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Antimicrobial susceptibility of *Salmonella*, 2016

Hospital and community laboratories are requested to refer all *Salmonella* isolated from human salmonellosis cases to ESR for serotyping and the laboratory-based surveillance of this disease. *Salmonella* from other sources, including food, animal and environmental sources, are also referred to ESR for typing. The antimicrobial susceptibility of a sample (approximately 20%) of non-typhoidal *Salmonella* isolates and all typhoidal isolates is routinely tested at ESR. In addition, the susceptibility of all isolates belonging to internationally recognised multidrug-resistant *Salmonella* clones is tested. These clones include *S.* Typhimurium phage types DT12, DT104, DT120, DT193 and U302, and *S. enterica* serovar 4,[5],12:i:-.

In 2016, antimicrobial susceptibility was determined by the European Committee on Antimicrobial Susceptibility (EUCAST) disc diffusion method.[1] Azithromycin, cephalothin, streptomycin, sulphonamide and tetracycline zone diameters were interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints,[2] as there are no EUCAST breakpoints for these antibiotics. EUCAST clinical breakpoints were used to interpret the zone diameters of all other antibiotics.[3] All cephalothin-resistant isolates were further tested for the production of extended-spectrum β-lactamase and plasmid-mediated AmpC β-lactamase. Multidrug resistance is defined as resistance to ≥3 antibiotic classes. Overseas travel history for human salmonellosis cases was obtained from information reported in the EpiSurv notifiable disease database supplemented with any additional travel information received when the isolate from the case was referred to ESR.

Non-typhoidal Salmonella

In 2016, the antimicrobial susceptibility of a representative sample of 370 non-typhoidal *Salmonella* was tested. The sample comprised 237 isolates from human sources and 133 food/animal/environmental isolates.

Resistance to each of the 10 antimicrobials tested and multidrug resistance is shown in Table 1. Antimicrobial resistance among *Salmonella* remains relatively low, with 90.0% (86.9% of human isolates and 95.5% of food/animal/environmental isolates) fully susceptible to all 10 antimicrobials.

None of the isolates tested in 2016 produced a β-lactamase that would confer resistance to 3rd-generation cephalosporins such as ceftriaxone. 4.1% of the *Salmonella* tested (5.9% from human sources and 0.8% from other sources) were categorised as ciprofloxacin resistant when tested with a 5 µg pefloxacin disc – a surrogate test to predict ciprofloxacin susceptibility and which detects low-level ciprofloxacin resistance more reliably than testing with a ciprofloxacin disc. Patients with systemic infections caused by *Salmonella* strains that have low-level ciprofloxacin resistance may fail fluoroquinolone treatment or have a delayed response to such treatment.

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Salmonella from human sources were significantly (p <0.05) more resistant to ampicillin, ciprofloxacin and sulphonamides than *Salmonella* from other sources (Table 1). When the same comparison was confined to only human salmonellosis cases who had no reported recent overseas travel, there were no significant differences in resistance between *Salmonella* from human sources and those from other sources.

Table 1. Antimicrobial resistance among non-typhoidal *Salmonella*, 2016

Antimicrobial	Percent resistant			P value for significance of any difference in resistance between human and other isolates[1]
	All isolates n = 370	Human isolates n = 237	Food/animal/ environmental isolates n = 133	
Ampicillin	4.1	5.9	0.8	0.016
Amoxicillin-clavulanate	1.1	1.3	0.8	1.000
Cephalothin	0.0	0.0	0.0	-
Chloramphenicol	1.6	2.5	0.0	0.092
Ciprofloxacin[2]	4.1	5.9	0.8	0.016
Co-trimoxazole	2.4	3.4	0.8	0.165
Gentamicin	0.3	0.4	0.0	1.000
Streptomycin	3.0	4.2	0.8	0.106
Sulphonamides	4.6	6.3	1.5	0.033
Tetracycline	5.1	6.8	2.3	0.060
Multiresistant to ≥3 antimicrobials	3.8	5.1	1.5	0.085

1 Chi-square test or Fisher's Exact test as appropriate.
2 Ciprofloxacin susceptibility was inferred from the results of pefloxacin 5 µg disc testing.

Table 2 shows a comparison of resistance among isolates from salmonellosis cases reported to have recently travelled overseas with isolates from cases for whom no recent overseas travel was reported. *Salmonella* from people who had travelled were consistently more resistant and multidrug resistant, and the differences were significant for all antimicrobials except amoxicillin-clavulanate and gentamicin (and cephalothin for which no resistance was detected).

