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Review Article

Epidemiology and Pathogenesis of *Providencia alcalifaciens* Infections

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Abstract. *Providencia alcalifaciens* is a member of the family Enterobacteriaceae that has been commonly implicated as a causative agent of diarrheal infection in humans and animals. Recent outbreaks of *P. alcalifaciens* in both developing and developed countries have raised public health concerns. Several studies have suggested that *P. alcalifaciens* can cause diarrhea by invading the intestinal mucosa, although its pathogenicity has not been well established. Often routine laboratory investigations that seek etiological agents of diarrhea do not actively pursue *P. alcalifaciens* detection. Therefore, routine laboratory diagnosis should be given more attention for better understanding the epidemiology and pathogenicity of *P. alcalifaciens*.

INTRODUCTION

Providencia, a genus of Gram-negative, rod-shaped bacteria, belongs to the family Enterobacteriaceae. Presently, it consists of nine species,[1] among which *Providencia stuartii*, *Providencia rettgeri*, *Providencia rustigianii*, and *Providencia alcalifaciens* are the causative agents of different types of infections, including urinary tract infections, wound infections, septicemia, nosocomial infections, and diarrhea.[2] Several studies have reported that *P. alcalifaciens* is the causative agent of diarrhea in humans and animals, as well as hemorrhagic pneumonia in piglets.[3–9] Strains of *P. alcalifaciens* isolated from diarrheal patients were able to develop diarrhea in the removal of ileal ties of adult rabbit diarrhea (RITARD) model and showed invasiveness to HEp-2 and other eukaryotic cell lines in vitro, although the pathogenicity of this organism remains poorly established.[7,10] Previously, three foodborne outbreaks caused by *P. alcalifaciens* were reported in Japan, Turkey, and Kenya, and have attracted considerable attention.[11–13] This article will present a brief review of the literature regarding the epidemiology and pathogenicity of *P. alcalifaciens*, and justify the importance of routine laboratory diagnosis.

EPIDEMIOLOGY

Providencia alcalifaciens is commonly found in soil, water, and sewage and has been isolated from a broad range of living organisms such as chickens, dogs, and cows.[14] The bacterium was first recognized in 1943 by Stuart, who identified it as "anaerogenic paracolon 29911" isolated from diarrheal patients.[15] Kauffmann labeled these anaerogenic paracolon 29911 strains as Providence group in 1951, based on the report published in Providence, RI by Stuart.[2] One year later in 1952, Brown found that the anaerogenic paracolon bacilli were responsible for gastroenteritis in children.[16] In 1955, Ridge reported a case with the Providence of paracolon bacillus type, similar to the organism previously published by Brown, in a residential nursery.[17] In the same year, Graber and Lincoln[18] also found an association between Proteus–Providence organisms and infantile diarrhea in Denver. Later, *Providencia*-associated diarrhea was reported in Nigeria and India.[19,20] In Bangladesh, Albert and others[10] were able to demonstrate the association between *P. alcalifaciens* and diarrheal disease in a case–control study by comparing the rates of isolation of the pathogen in children with and those without diarrhea. In 1989, Haynes and Hawkey[5] implicated *P. alcalifaciens* as a causative agent of traveler's diarrhea. They isolated *P. alcalifaciens* significantly more (7–8 times more frequently) among the returning British traveler's with diarrhea than among patients who had diarrhea but had no history of recent travel at the time. Subsequently, this was supported by the findings made by Yoh et al.,[6] in which the pathogen was isolated among traveler's returning to Japan. Furthermore, a cross-sectional study between 2009 and 2013 was able to isolate 3.2% of *P. alcalifaciens* from the stool samples of diarrheic children in Kenya.[21] Also, *P. alcalifaciens* is known to cause diarrhea in dogs and hemorrhagic pneumonia in piglets.[8,9]

