Prevalence of Capsular Serotype, Pilus Island Distribution, and Antibiotic Resistance in Pediatric and Adult Invasive *Group B Streptococcus* Isolates: Data From a Nationwide Prospective Surveillance Study in Germany

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Abstract: For neonates, group B Streptococcus is life threatening. Current prevention strategies remain insufficient, especially for cases of late-onset sepsis, where intrapartum antibiotic prophylaxis has demonstrated no benefit. One promising approach is the vaccination of pregnant women, which offers protective immunity via transplacental transmission of neutralizing antibodies. Our nationwide, prospective surveillance study aimed to characterize the prevalence of pilus antigen, capsular polysaccharide serotypes, and antibiotic resistance from invasive GBS infections in neonates and compare these results with those from children and adults in Germany. Our study includes 173 neonatal isolates of a total of 450 reported cases during the study period (incidence: 0.34/1000 live births), in addition to 2 pediatric and 803 adult isolates. The comparison between neonatal and adult isolates reveals age-dependent differences in capsular serotype and pilus type distribution and differences in antibiotic resistance patterns.

Key Words: group B Streptococcus, serotype, pilus island, antibiotic resistance

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Group B Streptococcus (GBS, also termed *Streptococcus agalactiae*) is one of the leading causative agents of neonatal sepsis and meningitis. Contributing substantially to perinatal mortality, GBS has an estimated 147,000 lethal cases annually, including stillbirths.[1] Adults, primarily those with underlying medical conditions such as diabetes mellitus, heart disease, or malignancies, also are at risk of life-threatening infections.[2] Elderly patients, which in our study were defined as >65 years of age, have an especially high incidence.[3] In a range of Western countries, morbidity and mortality are increasing in adults with underlying conditions.[4–6]

Standardized screening and antibiotic prophylaxis programs for colonized pregnant women have been successful in reducing the incidence of early-onset neonatal sepsis (EOD) (ie, the occurrence of symptoms during the first 7 days of life), but such measures have failed to provide benefits in relation to the incidence of late-onset sepsis in neonates and infants.[7,8] EOD prevention strategy relies on screening for GBS carrier status during late pregnancy and on intrapartum antibiotic prophylaxis in the event of a positive GBS screening result. However, the positive predictive value of the screening test can be as low as 70%, and sensitivity has been reported at approximately 90%.[9] These screening limitations frequently lead to unnecessary antibiotic treatment, as well as to missed cases (those that do not receive intrapartum antibiotic prophylaxis despite a positive carrier status). To further complicate the situation, a large proportion of newborns with GBS disease are born to mothers with documented negative carrier status.[10] This suggests that there are dynamic changes in GBS carrier status during late pregnancy.

It has been postulated that a vaccine against GBS during pregnancy may provide an efficient and safe way to reduce the disease burden in both early-onset and late-onset disease. Capsular polysaccharides are major virulence factors, as well as potential vaccine antigens.[11] Indeed, preliminary studies repeatedly have shown that multivalent conjugate vaccines of capsular polysaccharides linked to antigenic proteins such as tetanus toxoid lead to sustained antibody levels in both the mother and the neonate.[12] A vaccine also has been successfully tested in the elderly.[3] Pilus antigens have emerged as potential vaccine targets because they are conserved across all GBS strains and abundantly expressed on the surface of GBS.[13] Transplacental protection has been reported in mice,[14] and an inverse correlation between maternal antipilus antibodies and the risk of GBS transmission to the newborn has been described.[15] Pilus polymers have been described as relevant to colonization and also as a virulence factor in mouse models,[16,17] due to the polymers' ability to facilitate cellular adherence to the vaginal epithelium.[18] Nevertheless, a pathogenetically relevant role for human disease remains unknown. For this reason, we wanted to compare pilus island distribution among adults with neonatal disease. In doing so, we found a distinct prevalence. Potentially, this may indicate a different kind of pathogenic role for pilus polymers. In addition, by reporting on the pilus island epidemiology in Germany, our study helps to determine the epitopes that will be required for vaccine design.

Our study is based upon GBS samples collected during a prospective, nationwide, active surveillance study of invasive GBS infections during the years 2009–2010 in Germany. We recently reported on the development of neonatal sepsis incidence based upon the neonatal isolates of the same patient cohort.[7]

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MATERIALS AND METHODS

Case Definition

Our study defined a case of invasive GBS infection as a GBS isolate in any sample obtained from a normally sterile body site (eg, blood, cerebrospinal, or synovial fluid). Samples were collected from January 1, 2009, through December 31, 2010, in Germany.

Sample Acquisition

Our method of sample acquisition previously has been described in greater detail.[7,19] In brief, we collected GBS samples and patient characteristics data from clinical isolates obtained from 2 independent sources [ie, capture-recapture method[19]: from the German Pediatric Surveillance Unit/Survey of Rare Diseases (http://www.esped.uni-duesseldorf.de/)] and from microbiology laboratories across Germany. We did not apply any selection procedure with regard to geography or hospital size, thereby ensuring a representative patient cohort.

Before submitting data, laboratories undertook a pseudonymization of patient identities—a procedure approved by the RKI's [Robert Koch-Institut (German federal government agency for disease control and prevention)] data confidentiality officer. The study was approved by the ethics committee of Freiburg (No. 216/08 and 217/08).

The patient age range is shown in Figure 1A. The age at disease onset was applied for age classification.

