Le Infezioni in Medicina, n. 3, 217-221, 2016

Streptococcus agalactiae: prevalence of antimicrobial resistance in vaginal and rectal swabs in Italian pregnant women

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SUMMARY

Intrapartum antibiotic prophylaxis (IAP) reduces both the vertical transmission of *Streptococcus agalactiae* or *Group B Streptococcus* (GBS) and the early onset of neonatal sepsis. However, existing guidelines do not recommend that antimicrobial susceptibility testing (AST) be routinely performed. Penicillin or ampicillin are indicated as first-choice antibiotics, cefazolin being an alternative in the case of history of mild allergic reactions, and vancomycin or clindamycin an alternative in the event of severe reactions. We performed a cross-sectional analysis to identify the presence of any bacterial resistance towards the antibiotics most frequently used for IAP in pregnant women with GBS positive vaginal-rectal swabs, in the Pistoia area of central Italy. Of the 255 tested samples, 65 (25.5%) were positive for GBS. Sensitivity to glycopeptides was over 90%, but lower to ampicillin and penicillin (87.10% and 87.93% respectively). Resistance towards clindamycin and erythromycin was as high as 43.75% and 32.20%. All tested GBS proved susceptible to moxifloxacin, linezolid and tigecycline. Our observed prevalence is aligned or slightly higher than data reported in other series. The less than full effectiveness and low percentages of ampicillin and penicillin sensitivity observed give cause for concern. We confirmed the increase in clindamycin and erythromycin resistance. Glycopeptides can be used as second-line antibiotics, but the complete AST of GBS should always be performed before IAP. Given that gentamicin is used synergically with penicillin when treating chorioamnionitis, it needs to be always included in the AST. This is the first study on the GBS sensitivity profile in Tuscany. Further investigation on a larger scale is required prior to implementing any changes in the current guidelines.

Keywords: *Streptococcus agalactiae,* antimicrobial resistance, vaginal and rectal swab, intrapartum antibiotic prophylaxis, early-onset neonatal sepsis.

INTRODUCTION

Streptococcus agalactiae or *Group B Streptococcus* (GBS), which commonly host the intestinal and genital microflora, is one of the leading causes of invasive bacterial infections in the newborn, due mainly to a vertical transmission [1].

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In Italy, 10-20% of pregnant women carry GBS in the intestinal tract, from where it can colonize the uro-genital tract. Approximately 50-70% of infants born from GBS carrying women are colonized and, of these, 1-2% develop an early infection in case of no antibiotic treatment. Intrapartum Antibiotic Prophylaxis (IAP) reduces both GBS vertical transmission and the early onset of neonatal sepsis. Guidelines do not recommend to perform antimicrobial susceptibility testing (AST) routinely for isolates retrieved from antenatal screening and indicates in penicillin or ampicillin the first

choice antibiotics, being cefazolin an alternative in case of history of mild allergic reactions and vancomycin or clindamycin an alternative in case of severe reactions. Concerns about IAP pertain potential toxicity and, mainly, potential pressure towards antibiotic resistance among GBS strains [2].
The objective of this paper is to investigate the presence of GBS antibiotic resistance towards antibiotics used for IAP, chosen according to the guidelines implemented by the Public Health System [3].
The antibiotic resistance profile was also analyzed in order to explore possible association with some demographic and anamnestic characteristics of the study population.

PATIENTS AND METHODS

We performed a cross-sectional analysis to identify the presence of any bacterial resistance towards the antibiotics most frequently used for IAP, in pregnant women with GBS positive vaginal-rectal swab (VRS).
The ethical committee was informed about the study, which was done as part of the continuous quality assessment of the microbiology laboratory and women were not required to sign an informed consent.
From April to September 2013, we analyzed 255 VRSs, collected from pregnant women between 34th and 36th week +6 days of gestational age in Pistoia area, in Italy. Sixty-five (65) VRSs resulted to be positive to GBS and were further analyzed: AST and determination of the minimum inhibitory concentration (MIC), according to EUCAST guidelines 2013 [4], were performed for: benzylpenicillin; ampicillin, ampicillin/sulbactam; gentamicin; cefuroxime; levofloxacin; moxifloxacin; clindamycin; erythromycin; quinupristin/dalfopristin; linezolid; teicoplanin; tetracycline; tigecycline; nitrofurantoin; trimethoprim/sulfamethoxazole. Demographic data and medical history included in the analysis were: age, country of origin, level of education, employment, number of pregnancies, antibiotic therapy within 30 days prior to the execution of the VRS. The proportions were estimated by calculating the 95% confidence interval (95% CI). The odds ratio and the corresponding 95% CI were calculated to measure the association related to the relative probability of some selected variables, a first stage in the univariate approach and a second step in the multivariate approach; the latter analysis for confounding. The data were finally processed using the statistical software STATA 8.