Table 2. Antimicrobial resistance among non-typhoidal *Salmonella* from cases who had travelled overseas compared with non-travellers, 2016

Antimicrobial	Percent resistant		P value for significance of any difference in resistance between travellers and non-travellers[1]
	Cases who had travelled overseas n = 68	Cases who had not travelled overseas n = 169	
Ampicillin	19.1	0.6	<0.001
Amoxicillin-clavulanate	2.9	0.6	0.199
Cephalothin	0.0	0.0	-
Chloramphenicol	7.4	0.6	0.008
Ciprofloxacin	16.2	1.8	<0.001
Co-trimoxazole	10.3	0.6	0.001
Gentamicin	1.5	0.0	0.287
Streptomycin	13.2	0.6	<0.001
Sulphonamides	17.7	1.8	<0.001
Tetracycline	22.1	0.6	<0.001
Multiresistant to ≥3 antimicrobials	16.2	0.6	<0.001

1 Chi-square test or Fisher's Exact test as appropriate.

In 2016, several isolates belonging to internationally recognised multidrug-resistant *Salmonella* clones were identified and tested. These included:

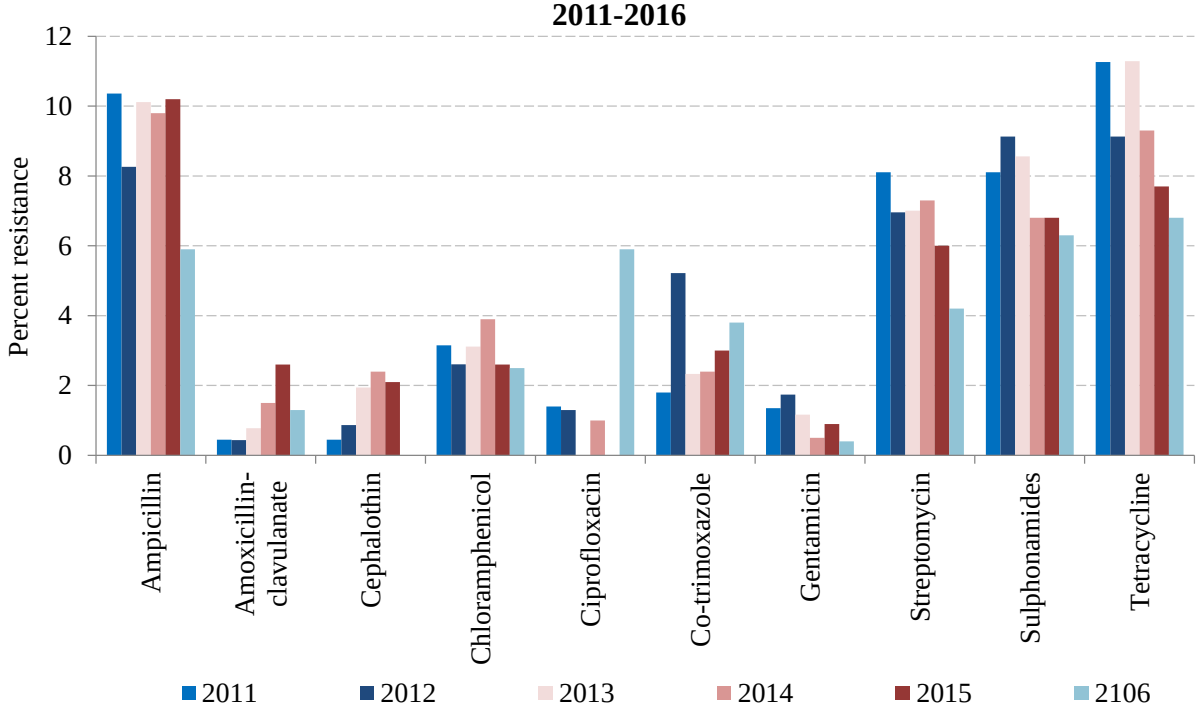
- 11 isolates of *S.* Typhimurium phage type DT12, all of which were fully susceptible.
- 9 isolates of *S.* Typhimurium phage type DT193, all of which were fully susceptible.
- 3 isolates of *S.* Typhimurium phage type DT120, two of which were fully susceptible but the third isolate, from a patient who had travelled to Southeast Asia, was multidrug resistant.
- 1 isolate of *S.* Typhimurium phage type U302, which was fully susceptible.

No isolates of the other internationally recognised multidrug-resistant *S.* Typhimurium clone, phage type DT104, were identified in 2016.

S. enterica serovar 4,[5],12:i:- is a monophasic variant of *S.* Typhimurium, and isolates are typically multiresistant to ampicillin, streptomycin, sulphonamides and tetracycline. This serovar is now one of the commonest *Salmonella* serovars isolated from humans in many countries in Europe. It was the seventh most common serovar in New Zealand in 2016, with 34 isolates, all from human salmonellosis cases, identified. Thirty (88.2%) of the 34 isolates were multidrug resistant, 27 of which had the resistance pattern typical of this serovar (ie, resistant to at least ampicillin, streptomycin, sulphonamides and tetracycline). The resistance pattern of four of the multidrug-resistant isolates also included ciprofloxacin resistance. Travel history was reported for 28 of the 30 multidrug-resistant cases, 20 of whom had recently travelled overseas: Thailand (10 cases), Indonesia (3), China (3), Vietnam (1), Philippines (1), Malaysia (1) and New Caledonia (1).

