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*Brief Original Article*

# Argentinian multicenter study on urinary tract infections due to *Streptococcus agalactiae* in adult patients

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## Abstract

Introduction: *Streptococcus agalactiae* (group B streptococcus, GBS) is a recognized urinary pathogen both in males and pregnant or non-pregnant women. Data regarding GBS serotypes recovered from urinary tract infections (UTIs) are scarce. The aim of this study was to describe the clinical and microbiological characteristics of UTIs caused by GBS in adult patients in Argentina.

Methodology: A prospective multicenter study involving 86 centers was conducted in Argentina between July 1st, 2014 and June 30th, 2015. Antimicrobial susceptibility and serotype distribution of GBS isolated from the urinary tract of adult patients were determined. Susceptibility tests were performed by the disk diffusion and/or agar dilution methods. Epidemiological and clinical characteristics of the patients were considered to identify associated comorbilities.

Results: Seven hundred and one GBS were sent to the reference laboratory in the above mentioned period, however, only 211 fulfilled our selection criteria (demographic data availability, underlying diseases reported, colony counts greater than $10^5$ CFU/mL, single organism isolated from the urine sample). No penicillin-resistant GBS was found but fluoroquinolone resistance was high (12.8%), especially among GBS isolated from men and non-pregnant women. UTIs due to GBS were associated to underlying diseases in men and non-pregnant women, particularly *diabetes mellitus*. Most of the isolates showed serotypes Ia and III.

Conclusions: GBS are still susceptible to penicillin but fluoroquinolone resistance is a growing concern, at least in Argentina. There are underlying conditions that could be associated to urinary infections caused by GBS.

**Key words:** Urinary tract infections; *Streptococcus agalactiae*; group B; Argentina.

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## Introduction

For the last four decades it has been known that *Streptococcus agalactiae*, also called group B streptococcus (GBS), is responsible for urinary tract infections (UTIs) in adult patients [1] and bacteriuria in pregnant women [2,3]. GBS is also the cause of other conditions, such as cystitis, pyelonephritis and urosepsis, with neurogenic bladder as a potential risk factor. In addition, a rare invasive infection has been described in one patient suffering from an unusual, massive abdominopelvic abscess secondary to acute GBS UTI [4].

The incidence of genitourinary infections due to GBS increased at the end of the last century [5]. GBS accounts for up to 2% of UTIs, and 4.5% of involved patients with type II *diabetes mellitus* [6,7].

It is known that different serotypes can be found in different regions. Serotype distribution also varies depending on the anatomical location of the infections.

Data regarding GBS serotypes recovered from UTIs is scarce [8]. Therefore, we decided to study the epidemiology of UTIs due to GBS in adult patients in the context of a one-year multicenter study, involving 86 health centers of Argentina. Specifically, we aimed to analyze the clinical characteristics of the enrolled patients such as age, gender, predisposing condition and underlying diseases. We also studied the serotypes and the antimicrobial susceptibility of GBS circulating in our country.

## Methodology

*Design*

The present work was a prospective, multicenter observational study of UTIs due to GBS carried out from July 1st, 2014 to June 30th, 2015, in which 86 health centers of 32 Argentinian cities participated (Figure 1). The data analyzed here represent all patients who presented significantly positive urine cultures (≥ $10^5$ CFU/mL of GBS) during the period studied.

*Patients and samples*

All adult patients (> 16 years of age) showing at least one sample of urine with ≥ $10^5$ CFU/mL of GBS, collected by the midstream clean-catch technique, were included in this study. CAMP and latex agglutination tests were used to identify isolates to the species level. All non-pregnant patients were symptomatic, while pregnant women (PW) were either symptomatic or asymptomatic. Patient data were obtained from their clinical charts.

The cases without accompanying complete clinical data (age, gender or pregnancy status) were excluded, as well as those cases with colony counts less than $10^5$ CFU/mL or without available colony count number, or instances in which GBS were isolated from a polymicrobial culture or those organisms that had been erroneously identified as GBS but belonged to other species.

Urine samples were cultured following the current procedures used in each center.

Urine pellets were obtained by centrifuging the urine samples for 10 minutes at 2,000 rpm. White blood cell (WBC) counts were considered significant if they were higher than 5 WBCs per high power field (HPF).

