Universidade Federal de São Paulo, Brazil; The Jones Group / JMI Laboratories, North Liberty, IA [www.jmilabs.com]



### **ABSTRACT**

**Background.** Diarrhea may be caused by a wide variety of agents. Usually its treatment does not require antimicrobials. However, in some clinical situations, antimicrobial therapy is required and the knowledge of the likelihood of resistance (R) to antimicrobials is important to clinicians. The objective of this study was to determine the pathogen frequency of occurrence and the antimicrobial susceptibility (S) profile of isolates capable of producing gastroenteritis in Latin American medical centers (LAMC) participating in the SENTRY Antimicrobial Surveillance Program.

Methods. The LAMC were guided by protocol to send the first 25 consecutive gastroenteritis pathogens isolated from stool or blood beginning in January 2001. The S to antimicrobial agents was tested by reference NCCLS methods. Quality control was performed using ATCC strains

Results. A total of 324 isolates, including 200 Shigella spp. (SHIG), 116 Salmonella spp. (SAL), were collected. Nearly 89.0% of the isolates were obtained from stool. The broad-spectrum cephalosporins, carbapenems and fluoroquinolones exhibited the highest S rates (100.0%). Ciprofloxacin (MIC<sub>50</sub>, 0.015 μg/ml) followed by levofloxacin or gatifloxacin (MIC<sub>50</sub>, 0.03 μg/ml) and meropenem (MIC<sub>50</sub>, 0.06 μg/ml) were the most potent compounds against the SAL and SHIG isolates. The S rate to ampicillin varied from 14.6% to 69.0% for SAL and SHIG isolates, respectively. Against SHIG, the lowest S rates were observed for

trimethoprim/sulfamethoxazole (26.5%), ampicillin (30.5%), and tetracycline (53.5%). None of the SAL or SHIG isolates exhibited the ESBL-phenotype according to the NCCLS criteria.

**Conclusions.** The results of this study show that SHIG isolates were more frequently R to antimicrobial agents than the SAL isolates; and that fluoroquinolones could constitute an excellent therapeutic option for treatment of gastroenteritis since quinolone R was not detected.

### INTRODUCTION

Diarrhea may be caused by a wide variety of agents. Outbreaks of salmonellosis have been reported for decades, but within the past 25 years an increase in the incidence of this pathogen has been observed on many continents. In the Western Hemisphere and Europe, Salmonella serotypes Enteritidis has become the predominant strain. In 1991, the introduction of Vibrio cholerae for the first time in the South American continent made it another important cause of infectious diarrhea. In addition, Escherichia coli serotype O157:H7 (E. coli) has also emerged rapidly as a major cause of bloody diarrhea and acute renal failure soon after its emergence in 1982.

Changes in microbial populations can lead to the emergence of new pathogens, development of new virulent mechanisms in old pathogens, and rise in antibiotic resistance. Furthermore, the population of highly susceptible people is expanding worldwide because of aging, malnutrition, HIV infections and other underlying medical conditions. Although the majority of the gastrointestinal infections result in asymptomatic or self-limited diarrheal disease, life-threatening bacteremias occur, particularly in immunocompromised hosts, neonates and elderly populations. Usually the treatment of diarrhea does not require antimicrobials. However, in some clinical situations, antimicrobial therapy is required and the knowledge of the likelihood of resistance to antimicrobials is important to

The objective of this study was to determine the frequency of occurrence and the antimicrobial susceptibility profile of isolates capable of producing gastroenteritis in the Latin American medical centers of the SENTRY Program.

### MATERIALS AND METHODS

Bacterial strains. A total of 324 bacterial isolates causing gastroenterirtis were collected from Latin America by the SENTRY Antimicrobial Surveillance Program between January/2001 and February/2001. The isolates were identified to the species level by the participant medical center and referred to the coordinating laboratory for identification confirmation and susceptibility testing. Just one isolate per patient was included in the current study. A summary description of demographic data such as patient's age, gender and hospitalization in intensive care unit was obtained for each isolate.

**Medical Centers.** The participating medical centers were guided by protocol to send the first 25 consecutive gastroenteritis pathogens isolated from stool or blood beginning on January 2001. The Latin American medical centers were distributed throughout five countries (nine cities) including São Paulo, Florianopolis, Brasilia and Porto Alegre in Brazil; Buenos Aires and San Isidro in Argentina; Santiago in Chile (2 medical centers); Mexico City in Mexico; and Caracas in Venezuela (Figure 1).

Susceptibility testing. Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the National Committee for Clinical Laboratory Standards. Antimicrobial agents were obtained from the respective manufacturers, and included ampicillin, amoxicillin/clavulanate, ceftriaxone, ceftazidime, cefepime, aztreonam, imipenem, meropenem, ciprofloxacin, levofloxacin, gatifloxacin, tetracycline, and trimethoprim/sulfamethoxazole. Quality control was performed by testing Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, Pseudomonas aeruginosa ATCC 27853, and Enterococcus faecalis ATCC 29212.

