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Antibiotic resistance of faecal *Escherichia coli* from healthy volunteers from eight developing countries

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***Objectives*: To determine the prevalence of antibiotic-resistant faecal *Escherichia coli* from adult volunteers from urban (U) areas in Kenya, Mexico, Peru and the Philippines, and non-urban (NU) locations in Curaçao, Mexico, Venezuela, Ghana, Zimbabwe and the Philippines.**

***Methods*: Faecal samples of adult volunteers (n = 1290) were analysed in one laboratory for the presence of antimicrobial-resistant *E. coli* using Eosin Methylene Blue agar plates containing, respectively, ampicillin, oxytetracycline, cefazolin, ciprofloxacin, gentamicin, chloramphenicol and trimethoprim at breakpoint concentrations.**

***Results*: The mean age of the volunteers was ~35 years; most of them were female. Ciprofloxacin resistance was in the range 1%–63%: the highest percentages were found in the urban populations of Asia and South America. In Peru and the Philippines (U and NU), the prevalence of gentamicin resistance was >20%. Cefazolin resistance was the highest in the urban Philippines (25%). Higher prevalences for ampicillin, oxytetracycline and trimethoprim were found for urban areas compared with non-urban ones of Asia, Africa and South America, respectively (P < 0.05).**

***Conclusions*: In the populations studied, antibiotic resistance in faecal *E. coli* from adult volunteers was emerging for cefazolin, gentamicin and ciprofloxacin and was high for the older drugs ampicillin, oxytetracycline, trimethoprim and chloramphenicol.**

Keywords: prevalence of antibiotic resistance, emerging resistance, ciprofloxacin

Introduction

In the last decades, an increase in antibiotic resistance, especially in the developing countries, has been observed. In these countries, the availability of antibiotics over the counter without prescription, the use of subtherapeutic doses that are often of substandard quality[1] and insufficient adherence of the medical profession to an antibiotic policy if available, are among factors that contribute to the emergence and spread of antibiotic-resistant strains.[2,3] Increasing international travel provides great opportunities for both pathogenic and non-pathogenic antibiotic-resistant bacteria to be carried over long distances to other countries, thus further aiding the dissemination of resistant bacteria.[3,4]

The faecal flora of the general population represents a potentially large reservoir of antimicrobial-resistant bacteria at sites where resistance genes can be transferred from the commensal flora to potentially pathogenic microorganisms.[3,4]

In this study, *Escherichia coli*, the predominant Gram-negative, facultative aerobic organism of the faecal flora, was used as indicator organism to determine the prevalence of antibiotic resistance in faecal samples of adult volunteers in eight developing countries in Africa, Asia and South America.

Materials and methods

Sample collection

Faecal samples were randomly collected by medical students of the University of Maastricht in towns and villages of eight developing countries between November 1998–July 2002 during door-to-door visits. Participating countries were for the urban (U) areas Mexico,

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Peru, Kenya and the Philippines, and for the non-urban (NU) regions Ghana, Zimbabwe, Venezuela, Curaçao, Mexico and the Philippines. Classification as urban or non-urban was based on information about the population size of the respective town. The cut-off value for the urban population was ≥150 000 inhabitants. Adult volunteers were asked to participate in the study by providing a fresh faecal sample (one sample per volunteer) and by filling out a questionnaire on age, gender and prior antibiotic use. Criteria for exclusion were hospitalization and presence of gastrointestinal disease in the past month. At least 90 evaluable volunteers were recruited from each sampling site.

