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Short Communication

Enterotoxigenicity of *Klebsiella pneumoniae* Associated with Childhood Gastroenteritis in Madras, India

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SUMMARY: *Klebsiella pneumoniae* was isolated as the predominant growth from 12 of 100 children under 3 years of age suffering from acute diarrhea. Of the 12 isolates, SA1, SA2, SA4, SA5, SA6 produced a secretogenic response in the ligated rabbit ileal loop, and one isolate, SA3, induced a diarrheagenic response in suckling mice. Two isolates, SA7 and SA8, were diarrheagenic in both assays. Strains SA9, SA10, SA11, and SA12 were found to be non-enterotoxigenic. These isolates belonged to serotypes K6, 16, 25, 30, 39, 46, 49, 53, 66, and 81. All eight enterotoxigenic strains were resistant to ampicillin, streptomycin, ceftazidime, cefuroxime, and cotrimoxazole. Only quinolones such as ciprofloxacin and norfloxacin appear to be effective against enterotoxigenic *K. pneumoniae*.

Although *K. pneumoniae* occurs as a commensal in the intestine, it has been reported to cause diarrhea since 1961, when it was first isolated from diarrheal infants in an epidemic in a nursery in Mexico, and serotype K19 was found to be responsible (1). Since then, the role of *K. pneumoniae* in causing watery diarrhea has been well established (2,3). Earlier records indicated that *K. pneumoniae* may induce diarrhea through the production of stable toxin (4,5). The enterotoxigenicity of these *Klebsiella* strains was established by assay of cell-free culture filtrates in rabbit ileal loops, Y1 adrenal cells, or Chinese hamster ovary tissue culture assays for heat labile toxin, and in the suckling mouse assay for heat stable toxin (6-8). *K. pneumoniae* appears to resemble *E. coli* in that certain strains elaborate ST alone and others produce both ST and LT (9,10). *K. pneumoniae* is usually ignored as native intestinal flora when isolated from cases of childhood diarrhea, even if no other enteric pathogens are demonstrated. During our investigations of childhood diarrhea in Madras, we found that12% of the stool samples yielded heavy growth of *K. pneumoniae* on the primary culture media. Since no other recognized enteric pathogens were recovered from these cases, we undertook this pilot study to study their diarrheagenic potential by investigating their enterotoxigenicity in ligated rabbit ileal loops and infant mice.

Rectal swabs from 100 children under 3 years of age with acute diarrhea attending the Institute of Child Health and Hospital for Children, Madras, were collected and transported in Cairy-Blair medium, and processed for enteric pathogens. The samples were inoculated onto a battery of media, and the *K. pneumoniae* isolates that showed heavy growth on MacConkey's agar and Salmonella-Shigella agar were included in this study. Among the100 diarrheal stool samples screened, 12 samples yielded *K. pneumoniae* as predominant growth on primary culture. The identity of these isolates was confirmed biochemically (11).

The *K. pneumoniae* isolates were serotyped by countercurrent immunoelectrophoresis (CCIE) (12,13). Strains with negative or doubtful CCIE reactions were investigated by the classical Quellung reaction (11). The 12 *K. pneumoniae* isolates belonged to serotypes K6, 16, 25, 30, 39, 46, 49, 53, 66 and 81 (Table I).

Enterotoxin was prepared as described by Wadstrom et al. (14) and stored in two portions at -20 C. One portion was assayed for LT in ligated rabbit ileal loops (15), and the other portion was heated to 100 C for 30 min in a water bath to inactivate the LT and then assayed for ST in suckling mice (16). Eight (66. 6%) of the 12 strains of *K. pneumoniae* isolate were found to be enterotoxigenic. Seven of these eight strains produced LT, as demonstrated by fluid accumulation in the rabbit ileal loop experiments, and three of the eight strains produced ST, as seen in the suckling mouse assay (Table I).

The susceptibility of the *K. pneumoniae* isolates to 14 antibiotics was tested on Mueller-Hinton Agar by the disk diffusion method of Bauer et al (17). A11 12 *K. pneumoniae* isolates were multidrug resistant, and the eight enterotoxigenic strains were found to be resistant to ampicillin, streptomycin, cefuroxime, ceftazidime, and cotrimoxazole, while sensitive to ciprofloxacin alone (Table I).