GBS Strain Characterization

Bacterial culture and proof of GBS purity were done via Christie–Atkins–Munch-Peterson testing, as described previously.[20] Capsular serotyping was performed by standardized latex agglutination tests (ImmuLex STREP-B-Kit, SSI Denmark, Hillerød, Denmark). A modified protocol was used as described previously.[21] Capsular genotypes of a predefined number of samples were determined by multiplex polymerase chain reaction as described previously[22]: first 50 isolates of capsular types Ia, III, V; 75 isolates of type Ib; and all isolates of types II, IV, VI–IX as well as all nontypeable isolates. The isolates were chosen according to their order of arrival in the study laboratory. In cases where latex agglutination and PCR returned conflicting results, the PCR result was used for further classification of the GBS serotype. Nineteen isolates (1.9%) yielded inconclusive results. These were classified as *not determinable*.

A predefined number of isolates was subjected to pilus island multiplex PCR analysis as described previously[15]: 20 isolates of capsular type Ia, V; 10 isolates of types Ib, II, IV; 30 isolates of type III; and all isolates of types VI–IX. The number of isolates to be tested was determined according to the expected frequencies of serotypes in the study population. The isolates were chosen according to their order of arrival. Due to limited testing resources, it was necessary to limit the selection.

Antibiotic Resistance

Resistance testing was performed according to the EUCAST standard procedure (http://www.eucast.org/ast_of_bacteria/calibration_and_validation/). Agar diffusion testing was performed with ampicillin, cefotaxime, linezolid, vancomycin, clindamycin, and erythromycin. E-testing (bioMérieux, Marcy l'étoile, France) was performed for penicillin G, gentamicin, cefotaxime, erythromycin, and clindamycin. Inducible clindamycin resistance (macrolide-lincosamide-streptogramin B-phenotype) was analyzed by double disk-testing for all isolates that tested resistant for erythromycin. All isolates were tested for antibiotic resistance. Because penicillin sensitivity implies ampicillin sensitivity, only a subset of isolates (386 of 978 isolates) was tested for ampicillin resistance. This was done to confirm the expected sensitivity. Of 219 erythromycin-resistant isolates, 217 were tested with a double-disk test.

Statistical Analyses

Association significance between different bacterial and patient features (eg, association between age and antibiotic resistance) was calculated using GraphPad's online tool "Quickcalcs" (https://www.graphpad.com/quickcalcs/), as well as using Fisher's exact test with two-tailed *P* values. *P* values below 0.05 were considered statistically significant. For comparison of more than 2 groups, χ^2 calculation was used, with *P* values below 0.05 being considered statistically significant. The association between serotype and pilus antigen was calculated as relative risk [including 95% confidence intervals (CI)]. This was done using GraphPad 8 with a Koopman asymptotic score and Fisher's exact test to calculate *P* values.

RESULTS

A total of 1088 GBS samples, isolated between January 1, 2009, and December 31, 2010, were sent to our research laboratory in Freiburg, Germany. Of those, 110 isolates (ie, 10.1 %) had to be excluded either due to missing clinical information or due to inadequate sample acquisition or preparation (eg, because of nonsterile body samples or due to contaminants). The remaining 978 invasive GBS isolates were specifically analyzed. Sample sizes are summarized in Figure (Supplemental Digital Content 1, http://links.lww.com/INF/E163).

The overall age distribution showed a peak during the first days of life. During the course of the first year of life, it decreased, showing just 17.7% of all isolates collected from patients <1 year of age (Fig. 1A). Of note, 73.3% (44/60) of all isolates from EOD cases were taken during the first 24 hours of life. Invasive GBS disease is rare during childhood over 1 year of age. Our study included only 2 samples from patients 1–17 years old. Invasive GBS disease is infrequent during young adulthood but incidence peaks again among the elderly >65 years of age. From this patient cohort, a total of 53.5% (523/978) of samples was collected (Fig. 1A). There were slightly more male patients than female patients (53.4% of all samples; Fig. 1B). The primary origin of the isolates were blood samples (85.5% of all isolates; Fig. 1C). In the infant cohort, cerebrospinal fluid was the second most common isolation site (7.5%), whereas in the adult cohort, GBS was found in the synovial fluid in 8.6% of all cases, followed by, in order of frequency, intraoperative swaps, ascites, abscess drainage, cerebrospinal fluid, bone biopsies, and pleural fluid (Fig. 1C).

Serotype III was the most frequently isolated serotype in the total cohort (31.5% of all isolates; primarily found in infant isolates [ie, 68.2% of infant isolates]; Fig. 2), followed by serotype V with 25.1% (primarily found in adult isolates [ie, 29.1%]), and serotype Ia with 21.5% (Fig. 2). Serotypes Ib and II mainly were present in the adult cohort (12.0% and 8.2%, respectively). The capsule polysaccharide serotype of the 2 isolates from the patients 1–17 years of age was Ia and V (not shown in Fig. 2).

