Surveillance of antimicrobial resistance in salmonella, shigella and *Vibrio cholerae* in Latin America and the Caribbean: A collaborative project

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Diarrheal disease is recognized as the most frequent cause of morbidity and mortality in children worldwide (1). Current estimates indicate that at least 3.5 million children under the age of five years die each year due to diarrhea (1). Indeed, the World Health Organization (WHO) and the Pan American Health Organization (PAHO) have identified acute gastroenteritis as a major health problem in all Latin American countries (2). Mortality associated with acute diarrhea is highest among infants younger than one year of age, and death rates average 20/1000 children born (2). The impact of lives lost, together with the high costs to local public health care systems associated with treatment of those afflicted, make prevention and control of diarrheal disease a priority health issue (2). Over the past decade, these concerns have been further reinforced by the emergence of antimicrobial resistance among the major groups of enteric pathogens causing disease.

Antimicrobial resistance is a problem of global concern that affects both developed and developing countries, and has rapidly spread among continents through increased international travel (3). The massive and ever increasing use of antibiotic agents on a worldwide scale in humans, animals and agriculture has created an emerging antibiotic resistance problem that is spreading more rapidly than in previous decades (3). Resistance is promoted in settings in which antibiotic use is frequent and inappropriate, thus, allowing for the selection of resistant strains (4). In addition, there is an in-

creasing incidence of multidrug resistance in enteric pathogens. This has been observed particularly in the foodborne pathogen *Salmonella typhimurium* phage type 104 (DT 104), showing the penta-resistance ACSSuT or resistance to antibiotic representing five common groups of antimicrobial agents (ampicillin, chloramphenicol, streptomycin, sulphadiazine and tetracycline). This may be related to the recent appearance of similar resistance patterns in *Salmonella enteritidis* phage type 4, the most frequently isolated *Salmonella* species in sporadic cases and outbreaks around the world (5,6). *Shigella* strains have shown progressively more resistance to multiple antimicrobial agents, with increased resistance appearing more commonly among strains of *Shigella dysenteriae* (4). Since appearing in Peru in 1991, *Vibrio cholerae* has spread to most countries in Latin America, and is showing increasing resistance to antibiotic therapy used to shorten the duration of diarrhea and reduce fluid loss in patients. Efforts to slow the pace of the emergence and spread of antimicrobial resistance are being addressed through surveillance activities to define the extent of resistance among pathogens in different geographical populations; this surveillance should help adjust treatment strategies, evaluate national drug policies and measure the success of intervention strategies (3). The recent change in antibiotic use policy in Chile from over-the-counter to physician-based prescribing is an example of just such a national response to the antibiotic efficacy crisis and the need to address this major problem of spreading antibiotic

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TABLE 1
Latin American and Caribbean countries participating in the surveillance of antimicrobial resistance project by year of joining

1996	1997	1999
Argentina	Caribbean Epidemiology Centre*	Bolivia
Brazil	Bahamas	Cuba
Chile	Barbados	Ecuador
Columbia	Jamaica	El Salvador
Costa Rica	Trinidad and Tobago	Guatemala
Mexico	St Lucia	Nicaragua
Peru		Paraguay
Venezuela		

*Coordinates the activities of the Caribbean members

resistance. Of key importance is the sharing of information and expertise as well as the creation of partnerships to optimize the use of resources and avoid duplication. These approaches are essential to combat this global threat (3).

In 1996, Health Canada, through its Laboratory Centre for Disease Control (LCDC) and under the direction of the National Laboratory for Enteric Pathogens (NLEP), launched a joint laboratory-based surveillance project in collaboration with the PAHO and participating Latin American countries to evaluate the occurrence and significance of antimicrobial resistance associated with enteric pathogens in the region. The Surveillance of Antimicrobial Resistance Project was developed as part of the global surveillance effort coordinated by the WHO, under its umbrella mandate to control the emergence and spread of antibiotic resistant pathogens, especially those that have multiple drug resistance (7). The major goal of this project was to improve surveillance capabilities by strengthening the laboratory-based capacity of the collaborating Latin American countries. The overall objective was to enable the implementation and development of effective prevention and control programs directed toward emerging antibiotic resistance among the three major groups of enteric pathogens *Salmonella* species, *Shigella* species and *Vibrio cholerae*.

THE PROJECT

Collaboration, cooperation, consensus: these three Cs form the pillars that support and nourish the program, and all its functions. The project is a collaborative one in that the participants work jointly in the direct exchange of intellectual information on the occurrence of enteric disease and the spread of antimicrobial resistance across Latin America and the Caribbean. Indeed, the member countries cooperate with each other for the mutual benefit of all in an effort to curtail the impact of these agents across the region. In the administration of the project, the NLEP in Winnipeg, takes the lead in organizing activities; however, the decision-making processes are performed in a consensus fashion, with input from all participants with agreement, before actions are initiated. The success of the project to date is largely due to the willingness of all groups to participate in the program in this collaborative, cooperative, consensus fashion.

