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EPIDEMIOLOGY OF *AEROMONAS* AND *PLESIOMONAS* DIARRHOEA

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Abstract

Aeromonas spp. and *Plesiomonas shigelloides* can be commonly isolated from stools of patients with diarrhoea in developing countries though much less commonly from such patients in developed countries. These bacteria are also common environmental water bacteria suggesting that persons who drink unpurified water will be ingesting these bacteria frequently. This paper reviews the studies which have attempted to determine if these agents are causes of diarrhoea. Although in some cases it seems likely that *Aeromonas* or *Plesiomonas* is the likely cause of the illness, it seems equally clear that these organisms can also be isolated from a great many people from whom these are not the causative agent. At present there is no clear case definition for the illness caused by either of these agents, and the lack of an adequate case definition inhibits epidemiological studies in determining the extent of the problem in a given geographical area. For both bacteria, specific virulence factors are being sought which would differentiate enteropathogenic from non-enteropathogenic bacterial isolates. Several such virulence factors are being proposed and will need to be validated in epidemiological studies. Because of suggestions from previous epidemiological studies, it is suggested that studies of *Aeromonas* should especially focus on persistent diarrhoea and that studies of *P. shigelloides* should focus on cases of mild dysentery.

Key words : Aeromonas; Pleiomonas; Plesiomonas shigelloides; Emdemiology; Diarrhoea; Virulence.

Introduction

The original purpose of this presentation was to describe the epidemiology of *Aeromonas* and *Plesiomonas* diarrhoea. The term epidemiology, however, implies the description of patterns of disease in population groups. This becomes difficult in the case of *Aeromonas* and *Plesiomonas* diarrhoea, because there is no acceptable case definition for these diseases. While there is much information on the organisms and their recovery from patients, the current epidemiological problem is to examine the available literature to determine if there is a strong evidence establishing these bacteria as causes of diarrhoea. This problem was summarized during the first *Aeromonas* and *Plesiomonas* workshop in 1986 when Drs. von Graevenitz and Turnbull in their concluding remarks stated, "While epidemiologic data point to these bacteria [*Aeromonas* and *Plesiomonas*] being etiologic agents of diarrhoea, it was the consensus of opinion at the workshop that the evidence was still not conclusive" (1).

Previous studies of *Aeromonas* and *Plesiomonas* diarrhoea have focused on differences in isolation rates of the organisms from diarrhoea patients and controls, but case definitions have not been strictly defined. The implied case definition was simply loose stools of any duration (acute or chronic) or type (watery or bloody), with or without other symptoms (e.g. vomiting, fever) in which the bacterium was isolated from a faecal specimen. As microbiological methods became more sensitive,

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isolation rates have increased, in some cases exceeding 70% for *Aeromonas* (G. Pazzaglia, unpublished). It seems likely that the *Aeromonas* is not the true pathogen in many if not most of these patients; hence, one must develop other criteria, in addition to a positive culture, to make the diagnosis of *Aeromonas* or *Plesiomonas* diarrhoea. Except for trends in symptoms, a "typical clinical syndrome" has not emerged for either of the aetiological agents nor have volunteer studies or common source outbreaks assisted in providing a clinical description. As will be described in this summary, studies are required which will more adequately define the clinical syndrome, thus assisting in future epidemiological descriptions.

In the attempt to define the diarrhoeogenic potential of these bacteria, case reports have been as important as the epidemiological studies. Especially crucial have been the reports of patients with persistent diarrhoea who responded to specific antibiotic treatment. A description of a series of such patients would assist even more in describing the case definition and the response to therapy of deseases caused by both *Aeromonas* and *Plesiomonas*.

Ecology of Aeromonas and Plesiomonas

The ecology of these bacteria has been reviewed elsewhere in the workshop. The point to be stressed here is that, unlike most other enteric pathogens, *Aeromonas* and *Plesiomonas* do not require a human or even a mammalian host for survival and spread. They survive well in the environmental waters and largely constitute the "normal flora" of surface waters. The faecal-oral cycle may contribute to the environmental bacteria; however, the numbers of these bacteria in surface waters will largely be determined by ecological factors rather than human contamination.