Pilus antigen distribution was determined in 173 of 978 isolates (see Methods section for selection algorithm). Across all ages, the combination of PI-1 + PI-2a was most frequent, with 37.6%, followed by the combination of PI-1 + PI-2b (24.9%). Of all isolates analyzed, 10.4% yielded either no results or else an inconclusive result. In the infant cohort, the combination of PI-1 + PI-2b dominated (n = 31; 43.1%), followed by PI-2a (n = 15; 20.8%), and the combination of PI-1 + PI-2a (n = 15; 20.8%; Fig. 3A). The pilus island antigens in the 2 isolates from children were not analyzed. In the adult cohort, PI-1 + PI-2a was the most abundant (n = 50;

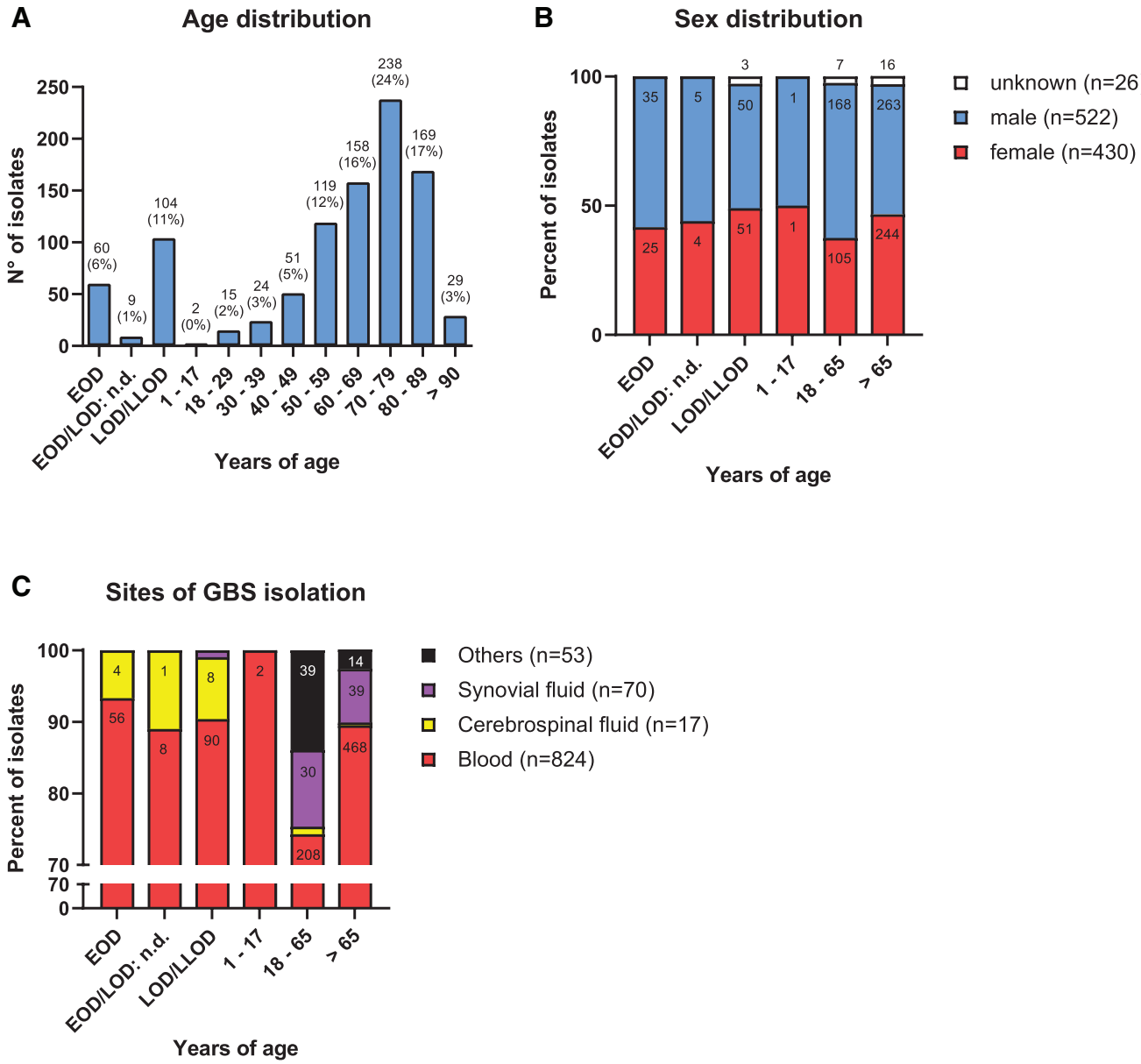

FIGURE 1. Patient and sample characteristics. A: Distribution of patient age at onset of invasive GBS disease, total numbers, and percentages in parentheses are indicated. B: Relative distribution of patient sex in different age groups, with absolute numbers indicated in the graph. C: Relative frequency of isolation sites of GBS in different age groups. "Others" comprises in order of frequency: intraoperative swaps, ascites, abscess fluid, bone biopsy, pleural fluid, and lymphocele fluid. In addition, absolute numbers are indicated inside the bars. EOD/LOD. n.d. indicates isolates from infant patients where the exact age of disease onset is not determinable, and thus the classification as EOD or LOD not possible. full color online

49.5%), especially among the elderly, followed by PI-1+PI-2b (n=12; 11.9%) and PI-2a (n=20; 19.8%; Fig. 3B). Correlating pilus islands with capsule polysaccharide serotypes revealed a statistically significant association between serotype Ia and pilus antigen 2a [relative risk (RR), 6.60; CI 4.06–10.80], between serotype Ib and PI-1+PI-2a (RR 3.49, CI: 1.44–8.49), between serotype II and PI-1+PI-2a (RR 2.99, CI: 1.29–6.94), between serotype V and PI-1+PI-2a (RR 2.78, CI: 1.44–5.41) as well as between serotype III and pilus antigen PI-1+PI-2b (RR 3.94, CI 2.67–5.82).

