

## Research Article

# In Vitro Resistance to Macrolides and Clindamycin by Group B *Streptococcus* Isolated from Pregnant and Nonpregnant Women

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**Background.** Despite the introduction of screening bases intrapartum prophylaxis, *Streptococcus agalactiae* is still an important etiological agent of perinatal infections. The increasing rate of resistance and the differences in resistance pattern among countries suggest that a program of surveillance at the institutional level is important in determining optimal prophylaxis. In contrast, knowledge on GBS epidemiology in Italy is limited, and no data are available in the Southern region of the country. We sought to determine the occurrence of resistance to macrolides and clindamycin of GBS isolates in pregnant and nonpregnant women. **Methods.** Between 2005 and 2008, 1346 vaginal and 810 rectovaginal swabs were obtained from pregnant and not-pregnant women. **Results.** The occurrence of macrolides and clindamycin resistance was 16.5% in 2005 increasing up to 69.9% in 2008. A high percentage of isolates was resistant to tetracycline through all the study period with no statistically significant annual. **Conclusions.** In our cohort, an increase of *in vitro* resistance of GBS to macrolides and clindamycin is clearly evident. The discordance with reports from different countries emphasize the crucial role of microbiological methods in setting possible therapeutic strategies.

## 1. Introduction

Among Gram-positive bacteria, *Streptococcus agalactiae*, also known as Group B *Streptococcus* (GBS) is considered a common commensal of the female urogenital tract and rectum [1] whose importance is referred to severe neonatal pathologies by perinatal transmission from women to newborns. Neonatal infections by GBS are usually distinguished in early onset (occurring in the first 7 days of life) and late onset (occurring between 7 days of life and 3 months of age): this temporal distinction reflects differences in the spectra of infection [2, 3].

In studies carried out in the 1970s, GBS emerged as the leading cause of neonatal morbidity and mortality, with a frequency of 2-3 cases per 1000 live births and case-fatality ratios of 50% [3, 4]. The vaginal colonization prevalence among pregnant women varies in European

countries between 10 and 20%, and the incidence of neonatal infections ranges from 0.5 to 2 per 1000 live births [5–8].

Between 1996 and 1997, the American College of Obstetricians and Gynecologists, the Centers for Disease Control and Prevention, and the American Academy of Pediatrics produced recommendations for prevention of perinatal GBS disease. These guidelines recommended the use of culture-based screening for GBS colonization between 35 and 37 weeks gestation and the antibiotic prophylaxis of all colonized women [9, 10].

The intrapartum antibiotic prophylaxis (IAP) for GBS carriers indicates the use of penicillin (or ampicillin). For penicillin-allergic women without a history of anaphylaxis, angioedema, respiratory distress, or urticaria, cefazolin is the preferred agent. Vancomycin and clindamycin are recommended for penicillin-allergic women at high risk for anaphylaxis. The introduction of IAP for GBS carriers has

been associated with a substantial decline in the incidence of early-onset neonatal infections [11].

The purpose of the present study was to assess the antibiotic susceptibility patterns of GBS isolates obtained from a heterogeneous female population (pregnant and nonpregnant) in a region of Southern Italy and to evaluate whether statistically significant changes in GBS antibiotic resistance regarding macrolides and clindamycin occurred in the years in order to generate local data for the development of rational interventions for prevention of GBS infection in our country.

## 2. Materials and Methods

**2.1. Study Population.** In the period from January 2005 up to December 2008, a total of 2156 biological samples (1346 vaginal swabs from nonpregnant women and 810 rectovaginal swabs from pregnant women at 35–37 weeks of gestation) were collected in the Microbiology Laboratory of University Hospital “Federico II”, Naples, Italy. All women gave their consent to take part in the study. Therapeutic protocols were not modified for women enrolled in the study.

**2.2. Processing of Samples, Culture of Microorganisms, and Identification Analysis.** All swabs were maintained in the Stuart transport medium and transported to the Microbiology Laboratory. Swabs were plated on several agar media, including Columbia colistin-nalidixic acid (CNA) agar with addition of 5% of sheep blood, MacConkey agar, Sabouraud agar, and chocolate agar and incubated at 37°C overnight in aerobic or microaerobic conditions and were examined microscopically to evaluate the preservation of *Lactobacillus* microbial status.