Participants and workshops: Currently, 20 Latin American and Caribbean countries participate in this project (Table 1). Under the coordination of staff from the NLEP (Canada), a workshop was held in November 1996 in Rio de Janeiro, Brazil at the Instituto Oswaldo Cruz-FIOCRUZ. The eight countries participating at the time were Argentina, Brazil, Chile, Colombia, Costa Rica, Mexico, Peru and Venezuela, along with Canada. A second workshop, held in July 1997 at the Caribbean Epidemiology Centre, in Trinidad, coordinated the addition of five Caribbean countries into the network: Bahamas, Barbados, Jamaica, Trinidad and Tobago, and St Lucia. The third workshop was held in October 1999 in Havana, Cuba at the Instituto Pedro Kuri. The seven participating countries were Bolivia, Cuba, Ecuador, El Salvador, Guatemala, Nicaragua and Paraguay.

Project goals: At the consensus meeting, the partnership countries identified five major goals for the project, the objectives of which were the following:

- to strengthen diarrheal disease surveillance across the region;
- to improve laboratory capabilities for serological identification and antimicrobial susceptibility testing in the laboratories of participating countries;
- to standardize laboratory methodologies for testing so that comparable data could be generated across the region;
- to establish a network of national reference laboratories in the individual countries; and
- to create a validated database on emerging antibiotic resistance among *Salmonella* species, *Shigella* species and *Vibrio cholerae* in the Americas.

Laboratory component – Methodology: The workshops held in Brazil, Trinidad and Cuba consisted of formal lectures, open discussion sessions and wet workshops. The laboratory component of each of these three workshops introduced laboratory representatives of each of the participating countries to the standardized methodology that would be used in the program for identifying, serotyping and antimicrobial susceptibility testing of *Salmonella* species, *Shigella* species and *V. cholerae* as part of the surveillance project. The methods, materials and protocols introduced to the participants in the workshop were: protocols for the isolation, identification and serotyping of the enteric pathogens selected; copies of the Kauffman-White official *Salmonella* species typing scheme; the procedures for the National Committee for Clinical Laboratory Standards (NCCLS) Kirby Bauer method for antimicrobial susceptibility testing; antibiotics to be used for testing; protocols for the exchange of strains as part of a proficiency testing and quality control program; methods for data collection and verification; criteria for determining the mechanism of antibiotic resistance; protocols for reporting results and data; and data storage, retrieval and dissemination.

There was consensus agreement that a proficiency panel of coded strains would be distributed by the NLEP on an annual basis to the 20 participating countries. The panel would con-

TABLE 2
Standard set of antibiotics for antimicrobial susceptibility testing for the surveillance of antimicrobial resistance project

<i>Salmonella</i> and <i>Shigella</i> species	<i>Vibrio cholerae</i>
Ampicillin	Ampicillin
Chloramphenicol	Chloramphenicol
Trimethoprim/ sulphamethoxazole	Trimethoprim/ sulphamethoxazole
Ciprofloxacin	Ciprofloxacin
Cefotaxime	Tetracycline
Gentamicin	Erythromycin

sist of five random *Salmonella* serotypes, five *Shigella* species of various serotype and five *V. cholerae* strains. In a like manner, each country would also send the same number of different strains selected from the routine specimens submitted by the regional laboratories in each country according to availability to the NLEP for quality control of their serological identification and antimicrobial susceptibility testing.

The serotyping antisera in use by the NLEP for serological identification was prepared at the NLEP in Winnipeg. Antisera used by the participating countries were prepared in either their respective laboratory production institutes or were purchased as commercial products. *Salmonella* species cultures were serotyped by determining somatic (O) and flagellar (H) antigens (8-10). All serotypes were identified using the Kaufmann-White salmonella serotyping scheme published by Institut Pasteur, Paris, France (10). *Shigella* species and *V. cholerae* cultures were serotyped by determining their respective somatic (O) antigens (8,11). Serotyping was conducted using slide agglutination. Antibiotic susceptibility testing was conducted using National Committee for Clinical Laboratory Standards guidelines (12), while the antibiotics selected as a standard set for use across the region to define the resistance patterns within the various *Salmonella* species, *Shigella* species and *V. cholerae* isolates were determined by consensus among the national groups (Table 2).

The serotyping and antimicrobial susceptibility testing results on the proficiency panel of strains used were sent to the NLEP by each of the participating countries. Subsequently, the NLEP sent out under code, the serological identification and antibiotic profiles on the strains used in the study. Similarly, results were also distributed on the quality control strains sent to the NLEP. Each country was identified by a specific number code for the purposes of reporting the test results and although each was aware of its own code, all other identifiers were held in confidence.

