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Metagenomic Exploration of Antibiotic Resistance of Neonatal Gut Under Intensive Care

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Abstract: The vicious spread of undesired antibiotic resistance among all the possible horizon of living world is a cause of great concern and requires immediate attention. As we are concerned about the prevalence of these antibiotic resistance genes (ARGs), studies have suggested the presence of antibiotic resistant determinants in highly controlled environments such as neonatal-intensive care units (ICU). The presence of ARGs in the typical neonatal facilities are a kind of modern nightmare. In the present work, neonatal gut resistome from infants under ICU care was analyzed by metagenomic approach, to examine the possibility of spread of ARGs in neonatal care units. All samples were found to be rich in ARGs and were containing 153 to 267 ARGs per sample and the abundance ranging from 7.68 to 12.86 copies of ARGs per copy of 16S rRNA gene. Among the all ARGs, Aminocoumarins (*mdtA, mdtC*), Aminoglycoside (*cpxA, APH(3'')-Ib*) and Bacitracin (*BacA*) were the most abundant. Analysis also found that chromosomal ARGs were having significantly higher abundance compared to plasmid ARGs ($p < 0.05$). While, taxonomy of ARGs carrying contigs showed majority of genera *Klebsiella* and *Enterobacter*. Present study showed, that the higher gut resistome in neonatal ICUs could be due to the compromised sterile conditions in the neonatal units and form mother, which present a greater risk to the neonates even in the controlled environment.

Keywords: Antibiotic resistance; Gut resistome; Infants; ICU's; Metagenome

Introduction

In last decade, many studies have shown us the presence of antibiotic resistance genes (ARGs) in human gut microbiota, specially metagenomic studies. Metagenomic studies of human fecal and oral samples have shown us the different antibiotic resistance genes in them such as, tetracycline resistance genes are the most abundant in the human gut microbiome. It is interesting to know that tetracycline is a one of the highly used antibiotic in animals, but not in humans [1]. Such metagenomic studies revealed that human gut microbiome acts as a reservoir of ARGs and paved foundation to stablish that human gut microbiome is a dynamic system that keep exchanging microbes form the external sources [1,2].

Studies have shown us that even neonatal-intensive care units (ICUs) are not free from these ARGs [3]. As, newly born human babies have higher risk of infection and diseases, specially those who born premature or underweight. Any such infection in infants is one of the major causes of mortality among them and treatment involves vast range of antibiotics, and most antibiotics which are used in the clinical practices are of broad-spectrum categories i.e. targets multiple types of bacteria [3,4]. Other than prescribed antibiotic treatments, other ARG sources are also there in infant-ICUs, affecting the infant resistome such as maternal ARGs passing through breast-feeding and feeding tubes for premature babies [5,6]. Studies also suggested that different genetic elements such as chromosomes and plasmids are actively involved in spread of ARGs [7], which can help us to understand the spread of ARGs, as ARGs placed on plasmids have higher probability to get

transferred by horizontal gene transfer [1]. Present study was designed to investigate the gut resistome and mobilome profile in the neonatal ICUs.

Material and Method

For study fecal metagenome (Illumina Hiseq) of six infants receiving ICU-care was, downloaded from the NCBI (SRA bioproject SRP160134). Quality control of metagenomic dataset was done using FastQ Quality Control Software (FaQCs) [8], by removing the reads that; contained more than 3 ambiguous nucleotides, low quality reads with length less than 100bp, adapter sequences and the reads with quality score below 30. All the clean metagenomic data reads were subjected for ARGs annotation against Antibiotic Resistance Database using Diamond Blastx [9], with filtering criteria of alignment length of 35 aa, similarity of 90% and e value of 1e-5 [10]. ARG abundance was calculated as "copy of ARGs per copy of 16S rRNA gene", where ARG like sequences were normalized with the corresponding ARG reference sequence length (nucleotide) and number of 16S rRNA genes [11]. Number of 16S rRNA subunits were checked by Metaxa2 by using default parameters for paired-end mode [12]. Clean metagenomic reads were subjected for bacterial taxonomy analysis with Metaphlan2 [13].

All the metagenomic reads were used for assembly with Megahit v1.2.9 (D. Li et al. 2015). Contigs with length less than 1000 bp were removed for downstream analysis. Contig coverage was calculated as Hits Per Million reads (HPM) by inhouse script of edge using samtools, bwa and bowtie2 algorithm [14]. Open reading frames (ORF) were predicted in contigs using Prodigal [15] and ORF were annotated against ARGs database using BLASTP, with filtering criteria of identity of 80 %, e value of 1e-10 and alignment length of 35 aa and were classified based on class of antibiotics [10]. Plasflow v 1.1 with default settings were used to predict the chromosomal and plasmid contigs carrying ARGs [16]. Taxonomic classification of ARGs carrying contigs were determined by Kaiju in greedy mode using NCBI RefSeq database [17].

