



**HAL**  
open science

## Antimicrobial resistance in colonizing group B Streptococci before the implementation of a Swedish intrapartum antibiotic prophylaxis program

M. Granlund, P. Axemo, K. Bremme, A.-L. Bryngelsson, M. Carlsson Wallin, C.-M. Ekström, S. Håkansson, B. Jacobsson, K. Källén, E. Spetz, et al.

► **To cite this version:**

M. Granlund, P. Axemo, K. Bremme, A.-L. Bryngelsson, M. Carlsson Wallin, et al.. Antimicrobial resistance in colonizing group B Streptococci before the implementation of a Swedish intrapartum antibiotic prophylaxis program. *European Journal of Clinical Microbiology and Infectious Diseases*, 2010, 29 (10), pp.1195-1201. 10.1007/s10096-010-0877-3 . hal-00614611

**HAL Id: hal-00614611**

**<https://hal.science/hal-00614611>**

Submitted on 13 Aug 2011

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Editorial Manager(tm) for European Journal of Clinical Microbiology & Infectious  
Diseases  
Manuscript Draft

Manuscript Number: EJCMIID-D-09-00523R1

Title: Antimicrobial resistance in colonizing Group B Streptococci before implementation of a Swedish intrapartum antibiotic prophylaxis program

Article Type: Article

Keywords: GBS, Antibiotic Susceptibility testing, Swedish parturients.

Corresponding Author: Dr Margareta Granlund, MD, PhD

Corresponding Author's Institution: Clinical Microbiology

First Author: Margareta Granlund, MD, PhD

Order of Authors: Margareta Granlund, MD, PhD; Pia Axemo, MD, PhD; Katarina Bremme, MD, PhD; Anna-Lena Bryngelsson, MD; Marie Carlsson Wallin, MD; Carl-Magnus Ekström, MD; Stellan Håkansson, MD, PhD; Bo Jacobsson, MD, PhD; Karin Källén, PhD; Eva Spetz, MD

Abstract: The prevalence of antibiotic resistance and their genetic determinants in colonizing group B streptococci (GBS) sampled in a Swedish nationwide survey was examined. In five GBS isolates (1,3 %) kanamycin/amikacin resistance and presence of the aphA-3 gene was identified. Three of these isolates carried the aad-6 gene and were streptomycin resistant. Screening with kanamycin and streptomycin 1000 µg disks enabled a rapid and easy detection of these isolates. In all 312/396 (79 %) GBS were tetracycline resistant and 95% of examined isolates harboured the tetM gene. Among the 22 (5.5 %) GBS resistant to erythromycin and/or clindamycin, the ermB gene was detected in nine isolates (41%) and erm(A/TR) in ten isolates (45%). High level of erythromycin and clindamycin resistance with MICs > 256mg/L was found in four serotype V isolates that harboured ermB. The erythromycin/clindamycin resistance was distributed among all the common serotypes Ia, Ib, II, III, IV and V but not present in any of 44 serotype III isolates, associated to clonal complex 17. Screening for penicillin resistance with 1µg oxacillin disks showed a homogenous population with a mean inhibition zone of 20mm. A change in the present oxacillin breakpoints for GBS is suggested.

Response to Reviewers: Dear Reviewers,

We have now tried to improve the paper: Antimicrobial resistance in colonizing Group B Streptococci before implementation of a Swedish intrapartum antibiotic prophylaxis program, according to your valuable suggestions.

Reviewer 1:

Introduction: the last five lines have been removed.

Material and methods: The abbreviation for SRGA is included. The sentence about aad-6 has been altered and the erroneous amount of Ampli-Taq Gold has been changed.

Results: The number of isolates with tetO and lack of tetO or tetM is given. Table I is rewritten and the number of ermA/TR is corrected.

The mobile elements GBSi1 and IS1548 is now presented in the Material and methods section, and the results of the characterization of the serotype III isolates in relation to the antibiotic resistance pattern is given in the result section and dicussed in the Discussion part.

The penicillin G MIC should be 0,032mg/L, which now is corrected. The gene product of aad-6 is hopefully now better described.

Discussion: Section 2; the part about the erroneous penicillin E-test is removed.

The Tables are rewritten and Figure 1 is omitted.

Reviewer 2.

We have tried to shorten the manuscript and have also made an effort to include data from AST with the EUCAST method on selected isolates.

Materials and methods: The part discussing the prevoiusly published material has been extended including the methods for serotyping and MLST.

Results: The paragraph on macrolide resistance has been rewritten and in addition to disk diffusion according to EUCAST MICs were determined. Since this gave new results, which showed a high-level of MLS resistance in four serotype V isolates these data are now presented in the result section. In the part regarding screening for penicillin resistance ceftibuten was included together with oxacillin in the disk diffusion made according to EUCAST. That test was made on 24 isolates with oxacillin inhibition zones on ISA-NAD <18,5 mm since ceftibuten disk diffusion results recently has been suggested to have 100% sensitivity and specificity for detection of penicillin resistance in GBS (Kimura et al, ref.25). Figure 1 is omitted, the tables I and II have been rewritten as well as the reference list.

