Long-term Comparison of Antibiotic Resistance in *Vibrio cholerae* O1 and *Shigella* Species Between Urban and Rural Bangladesh

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From 2000 to 2012, *Vibrio cholerae* O1 and *Shigella* species isolates from urban Dhaka and rural Matlab were tested for resistance to all clinically relevant antibiotics in Bangladesh. Resistances in urban and rural Bangladesh tended to rise and fall together, especially a few years after the introduction of new resistance.

Keywords. urban vs rural; antibiotic resistance; Bangladesh; *Vibrio cholerae*; *Shigella*.

Diarrheal disease is among the leading causes of morbidity and mortality in developing nations [1]. In Bangladesh, the most common bacterial causes of diarrheal disease are *Vibrio cholerae* O1 and *Shigella* species [2]. Left untreated, both pathogens are capable of producing life-threatening illness [3, 4]. However, aggressive rehydration combined with antibiotic use significantly reduces duration of illness and mortality rates [3, 4]. Because of the importance of antibiotics, it is imperative to monitor changing resistance patterns to effectively treat these illnesses. Whereas most academic medical centers in Dhaka are likely to follow similar antibiotic selection protocols, this may not be the case in rural areas where antibiotic resistance is less closely monitored. To address this concern, this study assessed antibiotic resistance trends in isolates of *V. cholerae* O1 and *Shigella* species recovered from patients over a 13-year period from urban Dhaka and rural Matlab.

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METHODS

Surveillance Sites

For the study period, 2000 through 2012, we reviewed surveillance records for patients diagnosed with laboratory-confirmed *V. cholerae* O1 or *Shigella* species infection at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b). Patients enrolled in the surveillance program were evaluated at 2 hospitals. One hospital, located in urban Dhaka, provides treatment to 140 000 diarrheal patients annually. The other hospital, located in rural Matlab, a 5-hour, 55-km drive to the southeast, treats 12 000–15 000 patients annually. In 1979, icddr,b began assessing the microbiologic etiology of diarrhea in every 25th patient as part of a program called the Diarrhoeal Disease Surveillance System (DDSS), a program authorized by the icddr,b Ethical Review Committee. In 1996, the proportion of patients enrolled in DDSS was reduced to every 50th individual to account for rising patient volumes. For each patient enrolled in the surveillance system, sociodemographic and clinical features of illness were collected using a standard questionnaire.

Microbiologic Evaluation

For each patient enrolled in DDSS during the study period, standard techniques were used to test stool specimens or rectal swabs for the presence of bacteria, viruses, and parasites known to cause diarrhea [2]. *Vibrio cholerae* O1 and *Shigella* species were tested for antibiotic resistance using the disc diffusion method following Clinical and Laboratory Standards Institute guidelines as previously described [2]. If anomalous results occurred, they were double-checked using standard Epsilometer test protocol.

Data Analysis

Data were analyzed using SPSS version 20.1 (IBM SPSS, Chicago, Illinois) and Microsoft Excel version 12.2.6 software. Differences in antibiotic resistance between Dhaka and Matlab for each year were determined using χ^2 tests, with $P \leq .05$ considered to confer statistical significance. Each *Shigella* species and *V. cholerae* O1 serotype was assessed separately for differences in trends, including the origin of resistance; because no

meaningful differences were found, they were grouped together for statistical analysis. A resistant isolate was defined as any isolate that displayed "resistance" or "intermediate resistance" using the disc diffusion method.

RESULTS

From 2000 to 2012, *V. cholerae* O1 was isolated from 5885 patients in Dhaka, and 1687 patients in Matlab, with the Ogawa serotype accounting for approximately two-thirds of the cases at each location. *Shigella* species were isolated from 1228 patients in Dhaka and 1727 patients in Matlab, with *Shigella flexneri* accounting for 55% and 75% of cases in Dhaka and Matlab, respectively. *Shigella boydii*, *Shigella sonnei*, and *Shigella dysenteriae* accounted for the remaining cases, with similar proportions at Dhaka and Matlab. *Shigella dysenteriae* accounted for the fewest cases (7%–9%), all of which were *S. dysenteriae* type 2.

Over the last 4 decades, a small number of antibiotics has been used in the treatment of shigellosis and cholera (Figure 1*A*). As resistance developed among these drugs, clinicians were forced to adopt alternative antibiotics, sometimes rather abruptly [5].