Transmission of *P. alcalifaciens* occurs primarily through ingestion of contaminated foods, which caused three foodborne outbreaks in Japan, Turkey, and Kenya.[11–13] In 1996, a large outbreak of foodborne infection caused by *P. alcalifaciens* occurred among children and teachers at two kindergartens and one high school and affected a total of 270 individuals in Fukui, Japan.[11] Here, no other recognized enteropathogens were detected in fecal samples except *P. alcalifaciens* and the isolates clonality was confirmed by pulsed-field gel electrophoresis. The source of the outbreak was the food eaten at lunch that was prepared and supplied by a single catering facility. In Turkey, *P. alcalifaciens* caused a diarrhea outbreak that affected 27 adults who had consumed potato salad and pork schnitzel during a festive meal.[12] In Kenya, *P. alcalifaciens* was associated with an outbreak of diarrheal disease in seven primary and four secondary cases where the sole factor was the mashed potatoes eaten by primary cases.[13] These outbreaks indicate that *P. alcalifaciens* is a potential foodborne pathogen. Thus, it reinforces the need to improve clinical surveillance and laboratory diagnosis.

CLINICAL FEATURES AND DIAGNOSIS

Diarrheal infection caused by *P. alcalifaciens* usually presents as watery, non-bloody diarrhea, or loose stool, occasionally with abdominal pain, vomiting, fever, and rarely tenesmus.[11–13]

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The incubation period for *P. alcalifaciens* gastroenteritis ranges from 1 to 4 days, and the frequency of diarrhea ranges from three to 10 episodes per day, although in some instances, up to 15 episodes have been reported.[11–13] The spectrum of disease severity varies depending on age groups, as observed during the outbreaks in Japan and Kenya. The attack rate was found higher in kindergarten children than in high school students and adults during the outbreak that occurred in Japan.[11] In addition, in Kenya, severe illness was observed among children compared with the adults who only reported mild diarrhea and required no hospitalization.[13]

Besides diarrheal infections in humans, *P. alcalifaciens* can cause enteritis in puppies, including the clinical signs of dehydration, hypothermia, vocalizing, and blood-tinged diarrhea.[8] After infection, puppies died within 1–2 days and diffuse superficial mucosal necrosis was observed in the small intestine after necropsies.

Recent studies demonstrated that *P. alcalifaciens* is not only limited to the intestinal infection. Wang and others[9] described *P. alcalifaciens*–associated hemorrhagic pneumonia in piglets. According to their report, the infected piglets died within 2–4 days after showing clinical symptoms of abdominal skin empurpling, difficulty in breathing, frothy nasal, and oral discharge. The lungs of infected piglets were friable, swollen, and dark brown caused by severe hemorrhage. Interstitial broadening and massive inflammatory cells infiltrated can be seen in histologic sections. However, Vieira et al.[22] also demonstrated the ability of *P. alcalifaciens* to translocate from the intestinal lumen to extraintestinal sites of the host using a small animal model. It was observed that both invasive and noninvasive *P. alcalifaciens* strains isolated from diarrheal patients were able to resist the human serum bactericidal activity and exhibited the ability to translocate from the gastrointestinal tract to mesenteric lymph nodes, liver, and spleen, which supports the potential role in causing disseminated infection.