# Antimicrobial resistance in colonizing Group B Streptococci before implementation of a Swedish intrapartum antibiotic prophylaxis program

**Authors:** M. Granlund MD, PhD<sup>1\*</sup>, P. Axemo, MD, PhD<sup>2</sup>, K. Bremme, MD, PhD<sup>3</sup>, A-L Bryngelsson, MD<sup>4</sup>, M. Carlsson Wallin, MD<sup>5</sup>, C-M Ekström, MD<sup>6</sup>, S. Håkansson, MD, PhD<sup>7</sup>, B. Jacobsson, MD, PhD<sup>8</sup>, K. Källén, PhD<sup>9</sup>, E. Spetz, MD<sup>10</sup>, I. Tessin, MD, PhD<sup>11</sup>,  
(The Swedish working group for prevention of perinatal group B streptococcal infections)

<sup>1</sup> Department of Clinical Bacteriology, Umeå University Hospital, Umeå

<sup>2</sup> Department of Obstetrics and Gynecology, Academic Hospital Uppsala

<sup>3</sup> Department of Obstetrics and Gynecology, Karolinska University Hospital, Stockholm

<sup>4</sup> Department of Obstetrics and Gynecology, University Hospital, Örebro

<sup>5</sup> Department of Obstetrics and Gynecology, Ystad Hospital, Ystad

<sup>6</sup> Department of Obstetrics and Gynecology, Vrinnevi Hospital, Norrköping

<sup>7</sup> Department of Pediatrics, University Hospital, Umeå

<sup>8</sup> Department of Obstetrics and Gynecology, Sahlgrenska University Hospital, Göteborg

<sup>9</sup> Department of Reproductive Epidemiology, Tornblad Institute, Lund University, Lund

<sup>10</sup> Department of Obstetrics and Gynecology, Östersund Hospital, Östersund

<sup>11</sup> Department of Pediatrics, Queen Silvia Children's Hospital, Göteborg.

## \*Corresponding author:

Margareta Granlund, MD, PhD

Phone: +46 (0)90 785 17 72

Department of Clinical Bacteriology,

Fax: +46 (0)90 785 35 91

Umeå University Hospital

Email: [Margareta.Granlund@climi.umu.se](mailto:Margareta.Granlund@climi.umu.se)

SE-901 85 Umeå

SWEDEN

## Keywords:

GBS, Antibiotic Susceptibility testing, Swedish parturients.

## Abstract

The prevalence of antibiotic resistance and their genetic determinants in colonizing group B streptococci (GBS) sampled in a Swedish nationwide survey was examined. In five GBS isolates (1, 3 %) kanamycin/amikacin resistance and presence of the *aphA-3* gene was identified. Three of these isolates carried the *aad-6* gene and were streptomycin resistant. Screening with kanamycin and streptomycin 1000 µg disks enabled a rapid and easy detection of these isolates. In all 312/396 (79 %) GBS were tetracycline resistant and 95% of examined isolates harboured the *tetM* gene. Among the 22 (5.5 %) GBS resistant to erythromycin and/or clindamycin, the *ermB* gene was detected in nine isolates (41%) and *erm(A/TR)* in ten isolates (45%). High level of erythromycin and clindamycin resistance with MICs > 256mg/L was found in four serotype V isolates that harboured *ermB*. The erythromycin/clindamycin resistance was distributed among all the common serotypes Ia, Ib, II, III, IV and V but not present in any of 44 serotype III isolates, associated to clonal complex 17. Screening for penicillin resistance with 1µg oxacillin disks showed a homogenous population with a mean inhibition zone of 20mm. A change in the present oxacillin breakpoints for GBS is suggested.

## Introduction

Group B streptococci (GBS) are recognized as a common cause of life-threatening infections in neonates. When the mother carries GBS in vagina and/or rectum, generally an asymptomatic condition, the infant is exposed to the bacteria during the passage in the birth canal. In an attempt to prevent GBS infection in neonates, most western countries have introduced programs for intrapartum antibiotic prophylaxis (IAP) (1, 2). The use of IAP has drastically reduced neonatal invasive GBS infections during the first week of life (1, 3). However, the increased use of antibiotics to women in labor has raised concerns about development of antibiotic resistance. For effective treatment and IAP it is important to monitor the prevalence of antibiotic resistance in GBS over time. Intravenous penicillin G is a first-choice drug for IAP and is given either to GBS colonized women or to women considered at risk. For treatment of established infections penicillin or ampicillin is often used in combination with an aminoglycoside as gentamicin (4). Streptococci are intrinsically resistant to aminoglycosides and not treatable with aminoglycosides given as single therapy. However, the combination of a cell-wall active antibiotic as penicillin or ampicillin and gentamicin has been shown to enhance killing of GBS (5). The synergistic effect between beta-lactam antibiotics and

1 aminoglycosides is abolished if the isolate is high-level gentamicin resistant, a resistance mechanism  
2 best described in enterococci but also in a few GBS isolates (6, 7, 8, 9). GBS have long been  
3 considered as uniformly penicillin susceptible. Data regarding sporadic reports of isolates with  
4 phenotypically reduced susceptibility to penicillin have been difficult to interpret due to methodological  
5 concerns and differences in recommended susceptibility breakpoints. However, two recent studies  
6 have reported alterations in the penicillin binding protein 2X gene (*pbp2x*) in GBS leading to  
7 decreased penicillin susceptibility (10,11).  
8  
9  
10  
11  
12  
13  
14  
15

16 The antibiotic most often used for IAP in case of penicillin allergy is clindamycin (12). Macrolides as  
17 erythromycin, clarithromycin and azithromycin have also been suggested as an alternative. The  
18 resistance rates for the macrolides-lincosamides-streptogramin B (MLS<sub>B</sub>) group of antibiotics in GBS  
19 exhibit geographic differences and vary over time (13, 14).  
20  
21  
22  
23  
24  
25