Result and Discussion

Analysis showed us the higher ARG load in all the samples as they were containing 153 to 267 ARGs per sample (**Figure 1**).

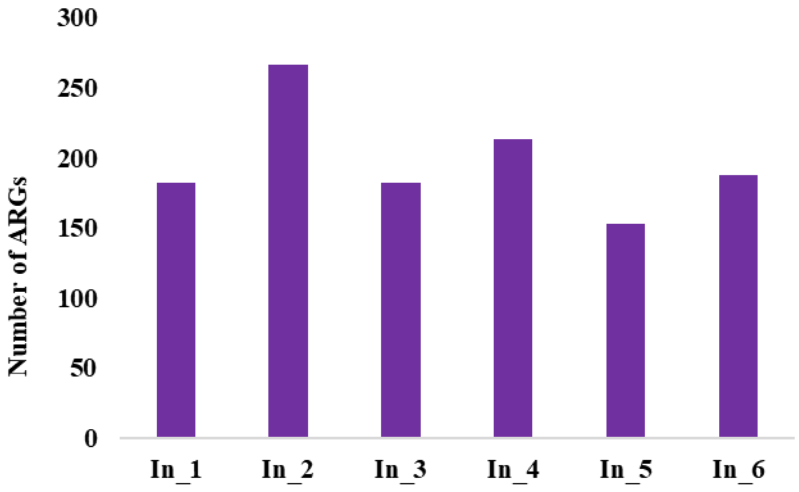

Figure 1. Total number of ARGs present in all six infants (In).

ARGs linked to class, aminocoumarins, multi drug-resistance (MDR), fluoroquinolones, betalactam and aminoglycoside were the most abundant in all six samples (**Figure 2**) (**Table 1**).

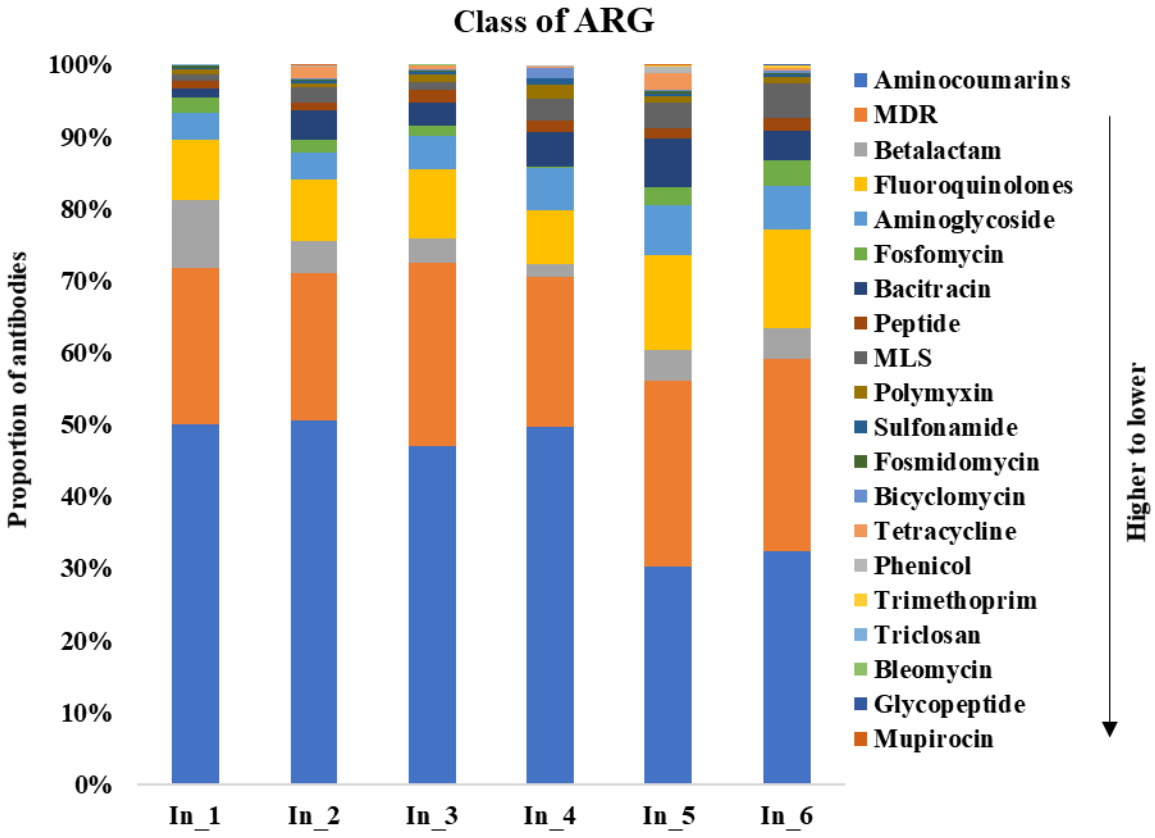

Figure 2. Proportion of top 25 class of ARGs present in all six infants (In).