Roles: A key component to the success of the program has been that the roles of all of the participants in this collaborative surveillance project were clearly defined. On joining, each Latin American or Caribbean country agreed to participate in the proficiency testing and quality control program for both serotyping and antibiotic susceptibility testing. Disk diffusion testing using standardized methods for susceptibility testing is performed on isolates received at the national reference centre within each country. Data are collected and sent to the NLEP

TABLE 3
Training workshops and annual meetings for the surveillance of antimicrobial resistance project

Location	Date	Title
Brazil (FioCruz)	November 1996	Identification, serotyping, antimicrobial resistance testing, transportation of dangerous goods and member induction
Venezuela (Caracas)	June 1997	Production of diagnostic reagents to enteric pathogens, absorptions and quality control
Trinidad and Tobago (CAREC)	October 1997	Identification, serotyping, antimicrobial resistance testing, transportation of dangerous goods and member induction.
Mexico (Mexico City)	January 1998	1st Annual Meeting and Country Reports
Canada (Winnipeg)	October 1998	<i>Escherichia coli</i> O157:H7 Workshop
Canada (Winnipeg)	February 1999	2nd Annual Meeting and Country Reports
Cuba (IPK Havana)	October 1999	Identification, serotyping, antimicrobial resistance testing, transportation of dangerous goods and member induction
Chile (Santiago)	February 2000	3rd Annual Meeting and Country Reports

for analysis. Participants attend the various workshops and meetings (Table 3) and agree to assist other centres in countries across the region.

The role of the PAHO is to supply the infrastructure to link participants to each other and to the NLEP. This in turn facilitates activities of the NLEP across the program within each country. The PAHO also supplies partial funding to ensure the functionality of the program.

The Health Canada/NLEP role is to provide proficiency testing and external quality control assurance. Training would be provided through workshops (Table 3). Provision of reference strains and assistance with outbreak investigations is also provided at the request of participating countries. The NLEP co-ordinates the project and provides database management with links to the PAHO. Data from proficiency testing and quality control testing, and data on isolates tested by participating countries are collated by NLEP and presented as a report at annual meetings (Table 3). PAHO and NLEP also conduct on site evaluations of participating countries.

Site visits and annual meetings: The overall goal of this international program is to strengthen the laboratory components of diagnostic and reference microbiology in collaborating countries and to enhance laboratory-based surveillance capabilities for enteric diseases. An integral component of this goal is being achieved by visiting institutions in participating countries to determine the current capabilities of both national and regional laboratories to take part in the surveillance network. The recommended actions, when adopted, contribute to efforts to address the problems of emerging antimicrobial resistance in selected enteric pathogens in each country and so enhance capacity in a broad-based fashion across the region.

To date, sites in five countries have been visited, including reference, regional, district and hospital laboratories within

TABLE 4
Site visits for the surveillance of antimicrobial resistance project*

Date	Country	City	Institutions visited
July 1998	Nicaragua	Managua	National Laboratory of Epidemiology and Hygiene
August 1998	Venezuela	Caracas	Instituto Nacional de Higiene "Rafael Rangel"
October 1998	Costa Rica	San José	Instituto Costarricense de Investigación y Enseñanza En Nutrición y Salud
May 1999	Peru	Lima	Instituto Nacional de Salud
November 1999	Brazil	Rio de Janeiro	LABENT, Instituto Oswaldo Cruz, FIOCRUZ

*In addition to the national facilities indicated, four to five regional and hospital laboratories were evaluated as a component of each site visit

each country. Staff from the NLEP, together with a PAHO representative and a senior laboratorian from a country in the region, but not from the one being visited, went to each country for five days. Countries that have had site visits are Nicaragua; Venezuela, Costa Rica, Peru and Brazil (Table 4). The site visits provided an excellent opportunity to make contact with individuals in participating laboratories and health authority officials, as well as with PAHO personnel in each country. A major objective is to evaluate the capacity to conduct identification, serotyping and surveillance of antimicrobial resistance in enteric pathogens. At the end of each visit, PAHO is provided with a comprehensive set of constructive recommendations for improvements to the laboratories visited and to the national programs described, with the objective of strengthening the project goals.

Since this project was launched in 1996, there have been four annual meetings. The inaugural meeting in 1996 was held in Brazil. The subsequent meetings were held in Mexico in 1998, Canada in 1999 and Chile in 2000 (Table 3). These annual meetings have provided the opportunity for participants in the network to meet, define roles and capabilities, exchange information and develop the consensus approach essential to the program. Problems have been identified and resolved more easily than would otherwise have occurred, and a time line for future activities was established.

