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BRIEF REPORT

High Prevalence of Carbapenem-resistant *Klebsiella Pneumoniae* in Fecal and Water Samples in Dhaka, Bangladesh

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We evaluated *Klebsiella pneumoniae* (Kp) gut carriage in healthy, unrelated adults and children living in separate households in Dhaka, Bangladesh. Average Kp prevalence in stool samples ranged from 61% in young children (15/25) to 81% in adults (21/26), with significantly higher abundance in adults ($P = .03$, *t*-test). Kp was also prevalent in household water (64%, 21/33) and standing water (85%, 23/27). The presence of Kp in household water was not strongly linked to stool Kp abundance among household members. Antimicrobial resistance was notable: 9% (6/69) of stool and 16% (7/44) of water isolates exhibited multidrug resistance. Carbapenem resistance was observed in 12% of stool isolates (8/69) and 14% of water isolates (6/44). These findings underscore the commonality of Kp in human and environmental reservoirs in Dhaka, Bangladesh, and highlight the emergence of drug-resistant Kp beyond healthcare settings.

Keywords. gut carriage; *Klebsiella pneumoniae*; multidrug resistant; antimicrobial resistance; gram-negative bacteria.

BACKGROUND

Klebsiella pneumoniae (Kp) is an important global pathogen associated with significant morbidity and mortality. Kp has contributed to the spread of antimicrobial-resistance (AMR) genes, including extended-spectrum β-lactamases (ESBLs) and carbapenemases. The burden of Kp is higher in low- and middle-income countries (LMICs) such as Bangladesh [1]. Kp is a frequent gastrointestinal colonizer of humans, and colonization may be an intermediate step before invasive infection and disease [2]. To date, most epidemiologic studies have focused on hospital-acquired Kp infections, and little is known about the prevalence and microbiological characteristics of asymptomatic carriage of Kp in the human gut or its presence in the environment. In this study, we quantified Kp in stool samples from healthy individuals in an urban community in Bangladesh; we also evaluated the presence of Kp in household water and in standing water in the same urban community. Our results shed light on the prevalence and potential transmission of Kp, including AMR Kp, in an urban community-based setting.

METHODS

Klebsiella pneumoniae Isolation From Stool and Water

One stool sample was collected from each participant for bacterial culture in sterile collection vials. After arrival at the laboratory, stool samples were homogenized using sterile phosphate-buffered saline (PBSG) (1× PBSG: 1× PBS, pH 7.2–7.6 supplemented with 15% [v/v] glycerol). PBSG was added depending on the stool volume: 200 mg of stool per 1 mL of PBSG. Homogenized stool samples were plated onto selective Simmons citrate (HiMedia, HiMedia Laboratories Private Limited, India) with 1% (w/v) inositol medium (Carl Roth, USA) for isolation of Kp [3]. Water samples were obtained from household taps where our study population resided, and standing water samples were collected from nearby sources adjacent to the households. These standing bodies of water included ponds, lakes, and bogs used for irrigation and for household practices, such as washing and bathing; standing bodies of water were within 0.5 to 2.5 km of participant households and were not known to be directly linked with sewage runoff. Household tap samples were concentrated (10× or 100×) and standing water samples were diluted (10-fold or 100-fold) using sterile PBS before plating; large, yellow, glossy colonies suspected of being Kp were identified using Kliger's iron agar test, the motility indole urea test, and the citrate test (HiMedia Laboratories Private Limited) [4] and confirmed by polymerase chain reaction (PCR) using primers for the *fiu* gene [5].

Evaluating Relative Abundance Using Quantitative PCR

An additional stool sample for PCR was collected at the same time as the specimen collected for culture, using a sterile container containing 10–12 mL of ≥95% molecular biology-grade ethanol. Bacterial DNA extraction was performed using QIAamp Fast

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DNA Stool Mini kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions. Real-time PCR was performed using a Kp multiplex quantitative PCR (qPCR) assay [5], which targets the Kp *fiu* gene and a pan-bacterial 23S rRNA gene. The *fiu* set detects members of the *K pneumoniae* complex (*K pneumoniae, K quasipneumoniae,* and *K variicola)* but not other *Klebsiella* species or other common bacteria in the gut microbiota. Primers (Integrated DNA Technologies, Coralville, IA, USA) and probes (Thermo Fisher Scientific, Waltham, MA, USA) with sequences and concentrations are listed in Supplementary Table 1.