A second ecological feature of *Aeromonas* important to its epidemiology is its greater resistance to chlorination relative to coliform bacteria, and the fact that treated water may actually be contaminated with *Aeromonas* (2). *Aeromonas* can then be consumed directly in the water and may also contaminate food which are washed with the water. Implicated food included both meats and vegetables. The ability of *Aeromonas* to multiply in cold temperature suggests that refrigeration does not protect from multiplication of the bacterium in food once contaminated.

These ecological considerations suggest that *Aeromonas* are consumed frequently. Persons in developed countries likely have less exposure than those in developing countries; still, consumption of contaminated food and water likely occurs frequently. In many developing countries consumption of large numbers of *Aeromonas* occurs daily.

Plesiomonas are also common bacteria of surface waters, though they usually occur in lower numbers than do *Aeromonas*. Now that selective media and growth conditions of bacteriological culture have been established for *Plesiomonas*, they are being isolated in every surface water sample tested in Bangladesh (A. Huq, unpublished). Unlike *Aeromonas*, they do not multiply in cold temperature, so refrigeration is effective in limiting their multiplication in food. Their survival in water with different levels of chlorine has not been tested.

Aeromonas as a diarrhoeal pathogen

Aeromonas has been suspected to cause diarrhoea for many years; however, except for case reports, there was little evidence for this until the report from Australia which described a statistically significant association of the organism to diarrhoea (3). In this study, *Aeromonas* was isolated from 10% of diarrhoea patients and rarely isolated from controls. Generally it was the only pathogen isolated. At least three enteric clinical presentations could be described in patients harbouring the *Aeromonas:* an acute watery diarrhoea, a persistent diarrhoea, and a bloody diarrhoea.

Similarly, studies of Peace Corps Volunteers (PCV) in Thailand showed a significant correlation of isolation of *Aeromonas* with symptoms (4). In two groups of PCV's, the rates of isolation of *Aeromonas* in the diarrhoea groups were 31 and 57% as compared with 9 and 15% in specimens from PCV's without diarrhoea. Unlike the traveller's diarrhoea, however, *Aeromonas* isolation was not associated with diarrhoea among residents of Thailand. In three different groups of

Thais, *Aeromonas* could be isolated frequently but with similar rates from both diarrhoea and non-diarrhoea groups.

In the United States in two epidemiological studies, again *Aeromonas* was associated with diarrhoea. In a hospital study from Michigan, *Aeromonas* was isolated from nearly 2% of all faecal samples sent to the clinical laboratory, and the percentage was about the same as *Salmonella*, though much less than with *Campylobacter* (5). In the same study, *Aeromonas* was almost never isolated from non-diarrhoeal patients.

Another case-control study, carried out by the Centers for Disease Control (CDC), USA, was focused on risk factors for infection with *Aeromonas* (6). In that study, 28 cases, defined as individuals with a faecal specimen containing *Aeromonas* identified by state health departments, were compared with selected controls. Faecal specimens of controls were not cultured because of the known low rates of isolation in U.S. population. Most of the patients were adults and a large proportion (64%) had persistent diarrhoea. Identified risk factors were the consumption of untreated water and the previous use of antibiotics to which *Aeromonas* are resistant. Of significance was the absence of association with eating fish and shellfish, and foreign travel. There was a suggestion of an association with swimming. Convalescent sera from cases did not show evidence of a rise in antibody titre with the infection.

In an attempt to clarify the diarrheogenic potential for *Aeromonas*, selected strains were given orally to volunteers in Texas (7). The study showed clearly that high inocula of these strains given to healthy volunteers did not cause diarrhoea. Two individuals did develop mild diarrhoea during the studies; however, higher doses of the same organism to other volunteers did not cause illness, suggesting that the few illnesses seen were probably not due to the *Aeromonas* isolate.

Work is now being focused on attempts to correlate virulence factors with diarrhoea symptoms. So far correlation between symptoms and specific virulence factors has not been established. A study from Australia did find a better association between the *Aeromonas* isolation and diarrhoea when counting only the isolates of *Aeromonas* shown to be enterotoxigenic (3). Others have not been able to confirm this (4), but it is hoped that further studies will show that enterotoxins, colonization factors or combinations of virulence factors will identify diarrheogenic from other aeromonads (8,9).

