Serial clustering of late-onset Group B streptococcal infections in the neonatal unit: a genomic re-evaluation of causality

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Introduction

Group B streptococcus (GBS, *Streptococcus agalactiae*) dominates as a cause of serious neonatal early-onset (EOD) and late-onset disease (LOD). The incidence of EOD is 0.44 per 1,000 live births and LOD 0.23 per 1,000 live births in 2014 (1). In the absence of an effective vaccine, current prevention strategies that target EOD do not impact on LOD (2). GBS are asymptomatically carried as part of the enteric and vaginal microbiota in 20-30% of healthy women worldwide (3, 4). The research on LOD transmission and pathogenesis is scares; because of LOD cases are assumed to be largely sporadic. Although nosocomial outbreaks of GBS have been described elsewhere (5,6), the frequency of such outbreaks is presumed to be low (5).

We used genomic analyses to investigate a cluster of four LOD cases that arose within 4 weeks in a single neonatal intensive care unit (NICU). This prompted prospective enhanced surveillance and genomic analysis of all GBS LOD over 24 months. We found that almost all LOD arising in the NICU could be linked.

Summary and Conclusions

- Transmission routes for GBS in the nosocomial setting are still poorly understood, cases might not necessary be sporadic.
- Within this neonatal ICU, our data suggest that a single case of LOD GBS sepsis should be considered a potential nosocomial transmission event warranting prompt investigation, heightened infection prevention vigilance and action where required.
- Large-scale genomic databases, which include longitudinally collected data from both disease-associated and carriage isolates can provide a very useful context for outbreak.
- Macrolide resistance genes, *emrB* or *mefA* and *msrD*, were identified in two out of four outbreaks, underlining the increasing hazards associated with use of macrolides in obstetric antimicrobial guidelines.
- GBS isolates that cause adult disease are similar to those causing neonatal infection, supporting a common human reservoir of disease isolates.

Results

Cluster 1

- Four serotype V GBS (ST1) were identified from 4 LOD cases in 4 weeks.
- All isolates were carrying *tetM* (confer resistance to tetracycline) and *ermB* (confer resistance to macrolides and lincosamides).
- Comparison with contemporaneous UK ST1 GBS isolates (n=18) indicated that neonatal and adult invasive strains were intermixing (Figure 1a).
- Serotype V isolates associated with cluster were identical with 1 SNP difference.
- Comparison with 40 serotype V ST1 invasive isolates from North America (7) showed that there was no geographical clustering determining N. American or UK strains, also, adult and neonatal isolates were intermixed; isolates carrying *tetM*- and *ermB*- genes were clustering together (Figure 1b).

Cluster 2

- Two LOD cases were caused by serotype III (ST17) that arose within 2 weeks.
- Both isolates had *tetM* gene, but no macrolide resistance genes.
- Genomic analysis indicated that these two serotype III GBS isolates differed by a single SNP, indicating a very recent common ancestor (Figure 2a).
- Comparison to contemporaneous ST17 isolates (n=98) indicated intermixing of isolates that originated from causing disease in adults and neonates.
- A further ST17 serotype III LOD occurred 9 months later; this was 91-92 SNPs different to Cluster 2 isolates.