26 During 2005 a Swedish, population-based study of GBS carriage in parturients and infants, and risk  
27 factors for colonization, was performed before a regular national IAP program had been implemented  
28 (15). Of 1,569 mother/infant pairs examined the vaginal/rectal carriage rate of the mothers was 25,4%.  
29 With phenotypic antibiotic susceptibility tests 5% of these isolates were found resistant to clindamycin  
30 and erythromycin. None of the isolates were resistant to penicillin G or showed high-level gentamicin  
31 resistance (15). In the present study 396 colonizing GBS were further examined for genetic resistance  
32 determinants of clindamycin, erythromycin, kanamycin, streptomycin and tetracycline.  
33  
34  
35  
36  
37  
38  
39  
40  
41

## 42 **Material and methods**

43  
44

45 **GBS isolates** (n= 396), were from a nationwide surveillance study conducted in Sweden during 2005  
46 (15). The isolates were from vagina/rectum from parturient women, and from surface cultures from 44  
47 newborns in whom the cultures from the mothers were negative. Since the surface cultures of the  
48 infants were taken immediately after birth, the vaginal samples in these mothers were assumed to be  
49 false negative. All GBS isolates were serotyped (types Ia, Ib, II-VIII) by co-agglutination (Essum AB,  
50 Umeå, Sweden) In non-typable isolates a PCR-based typing method was used (16). By multilocus  
51 sequence typing (MLST) a serotype III lineage, CC17, containing sequence type 17 (ST17),  
52 associated with neonatal invasive infections has been identified (17, 18). The presence of the mobile  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 genetic elements GBSi1 and IS 1548 in serotype III isolates correlate with the phylogenetic lineages of  
2 clonal complexes 17 (CC17) and 19 (CC19) respectively (17). Serotype III isolates of the study were  
3 further characterized by PCR for the presence of these mobile genetic elements. The serotypes of  
4 mother/infant pair isolates were congruent. The distribution of serotypes among the GBS was as  
5  
6 follow: Ia 11%, Ib 13%, II 16%, III 24% (CC19 11%, CC17 11%), IV 15%, V 19%, VI 0.5%, VII 1%, and  
7  
8 0.5% non-typable. No serotype VIII isolates was found (15).  
9  
10

11  
12  
13 **Phenotypic antibiotic susceptibility testing** The isolates were cultured on blood agar plates  
14 containing Columbia agar base (Acumedia, Svenska Labfab, Ljusne, Sweden) and 5% defibrinated  
15 horse blood and were identified as GBS as previously described (17). Disk diffusion was performed on  
16 Iso-Sensitest Agar (ISA) (Oxoid Ltd, Basingstoke, United Kingdom) with 5% defibrinated horse blood  
17 and 20 mg/L of nicotinamide adenine dinucleotide (NAD) with a semi-confluent inoculum according to  
18 the Swedish Reference Group of Antibiotics (SRGA) ([www.srga.org](http://www.srga.org)). Twenty-two isolates with MLS<sub>B</sub>  
19 resistance and 24 isolates with oxacillin inhibitions zones less than 18.5 mm on ISA-NAD agar were  
20 also tested with the disk diffusion method that during 2010 will be recommended by the European  
21 Committee on Antimicrobial Susceptibility Testing (EUCAST) ([www.eucast.org](http://www.eucast.org)). In this method  
22 streptococci are tested on Mueller-Hinton agar (MH) supplemented with 5% horse blood and 20 mg/L  
23 NAD with a confluent inoculum. *Streptococcus pneumoniae* ATCC 49619 was used for validation of  
24 the susceptibility tests. Screening for penicillin resistance was performed with 1 µg oxacillin disks and  
25 with the EUCAST method also with 30 µg ceftibuten disks. Minimum inhibitory concentrations (MICs)  
26 were tested with E-tests (AB Biodisk, Solna, Sweden). The plates were incubated for 18 h at 35°C in 5  
27 % CO<sub>2</sub>. To identify isolates with inducible MLS<sub>B</sub> resistance (iMLS<sub>B</sub>) the double-disk diffusion test with  
28 an erythromycin disk and a clindamycin disk, both of 15 µg, placed 25 mm apart was used. Flattening  
29 of the clindamycin growth inhibition zone indicate iMLS<sub>B</sub>. To screen for high-level aminoglycoside-  
30 resistance disks prepared with 1000 µg kanamycin and streptomycin respectively was used (8).  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50

51  
52 **PCR amplification of resistance genes** The occurrence of the tetracycline resistance genes *tet(O)*  
53 and *tet(M)*, the MLS<sub>B</sub> resistance genes *erm(B)*, *erm(A/TR)* and *mef(A)*, and the genes *aad-6* and  
54 *aphA-3* encoding aminoglycoside-modifying enzymes were determined with PCR according to Poyart  
55 et al (19). The *aad-6* gene is also known as *ant-6*, and is coding for a streptomycin  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 adenylyltransferase. DNA was extracted by the guanidium thiocyanate metod (20) for the PCR  
2 reactions with the *aad-6* and *aphA-3* primers, for the other reactions the samples were boiled in water  
3 for 10 min. The PCR mixtures of 50 µl contained 0,2 µM forward and reverse primers (DNA  
4 Technology A/S, Århus, Denmark), 1U of *Ampli Taq Gold* (Applied Biosystems, Stockholm, Sweden),  
5 0.2 mM of each deoxynucleoside triphosphate (Amersham Biosciences, Buckinghamshire, UK), 1.5  
6 mM MgCl<sub>2</sub>, and 100 ng of template DNA in PCR buffer II with bovine serum albumin (Applied  
7 Biosystems). DNA amplification was carried out by initial denaturation at 95°C for 10 min and then  
8 cycling at 95°C for 1 min, 60°C for 30s, and 72°C for 30s for 35 cycles, with a final incubation at 72°C  
9 for 5 min in a DNA Engine PTC-200 (MJ Research Inc., Watertown. Mass. USA). The amplicons were  
10 visualized by UV transillumination of ethidium bromide stained 1.75% agarose gels. PCR products  
11 were sequenced with the BigDye terminator cycle sequencing ready reaction kit (PE Applied  
12 Biosystems) on an ABI 377 (ABI prism sequencer, Applied Biosystems).  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27