Table 1. Table of top 25 ARGs along with their class and positioning.

Class	Type	Position
MDR	AcrA	Chromosome
MDR	AcrB	Chromosome

MDR	acrF	Chromosome, Plasmid
Aminoglycoside	APH(3")-Ib	Chromosome, Plasmid
Bacitracin	**BacA**	Chromosome
Aminoglycoside	baeR	Chromosome, Plasmid
Bicyclomycin	bcr-1	Chromosome
Aminoglycoside	cpxA	Chromosome
MDR	CRP	Chromosome
Fluoroquinolones	emrA	Chromosome, plasmid
Fluoroquinolones	emrB	Chromosome, Plasmid
MDR	EmrD	Chromosome
MDR	hmrM	Chromosome
MLS	macA	Chromosome
MDR	MacB	Chromosome, Plasmid
MDR	marA	Chromosome
Aminocoumarins	**mdtA**	Chromosome
Aminocoumarins	mdtB	Chromosome
Aminocoumarins	**mdtC**	Chromosome
Fluoroquinolones	MdtH	Chromosome
Fluoroquinolones	mfd	Chromosome
Fluoroquinolones	parC	Chromosome, Plasmid
MDR	sdiA	Chromosome
MDR	tolC	Chromosome
Peptide	YojI	Chromosome

Additionally, all six samples were rich in the numerous ARGs, specially for *mdtA, mdtC* (Aminocoumarins), *cpxA, APH(3'')-Ib* (Aminoglycoside) and *BacA* (Bacitracin) (**Figure 3**).

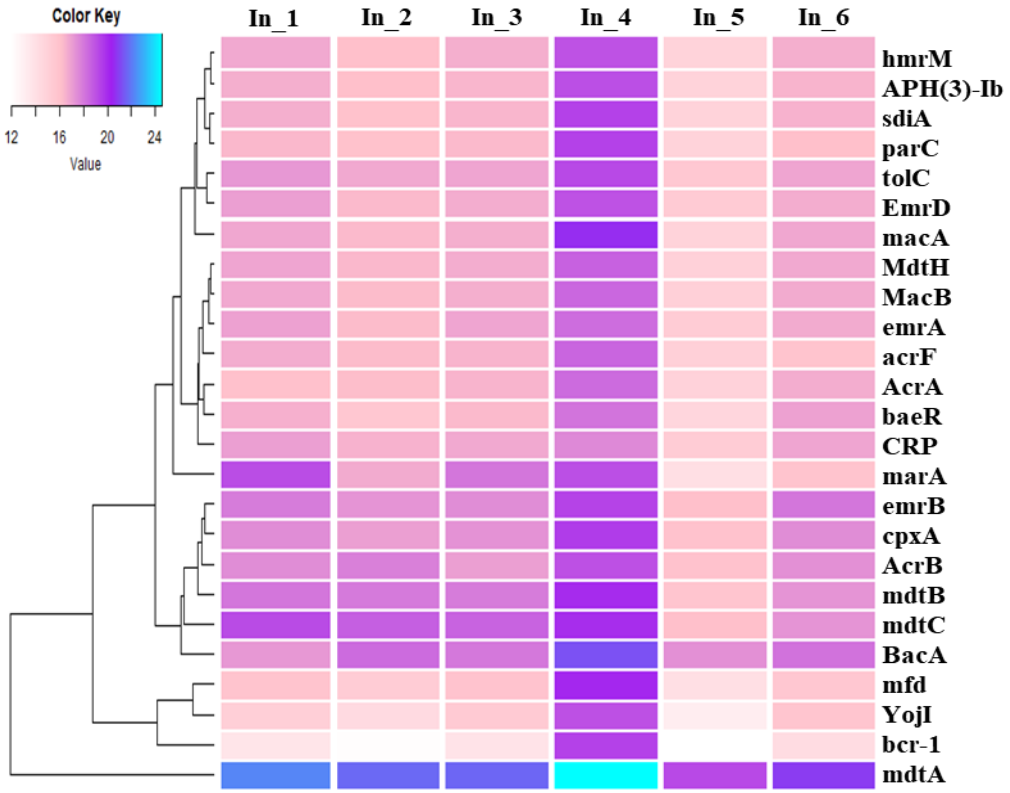

Figure 3. Distribution of top twenty-five most prevalent ARG type in all six infants (In).