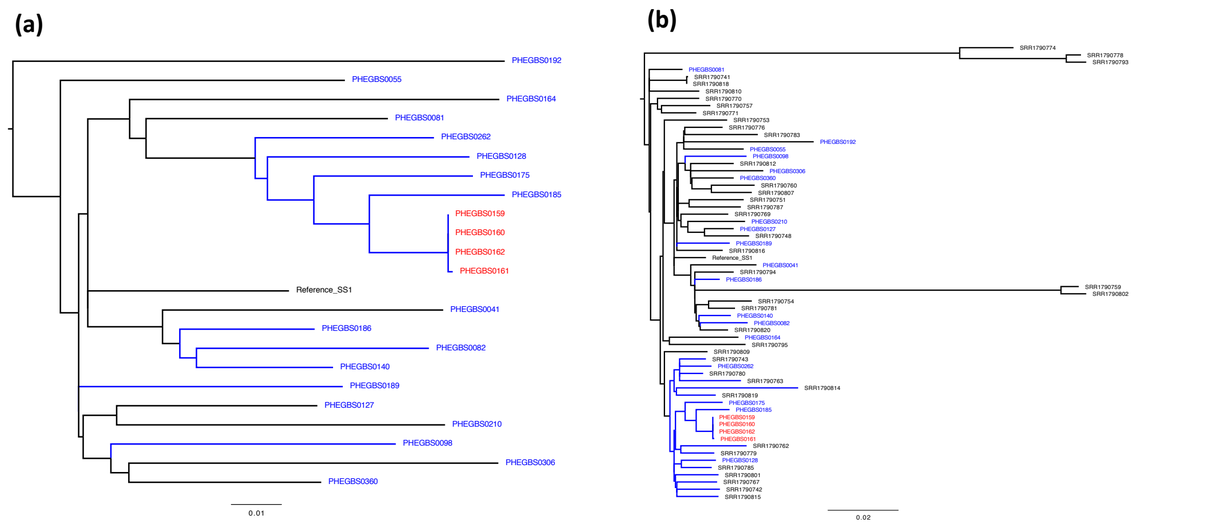

Figure 1. Phylogenetic analysis of Cluster 1, serotype V strains. Phylogenetic analysis of outbreak cluster strains in the context of UK and North America isolates. Single-nucleotide polymorphism (SNP)–based approximate maximum likelihood phylogeny trees were constructed using FastTree. Isolates with 0–2-SNPs differences were considered to share a common source; putative outbreaks are colored red. Scale bars indicate the nucleotide substitutions per site. Blue tree branches indicate isolates with tetracycline as well as macrolide/lincosamide resistance genes. This phylogeny tree based on 4 putative outbreak isolates and 18 contemporaneous serotype V sequence type (ST) 1 isolates from the same year with reference sequence *S. agalactiae* SS1 (NZ_CP010867.1). There was 1-SNP difference between outbreak isolate PHEGBS0161 and the other 3 outbreak isolates, PHEGBS0159, PHEGBS0160, and PHEGBS0162.

Cluster 3

- Two LOD cases due to GBS serotype Ib (ST139).
- Two additional rectal serotype Ib (ST139) carriage isolates from neonates in same NICU.
- Genomic analyses indicated all four isolates were identical with a single SNP difference between two invasive isolates;
- All four isolates clustered together when compared to contemporaneous genetically related GBS isolates (n=27) (Figure 2b).

Cluster 4

- Three LOD cases due to serotype Ia (ST23) occurred in 3 month period (all macrolide resistant).
- Two ST23 carriage isolates: 1 from LOD case, 1 from un-related neonate were also isolated.
- Genomic analysis showed all five isolates clustered together.
- All isolates carrying *mefA* and *msrD* clustered togetherin the tree with contemporaneous serotype Ia ST23 isolates (n=37) (Figure 2c).

All isolates of serotypes V, Ib, III and Ia that were associated with clusters had *tetM* conferring resistance to tetracycline. Serotype V and Ia isolates additionally carried elements conferring resistance to macrolides.

