Imbalance of the Gut microbiota possess a potential risk for developing sepsis in neonates

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INTRODUCTION

- Sepsis is a critical condition that mostly happens due to the unregulated body's immune response to an systemic or other infections.
- Infants younger than 90 days develop this condition with a mortality rate of 11-19% worldwide.

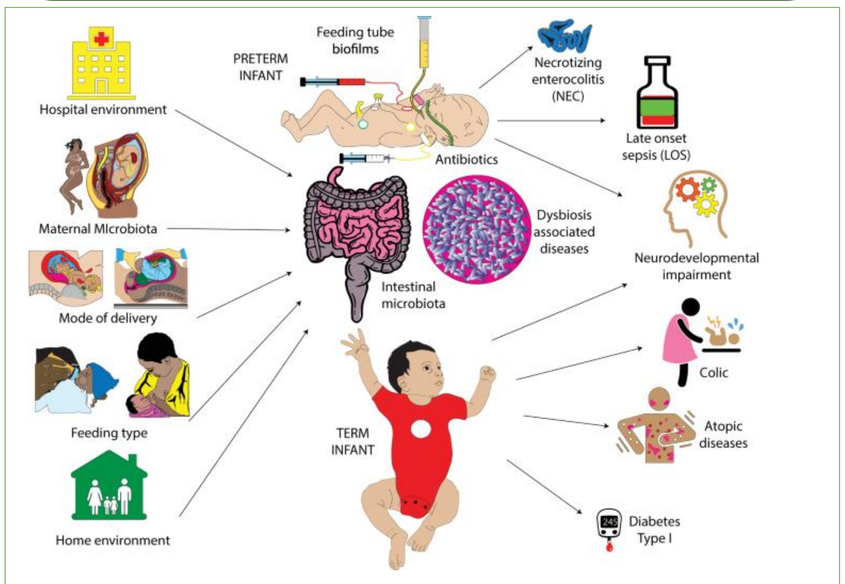

Fig1: Factors influencing the intestinal microbiota in infants and diseases associated with infant's intestinal dysbiosis, Underwood et al, 2020.

OBJECTIVE

In this longitudinal, multi-centric study, we aim to identify taxonomic and functional signatures of the gut microbiome associated with systemic infections and sepsis in Indian neonates.

METHODOLOGY

- Participating sites: Safdarjung Hospital, Lady Hardinge Medical college, Baba Saheb Ambedkar Hospital and Guru Teg Bahadur Hospital.
- Neonatal blood, meconium and stool samples from the sepsis-positive, and healthy neonates were collected. Metagenomic bacterial and fungal DNA isolation were done for gut samples.
- Sequencing was performed for 193 samples to estimate the bacterial (16S rRNA-V3-V4 regions) and fungal (ITS2-ITS4) abundance, respectively. Sequencing reads were analyzed using Dada2, and Phyloseq R package.
- Whole genome sequencing (n=127) was performed for bacterial isolates from blood and Stool samples.
- Genotypes of bacterial species were determined by MLST Schemes and AMR Genes were screened using Abricate.