All analyzed isolates were sensitive against ampicillin, cefotaxime, linezolid, and vancomycin, as determined by the agar disk diffusion test. Using an E test, all isolates tested sensitive against benzylpenicillin, cefotaxime, and gentamicin. In addition, all 978 isolates were tested for erythromycin and clindamycin resistance. Of these, 219 isolates (22.4%) were classified as erythromycin resistant after E-test confirmation, while 3 isolates were classified intermediate resistant. An additional 138 isolates (14.1%) tested clindamycin resistant with E-test confirmation. Erythromycin-resistant isolates were tested with a double-disk test for the presence of an inducible MLS_B-phenotype, that is, the induction of clindamycin resistance by erythromycin exposure. The test revealed 46 isolates (21.2%) with an inducible MLS_B-phenotype,

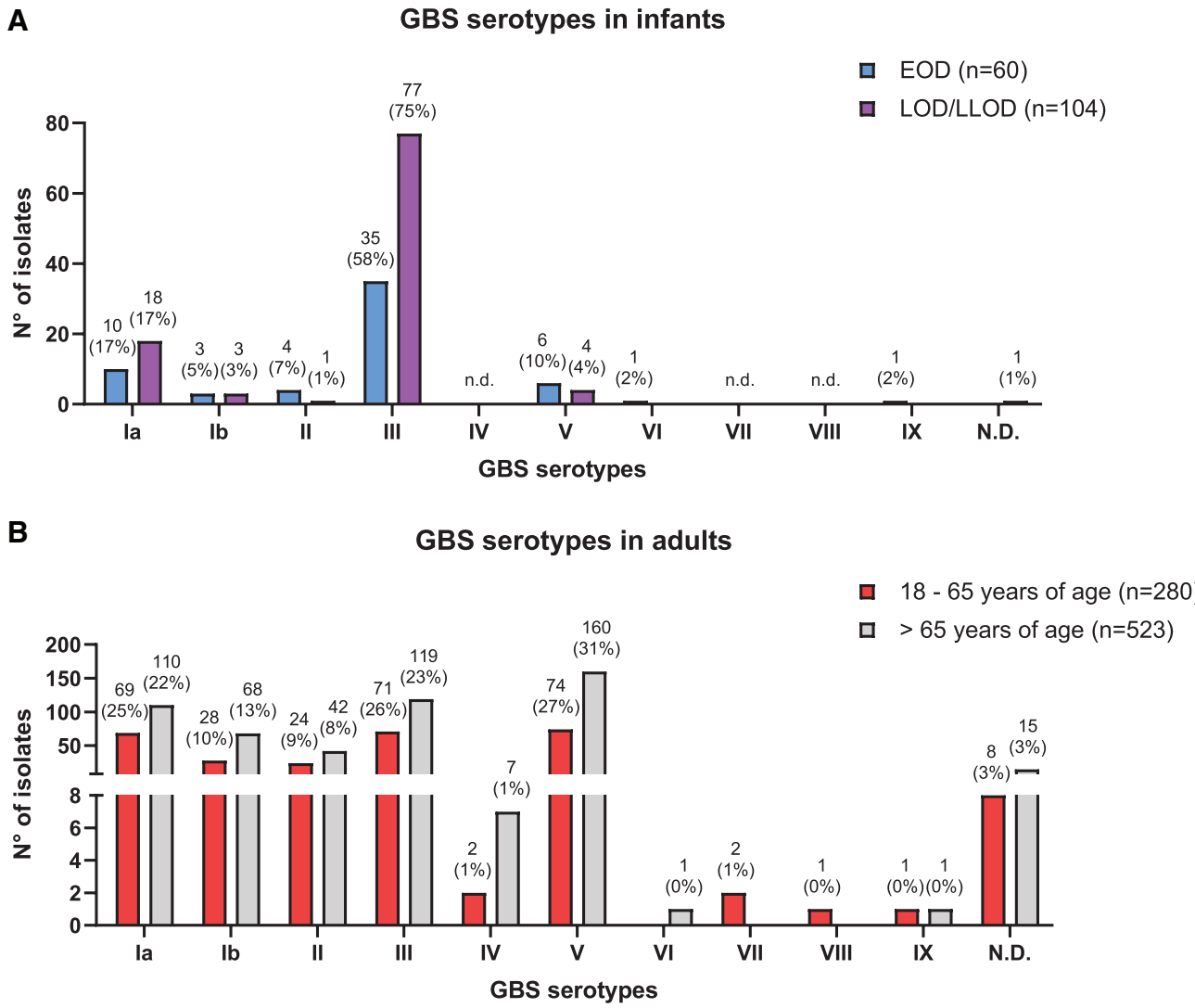

FIGURE 2. Relative prevalence of capsule polysaccharide serotypes. A: Capsule polysaccharide serotype in isolates from EOD and LOD/LLOD patients. In addition, the 9 isolates from infants with unknown age at disease onset are 2× serotype 1a, 6× serotype 3, 1× serotype 1b (not shown in this figure). B: Capsule polysaccharide serotype in isolates from adult and elderly patients. Capsule polysaccharide serotype was determined by latex agglutination and genotyping. Overall, the serotype of 97.5% of all isolates was determined. The capsule polysaccharide serotype of the 2 isolates from patients 1–17 years of age where Ia and V (not shown in this figure). Total numbers and percentages in parentheses are indicated in A and B. Isolates that could not be assigned to a specific serotype are indicated as N.D. LLOD indicates late-late-onset disease; LOD, late-onset disease; n.d., not detected; N.D., not determinable. full color online