Some unusual epidemiological features should be noted about *Aeromonas* which are different from many other diarrheogenic bacteria. First, common source outbreaks have not been reported with this organism. Perhaps these have occurred and not been detected. Alternatively, this might sugest that host factors rather than bacterial virulence factors play the major role. Studies are needed in especially vulnerable groups (i.e. very young, immunosuppressed, or aged populations) to determine if such groups are at higher risk.

Secondly, the age distribution of many published reports is unusual for an enteric pathogen. With other enteropathogens, the highest rates of illness are seen in the youngest age group. By contrast, in one area with high prevalence rates for *Aeromonas* infection (Thailand), the highest rates were seen in adults (4).

Thirdly, rates of isolation in control populations have been extremely high as illustrated in Thailand (4), Bangladesh (10), and Peru (G. Pazzaglia, unpublished). This seems unusual for a true enteropathogen.

Fourthly, the clinical syndrome is unusually varied in that both acute and persistent diarrhoea as well as bloodly diarrhoea have been described with *Aeromonas*. Generally (and there are exceptions) a given pathogen will lead to a certain clinical presentation. This non-specificity of symptoms suggests that for many of the disease episodes attributed to *Aeromonas*, this bacterium may not be the causative agent.

Fifthly, the absence of a serological response to the bacteria in a convalescing patient is unusual for an enteric pathogen. Although every patient may not have a serological response to an enteric pathogen, in general, it would seem that some patients should develop serum antibodies following infection. In the CDC study (6) where this was sought, a bacterial agglutination test was used, and this relatively insensitive test might have missed probably low-grade serological responses.

A confounding factor in evaluating *Aeromonas*

as a pathogen is the high numbers of *Aeromonas* in surface waters. When diarrhoea occurs, patients presumably continue to drink (the same contaminated) water; hence, recovery of *Aeromonas* in the faeces would be expected, especially when one considers the rapid intestinal transit time in diarrhoea. Thus the rate of recovery of *Aeromonas* could be expected to correlate with diarrhoea, since diarrhoea, consumption of contaminated water, rapid intestinal transit, and recovery of *Aeromonas* are all inter-related. This would result in a statistical association between diarrhoea and isolation of the bacteria even if *Aeromonas* were not the aetiological agent. This epidemiological phenomenon will always confound case-control studies on *Aeromonas* diarrhoea in developing countries.

Of particular interest is the frequent association of *Aeromonas* with persistent diarrhoea and the clinical recovery of patients treated with antibiotics directed against the *Aeromonas*. In view of the realization of the importance of persistent diarrhoea and the difficulty in treating it, evaluation of the linkage between *Aeromonas* and persistent diarrhoea, should be a high research priority.

Some have suggested that there is a difference in pathogenicity between the different species of *Aeromonas*. Though this seems well established in animal models of virulence (mouse lethality following intra-peritoneal injection) (8), a differential diarrheogenic potential between the different species did not seem to be confirmed in studies from Bangladesh in which the three species were isolated from diarrhoea patients in about equal numbers (10). This aspect needs further study.

Plesiomonas as a diarrhoeal pathogen

The epidemiology of infections due to *P. shigelloides* has recently been reviewed (11). *Plesiomonas* also had been considered as a potential pathogen earlier, and the report from Japan suggesting this to be the cause of two diarrhoea outbreaks seemed to provide more evidence (12). In the first outbreak 978 young people attending a youth gathering developed diarrhoea, and *Plesiomonas* was isolated from stool specimens as well as from water and oyster samples. In the second outbreak, a much smaller group on a tour developed diarrhoea. The description of the outbreaks is highly suggestive; however, only a few of the those ill were actually cultured; so, although the outbreaks were large, the number of recovered isolates were small. During the first outbreak, three serotypes of *Plesiomonas* were recovered, with one (017) being most common. All strains during the second outbreak were of the same serotype (024).

This was followed by other descriptions of outbreaks such as that reported in a letter to *Lancet* (13). Following an oyster roast, 36 of 102 persons became ill during the next few days, with a mean incubation of 50 hours. *Plesiomonas* was isolated from a faecal specimen of the index case, but unfortunately other specimens were not available. A similar isolate was found in oysters from the same location. Outbreaks like this suggest that *Plesiomonas* was the cause, but because of the small number of isolates, they do not prove it.