## 28 Results

29 Phenotypic tetracycline resistance was identified in 313 isolates (79%). Every fourth of these isolates  
30 were tested for the presence of *tetO* and *tetM* by PCR. In 89 of 94 isolates (94.7%) *tetM* was found and  
31 in 3 of 94 (3%) *tetO*. Neither *tetO* nor *tetM* could be identified in two of 94 isolates (2%).  
32  
33

34 MLS<sub>B</sub> resistance was detected in 22 of the 396 isolates (5, 5%). According to the breakpoints for  
35 erythromycin from SRGA beta-haemolytic streptococci with growth-inhibition zones of ≥ 23 mm are  
36 interpreted as susceptible and isolates with zones ≤ 19 mm as resistant. Nineteen GBS met the criteria  
37 for intermediate susceptibility or resistance with disk diffusion on ISA-NAD agar and had erythromycin  
38 MICs ≥ 1 mg/L indicating resistance (Table I). No cut-off values for the disk diffusion have been  
39 determined by EUCAST, but with the use of the EUCAST method those 19 GBS had erythromycin  
40 inhibition zones < 18 mm. In seven of these isolates a flattened clindamycin inhibition zone indicated  
41 iMLS<sub>B</sub>. The *erm* (A/TR) gene was amplified in all iMLS<sub>B</sub> isolates and in three additional isolates, which  
42 lacked clindamycin inhibition zones. The *ermB* gene was identified in nine isolates (Table I). GBS with  
43 MLS<sub>B</sub> resistance caused by an active efflux mediated by *mef* genes have been described. The M-  
44 phenotypes of such isolates, are erythromycin resistant and clindamycin susceptible without flattening of  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 the clindamycin inhibition zone in the double diffusion test (21). The *mef(A)* gene could not be identified  
2 in any of the isolates of this study, which is in agreement with a lack of isolates with the M-phenotype.  
3 Neither *erm (A/TR)*, *ermB* nor the *mef(A)* gene could be identified in three isolates that were susceptible  
4 to erythromycin but with clindamycin inhibition zones 1.5 -0.5 mm below the present breakpoint for  
5 resistance set at 24mm. The clindamycin MIC was 2 mg/L, which indicated resistance, while the  
6 erythromycin MICs were low (Table I). Seven of the 64 serotype II isolates (11%), five of 76 serotype V  
7 isolates (7%) and six of 92 serotype III isolates (6,5%) exhibited MLS<sub>B</sub> resistance but only single isolates  
8 of serotype Ia, Ib and IV. Four serotype V isolates harboring *ermB*, isolated from women from four  
9 different cities in Sweden, were the only isolates with MICs > 256 mg/L for both erythromycin and  
10 clindamycin. Tetracycline resistance was found in 89% of the 44 serotype III isolates that harbored the  
11 GBSi1 intron, corresponding to CC17 (17), but none of these isolates were erythromycin or clindamycin  
12 resistant.  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25

26 All GBS had penicillin MICs ≤ 0.064 mg/L (15). The distribution of the inhibition zones from the screening  
27 of penicillin resistance with 1µg oxacillin disks suggested that all the isolates could belong to the wild  
28 type population (Table II). However, three GBS isolates gave inhibition zones ≤17 mm, the breakpoint  
29 set by SRGA to guard for penicillin resistance in beta-haemolytic streptococci. The oxacillin MIC<sup>90</sup> for the  
30 GBS isolates of the study was 1 mg/L. The three isolates with oxacillin inhibitory zones repeatedly less  
31 than 17 mm (range 16.2-16.9 mm) were further tested and showed the following MICs: oxacillin 1 mg/L,  
32 penicillin G 0,032-0,064mg/L, ampicillin 0,064-0,125mg/L, cefuroxime 0,064mg/L, cefotaxime 0,064-  
33 0,125mg/L, imipenem 0,032-0.125 mg/L and ceftiofloxacin 2-4 mg/L. The identity of these isolates as GBS  
34 was confirmed with API-Strep (bioMérieux AB, Askim, Sweden). When tested with the EUCAST method  
35 the inhibition zone ranges of the three isolates were 15,7-18,5 mm and 16,2-19,2 mm for oxacillin and  
36 ceftibuten respectively, in agreement with results of the other isolates tested (Table II).  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49

50 In 28 isolates with inhibitory zones for gentamicin of ≤ 8 mm the MICs ranged between 24-96 mg/L; i.e.  
51 32 -128 mg/l when the results are rounded up to the next highest twofold dilution, indicating that none of  
52 the isolates had high-level gentamicin resistance ( www.srga.org). One of these 28 isolates, which had a  
53 gentamicin MIC of 64 mg/L, lacked an inhibition zone around the 1000 µg kanamycin disk and  
54 harboured the *aphA-3* gene. Four additional isolates, with gentamicin inhibition zones > 8 mm, lacked  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 inhibition zones around the highly loaded kanamycin disk and three of these also lacked inhibition zones  
2 around the 1000 µg streptomycin disk (Fig 1). The kanamycin phosphorylase gene *aphA-3* was present  
3 in all of the kanamycin resistant isolates. In addition the *aad-6* gene, encoding a nucleotidyltransferase  
4 that adenylylate streptomycin, was identified in the three streptomycin resistant isolates. The kanamycin  
5 phosphorylas mediate resistance against amikacin as well and accordingly the amikacin MICs in all of  
6 the kanamycin resistant isolates were >256mg/L. No zone around the 1000 µg kanamycin disk is  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