whereas 137 isolates (63.1%) were double-resistant and therefore classified as constitutive MLS_B-phenotype. Further, 36 isolates (16.6%) were erythromycin-resistant, clindamycin-sensitive, and had a negative double-disk test result. Labeled M-type, this resistance pattern is conveyed by the *mefA* gene.[23] Erythromycin-resistance is slightly more frequent in isolates from EOD patients than late-onset disease (LOD) patients, although this difference is not significant (RR 1.57, CI: 1.01–2.30; Fig. 4). Correlating resistance to capsule polysaccharide serotype showed a significant association between serotype V and both erythromycin (53.0% of all resistant isolates, RR 3.12, CI: 2.55–3.80) and clindamycin (66.7% of all resistant isolates, RR 3.66, CI: 3.03–4.39) resistance. This observation remained statistically significant in all age-related subgroup analyses.

DISCUSSION

This study reports on microbiologic characteristics of 978 invasive GBS isolates, including both infant and adult patients. By providing a direct comparison of characteristics of infant and adult isolates, it confirms distinct distribution patterns of serotype and pilus antigen in these 2 age groups.

Elderly patients increasingly have become recognized as an at-risk population. Incidence of invasive GBS infections in adults over 60 years old has doubled during the time period 2000–2010 in a range of Western countries.[6] Risk factors for adult invasive GBS infections are well-known and include liver cirrhosis, diabetes mellitus, or bladder dysfunction.[24]

For infants and adult invasive GBS disease, the vaccine development seems promising for decreasing the disease burden

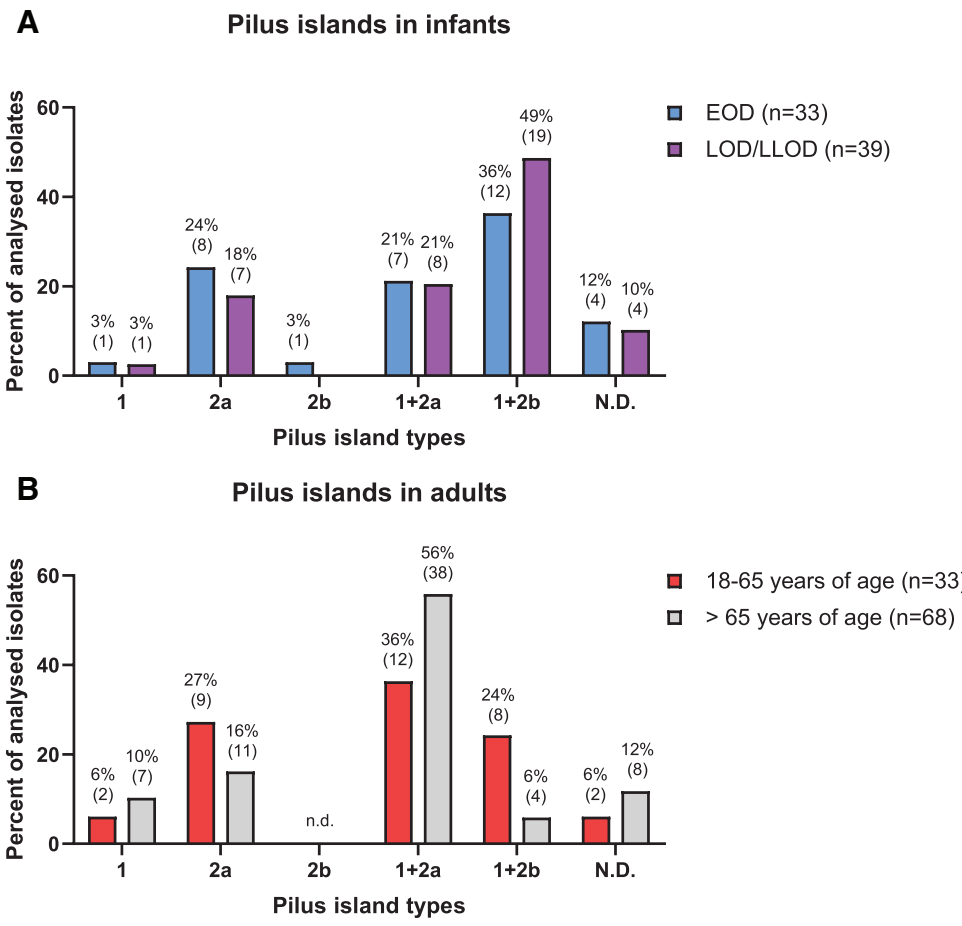

FIGURE 3. Pilus island distribution stratified according to patient age at disease onset. A: Pilus island distribution in isolates from patients with EOD and LOD/LLOD. One isolate from an infant with unknown age at disease onset is included in the LOD population (pilus type 2a). B: pilus island distribution in isolates from adult and elderly patients. Overall, 18 of 173 analyzed isolates (10.4%) showed either no or an inconclusive result (eg, both type 2a and 2b) and were marked as N.D. N.D. in the above graph. The 2 isolates from children were not analyzed regarding pilus island distribution. Percentages and total numbers in parentheses are indicated in (A) and (B). LLOD indicates late-late-onset disease; LOD, late-onset disease; N.D., not determinable. full color online