RESULTS

Of the total 255 tested samples, 65 (25.5%) were positive for GBS, 41 of which coming from Public consulting room and 24 from private structures. Anamnestic information were available only for the first subgroup (Public structures). The descriptive analysis on the subgroup of 41 subjects is reported in Table 1. The 65 samples (non-random sample) analyzed for susceptibility tests were tested towards different pattern of antibiotics, but ampicillin/sulbactam, cefuroxime and gentamicin were tested only for one sample and showed high sensitivity. Sensitivity and resistance to the all other antibiotics tested were reported in Table 2. All GBS resulted susceptible to moxifloxacin,

Table 1 - Demographic and anamnestic characteristics of the study population.

Characteristics	*No.*	*%*
Country of origin		
Italy	21	51.22
Northern Europe	2	4.88
East Europe	14	34.15
Africa	3	7.32
America	1	2.43
Total	**41**	**100.00**
Level of education		
Middle school	23	56.10
High school	12	29.27
University degree	6	14.63
Total	**41**	**100.00**
Employment		
Unemployed	19	46.34
Employed	22	53.66
Total	**41**	**100.00**
First pregnancy		
Uni or multiparous	23	56.10
Nulliparous	18	43.90
Total	**41**	**100.00**

Table 2 - Distributions of resistance to the antibiotics tested in GBS isolated.

Antibiotics	*Sensitivity*		*Resistance*		*Total*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Clindamycin	36	56.25	28	43.75	64	100.00
Erythromycin	40	67.80	19	32.20	59	100.00
Tetracycline	54	84.38	10	15.62	64	100.00
Ampicillin	54	87.10	8	12.90	62	100.00
Benzylpenicillin	51	87.93	7	12.07	58	100.00
Levofloxacin	59	90.77	6	9.23	65	100.00
Vancomycin	59	92.19	5	7.81	64	100.00
Teicoplanin	59	95.16	3	4.84	62	100.00
Nitrofurantoin	60	95.24	3	4.76	63	100.00
Trimethoprim/Sulfamethoxazole	62	95.49	3	4.62	65	100.00
Moxifloxacin	60	100.00	0	0.00	60	100.00
Linezolid	62	100.00	0	0.00	62	100.00
Tigecycline	62	100.00	0	0.00	62	100.00

linezolid and tigecycline. Sensitivity to glycopeptides was over 90%, but that to ampicillin and penicillin was lower: 87,10% and 87,93% respectively. Resistance towards clindamycin and erythromycin was as high as 43,75% and 32,20%. Moreover, both by univariate analysis using either the multivariate analysis, it was observed that there are no significant correlations between antibiotic resistance and the subjects anamnestic characteristics (Table 3).

DISCUSSION

The study results support the known prevalence of GBS positive VRS in pregnant women: observed prevalence of 25.5% is higher than data reported in other series [5] (Table 4). Worrying is the not full effectiveness and low percentages of ampicillin and penicillin (first-line choice) sensitivity observed and, noteworthy, the percentage of clindamycin resistant strains was significantly higher than the 20-30% of resistance found in the reviewed literature concerning other European and non-European countries apart from a recent study from Beijng, China [6-10]. The same, erythromycin resistance confirms the increase and if the isolate is sensitive to clindamycin but resistant to erythromycin, clindamycin may be used only if testing for inducible clindamycin resistance is negative. Resistance of GBS to erythromycin is related to different mechanisms: methylation of 23S rRNA by specific methylase enzymes and the activation of an efflux pump; the methylation blocks the binding of erythromycin with the 50S ribosomal subunit, determining the resistance to other antibacterial drugs, specifically lincosamides and streptogramin. Cephalosporins as alternative agent, given their efficacy is inferred from the penicillin susceptibility, should be used only after antimicrobial susceptibility testing (AST). The glycopeptides can be used as second-line antibiotics, but the complete AST of GBS should be always performed before IAP. Given that Gentamicin used as synergic with penicillin when treating chorioamnionitis, needs to be always included in the AST.

Other antibiotics (*e.g.* linezolid) are not recommended as their placental transfer is not known.

An overview of our data and of data from similar studies, carried out from 2007 to 2015, is presented in Table 4, where the use of different interpreta-

Table 3 - Univariate and multivariate analysis of ampicillin resistance according to the variables considered.

