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# Fluoroquinolone-Resistant *Streptococcus agalactiae* Invasive Isolates Recovered in Argentina

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***Background:*** *Streptococcus agalactiae* or group B *Streptococcus* (GBS) is an important pathogen in neonates and nonpregnant individuals. Epidemiological studies of GBS resistance to fluoroquinolones (FQs) in Latin America are scarce. This study aimed to determine the local prevalence of FQ resistance in the frame of a national, prospective multicenter study of invasive GBS infections and to investigate mechanisms of resistance, serotype distribution, and clonal relationships among resistant isolates.

***Methods:*** From July 2014 to July 2015, 162 invasive GBS isolates were collected from 86 health care centers in 32 Argentinean cities. All isolates were screened for FQ nonsusceptibility using a five-disc scheme: levofloxacin (LVX), ciprofloxacin, norfloxacin (NOR), ofloxacin, and pefloxacin (PF). LVX minimal inhibitory concentration (MIC) was determined by the agar dilution method. Sequencing of internal regions of *gyrA* and *parC* genes was performed. Capsular typing and genetic characterization of nonsusceptible isolates were assessed by latex agglutination, pulsed-field gel electrophoresis (PFGE), and multilocus sequence typing.

***Results:*** Twenty-four of one hundred sixty-two GBS isolates exhibited no inhibition zones to all tested FQs with an MIC range of 16–32 mg/L for LVX, and one isolate with MIC = 1 mg/L showed no inhibition zones around NOR and PF discs. In all resistant isolates, point mutations were detected in both genes. Serotype Ib was prevalent (88%). One PFGE type accounted for 84% of the FQ-resistant isolates and belonged to serotype Ib, sequence type 10.

***Conclusions:*** The prevalence of FQ resistance was 14.8% likely to be associated with dissemination of an ST10/serotype Ib clone. The unexpected high rate of resistance emphasizes the relevance for continuous surveillance of GBS epidemiology and antibiotic susceptibility.

**Keywords:** antibiotic resistance, epidemiology, fluoroquinolones, *Streptococcus agalactiae*



## Introduction

*Streptococcus agalactiae* or group B *Streptococcus* (GBS) is the leading cause of neonatal sepsis and meningitis and it has been recognized as an important pathogen in nonpregnant individuals, especially elderly people and those suffering from underlying medical disorders.[1,2]

Penicillin (PEN) remains the first choice to treat GBS infections, although strains with reduced susceptibility to this antibiotic have been recently described.[3,4] Alternative therapies are the use of macrolides and lincosamides; unfortunately, resistance to this class of antibiotics has emerged in the last decades.[5,6] Resistance to fluoroquinolone (FQ) in GBS was first described in Japan in 2003 and then it became a growing problem.[7] However, reports describing the increase of resistance rates to FQs in GBS are scarce, especially in isolates recovered from invasive infections. Resistance has been documented in Spain, United States of America, and Taiwan, but the highest levels of rates were found in Korea.[8–11] In Argentina, FQ resistance in GBS was first reported after a national surveillance study of noninvasive infections performed between 2005 and 2007; the prevalence of FQ resistance was 0.9%.[12] In other countries of Latin America, similar studies found low FQ resistance levels.[5,13]

The major mechanism of FQ resistance among GBS is related to point mutations acquired through a stepwise process in the quinolone resistance-determining region (QRDR) of *gyrA* and *parC* genes, which encode DNA gyrase and topoisomerase IV. These substitutions weaken

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the interaction between quinolones and enzymes. The *parC* gene is the primary target, which substantially increases the probability of a second mutation in *gyrA*, resulting in high-level quinolone resistance.[10] Frequently described mutations in *parC* are S79Y and S79F and in *gyrA* occurring predominantly at amino acid position 81 (S81L).[9] Infrequently, active efflux was described in GBS isolates with no mutations in the QRDRs.[14]

In the context of a national, prospective multicenter study of invasive infections of GBS, our purpose was to determine the local prevalence of FQ resistance. In addition, we sought to comprehensively characterize mechanisms of resistance, serotype distribution, and clonal relationships among resistant isolates.