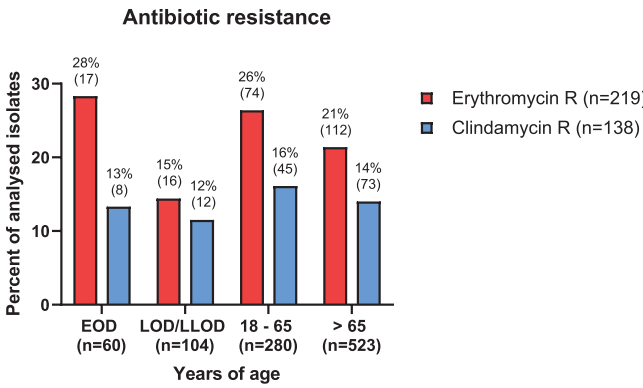

FIGURE 4. Antibiotic resistance stratified according to patient age at disease onset. Resistance against erythromycin and clindamycin was determined by agar diffusion test and confirmed, in case of resistance, by E test. Percentages and total numbers in parentheses are indicated. Three isolates from adult patients were classified as intermediate against erythromycin; they are included within the "sensitive" population in the above graph. Differences in resistance frequencies between the age groups are not significant, as determined by Fisher's exact test. Percentages and total numbers in parentheses are indicated. full color online

in all at-risk populations. Conjugated capsular polysaccharide vaccines currently are the most promising vaccine candidates, especially for infants.[11] Nevertheless, several limitations exist, including higher costs.[25] In addition, GBS is able to switch serotype by horizontal gene transfer[26] and may have several capsular polysaccharide serotypes,[27] as well as different levels of capsular polysaccharide expression.[28] Additional alternative preventive strategies therefore are needed. Due to their ubiquitous expression and highly conserved antigen structure, pilus antigens previously have been suggested as promising vaccine candidates.[13]

For designing GBS vaccines, knowledge of the prevalence of immunization targets such as capsular serotypes and pilus island types is essential. Our study confirmed previously published results showing that in contrast to the distribution among infants, serotype distribution among adults is spread evenly among the 5 most abundant serotypes (ie, Ia, Ib, II, III, and V). In our study, serotype V was the most frequent serotype in the adult population. This also has been noted in the United States[29] and in Sweden,[30] although in Iceland[31] and Portugal,[32] serotype Ia was most common. Serotype III predominance is a key feature of EOD and LOD/late-late-onset disease.[15,33] It also reflects the previously described increased capacity for invasiveness of this serotype.[34] The predominance of serotype III most likely encompasses clonal complex 17, a GBS cluster with several genetic modifications that lead to enhanced virulence in EOD and, to a lesser degree, LOD pathogenesis.[35] Serotype V tends to be associated with clonal complex 1.[36] Although 10 isolates had divergent results in the serotype and genotype analysis,

this finding either may be attributed to technical limitations or to multiple serotype carriage.[27]

Apart from the occurrence of pilus island type 1 alone, without the combination with pilus island type 2a or 2b, the distribution of pilus types in our study was similar to GBS collections from the United States and Italy,[13] as well as from Spain and Portugal,[37] although these studies included colonizing isolates. Interestingly, the distribution of pilus antigens differed between infant and adult isolates. This could hint at different roles for the pathogenicity or immunogenicity in the different age groups. Furthermore, both the different serotypes and the different pilus antigen distribution need to be taken into account when designing a vaccine with potential applications in different age groups.

In a different study, we have compared the pilus typing results of 72 infant isolates from this study with pilus typing results conducted by a newly developed DNA microarray assay in a larger collection of infant isolates.[38] For 60 of those infant isolates, microarray data were available. The 8 infant isolates that could not be assigned to a pilus type by PCR were able to be identified by microarray as harboring pilus islands 1 and 2a (4 isolates), pilus island 2a, or pilus islands 1 and 2b (2 isolates each, respectively). Interestingly, the 2 isolates classified by PCR as being pilus island type 1 alone showed a clear signal in the microarray for a combination of pilus island type 1 with type 2a or 2b. Three other isolates showed discordant pilus island typing results for the PCR and the microarray assay. This may be due to different target sequences within the gene.

The occurrence of pilus island type 1 alone rarely has been reported in the literature. This leads us to the conclusion that the distribution of pilus island types mainly consists of just 3 combinations: type 2a, type 1 combined with 2a, and type 1 combined with 2b. This makes it an appealing candidate for vaccine design, as a limited number of epitopes would be able to cover the vast majority of strains. In addition to pilus antigens, other nonpilus, cell wall-anchored proteins have been proposed as potential vaccine epitopes.[39] This has been based upon the observation that antibody levels against other surface markers inversely correlate with colonization likelihood during pregnancy.[40]

In our study cohort, resistance rates against erythromycin and clindamycin were similar between infant and adult invasive strains. However, analogous to trends in other countries,[32] in our study cohort of infant invasive isolates, resistance rates against both antibiotics have doubled in comparison to a similar national cohort from 2001 to 2003.[19]

In summary, our study assessed the prevalence of capsular polysaccharide type and pilus antigen type, as well as antibiotic resistance patterns, of a large, nationwide collection of both infant and adult invasive GBS isolates during a 2-year period in Germany. This information is critical for the purpose of designing potential vaccines.