inhibition zones around the highly loaded kanamycin disk and three of these also lacked inhibition zones around the 1000 µg streptomycin disk (Fig 1). The kanamycin phosphorylase gene *aphA-3* was present in all of the kanamycin resistant isolates. In addition the *aad-6* gene, encoding a nucleotidyltransferase that adenylylate streptomycin, was identified in the three streptomycin resistant isolates. The kanamycin phosphorylas mediate resistance against amikacin as well and accordingly the amikacin MICs in all of the kanamycin resistant isolates were >256mg/L. No zone around the 1000 µg kanamycin disk is indicative for resistance. None of 20 isolates with kanamycin inhibition diameters ranging from 12 to 15 mm harboured the *aphA-3* or *aad-6* gene. The 5 kanamycin resistant isolates were also macrolide resistant and had the *erm(A/TR)* gene (1 isolate) or the *erm(B)* gene (Table I).

## Discussion

This study is, to our knowledge, the first report of high-level aminoglycoside resistance in GBS isolates from Scandinavia. Five of 396 GBS isolates (1.3%) from a nation wide study of colonizing GBS were kanamycin and amikacin resistant. Screening with kanamycin 1000 µg disks enabled a rapid and easy detection of these isolates. The breakpoint for the susceptible isolates was originally proposed as 17 mm (8). However, the distribution of the GBS population from this study suggests that a lower breakpoint, possibly at 12 mm would be more appropriate (Fig 1).

The kanamycin resistance gene *aphA-3*, present in these isolates, is in *S. pneumoniae* and *Enterococcus faecalis* located on transposons and plasmids, but the exact genetic organisation of the gene in these GBS isolates is not known. No isolates with high level gentamicin resistance was found. Despite this, keeping the evolution in enterococci in mind, in which several aminoglycoside modifying enzymes have been described (22), an active surveillance on the aminoglycoside resistance in GBS is warranted. The first gentamicin high level resistant (HLR) GBS, strain B128, was described in France in 1990 (6). The gene of the bifunctional aminoglycoside modifying enzyme Aac(6')-Aph(2'') that mediate resistance to gentamicin and all other aminoglycosides besides streptomycin as well as homologes to *aphA-3* and *aad-6* genes was later identified in B128 (9). The aminoglycoside resistant determinants were carried by the transposon Tn3706 located on the chromosome. Tn3706 could be transposed from its chromosomal location onto the plasmid IP501, a conjugative multiple antibiotic resistance plasmid that can be transferred and stably maintained in a variety of Gram-positive genera, including GBS (23). However, the transposition lead to a structural instability of the plasmid and the

1 authors concluded that pIP501-like plasmids probably do not constitute appropriate vectors for  
2 dissemination of gentamicin HLR in GBS. The clinical impact of HLR aminoglycoside resistance is a  
3 loss of the synergistic bactericidal effect achieved in conjunction with cell wall active antibiotics as  
4 penicillin, which will compromise the effectiveness of therapy (5). The combination of a cell wall active  
5 antibiotic and an aminoglycoside has been shown to greatly enhance the killing of GBS *in vitro* (5, 24).  
6  
7  
8  
9

10  
11 Penicillin is the main choice for prophylaxis and therapy of GBS infections. The methodology used for  
12 the antibiotic susceptibility test is of vital importance when resistance data are interpreted. An  
13 additional important factor for the comparison of susceptibility among isolates is the breakpoints set for  
14 classification of isolates. Screening for penicillin resistance with 1 µg oxacillin disks is recommended in  
15 *Streptococcus pneumoniae* and group A, B, C, and G streptococci by SRGA. The breakpoint to guard  
16 for penicillin resistance is at present set at 17mm. However, compared to the population distributions  
17 seen in pneumococci and group A, C, G streptococci, with a majority of isolates with oxacillin inhibition  
18 zones of 27-28 mm (www.srga.org), the GBS of this study exhibited inhibition zones with a mean zone  
19 size of 20mm (Table II). This is in agreement with data, presented by Baker et al almost thirty years  
20 ago, which showed that higher penicillin concentrations were needed for growth inhibition and killing of  
21 GBS than for group A streptococci (5). The oxacillin breakpoint of 17mm divides what seems to be a  
22 susceptible (native) GBS population and possibly could a breakpoint of 14 or 15 mm be more suited  
23 for GBS. The results of the oxacillin screening according to the EUCAST method were in agreement  
24 with that suggestion. Recently, high sensitivity and specificity for detection of reduced penicillin  
25 susceptibility in GBS was reported with a provisional cut off value of 20 mm for ceftibuten in disk  
26 diffusion (25). The tests were performed in accordance with the recommendation from the American  
27 Clinical and Laboratory Standards Institute (CLSI). From the results of our study a ceftibuten  
28 breakpoint for susceptibility of  $\geq 15$ -16 mm seems to be more appropriate, a discrepancy possibly  
29 caused by the differences in methods used. However, studies on the alteration of Pbp genes in  
30 relation to penicillin susceptibility are needed to fully evaluate the isolates with smaller inhibition zones.  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51

52  
53 During 2008 a risk based program for IAP has been recommended by the National board of health in  
54 Sweden. Since this will lead to increased antibiotic use in the future it is of importance to follow the  
55 epidemiology of antibiotic resistance in GBS to be able to give guidance for the prophylaxis used. This  
56 is especially important when a risk based IAP program is used since no individual GBS isolates are  
57  
58  
59  
60  
61  
62  
63  
64  
65