Trends in resistance among *Salmonella* from human cases since 2011 are shown in Figure 1. Except for ciprofloxacin, there have been no significant (p <0.05) changes in resistance over the last 6 years. The apparent increase in ciprofloxacin resistance is likely due to the change in test methods (ie, use of the surrogate pefloxacin disc which detects more low-level ciprofloxacin resistance than testing with ciprofloxacin itself).

Figure 1. Resistance among non-typhoidal *Salmonella* from human cases, 2011-2016



The ciprofloxacin resistance rates for the years 2011 to 2015 are based on ciprofloxacin disc susceptibility testing and the current CLSI breakpoints. The rate for 2016 is based on testing with the surrogate pefloxacin disc and EUCAST breakpoints. Testing with a pefloxacin disc is more likely to detect low-level ciprofloxacin resistance than ciprofloxacin disc susceptibility testing. This change in test procedures is likely to account for the apparent increase in ciprofloxacin resistance in 2016.

Typhoidal Salmonella

In 2016, 41 *S.* Typhi, 12 *S.* Paratyphi A and 3 *S.* Paratyphi B isolates were referred to ESR and available for susceptibility testing. Resistance among these typhoidal *Salmonella* to each of the 11 antimicrobials tested is shown in Table 3.

Over half of the 41 *S.* Typhi isolates were categorised as ciprofloxacin resistant when tested with a 5 µg pefloxacin disc. As noted above, testing with a pefloxacin disc detects low-level ciprofloxacin resistance more reliably than testing with a ciprofloxacin disc. This detection of low-level resistance is likely to account for the large increase in ciprofloxacin resistance among *S.* Typhi recorded in 2016 (61.0% vs 4.3% in 2015). With one exception, where the patient's travel history was known, ciprofloxacin-resistant *S.* Typhi were isolated from patients who had been in the Indian subcontinent.

Due to the emergence of ciprofloxacin non-susceptibility among *S.* Typhi in the Indian subcontinent and Southeast Asia, azithromycin is now the recommended treatment for typhoid fever. No azithromycin resistance was detected among the *S.* Typhi in 2016.

Two (4.9%) of the *S.* Typhi isolates were multidrug resistant, and both had an identical pattern of resistance to ampicillin, amoxicillin-clavulanate, chloramphenicol, ciprofloxacin, co-trimoxazole, streptomycin and sulphonamides. Both patients with multidrug-resistant *S.* Typhi has recently travelled to Pakistan.

None of the *S.* Paratyphi A or *S.* Paratyphi A were multidrug resistant, however, a high proportion were ciprofloxacin resistant (Table 3).

Table 3. Antimicrobial resistance among *Salmonella* Typhi and *S*. Paratyphi, 2016[1]

Antimicrobial	Percent (number) resistant		
	***S*. Typhi n = 41**	***S*. Paratyphi A n = 12**	***S*. Paratyphi B[1] n =3**
Ampicillin	4.9 (2)	0.0 (0)	33.0 (1)
Amoxicillin-clavulanate	4.9 (2)	0.0 (0)	0.0 (0)
Azithromycin	0.0 (0)	-[2]	-[2]
Cephalothin	0.0 (0)	0.0 (0)	0.0 (0)
Chloramphenicol	4.9 (2)	0.0 (0)	0.0 (0)
Ciprofloxacin	61.0 (25)	83.3 (10)	33.0 (1)
Co-trimoxazole	4.9 (2)	0.0 (0)	0.0 (0)
Gentamicin	0.0 (0)	0.0 (0)	0.0 (0)
Streptomycin	17.1 (7)	0.0 (0)	0.0 (0)
Sulphonamides	4.9 (2)	0.0 (0)	0.0 (0)
Tetracycline	0.0 (0)	0.0 (0)	0.0 (0)
Multiresistant to ≥3 antimicrobials	4.9 (2)	0.0 (0)	0.0 (0)

1 *S.* Paratyphi B var Java isolates are not included with the *S.* Paratyphi isolates, as they are no longer considered to belong to the typhoidal Salmonella.

2 There are no CLSI azithromycin interpretive standards for *S.* Paratyphi.

[1] European Committee on Antimicrobial Susceptibility Testing. Antimicrobial susceptibility testing. EUCAST disk diffusion method. Available at http://eucast.org/.

[2] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 26th ed. CLSI supplement M100S. Wayne, PA, USA: CLSI; 2016.

[3] European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 6.0; 2016 Jan. Available from: URL: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_6.0_Breakpoint_table.pdf.