

# Occurrence and Susceptibility Profiles of Pathogens Causing Gastroenteritis in North America and Europe: Report from SENTRY Antimicrobial Surveillance Program

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## AMENDED ABSTRACT

**Background:** The SENTRY Program initiated surveillance of gastroenteritis (GE) pathogens in 2001, documenting pathogen frequency and antibiogram profiles among the most common species causing GE in North America (NA) and Europe (EU). **Methods:** Participants collected 750 stool or blood culture isolates from EU (18 sites) and 439 isolates from NA (23 sites). Identification confirmation included Vitek, hippurate hydrolysis for *Campylobacter* (CAMP) and latex agglutination for *E. coli* (EC) 0157:H7. The reference site tested isolates by broth microdilution using NCCLS methods or Etest (CAMP). ESBL screen (+) were confirmed by Etest on a subset of *Salmonella* spp. (SAL). PCR methods were used to determine the  $\beta$ -lactamase types. **Results:** Among 1,189 isolates, 684 SAL included *S. enteritidis* (196), *S. typhimurium* group B (121), *S. typhimurium* group D (137); 225 *Shigella* spp. (SHIG) included *S. sonnei* (151) and *S. flexneri* (39); 168 CAMP; 51 *Aeromonas* spp. (AERO); and 23 EC 0157:H7 were tested. Ampicillin (AMP) resistant (R) SAL was comparable in NA and EU (18.3 - 21.5%). Fluoroquinolones (FQ), aminoglycosides and trim/sulfa (T/S) were very active ( $\geq 95\%$  susceptible [S]). Potential FQ target mutations (ciprofloxacin MICs, 0.12 - 0.5  $\mu\text{g/ml}$ ) were detected in EU (14.0%) > NA (2.8%). Tetracycline (TET)-R was 22.1%. All, but one tested SAL isolates were negative by ESBL confirmation, but PCR revealed CMY-like enzymes. SHIG isolated from NA was significantly more AMP-R (87.4%) vs EU (35.2%), but TET- and T/S-R were higher in EU 72.7 and 79.5%, than in NA 61.2 and 51.5%, respectively. FQ-R CAMP was 23.5% in EU > 9.1% in NA. AERO were S ( $\geq 98\%$ ) to FQ, cephalosporins and carbapenems but less S to T/S (90.2%) and TET (84.3%). EC isolates were more S to AMP and TET in NA (94.4%) > EU (80%). **Conclusions:** Regional variation of antimicrobial S was documented among SAL, SHIG, CAMP and EC 0157:H7. FQ-R CAMP, AMP- and TET-R EC was much higher in EU. SAL isolates with CMY enzymes and AMP-R (TEM-1) SHIG were higher in NA, revealing the value of international surveillance programs.

## INTRODUCTION

Organisms known to cause gastroenteritis are prevalent in all regions of the world, particularly in developing countries. However, epidemic occurrences of food/water borne illness due to organisms capable of producing diarrheal symptoms can occur even in countries with more acceptable sanitation practices. Depending on the risk factors of the individual, including age and nutritional status, the invasiveness of the responsible pathogen can lead to serious disease including haemolytic uremic syndrome, typhoid fever, sepsis, and although uncommon, meningitis. Usually, when the disease is self-limiting and the infected individual has good health status, no antimicrobial therapy is prescribed. Even if a bacterial pathogen is cultured and identified, most often the susceptibility profile of the offending organism is not determined. Although it is acceptable that most individuals do not require antimicrobial therapy for non-invasive gastroenteritis when host defenses are capable of cure, it is a concern to the medical community that the prevalence of antimicrobial resistance among common gastroenteritis pathogens is not well understood.

In 2001, the SENTRY Antimicrobial Surveillance Program initiated a focused objective to determine the frequency of organism occurrence and antimicrobial resistance patterns of pathogens which cause gastroenteritis. This study monitored medical centers within the SENTRY Program network in North America, Latin America and Europe in order to analyze potential regional variations in susceptibility patterns of species associated with food/water borne illness. Isolates forwarded for this objective were members of the family Enterobacteriaceae including *Salmonella* spp., *Shigella* spp., "enteropathogenic" *E. coli* including 0157:H7, *Yersinia enterocolitica* as well as, *Campylobacter* spp. and *Aeromonas* spp. The isolates were tested against numerous therapeutic agents and this report focuses on pathogens isolated from centers in the Northern Hemisphere.