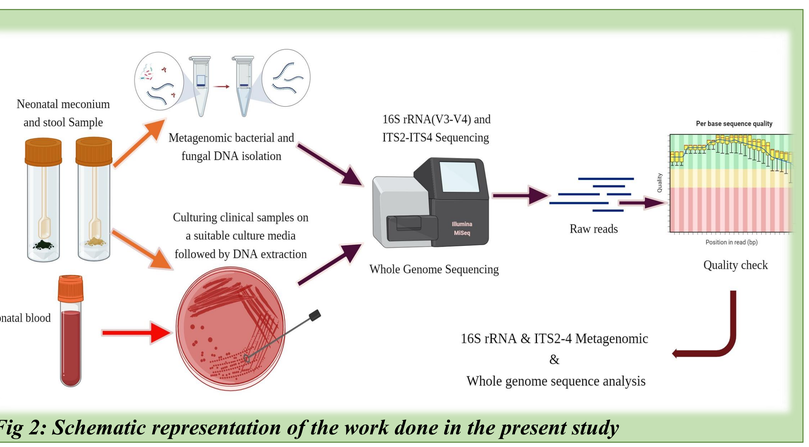

Fig 2: Schematic representation of the work done in the present study

DISCUSSION

- The study data serve as baseline information for understanding the diversity and composition of bacteria and fungus and bacterial heterogeneity in neonatal gut.
- Initial findings indicates that the sepsis positive host's gut and blood can be the potential reservoir for *Klebsiella, Escherichia,* and *Acinetobacter.*

CONCLUSION

Gut could be a potential source for systemic infections. MLST and core genome SNP analysis indicates that there is significant heterogeneity in the critical priority pathogens associated with neonatal sepsis which needs to be explored further.

RESULTS

- In case of 16S metagenomics, in the infant's gut, *Enterococcus* (25.88%), *Klebsiella* (24.27%) and *Escherichia* (22.3%) are dominant genera **(*Fig 3A*)**.
- ITS2-4 analysis depicts *Candida* (47.18%), *Aspergillus* (16.77%) and *Cladosporium* (7.99%), as major genera **(*Fig 3B*)**.
- Among the 127 pathogens sequenced for WGS, subset analysis revealed *E. coli* belonging to ST73 and *A. baumannii* belonging to ST1 and ST2 were commonly found both in neonatal blood and gut.
- The majority of the isolates harboured multiple AMR genes encoding for beta-lactamases ($bla_{\text{CTX-M-15}}$, bla_{NDM}, $bla_{\text{Oxa-1}}$), aminoglycoside-modifying enzymes (*aac(3)-IIa, aph(3")-Ib, aph(6)-Id,*), 16S RMTases (*rmtC*) mostly by *Klebsiella pneumoniae*, macrolides resistance (*mphA*, *mphE, mrx*).
- Analysis of multiple colonies (n=10) from the same samples revealed clonal heterogeneity in 31 *E. coli*, 21 *K. pneumoniae and* 3 *A. baumannii* isolates which harboured various distinct ARGs some of which were also identified to be pathogen-specific **(*Fig 9: A, B, C)*** .

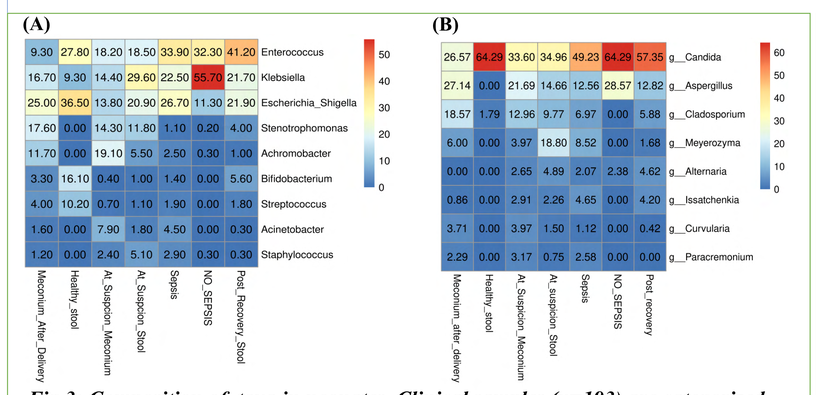

Fig 3: Composition of taxa in neonates. Clinical samples (n=193) are categorized based on clinical conditions A) Bacterial Genera B) Fungal Genera