Bacteria were identified by conventional methods (Gram stain, catalase test) and automated system (Vitek II, bioMérieux, France). The identification of the Lancefield antigen was obtained by Streptococcal Grouping Kit (Oxoid, Hampshire, England).

**2.3. Antimicrobial Susceptibility Testing Method.** To check the sensitivity to antimicrobial agents, an automated microdilution method (Vitek II) was utilized. The susceptibility criteria were in accordance with the National Committee for Clinical Laboratory Standards Interpretative Criteria [12]. Antibiotics tested were as follows: amoxicillin/clavulanic acid, ampicillin, cefaclor, cefotaxime, ceftriaxone, clindamycin, erythromycin, penicillin, teicoplanin, tetracycline, trimethoprim/sulfamethoxazole, vancomycin, levofloxacin, azithromycin, clarithromycin, quinupristin/dalfopristin, and linezolid.

**2.4. Statistical Analysis.** Statistical analysis, including comparison of proportions and chi-squared test, was applied throughout the study. A  $P < 0.05$  was considered statistically significant.

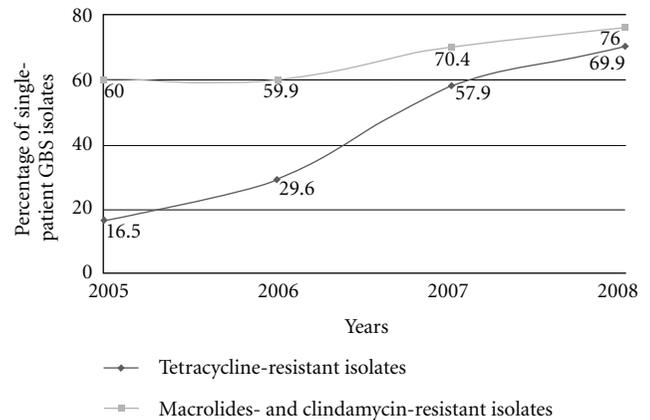


FIGURE 1: Annual increment (%) of resistant isolates during the study period.

## 3. Results

In the study period, a total of 879 GBS from all samples (2156 between vaginal and rectal-vaginal swabs) were isolated. The distributions of swabs, positive cultures, and patients in the period of study are indicated in Table 1.

The distribution of single-patient resistant GBS isolates is showed in Table 2 as well as the susceptibility pattern over the study period. The antibiotic susceptibility profiles indicate that isolates showed sensitivity to beta-lactams, glycopeptides, quinolones, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole, and linezolid.

The number of isolates resistant to tetracycline was high through all the study period, indicating not statistically significant fluctuations. Instead, the increment of resistance to macrolides and clindamycin was statistically significant through the study period ( $X_2$  for trend = 8.100,  $P = 0.004$ ). The annual increment in percentage of macrolides- and clindamycin-resistant isolates during the study period is indicated in Figure 1.

The GBS isolates resistant to tetracycline showed a MIC value  $\geq 16 \mu\text{g/mL}$  through all the study period. The MICs obtained for macrolides and clindamycin range from  $\leq 0.25 \mu\text{g/mL}$  to  $\geq 8 \mu\text{g/mL}$ .

## 4. Discussion

In our experience, in accordance with CDC 2010 guidelines, penicillin and ampicillin are still the first choice for IAP, followed by first-generation cephalosporins as cefazolin in penicillin allergic women. In fact all GBS isolates were susceptible to these antibiotics. The prevalence of isolates resistant to macrolides and clindamycin is considerably high (55%) and has increased significantly from 16.5% to 70% during the study period ( $P < 0.05$ ).

Although very few women GBS positive give birth to babies who are infected with GBS, antenatal screening is routinely performed to reduce the rate of early-onset infections in newborns. However, there is still controversy about its prevention since antenatal screening and treatment

TABLE 1: Distribution of swab type, positive cultures, and number of infected patients.