## MATERIALS AND METHODS

A total of 1,183 bacterial isolates were collected for this analysis which were considered species documented to produce clinically significant gastroenteritis with or without systemic symptoms. Organisms were collected by participant laboratories in the SENTRY Program during 2001 and forwarded to the central laboratory (JMI Laboratories, North Liberty, Iowa). The medical center laboratories were instructed to forward between 25 and 50 consecutively collected isolates from blood or stool specimens. The species of most interest included *Salmonella* spp., *Shigella* spp., *Aeromonas* spp., *Campylobacter* spp., *Yersinia* spp., *Vibrio* spp., and enteropathogenic *E. coli* especially 0157:H7 strains. Participants compliant to the protocol design included European sites in France (four), the United Kingdom (four), Spain (three), Italy (two), and Belgium, as well as two sites in Turkey and one in Israel. The North American centers included 18 sites from 15 states and five sites in Canada (different provinces). The North American sites forwarded 463 isolates and the European sites referred 657 strains for analysis.

Isolates were subcultured for purity upon arrival, identified (*E. coli* 0157:H7 and *Campylobacter*) and tested for antimicrobial susceptibility using broth microdilution methods recommended by the NCCLS. *Campylobacter* spp. were tested using Etest (AB BIODISK) due to the fastidious nature of this species and the accuracy of this method compared to reference tests. MIC values were determined for over 20 antimicrobial agents (10 - 14 reported here) which included penicillins, cephalosporins, carbapenems, old and new generation quinolones, aminoglycosides, tetracyclines, and trimethoprim/sulfamethoxazole. Erythromycin and chloramphenicol were also tested against *Campylobacter* spp. *Salmonella* spp. strains which met NCCLS criteria for potential ESBL production (cefazidime, ceftriaxone or aztreonam MICs,  $\geq 2 \mu\text{g/ml}$ ) were further characterized using the Etest ESBL strip. When a clonal outbreak of strains was suspected, isolates were tested by automated ribotyping (RiboPrinter, Qualicon, Wilmington, DE) and PFGE.

- The occurrence of strains causing gastroenteritis (Table 1) demonstrates that among the *Salmonella* spp., *S. enteritidis* (39.4%) and *S. typhi* (serotype D, 22.6%) strains are more commonly isolated in Europe compared to North America (5.1% and 14.5%, respectively). The most commonly isolated species in North America was *S. typhimurium* (serotype B, 27.6%).
- The occurrence rates of *Shigella* spp. (18.6 - 22.2%) was similar on both continents with the majority of isolates speciated as *S. sonnei* (62.1 - 71.3%). The occurrence of *Campylobacter* spp. (14.3 - 15.6%) was also similar and *C. jejuni* isolates were most often identified.
- In North America, resistance to ampicillin among *Salmonella* spp. was 21.5% and 5.14% of isolates from North America met NCCLS criteria as potential ESBL producing strains (Table 2). All 11 ESBL phenotypes were negative by Etest. The isolation of ampicillin-resistant *Salmonella* spp. was similar (18.3%) in Europe, however, ESBL phenotypes were rare (0.2%, one isolate was positive by Etest confirmation).

- As a predictive marker, nalidixic acid resistance suggests that nearly 3% of the *Salmonella* spp. isolates from North America may have point mutations in the QRDR. The potential for fluoroquinolone therapy failure was much higher among European isolates (18.3% nalidixic acid resistance). Tetracycline-resistance was common (20.6 - 25.7%) among *Salmonella* spp. on both continents.

- Table 2 shows that with the exception of ampicillin, tetracycline and trimethoprim/sulfamethoxazole, *Shigella* spp. isolates from North America are very susceptible to the tested agents ( $\geq 98.0\%$ ). Only one ampicillin-resistant isolate of *E. coli* 0157:H7 with co-resistances to gentamicin and tetracycline was detected, all other compounds were active.

