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# Antibiotic Resistance in Diarrheagenic *Escherichia coli* and *Shigella* Strains Isolated from Children in Hanoi, Vietnam

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**The MICs for 162 diarrheagenic *Escherichia coli* strains and 28 *Shigella* strains were determined on the basis of NCCLS guidelines. More than 75% of the strains were resistant to ampicillin, chloramphenicol (53.6% of *Shigella* strains), and trimethoprim-sulfamethoxazole. Multiresistance was detected in 89.5% of *E. coli* strains and 78.6% of *Shigella* strains.**

Antibiotics have revolutionized the treatment of common bacterial infections and play a crucial role in reducing mortality. Antimicrobial therapy should be used in severe cases of diarrheal disease to reduce the duration of illness and may be used to prevent traveler's diarrhea (12). However, the progressive increase in antibiotic resistance among enteric pathogens in developing countries is becoming a critical area of concern. In addition, the overuse and misuse of antibiotics in the treatment of diarrhea could lead to an increase of antibiotic resistance.

In 1986, an economic reform, known as Doi Moi, was launched in Vietnam, introducing a market economy with privatization in all sectors, including drug provision (5). People could easily buy antibiotics without a doctor's prescription for treating themselves. One study performed in 1999 in Hanoi showed that 90% of drug dispensing was without a prescription and 94.9% of customers decided by themselves which drugs to buy (6). As a result, many children with symptoms of illnesses (respiratory infections, diarrhea, etc.) may have been empirically treated with antibiotics without advice from medical staffs (10, 16, 21). According to epidemiological studies in Vietnam, *Escherichia coli* is most commonly isolated in clinical samples from patients with diarrhea and shows a high prevalence of resistance to antibiotics (2, 9). Knowledge of local antimicrobial therapy is important in selecting the appropriate therapy. Moreover, there are few recently reported studies about antimicrobial resistance among diarrheagenic *E. coli* and *Shigella* strains in children in Vietnam (2, 9).

In this study, the susceptibilities of 28 *Shigella* strains and 162 diarrheagenic *E. coli* strains to different antibiotics were evaluated. Specifically, 28 *Shigella* strains, including 1 *Shigella boydii* strain, 7 *Shigella flexneri* strains, 20 *Shigella sonnei* strains, and 162 diarrheagenic *E. coli* strains belonging to 86 enteroaggregative *E. coli* (EAEC) strains, 12 enteroinvasive *E. coli* (EIEC) strains, 50 enteropathogenic *E. coli* (EPEC) strains, and 14 enterotoxigenic *E. coli* (ETEC) strains were examined. *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 29213) were used for quality control of antibiotic susceptibility testing.

The following antibiotics were used for susceptibility testing: ampicillin (AMP; AstraZeneca), chloramphenicol (CHL; Sigma), trimethoprim-sulfamethoxazole (SXT; Sigma), imipenem (IPM; Merck Shark & Dohme B.V.), cefuroxime (CXM; Sigma), cefotaxime (CTX; Sigma), nalidixic acid (NAL; Sigma), and ciprofloxacin (CIP; Bayer AG).

MICs were determined by the agar dilution method following the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (14, 15). Data from antibiotic susceptibility testing were analyzed using WHONET 5.1 software. The data were analyzed using Kruskal-Wallis H test for multiple comparison and Mann-Whitney U test for comparing two groups of *E. coli* in terms of antibiotic resistance to each antibiotic. A $P$ value of $<0.05$ was considered significant.

The project was approved by the Ethical Committees of both the Karolinska Institutet, Stockholm, Sweden, and Hanoi Medical University, Hanoi, Vietnam.

The antibiotic susceptibility testing data are shown in Table 1. Of the 162 diarrheagenic *E. coli* isolates, 86.4% were resistant to AMP, 77.2% were resistant to CHL, 29.6% were resistant to CXM, 24.1% were resistant to CTX, 19.1% were resistant to NAL, 3.7% were resistant to CIP, 88.3% were resistant to SXT, and all were sensitive to IPM. Of the few *E. coli* strains resistant to CIP, five strains were EPEC and one strain was an ETEC. The traditional antibiotics, including AMP, CHL, and SXT, showed low activity against the diarrheagenic *E. coli* strains (MIC at which 90% of the isolates tested are inhibited [$MIC_{90}$] of 1,024 mg/liter for AMP and CHL; $MIC_{90}$ of 4/76 mg/liter for SXT). CXM, CTX, and NAL showed moderate activity. However, $MIC_{90}$s of CTX and CXM were rather high at >1,024 mg/liter. They were still more active than the traditional antibiotics but less active than NAL with a $MIC_{90}$ of 128 mg/liter.