1 cultured for examination of the antibiotic susceptibility in this regime. The overall degree of antibiotic  
2 resistance in the Swedish colonizing GBS isolates was low, with the exception for 79 % resistance  
3 against tetracycline. Only 5 % of the GBS were resistant to clindamycin, which can be compared to  
4 MLS<sub>B</sub> resistance rates around 15% in several European and Australian reports (18, 26), and 5-32 % in  
5 the American continent (8, 27).  
6  
7

8  
9 An increased degree of MLS<sub>B</sub> resistance in serotype V isolates has been described (19, 26). This has  
10 also been shown in recent Swedish and Norwegian studies of invasive GBS isolates (28, 29).  
11

12 However, in this nation wide collection of colonizing GBS from parturient women no serotype  
13 dominated among the clindamycin and/or erythromycin resistant isolates. Whether the high-level of  
14 MLS<sub>B</sub> resistance found in the four serotype V isolates that carried the *ermB* gene indicate that they  
15 have an additional resistance mechanism has to be further evaluated.  
16  
17

18 The serotype III lineage, CC17, containing sequence type 17 (ST17), is strongly associated with  
19 neonatal invasive infections (17, 18). In a recent study serotype III isolates of ST17 were shown to  
20 carry less antibiotic resistance genes than the other GBS serotypes (14). The lack of MLS<sub>B</sub> resistance  
21 in this lineage was corroborated in our study.  
22

23 We here provide a baseline description of resistance levels and resistance genes present among  
24 colonizing GBS in Sweden before implementation of a novel prophylactic regimen that will increase  
25 the use of intrapartum antibiotics. This kind of information will be useful in future evaluations of  
26 changes of resistance in GBS. We also suggest that a change of breakpoints for GBS oxacillin  
27 susceptibility tests should be considered. The results of this study partially disagree with some  
28 previous studies. We found no particular serotype to dominate among the MLS<sub>B</sub> resistant isolates. In  
29 contrast, other studies, in particular those that have included invasive isolates (14, 27, 28), indicate  
30 serotype V to be predominant among the MLS<sub>B</sub> resistant GBS. In Norway an increase in fatality rate  
31 among neonates with GBS infection has been observed which coincided with an increase in MLS<sub>B</sub>  
32 resistance; predominantly in serotype V isolates (29). A possible explanation for the discrepancy  
33 between our study and others is that a clonal dissemination of MLS<sub>B</sub> resistant serotype V strains  
34 account for the increase among the invasive cases. We conclude that in this Swedish colonizing  
35 material, serotype V is a poor predictor of antibiotic resistance. However, the occurrence of high level  
36 erythromycin and clindamycin resistance found in all the four serotype V isolates containing the *ermB*  
37 gene merits further examination. In summary, among Swedish colonizing GBS isolates 5, 5% MLS<sub>B</sub>  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

1  
2 resistance and 1, 3% aminoglycoside resistance was found among the evenly distributed serotypes Ia  
3  
4  
5  
6  
7  
8 to V.

## 9 **Acknowledgments**

10 Åsa Hedlund and Helén Edebro for technical assistance.  
11 This study was supported by a grant (ALF) from Västerbottens County Council.  
12  
13  
14  
15  
16  
17  
18  
19

## 20 **References**

21  
22  
23  
24 1. Schrag SJ, Zywicki S, Farley MM, Reingold AL, Harrison LH, Lefkowitz LB, Hadler JL, Danila R,  
25 Cieslak PR, Schuchat A (2000) Group B streptococcal disease in the era of intrapartum antibiotic  
26 prophylaxis. *N Engl J Med* 342:15–20  
27  
28  
29

30  
31  
32 2. Berardi A, Lugli L, Rossi C, Morini MS, Vagnarelli F, Ferrari F (2008) Group B streptococcus and  
33 preventive strategies in Europe. *Arch Dis Child Fetal Neonatal Ed* 93:F249.  
34  
35  
36 doi:10.1136/adc.2007.135392  
37  
38  
39

40  
41 3. Law MR, Palomaki G, Alfirevic Z, Gilbert R, Heath P, McCartney C, Reid T, Schrag S (2005) The  
42 prevention of neonatal group B streptococcal disease: a report by a working group of the Medical  
43 Screening Society. *J Med Screen* 12:60-68  
44  
45  
46

47  
48 4. Isaacs D, Wilkinson AR (1987) Antibiotic use in the neonatal unit. *Arch Dis Child* 62:204–208  
49  
50  
51

52  
53 5. Baker CN, Thornsberry C, Facklam RR (1981) Synergism, killing kinetics, and antimicrobial  
54 susceptibility of group A and B streptococci. *Antimicrob Agents Chemother* 19:716-725  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65  
6. Buu-Hoï A, Le Bouguenec C, Horaud T (1990) High-level chromosomal gentamicin resistance in *Streptococcus agalactiae* (group B). *Antimicrob Agents Chemother* 34:985–988
7. Liddy H, Holliman R (2002) Group B *Streptococcus* highly resistant to gentamicin. *J Antimicrob Chemother* 50:142-143
8. Lopardo HA, Vidal P, Jeric P, Centron D, Paganini H, Facklam RR, Elliott J; Argentinian *Streptococcus* Study Group (2003) Six-month multicenter study on invasive infections due to group B streptococci in Argentina. *J Clin Microbiol* 41:4688-4694
9. Kaufhold A, Podbielski A, Horaud T, Ferrieri P (1992) Identical genes confer high-level resistance to gentamicin upon *Enterococcus faecalis*, *Enterococcus faecium*, and *Streptococcus agalactiae*. *Antimicrob Agents Chemother* 36:1215-1218
10. Kimura K, Suzuki S, Wachino J, Kurokawa H, Yamane K, Shibata N, Nagano N, Kato H, Shibayama K, Arakawa Y (2008) First molecular characterization of group B streptococci with reduced penicillin susceptibility. *Antimicrob Agents Chemother* 52:2890-2897
11. Dahesh S, Hensler ME, Van Sorge NM, Gertz RE Jr, Schrag S, Nizet V, Beall BW (2008) Point mutation in the group B streptococcal *pbp2x* gene conferring decreased susceptibility to beta-lactam antibiotics. *Antimicrob Agents Chemother* 52:2915-2918
12. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A (2002) Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. *MMWR Recomm Rep* 51:1-22
13. Andrews JI, Diekema DJ, Hunter SK, Rhomberg PR, Pfaller MA, Jones RN, Doern GV (2000) Group B streptococci causing neonatal bloodstream infection: antimicrobial susceptibility and serotyping results from SENTRY centers in the Western Hemisphere. *Am J Obstet Gynecol* 183:859-862