- Table 3 shows that ampicillin resistance was lower among European *Shigella* spp. isolates (35.2%) and resistance to tetracycline and trimethoprim/sulfamethoxazole was high (72.7 - 79.5%). Potential QRDR mutations were more prevalent (40%) in *E. coli* 0157:H7 compared to North American isolates. *Y. enterocolitica* from Europe were not susceptible to ampicillin and the utility of piperacillin/tazobactam, fluoroquinolones, and trimethoprim/sulfamethoxazole may be compromised among some isolates.

Table 1. Occurrence of gastroenteritis pathogens among significant species (1,183 strains) isolated during 2001 in North America and Europe: A SENTRY Report.

Organism species	North America (no.)	Europe (no.)
<i>Salmonella</i> spp.		
<i>S. typhimurium</i> (serotype B)	59	62
<i>S. typhi</i> (serotype D)	16	106
<i>S. choleraesuis</i> (serotype C)	15	21
<i>S. enteritidis</i>	11	185
<i>S. enterica</i>	-	9
Other species or unspiculated/not serotyped <sup>a</sup>	98	87
Total	214	470
<i>Shigella</i> spp.		
<i>S. sonnei</i>	64	87
<i>S. flexneri</i>	16	23
<i>S. boydii</i>	5	5
<i>S. dysenteriae</i>	4	2
Unspiculated	14	5
Total	103	122
<i>Campylobacter</i> spp. <sup>b</sup>	66	102
<i>E. coli</i> 0157:H7	18	5
<i>Aeromonas</i> spp.	18	33
<i>Y. enterocolitica</i>	-	10
<i>Vibrio</i> spp.	6	2
<i>Plesiomonas shigelloides</i>	4	-

a. Includes 129 unspiculated/not serotyped strains and 16 other speciated strains not listed above.  
b. Includes *C. jejuni* (104 strains), *C. coli* (three strains) and unspiculated *Campylobacter* spp. (61 strains).

## CONCLUSIONS

- This large sample of bacterial pathogens which cause gastroenteritis show that the prevalence of *Salmonella* species and serotypes are variable in occurrence between North America and Europe. *S. enteritidis* is most common in Europe and *S. typhimurium* (serotype B) is more common in North America.
- ESBL phenotypes are more common among *Salmonella* spp. in North America, but were not confirmed by MIC reduction using clavulanic acid (CMY-type enzymes). In contrast, potential mutations in the QRDR among *Salmonella* spp. were much higher among European isolates.
- Most enteric pathogens which cause gastroenteritis are susceptible to piperacillin/tazobactam, cephalosporins, carbapenems, fluoroquinolones and aminoglycosides.
- Campylobacter* spp. isolated in Europe are much more resistant to fluoroquinolones and erythromycin compared to those in North America.

## RESULTS

- The fluoroquinolones were the most potent compounds tested against *Campylobacter* spp. from North America (MIC<sub>50</sub>, 0.5 - 1  $\mu\text{g/ml}$ ) although 9.1% of strains were resistant to ciprofloxacin. Only one isolate showed resistance to erythromycin. In contrast, *Campylobacter* spp. in Europe were more resistant to ciprofloxacin and erythromycin (23.5%).

- An outbreak of eight strains of *S. typhimurium* was detected in France, all of which were resistant to ampicillin and tetracycline (possible DT104). The strains were all stool isolates from symptomatic patients ages 1 - 47, the oldest and youngest of which required hospitalization. All strains had identical ribotype and PFGE patterns.

Table 2. Antimicrobial susceptibility of gastroenteritis pathogens from isolates collected in North American medical centers participating in the SENTRY Antimicrobial Surveillance Program during 2001.