Sample processing

On the day of collection, the faecal samples were diluted 1:10 in sterile saline [0.9% (w/v) NaCl] with 20% (v/v) glycerol at the local laboratory and stored at −20°C until transportation to the Medical Microbiology Laboratory of the University Hospital Maastricht, The Netherlands, in dry ice using shipping services for microbiological analysis. Weekly Eosin Methylene Blue (EMB) agar (Oxoid, CM 69, Basingstoke, UK) plates with and without antibiotics were prepared and stored at 4°C until further use within 1 week. Ampicillin- and oxytetracycline-containing agar plates were prepared daily. The antibiotic concentrations were based on the resistance breakpoints of the National Committee for Clinical Laboratory Standards (NCCLS) guidelines and modified to make comparison with previous studies possible.[5] The antibiotics tested and concentrations used were: ampicillin, 25 mg/L; cefazolin, 32 mg/L; ciprofloxacin, 4 mg/L; chloramphenicol, 25 mg/L; gentamicin, 16 mg/L; oxytetracycline, 25 mg/L; and trimethoprim, 8 mg/L. When using trimethoprim, 5% (v/v) lysed horse blood was added to the EMB agar. Faecal samples were 10-fold diluted and 40 μL from each dilution was inoculated on the EMB agar plates with and without antibiotics, using a spiral plater (Eddy Jet; IUL Instruments, Leerdam, The Netherlands). After 18–24 h of incubation at 37°C, *E. coli* appeared as purple colonies with a dark centre and a metallic green sheen. One colony of the dominant flora was randomly picked from each plate for identification using the indole and β-glucuronidase (β-glucuronidase discs; Rosco, Denmark) test. When both tests were positive, the colony was considered to be *E. coli*; if there remained any doubt, further biochemical tests were performed using API 20E strips (bioMérieux, Plainview, NY, USA). The minimum detection level by this method was 300 cfu/g faeces and it has been shown that >95% of the presumptively identified colonies are *E. coli*.[6]

The prevalence of resistance to a certain antibiotic was defined as the proportion of faecal samples showing growth of *E. coli* on agar plates containing that antibiotic compared with the total number of samples tested.

Statistical analysis

Statistical differences in the prevalences of antibiotic resistance between the different populations were determined using the Mann–Whitney *U*-test.

Results

Study populations

In total, 1290 healthy volunteers participated in the study: Venezuela, $n = 230$; Curaçao, $n = 149$; Mexico U, $n = 99$; Mexico NU, $n = 99$; Peru, $n = 95$; Zimbabwe, $n = 207$; Ghana, $n = 100$; Kenya, $n = 100$; the Philippines U, $n = 105$; and the Philippines NU, $n = 106$. The populations studied were derived from four urban (Mexico, Peru, Kenya and the Philippines) and six non-urban regions (Table 1).

The majority of the volunteers were in the range 18–50 years old, and were mostly female (56%–76%), except in Ghana and Kenya (59% and 75% male, respectively). Previous antibiotic use reported by the volunteers was in the range 1%–15%.

Prevalence of resistance

Five percent (70/1290) of all volunteers showed no growth of faecal *E. coli*. Resistance to cefazolin, gentamicin and ciprofloxacin was emerging in most of the populations tested (Table 2). There was considerable variation in the prevalence of ciprofloxacin resistance. It ranged from 2% or lower in the African countries, Curaçao and Venezuela to >30% in Mexico, Peru and the Philippines. Gentamicin resistance was the lowest in

Table 1. General information about the ten populations studied

Country	Town or village	Population[a]	Demographic category	Year of collection	Number of volunteers
South-America					
Venezuela	Pueblo Llano	15 000	NU	1998	230
Netherlands Antilles	Curaçao	130 627	NU	2000	149
Mexico U	Mexico City	8 605 239	U	2001	99
Mexico NU	Puerto Angel	<50 000	NU	2002	99
Peru	San Juan de Miraflores, Lima	329 023	U	2002	95
Sub-Saharan Africa					
Zimbabwe	Musami	<10 000	NU	1999	207
Ghana	Duayaw Nkwanta	<20 000	NU	2001	100
Kenya	Nairobi	2 143 254	U	2001	100
Asia					
The Philippines U	Antipolo	470 000	U	2000	105
The Philippines NU	Sapang Palay	<50 000	NU	2000	106

U, urban; NU, non-urban.
[a]Population size derived from www.citypopulation.de.