In Thailand, a study similar to the *Aeromonas* study (discussed earlier) was carried out (4). Among Thai residents with diarrhoea, *Plesiomonas* was found frequently, but a statistical association was not found between diarrhoea and *Plesiomonas*. In the same group of PCV's described earlier, *Plesiomonas* was also isolated frequently, and with a higher rate in the patients with "traveller's diarrhoea" than in the controls. The differences in isolation rates were impressive and suggest an aetiological role of *Plesiomonas;* however, they proved not to be statistically different.

Using a study design similar to that used for *Aeromonas*, CDC likewise carried out a case-control study to determine risk factors for *Plesiomonas* infection (14). In contrast to *Aeromonas*, *Plesiomonas* infection was associated with consumption of uncooked shellfish and with foreign travel, and especially with consumption of raw shellfish while in a foreign country. Many of the cases in this study had a dysentery illness suggesting an invasive process.

As with *Aeromonas*, *Plesiomonas* was also tested in volunteers to determine if it would cause diarrhoea (15). In spite of increasing doses and even pre-treating some volunteers with ampicillin, none of the volunteers became ill, nor did samples

of paired sera show a rise in antibody titre to the *Plesiomonas*.

Though volunteers did not become ill, case reports have been convincing as to the ability of *Plesiomonas* to cause colitis. Recently, a bisexual man developed proctitis and fatal sepsis with *P. shigelloides* (16). Unfortunately, faeces were not cultured; however, it seemed likely that the *Plesiomonas* caused the colitis and that the gastrointestinal tract was the portal of entry for the *Plesiomonas*. Other cases of colitis using biopsy examinations have been associated with the isolation of *Plesiomonas* in Bangladesh (unpublished, K.A. Choudhury).

The ability of *Plesiomonas* to cause an inflammatory mucosal lesion is further confirmed by oral inoculations of conditioned rabbits. When three different strains of *Plesiomonas*, two isolated from patients with diarrhoea and one from river water, were given to adult rabbits orally in high doses, none of them developed illness; however, microscopical examinations of biopsy specimens of the intestine did show mild acute inflammatory lesions similar to those seen in the intestines of patients (unpublished, K.A. Choudhury).

Relation of Plesiomonas infection to epidemiology of shigellosis

Some serotypes of *Plesiomonas* agglutinate with antisera to specific serotypes of *Shigella*. The most frequently isolated such *Plesiomonas* serotype is 017 which agglutinates with *Shigella sonnei* antisera. In past studies, this serotype has constituted about 25% of all *Plesiomonas* isolates (17). The lipopolysaccharide (LPS) of *Shigella* is thought to be a protective antigen (18), and one might hypothesize that exposure to *Plesiomonas* from contaminated water might immunize against infeciton with *S. sonnei*.

Two lines of evidence suggest that this hypothesis may be true. First, the proportion of shigellosis due to *S. sonnei* vs *S. flexneri* appears to be directly related to the improvement in water supplies. As a society develops economically and as the water supply improves, the *sonnei* to *flexneri* ratio increases, suggesting that there is a loss of immunity to *S. sonnei* antigens with the improvement in water. Secondly, experimental data from the rabbit shigellosis model shows that immunization with a strain of 017 *Plesiomonas* does solidly protect against a lethal *S. sonnei* challenge (19). The hypothesis has not been tested directly in humans but could be tested in volunteers in the future.

In summary, *Aeromonas* and *Plesiomonas* are ubiquitous environmental bacteria which commonly are isolated from patients' faeces during episodes of diarrhoea. Although epidemiological data are strong showing a statistical association with diarrhoeal symptoms, the epidemiological studies have not yet proved causality. If volunteers given the bacteria had developed diarrhoea, the aetiological role of these bacteria would have been secure. Since the volunteer studies were negative, further studies are needed to establish pathogenicity based on careful case definitions, age-specific differences, and case reports. Available data suggest that the syndrome of persistent diarrhoea may be especially important for *Aeromonas*, while a mild dysentery may be related to *Plesiomonas*. Further studies of these two diseases are especially needed. As future studies are carried out, virulence markers must be correlated with the syndrome. The epidemiology of the *Plesiomonas* suggests that the pattern of infection with this bacterium may affect the pattern of infection with *Shigella*, and this relationship may be even more important than the disease resulting from the *Plesiomonas* infection itself.

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