*Antimicrobial susceptibility tests*

Antimicrobial susceptibility to levofloxacin (LEV) and norfloxacin (NOR) was tested by the disk diffusion method according to CLSI guidelines. The screening for susceptibility to penicillin (PEN) was performed based on the scheme proposed by Kimura *et al.* [9] using oxacillin (OXA), ceftibuten (CBT) and ceftizoxime (CZX) disks and interpreted in accordance with their criteria. The interpretation of results for NOR was performed using the breakpoints of the Societé Francaise de Microbiologie [10] and those for LEV by using CLSI breakpoints [11].

MICs of PEN, erythromycin (ERY), clindamycin (CLI) and LEV were obtained by using the agar dilution method according to CLSI guidelines [12]. Phenotypes were characterized by the D test [12].

*Serotyping*

Serotyping was performed by latex agglutination using the Strep B latex kit (Statens Serum Institut, Denmark).

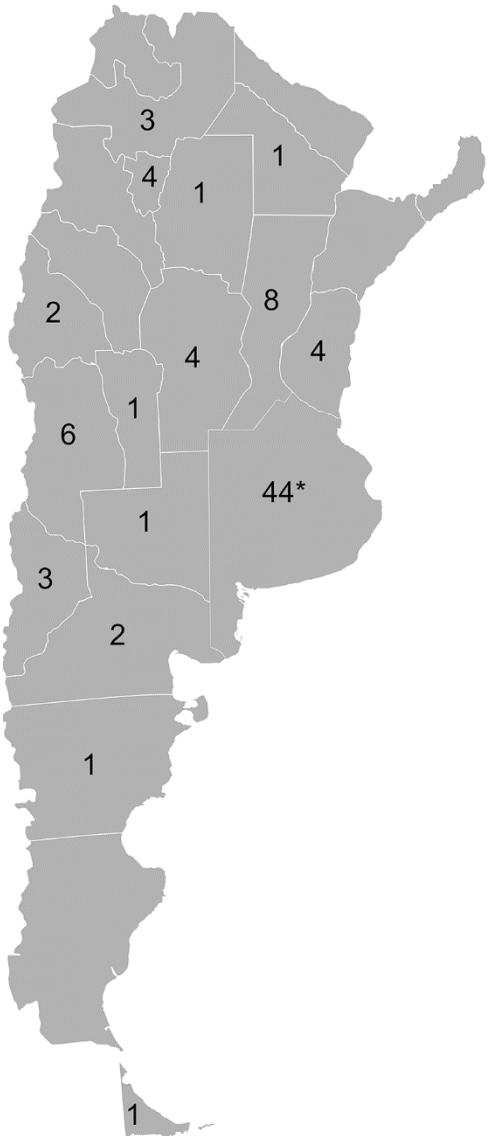
*Statistical analysis*

VassarStats was used to determine 95% confidence intervals and p-values using a chi-square test of association according to Yates'correction or the Fisher's test, as required.

*Ethics approval*

This study fulfilled the requirements for a low risk research (National Health and Medical Research

**Figure 1.** Health center distribution (32 cities).




* 25/44 centers belong to the Federal District (Buenos Aires City); The map source is "source =Argentina Blank Map.svg".

Council, NHMRC, 2015). Ethics approval was provided by the Ethics Committee of the Faculty of Pharmacy and Biochemistry, Universidad de Buenos Aires, Res (D) N4467/14.

## Results

*Background and clinical characteristics*

Seven hundred and one adult patients were studied during the above mentioned period. According to previous criteria, the total number of cases included were 211. Patients were stratified in males (M) (n = 28), non-pregnant women (NPW) (n = 104) and pregnant women (PW) (n = 79).

An equal number of GBS obtained from mid stream clean-catch urine samples from adult patients (> 16 years of age) were isolated and characterized.

Underlying conditions are listed in Table 1. As expected, M and NPW showed more underlying diseases than PW. *Diabetes mellitus* was the most frequent comorbidity associated with UTIs due to GBS. Cardiovascular diseases were also frequently found, among which, arterial hypertension is one of the most common conditions, also associated with normal aging.

Age distribution was bimodal for NPW with peaks in the elderly (> 50 y-o) and younger patients (16 - 30 y-o) and not so defined for M. As expected , most PW showed ages between 16 and 30 y-o (Figure 2).

*Urine pellet results*

WBC counts ≥ 5 WBC/HPF in urine samples from M (28/32; 87.50%) and NPW (101/123; 82.11%) were significantly higher than those from PW (33/86; 38.37%), ($p \leq 0.05$).