Organism/antimicrobial agent (no.)	MIC ( $\mu\text{g/ml}$ ):			% susceptible <sup>a</sup>	% resistant
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range		
<i>Salmonella</i> spp. (214)					
Ampicillin	$\leq 2$	>16	$\leq 2$ ->16	78.5	21.5
Piperacillin/Tazobactam	2	8	$\leq 0.5$ ->64	97.2	1.4
Cefoxitin	2	4	1-8	100.0	5.1
Ceftriaxone	$\leq 0.25$	$\leq 0.25$	$\leq 0.25$ ->32	96.2	1.9(5.1) <sup>b</sup>
Ceftazidime	$\leq 2$	$\leq 2$	$\leq 2$ ->16	94.9	3.3(5.1) <sup>b</sup>
Cefepime	$\leq 0.12$	$\leq 0.12$	$\leq 0.12$ -1	100.0	0.0
Imipenem	0.12	0.25	$\leq 0.05$ -0.25	100.0	0.0
Nalidixic acid	4	8	2->32	97.2	2.8
Ciprofloxacin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$ -0.5	100.0	0.0
Gatifloxacin	$\leq 0.03$	0.06	$\leq 0.03$ -0.5	100.0	0.0
Garenoxacin	0.06	0.12	$\leq 0.03$ -4	100.0	0.0
Gentamicin	$\leq 2$	$\leq 2$	$\leq 2$ ->8	99.1	0.9
Tetracycline	$\leq 4$	>8	$\leq 4$ ->8	74.3	25.7
Trimethoprim/Sulfamethoxazole	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$ ->2	95.8	4.2
<i>Shigella</i> spp. (103)					
Ampicillin	>16	>16	$\leq 2$ ->16	12.6	87.4
Piperacillin/Tazobactam	2	4	$\leq 0.5$ ->64	99.0	1.0
Cefoxitin	2	4	1-8	100.0	0.0
Ceftriaxone	$\leq 0.25$	$\leq 0.25$	$\leq 0.25$ -0.5	100.0	0.0
Ceftazidime	$\leq 2$	$\leq 2$	$\leq 2$ -4	100.0	0.0
Cefepime	$\leq 0.12$	$\leq 0.12$	$\leq 0.12$ -1	100.0	0.0
Imipenem	0.12	0.25	$\leq 0.06$ -0.25	100.0	0.0
Nalidixic acid	2	4	2->32	98.1	1.9
Ciprofloxacin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$ -0.12	100.0	0.0
Gatifloxacin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$ -0.25	100.0	0.0
Garenoxacin	$\leq 0.03$	$\leq 0.03$	$\leq 0.03$ -0.25	100.0	0.0
Gentamicin	$\leq 2$	$\leq 2$	$\leq 2$ ->8	100.0	0.0
Tetracycline	>8	>8	$\leq 4$ ->8	38.8	61.2
Trimethoprim/Sulfamethoxazole	>2	>2	$\leq 0.5$ ->2	48.5	51.5
<i>E. coli</i> 0157:H7 (18)					
Ampicillin	4	8	4->16	94.4	5.6
Piperacillin/Tazobactam	2	2	1-2	100.0	0.0
Cefoxitin	2	4	4-8	100.0	0.0
Ceftriaxone	$\leq 0.25$	$\leq 0.25$	$\leq 0.25$ -0.5	100.0	0.0
Ceftazidime	$\leq 2$	$\leq 2$	$\leq 2$ -8	100.0	0.0
Cefepime	$\leq 0.12$	0.25	0.12-0.25	100.0	0.0
Imipenem	0.12	0.25	0.12-0.25	100.0	0.0
Nalidixic acid	4	4	4-8	100.0	0.0
Ciprofloxacin	$\leq 0.015$	$\leq 0.015$	$\leq 0.015$ -0.12	100.0	0.0
Gatifloxacin	$\leq 0.03$	0.06	$\leq 0.03$ -0.06	100.0	0.0
Garenoxacin	$\leq 0.03$	0.06	$\leq 0.03$ -0.06	100.0	0.0
Gentamicin	$\leq 1$	2	$\leq 1$ -2	94.4	5.6
Tetracycline	$\leq 4$	$\leq 4$	$\leq 4$ ->8	94.4	5.6
Trimethoprim/Sulfamethoxazole	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$ ->2	100.0	0.0
<i>Aeromonas</i> spp. (18)					
Ampicillin	>16	>16	>16	0.0	100.0
Piperacillin/Tazobactam	4	>64	1->64	83.3	16.7
Ceftriaxone	$\leq 0.25$	2	$\leq 0.25$ -4	100.0	0.0
Ceftazidime	$\leq 2$	4	$\leq 2$ -8	100.0	0.0
Cefepime	$\leq 0.12$	0.25	$\leq 0.12$ -1	100.0	0.0
Imipenem	0.25	2	$\leq 0.06$ -4	100.0	0.0
Nalidixic acid	2	>32	2->32	83.3	16.7
Ciprofloxacin	$\leq 0.015$	0.12	$\leq 0.015$ -0.25	100.0	0.0
Gatifloxacin	$\leq 0.03$	0.12	$\leq 0.03$ -0.5	100.0	0.0
Garenoxacin	0.12	1	$\leq 0.03$ -2	100.0	0.0
Gentamicin	$\leq 2$	$\leq 2$	$\leq 2$ -8	100.0	0.0
Tobramycin	1	2	0.5-8	94.4	0.0
Tetracycline	$\leq 4$	$\leq 4$	$\leq 4$ ->8	89.9	5.6
Trimethoprim/Sulfamethoxazole	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$ ->2	94.4	5.6
<i>Campylobacter</i> spp. (66)					
Ampicillin	2	8	0.12-32	-	-
Ceftriaxone	>32	>32	0.5->32	-	-
Ciprofloxacin	0.12	1	0.016->32	-	9.1 <sup>c</sup>
Gatifloxacin	0.06	0.5	0.016->32	-	-
Garenoxacin	0.06	0.5	0.03->32	-	-
Erythromycin	1	2	0.12-32	-	1.5 <sup>d</sup>
Gentamicin	0.5	1	0.125-2	-	-
Chloramphenicol	1	4	0.25-8	-	-
Tetracycline	0.12	>256	0.03->256	-	-
Trimethoprim/Sulfamethoxazole	0.25	1	0.008-32	-	-