## Methods

### *Study design*

A prospective observational study was designed to evaluate the prevalence and phenotypic and molecular features of FQ-resistant GBS isolates recovered from patients suffering from invasive disease caused by *S. agalactiae*. This study was accomplished during one complete year, from July 1, 2014, to June 30, 2015, in 86 health care centers from 32 Argentinean cities. Ethical approval was provided by the Ethics Committee of the Faculty of Pharmacy and Biochemistry, Universidad de Buenos Aires, Res (D) N4467/14.

### *Antibiotic and susceptibility tests*

A five-disc scheme was designed using levofloxacin (LVX 5-μg discs), ciprofloxacin (CIP 5-μg discs), norfloxacin (NOR 10-μg discs), ofloxacin (5-μg discs), and pefloxacin (PF 5-μg discs) based on the Clinical and Laboratory Standards Institute (CLSI) and Société Française de Microbiologie recommendations.[15,16] The use of PF was included as it was proposed by Varon *et al.*, and the combination of discs allows to predict the presence of *gyrA* and *parC* mutations.[17] The minimal inhibitory concentration (MIC) of LVX was determined by the agar dilution method. PEN (10-μg discs), erythromycin (15-μg discs), clindamycin (2-μg discs), and aminoglycosides (Streptomycin 300-μg and Gentamicin 120-μg discs to detect high-level resistance) were tested by the disc diffusion method.[15]

### *Amplification and sequencing of* gyr*A and* par*C fragments*

Amplification and sequencing of internal regions of *gyrA* and *parC* genes were accomplished as described elsewhere.[6] Analysis of deduced amino acid sequences was performed using the software Vector NTI Express, InforMax Inc. The *S. agalactiae* strain GD201008-001 (Accession number: cp003810.1 GenBank: https://ncbi.nlm.nih.gov/nuccore/cp003810.1) was used as a reference strain for comparative analysis.

### *Typing*

Capsular typing was done using the Strep-B Kit (Statens Serum Institut, Copenhagen, Denmark). Pulsed-field gel electrophoresis (PFGE) was performed as it was described previously using *Apa*I, with minor modifications.[18] Comparison of fingerprints was carried out by the unweighted pair-group method applying the Dice correlation coefficient. A similarity of 80% was used to define a PFGE group and with this cutoff value, profiles with differences in 1–6 bands were assigned to the same clonal type and coded with capital letters. Multilocus sequence typing (MLST) was performed as described elsewhere (http://pubmlst.org/sagalactiae).

## Results

A total of 162 isolates were recovered from invasive infections. According to the CLSI breakpoint, the resistance rate to LVX was 14.8%.[15] By the five-disc method, 24 isolates exhibited no inhibition zones to all tested FQs. One isolate, denominated GBS30, showed no inhibition zones to NOR and PF discs. The MIC range of LVX for the aforementioned isolates (24/25) was 16–32 mg/L and the MIC value for GBS30 was 1 mg/L.

In this study, resistant isolates proceeded from 13 different health care centers sited in 10 cities from 7 provinces across the country. The analysis of epidemiological data (Table 1) showed that 16 isolates were recovered from adult women. Eleven of them had at least one underlying condition and diabetes mellitus was the most frequent one. Moreover, 11/23 adults reported previous antibiotic use during the 10-day period before GBS infection was confirmed. In six of these eleven cases, CIP was employed. Only two isolates proceeded from early onset neonatal sepsis (24 and 48 h after birth, respectively). Nine of twenty-five isolates were recovered from skin or soft tissue infections and 8/25 from bacteremia.

All resistant isolates had amino acid changes within the QRDRs of *gyrA* and *parC* compared with the reference sequence. The mutations were S79F (23/24) or S79Y (1/24) in the product of *parC* and S81L in the product of *gyrA* (24/24). The LVX MIC range of 16–32 mg/L was associated with the presence of double mutations. The GBS30 strain exhibited a single-nucleotide substitution exclusively in *parC*, resulting in S79C change. To our knowledge, this is the first description of this novel mutation in the *S. agalactiae parC* gene. Moreover, several silent base substitutions, especially in *parC*, were detected (data not shown).

All isolates were susceptible to penicillin. Coresistance to macrolides was found in three isolates (Table 1).

Serotype Ib was prevalent among FQ-resistant isolates (22/25, 88%), followed by V (8%) and III (4%).

