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Extraintestinal Pathogenic *Escherichia coli* and Antimicrobial Drug Resistance in a Maharashtrian Drinking Water System

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Abstract. Although access to piped drinking water continues to increase globally, information on the prevalence and clonal composition of coliforms found in piped water systems in low-resource settings remains limited. From June to July 2016, we examined *Escherichia coli* isolates in domestic water from the distribution system in Alibag, a small town in India. We analyzed the isolates for drug resistance and genotyped them by multilocus sequence typing. Of 147 water samples, 51 contained coliforms, and 19 (37%) of the 51 were biochemically confirmed to contain *E. coli*. These samples contained 104 *E. coli* isolates—all resistant to ampicillin. Resistance to ceftazidime was observed in 52 (50%) isolates, cefotaxime in 59 (57%), sulfamethoxazole–trimethoprim in 46 (44%), ciprofloxacin in 30 (29%), and gentamicin in two (2%). Thirty-eight (36%) belonged to sequence types recognized as extraintestinal pathogenic *E. coli* (ExPEC); 19 (50%) of these 38 ExPEC belonged to known uropathogenic *E. coli* lineages. This exploratory field research shows the extent to which "improved" drinking water is a potential source of *E. coli* strains capable of causing extraintestinal infections.

The prevalence of bacteria resistant to antimicrobial agents is a serious threat to global public health. Studies have shown that human activity is correlated with increased prevalence of genes conferring resistance to antimicrobial agents in the environment.[1] Specifically, this increase in resistance is correlated with the introduction of antimicrobial agents and bacteria resistant to antimicrobial agents into the environment through activities known to occur in low-resource settings, such as wastewater dumping.[2] When piped drinking water contains agents such as New Delhi metallo-beta-lactamase 1 (NDM-1), even the highest rung of the Joint Monitoring Program's "improved" water ladder is not safe.[3,4] The risks are potentially high in small towns of the Global South, where water treatment and water quality data are both limited.

As *Escherichia coli* is easily eliminated from drinking water, researchers use it as an indicator bacterium to determine whether water has recently been exposed to feces and whether it is safe for consumption. Its presence in more than 5% of drinking water samples indicates that the water treatment (if any) is inadequate to eliminate more harmful bacteria such as *Campylobacter* or *Salmonella*.[5] Detection of *E. coli* can also indicate either treatment inadequacy or posttreatment contamination. When considering an intermittent system, the possibility of posttreatment contamination is high. Few researchers have conducted in-depth microbiological studies of drinking water distribution systems; their focus has largely been on general bacterial community analysis or calculating the number of colony-forming units of *E. coli*.[6–8] The use of *E. coli* solely as a fecal indicator bacterium prevents researchers from understanding the public health impact of its antimicrobial drug resistance and its potential to be a human pathogen.

A subgroup of *E. coli* causes diarrhea and is responsible for foodborne diseases in both high-income and low-income countries.[9] Another group of *E. coli* causes extraintestinal infections, referred to as extraintestinal pathogenic *E. coli* (ExPEC). It is the leading cause of Gram-negative bacteremia and the most common cause of urinary tract infections (UTI), an infection primarily affecting women; both are potentially lethal if left untreated.[10,11] This exploratory study in a "typical" small town in India sought to determine what proportion of *E. coli* strains used as an indicator bacterium in field drinking water tests are drug-resistant, and are potential human pathogens.

Alibag, Maharashtra, is a coastal tourist city with a population of 20,743.[12] Its piped drinking water system is intermittently supplied with water by the Maharashtra Industrial Development Corporation (MIDC). The MIDC drinking water treatment plant sources drinking water from the Amba River and treats the raw water using liquid alum sulfate, flash mixing, flocculation/settling, sand filtration, and chlorination with Cl_2 gas to 0.2 ppm. The treated water is then tested four times a day by an MIDC chemist for multiple contaminants.

Water samples were collected from the water distribution system over an 8-week period from June to July 2016, which evenly captured the end of summer and the onset of the monsoon season. Samples were collected once a week from the treated water at the MIDC and from one of the three elevated storage reservoirs from which water is piped to households. Many households stored water in rooftop tanks connected to the distribution system to cope with its intermittent deliveries. Point-of-use samples were taken from households with in-home taps; for households collecting water from a public tap connected to the distribution system, points-of-collection samples were taken during their scheduled water allocations. Households were sampled such that the service area of the drinking water system was adequately covered.

Water samples for quantification of bacteria were collected and processed with the compartment bag test (CBT) (Aquagenx, Chapel Hill, NC), which uses a β-D-glucuronide *E. coli* indicator.[13] As per the CBT protocol, drinking water was collected in presterilized 100-mL pouches with a sodium thiosulfate tablet to neutralize any residual chlorine—samples

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were processed within 6 hours of collection. Duplicates were not collected because of limited resources in the field.