a. Susceptibility percentage based on NCCLS recommendations [NCCLS, 2002].  
b. Percentage of isolates meeting NCCLS criteria for ESBL phenotype [NCCLS, 2002], also cefotaxim-resistant.  
c. Percentage at  $\geq 4 \mu\text{g/ml}$  [NCCLS, 2002].  
d. Percentage at  $\geq 8 \mu\text{g/ml}$  [Baker et al., 1992], one strain.

Table 3. Antimicrobial susceptibility of gastroenteritis pathogens from isolates collected in European medical centers participating in the SENTRY Antimicrobial Surveillance Program during 2001.

Organism/antimicrobial agent (no.)	MIC ( $\mu\text{g/ml}$ ):			% susceptible <sup>a</sup>	% resistant
	MIC <sub>50</sub>	MIC <sub>90</sub>	Range		
<i>Salmonella</i> spp. (470)					
Ampicillin	$\leq 2$	>16	$\leq 2$ ->16	81.2	18.3
Piperacillin/Tazobactam	2	4	$\leq 0.5$ ->64	98.9	0.4
Cefoxitin	2	4	0.5->32	99.1	0.6
Ceftriaxone	$\leq 0.25$	$\leq 0.25$	$\leq 0.25$ ->32	99.8	0.2(0.2) <sup>b</sup>
Ceftazidime	$\leq 2$	$\leq 2$	$\leq 2$ -16	99.8	0.0(0.2) <sup>b</sup>
Cefepime	$\leq 0.12$	$\leq 0.12$	$\leq 0.12$ -16	99.8	0.0
Imipenem	0.25	0.25	0.12-0.5	100.0	0.0
Nalidixic acid	4	>32	2->32	85.7	14.3
Ciprofloxacin	$\leq 0.03$	0.12	$\leq 0.03$ -0.5	100.0	0.0
Gatifloxacin	0.06	0.25	$\leq 0.03$ -1	100.0	0.0
Garenoxacin	0.06	0.25	$\leq 0.03$ -1	100.0	0.0
Gentamicin	$\leq 2$	$\leq 2$	$\leq 2$ ->8	98.7	1.3
Tetracycline	$\leq 4$	>8	$\leq 4$ ->8	79.2	20.6
Trimethoprim/Sulfamethoxazole	$\leq 0.5$	$\leq 0.5$	$\leq 0.5$ ->2	94.9	5.1
<i>Shigella</i> spp. (122)					
Ampicillin	4	>16	$\leq 2$ ->16	63.9	35.2
Piperacillin/Tazobactam	1	4	$\leq 0.5$ -8	90.0	0.0
Cefoxitin	2	4	1-8	100.0	0.0
Ceftriaxone	$\leq 0.25$ </				