# Prevalence and Antimicrobial Spectrum of Oral Agents Tested Against Gastroenteritis Isolates Recovered in Europe and Latin America: Report from the SENTRY Antimicrobial Surveillance Program (2003)


C2-1721

ICAAC 2004
The JONES Group/JMI Laboratories
North Liberty, IA, USA; www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

TR FRITSCHE, PR RHOMBERG, LS STRATCHOUNSKI, RN JONES, JM STREIT, MA TOLEMAN

The JONES Group/JMI Laboratories, North Liberty, IA; Smolensk Reg Hospital, Smolensk, Russia; and BCARE, Bristol, UK


## AMENDED ABSTRACT


**Background**: Gastroenteritis (GE) pathogens produce significant morbidity and mortality with some species recently displaying increased antimicrobial resistance (R). This report summarizes prevalence and susceptibility (S) profiles of GE species in Europe (EU) and Latin America (LA).

**Methods**: GE pathogens were isolated in EU (27 sites) and LA (10); 50 consecutive isolates/site. Antimicrobial S testing was performed using NCCLS methods. Ribotyping and PFGE were used to confirm clonality, and PCR with product sequencing was used to identify ß-lactamase (BL) genes.

**Results**: 1,479 isolates (no./prevalence %) were: *Salmonella* spp. (SAL; 834/56), *Shigella* spp. (SHI; 311/21), *Campylobacter* spp (CAMPY; 182/12), *Aeromonas* spp. (AER; 72/5), enteropathogenic *E. coli* (58/4), and *Yersinia* spp. (YER; 22/2). No differences were noted between regions. Ciprofloxacin (CIP) was the most potent ($MIC_{50}$, ≤0.03 µg/ml ) agent tested. CIP-R SAL in EU/LA was 0.3/0.0%, however 16.5/15.3% were R to nalidixic acid (NAL), indicating first step (QRDR) mutations. % tetracycline R among SAL, SHI and AER in EU was higher (19.9, 80.6, 16.0, respectively) than LA (18.8, 56.3, 9.1). A cluster of 18 ESBL screen + SAL isolates from Russia were seen and a CTX-M5 BL was detected in 15 strains, identical by ribotype/PFGE (189.1/A). SAL (107) isolated from bloodstream infections displayed near-identical antibiograms, and included one CIP-R strain.

**Conclusions**: SAL, SHI and CAMPY remain primary causes of GE with similar global distribution. A variety of oral agents retain activity for use in serious infections, however NAL-R indicated the potential of CIP therapy failure for 1 in 6 SAL cases. Surveillance programs provide meaningful data regarding dissemination of R in GE isolates.


## INTRODUCTION

Gastroenteritis pathogens produce significant morbidity and mortality with some species recently displaying increased antimicrobial resistance. The rapid increase in resistance to the fluoroquinolones in non-typhoidal *Salmonella* has been extremely worrisome. Resistance to nalidixic acid has been shown to indicate elevated ciprofloxacin MICs and probable therapeutic failure.

The recent emergence of extended-spectrum ß-lactamase (ESBL) enzymes in *Salmonella* threatens to compromise the clinical use of "third-generation" cephalosporins and monobactams. ESBL's associated with *Salmonella* have been reported increasingly in the last few years with a variety of enzymes being described (principally TEM, SHV and CTX-M types). Previous studies have shown that ESBL plasmids are highly mobile and may also carry elements for tetracycline resistance.

This report summarizes prevalence and susceptibility profiles of bacterial species producing gastroenteritis (*Salmonella, Shigella, Aeromonas* and *Yersinia* spp.) in Europe and Latin America, studied as part of the 2003 SENTRY Antimicrobial Surveillance Program.

## MATERIALS AND METHODS

Bacterial isolates. A total of 1,479 isolates from gastroenteritis infections were collected from 27 medical centers in Europe and 10 sites in Latin America. Each site forwarded up to 50 consecutive non-duplicate clinical isolates to a central monitor (JMI Laboratories, North Liberty, IA) for susceptibility testing. The rank order of pathogens (number; prevalence) included *Salmonella* spp. (834; 56%), *Shigella* spp. (311; 21%), *Campylobacter* spp. (182; 12%), *Aeromonas* spp. (72; 5%), enteropathogenic *E. coli* (58; 4%) and *Yersinia* spp. (22; 1.5%).

Susceptibility testing. All isolates (except for *Campylobacter*) were tested by the National Committee for Clinical Laboratory Standards (NCCLS) M7-A6 broth microdilution methods. Dry-form broth microdilution panels manufactured by TREK Diagnostics (Cleveland, OH) were used to test over 20 antimicrobial agents. Cation-adjusted Mueller-Hinton broth was used as the test medium. The following quality control organisms were tested concurrently: *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, *E. coli* ATCC 25922 and 35218 and *P. aeruginosa* ATCC 27853. Interpretive criteria were those of NCCLS [2004].

