Streptococcus agalactiae causing human infections: genetic diversity and capsular switching

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SUMMARY

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*Streptococcus agalactiae* (group B streptococci, GBS) is primarily a colonizing agent of the genitourinary and gastrointestinal tracts of a significant proportion of the human population. It is, however, well established as a leading cause of bacterial sepsis and meningitis in neonates and is increasingly associated with invasive infections in adults.

While vertical transmission is commonly accepted to be the cause of early-onset disease, the source of bacterial strains causing infection in the late-onset period is less well understood. Administration of intrapartum antimicrobial prophylaxis to colonized women has resulted in a striking decline in early-onset and maternal GBS disease, but late-onset infections have mostly remained unchanged. Moreover, antimicrobial prophylaxis raised concerns as to selection and emergence of GBS resistant strains and alternative prevention strategies have focused on the development of vaccines that hold promising, although still preliminary results.

The aim of the work presented in this thesis was to characterize the population structure of GBS in Portugal, and to assess the genetic diversity of isolates recovered from vaginal colonization and invasive disease in different age groups, to contribute to the global epidemiology of GBS and our understanding of GBS population biology. To this end a set of common techniques was chosen, including serotyping, antimicrobial susceptibility testing, pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and surface protein gene profiling. In combination, these methods allowed the identification of the main genetic lineages circulating in Portugal and Barcelona, providing the means for an appropriate comparison of both.

These studies started with the comparison of 64 isolates recovered from invasive infections in newborns in the Lisbon area with 269 isolates colonizing women in the third trimester of pregnancy, from the same period. The genetic lineages defined by both PFGE and MLST identified very diverse populations with reported differences in the prevalence of serotypes and clones in carriage and invasive disease. A major finding concerned the identification of an unusually high proportion of ST24 isolates among serotype Ia, further strengthened by the independent study of another population (212 neonatal isolates) from the Barcelona area. Despite the geographic distance, both studies
from Barcelona and Lisbon revealed extensive similarity in terms of clonal structure and genetic lineages. The high prevalence in both the studies of a particular lineage serotype Ia, defined by ST24 and the surface protein gene \textit{bca}, highlighted the importance of local dynamics, indicating that genetic evolution of GBS presents with a geographic structure and may depend on local factors.

The subsequent analysis of 225 isolates recovered from non-pregnant adults in Portugal revealed a GBS population dominated by a more diverse clonal composition when compared to that of neonates, consistent with the broader spectrum of disease presentation in these patients and consequent multiplicity of genetic lineages. Invasive disease in this population increased with age and was more frequent among men. The dominance of serotype Ia in this population, regardless of age, highlighted the importance of this serotype in GBS pathogenesis as a leading cause of invasive infections in adults, not reported elsewhere but already noted among neonatal infections in the Iberian Peninsula. Furthermore, the high prevalence of ST24 in all these studies, as opposed to rare descriptions elsewhere, suggested that this lineage had enhanced invasiveness and was probably expanding as a regionally successful clone that may disseminate more globally.

Macrolide resistance rates in Portugal did not show significant trends, even if macrolides have been used in intrapartum prophylaxis increasing the selective pressure on GBS. Macrolide resistance is disseminated in Portugal by both a multiclonal mechanism resulting from the spread of resistance genes throughout most serotypes and genetic backgrounds, as well as by clonal expansion of particular lineages, such as the serotype V ST1/\textit{alp3}.

One of the main purposes of the analysis of a significant number of GBS isolates was their classification into lineages sharing the same genetic background, which would allow the inference of genetic relationships between strains and their contextualization in the global epidemiology of GBS. However, the associations of phenotype-genotype or between different genetic traits were never absolute, highlighting the role of horizontal genetic transfer in the evolution of GBS. Capsular switching was anticipated to occur frequently within GBS, even though this species is not recognized to be naturally competent for the acquisition of foreign DNA. Substantial evidence provided by the epidemiological studies performed on the Portuguese GBS collections drove the search for capsular transformants within these populations. The results obtained confirmed the existence of capsular switching in GBS, but questioned the high
frequency of these events estimated from previous studies. Serotyping errors probably justified the overrepresentation of capsular switching in epidemiological studies. The mechanism for these genetic transfer events involved the replacement of the whole capsular locus instead of the previously proposed genetic transfer of only the serotype-specific genes.

Globally, the results presented in this thesis suggest that GBS has an apparent remarkably stable, both temporally and geographically, clonal structure. Against this, background diversification is ongoing and can depend on local factors. Capsular switching is likely contributing to diversification, however not as frequently as initially thought and may impact on the vaccine formulations currently under development. Despite increasing information on maternal colonization and invasive disease, a better understanding of colonization in adults and natural reservoirs of GBS is required for the appropriate management of the GBS infections.