Year	Total swabs N (%)		Total positive cultures N (%)	
	Vaginal-rectal	Vaginal	Vaginal-rectal	Vaginal
2005	76 (7.7%)	153 (13%)	23 (6.4%)	62 (11.9%)
2006	192 (19.5%)	212 (18.1%)	51 (14.3%)	97 (18.6%)
2007	345 (35.1%)	357 (30.4%)	134 (37.5%)	146 (28%)
2008	370 (37.6%)	451 (38.4%)	149 (41.7%)	217 (41.6%)
	Total 2156		Total 879	

TABLE 2: Distribution (number and percentage) of resistant GBS strains during the 4-year study period.

	2005		2006		2007		2008	
	positive cultures (85)		positive cultures (162)		positive cultures (280)		positive cultures (366)	
	N°	%	N°	%	N°	%	N°	%
AMC	0	—	0	—	0	—	0	—
AMP	0	—	0	—	0	—	0	—
CEC	0	—	0	—	0	—	0	—
CTX	0	—	0	—	0	—	0	—
CRO	0	—	0	—	0	—	0	—
CLI	14	16.5	48	29.6	162	57.9	256	69.9
ERY	14	16.5	48	29.6	162	57.9	256	69.9
PEN	0	—	0	—	0	—	0	—
TEC	0	—	0	—	0	—	0	—
TET	51	60	97	59.9	197	70.4	278	76
SXT	0	—	0	—	0	—	0	—
VAN	0	—	0	—	0	—	0	—
LVX	0	—	0	—	0	—	0	—
AZM	14	16.5	48	29.6	162	57.9	256	69.9
CLR	14	16.5	48	29.6	162	57.9	256	69.9
Q-D	0	—	0	—	0	—	0	—
LZD	0	—	0	—	0	—	0	—

AMC = amoxicillin-clavulanic acid; AMP = ampicillin; CEC = cefaclor; CTX = cefotaxime; CRO = ceftriaxone; CLI = clindamycin; ERY = erythromycin; PEN = penicillin; TEC = teicoplanin; TET = tetracycline; SXT = trimethoprim-sulfamethoxazole; VAN = vancomycin; LVX = levofloxacin; AZM = azithromycin; CLR = clarithromycin; Q-D = quinupristin-dalfopristin; LZD = linezolid.

may carry disadvantages for the mother and the baby. The usual recommendation for prevention of GBS transmission from colonized women to their infants during labour is to administer intravenous penicillin or ampicillin every 4 h for the duration of labour [11].

On the maternal side, IAP's risks are allergic reactions. Even if there are some anecdotal reports of maternal mortality due to anaphylaxis, usually allergic reactions are not severe and mainly with maculopapular rashes [11, 13, 14].

On the fetal/neonatal side, there is no risk for anaphylaxis resulting from IAP, but there is a growing concern about the development of antibiotic resistance among GBS isolates and other pathogens. The increased resistance may have two effects: exposure of neonates to antibiotic-resistant pathogens with development of intractable sepsis and reduction of the chance to prevent maternal fetal transmission by GBS.

Two recent published surveys have demonstrated that in England and France neonatal infections are still mainly

caused by GBS, and the current policy of GBS maternal prophylaxis is not associated with an excessive risk of pathogen resistance [15, 16]. The incidence of early-onset sepsis (EOS) ranged among 0.9 to 1.9/1000 live births, and GBS (58–62%) and *Escherichia coli* (18–25%) were the most common organisms. About the antibiotic resistance, the majority of pathogens (95%) causing EOS were susceptible to commonly used empiric first-line antibiotic combinations.

About the risk of reduced efficacy of IAP for GBS, data are reassuring. Worldwide, there have been only a few reports of penicillin resistance [17, 18] or elevated MIC [19, 20] secondary to the alterations in penicillin-binding proteins (PBP). In the majority of isolates with alteration of PBP, the measured MICs were just at the threshold of susceptibility, but the clinical significance of higher MIC values remains unclear. Elevated MICs to ceftazolin also were reported, but as penicillin/ampicillin, the clinical significance of higher MICs to ceftazolin among GBS isolates remains unclear [11]. In our experience GBS isolates have not yet developed

