0.7%), ($p < 0.05$)[1]. The isolation rates were highest in children ill with diarrhea between the ages of seven months to five years (about 14%). There was also a striking seasonality, with isolations predominantly being made in the warm summer and autumn months.

Studies from a clinic population in Italy[4] by way of contrast showed low isolation rates (about 1%) with no significant differences between diarrhea cases and matched controls.

Review of preliminary and unpublished data

Studies from the International Centre for Diarrheal Disease Research, Bangladesh[6] in which enrichment methods and selective media were used revealed a high rate of isolation among patients admitted with acute diarrhea (33%). In about one-third of these cases *Aeromonas* were the only potential pathogens isolated. The three species of *Aeromonas* were isolated with roughly equal frequency. Data are now being collected from non-diarrheal patients. Isolation rates were generally highest during the early warm season (March and April), with isolations being made throughout the year.

In Peru, we have carried out diarrhea surveillance among 400 families (circa 2000 people) of low socio-economic status, living in shanty-towns on the outskirts of Lima, with twice weekly home visitations. Diarrheal stools, as well as normal stools taken randomly throughout the year from all persons, were cultured for *Aeromonas* using selective techniques of enrichment in alkaline peptone water and streaking on ampicillin-blood agar[5]. Data from two years of observations revealed higher rates in the diarrhea samples than in the control samples. In 1984 when entire families were under surveillance, the isolation rate for *Aeromonas* was 18.2% in diarrhea samples and 14.2% in controls. The largest differences seen were in the group < 6 months of age where the rates were 16% and 9% in diarrhea and control samples, respectively. In 1985, when only children < 3 years of age were under surveillance, the isolation rates were 9% in diarrheal stools and 4.3% in normal stools. These differences in both years were significant. No clear-cut seasonal patterns were found in *Aeromonas* isolations. *Aeromonas* was also found regularly in the drinking water of this community throughout the year (personal communication, Dr R. H. Gilman).

These findings are compatible with other clinical and laboratory data suggesting that at least some strains of this ubiquitous organism are diarrhogenic. They suggest that primary exposures