*Antimicrobial susceptibility*

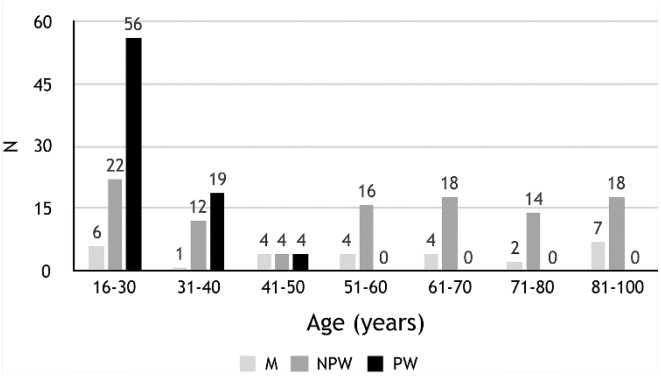
Antimicrobial susceptibility to LEV and NOR was tested for 194 isolates by using the disk diffusion method. LEV and NOR resistance was observed in 12.8% of the tested isolates as a whole, while significant differences ($p < 0.05$) were found between M and NPW (16.0 and 18.7%) with respect to PW (3.8%), (Table 2).

MICs of PEN, ERY, CLI and LEV were obtained by using the agar dilution method. A hundred eighty eight isolates were tested for susceptibility to PEN, 194 isolates to fluoroquinolones and 182 to macrolides.

All GBS tested were susceptible to PEN (MIC90 = 0.06 µg/mL, MIC50 = 0.03 µg/mL, range ≤ 0.015 µg/mL - 0.06 µg/mL) despite some false positive results using the screening test of Kimura *et al*. on 188 isolates. CBT yielded more false positive results than CZX and OXA (Table 2).

ERY resistance rate (R + I) was 25.3% (46/182) while the CLI resistance rate was 13.19% (24/ 182). The prevalent phenotype was $cMLS_B$ with 10.99% (20/182), followed by $iMLS_B$ with 4.95% (9/182), M with 3.85% (7/182) and L with 2.2% (4/182).

**Figure 2.** Age distribution of adult patients with UTI due to GBS.




NPW: non-pregnant women, M: males, PW: pregnant women.

**Table 1.** Underlying conditions of adult patients with UTI due to GBS.

| Underlying conditions | Males | Non-pregnant women | Pregnant women | Total |
|---|---|---|---|---|
| *Diabetes mellitus* | 6 | 8 | 1 | 15 |
| Malignancy | 3 | 4 | 0 | 7 |
| Cardiovascular disease | 2 | 10 | 0 | 12 |
| Renal or urologic pathology | 5 | 2 | 0 | 7 |
| Central nervous system condition | 0 | 2 | 0 | 2 |
| Gastrointestinal complaint | 0 | 2 | 1 | 3 |
| Others | 1 | 7 | 0 | 8 |
| Without data | 8 | 33 | 18 | 59 |
| None | 5 | 44 | 59 | 108 |
| One predisposing condition | 13 | 19 | 2 | 34 |
| More than one | 2 | 8 | 0 | 10 |
| **Total** | **28** | **104** | **79** | **211** |

**Table 2.** Antimicrobial susceptibility of GBS isolated from the urinary tract of adult patients.

| Antibiotic | Breakpoint (mm) | N (%) | MIC (µg/mL) |
|---|---|---|---|
| Ceftibuten | < 20 | 178 (94.7) | |
| Oxacillin | < 17 | 8 (4.2) | PEN |
| Ceftizoxime | < 29 | 17 (9.0) | < 0.03 – 0.06 |
| Norfloxacin | < 12 [a] | 24 (12.8) | |
| Levofloxacin (total) | < 17 [b] | 24 (12.8) | LEV R |
| Levofloxacin (PW) *, ** | < 17 [b] | 3/78 (3.8) | 16 -32 |
| Levofloxacin (NPW)* | < 17 [b] | 17/91 (18.7) | |
| LEV M ** | < 17 [b] | 4/25 (16.0) | LEV S 0.5 -1 |

Breakpoint: below this breakpoint for each antibiotic, the isolate is considered resistant; [a] Committee of the antibiogram of the French Society of Microbiology. (2015), [b] Clinical and Laboratory Standards Institute (2015); *chi square, Yates: p = 0.005; ** Fisher: p = 0.05.

*Serotyping*

One hundred ninety one isolates could be serotyped. Serotype Ia was the most frequent one among the GBS isolated from UTIs in adult patients (41.36%). Serotype III was detected more frequently among PW than in NPW ($p < 0.05$) (Table 3).