By PFGE, five major types were defined, type A accounted for 84% of isolates (21/25). The remaining four isolates presented unrelated profiles (designated as type B to E). All PFGE type A isolates belonged to serotype Ib and were recovered from 12 hospitals distributed in nine different cities of Argentina. The isolates included in PFGE types D and E, GBS25 and GBS30, respectively, belonged to serotype V. One representative isolate from PFGE type A was studied by MLST and it belonged to sequence type 10.

## Discussion

The rate of FQ resistance recorded in this study (14.8%) is higher than the latest reports from our country and even those from other countries of Latin America and the rest of

TABLE 1. PULSED-FIELD GEL ELECTROPHORESIS TYPE, SEROTYPE, AND CLINICAL DATA OF GROUP B *STREPTOCOCCUS* ISOLATES ($N=25$) DESCRIBED IN THIS STUDY

| | | | | *Clinical data* | | | | |
|---|---|---|---|---|---|---|---|---|
| *Isolate* | *PFGE type* | *Serotype* | *Resistance profile* | *Gender* | *Age* | *Specimen* | *Underlying medical condition* | *City* |
| GBS22 | A | Ib | FQ | F | 51 years | SST | DM | Tierra del Fuego |
| GBS8 | A | Ib | FQ | M | 48 years | Blood | KD | Santa Fe |
| GBS14 | A | Ib | FQ | M | 55 years | SST | DM/HBP/KD | Mar del Plata |
| GBS2 | A | Ib | FQ | F | 34 years | SST | Schizophrenia | San Miguel de Tucumán |
| GBS13 | A | Ib | FQ | F | 31 years | Genital and perinatal | ND | Buenos Aires |
| GBS21 | A | Ib | FQ | F | 26 years | SST | DM/hypothyroidism | Santa Fe |
| GBS1 | A | Ib | FQ | M | 87 years | Blood | WO | Río Negro |
| GBS20 | A | Ib | FQ | F | 32 years | Genital and perinatal | ND | Buenos Aires |
| GBS26 | A | Ib | FQ | M | 2 days | Blood | WO | Avellaneda |
| GBS27 | A | Ib | FQ | F | ND | Genital and perinatal | Ovarian cancer | Buenos Aires |
| GBS24 | A | Ib | FQ | F | ND | Genital and perinatal | ND | Buenos Aires |
| GBS11 | A | Ib | FQ | F | 39 years | SST | WO | Maipú |
| GBS7 | A | Ib | FQ, $iMLS_B$ | M | 45 years | SST | DM | Santa Fe |
| GBS31 | A | Ib | FQ | F | 51 years | SST | WO | Maipú |
| GBS23 | A | Ib | FQ | F | 61 years | Blood | HBP | Buenos Aires |
| GBS19 | A | Ib | FQ | F | 92 years | Blood | HBP | Buenos Aires |
| GBS12 | A | Ib | FQ | M | 66 years | Bone and joint | DM | Buenos Aires |
| GBS6 | A | Ib | FQ | F | 18 years | Genital and perinatal | WO | Buenos Aires |
| GBS10 | A | Ib | FQ | F | 55 years | Blood | Tumor | Maipú |
| GBS18 | A | Ib | FQ | M | 58 years | SST | ND | Maipú |
| GBS3 | A | Ib | FQ | M | 73 years | SST | WO | Buenos Aires |
| GBS9 | B | Ib | FQ, AG | F | 1 day | Blood | WO | Granadero Baigorria |
| GBS5 | C | III | FQ, AG, $cMLS_B$ | F | 36 years | Blood | WO | Córdoba |
| GBS25 | D | V | FQ, $iMLS_B$ | F | 29 years | Abdominal | ND | Buenos Aires |
| GBS30 | E | V | [a] | F | 36 years | Genital and perinatal | WO | Buenos Aires |

[a]Resistant to norfloxacin and pefloxacin and susceptible to levofloxacin, ciprofloxacin, and ofloxacin.

AG, aminoglycosides; FQ, fluoroquinolone; GBS, group B *Streptococcus*; PFGE, pulsed-field gel electrophoresis; ND, no available data; SST, skin and soft tissue; DM, diabetes mellitus; KD, kidney disease; HBP, high blood pressure; WO, without underlying condition; $iMLS_B$, inducible phenotype; $cMLS_B$, constitutive phenotype.

the world, except Korea.[9,12] Differences in antibiotic use might be the major factors contributing to geographic differences in FQ resistance.