Bio-Rad iQ Powermix without ROX (Bio-Rad, Hercules, CA, USA) was used for the PCR. A total of 5 µL of template was used in a 20-µL final reaction volume. PCR conditions were: 1 cycle of 95 °C for 2 minutes, followed by 40 cycles of 95 °C for 10 seconds, and 60 °C for 1 minute, on a CFX96 real-time PCR detection system (Bio-Rad). A control stock of Kp genomic DNA was used as a positive control in each run and as a baseline for the relative abundance calculations, using the $\Delta\Delta C_T$ method, ie,

$$\text{relative abundance} = 2^{((\text{23S Ct value of sample} - \text{fiu Ct value of sample}) - (\text{23S Ct value of positive control} - \text{fiu Ct value of positive control}))} \times 100\%$$

Antimicrobial Susceptibility Testing

We evaluated the susceptibility of Kp isolates to 12 antimicrobial agents (ampicillin, piperacillin + tazobactam, ceftazidime, cefepime, ceftriaxone, imipenem, ertapenem, gentamicin, amikacin, ciprofloxacin, levofloxacin, trimethoprim + sulfamethoxazole) using the disk diffusion method, interpreted according to CLSI M100 guidelines [6]. ESBL isolates were identified using the double-disk synergy test (Thermo Fisher Scientific): ceftazidime and ceftazidime/clavulanic acid, cefotaxime, and cefotaxime/clavulanic acid and interpreted according to CLSI M100 [6].

Statistical Analyses

Statistical analyses were performed using GraphPad Prism version 8.4.2. We compared the relative abundance of Kp carriage among different age groups and the relative abundance of Kp between standing and household water using *t*-tests. We used Fisher exact test to compare the presence of Kp in standing and household water samples. We used the Mann-Whitney test to associate the presence of Kp in household water with Kp carriage (relative abundance) in study participants. All statistical tests were 2-tailed, and the significance level was set at $P < .05$.

Ethical Approval and Patient Consent

We obtained written consent from study participants or their parent/guardian. The study was approved by the Bangladesh Medical Research Council National Research Ethics Committee.

RESULTS

Kp Carriage in Healthy Individuals

We enrolled 76 unrelated healthy participants from separate households in an urban locality of Dhaka, Bangladesh, between August 2022 and January 2023 (28 children younger than age 5 years, 22 children aged 5–17 years, and 26 adults). We collected stool samples at 4 time points over 60 days of follow-up (days 0, 7, 30, and 60). Using culture-based methods, we found a carriage rate of Kp of 54% (15/28) in children younger than age 5 years, 68% (15/22) in children aged 5–17 years, and 81% (21/26) in adults (Figure 1*A*). Kp carriage rates were generally stable over the time points within the study population, except among children younger than age 5 years, where it varied from 75% (21/28) at day 0% to 32% (9/28) at day 60 (Supplementary Figure 1). We did not identify a sex-based difference in carriage rates (19/29 males [66%] and 32/47 females [68%]). The Kp detection rate was higher using qPCR assay than by culture; average carriage rates by qPCR were 75% (21/28) in children younger than age 5 years, 81% (18/22) in children aged 5–17 years, and 99% (25.75/26) in adults (Figure 1*A*). When we evaluated relative abundance with qPCR, adults had a significantly higher relative abundance of Kp compared to children younger than age 5 years ($P = .03$) (Figure 1*B*).

Prevalence of Kp in Standing Water and Household tap Samples and Relationship to Kp in Study Participants

We collected 60 standing water samples from the same urban community where our study population resided. The samples were collected at one of the 3 follow-up time points: 33 samples were from the household taps of study participants, and 27 samples were from standing water (ponds, lakes, and bogs) near the study participants' households. Using culture-based isolation with PCR confirmation (using *fiu* primers), we identified Kp in 85% (23/27) of standing water samples and 64% (21/33) of household tap samples (*P*-value not significant by Fisher exact test). Kp abundance in standing water was significantly higher (10^3–10^4 CFU/mL) than in household water (10^1–10^3 CFU/mL), *P*-value <.0001 by *t*-test. (Figure 2).