- 1  
2  
3  
4  
5  
6  
7  
8  
9  
10  
11  
12  
13  
14  
15  
16  
17  
18  
19  
20  
21  
22  
23  
24  
25  
26  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65
14. Zhao Z, Kong F, Zeng X, Gidding HF, Morgan J, Gilbert GL (2008) Distribution of genotypes and antibiotic resistance genes among invasive *Streptococcus agalactiae* (group B streptococcus) isolates from Australasian patients belonging to different age groups Clin Microbiol Infect 14:260-267
  15. Håkansson S, Axemo P, Bremme K, Bryngelsson AL, Wallin MC, Ekström CM, Granlund M, Jacobsson B, Källén K, Spetz E, Tessin I; Swedish Working Group For The Prevention of Perinatal Group B Streptococcal Infections (2008) Group B streptococcal carriage in Sweden: a national study on risk factors for mother and infant colonisation. Acta Obstet Gynecol Scand 87:50-58
  16. Kong F, Gowan S, Martin D, James G, Gilbert GL (2002) Serotype identification of group B streptococci by PCR and sequencing. J Clin Microbiol 40:216-26.)
  17. Luan SL, Granlund M, Sellin M, Lagergård T, Spratt BG, Norgren M (2005) Multilocus sequence typing of Swedish invasive group B streptococcus isolates indicates a neonatally associated genetic lineage and capsule switching. J Clin Microbiol 43:3727-3733
  18. Jones N, Bohnsack JF, Takahashi S, Oliver KA, Chan MS, Kunst F, Glaser P, Rusniok C, Crook DW, Harding RM, Bisharat N, Spratt BG (2003) Multilocus sequence typing system for group B streptococcus. J Clin Microbiol 41:2530-2536
  19. Poyart C, Jardy L, Quesne G, Berche P, Trieu-Cuot P (2003) Genetic basis of antibiotic resistance in *Streptococcus agalactiae* strains isolated in a French hospital. Antimicrob Agents Chemother 47:794-797
  20. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28:495-503.
  21. Arpin C, Daube H, Tessier F, Quentin C (1999) Presence of *mefA* and *mefE* genes in *Streptococcus agalactiae*. Antimicrob Agents Chemother 43:944-946

22. Chow JW (2000) Aminoglycoside resistance in enterococci. Clin Inf Dis 31:586-589

23. Horaud T, de Céspedes G, Trieu-Cuot P (1996) Chromosomal gentamicin resistance transposon Tn3706 in *Streptococcus agalactiae* B128. Antimicrob Agents Chemother 40:1085-1090

24. Cooper MD, Keeney RE, Lyons SF, Cheatle EL (1979) Synergistic effects of ampicillin-aminoglycoside combinations on group B streptococci. Antimicrob Agents Chemother 15:484-486

25. Kimura K, Wachino J, Kurokawa H, Suzuki S, Yamane K, Shibata N, Arakawa Y (2009) Practical disk diffusion test for detecting group B streptococcus with reduced penicillin susceptibility. J Clin Microbiol 47:4154-4157

26. Zeng X, Kong F, Wang H, Darbar A, Gilbert GL (2006) Simultaneous detection of nine antibiotic resistance-related genes in *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization assay. Antimicrob Agents Chemother 50:204-209

27. Phares CR, Lynfield R, Farley MM, Mohle-Boetani J, Harrison LH, Petit S, Craig AS, Schaffner W, Zansky SM, Gershman K, Stefonek KR, Albanese BA, Zell ER, Schuchat A, Schrag SJ; Active bacterial core surveillance/emerging infections program network (2008) Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. JAMA 299:2056-2065

28. Persson E, Berg S, Bergseng H, Berg K, Valsö-Lyng R, Trollfors B (2008) Antimicrobial susceptibility of invasive group B streptococcal isolates from south-west Sweden 1988-2001. Scand J Infect Dis 40:308-313

29. Bergseng H, Afset JE, Radtke A, Loeseth K, Lyng RV, Rygg M, Bergh K (2009) Molecular and phenotypic characterization of invasive group B streptococcus strains from infants in Norway 2006–2007. Clin Microbiol Infect 15:1182-1185

Figure 1.