According to the CLSI interpretative breakpoint, the isolate GBS30 is categorized as susceptible. However, the absence of an inhibition zone around the disc of NOR indicates that it should be interpreted as decreased susceptibility with the remark that there is a high risk of clinical failure due to *in vivo* selection of FQ-resistant mutants.[16] The scheme of five discs was able to detect GBS30, with no inhibition zones to two FQs (NOR and PF) and exhibiting a single-nucleotide substitution exclusively in *parC*. The lack of an inhibition zone could represent an alert of first-step mutation and also a high risk to select an FQ-resistant mutant *in vivo*, with consequent treatment failure. The use of a 10-μg NOR disc test as a surrogate marker, by which to detect low-level FQ-resistant mutants harboring only topoisomerase IV mutations, was previously proposed in *S. pneumoniae*.[17] Herein, we demonstrate that this strategy could detect a GBS single-step mutant in *parC*.

Serotype Ib was the most frequent serotype among FQ-resistant isolates. Considering the entire collection of 162 invasive isolates, serotypes Ia and Ib were more frequent (28.4% and 28.4%, respectively, data not shown). Moreover, serotype distribution in FQ-resistant isolates is significantly different from the rest of the isolates that were susceptible to FQ ($p < 0.005$, chi-square analysis).

Reduced susceptibility to penicillin among FQ-resistant GBS isolates was described by Kimura *et al.*, but in our collection, all FQ-resistant isolates were susceptible to penicillin.[19]

In this study, as described by other authors, the relationship between the serotype and PFGE profiles was detected.[5] The prevalence of clonal complex 10 among LVX-resistant *S. agalactiae* was previously reported in Korea[9] and Japan.[20]

In conclusion, the increase in FQ resistance may be explained by dissemination of a single clone serotype Ib that accounts for 84% of the GBS FQ-resistant isolates. Our results emphasize the need for careful epidemiologic investigation. Furthermore, this study reinforces the importance of monitoring antibiotic susceptibility to quinolones. The inadvertent consequences of the use of FQs, particularly for treatment of urinary tract infections, may contribute to selection of resistant strains, especially considering that GBS is a frequent uropathogen.