## • In the SENTRY Antimicrobial Surveillance Program study, Shigella spp. (200/61.7%) was the most frequent pathogen causing gastroenteritis in the Latin American centers, followed by Salmonella spp. (116/35.8%; Table 1).

Frequency of Occurrence and Antimicrobial Resistance Patterns for Gastroenteritis Pathogens Isolated from Stool and Blood Cultures in Latin America:

- Among the Shigella spp. isolates collected, S. sonnei (34.6%) was the most frequent species followed by S. flexneri (22.5%). More than 26% of the Salmonella spp. causing gastroenteritis in the Latin American centers were not identified to the species level by the local medical centers (Table 1)
- Salmonella spp. were more frequently isolated than Shigella spp. strains in only three medical centers, which were located in Buenos Aires (Argentina), Santiago (Chile), and São Paulo (Brazil) (Table 2).

### Frequency of occurrence of pathogens causing gastroenteritis in the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Species	No. (%)	Species distribution	No. (%)
<i>Shigella</i> spp.	200 (61.7)	Shigella sonnei Shigella flexneri Shigella spp. Shigella boydii	112 (34.6) 73 (22.5) 14 (4.3) 1 (0.3)
Salmonella spp.	116 (35.8)	Salmonella spp. Salmonella group B Salmonella enterica Salmonella typhimurium Salmonella group C Salmonella enteritidis Salmonella typhi Salmonella hadar Salmonella parathyphi	85 (26.2) 10 (3.1) 7 (2.2) 6 (1.9) 2 (0.6) 2 (0.6) 2 (0.6) 1 (0.3) 1 (0.3)
Escherichia coli	7 (2.2)	Escherichia coli	7 (2.2)
Campylobacter spp.	1 (0.3)	Campylobacter spp.	1 (0.3)
Total	324 (100.0)	Total	324 (100.0)

# Table 2. Number of pathogens causing gastroenteritis in the Latin American medical centers (SENTRY Antimicrobial Surveillance Program,

			Total			
Medical center	Nation	E. coli	Salmonella spp.	Shigella spp.		
39	Argentina		19 (90.5)	2 (9.5)	21 (6.5)	
40	Argentina		8 (33.3)	16 (66.7)	24 (7.4)	
42	Chile		13 (15.3)	72 (84.7)	85 (26.2)	
43	Chile	6 (25.0)	17 (70.8)	1 (4.2)	24 (7.4)	
46	Brazil		20 (34.5)	38 (65.5)	58 (17.9)	
48	Brazil		20 (74.1)	7 (25.9)	27 (8.3)	
49	Venezuela	1 (2.7)	10 (27.0)	26 (70.3)	37 (11.4)	
57	Brazil		7 (15.6)	38 (84.4)	45 (13.9)	
101	Brazil		1 (33.3)	2 (66.7)	3 (0.9)	
Total		7 (2.2)	116 (35.8)	200 (61.7)	324 (100.0)	

### RESULTS

- The fluoroquinolones tested (MIC<sub>50</sub>, 0.03 μg/ml) and meropenem (MIC<sub>50</sub>, 0.06 μg/ml) were the most potent compounds against the pathogens causing gastroenteritis in the Latin America (Tables 4 and 5).
- The broad-spectrum cephalosporins (MIC<sub>90</sub>, 0.25-2 μg/ml) exhibited excellent in vitro activity against the gastroenteritis pathogens and, in addition, the carbapenems and fluoroquinolones exhibited the highest susceptibility rates (100.0%; Tables 4 and 5).
- Among Salmonella spp. isolates, the highest resistance rates were observed for tetracycline (16.4%) followed by ampicillin (14.7%) and trimethoprim/sulfamethoxazole (5.2%). The Shigella spp. strains demonstrated higher resistance rates than the Salmonella spp. strains and, the highest resistance rates were observed for trimethoprim/sulfamethoxazole (73.5%), followed by ampicillin (69.0%) and tetracycline (46.5%).

### Table 3. Number of pathogens causing gastroenteritis according to the body site of isolation in the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Species		Total				
	Blood culture	Stool	Urine	Wound	Other	
E. coli		7 (100.0)				7 (2.2)
Salmonella spp.	20 (17.2)	90 (77.6)	1 (0.8)	1 (0.8)	4 (3.6)	116 (35.8)
Shigella spp.	2 (1.0)	190 (95.0)			8 (4.0)	200 (61.7)
Total	22 (6.8)	288 (88.9)	1 (0.3)	1 (0.3)	12 (3.7)	324 (100.0)