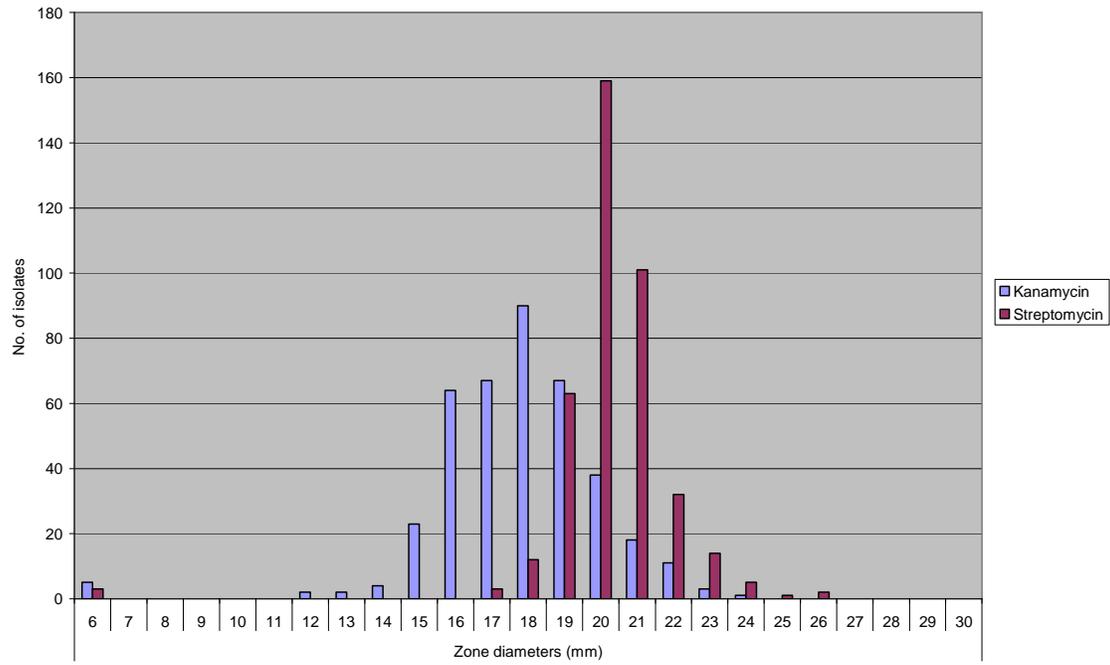


Fig.1. Zone diameter distributions for 1000 µg disks of kanamycin and streptomycin against 396 group B streptococci.

Table I

Sample number	Resistance genes					Disk-diffusion, inhibition zones (mm)				Etest, MIC (mg/L)		Serotype
	<i>aad-6</i>	<i>aphA-3</i>	<i>ermB</i>	<i>ermA/TR</i>	<i>mefA</i>	ISA-NAD E	ISA-NAD DA	MH-NAD E	MH-NAD DA	MH-NAD EM	MH-NAD CM	
2321	pos	pos	pos	neg	neg	12.8	17.3	11.9	14	2	0.5	III
2051	neg	pos	pos	neg	neg	14.2	21.1	11.1	12.8	2	0.5	II
1944	pos	pos	pos	neg	neg	15	19.3	12.5	14.3	4	0.5	II
302	neg	pos	pos	neg	neg	13.8	16.2	12.3	14.2	4	0.5	III
2655			pos	neg	neg	6	23.7	6	21	>256	1	III
1057			pos	neg	neg	6	6	6	6	>256	>256	V
1540			pos	neg	neg	6	6	6	6	>256	>256	V
3066			pos	neg	neg	6	6	6	6	>256	>256	V
2920			pos	neg	neg	6	6	6	6	>256	>256	V
188	pos	pos	neg	pos	neg	20.1	31,6 D	10.8	22,7D	1	0.25	III
3107			neg	pos	neg	19.8	33,5 D	12.6	23,4D	1	0.12	III
2988			neg	pos	neg	15	31,2 D	12.9	29,5D	2	0.12	II
1456			neg	pos	neg	15.5	31,9 D	11.8	28,0D	4	0.12	II
2107			neg	pos	neg	16.7	32,4 D	11.8	17,3D	4	0.12	V
846			neg	pos	neg	17.7	34,8 D	12.8	30,1D	4	0.12	Ia
2280			neg	pos	neg	20.2	32,6 D	NT	NT	NT	NT	III
2710			neg	pos	neg	18.6	6	17	6	4	>256	II
1511			neg	pos	neg	21.3	6	17.2	6	4	>256	Ia
2040			neg	pos	neg	13.9	6	6	6	8	>256	Ib
2292			neg	neg	neg	29.1	23.5	27.8	24	0.12	2	II
2225			neg	neg	neg	30.4	23.1	26.8	23.5	0.12	2	II
865			neg	neg	neg	31	22.5	26.8	22.8	0.25	2	IV

The genes *aad-6* and *aphA-3* encode aminoglycoside-modifying enzymes.

The genes *erm*(B), *erm*(A/TR) and *mef*(A) mediate resistance to the macrolides-lincosamides-streptogramin B group of antibiotics.

ISA-NAD is Iso-Sensitest Agar with 5% blood and 20 mg/L of nicotinamide adenine dinucleotide.

MH-NAD is Mueller-Hinton agar with 5% horse blood and 20 mg/L nicotinamide adenine dinucleotide

E and EM: erythromycin

DA and CM:clindamycin

Table II

Oxacillin Zone diameter range ISA-NAD (mm)	Penicillin G MIC (Number of isolates)			Oxacillin Zone diameter range MH-NAD (mm)	Ceftibuten Zone diameter range MH-NAD (mm)
	0,016 mg/L	0,032 mg/L	0,064 mg/L		
16,3-16,9	0	0	3	15,7-18,5	16,2-19,2
17,7-18,4	0	10	11	16,1-20,7	16,9-20,5
18,5-19,4	0	71	44	NT	NT
19,5-20,4	2	73	33	NT	NT
20,5-21,4	0	68	18	NT	NT
21,5-22,4	7	38	2	NT	NT
22,5-23,4	1	10	0	NT	NT
23,5-24,4	1	0	0	NT	NT
24,5-25,4	2	1	0	NT	NT