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## Disclosure Statement

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## References

1. Baker, C.J. 2000. Group B streptococcal infections. In D.L. Stevens and E.L. Kaplan (ed.), Streptococcal infections. Oxford University Press, New York, pp. 222–237.
2. Skoff, T.H., M.M. Farley, S. Petit, A.S. Craig, W. Schaffner, K. Gershman, L.H. Harrison, R. Lynfield, J. Mohle-Boetani, S. Zansky, B.A. Albanese, K. Stefonek, E.R. Zell, D. Jackson, T. Thompson, and S.J. Schrag. 2009. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990–2007. Clin. Infect. Dis. 49: 85–92.
3. Kimura, K., Y. Nishiyama, S. Shimizu, J. Wachino, M. Matsui, S. Suzuki, K. Yamane, K. Shibayama, and Y. Arakawa. 2013. Screening for group B streptococci with reduced penicillin susceptibility in clinical isolates obtained between 1977 and 2005. Jpn. J. Infect. Dis. 66:222–225.
4. Kimura, K., S. Suzuki, J. Wachino, H. Kurokawa, K. Yamane, N. Shibata, N. Nagano, H. Kato, K. Shibayama, and Y. Arakawa. 2008. First molecular characterization of group B streptococci with reduced penicillin susceptibility. Antimicrob. Agents Chemother. 52:2890–2897.
5. Dutra, V.G., V.M. Alves, A.N. Olendzki, C.A. Dias, A.F. de Bastos, G.O. Santos, E.L. de Amorin, M.A. Sousa, R. Santos, P.C. Ribeiro, C.F. Fontes, M. Andrey, K. Magalhaes, A.A. Araujo, L.F. Paffadore, C. Marconi, E.F. Murta, P.C Fernandes, Jr., M.S. Raddi, P.S. Marinho, R.B. Bornia, J.K. Palmeiro, L.M. Dalla-Costa, T.C. Pinto, A.C. Botelho, L.M. Teixeira, and S.E. Fracalanzza. 2014. *Streptococcus agalactiae* in Brazil: serotype distribution, virulence determinants and antimicrobial susceptibility. BMC Infect. Dis. 14:323.
6. Lee, W.T., and M.C. Lai. 2015. High prevalence of *Streptococcus agalactiae* from vaginas of women in Taiwan and its mechanisms of macrolide and quinolone resistance. J. Microbiol. Immunol. Infect. 48:510–516.
7. Kawamura, Y., H. Fujiwara, N. Mishima, Y. Tanaka, A. Tanimoto, S. Ikawa, Y. Itoh, and T. Ezaki. 2003. First *Streptococcus agalactiae* isolates highly resistant to quinolones, with point mutations in *gyrA* and *parC*. Antimicrob. Agents Chemother. 47:3605–3609.
8. Miro, E., M. Rebollo, A. Rivera, M.T. Alvarez, F. Navarro, B. Mirelis, and P. Coll. 2006. [*Streptococcus agalactiae* highly resistant to fluoroquinolones]. Enferm. Infecc. Microbiol. Clin. 24:562–563 (Article in Spanish).
9. Ryu, H., Y.J. Park, Y.K. Kim, J. Chang, and J.K. Yu. 2014. Dominance of clonal complex 10 among the levofloxacin-resistant *Streptococcus agalactiae* isolated from bacteremic patients in a Korean hospital. J. Infect. Chemother. 20:509–511.
10. Wehbeh, W., R. Rojas-Diaz, X. Li, N. Mariano, L. Grenner, S. Segal-Maurer, B. Tommasulo, K. Drlica, C. Urban, and J.J. Rahal. 2005. Fluoroquinolone-resistant *Streptococcus agalactiae*: epidemiology and mechanism of resistance. Antimicrob. Agents Chemother. 49:2495–2497.
11. Wu, H.M., R.P. Janapatla, Y.R. Ho, K.H. Hung, C.W. Wu, J.J. Yan, and J.J. Wu. 2008. Emergence of fluoroquinolone resistance in group B streptococcal isolates in Taiwan. Antimicrob. Agents Chemother. 52:1888–1890.
12. Faccone, D., L. Guerriero, E. Mendez, L. Errecalde, H. Cano, N. Yoyas, A. Togneri, V. Romanowski, M. Galas, R. Whonet, and A. Corso. 2010. Fluoroquinolone-resistant *Streptococcus agalactiae* isolates from Argentina. Rev. Argent. Microbiol. 42:203–207.

13. Crespo-Ortiz, M.del.P., C.R. Castaneda-Ramirez, M. Recalde-Bolanos, and J.D. Velez-Londono. 2014. Emerging trends in invasive and noninvasive isolates of *Streptococcus agalactiae* in a Latin American hospital: a 17-year study. BMC Infect. Dis. 14:428.
14. Dang, T.N., U. Srinivasan, Z. Britt, C.F. Marrs, L. Zhang, M. Ki, and B. Foxman. 2014. Efflux-mediated resistance identified among norfloxacin resistant clinical strains of group B *Streptococcus* from South Korea. Epidemiol. Health 36:e2014022.
15. Clinical and Laboratory Standards Institute. 2016. Performance Standards for antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA.
16. Société Francaise de Microbiologie, Comité de l'Antibiogramme. París; 2018. Recommendations 2018. Available at https://www.sfm-microbiologie.org/userfiles/files/CASFM/CASFMV2 (accessed September 2018).
17. Varon, E., S. Houssaye, S. Grondin, and L. Gutmann. 2006. Nonmolecular test for detection of low-level resistance to fluoroquinolones in *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. 50:572–579.
18. Bonofiglio, L., M. Regueira, J. Pace, A. Corso, E. Garcia, and M. Mollerach. 2011. Dissemination of an erythromycin-resistant penicillin-nonsusceptible *Streptococcus pneumoniae* Poland$^{6B}$-20 clone in Argentina. Microb. Drug Resist. 17:75–81.
19. Kimura, K., N. Nagano, Y. Nagano, S. Suzuki, J. Wachino, K. Shibayama, and Y. Arakawa. 2013. High frequency of fluoroquinolone- and macrolide-resistant streptococci among clinically isolated group B streptococci with reduced penicillin susceptibility. J. Antimicrob. Chemother. 68:539–542.
20. Morozumi, M., T. Wajima, M. Takata, S. Iwata, and K. Ubukata. 2016. Molecular characteristics of group B streptococci isolated from adults with invasive infections in Japan. J. Clin. Microbiol. 54:2695–2700.

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