### **Table 4.** Antimicrobial activity of diverse antimicrobial agents against 116 Salmonella spp. isolates causing gastroenteritis in the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Antimicrobial	Cumulative Percent inhibited at (μg/ml)									MIC <sub>50/90</sub> <sup>a</sup> % susc				
Agents	0.12	0.25	0.5	1	2	4	8	16	32					
ß-lactams														
Ampicillin	_c	-	-	-	81.0	84.5	84.5	85.3	-	≤2/>16	84.5			
Amox/Clav <sup>d</sup>	-	-	-	-	84.5	84.5	92.2	97.4	-	≤2/8	92.2			
Ceftriaxone	-	99.1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	≤0.25/≤0.25	100.0			
Ceftazidime	-	-	-	-	100.0	100.0	100.0	100.0	-	≤2/≤2	100.0			
Cefepime	98.3	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-	≤0.12/≤0.12	100.0			
Aztreonam	97.4	99.1	100.0	100.0	100.0	100.0	100.0	100.0	-	≤0.12/≤0.12	100.0			
Imipenem	39.9	95.7	100.0	100.0	100.0	100.0	100.0		-	0.25/0.5	100.0			
Meropenem	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-	-	≤0.06/≤0.06	100.0			
Quinolones														
Ciprofloxacin	95.7	100.0	100.0	100.0	100.0	-	-	-	_	≤0.015/0.12	100.0			
Levofloxacin	87.1	97.4	100.0	100.0	100.0	100.0	-	-	_	≤0.03/0.25	100.0			
Gatifloxacin	91.4	100.0	100.0	100.0	100.0	100.0	-	-	-	≤0.03/0.12	100.0			
Others														
Tetracycline <sup>e</sup>	_	-	-	-	-	83.6	84.5	_	-	≤4/>8	83.6			
Trim/Sulfa <sup>e</sup>	-	-	94.8	94.8	94.8	-	-	-	-	≤0.5/≤0.5	94.8			

e. The antimicrobial concentrations tested were 4 and 8 μg/ml for tetracycline and 0.5 and 1 μg/ml for trimethoprim/sulfamethoxazole (Trim/Sulfa).

b. Susceptibility rates calculated according to the criteria published by the NCCLS [2002].

c. - = untested concentration.

d. Amox/Clav = amoxicillin/clavulanate.

# Geographic distribution of the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).



Table 5. In vitro antimicrobial activity of diverse antimicrobial agents against 200 Shigella spp. isolates causing gastroenteritis in the Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2001).

Antimicrobial	Cumulative Percent inhibited at (μg/ml)									MIC <sub>50/90</sub> <sup>a</sup>	% susc. <sup>b</sup>
Agents	0.12	0.25	0.5	1	2	4	8	16	32		
ß-lactams											
Ampicillin	_c	-	-	-	17.5	30.0	30.5	31.0	-	≤16/>16	30.5
Amox/Clav <sup>d</sup>	-	-	-	-	16.0	37.0	77.0	100.0	-	16/16	77.0
Ceftriaxone	-	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	≤0.25/≤0.25	100.0
Ceftazidime	-	-	-	-	100.0	100.0	100.0	100.0	-	≤2/≤2	100.0
Cefepime	88.5	98.5	100.0	100.0	100.0	100.0	100.0	100.0	-	≤0.12/0.25	100.0
Aztreonam	98.5	99.5	99.5	100.0	100.0	100.0	100.0	100.0	-	≤0.12/≤0.12	100.0
Imipenem	79.5	99.0	99.5	100.0	100.0	100.0	100.0	-	-	0.12/0.5	100.0
Meropenem	100.0	100.0	100.0	100.0	100.0	100.0	100.0	-	-	≤0.06/≤0.06	100.0
Quinolones											
Ciprofloxacin	100.0	100.0	100.0	100.0	100.0	-	-	-	-	≤0.015/≤0.15	100.0
Levofloxacin	100.0	100.0	100.0	100.0	100.0	100.0	-	-	-	≤0.03/≤0.03	100.0
Gatifloxacin	100.0	100.0	100.0	100.0	100.0	100.0	-	-	-	≤0.03/≤0.03	100.0
Others											
Tetracycline <sup>es</sup>	-	-	-	-	-	53.5	53.5	-	-	≤4/>8	53.5
Trim/Sulfa <sup>e</sup>	-	-	26.0	26.5	26.6	-	-	-	-	>2/>2	26.5
a. Minimal inhibitory	a. Minimal inhibitory concentration (MIC) was determined by broth microdilution technique.										

- Susceptibility rates calculated according to the criteria published by the NCCLS [2002].
- Amox./Clav., amoxicillin/clavulanate.
- The antimicrobial concentrations tested were 4 and 8 µg/ml for tetracycline and 0.5 and 1 µg/ml for trimethoprim/ sulfamethoxazole

### CONCLUSIONS

- Our results demonstrate that multidrug resistance, especially fluoroquinolone resistance, does not represent a serious problem among gastroenteritis pathogens collected from the Latin American medical centers by the SENTRY Program.
- Results indicate that control measures implemented now could prevent the emergence and spread of multi-drug resistant gastroenteritis pathogens in the Latin American region.