The differences in the distributions of resistance and MICs were seen for individual antibiotics and each category of *E. coli*. The multiple comparison showed a significant difference in resistance to CHL and CIP ($P = 0.004$ and 0.021, respectively). For SXT, the $P$ value was borderline significant ($P = 0.062$). When the comparisons of antibiotic resistance of two groups of *E. coli* were performed, EAEC were significantly

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TABLE 1. Antibiotic susceptibilities of diarrheagenic *E. coli* and *Shigella* strains

| Organism and agent | No. of isolates | % not susceptible | MIC | | |
|---|---|---|---|---|---|
| | | | $MIC_{50}$ | $MIC_{90}$ | Range |
| Diarrheagenic *E. coli*[a] | 162 | | | | |
| AMP | | 86.4 | >1,024 | >1,024 | 2–>1,024 |
| CHL | | 77.2 | 256 | 1,024 | 2–1,024 |
| CXM | | 29.6 | 4 | >1,024 | 0.032–>1,024 |
| CTX | | 24.1 | 0.064 | >1,024 | 0.032–>1,024 |
| NAL | | 19.1 | 2 | 128 | 1–>1,024 |
| CIP | | 3.7 | 0.016 | 0.25 | 0.008–128 |
| IPM | | 0 | 0.125 | 0.125 | 0.064–1 |
| SXT | | 88.3 | >4/76 | >4/76 | 0.032–>4/76 |
| EAEC | 86 | | | | |
| AMP | | 90.7 | >1,024 | >1,024 | 2–1,024 |
| CHL | | 87.2 | 512 | 1,024 | 2–1,024 |
| CXM | | 36 | 8 | >1,024 | 0.032–1,024 |
| CTX | | 26.7 | 0.064 | >1,024 | 0.032–1,024 |
| NAL | | 18.6 | 2 | 64 | 1–1,024 |
| CIP | | 0 | 0.016 | 0.125 | 0.008–1 |
| IPM | | 0 | 0.125 | 0.125 | 0.064–1 |
| SXT | | 91.9 | >4/76 | >4/76 | 0.032–16 |
| EIEC | 12 | | | | |
| AMP | | 91.7 | 1,024 | >1,024 | 8–1,024 |
| CHL | | 83.3 | 256 | 512 | 2–1,024 |
| CXM | | 16.7 | 4 | >1,024 | 0.064–1,024 |
| CTX | | 25 | 0.064 | >1,024 | 0.032–1,024 |
| NAL | | 8.3 | 2 | 4 | 1–64 |
| CIP | | 0 | 0.016 | 0.032 | 0.016–0.064 |
| IPM | | 0 | 0.125 | 0.125 | 0.064–0.125 |
| SXT | | 100 | >4/76 | >4/76 | 8–16 |
| EPEC | 50 | | | | |
| AMP | | 82 | 1,024 | >1,024 | 2–1,024 |
| CHL | | 64 | 256 | 1,024 | 4–1,024 |
| CXM | | 24 | 8 | 1,024 | 0.064–1,024 |
| CTX | | 20 | 0.064 | 64 | 0.032–1,024 |
| NAL | | 24 | 2 | 128 | 2–1,024 |
| CIP | | 10 | 0.016 | 1 | 0.016–128 |
| IPM | | 0 | 0.125 | 0.25 | 0.064–1 |
| SXT | | 84 | >4/76 | >4/76 | 0.032–16 |
| ETEC | 14 | | | | |
| AMP | | 71.4 | 512 | >1,024 | 2–1,024 |
| CHL | | 57.1 | 128 | 1,024 | 4–1,024 |
| CXM | | 21.4 | 4 | 1,024 | 0.064–1,024 |
| CTX | | 21.4 | 0.032 | 128 | 0.032–128 |
| NAL | | 14.3 | 2 | >1,024 | 2–1,024 |
| CIP | | 7.1 | 0.016 | 1 | 0.008–32 |
| IPM | | 0 | 0.125 | 0.125 | 0.064–0.25 |
| SXT | | 71.4 | >4/76 | >4/76 | 0.064–16 |
| *Shigella*[b] | 28 | | | | |
| AMP | | 75 | 256 | 1,024 | 2–1,024 |
| CHL | | 53.6 | 16 | 256 | 2–1,024 |
| CXM | | 14.3 | 2 | 128 | 0.032–1,024 |
| CTX | | 10.7 | 0.064 | 128 | 0.016–256 |
| NAL | | 7.1 | 2 | 8 | 1–32 |
| CIP | | 3.6 | 0.016 | 0.064 | 0.016–2 |
| IPM | | 10.7 | 0.125 | 64 | 0.064–128 |
| SXT | | 89.3 | 16 | 16 | 0.064–16 |
| *S. sonnei* | 20 | | | | |
| AMP | | 70 | 128 | >1,024 | 2–>1,024 |
| CHL | | 40 | 4 | 256 | 2–1,024 |
| CXM | | 15 | 0.064 | 128 | 0.032–1,024 |
| CTX | | 10 | 0.064 | 0.25 | 0.032–128 |
| NAL | | 5 | 2 | 8 | 1–32 |
| CIP | | 0 | 0.016 | 0.032 | 0.016–0.25 |
| IPM | | 10 | 0.125 | 0.25 | 0.064–128 |
| SXT | | 90 | >4/76 | >4/76 | 0.064–>4/76 |
| *S. flexneri* | 7 | | | | |
| AMP | | 85.7 | 512 | >1,024 | 4–>1,024 |
| CHL | | 85.7 | 128 | 256 | 8–128 |
| CXM | | 0 | 0.125 | 2 | 0.064–4 |
| CTX | | 0 | 0.064 | 0.25 | 0.016–0.25 |
| NAL | | 0 | 2 | 2 | 1–2 |
| CIP | | 0 | 0.016 | 0.016 | 0.016–0.016 |
| IPM | | 0 | 0.125 | 0.25 | 0.064–0.25 |
| SXT | | 85.7 | >4/76 | >4/76 | 1–>4/76 |
| *S. boydii*[c] | 1 | | | | |