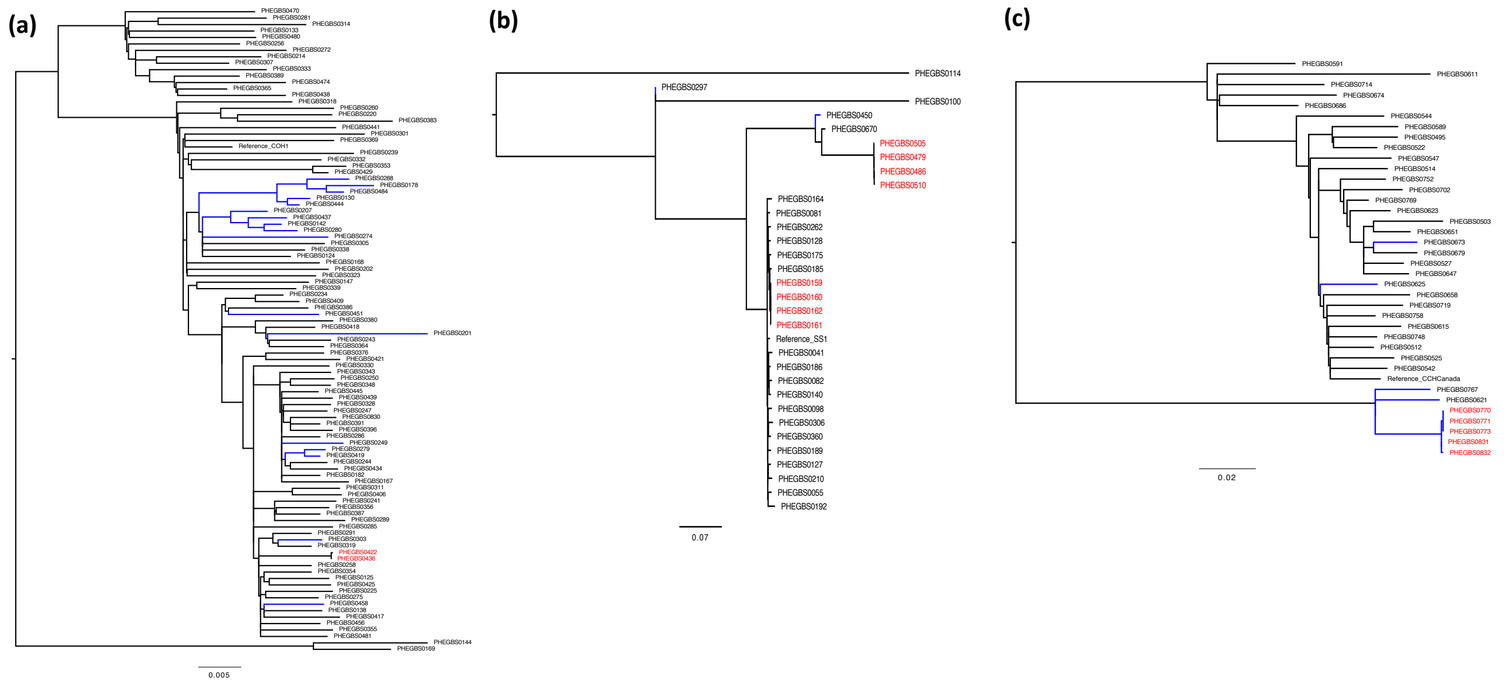

Figure 2. Phylogenetic analysis of clusters 2, 3 and 4 in context of other contemporaneous UK isolates. (a) Cluster 2: Phylogeny tree based on 2 serotype III putative outbreak isolates, a single serotype III sporadic late-onset disease (LOD), and 98 available whole-genome sequences of invasive serotype III ST17 from the same year with Refseq S. agalactiae COH1 (NZ_HG939456). There was 1-SNP difference between putative outbreak isolates PHEGBS0422 and PHEGBS036, whereas there were 91–92 SNPs between the outbreak isolates and the sporadic serotype III LOD isolate. **(b) Cluster 3:** Phylogeny tree based on serotype Ib ST139 isolates (n = 4) from putative outbreak and single-locus variants (ST1 and ST3) of serotypes Ib, II, V, and VI (n = 27) with ST1 reference sequence S. agalactiae SS1 (NZ_CP010867.1); the phylogeny tree includes all ST1 isolates shown in A, including cluster 1. Two invasive isolates from the putative 1b outbreak were identical, with no SNP difference. The rectal swab sample isolate PHEGBS0505 differed by 5 SNPs from blood isolates PHEGBS0510 and PHEGBS0486. **(c) Cluster 4:** Phylogeny tree based on 37 serotype Ia ST23 isolates with Refseq S. agalactiae CCH210801006 (ERS337511). Blood and colonization isolates (n = 4) from the putative serotype Ia outbreak differed from each other by just 0–2 SNPs.

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Footnote

ST – sequence type based on multi locus sequence typing (MLST), a molecular biology technique that helps to characterize microbial species using DNA sequences of internal fragments of housekeeping genes.

How to read a phylogenetic tree – phylogenetic tree represents the evolutionary relationships among a set of strains. The tips of the tree represent groups of descendent strains and the nodes on the tree represent the common ancestors of those descendants. The longer the tree branch the more genetic differences there are between the strains at the tips of the tree and their common ancestor.