any resistance against penicillin and ampicillin and first-generation cephalosporin. This aspect is very reassuring if we consider that these antibiotics are constantly indicated as first choice in women positive for GBS. Their efficacy as IAP was demonstrated for the first time in clinical trials by Boyer and Gotoff [21] in 1986 and by Garland and Fliegner [22] in 1991. On the contrary the efficacy of alternatives to penicillin/ampicillin for allergic women (including cefazolin, clindamycin, erythromycin, and vancomycin) has not been tested in controlled trials. About cephalosporin, it has been supposed that, given the similar activity, pharmacokinetics, and dynamics of cefazolin to penicillin/ampicillin, it could be a second-line antibiotic in penicillin allergic women with low risk of anaphylaxis. As long as allergic women with high risk of anaphylaxis, the guidelines suggest the use of clindamycin/erythromycin or vancomycin although their ability to reach bactericidal levels in the fetal circulation and amniotic fluid are very limited [23–25]. The choice of one or another antibiotics is made on the results of antimicrobial susceptibility testing. These women should receive clindamycin if their GBS isolate is susceptible to clindamycin and erythromycin or if it is resistant to erythromycin but sensitive to clindamycin with negative testing for inducible clindamycin resistance. Otherwise, if susceptibility to both agents is unknown, these women should receive vancomycin. At the moment, erythromycin is no longer considered an alternative for IAP in penicillin-allergic women at high risk for anaphylaxis [11].

Starting from our data, in the next future the problems related with antibiotic resistance will become bigger and bigger and the treatment of allergic women will be a major obstacle. In fact during the study period, the number of colonized women is increased from 85 to 366. If we consider stable the number of allergic women at risk for anaphylaxis, we will have that more and more women will be treated with alternative antibiotics which will be potentially ineffective or will increase the spectrum of resistance.

In reports published, the prevalence of resistance among GBS ranged from 7% to 25% for erythromycin and from 3% to 21% for clindamycin [26, 27]. Resistance to erythromycin was frequently but not always associated with clindamycin resistance. In our series resistance was always to both erythromycin and clindamycin, and the prevalence was considerably higher, ranging from 16.4% to 70% in the study period, showing a statistically significant increment ( $P < 0.05$ ). This finding is very far from previous reports from other nations indicating significant country variations and supporting the usefulness of research about GBS in each population. The increased resistance to macrolides, particularly to erythromycin observed all over the world, can be ascribable to the treatment of *Chlamydia* infections of the lower reproductive tract [28]; however we have no explanation for the higher rate of resistance in our population. An hypothesis is that the variation may be due to differences in techniques as well as characteristics of the population investigated.

The prevalence of GBS-positive women observed in our population is higher even when compared with other Italian studies. In the study of Savoia et al. [29], among 300

pregnant women screened, 73 single-patient GBS isolates were collected and only 3 out of 73 (4.1%) were resistant to erythromycin. Also the lincosamides (lincomycin) were less efficient. Overall the infection prevalence was 18.2% versus 41% observed in our population (879 infected patients out of 2156 patients). In another Italian study by Sensini et al. [6], the prevalence of GBS was even lower (11%). Comparing the numbers, an hypothesis is that the prevalence of infection is growing over the years (11% versus 18.2 versus 41%).

The main limitations of our study are the difference in surveillance population (pregnant and nonpregnant) and, for pregnant women, the lack of clinical data about the pregnancy and neonatal outcome. This aspect could be a starting point for new research since to date whether *in vitro* resistance of GBS has direct clinical implications remains unclear.

## 5. Conclusion

Antibiotics are used for both GBS prevention and treatment. The introduction of IAP for GBS carriers has been associated with a substantial decline in the incidence of early-onset neonatal infections. However, the potential side effect of the protocol is the risk of development of pathogen resistance to antibiotics. Until now GBS isolates remain susceptible to penicillin and ampicillin and first-generation cephalosporin, but resistance to alternative agents as erythromycin and clindamycin is an increasing concern. In fact these agents are suggested in women with high risk of anaphylaxis although their ability to reach bactericidal levels in the fetal circulation and amniotic fluid is very limited. Comparing reports of the literature, epidemiology of infection, and resistance pattern change substantially among countries suggesting the need of local study to map the prevalence of resistant isolates.

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