JOURNAL OF MEDICAL MICROBIOLOGY

SHORT COMMUNICATION

Guzmán-Martín *et al.*, *Journal of Medical Microbiology* 2018;67:965–967

DOI 10.1099/jmm.0.000755

MICROBIOLOGY SOCIETY

Nalidixic acid surrogate test for susceptibility to ciprofloxacin in *Salmonella*. Revisiting the question

José Luis Guzmán-Martín,[1] José María Navarro-Marí,[2] Manuela Expósito-Ruiz[3] and José Gutiérrez-Fernández[1,2,*]

Abstract

We investigated the reliability of nalidixic acid (NA) susceptibility as a marker of ciprofloxacin susceptibility in *Salmonella*, analysing 302 stool isolates. NC53 of the MicroScan system was used for NA susceptibility tests and the E-test was used for ciprofloxacin susceptibility tests. Among the isolates, 178 (58.9 %) were serogroup B, 74 (24.5 %) were serogroup D, 27 (8.9 %) were serogroup C and 23 (7.6 %) were from other minor serogroups. Globally, susceptibility to NA correctly predicted the susceptibility of *Salmonella* to ciprofloxacin, with a sensitivity of 81.5 %, a specificity of 97.6 %, and positive and negative predictive values of 88 and 96 %, respectively. However, there were differences among the serogroups in terms of sensitivity (P<0.001) and positive predictive values (P=0.013). NA is a reliable marker for serogroup D, but not for serogroups B or C. According to these findings, NA susceptibility measured with the MicroScan system can be used as a marker of ciprofloxacin resistance in some serogroups in our setting.

Salmonella is responsible for a wide range of human diseases and is one of the leading causes of food-borne disease in our region [1, 2]. Nontyphoidal *Salmonella* causes gastroenteritis with self-limiting diarrhoea, cramping and acute fever [3], and is the second most frequently isolated bacteria from diarrhoea faeces after *Campylobacter*. The high incidence of nontyphoidal salmonellosis has considerable economic impact, and the total annual cost of the disease is estimated to be $4.4 billion in the USA alone [4]. There is a need for more economical methods to detect the infection and perform antibiotic susceptibility tests for identified strains. In a recent study of stool cultures in our region [5], 54 (59.3 %) out of 91 isolates of *Salmonella enterica* belonged to *Salmonella* serogroup D, which is susceptible to cefotaxime (100 %), ciprofloxacin (55.6 %), ampicillin (94.3 %) and trimethoprim/sulfamethoxazole (100 %), while 33 (36.3 %) belonged to serogroup B, which is susceptible to cefotaxime (100 %), ciprofloxacin (78.8 %), trimethoprim/sulfamethoxazole (86.9 %) and ampicillin (9.1 %), and 2 (2.2 %) belonged to serogroup C, which is susceptible (100 %) to all of the above antibiotics. Empirical treatment with ciprofloxacin is therefore not valid due to the high resistance rates, and it is recommended that the susceptibility of antibiotics be studied before treatments are selected.

The MicroScan system (Siemens Healthcare Diagnostics, USA) is widely used to evaluate antibiotic susceptibility, but most of the susceptibility panels in this system cannot detect ciprofloxacin susceptibility; therefore, the minimum inhibitory concentration (MIC) must be established separately with an E-test, increasing the expenditure and diagnostic delay. Our hypothesis was that nalidixic acid (NA) susceptibility, determined using the MicroScan system, could be used as a surrogate test when a ciprofloxacin MIC test is not possible, avoiding this additional step. The objective of this study was to determine the predictive capacity of NA susceptibility as a marker of ciprofloxacin susceptibility in our setting.

A total of 302 stool isolates of *Salmonella* spp. were analysed in the microbiology laboratory of the Virgen de las Nieves University Hospital in Granada (Spain) from 2012 through 2016. The stool samples were from paediatric and adult patients (age 0–86 years old) from the Emergency, Paediatrics and Digestive departments. All isolates underwent NA and ciprofloxacin susceptibility tests. *Salmonella* isolates were identified and grouped as previously reported [6], with serogroups being determined by slide agglutination using specific antisera. Because phase 1 or phase 2 flagellar antigens were not studied, serotypes were not considered. Panel NC53 of the MicroScan system was used to determine

Received 6 February 2018; Accepted 28 April 2018

Author affiliations: [1]Departamento de Microbiología-Instituto de Investigación Biosanitaria de Granada, Universidad de Granada, Granada, Spain; [2]Laboratorio de Microbiología, Hospital Universitario Virgen de las Nieves- Instituto de Investigación Biosanitaria de Granada, Granada, Spain; [3]Metodología de la Investigación y Bioestadística de la Fundación FIBAO, Granada, Spain.

***Correspondence:** José Gutiérrez-Fernández, josegf@go.ugr.es

Keywords: enteritis; *Salmonella*; nalidixic acid; ciprofloxacin; stools.