Shigella

Over the study period, ampicillin and co-trimoxazole displayed a high and steady rate of resistance at both Dhaka and Matlab (Figure 1*B*). At the same time, resistance to naladixic acid gradually increased at both sites. Because of these trends, mecillinam and ciprofloxacin were the main antibiotics used in this study period. However, as can be seen, resistance to each of these antibiotics increased notably, beginning in 2006–2007. *Shigella* was highly susceptible to ceftriaxone (>98%), which was introduced during the latter part of the study period as a result of growing resistance to mecillinam and ciprofloxacin (data not shown). Three important subtrends should be noted. First, resistance to ciprofloxacin and naladixic acid grew at a similar and gradual rate at Dhaka and Matlab. Second, the rise in resistance to mecillinam occurred simultaneously at both sites, but was more pronounced at Dhaka before falling to rates similar to those seen at Matlab. Third, as demonstrated with mecillinam from 2009 to 2012 and ampicillin from 2002 to 2005, levels of resistance dropped gradually but significantly ($P < .001$) at the site with the higher resistance.

Vibrio cholerae

After several years in which *V. cholerae* was uniformly susceptible to tetracycline, resistance levels abruptly increased in 2004–2005 (Figure 1*C*). In the years that followed this increase, resistance levels fluctuated dramatically in similar fashion at both study sites. Erythromycin resistance arose at the same time as tetracycline resistance, but exhibited early and large, albeit temporary, differences between study sites. *V. cholerae* has shown high susceptibility (>99%) to ciprofloxacin and azithromycin, which were introduced as a result of the abrupt rise in tetracycline resistance (data not shown). Finally, icddr,b stopped consistently testing for resistance to furazolidone due to high resistance levels (data not shown).

DISCUSSION

For both *Shigella* and *V. cholerae*, growing resistance caused clinicians to adopt new antibiotics in both Dhaka and Matlab to replace others that had lost effectiveness. In the case of *Shigella*, mecillinam and ciprofloxacin replaced ampicillin, co-trimoxazole, and naladixic acid. For the quinolones, resistance grew gradually, steadily, and uniformly at Dhaka and Matlab. In contrast, mecillinam resistance developed faster at Dhaka than Matlab, but this divergence was short-lived. The reasons for these discrepant patterns of resistance are unclear. It is unusual for *Shigella* to regain susceptibility to antibiotics following the development of resistance [9]. However, we observed this phenomenon with both mecillinam and ampicillin across all *Shigella* species during the study period. Although these declines were statistically significant, *Shigella* never reverted to full susceptibility to these agents, making the declines less significant clinically. The persistence of resistance to nalidixic acid, co-trimoxazole, and ampicillin during the study period may have resulted from the lingering use of these older, less expensive antibiotics in agriculture and over-the-counter self-treatment. In addition, *Shigella* species can acquire resistance plasmids from other Enterobacteriaceae such as *Escherichia coli* [9].

In contrast to *Shigella*, resistance patterns in *V. cholerae* are known to fluctuate rapidly [9]. This occurs because *V. cholerae* cannot stably carry plasmids that confer resistance, and because the organism naturally resides in aquatic environments devoid of selective pressure from antibiotics [6, 9]. In this study and an earlier one [5], this phenomenon was prominently illustrated with tetracycline resistance, which oscillated substantially after a rapid rise in 2004–2005. Although this phenomenon was also seen with erythromycin resistance at Matlab, it was contrasted by a sharp and permanent rise in resistance at Dhaka.

With regard to differences in resistance between urban and rural areas, the findings of our study are consistent with earlier findings that statistically significant differences can occur [6]. However, our study shows that differences are unlikely to be clinically relevant, and over time, appear to regress. The ramifications of these findings are that physicians in rural Bangladesh are encouraged to follow treatment protocols established in urban Bangladesh unless presented with evidence to the contrary. Ongoing surveillance throughout the country is important to detect clinically meaningful differences in resistance should they appear.