Limitations of our study include the following: First, capsular polysaccharide typing primarily was derived from serotyping and only a subset of isolates was genotyped. However, our rate of accordance between serotyping and genotyping was similar to international standards.[20] Second, due to limited financial resources, pilus island typing was done only for a subset of isolates. By predefining the isolates to be typed, we generated a representative sample selection. Corresponding well to previously published pilus island distributions, our results confirmed this representativeness. Third, 10.4% of GBS isolates were not able to be assigned to a pilus type by PCR. Therefore, we tested all infant invasive GBS isolates using a newly developed GBS microarray method.[38] Within this subset, 99% of strains could be typed by pilus antigen, and the overall ratio of pilus island typing was highly concordant with our PCR typing results, as described above. Of note, our study did not analyze pilus islands expression levels. To validly identify vaccine targets, doing so would be essential because expression levels can differ significantly. Finally, we cannot fully exclude the possibility that contamination occurred during sampling. If this occurred, a strain could have been falsely classified as invasive. This may have particular relevance for intraoperative swabs.

To date, no satisfactory control of severe GBS disease has been achieved in all 3 of the vulnerable populations identified—that is, newborns/infants, women near the time of child delivery, and elderly persons who have underlying medical conditions. Therefore, follow-up efforts are needed, including but not limited to the development of vaccines against promising targets such as the pilus islands.

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REFERENCES

1. Seale AC, Bianchi-Jassir F, Russell NJ, et al. Estimates of the burden of group B streptococcal disease worldwide for pregnant women, stillbirths, and children. *Clin Infect Dis.* 2017;65(suppl_2):S200–S219.
2. Schwartz B, Schuchat A, Oxtoby MJ, et al. Invasive group B streptococcal disease in adults. A population-based study in metropolitan Atlanta. *JAMA.* 1991;266:1112–1114.
3. Palazzi DL, Rench MA, Edwards MS, et al. Use of type V group B streptococcal conjugate vaccine in adults 65-85 years old. *J Infect Dis.* 2004;190:558–564.
4. Skoff TH, Farley MM, Petit S, et al. Increasing burden of invasive group B streptococcal disease in nonpregnant adults, 1990-2007. *Clin Infect Dis.* 2009;49:85–92.
5. McLaughlin JM, Peyrani P, Furmanek S, et al. Burden of adults hospitalized with group B streptococcal infection. *J Infect Dis*. 2020;jiaa110. [Online ahead of print]
6. Ballard MS, Schønheyder HC, Knudsen JD, et al; International Bacteremia Surveillance Collaborative. The changing epidemiology of group B streptococcus bloodstream infection: a multi-national population-based assessment. *Infect Dis (Lond).* 2016;48:386–391.
7. Wicker E, Lander F, Weidemann F, et al. Group B streptococci: declining incidence in infants in Germany. *Pediatr Infect Dis J.* 2019;38:516–519.
8. Madrid L, Seale AC, Kohli-Lynch M, et al; Infant GBS Disease Investigator Group. Infant group B streptococcal disease incidence and serotypes worldwide: systematic review and meta-analyses. *Clin Infect Dis.* 2017;65(suppl_2):S160–S172.
9. Kovavisarach E, Jarupisarnlert P, Kanjanaharuetai S. The accuracy of late antenatal screening cultures in predicting intrapartum group B streptococcal colonization. *J Med Assoc Thai.* 2008;91:1796–1800.
10. Stoll BJ, Hansen NI, Sánchez PJ, et al; Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. Early onset neonatal sepsis: the burden of group B streptococcal and *E. coli* disease continues. *Pediatrics.* 2011;127:817–826.
11. Heath PT. Status of vaccine research and development of vaccines for GBS. *Vaccine.* 2016;34:2876–2879.
12. Baker CJ, Rench MA, McInnes P. Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine. *Vaccine.* 2003;21:3468–3472.
13. Margarit I, Rinaudo CD, Galeotti CL, et al. Preventing bacterial infections with pilus-based vaccines: the group B streptococcus paradigm. *J Infect Dis.* 2009;199:108–115.
14. Maione D, Margarit I, Rinaudo CD, et al. Identification of a universal group B streptococcus vaccine by multiple genome screen. *Science.* 2005;309:148–150.
15. Fabbrini M, Rigat F, Rinaudo CD, et al. The protective value of maternal group B streptococcus antibodies: quantitative and functional analysis of naturally acquired responses to capsular polysaccharides and pilus proteins in European maternal sera. *Clin Infect Dis.* 2016;63:746–753.