We assessed the relationship between the presence of Kp in household tap samples and the relative abundance of Kp in stool samples from study participants. For this analysis, we focused on 2 groups of participants that had presence (n = 29) or

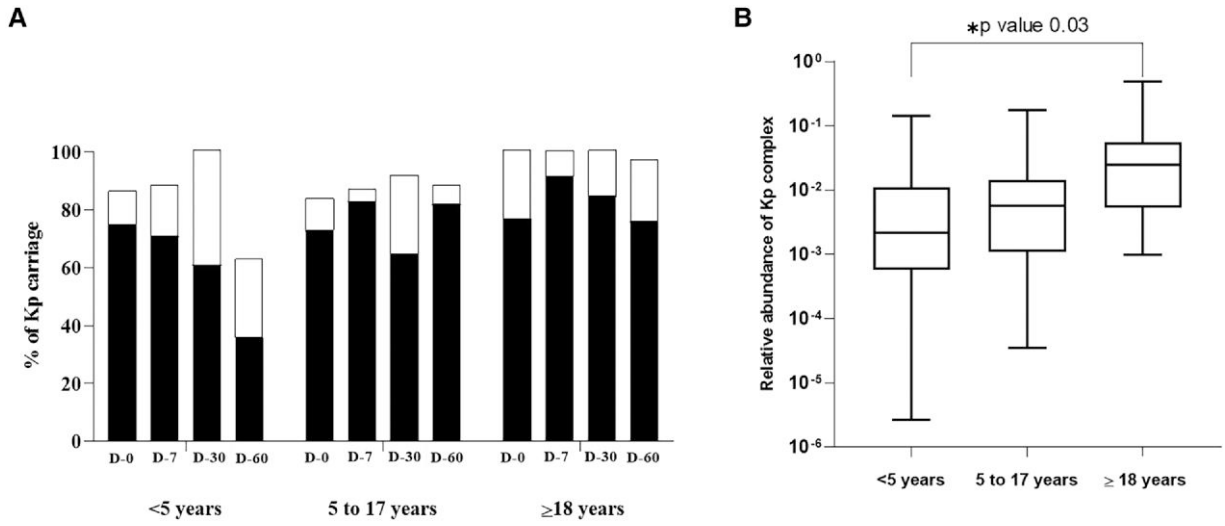

Figure 1. *A*, Prevalence of intestinal Kp colonization in 3 age groups (<5 y, 5–17 y, ≥18 y) using culture-based methods (black) and qPCR assay (combined black and white). No significant differences in Kp colonization were observed between time points. *B*, Kp relative abundance by Kp qPCR in 3 age groups, with significance (*$P = .03$ by *t*-test) in the comparison between <5 years versus ≥18 years.

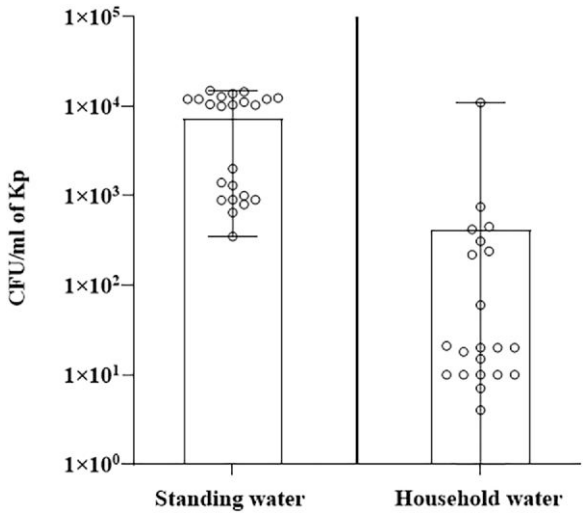

Figure 2. Comparative colony forming units (CFU)/mL of Kp in standing water samples (n = 23/27) and household water samples (n = 21/33).

absence (n = 20) of Kp in household tap water specimens that were collected at the same time point as the stool sample. The median relative abundance of Kp in stool by qPCR was higher (0.0005%) in the group with Kp-positive household water compared to the group with Kp-negative household water (0.0001%), but these differences were not significant by the Mann-Whitney test (Supplementary Figure 2).