Molecular epidemiology. Isolates identified as being part of potential clusters were subjected to ribotyping using the Riboprinter Microbial Characterization System (Qualicon, Inc., Wilmington, DE) as recommended by the manufacturer. Matching isolates were subsequently analyzed by pulsed-field gel electrophoresis (PFGE) using XbaI as the restriction enzyme and the electrophoresis performed with a CHEF DRII (Bio-Rad, Hercules, CA) apparatus.

ß-lactamase typing. Plasmid extraction was carried out with the QIAprep Spin Mini kit (Qiagen, West Sussex, United Kingdom). *bla* genes were amplified using published primers for $bla_{CTX-M}$ series enzymes. PCR fragments were sequenced using DuPont Automated Systems. The nucleotide and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, WI) and compared to sequences available from reference sources.

## RESULTS

- The activity of nine antimicrobial agents tested against 1,239 pathogenic gastroenteritis organisms collected from Europe and Latin America, respectively, are summarized in Tables 1 and 2.

- The fluoroquinolones showed the greatest activity with only three strains being non-susceptible using NCCLS breakpoint criteria. Ciprofloxacin was the most potent agent tested ($MIC_{50}$, ≤0.03 µg/ml). Ciprofloxacin resistance in Europe and Latin America was 0.03 and 0.0%, respectively, however, 16.5 and 15.3% were resistant to nalidixic acid (data not shown), respectively, indicating first step *gyrA* mutations.

**Table 1.** Activity of antimicrobial compounds tested against 903 strains recovered from gastroenteritis infections found in patients in Europe, Israel and Turkey (SENTRY Program, 2003).

| | MICs (µg/ml) | | | | |
|---|---|---|---|---|---|
| Organism/antimicrobial agent (no. tested) | 50% | 90% | Range | % susceptible | % resistant |
| *Aeromonas* spp. (50) | | | | | |
| Ampicillin | >16 | >16 | 2->16 | -[a] | -[a] |
| Amoxicillin/Clavulanate | 16 | >16 | ≤1->16 | -[a] | -[a] |
| Cefuroxime | 2 | 8 | ≤0.12->16 | -[a] | -[a] |
| Ceftriaxone | ≤0.25 | 1 | ≤0.25-2 | 100.0[b] | 0.0[b] |
| Ciprofloxacin | ≤0.03 | 0.12 | ≤0.03-0.5 | 100.0[b] | 0.0[b] |
| Gatifloxacin | ≤0.03 | 0.25 | ≤0.03-0.25 | 100.0[b] | 0.0[b] |
| Levofloxacin | ≤0.03 | 0.25 | ≤0.03-0.25 | 100.0[b] | 0.0[b] |
| Tetracycline | ≤2 | >8 | ≤2->8 | 80.0[b] | 16.0[b] |
| Trimethoprim/Sulfamethoxazole | ≤0.5 | >2 | ≤0.5->2 | 80.0[b] | 20.0[b] |
| *Salmonella* spp. (664) | | | | | |
| Ampicillin | 2 | >16 | ≤1->16 | 74.3 | 25.2 |
| Amoxicillin/Clavulanate | 2 | 16 | ≤1->16 | 85.4 | 3.5 |
| Cefuroxime | 4 | 8 | 0.25->16 | 50.5 | 3.3 |
| Ceftriaxone | ≤0.25 | ≤0.25 | ≤0.25->32 | 97.6 | 2.4 |
| Ciprofloxacin | ≤0.03 | 0.12 | ≤0.03-4 | 99.7 | 0.2 |
| Gatifloxacin | 0.06 | 0.12 | ≤0.03-2 | 100.0 | 0.0 |
| Levofloxacin | 0.06 | 0.25 | ≤0.03-4 | 99.7 | 0.0 |
| Tetracycline | ≤2 | >8 | ≤2->8 | 79.8 | 19.9 |
| Trimethoprim/Sulfamethoxazole | ≤0.5 | ≤0.5 | ≤0.5->2 | 93.3 | 6.7 |
| *Shigella* spp. (167) | | | | | |
| Ampicillin | >16 | >16 | ≤1->16 | 24.0 | 76.0 |
| Amoxicillin/Clavulanate | 16 | 16 | ≤1->16 | 29.3 | 0.6 |
| Cefuroxime | 2 | 4 | 1-8 | 95.2 | 0.0 |
| Ceftriaxone | ≤0.25 | ≤0.25 | ≤0.25-1 | 100.0 | 0.0 |
| Ciprofloxacin | ≤0.03 | ≤0.03 | ≤0.03-1 | 100.0 | 0.0 |
| Gatifloxacin | ≤0.03 | ≤0.03 | ≤0.03-1 | 100.0 | 0.0 |
| Levofloxacin | ≤0.03 | ≤0.03 | ≤0.03-1 | 100.0 | 0.0 |
| Tetracycline | >8 | >8 | ≤2->8 | 19.4 | 80.6 |
| Trimethoprim/Sulfamethoxazole | >2 | >2 | ≤0.5->2 | 34.1 | 65.9 |
| *Yersinia enterocolitica* (22) | | | | | |
| Ampicillin | >16 | >16 | ≤1->16 | 9.1 | 81.8 |
| Amoxicillin/Clavulanate | 8 | 16 | ≤1->16 | 86.4 | 9.1 |
| Cefuroxime | 4 | 4 | 1-8 | 95.5 | 0.0 |
| Ceftriaxone | ≤0.25 | ≤0.25 | ≤0.25 | 100.0 | 0.0 |
| Ciprofloxacin | ≤0.03 | ≤0.03 | ≤0.03-0.5 | 100.0 | 0.0 |
| Gatifloxacin | ≤0.06 | 0.06 | ≤0.03-0.5 | 100.0 | 0.0 |
| Levofloxacin | ≤0.06 | 0.06 | ≤0.03 | 100.0 | 0.0 |
| Tetracycline | ≤2 | ≤2 | ≤2-4 | 100.0 | 0.0 |
| Trimethoprim/Sulfamethoxazole | ≤0.5 | 1 | ≤0.5-2 | 100.0 | 0.0 |