[a] Includes EAEC, EIEC, EPEC, and ETEC strains.
[b] Includes *S. sonnei*, *S. flexneri*, and *S. boydii* strains.
[c] One *S. boydii* strain was resistant to all antibiotics tested.

more susceptible to CIP than EPEC and ETEC ($P = 0.003$ and 0.013, respectively). However, EAEC were more resistant to CHL ($P = 0.002$ and 0.006, respectively). This group of *E. coli* also showed higher resistance to AMP and SXT ($P = 0.041$ and 0.024, respectively) compared to ETEC. The other comparisons among *E. coli* types to antibiotics did not appear to be statistically different.

Different resistance patterns were defined in the four categories of diarrheagenic *E. coli*. The most prevalent multiresistance pattern (resistance to at least two antibiotics) was $Amp^r$ $Chl^r$ $Cxm^s$ $Ctx^s$ $Nal^s$ $Cip^s$ $Ipm^s$ $Sxt^r$ in all types of *E. coli*, namely, 34.8, 16.6, 28, and 21.4% for EAEC, EIEC, EPEC, and ETEC, respectively. The $Amp^r$ $Chl^r$ $Cxm^r$ $Ctx^r$ $Nal^s$ $Cip^s$ $Ipm^s$ $Sxt^r$ pattern in EAEC strains and the $Amp^r$ $Chl^s$ $Cxm^s$ $Ctx^s$ $Nal^s$ $Cip^s$ $Ipm^s$ $Sxt^r$ pattern in EPEC strains were the second most prevalent multiresistance patterns with a prevalence of 17.4 and 18% in each category, respectively. Multiantibiotic resistance was detected in 89.5% of all diarrheagenic *E. coli* strains, 91.8% of EAEC strains, 100% of EIEC strains, 86% of EPEC strains, and 78.6% of ETEC strains. There was no significant difference in antibiotic resistance in diarrheagenic *E. coli* strains isolated from children with diarrhea compared to the strains from healthy control children (data not shown).

Twenty-eight *Shigella* strains were isolated from children with diarrhea only. AMP and SXT showed very low activity against *Shigella* strains with $MIC_{90}$s of >1,024 and >4/76 mg/liter, respectively. One strain was susceptible to all tested antibiotics. There were 22 of 28 (78.6%) multiantibiotic-resistant *Shigella* strains. One *S. boydii* strain was resistant to all tested antibiotics. The most common multiresistance pattern was $Amp^r$ $Chl^s$ $Cxm^s$ $Ctx^s$ $Nal^s$ $Cip^s$ $Ipm^s$ $Sxt^r$ with a prevalence of 35%.