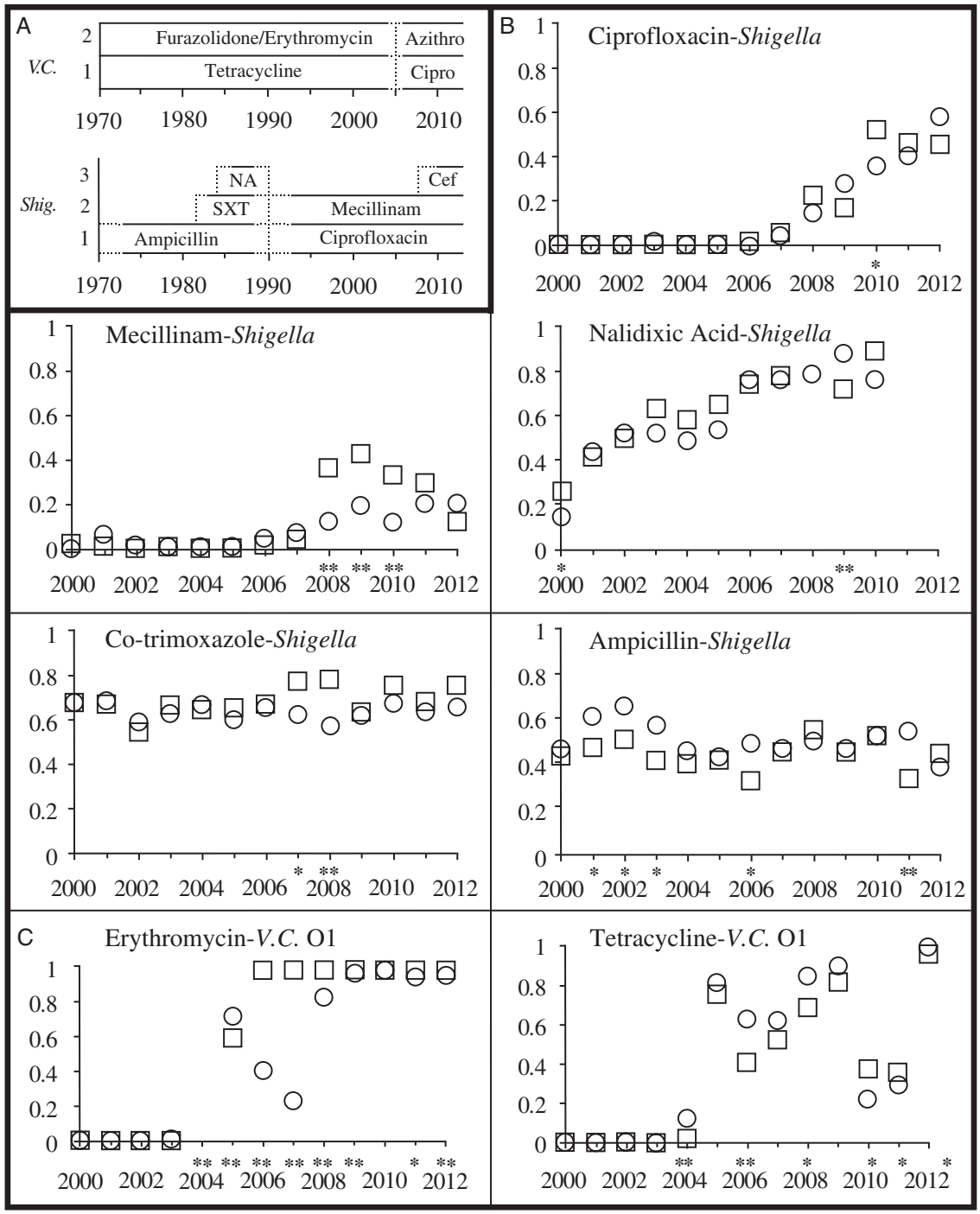

Figure 1. *A*, Approximate timeline for antibiotics used to treat cholera and shigellosis, Bangladesh, 1970–2013 [5–8]. Y-axis shows antibiotics. *B*, Antibiotic resistance, *Shigella* species, Bangladesh, 2000–2012. Y-axis shows fraction of isolates resistant; ○, Matlab; □, Dhaka. $^{*}P \leq .05$, $^{**}P \leq .01$ by χ^2 test. *C*, Antibiotic resistance, *Vibrio cholerae* O1, Bangladesh, 2000–2012. Y-axis shows fraction of isolates resistant; ○, Matlab; □, Dhaka. $^{*}P \leq .05$, $^{**}P \leq .01$ by χ^2 test. Abbreviations: Azithro, azithromycin; Cef, ceftriaxone; Cipro, ciprofloxacin; NA, naladixic acid; *Shig.*, *Shigella*; SXT, co-trimoxazole; *V.C.*, *Vibrio cholerae*.

This study has several limitations. First, it considered diarrheal disease caused by only 2 pathogens, *V. cholerae* O1 and *Shigella* species in Bangladesh. The same conclusions may not hold for other bacteria and/or countries. Second, Dhaka and Matlab were used as proxies for urban and rural populations, respectively, and may not be representative of all urban and rural areas nationally. These limitations call for further efforts to compare antibiotic resistance in urban and rural areas. Although it is impractical to

monitor antibiotic resistance in all of rural Bangladesh, the fact that roughly 70% of the population resides in rural areas makes it important to determine when and where rural populations can follow treatment guidelines established at urban research centers.

Notes

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