Routine laboratory investigations for the identification of etiologic agents of diarrhea do not actively pursue *P. alcalifaciens* because most of the clinical laboratories do not recognize *P. alcalifaciens* as a causative agent of diarrheal disease. Also, it may not be easy to identify or distinguish *Providencia* species from other non-lactose fermenters such as *Salmonella* or *Shigella* species using media such as deoxycholate hydrogen sulfide lactose, MacConkey, and Salmonella-Shigella agar which are often used as selective media for the isolation of bacteria in feces. To circumvent the challenge of isolation and differentiation of *Providencia* species from other bacteria in culture media, Senior developed *P. alcalifaciens* medium (PAM) in 1997.[23] *Providencia alcalifaciens* medium was found to be a suitable differential medium for *P. alcalifaciens* because it yields red colonies as opposed to yellow or white colonies observed in other Enterobacteriaceae. This is due to the inability of *P. alcalifaciens* to ferment mannitol, xylose, or galactose present in the PAM medium, unlike other fecal bacteria which can ferment one or more of these sugars. *Pseudomonas aeruginosa* was also found to yield red colonies similar to *P. alcalifaciens*, but they can be easily differentiated using the oxidase test. *Providencia aeruginosa* is oxidase positive, whereas *P. alcalifaciens* is oxidase negative. *Morganella morganii* also shows similar growth to *P. alcalifaciens* on PAM medium. Subsequently, Yoh et al.[6] modified PAM medium by substituting mannitol and galactose with maltose by adding polymyxin-B to establish a selective culture medium known as the polymyxin–mannitol–xylitol medium for *Providencia* (PMXMP). Both the PAM and PMXMP media showed promising results for the isolation and differentiation of *Providencia* species from other Enterobacteriaceae. In addition, *P. alcalifaciens* can be detected from cultures using commercial API-20E biochemical strip kit (API System; BioMérieux, Marcy-l'Étoile, France) and automated systems such as Vitek-II (BioMérieux). For sensitive and rapid methodologies, polymerase chain reaction–based 16S rRNA and *dnaJ* gene sequences have been used for identification.[11,13]

PATHOGENESIS

Providencia alcalifaciens–causing diarrheal pathogenicity was first studied by Albert and others[10] with RITARD model. Studies have shown that the RITARD model has been used to examine diarrheagenic properties in various microorganisms.[24–26] However, in this study, *P. alcalifaciens* was isolated from a child and two adults with diarrhea where no other recognized enteric pathogen was detected. All three strains of *P. alcalifaciens* were able to develop diarrhea, whereas some differences were observed in disease-producing abilities.[10] Two of the three strains developed diarrhea in 80–91% of experimental animals and colonized the intestinal wall, whereas one strain developed diarrhea in 40% of the animals and was not recovered from the small intestine of one rabbit during the time of sacrifice. These data indicate the differing disease-producing abilities among various strains of *P. alcalifaciens*. The electron microscopy result of the ileal mucosa indicated two modes of entry into intestinal epithelial cells: one by endocytosis with polymerization of cytoskeletal components and the other by disruption of tight junctions.[27] On the other hand, none of the strains were able to produce heat-stable enterotoxin in suckling mice, but they develop hindlimb paralysis among rabbits in RITARD model although its relevance with human disease is not clear.[10]

In addition, these three *P. alcalifaciens* isolates showed negative results for heat-labile enterotoxicity in mouse adrenal tumor Y1 cells and cytotoxicity in HeLa cells. They also exhibited adherence to cultured HEp-2 cells and were able to invade cell monolayers with actin condensation. The pattern of this actin condensation was similar to that produced by another invasive organism, *Shigella flexneri*, but different from enteropathogenic *Escherichia coli*. Subsequently, a similar type of experiment carried out with 14 additional strains isolated from diarrheal stools confirmed their invasive character and suggested that prior growth at 37°C is the most effective for optimal invasiveness.[28] Khashe et al.[29] observed that highly invasive *P. alcalifaciens* strains could attach to HEp-2 cell within 2 hours of postinfection, whereas weak or noninvasive strains were found non-adherent, suggesting that the differential attachment may be linked to the key adhesin factors on the cell surface of invasive strains. HeLa cell invasion assay showed that seven of 11 *P. alcalifaciens* strains isolated in the São Paulo City, Brazil, have invasive character and actin condensation ability, and no difference was observed when compared with HEp-2 cells.[30] In addition, Maszewska et al.[31] demonstrated the invasive phenomenon of *P. alcalifaciens* in Caco-2 cell lines and compared with HEp-2 cells. The purpose of using Caco-2 cell lines was to understand the in vivo situation more closely because they can differentiate and form polarized monolayers with a phenotypical resemblance to

enterocytes of the small intestine.[32] However, nearly 60% of *P. alcalifaciens* isolates were invasive into Caco-2 cells, whereas HEp-2 cells showed 95% invasiveness, suggesting that the level of invasion depends on not only a strain but also on the type of epithelial cells.[31]