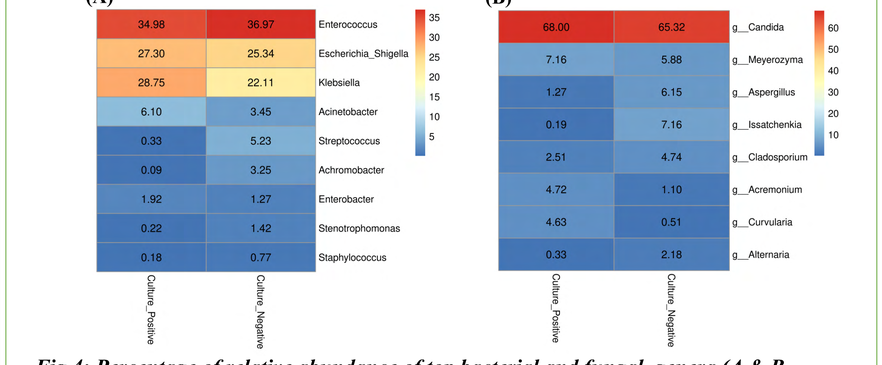

Fig 4: Percentage of relative abundance of top bacterial and fungal genera (A & B respectively) in culture positive (n=14) and culture negative (n=53) sepsis