a. No breakpoints have been established by NCCLS [2004]
b. Breakpoints used are those for *P. aeruginosa* and other non-Enterobacteriaceae [NCCLS, 2004].

- Tetracycline resistance among *Salmonella* spp., *Shigella* spp. and *Aeromonas* spp. in Europe was higher (19.9, 80.6, 16.0%, respectively) than in Latin America (18.8, 56.3, 9.1%).

- *Salmonella* spp. isolated from bloodstream infections (107 strains) displayed antibiograms that were nearly identical to those producing gastroenteritis; and included one ciprofloxacin-resistant strain (data not shown).

- A marked difference between regions was seen only with amoxicillin/clavulanate and tetracycline susceptibility in *Shigella* spp. Strains from Europe were 29.5% and 19.4% susceptible for amoxicillin/clavulanic and tetracycline, respectively, whereas Latin American strains were 72.9 and 43.1% susceptible.

- A cluster of 18 ESBL screen-positive *Salmonella typhimurium* isolates originating from Russia were identified and found to be identical by both ribotyping and PFGE. A CTX-M5 ß-lactamase was detected in 15 of these strains and are considered to be part of an ongoing clonal outbreak that began in 1994 [M. Edelstein; Antimicrob. Agents Chemother. 2004; 48:2808].

**Table 2.** Activity of antimicrobial compounds tested against 336 strains recovered from gastroenteritis infections found in patients in Latin America (SENTRY Program, 2003).