Globally, serotypes Ia, Ib, and III, accounted for 85.3% of the isolates. Serotypes IV, VI, VII, VIII, and IX were not found among these 191 GBS isolates. A significant association between serotype Ib and fluoroquinolone resistance was found ($p < 0.001$). No association between serotypes and patient groups was detected.

## Discussion

The importance of this study lies in the representativeness of the epidemiological data on the spread of GBS bacteria throughout the country. Prior to this study, GBS serotypes and antimicrobial susceptibility were seldom described by a few centers and often focalized in a limited number of invasive or colonizing isolates.

It is remarkable that, despite the limitations of this study (bias due to lack of information of several patients), M and NPW frequently presented underlying conditions, especially *diabetes mellitus*.

GBS continued to be susceptible to PEN in Argentina. Fluoroquinolone resistance (12.8%) was higher than in the latest reports from our country and even from other countries of Latin America and the rest of the world, except Korea [13] and China, where the prevalence is near 50% in isolates recovered from UTIs [14]. The fluoroquinolone resistance rate found in this study was similar to that determined in invasive GBS isolates obtained from the same centers in a study of invasive disease caused by GBS conducted by our group during the same period (unpublished results). GBS isolates recovered from NPW and M were significantly more resistant to fluoroquinolones than those isolated from PW. The cause of this difference might be that these antibiotics are not used for the treatment of infections in PW. However, it may also be associated with the clonal spread of a fluoroquinolone-resistant strain among NPW and/or M, an issue that will be further investigated.

Studies reporting antimicrobial resistance of GBS recovered from UTIs are scarce. In Brazil, ERY and CLI resistance rates vary from 5% to 19%, while in

**Table 3.** GBS Serotypes isolated from the urinary tract of adult patients.

| Serotype | Urinary tract isolates (%) | | | |
|---|---|---|---|---|
| | Total N (%) | M | NPW | PW |
| Ia | 79 (41.36) | 10 | 36 | 33 |
| Ib | 25 (13.09) | 2 | 16 | 7 |
| II | 6 (3.14) | 1 | 4 | 1 |
| III | 59 (30.89) | 8 | 20 | 31 |
| IV | 0 | 0 | 0 | 0 |
| V | 14 (7.33) | 4 | 5 | 5 |
| VI | 0 | 0 | 0 | 0 |
| VII | 0 | 0 | 0 | 0 |
| VIII | 0 | 0 | 0 | 0 |
| IX | 0 | 0 | 0 | 0 |
| NT | 8 (4.19) | 0 | 5 | 3 |
| Total | 191 | 25 | 86 | 80 |

NPW: non-pregnant women, M: males, PW: pregnant women.

USA, ERY resistance was observed in 39.5% of the isolates [7,15,16]. In the present study, almost 25% resistance to ERY was detected, half of which was associated with resistance to CLI. Considering that different studies were carried out in different time periods, it is difficult to reliably compare our scenario. We can only say that the observed resistance to CLI and ERY does not differ much from that found in Brazil [16] and Turkey [17] and that it is slightly lower than that reported in the USA [7].

In a recent Brazilian study [18], CLI resistance levels in bacteriuria were reported to be slightly lower (9.3%) than those herein detected; however, ERY resistance was considerably lower (11.3%). Notably, no LEV-resistant strains were detected. The level of LEV resistance in strains from adult patients in our country (12.8%) is alarming and should be an incentive for neighbouring regions to continue an active screening of fluoroquinolone sensitivity.

Serotypes Ia, III and Ib (in this order) were the most frequently recorded among GBS from the urinary tract, while serotypes, Ia, Ib, V and III (in this order) were prevalent in invasive isolates obtained during the same period (unpublished results). Serotype III was especially more frequently found in GBS from PW than in the isolates from NPW.

Serotype distribution differs from that reported in Brazil [16,18]. In a study of bacteriuria samples, the prevalent serotype was V, followed by Ia, II and III. In a recent study of symptomatic urinary tract infections, serotype V was also prevalent, followed by II, Ia and III. This difference in serotype distribution emphasizes the importance of gaining awareness about the current status of regional epidemiology.

## Conclusion

UTIs due to GBS were associated to underlying diseases in M and NPW, especially *diabetes mellitus*. Serotypes Ia and III were the most prevalent among the isolates studied. GBS are still susceptible to penicillin but fluoroquinolone resistance is a growing health concern, at least, in Argentina.

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