Metagenomic analysis showed that phylum Proteobacteria, Firmicutes and Actinobacteria were majorly present in the samples, while phylum Bacteroidetes was present in the only one sample (In_2), and in one sample (In_4) Firmicutes covered the most (**Figure 4**).

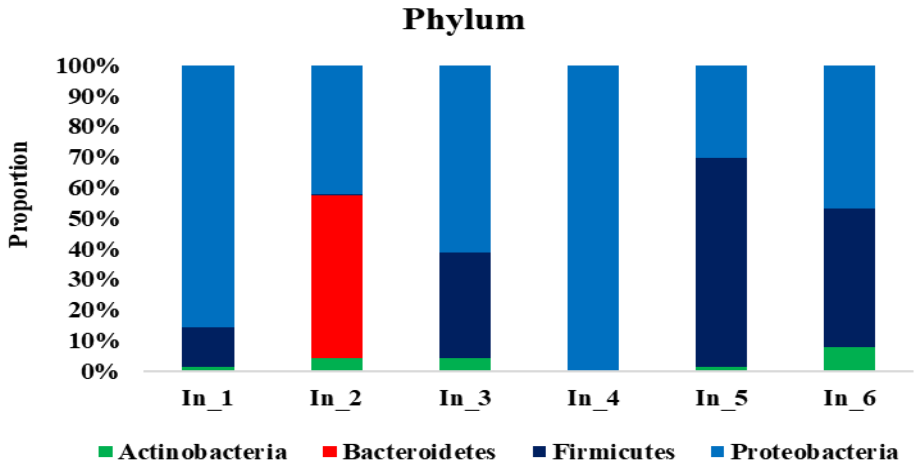

Figure 4. Distribution of microbiota among six infants (In), at phylum level.

Gut resistome is a dynamic structure which grows with time, as infants are very young, we can understand this variation [1]. While, genera *Enterococcus* and *Klebsiella* were in the majority (**Figure 5**), as similar previous studies also confirmed their prevalence in gut resistome [18].