Studies have demonstrated that enterobacteria such as *Shigella* spp., enteroinvasive *E. coli*, and *Yersinia enterocolitica* harbored plasmid correlates with invasive ability.[33–36] By contrast, most of the *P. alcalifaciens* studies found no correlation between the presence of plasmids and invasiveness, indicating that not plasmid genes but chromosomal genes are involved in the invasion.[4,31,37] To determine the gene(s) responsible for invasion, TnphoA insertion mutagenesis has been used successfully to screen encoding virulence determinants in a variety of pathogenic bacteria, including *P. alcalifaciens*.[38–41] Four diarrheal TnphoA mutant *P. alcalifaciens* strains were compared with the parent strain and negligible invasion, and actin condensation in HEp-2 cells was observed.[38]

Beside invasiveness, recently Shima et al.[42] reported that *P. alcalifaciens* strains could produce the cytolethal distending toxin (CDT) which causes cell elongation, cell distention, and blocks eukaryotic cell proliferation at the G2/M phase, leading to cell death. Since 1987, CDT toxin has been reported in various Gram-negative bacteria such as *E. coli*, *Haemophilus ducreyi*, *Helicobacter*, *Campylobacter*, and *Shigella* spp., which are composed of three polypeptides, namely, CdtA, CdtB, and CdtC, that form a complex structure required for the toxin activity.[43] Some studies have documented the isolation and characterization of CDT-producing bacteria from patients with diarrhea.[44–47] However, CDT of *P. alcalifaciens* showed some homology with the CDT of *Shigella boydii*, suggesting that the CDT gene cluster of *P. alcalifaciens* might be horizontally transferable. This suggests that *P. alcalifaciens* may be similar in terms of pathogenicity with *S. boydii* and this could explain, in part, why *P. alcalifaciens* may not be overly invasive. It is however important to note that only some of the *P. alcalifaciens* studied were shown to harbor the CDT genes. Moreover, further characterization of the identified *P. alcalifaciens* CDT would be required to confirm it as a virulence factor.

Another study suggested that *P. alcalifaciens* uses manganese superoxide dismutase (Mn-SOD) for intra-phagocytic survival, whereas sodA-deleted mutant strain showed lower virulence properties than wild type.[48] Also, Mn-SOD protects *P. alcalifaciens* from murine macrophage cells. This result provides evidence that Mn-SOD of *P. alcalifaciens* is involved in invasive activity, resulting in intracellular survival. Recently, it has been shown that lipopolysaccharides were a potent inducer of epithelial barrier dysfunction and endothelial cytotoxicity during *P. alcalifaciens* infection.[49]

CONCLUSION

In recent years, several foodborne outbreaks caused by *P. alcalifaciens* have confirmed the increasing incidence of diarrhea due to *P. alcalifaciens* in the world, regardless of the socioeconomic strata. And this incidence is probably higher than that reported because it is mostly implicated as a self-limiting diarrheagenic pathogen and usually causes moderate morbidity. In addition, most studies conducted within the tropics do not properly consider *P. alcalifaciens* as a possible etiologic agent of diarrhea, and thus do not actively detect the organisms as well as focus on epidemiological aspects. Invasiveness remains the most established mode of pathogenesis in *P. alcalifaciens*, and future research on this matter is needed. The incidence of *P. alcalifaciens*–causing gastroenteritis can be reduced by good hygiene and food preparation. Detection of the pathogen routinely at clinical laboratories is strongly recommended; this along with integrated surveillance programs can be effective for the control of *P. alcalifaciens* infections.

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