



Emergence of multidrug-resistant and -hypervirulent *Streptococcus agalactiae* in Bulgarian patients

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To the Editor,

Group B Streptococcus (GBS) (*Streptococcus agalactiae*) is the leading etiological agent of neonatal meningitis and sepsis but also causes soft-tissue and urogenital infections.¹ In streptococcal infections complicated by allergy or poor therapeutic response to penicillin, the first alternative choice is macrolides.^{1,2} The rate of macrolide resistance worldwide has increased recently, sometimes in combination with lincosamide resistance in GBS.² The most common serotype-specific antigens for human GBS isolates are Ia, Ib, II, III, and V.³ A hypervirulent clone of GBS serotype III that causes invasive disease, pregnancy-associated infections, and urinary tract infections emerged in Hong Kong between 1993-2012.⁴ In 2018-2019, 107 nonduplicate GBS strains were isolated from 792 Bulgarian patients aged between 22-68 years: group I patients (n = 93) had genital secretions (vaginal and prostate fluid samples), and group II patients (n = 14) urinal samples (Table 1). Antimicrobial susceptibility testing to 5 agents was based on minimum inhibitory concentration (MIC) according to the European Committee

on Antimicrobial Susceptibility Testing, version 10.0, 2020 criteria (<http://www.eucast.org>). Capsular serotypes Ia, Ib, II, III, IV, V, VI, VII, and VIII were detected by multiplex polymerase chain reaction⁵, with a denaturation step for 5 minutes at 95°C, followed by 30 cycles of 95°C for 30 seconds, 59°C for 30 seconds, 72°C for 60 seconds, and a final extension of 72°C for 5 minutes.

All the collected GBS were susceptible to penicillin but with an MIC₉₀ of 0.12 mg/L, near the limit value (0.25 mg/L); 58.88% showed resistance to erythromycin, and 15.89% showed resistance to clindamycin, both with an MIC₉₀ of 2 mg/L. Levofloxacin resistance occurred in 10.28% of the isolates (MICs₉₀ of 3 mg/L); it occurred in the urinal samples (42.86%) more than in the genital ones (5.38%; *P* < 0.05), probably because of frequent quinolone use in uroinfections. The tetracycline resistance rate of all tested isolates was 94.62% with an MICs₉₀ > 256 mg/L. Multidrug resistance (MDR) to at least 3 classes of drugs occurred in 22.22% of the GBS strains. The most prevalent serotypes were Ia and V, followed by serotype III. Hypervirulent serotype III, causing invasive infections in other countries,⁴

TABLE 1. Distribution of capsular types in 107 studied isolates GBS according clinical diagnoses and the source of samples

Capsulartype	I. group: genital secretions (n = 93)									
	A. Samples from pregnant women with vaginitis (n = 25)				B. Samples from non-pregnant women with vaginitis (n = 57)		C. Prostatic fluid from patients with prostatitis (n = 11)		II. group: samples of urine from patients with uro-infections (n = 14)**	
	n	%	n	%	n	%	n	%	n	%
IA	7	28	26	45.6	0.1511	3	27.3	6	49.9	
IB	0	0	3	5.3	0.5495	2	18.2	4	28.6	
II	1	4	8	14	0.2638	1	9.1	1	7.1	
III	9	36	6	10.5	0.0112	0	0	0	0	
IV	1	4	3	5.3	1.000	0	0	0	0	
V	7	28	11	19.3	0.3973	5	45.5	3	21.4	
TOTAL	25	100	57	100		11	100	14	100	

*Underlined text considered to be statistically significant; ***P* value was not calculated because small number of samples were presented.

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was identified in 14.0% of the Bulgarian GBS isolates. This serotype was isolated more often ($P < 0.05$) from pregnant women with vaginitis (36.0%) than from nonpregnant ones (10.5%). It was not detected in patients with uroinfection and prostatitis.

To the best of our knowledge, this is the first report of MDR and hypervirulent GBS isolates in Bulgaria with such a high frequency of resistant GBS. Although the conventional antimicrobial agent penicillin was most effective *in vitro*, the alternative treatment options for patients with allergy or therapeutic failures after the first-line penicillin treatment were unpromising. There is a concerning trend toward the rapid development of macrolide resistance. That reflects on the use of lincosamides, especially in more severe infections, making their therapeutic effect unsafe.² The resistance to tetracyclines was nearly absolute. Quinolone resistance also occurred, albeit to a lesser extent but often in MDR isolates. Alarming, over 22% of the tested Bulgarian GBS isolates were MDR, and at least 3 antimicrobial classes considered appropriate for treatment are becoming ineffective. In addition, 14.0% of all GBS isolates and 36% of those from pregnant women harbored genes encoding the hypervirulent serotype III, which increases the risk of invasive streptococcal infections. To prevent streptococcal MDR from increasing, macrolides, lincosamides, tetracyclines, and quinolones must be used only after antimicrobial susceptibility testing. A correct and timely GBS screening in pregnant women can facilitate proper treatment to reduce maternal and perinatal GBS disease.

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