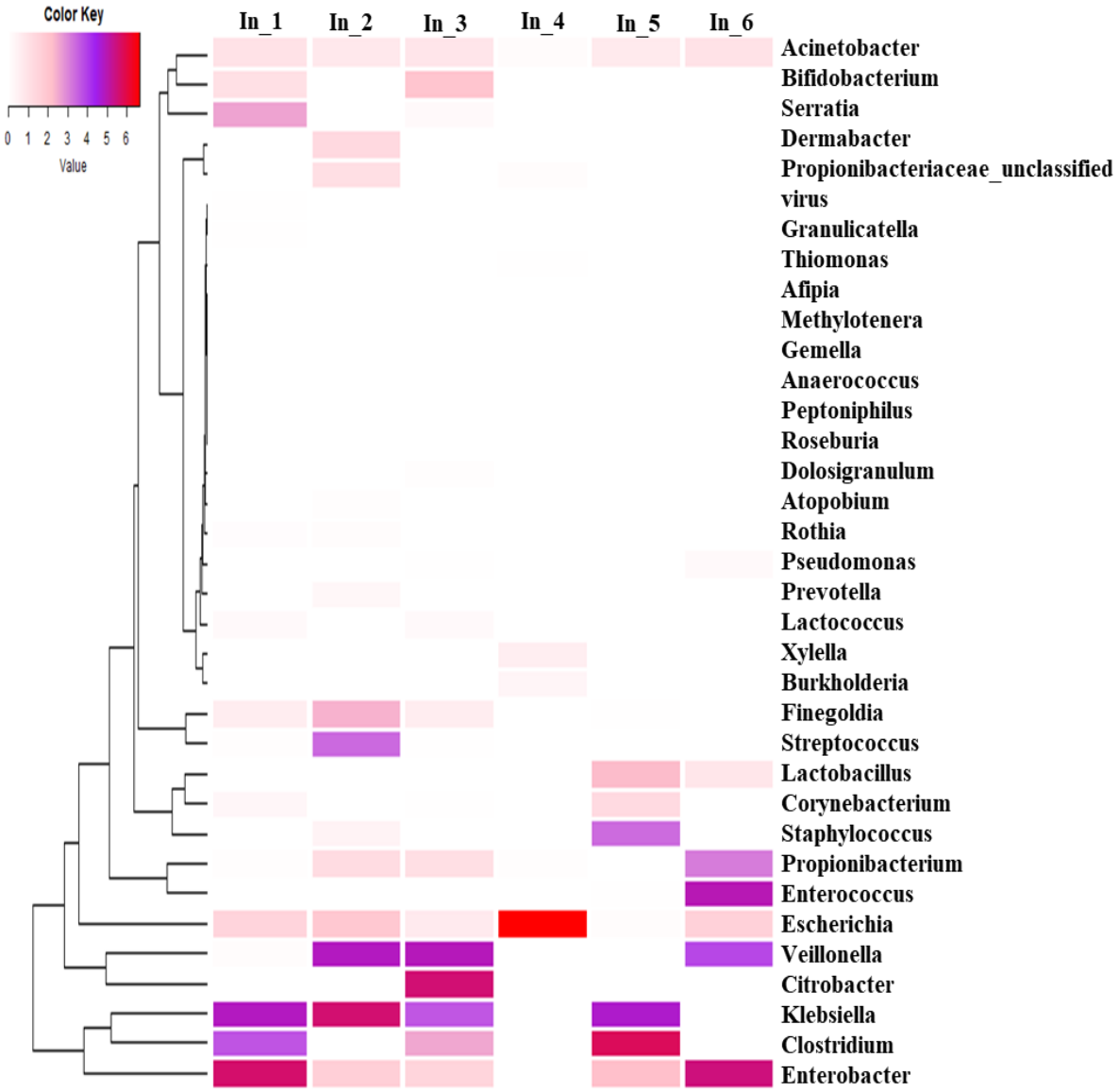

Figure 5. Distribution of microbiota among six infants (In) at genus level (log2 scale).

Further, Plasflow analysis showed that most of the ARGs present in all six infants were situated on chromosome and very few were on plasmid (**Figure 6**), suggesting most of the ARGs maybe driven from mothers.

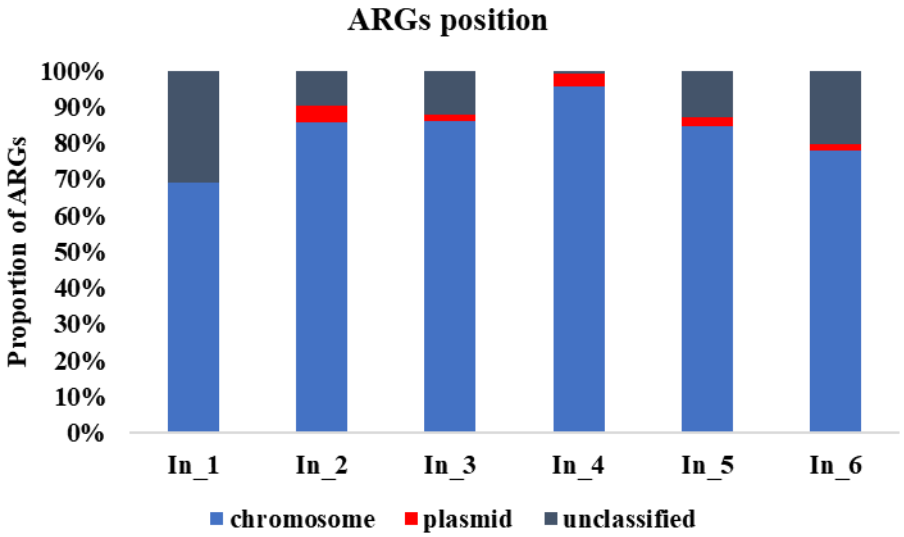

Figure 6. Position of ARGs on the basis of genetic element.

As previous studies also confirm the possibility of vertical gene transfer and involvement of maternal breast milk microbiota's ARGs to enhance the gut resistome of the infants.

Conclusion

Present study illustrated that gut-resistome starts growing from the very age of infants, where Aminocoumarins (*mdtA, mdtC*) and Aminoglycoside (*cpxA, APH(3'')-Ib*) were the most prevalent ARGs. Additionally, *Enterococcus* and *Klebsiella* were the dominant genera, which are often associated with hospital acquired infections. The study also found that majority of ARGs present in neonatal gut-resistome were located on the chromosome, not on the plasmid, which they might have obtained from their mother.

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Conflict of interest: Authors declare no conflict of interest.
Author Contributions: VS performed the analysis and drafted the final manuscript. SR participated in the analysis. UT reviewed the analysis methodology and manuscript.

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