Table 2. Prevalence of antibiotic-resistant faecal *E. coli* from healthy volunteers in the 10 populations studied

Antibiotic	conc (mg/L)	Venezuela *n* = 230	Curaçao *n* = 149	Mexico U *n* = 99	Mexico NU *n* = 99	Peru *n* = 95	Zimbabwe *n* = 207	Ghana *n* = 100	Kenya *n* = 100	The Philippines U *n* = 105	The Philippines NU *n* = 106
Ampicillin	25	43	48	78	94	95	49	89	89	87	75
Cefazolin	32	12	7	0	4	13	0	4	2	25	18
Ciprofloxacin	4	1	1	15	51	36	1	8	1	63	35
Chloramphenicol	25	30	8	45	75	52	19	82	45	68	60
Gentamicin	16	3	4	10	14	33	0,5	2	2	46	20
Oxytetracycline	25	52	56	86	97	93	59	90	92	94	85
Trimethoprim	8	33	32	76	96	93	64	89	88	93	86

U, urban; NU, non-urban.

the African countries, Curaçao, Venezuela and both Mexican populations. In Peru and both areas in the Philippines, the percentage was 20% and higher.

The prevalences of resistance for cefazolin and gentamicin, respectively, in the urban areas of Asia and South America were higher compared with the resistance in the non-urban areas. For both continents, ciprofloxacin resistance prevalence was higher in the urban areas compared with the non-urban areas. The prevalences of resistance to ampicillin, oxytetracycline and trimethoprim were higher in the urban areas compared with the non-urban areas from the three continents ($P < 0.05$).

In each population, the highest prevalences of resistance were found for oxytetracycline, ampicillin and trimethoprim. In >30% of the faecal samples, *E. coli* was resistant to chloramphenicol, with the exception of those from Curaçao and Zimbabwe. Samples from the Philippines had the highest prevalence of resistance for three out of seven antibiotics tested.

Discussion

The prevalence of resistance of faecal *E. coli* isolated from 10 populations of adult volunteers living in eight different developing countries was determined in one laboratory using the same method. Ciprofloxacin resistance prevalence showed large variations between the populations and was higher in the urban areas of Asia and South America compared with the non-urban areas, but was similar in urban and non-urban regions in Africa. This high prevalence in Asia and South America might be due to the food supply. In both continents, poultry in particular are intensively raised and fluoroquinolones are commonly used during production; they are mixed through the water supply of the whole flock.[7] Similar to the study of van de Mortel *et al.*,[5] significant differences in the prevalence of resistance for the three continents between non-urban and urban areas for ampicillin, oxytetracycline and trimethoprim were found ($P < 0.01$). These differences in resistance between urban and non-urban areas might be due to the availability of antibiotics,[8] as in cities a large variety of often inexpensive antibiotics are available in pharmacies, over the counter and in market stalls.[3] Furthermore, crowding together, with poor hygiene and poor sanitary facilities for sewage disposal in the cities might encourage the exchange of antibiotic-resistant bacteria in a population.[3,4,9] It is not surprising that the highest prevalences of resistance were found for ampicillin, oxytetracycline and trimethoprim. The combination of resistance to these three antibiotics is very likely because the genes encoding resistance to these antimicrobials are located on the same plasmid.[10]

To our knowledge, the present study was the first in which the prevalence of antibiotic-resistant faecal *E. coli* from adult volunteers from eight developing countries on three continents was determined in one laboratory. Although time bias could not be avoided, the methods used for collecting the faecal samples and determining the prevalence of antibiotic-resistant *E. coli* were similar for the five successive years.

In conclusion, an emerging prevalence of resistance to the newer antibiotics was found among faecal *E. coli* from adult individuals in developing countries. Moreover, the resistance was more common in urban than in rural areas. The resistance prevalences to the older drugs were, as expected, high. Unfortunately, we could not correlate these resistance prevalences to data on antibiotic use due to the lack of official data on the use of antibiotics and over-the-counter sales.

More surveillance studies are necessary both in developed and developing countries to monitor antibiotic use and antibiotic resistance over time. These data are important as a basis for the implementation of an antibiotic policy. In addition, education of healthcare workers, patients and parents is also important, as is control of antibiotic use and resistance percentages in food animals in order to control antibiotic resistance over time.

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