16. Jiang S, Park SE, Yadav P, et al. Regulation and function of pilus island 1 in group B streptococcus. *J Bacteriol.* 2012;194:2479–2490.
17. Lazzarin M, Mu R, Fabbrini M, et al. Contribution of pilus type 2b to invasive disease caused by a *Streptococcus agalactiae* ST-17 strain. *BMC Microbiol.* 2017;17:148.
18. Sheen TR, Jimenez A, Wang NY, et al. Serine-rich repeat proteins and pili promote *Streptococcus agalactiae* colonization of the vaginal tract. *J Bacteriol.* 2011;193:6834–6842.
19. Fluegge K, Supper S, Siedler A, et al. Antibiotic susceptibility in neonatal invasive isolates of *Streptococcus agalactiae* in a 2-year nationwide surveillance study in Germany. *Antimicrob Agents Chemother.* 2004;48:4444–4446.
20. Afshar B, Broughton K, Creti R, et al; Members of the DEVANI Study Group. International external quality assurance for laboratory identification and typing of *Streptococcus agalactiae* (Group B streptococci). *J Clin Microbiol.* 2011;49:1475–1482.
21. Slotved HC, Elliott J, Thompson T, et al. Latex assay for serotyping of group B streptococcus isolates. *J Clin Microbiol.* 2003;41:4445–4447.
22. Imperi M, Pataracchia M, Alfarone G, et al. A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*. *J Microbiol Methods.* 2010;80:212–214.
23. Clancy J, Petitpas J, Dib-Hajj F, et al. Molecular cloning and functional analysis of a novel macrolide-resistance determinant, mefA, from *Streptococcus pyogenes*. *Mol Microbiol.* 1996;22:867–879.
24. Jackson LA, Hilsdon R, Farley MM, et al. Risk factors for group B streptococcal disease in adults. *Ann Intern Med.* 1995;123:415–420.
25. Chen C, Cervero Liceras F, Flasche S, et al. Effect and cost-effectiveness of pneumococcal conjugate vaccination: a global modelling analysis. *Lancet Glob Health.* 2019;7:e58–e67.
26. Martins ER, Melo-Cristino J, Ramirez M. Evidence for rare capsular switching in *Streptococcus agalactiae*. *J Bacteriol.* 2010;192:1361–1369.
27. Ramaswamy SV, Ferrieri P, Flores AE, et al. Molecular characterization of nontypeable group B streptococcus. *J Clin Microbiol.* 2006;44:2398–2403.
28. Sendi P, Johansson L, Dahesh S, et al. Bacterial phenotype variants in group B streptococcal toxic shock syndrome. *Emerg Infect Dis.* 2009;15:223–232.
29. Kothari NJ, Morin CA, Glennen A, et al. Invasive group B streptococcal disease in the elderly, Minnesota, USA, 2003-2007. *Emerg Infect Dis.* 2009;15:1279–1281.
30. Gudjónsdóttir MJ, Hentz E, Berg S, et al. Serotypes of group B streptococci in western Sweden and comparison with serotypes in two previous studies starting from 1988. *BMC Infect Dis.* 2015;15:507.
31. Björnsdóttir ES, Martins ER, Erlendsdóttir H, et al. Changing epidemiology of group B streptococcal infections among adults in Iceland: 1975-2014. *Clin Microbiol Infect.* 2016;22:379.e9–379.e16.
32. Lopes E, Fernandes T, Machado MP, et al; The Portuguese Group For The Study Of Streptococcal Infections. Increasing macrolide resistance among *Streptococcus agalactiae* causing invasive disease in non-pregnant adults was driven by a single capsular-transformed lineage, Portugal, 2009 to 2015. *Euro Surveill.* 2018;23:1700473.
33. Edmond KM, Kortsalioudaki C, Scott S, et al. Group B streptococcal disease in infants aged younger than 3 months: systematic review and meta-analysis. *Lancet.* 2012;379:547–556.
34. Ip M, Ang I, Fung K, et al. Hypervirulent clone of group B streptococcus serotype III sequence type 283, Hong Kong, 1993-2012. *Emerg Infect Dis.* 2016;22:1800–1803.
35. Jones N, Oliver KA, Barry J, et al; Oxford Group B Streptococcus Consortium. Enhanced invasiveness of bovine-derived neonatal sequence type 17 group B streptococcus is independent of capsular serotype. *Clin Infect Dis.* 2006;42:915–924.
36. Luan SL, Granlund M, Sellin M, et al. Multilocus sequence typing of Swedish invasive group B streptococcus isolates indicates a neonatally associated genetic lineage and capsule switching. *J Clin Microbiol.* 2005;43:3727–3733.
37. Martins ER, Andreu A, Melo-Cristino J, et al. Distribution of pilus islands in Streptococcus agalactiae that cause human infections: insights into evolution and implication for vaccine development. *Clin Vaccine Immunol.* 2013;20:313–316.
38. Zürn K, Lander F, Hufnagel M, et al. Microarray analysis of group B streptococci causing invasive neonatal early- and late-onset infection. *Pediatr Infect Dis J.* 2020;39:449–453.
39. Pietrocola G, Arciola CR, Rindi S, et al. *Streptococcus agalactiae* non-pilus, cell wall-anchored proteins: involvement in colonization and pathogenesis and potential as vaccine candidates. *Front Immunol.* 2018;9:602.
40. Dzanibe S, Kwatra G, Adrian PV, et al. Association between antibodies against group B streptococcus surface proteins and recto-vaginal colonisation during pregnancy. *Sci Rep.* 2017;7:16454.

Supplemental digital content 1. Overview of sample sizes. 110 isolates were excluded from the study either due to missing clinical information or due to inadequate sample acquisition or preparation (e.g., because of samples from non-sterile sites or due to contaminants). All isolates were tested for antibiotic resistance and capsular phenotype by latex agglutination. A predefined number of isolates was subjected to capsular genotyping and to pilus island multiplex PCR analysis, respectively (for details see Materials and methods section). Because penicillin sensitivity implies ampicillin sensitivity, only a subset of isolates was tested for ampicillin resistance.