Project challenges: A number of key issues identified by all participants are challenges to this project. Among these, the availability of high quality, diagnostic serotyping reagents across the region was identified as the single most important problem facing the group. Commercial reagents were noted as being expensive, and have both limited availability and quality. Additionally, a number of critical serotyping reagents are not available commercially. There is a need for commonly used antisera to be equitably distributed based on a needs assessment. Recommendations were also made that countries within the network that have well developed diagnostic reagent production programs, such as Argentina, Brazil, Mexico and Peru, prepare and distribute these on a cost basis to participating countries. The NLEP would provide technical support through PAHO for both training and quality control of antisera production. The majority of laboratories need good quality *Salmonella* species, *Shigella* species and *V. cholerae* antisera. Accurate biochemical and serological identification of enteric pathogens are key components to meaningful laboratory-based surveillance programs (4-7). The standardization of antimicrobial susceptibility testing methodology for antibiotic

disks and media as well as accurate measurement of zone sizes form some of the critical thrusts of the program. There is a need for standardized procedures to be adopted across laboratories in all participating countries to obtain consistent and accurate data on the emergence of antimicrobial resistance in enteric pathogens within the Americas. These procedures must be conducted according to standard National Committee for Clinical Laboratory Standards guidelines (12). For national and regional laboratories, the establishment of training programs within each country is essential to strengthen individual laboratory capabilities for the identification of enteric pathogens and determination of the criteria for antimicrobial susceptibility testing. As part of the laboratory component of the project, all biochemical and serological identification are to be conducted from a single colony selected using a stereoscopic microscope to ensure that pure cultures are identified. Strains exchanged for quality control are prepared in duplicate sets, with one set sent to the laboratory conducting the investigation and the second set maintained in the original laboratory to allow for strain verification if discrepancies occur. A major challenge posed to all participants has been the transport of strains under the International *Transportation of Dangerous Goods Act* (13); complying with the act ensures that all dangerous goods are properly classified, packaged, labelled, marked and documented before shipping. To meet this challenge, extensive information was supplied at workshops and training with certification has also been provided to the countries independent of this project.

IMPACT ON PUBLIC HEALTH

The success of this surveillance project has been built heavily on a spirit of cooperation, collaboration and consensus by all participants. The targeted agreement from the onset was a requirement to develop strong population-based laboratory surveillance systems to detect the emergence of antimicrobial resistance in *Salmonella* species, *Shigella* species and *V. cholerae*.

In 1992, an expert committee of the Institute of Medicine, Washington, DC, issued a report entitled, *Emerging Infections: Microbial Threats to Health in the United States* (14), that defined emerging infections broadly as "new, reemerging or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future" (14). Emerging antibiotic resistance, especially resistance to multiple antibiotics, is a matter for concern with many microorganisms, especially

enteric pathogens (7). The use of antibiotics in animal feeds for the purposes of growth promotion is widespread in many parts of the world (15). The constant use of antibiotics in antibacterial therapy within hospital and veterinary practices has led to a marked increase in the incidence of antimicrobial drug resistance (15). The unregulated use of antibiotics in many Latin American countries in a nonprescribed, self-administered, over-the-counter purchase mode exacerbates the problem of emerging resistance across the region. All of these practices contribute to the development of antibiotic-resistant strains, which in humans often cause life-threatening infections that no longer respond to antibiotic therapy. This translates into a requirement for a more rational use of antibiotics. In 1999, the European Union banned the use of four antibiotics as animal feed additives as a precautionary measure to minimize the risk of the development of resistant bacteria, and to preserve the efficacy of certain antibiotics currently used in human medicine (16).

Global cooperation is essential for the exchange of information on emerging infections and to develop reliable surveillance systems to detect these infections (17). An important component of the surveillance of antimicrobial resistance project has been to strengthen surveillance capabilities within Latin American countries. Accomplishing this will enable health authorities in partner countries to do the following: provide timely data both nationally and regionally on emerging antimicrobial resistance in *Salmonella* species, *Shigella* species and *V. cholerae*; provide an early warning system to detect, identify and reduce the risk of outbreaks of disease across the region; provide rapid information exchange to target and control outbreaks of enteric disease; develop intervention strategies for containment, prevention and control of enteric pathogens associated with outbreaks of disease in Latin America and the Caribbean; reduce the risk of disease burden, medical costs and economic loss; reduce the spread of multiresistant organisms on a worldwide basis; and provide information to increase public awareness and education of these critical problems.

The PAHO/NLEP/Latin America collaborative surveillance project on antimicrobial resistance is strongly supported as a global initiative by the World Health Organization. The rational use of antibiotics and reliable surveillance systems for detecting the emergence of antibiotic-resistant infections support the development of control programs for the increasing problem of antimicrobial resistance among enteric pathogens.

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