| | MICs (µg/ml) | | | | |
|---|---|---|---|---|---|
| Organism/antimicrobial agent (no. tested) | 50% | 90% | Range | % susceptible | % resistant |
| *Aeromonas* spp. (22) | | | | | |
| Ampicillin | >16 | >16 | >16 | -[a] | -[a] |
| Amoxicillin/Clavulanate | 16 | >16 | 16->16 | -[a] | -[a] |
| Cefuroxime | 2 | 8 | ≤0.12-8 | -[a] | -[a] |
| Ceftriaxone | ≤0.25 | 4 | ≤0.25-16 | 95.5[b] | 0.0[b] |
| Ciprofloxacin | ≤0.03 | 0.06 | ≤0.03-0.12 | 100.0[b] | 0.0[b] |
| Gatifloxacin | ≤0.03 | 0.06 | ≤0.03-0.12 | 100.0[b] | 0.0[b] |
| Levofloxacin | ≤0.03 | 0.06 | ≤0.03-0.12 | 100.0[b] | 0.0[b] |
| Tetracycline | ≤2 | ≤2 | ≤2->8 | 90.9[b] | 9.1[b] |
| Trimethoprim/Sulfamethoxazole | ≤0.5 | >2 | ≤0.5->2 | 77.3[b] | 22.7[b] |
| *Salmonella* spp. (170) | | | | | |
| Ampicillin | 2 | >16 | ≤1->16 | 87.1 | 12.9 |
| Amoxicillin/Clavulanate | ≤1 | 8 | ≤1->16 | 94.1 | 2.4 |
| Cefuroxime | 4 | 8 | ≤0.12->16 | 56.5 | 1.8 |
| Ceftriaxone | ≤0.25 | ≤0.25 | ≤0.25->32 | 98.8 | 0.6 |
| Ciprofloxacin | ≤0.03 | 0.12 | ≤0.03-0.5 | 100.0 | 0.0 |
| Gatifloxacin | ≤0.03 | 0.25 | ≤0.03-1 | 100.0 | 0.0 |
| Levofloxacin | 0.06 | 0.25 | ≤0.03-1 | 100.0 | 0.0 |
| Tetracycline | ≤2 | >8 | ≤2->8 | 81.2 | 18.8 |
| Trimethoprim/Sulfamethoxazole | ≤0.5 | ≤0.5 | ≤0.5->2 | 91.1 | 8.9 |
| *Shigella* spp. (144) | | | | | |
| Ampicillin | >16 | >16 | ≤1->16 | 26.4 | 73.6 |
| Amoxicillin/Clavulanate | 8 | 16 | ≤1->16 | 72.9 | 0.7 |
| Cefuroxime | 4 | 4 | 0.5-8 | 96.5 | 0.0 |
| Ceftriaxone | ≤0.25 | ≤0.25 | ≤0.25 | 100.0 | 0.0 |
| Ciprofloxacin | ≤0.03 | ≤0.03 | ≤0.03->4 | 99.3 | 0.7 |
| Gatifloxacin | ≤0.03 | ≤0.03 | ≤0.03-4 | 99.3 | 0.0 |
| Levofloxacin | ≤0.03 | ≤0.03 | ≤0.03->4 | 99.3 | 0.7 |
| Tetracycline | >8 | >8 | ≤2->8 | 43.1 | 56.2 |
| Trimethoprim/Sulfamethoxazole | >2 | >2 | ≤0.5->2 | 34.7 | 65.3 |

a. No breakpoints have been established by NCCLS [2004].
b. Breakpoints used are those for *P. aeruginosa* and other non-Enterobacteriaceae [NCCLS, 2004].

## CONCLUSIONS

- *Salmonella* spp., *Shigella* spp. and *Campylobacter* spp. remain primary (89%) causes of bacterial gastroenteritis infections within the two studied continents.

- While antibiograms for *Salmonella* spp. are similar, those for *Shigella* spp. display greater variability with isolates from Latin America being more susceptible to tetracyclines and amoxicillin/clavulanate.

- Many oral antimicrobial agents remain active for use in serious gastroenteritis infections. The finding of nalidixic acid resistance in one of six *Salmonella* spp. strains evaluated indicates, however, the potential for subsequent mutations and failure with fluoroquinolone therapy.

- One ESBL (CTX-M5) cluster of *S. typhimurium* was identified that appears to be an extension of an ongoing outbreak in Russia and Belarus.

- Longitudinal worldwide surveillance programs such as the SENTRY Program continue to provide both meaningful data regarding dissemination of resistance and guidance for empirical therapy in gastroenteritis infections.

## SELECTED REFERENCES


Edelstein M, Pimkin M, Dmitrachenko T, Semenov V, Kozlova N, Gladin D, Baraniak A, Stratchounski L. (2004). Multiple outbreaks of nosocomial Salmonellosis in Russia and Belarus caused by a single clone of *Salmonella enterica* serovar Typhimurium producing an extended-spectrum ß-lactamase. *Antimicrobial Agents and Chemotherapy* 48:2808-2815.

Mhand RA, Soukri A, Amarouch H, Mdaghri NE, Benbachir M. (1999). Typing of extended-spectrum ß-lactamase-producing *Salmonella typhimurium* strains isolated in a pediatric unit. *Sante* 9:341-344.

National Committee for Clinical Laboratory Standards. (2003). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6.* Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2004). *Performance standards for antimicrobial susceptibility testing, 14th information supplement M100-S14*. Wayne, PA:NCCLS.

Paterson DL. (2001). Extended-spectrum ß-lactamases: The European experience. *Current Opinion Infectious Disease* 14:697-701.

Romero L, Lopez L, Martinez-Martinez L, Guerra B, Hernandez JR, Pascual A. (2004). Characterization of the first CTX-M-14-producing *Salmonella enterica* serotype Enteritidis isolate. *Journal of Antimicrobial Chemotherapy* 53:1113-1114.

Stephen JM, Toleman MA, Walsh TR, Jones RN, The SENTRY Program Participants Group. (2003). *Salmonella* bloodstream infections: Report from the SENTRY Antimicrobial Surveillance Program (1997-2001). *International Journal of Antimicrobial Agents* 22:395-405.

Walther-Rasmussen H, Hoiby N. (2004). Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum ß-lactamases. *Canadian Journal of Microbiology* 50:137-165.