Compartment bag test samples were incubated at ambient temperature for 48 hours to account for the temperature variance, after which a sterile needle was used to inoculate samples in Luria-Bertani (LB) agar stabs in 1.5-mL tubes (Fisher Scientific, Hampton, NH). Inoculated stabs were incubated at ambient temperature overnight, wrapped in Para Film®, and stored at 4°C until processing after the end of the study period. The samples were transported to the University of California, Berkeley, in August 2016, with permissions from the customs office in Mumbai, Maharashtra, and with a CDC import permit (Permit Number: 2016-05-167).

Bacteria were isolated on MacConkey agar plates. Bacterial stabs were streaked on ampicillin (100 µg/mL)-containing and drug-free plates and incubated overnight. Only ampicillin-resistant colonies were processed for further characterization. Isolates were tested for indole positivity as a biochemical confirmatory test for *E. coli*.

Five-to-ten lactose-fermenting colonies per plate were inoculated into 2 mL LB broth and incubated for 12–15 hours at 37°C. A 1-mL suspension of the culture was pelleted, and DNA was extracted from the pellet following established procedures.[14]

All biochemically confirmed *E. coli* isolates were screened by an enterobacterial repetitive intergenic consensus (ERIC) polymerase chain reaction (PCR) fingerprinting assay.[14] The samples were visualized by gel electrophoresis to compare banding patterns. Strains with different ERIC banding patterns were further analyzed by multilocus sequence typing (MLST). Multilocus sequence typing was performed according to established procedures.[15] All isolates were tested for drug susceptibility by disc diffusion based on the Clinical and Laboratory Standards Institute interpretative criteria.[16] The antimicrobial agents tested included ampicillin, ciprofloxacin, cefotaxime, ceftazidime, gentamicin, trimethoprim–sulfamethoxazole, and imipenem (VWR International, Radnor, PA). Multidrug resistance (MDR) was defined as resistance to three or more of the aforementioned drug classes.

Data were analyzed in Stata 13.[17] Our null hypothesis was that the frequency of resistance to two or more drugs is proportionally distributed across all sequence types (STs). All recovered *E. coli* isolates were resistant to ampicillin. We used a Pearson $\chi 2$ test for categorical variables to compare the frequency of resistance to only ampicillin (AmpR) versus resistance to two or more drugs. Institutional review board approval was granted by the Office for Protection of Human Subjects at the University of California at Berkeley (Protocol ID 2016-04-8702).

Of the 51 water samples that tested positive for *E. coli* by the CBT (Aquagenx), 38 (75%) showed growth on MacConkey agar plates. Indole testing biochemically confirmed 19 samples to contain *E. coli*, and 104 *E. coli* isolates were analyzed from these samples.

Table 1 compares the ST and ST complexes (STCplx) with the resistance profiles of the isolates. Resistance to two or more drugs was observed in 27 isolates and MDR was found in 28 isolates. Two were resistant to gentamicin and none were resistant to imipenem. Fifty-nine (58%) and 52 (50%) isolates were resistant to cefotaxime and ceftazidime, respectively. Co-resistance to ceftazidime and cefotaxime was found in 52 (50%) isolates, which would be considered to be possible extended-spectrum beta-lactamase (ESBL) producers. Thirty-six (35%) isolates were resistant to trimethoprim–sulfamethoxazole, and 17 (16%) were resistant to ciprofloxacin.

Of the 104 *E. coli* isolates, 71 (68%) belonged to 17 unique STs or STCplx. Thirty-three (32%) of the genotyped isolates were not included in the Enterobase database (http://enterobase.warwick.ac.uk). Sequence type complex 155 represented 17 (16%) of the genotyped isolates and contained three different STs (ST58, ST155, and ST616). Table 2 shows that the most prevalent STs were ST58, ST224, ST155, ST1588, STc165, and ST1519.

Escherichia coli strains that cause diarrhea belong to intestinal pathogenic *E. coli* (IPEC), and those that cause infection outside of the intestinal tract are referred to as ExPEC. Several classes of IPEC are recognized in Table 2. Of the 17 recognized STs and STCplx, five have been reported to cause

TABLE 1
Distribution of *Escherichia coli* genotypes by multilocus sequence type and resistance to antimicrobial agents