Antibiotic Resistance in Human Stool and Water Kp Isolates

We selected the first Kp colony isolated from study participants (n = 69) and assessed their susceptibility to 12 commonly used antibiotics. Of the corresponding 69 isolates, 6/69 (9%) were multidrug resistant (MDR). MDR was defined as resistance to at least 1 agent in 3 or more antimicrobial categories except ampicillin. We identified 19/69 (28%) as resistant to fluoroquinolones (ciprofloxacin or levofloxacin), 8/69 (12%) as resistant to 1 or more carbapenem drugs (imipenem or ertapenem), 5/69 (7%) as ESBL producers, and 4/69 (6%) as resistant to third-/fourth-generation cephalosporins (ceftriaxone, ceftazidime, or cefepime). Additionally, we observed several isolates that were susceptible in a dose-dependent manner (SDD) according to the CLSI definition. In this study, 10% (7/69) SDD was observed for cefepime (7/69, 10%) and 54% (37/69) for piperacillin/tazobactam (Supplementary Table 2). Rates of antimicrobial resistance were similar between different age groups (Supplementary Table 3).

We also performed antibiotic susceptibility testing on the 44 Kp isolates from water samples. Among these, 17% (4/23) of standing water isolates and 19% (4/21) of household water isolates were MDR. We identified 17% (4/23) of Kp isolates from standing water samples and 14% (3/21) of Kp isolates from household water samples as ESBL producers. Moreover, 1/21 (5%) of household water isolates were resistant to 1 or more carbapenems, whereas 5/21 (24%) were resistant to fluoroquinolones and 5/21 (24%) were resistant to third-generation cephalosporins. In standing water isolates, the resistance percentages were even higher: 5/23 (22%) were resistant to 1 or more carbapenems and 7/23 (30%) were resistant to

fluroquinolones. Thirty-six percent (16/44) of all water isolates exhibited SDD to piperacillin/tazobactam (Supplementary Table 2).

DISCUSSION

We found a high level of MDR Kp colonization in the gut of community residents, as well as in standing and household water in a survey of an urban community in Dhaka, Bangladesh. To our knowledge, this is the first report to assess Kp prevalence in this setting. Our findings contribute to a small but growing body of evidence that, in LMICs, AMR Kp is a common community organism and that acquisition is not restricted to nosocomial environments [7, 8]. The commonality of this organism may explain why high rates of life-threatening pediatric infections with Kp are observed in certain settings in LMICs, where children with undernutrition may be more susceptible to Kp as an opportunistic pathogen [9]. Moreover, the high prevalence of SDD isolates poses a significant challenge and could be associated with the therapeutic inefficacy of antibiotics such as cefepime and piperacillin/tazobactam in the treatment of infections.

Notably, adults in our study had a higher Kp relative abundance in their gut than children, possibly because of factors like chronic diseases, antibiotic use, diet, or oral hygiene [10]. Factors such as dietary habits, food sources, and sanitation systems may contribute to the high prevalence of Kp carriage [11].

This study has limitations. Our sampling scheme was restricted to a single urban area and might not be representative of other geographic locations. We were also limited to phenotypic analysis of samples and Kp isolates; comparative genomics of Kp isolates from fecal and water sources would be an important next step to explore the transmission risk of Kp isolates and the presence of virulence and AMR genes. We performed antibiotic susceptibility testing on single colonies of Kp isolated from study participants and water samples; it is possible that this approach might have missed subpopulations of Kp with different antibiotic susceptibility patterns.

Despite the limitations, our study's findings emphasize that Kp is present in both the environment and the human gut in Dhaka and is accompanied by high levels of antibiotic resistance, including to last-resort antibiotics like carbapenems.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential Conflict of interest. J.B.H. and R.C.L. have received royalties from UpToDate. All other authors report no potential conflicts.

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