Variable	*Univariate*		*Multivariate*	
	OR	$IC_{95\%}$	*OR*	$IC_{95\%}$
Origin	0.75	0.34 - 1.63	1.16	0.43 - 3.14
Instruction	3.20	0.93 - 11.03	1.42	0.56 - 4.76
Employment	1.80	0.26 - 12.67	0.75	0.66 - 8.54
Nulliparous	0.43	0.06 - 3.09	0.37	0.47 - 2.92

Table 4 - Summary of relevant GBS studies published worldwide from 2007 to 2015.

References *Year of Publication*	*Our data* *2016*	*Wang [10]* *2015*	*Frohlicher [14]* *2014*	*Dutra [11]* *2014*	*Abarzúa [13]* *2014*	*Capanna [6]* *2013*	*Muller [12]* *2008*	*Busetti [5]* *2007*
Study period (months)	2013 (6)	2012-2013 (24)	2009-2010 (17)	2005-2009 (58)	2010-2012 (26)	2011 (3)	2002-2003 (13)	2002-2005 (48)
Country	Italy (Pistoia)	China (Beijing)	Switzerland (Bern)	Brasil (5 regions)	Chile (South)	Swiss (Geneva)	Netherlands (Hague)	Italy (Trieste)
Prevalence (%)	25.5	6.48	na	na	14.4	16.3	21.4	17.9
No. of total swabs	255	863	na	na	1181	760	1702	5020
No. of GBS positive swabs	65	56	364	434	167	124	365 (107 for AST)	901
Colonization (C) and/or Infection (I)	C	C	C	C and I	C	C	C	C
Antibiotic Interpretation System	EUCAST 2013	CLSI 2012	EUCAST (year na)	CLSI 2010	CLSI 2010	CLSI 2010	NCCLS 2004	na
Antibiotic and Resistance %								
Clindamycin	43.75	64.30	14.00	3.00	13.70	28.00	6.00	na
Erythromycin	32.2	78.60	14.50	4.10	9.50	30.00	7.00	na
Tetracyclin	15.62	83.90	na	97.00	na	89.00	na	na
Ampicillin	1.9	na	na	0.00	na	na	na	na
Benzylpenicillin	12.07	0.00	0.00	na	na	0.00	0.00	na
Levofloxacin	9.23	35.70	na	0.00	na	1.60	na	na
Vancomycin	7.81	na	na	na	na	0.00	na	na
Teicoplanin	4.84	na	na	na	na	na	na	na
Nitrofurantoin	4.76	na	na	na	na	na	na	na
Trimethoprim/sulfamethoxazole	4.62	na	na	na	na	na	na	na
Moxifloxacin	0	na	na	na	na	na	na	na
Linezolid	0	na	na	na	na	0.00	na	na
Tigecycline	0	na	na	na	na	na	na	na
Ceftriaxone	na	0.00	na	na	na	na	na	na
Cefotaxime	na	na	na	0.00	na	na	na	na
Clarithromycin	na	78.60	na	na	na	na	na	na
Azithromycin	na	87.50	na	na	na	na	na	na
CAF	na	na	na	0.00	na	na	na	na
Gentamicin	na	na	na	na	na	0.80	na	na
Moxifloxacina	na	na	na	na	na	1.60	na	na
Rifampicina	na	na	na	na	na	0.00	na	na
Cephalothin	na	na	na	na	na	na	0.00	na

na: not available.
EUCAST: The European Committee on Antimicrobial Susceptibility Testing.
CLSI: Clinical and Laboratory Standards Institute.
NCCLS: National Committee for Clinical Laboratory Standards.

tion criteria (CLSI and EUCAST) and the different settings precludes a univocal comparison. Nonetheless our results show a high prevalence of resistance to clindamycin, erythromycin, benzylpenicillin, levofloxacin and vancomycin likely due to the increased use of antibiotics for prophylaxis in Tuscany. Moreover, we observe a lower percentage of resistance to tetracycline while teicoplanin, nitrofurantoin and trimethoprim/sulfamethoxazole are not been examined in other studies.
There were no correlations between antibiotic resistance and the demographic and medical history. The limit of this study is the small sample size, but this is the first study on the GBS sensitivity profile in Tuscany. In conclusion, we believe that our results deserve further investigation on a larger scale before implementing any changes in the current guidelines and a surveillance system of antibiotic sensitivity patterns in Tuscan Public Health System.

ACKNOWLEDGMENTS
The authors wish to acknowledge Dr Ilaria Galgani (Siena, Italy) for her scientific and linguistic review, and all the women who agreed to be screened and included in the study.

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