Abbreviations: MIC, minimum inhibitory concentration; NA, nalidixic acid; NPV, negative predictive value; PPV, positive predictive value.

All authors meet the ICMJE authorship criteria.

Table 1. Comparison of nalidixic acid (NA) and ciprofloxacin susceptibility tests in different *Salmonella* serogroups

Serogroups	Susceptibility to NA and ciprofloxacin				Reliability of NA for the detection of ciprofloxacin resistance			
	SS	SR	RS	RR	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Serogroup B	159	9	4	6	40 %	97.5 %	60 %	94.6 %
Serogroup C	22	1	1	3	75 %	95.6 %	75 %	95.6 %
Serogroup D	43	0	1	30	100 %	97.7 %	96.8 %	100 %
Others	18	0	0	5	100 %	100 %	100 %	100 %
Total	242	10	6	44	81.5 %	97.6 %	88 %	96 %
P-value	Fisher's exact test				<0.001	0.870	0.013	0.411

SS, nalidixic acid-susceptible and ciprofloxacin-susceptible; SR, nalidixic acid-susceptible and ciprofloxacin-resistant; RS, nalidixic acid-resistant and ciprofloxacin-susceptible; and RR, nalidixic acid-resistant and ciprofloxacin-resistant.

NA susceptibility and the E-test (Liofilchem, Roseto degli Abruzzi, Italy) was used to determine ciprofloxacin susceptibility. The MIC breakpoints selected for reduced susceptibility were $>0.125\,\mu g\,ml^{-1}$ for ciprofloxacin and $>16\,\mu g\,ml^{-1}$ for NA, based on previous studies [7]. We performed Fisher's exact test to compare sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) among the serogroups [8].

Out of the 302 isolates of *Salmonella* spp., 178 (58.9 %) were serogroup B, 74 (24.5 %) were serogroup D, 27 (8.9 %) were serogroup C and 23 (7.6 %) were from other minor serogroups. Table 1 presents the ciprofloxacin and NA susceptibility test results for the different isolates. Statistically significant differences were found among the serogroups in terms of sensitivity ($P<0.001$) and PPV ($P=0.013$).

Various studies have described a correlation between NA resistance and ciprofloxacin resistance in *Salmonella* spp. [9–12]. The performance of NA as a ciprofloxacin resistance marker has been widely reviewed in *S. enterica* var. Typhi [13, 14], but little studied in other serovars. Overall, susceptibility to NA accurately predicted the susceptibility of *Salmonella* to ciprofloxacin in the present study. Our global result is consistent with previous findings indicating that the identification of NA resistance had a sensitivity of 100 % and specificity of 87.3 % to detect isolates for which the ciprofloxacin MIC was $\geq 0.125\,\mu g\,ml^{-1}$ [10]. However, the sensitivity for the detection of ciprofloxacin resistance in serogroup B *Salmonella* was low (40 %) in our study, with a PPV of only 60 %. The majority of our cases were serogroup B, and the fact that only 6 out of 15 (40 %) ciprofloxacin resistances were correctly detected by NA resistance in this serogroup is a major limitation of NA screening in *Salmonella* species other than *S. Typhi*. Therefore, NA is not reliable as a surrogate marker to predict ciprofloxacin resistance in serogroup B, but it did achieve a markedly superior PPV for the other serogroups. However, the specificity (97.5 %) and NPV (94.6 %) of NA for the detection of ciprofloxacin resistance in serogroup B remained high, indicating that susceptibility values are more reliable than resistance values. In 2005, Hakanen *et al.* [15] reported the emergence of cases of the Southeast Asia strain that were susceptible to NA but resistant to ciprofloxacin, in line with the present findings. One possible reason for the reduced sensitivity of NA in serogroup B in our setting may be that this serogroup accumulates other resistance mechanisms that are not detected by the study of susceptibility.

Salmonella serogroup D and other serogroups are much more resistant to fluoroquinolone than serogroups B and C. Thus, NA is a useful marker to predict fluoroquinolone resistance in serogroup D but not serogroups B or C.

Finally, MicroScan recently incorporated a new panel (Negative MIC EN 47) that includes a well with $0.12\,mg\,l^{-1}$ ciprofloxacin and may serve to detect *Salmonella* resistance, with no need for the E-test, although it also contains antibiotics that may not be relevant for other bacteria isolated in the microbiology laboratory. The results for MicroScan reliability may vary according to the characteristics of the strains in different regions. In Spain, NA susceptibility tested with the MicroScan system can be used as a ciprofloxacin resistance marker in *Salmonella* serogroup D. According to the present findings, although no single test detects resistance due to all of the possible ciprofloxacin resistance mechanisms in *Salmonella* spp., as indicated by the CLSI [7], NA in MicroScan panels could be used as a marker of ciprofloxacin resistance for some serogroups in our setting. In conclusion, MicroScan panels with NA can be applied with care to predict ciprofloxacin resistance in *Salmonella* species, with the exception of serogroup B, the most frequent serogroup in our series.