ST (STCplx)	No.	No. AmpR only† (%)	No. resistant‡ (%)	No. MDR§ (%)	*P*-value‖
ST58 (ST155 Cplx)	9	0 (0)	9 (100)	0 (0)	1.0
ST155 (ST155 Cplx)	7	1 (14)	1 (14)	5 (71)	0.008**
ST616 (ST155 Cplx)	1	1 (100)	0 (0)	0 (0)	–
(ST648 Cplx)	1	0 (0)	1 (100)	0 (0)	–
(ST165 Cplx)	6	0 (0)	0 (0)	6 (100)	1.0
ST349 (ST349 Cplx)	1	0 (0)	0 (0)	1 (100)	–
ST224	8	6 (75)	0 (0)	2 (25)	1.0
ST181 (ST168 Cplx)	1	0 (0)	1 (100)	0 (0)	–
ST196	1	0 (0)	1 (100)	0 (0)	–
ST1720	5	3 (60)	2 (40)	0 (0)	0.025*
ST1519	6	0 (0)	4 (66)	2 (33)	1.0
ST1163	3	2 (66)	1 (33)	0 (0)	0.083
ST1588	7	1 (14)	5 (71)	1 (14)	0.008**
ST1598	4	0 (0)	1 (25)	3 (75)	1.0
ST3541	2	0 (0)	0 (0)	2 (100)	1.0
ST92	4	2 (50)	1 (25)	1 (25)	0.0046**
(ST23 Cplx)	5	0 (0)	0 (0)	5 (100)	1.0

ST = sequence type; STCplx = ST complexes.
* Significance at 0.05; ** Significance at 0.01.
† Resistant to ampicillin (baseline).
‡ Resistant to two classes of antimicrobial agents.
§ Resistant to three to four classes of antimicrobial agents (multidrug resistance [MDR]).
‖ *P*-value compares ampicillin resistance (AmpR only, baseline) and any co-resistance.

TABLE 2
Distribution of multilocus sequence typing isolates by reported* pathogenic *Escherichia coli* classes

ST (STCplx)	No.	*E. coli* class(es)
ST58 (ST155 Cplx)	9	EIEC, EAEC, ExPEC
ST224	8	ExPEC
ST155 (ST155 Cplx)	7	EAEC
ST1588	7	ND
ST1519	6	ND
(ST165 Cplx)	6	EAEC, EPEC
(ST23 Cplx)	5	EHEC, ETEC, EAEC, ExPEC
ST1720	5	ND
ST92	4	ExPEC
ST1598	4	EAEC
ST1163	3	ND
ST3541	2	ND
ST 616 (ST155 Cplx)	1	ND
(ST648 Cplx)	1	EAEC, EPEC, ExPEC
ST349 (ST349 Cplx)	1	EAEC, EPEC
ST181 (ST168 Cplx)	1	ND
ST196	1	ND

EAEC = enteroaggregative *E. coli*; EHEC = enterohemorrhagic *E. coli*; EIEC = enteroinvasive *E. coli*; EPEC = enteropathogenic *E. coli*; ETEC = enterotoxigenic *E. coli*; ExPEC = extraintestinal pathogenic *E. coli*; ND = no class determined; ST = sequence type; STCplx = ST complexes.
* As reported by the Enterobase Database (http://enterobase.warwick.ac.uk).

extraintestinal infections such as bloodstream infections and four were reported to cause intestinal infections in the Enterobase database; four (ST648, ST92, STc23, and ST58) belonged to STs reported to cause UTI. The remaining eight (19%) were not reported as human pathogens or animal pathogens in the Enterobase database. Of the 19 isolates identified by the ST database to cause UTI, two (10%) were solely ampicillin resistant, 11 (58%) were resistant to two classes of antimicrobial agents, and six (32%) were MDR (Table 2). Five of these were potential ESBL-producing strains (ST58, ST155, ST349, ST1598, and ST168 complex). Overall, of the 104 genotyped *E. coli* isolates recovered from 19 drinking water samples, 45 (43%) belonged to STs archived as either ExPEC or IPEC strains. The presence of extraintestinal pathogenic strains indicates that *E. coli* in drinking water should be investigated beyond its role as an indicator of fecal contamination.

The primary limitation of this exploratory study was the small sample size. In addition, 32% of the MLST profiles in our samples were not archived in the Enterobase database, further restricting the sample size. Furthermore, this study did not include any human subjects who may have developed extraintestinal or intestinal infections with the *E. coli* strains found to belong to ExPEC or IPEC STs. Future work should use clinical case data to link *E. coli* found in drinking water samples to cases of *E. coli* infections, such as UTIs. Finally, this study was performed during only two seasons, late summer and early monsoon; levels of contamination by *E. coli* may vary during other seasons.

Although there are many research studies focused on drinking water quality, the majority use tests that only indicate the presence or absence of *E. coli*. Using *E. coli* only as an indicator precludes the opportunity to identify potentially pathogenic *E. coli* strains. This study characterized the *E. coli* genotypic community and their antimicrobial drug resistance in the drinking water system in Alibag, India, to better understand and inform further research on potential exposure risks in low-resource small-town settings. Alibag has a fairly advanced drinking water treatment scheme. However, Alibag's poor sanitation likely has negative impacts on the drinking water quality, negating the benefit of their MIDC. Looking more broadly to the sustainable development goals, this work shows how drinking water and sanitation are truly interlinked, wherein there can be no safely managed drinking water without safely managed sanitation.

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