Enterotoxigenic *K. pneumoniae* strains are occasionally isolated from children with acute diarrhea presenting with classical cholera-like illness (1,18). These organisms have been reported in sporadic cases of diarrhea in India (2), South Africa (7), Brazil (3), Ethiopia (14), and even in the United States (6). In the present study, *K. pneumoniae* was isolated as the predominant growth on primary culture from 12 patients, and none of the stool samples from these cases yielded enteropathogens such as *Vibrio cholerae*, *Salmonella*, or *Shigella*. Since klebsiellae are usually found as commensal microflora in stool samples, it becomes difficult to implicate these organisms as causal organisms of diarrhea in a given case. However, in the absence of a recognized enteropathogen, heavy growth of *K. pneumoniae* from diarrheic stool suggests a causal rather than a casual association (2). Eight of the *K. pneumoniae* isolates obtained in the present study were demonstrated to be enterotoxigenic in the classical rabbit ileal loop and infant mouse assays. These results indicate the prevalence of enterotoxigenic *K. pneumoniae* in the southern part of India responsible for sporadic cases of diarrhea in children. Similar observations were earlier reported by Panigrahi et al (5).

Klebsiella spp. are presently classified into 77 K serotypes (19), among which *K. pneumoniae* strains possessing K1 or K2 capsular antigens have been frequently isolated from extraintestinal infections such as sepsis and bacteremia. Among the various serotypes of *Klebsiella* strains from such sources, *K. pneumoniae* serotype O1:K2 strains are reported to be more virulent in the mouse intraperitoneal model (20). Data on the serotypes of *K. pneumoniae* associated with diarrhea are lacking. The results of this study have indicated that several serotypes are associated with diarrheal illness (Table I), and the data show that two K-39 serotypes, two K-66 serotypes and one K-16, K-30, K-53, and K-81 serotype each were diarrheagenic.

Klebsiellae are known to usually be resistant to β-lactams such as ampicillin. The occurrence of klebsiellae drug-resistant to the commonly used antibiotics such as ampicillin, kanamycin, tetracycline, gentamycin, and nalidixic acid has earlier been reported (21,22). The eight isolates of enterotoxigenic *K. pneumoniae* obtained in this study were resistant to cotrimoxazole, in addition to ampicillin, amoxicillin, streptomycin, cefuroxime, and ceftazidime. A combination of ampicillin and gentamycin or cotrimoxazole is usually administered in the treatment of cases of childhood gastroenteritis that require antibiotic therapy in hospitals in tropical countries. In light of the prevalence of enterotoxigenic strains of *K. pneumoniae* resistant to commonly used antibiotics, as observed in

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Table 1. Toxin and antibiotic resistance profile of various *Klebsiella pneumoniae* serotypes

Strain No	K-type	LT	ST	Drug resistance
SA1	K-30	+	-	A Ca Co Cp Cu G S
SA2	K-53	+	-	A Am Ca Ci Co Cp Cu G S
SA3	K-81	-	+	A Am Ca Ce Co Cu G S Tb
SA4	K-39	+	-	A Ca Ce Ci Co Cu G S Tb
SA5	K-39	+	-	A Am Ca Ce Ci Co Cu G S Tb
SA6	K-16	+	-	A Ak Ca Ce Ci Cu G Nx S Tb
SA7	K-66	+	+	A Ak Ca Ce Ci Co Cu G S Tb
SA8	K-66	+	+	A Am Ca Ce Ci Co Cu G Nf S Tb
SA9	K-46	-	-	A Am Ca Ce Cp G S Tb
SA10	K-25	-	-	A Ca Ci Co Cp Nf S Tb
SA11	K-49	-	-	A Am Ci Cp Cu G S Tb
SA12	K-6	-	-	A Ak Ca Cp Cu Nf S

LT: labile toxin, ST: Stable toxin, A: Ampicillin, Ak: Amikacin, Am: Amoxicillin, Ca: Ceftazidime, Ce: Cefatoxime, Ci: Cefriaxone, Co: Cotrimoxazole, Cp: Ciprofloxacin, Cu: Cefuroxime, G: Gentamycin, Nf:Nitrofurantoin, Nx: Norfloxacin, S: Streptomycin, Tb: Tobramycin

this study, heavy growth of these organisms in primary culture, especially from pediatric cases should not be ignored as commensals.

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