Funding information
Parts of this work were supported by the CTS-521 research group. The authors are affiliated to the Granada Institute for Biomedical Research (IBS), the University Hospital Complex Virgen de las Nieves and the University of Granada. J. G. F. is the Director of the Culture Unit and coordinates the activities of Research Group CTS-521 of the Ministry of Economy, Innovation and Science of the Andalusian regional government in Granada.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The study protocol was carried out in accordance with the Declaration of Helsinki. This was a non-interventional study with no investigation

in addition to routine procedures. Biological material was only used for standard enteric infection diagnostics following physicians' prescriptions. No additional sampling or modification of the routine sampling protocol was performed. Data analyses were carried out using an anonymous database. Therefore, ethics committee approval was considered unnecessary according to national guidelines (Law on Data Protection – Organic Law 15/1999 of 13 December on the protection of data of a personal nature, https://www.boe.es/buscar/doc.php?id=BOE-A-1999-23750).

References

1. **Crum-Cianflone NF.** Salmonellosis and the gastrointestinal tract: more than just peanut butter. *Curr Gastroenterol Rep* 2008;10:424–431.
2. **Sánchez-Capilla AD, Sorlózano-Puerto A, Rodríguez-Granger J, Martínez-Brocal A, Navarro-Marí JM *et al.*** Infectious etiology of diarrheas studied in a third-level hospital during a five-year period. *Rev Esp Enferm Dig* 2015;107:89–97.
3. **Hohmann EL.** Nontyphoidal salmonellosis. *Clin Infect Dis* 2001;32:263–269.
4. **Cummings KJ, Warnick LD, Gröhn YT, Hoelzer K, Root TP *et al.*** Clinical features of human salmonellosis caused by bovine-associated subtypes in New York. *Foodborne Pathog Dis* 2012;9:796–802.
5. **Jiménez-Guerra G, Foronda García-Hidalgo C, Moreno-Torres IS, Gutiérrez Fernández J, Navarro Marí JM.** Sensibilidad antibiótica de Campylobacter spp. y de Salmonella enterica en pacientes con enteritis. *Rev Soc Andaluza Microbiol Parasitol Clín* 2017;1:10.
6. **Gómez-Camarasa C, Gutiérrez-Fernández J, Rodríguez-Granger JM, Sampedro-Martínez A, Sorlózano-Puerto A *et al.*** Evaluation of the rapid RIDAQUICK Campylobacter® test in a general hospital. *Diagn Microbiol Infect Dis* 2014;78:101–104.
7. **CLSI.** *Performance Standards for Antimicrobial Susceptibility Testing*, 27th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2017.
8. **Mehta CR, Patel NR.** Algorithm 643: FEXACT: a FORTRAN subroutine for Fisher's exact test on unordered r×c contingency tables. *ACM Trans Math Softw* 1986;12:154–161.
9. **Angelo KM, Reynolds J, Karp BE, Hoekstra RM, Scheel CM *et al.*** Antimicrobial resistance among nontyphoidal *Salmonella* isolated from blood in the United States, 2003–2013. *J Infect Dis* 2016;214:1565–1570.
10. **Ryan MP, Dillon C, Adley CC.** Nalidixic acid-resistant strains of *Salmonella* showing decreased susceptibility to fluoroquinolones in the midwestern region of the Republic of Ireland due to mutations in the gyrA gene. *J Clin Microbiol* 2011;49:2077–2079.
11. **Crump JA, Barrett TJ, Nelson JT, Angulo FJ.** Reevaluating fluoroquinolone breakpoints for *Salmonella enterica* serotype Typhi and for non-Typhi *Salmonellae*. *Clin Infect Dis* 2003;37:75–81.
12. **Hakanen A, Kotilainen P, Jalava J, Siitonen A, Huovinen P.** Detection of decreased fluoroquinolone susceptibility in Salmonellas and validation of nalidixic acid screening test. *J Clin Microbiol* 1999;37:3572–3577.
13. **Ray P, Sharma J, Marak RS, Garg RK.** Predictive efficacy of nalidixic acid resistance as a marker of fluoroquinolone resistance in *Salmonella enterica* var Typhi. *Indian J Med Res* 2006;124:105–108.
14. **Kapil A, Renuka, Das B.** Nalidixic acid susceptibility test to screen ciprofloxacin resistance in *Salmonella typhi*. *Indian J Med Res* 2002;115:49–54.
15. **Hakanen AJ, Lindgren M, Huovinen P, Jalava J, Siitonen A *et al.*** New quinolone resistance phenomenon in *Salmonella enterica*: nalidixic acid-susceptible isolates with reduced fluoroquinolone susceptibility. *J Clin Microbiol* 2005;43:5775–5778.