Research Article

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

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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 mastiff 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, 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.

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 (\( \chi^2 \) 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 \( \geq 16 \mu g/mL \) through all the study period. The MICs obtained for macrolides and clindamycin range from \( \leq 0.25 \mu g/mL \) to \( \geq 8 \mu g/mL \).

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.

<table>
<thead>
<tr>
<th>Year</th>
<th>Vaginal-rectal Total swabs N (%)</th>
<th>Vaginal Total positive cultures N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaginal-rectal</td>
<td>Vaginal</td>
</tr>
<tr>
<td>2005</td>
<td>76 (7.7%)</td>
<td>153 (13%)</td>
</tr>
<tr>
<td>2006</td>
<td>192 (19.5%)</td>
<td>212 (18.1%)</td>
</tr>
<tr>
<td>2007</td>
<td>345 (35.1%)</td>
<td>357 (30.4%)</td>
</tr>
<tr>
<td>2008</td>
<td>370 (37.6%)</td>
<td>451 (38.4%)</td>
</tr>
</tbody>
</table>

Total 2156  Total 879

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

<table>
<thead>
<tr>
<th></th>
<th>2005 positive cultures (85)</th>
<th>2006 positive cultures (162)</th>
<th>2007 positive cultures (280)</th>
<th>2008 positive cultures (366)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N*</td>
<td>%</td>
<td>N*</td>
<td>%</td>
</tr>
<tr>
<td>AMC</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>AMP</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>CEC</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>CTX</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>CRO</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>CLI</td>
<td>14</td>
<td>16.5</td>
<td>48</td>
<td>29.6</td>
</tr>
<tr>
<td>ERY</td>
<td>14</td>
<td>16.5</td>
<td>48</td>
<td>29.6</td>
</tr>
<tr>
<td>PEN</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>TEC</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>TET</td>
<td>51</td>
<td>60</td>
<td>97</td>
<td>59.9</td>
</tr>
<tr>
<td>SXT</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>VAN</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>LVX</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>AZM</td>
<td>14</td>
<td>16.5</td>
<td>48</td>
<td>29.6</td>
</tr>
<tr>
<td>CLR</td>
<td>14</td>
<td>16.5</td>
<td>48</td>
<td>29.6</td>
</tr>
<tr>
<td>Q-D</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>LZD</td>
<td>0</td>
<td>—</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

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 rushes [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 cefazolin also were reported, but as penicillin/ampicillin, the clinical significance of higher MICs to cefazolin 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
[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 anti-
microbial 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

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
erthyromycin. 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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