Acute or chronic diarrhea caused by the different categories of *E. coli* is an emerging problem in many parts of the world (13). In developing countries, AMP, CHL, and SXT are widely used to treat diarrhea because of their low cost and ready availability. However, some previous studies have shown high prevalences of resistance to these antibiotics in enteric pathogens, especially diarrheagenic *E. coli* (8, 17). In our study, more than 77% of diarrheagenic *E. coli* strains were resistant to the commonly used antibiotics with high $MIC_{90}$s. The percentages of ETEC strains susceptible to AMP, CHL, and SXT were

higher than those of other *E. coli* types. NAL, CIP, and IPM were the most active agents overall. Compared to other *E. coli* types, EAEC seemed highly resistant not only to AMP, CHL, and SXT but also to CXM, CTX, and NAL. According to our study, AMP, CHL, and SXT should not be used for the treatment of diarrhea in this population. Therefore, local information about antibiotic resistance should be used in clinical management, and treatment guidelines should be updated (23).

Although the cephalosporins (CXM and CTX) and IPM are not indicated to treat diarrhea, we have tested the susceptibilities of diarrheagenic *E. coli* strains to these antibiotics, since they could be empirically or incidentally used. According to the national household survey in Hanoi, Vietnam, in 1997 to 1998, on average, about two-thirds of those who are ill treated themselves (4). In our study, the results showed that CXM and CTX had moderate activity against *E. coli* strains. All *E. coli* strains were susceptible to IPM. The fluoroquinolone (CIP) and quinolone (NAL) antibiotics are now commonly used to treat infections, including diarrhea. They have also been recommended for prophylaxis and treatment of traveler's diarrhea. Compared to other studies (7, 22), our study showed a higher prevalence of resistance to NAL and CIP in diarrheagenic *E. coli*. We have isolated five *E. coli* strains (four EPEC strains and one ETEC strain) resistant to both NAL and CIP. If these antibiotics are used widely as the first choice of treatment of diarrhea, especially in developing countries, where the usage of antibiotics is not effectively controlled, a rapid emergence of antibiotic resistance most likely will occur.

Four categories of diarrheagenic *E. coli* had a high prevalence of multiresistance. The multiresistance to AMP, CHL, and SXT was the most prevalent in diarrheagenic *E. coli* strains. Multiresistance has been reported in previous studies (8, 11, 22). Sang et al. (20) described four cases of diarrhea caused by multiantibiotic-resistant EAEC. Moreover, these *E. coli* also had other multiresistance patterns with different prevalences. In particular, there were three EPEC strains and one ETEC strain resistant to all tested antibiotics except IPM. As mentioned, NAL and CIP can be used as alternative antibiotics in case the enteropathogens are resistant to the traditional antibiotics. Our study showed that 30 diarrheagenic *E. coli* strains (18.5%) were resistant to AMP, CHL, SXT, and either NAL or CIP. That means that the patients infected with these *E. coli* strains could risk a treatment failure. It is also indicated that the multiresistance of different categories of diarrheagenic *E. coli* strains is emerging in different developing countries where these antibiotics (both classical and new) have been widely used (7, 22).

Antibiotic treatment is indicated for dysentery caused by *Shigella* species because it can limit both the clinical course of illness and the duration of fecal excretion of the causative organism (18). However, *Shigella* bacteria are becoming increasingly resistant to most antibiotics commonly used in the treatment of diarrhea (8, 19). In the present study, the prevalences of resistance to SXT and AMP were similar to those of other studies in developing countries (1, 3, 8). In contrast, the study of Replogle et al. in United States showed lower prevalence of resistance of *Shigella*, with 59% resistant to SXT and 63% resistant to AMP (18). The lower resistance rates to these two antibiotics have also been reported in the study by Prats et al. in Spain (17). The resistance rates may be lower because of the more appropriate usage of antibiotics in developed countries compared to the developing countries.

Many studies have reported multiresistance in *Shigella* especially to AMP and SXT, which are commonly used to treat shigellosis (1, 11). In our study, 21 of 28 (75%) of *Shigella* strains were resistant to both AMP and SXT. Except for CHL that showed moderate activity with shigella, other antibiotics were still active against this pathogen. However, these antibiotics may appear active in vitro but may not be effective clinically. The wide usage of these antibiotics and the increasing prevalence of resistance are matters of concern to general practitioners and pediatricians. Nearly 60% of *Shigella* isolates in the study of Chu et al. in Hong Kong were $Nal^r$ in the period of 1994 to 1995 (3). In the present study, we found one *S. sonnei* strain that was resistant to AMP, CHL, SXT, and NAL and one *S. boydii* strain that was resistant to all antibiotics tested. The progressive increase in antibiotic resistance among enteric pathogens, particularly in developing countries, is becoming a special concern. Of greatest immediate concern is the need for an effective, inexpensive antimicrobial agent that can be used safely for treatment of children with diarrhea, especially in developing countries, such as Vietnam.

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