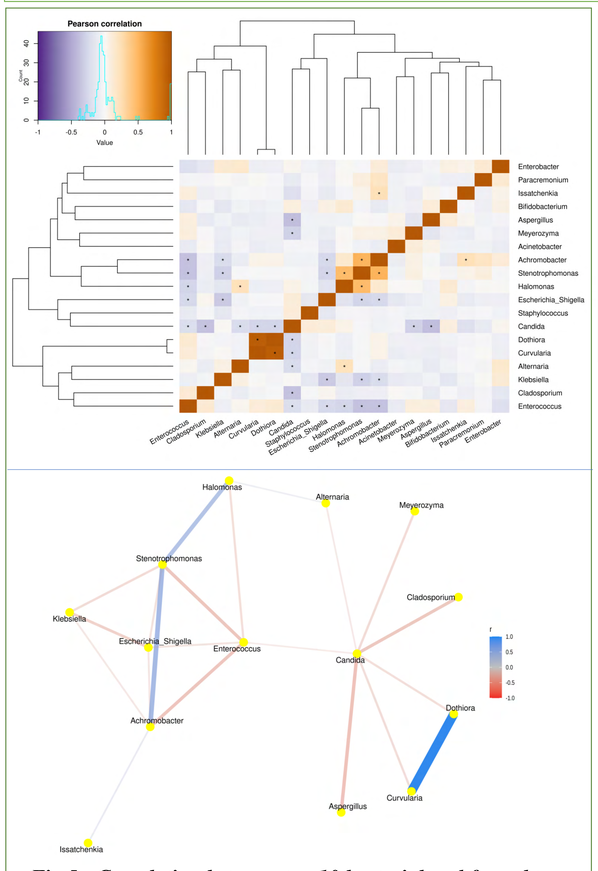

Fig 5 : Correlation between top 10 bacterial and fungal taxa

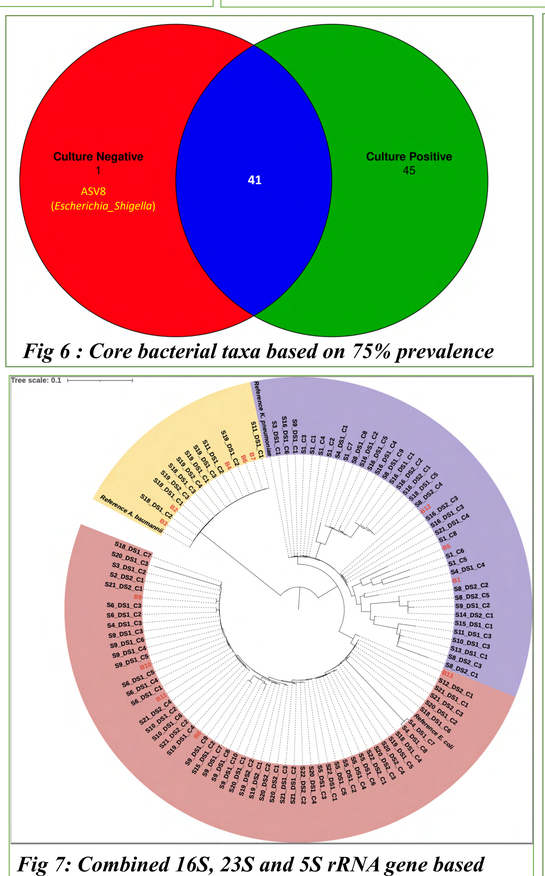

Fig 6 : Core bacterial taxa based on 75% prevalence

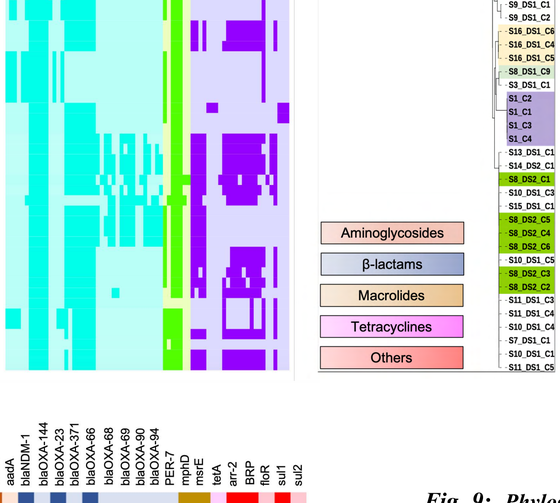

Fig 7: Combined 16S, 23S and 5S rRNA gene based Phylogenetic tree of 127 bacterial isolates

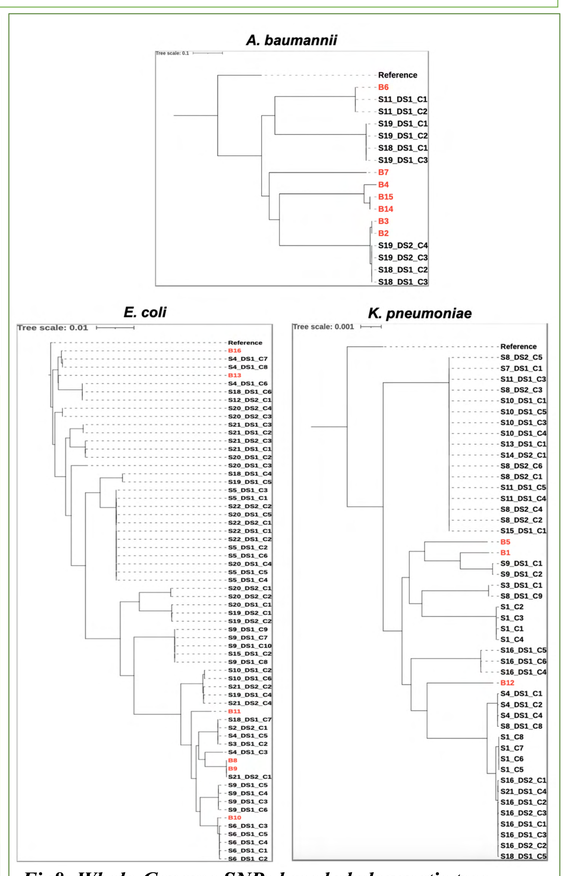

Fig8: Whole-Genome SNPs based phylogenetic tree

Fig 9: Phylogenetic analysis based on seven house-keeping genes (MLST) of 63 E. coli (A), 47 K. pneumoniae (B), 17 A. baumannii (C) from neonatal blood (represented by 'B') and stool (represented by 'S'). The highlighted samples show intraclonal heterogeneity.
Heat map represents the presence (shaded dark) or absence (shaded light) of antimicrobial resistant genes categorized based on the antimicrobial classes and virulence factors.

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