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Maternal-neonatal transmission of *Enterococcus* strains during delivery

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Abstract

Background: Cesarean delivery (c-section) is on the rise globally. C-section is generally preferred if complications prevail in a vaginal delivery. Some private institutions conduct it unnecessarily for economic gains. This has led to a severe risk to a child's health. The neonate's gut microbiota has been observed to be influenced by the mode of delivery. Lactobacilli and other bacteria are very vital for developing healthy gut flora. An attempt has been made to investigate the passage of lactic acid bacteria to the infant via delivery. The vaginal swabs of the mothers and fresh meconium samples of the newborns post-delivery were examined for the presence of lactobacilli through culture techniques.

Results: The present investigation revealed that c-section-delivered infants lacked these bacteria in comparison with vaginally delivered infants. Biochemical tests and 16S rRNA sequencing showed similarity with groups 1, 2, 3, and 5 of *Enterococcus* genus. Phylogenetic analysis revealed more than 98% similarity with several *Enterococcus* species. *E. canintestini*, *E. rivorum*, and *E. dispar* were transferred frequently from mother to infants born via vaginal delivery. The strains were submitted to the NCBI GenBank database under accession numbers KX830968–KX830982.

Conclusion: The study supports the hypothesis of maternal-neonatal transmission of bacteria during delivery and also possibilities of isolating newer strains for probiotic use.

Keywords: Delivery, Gut flora, *Enterococcus transmission*, Probiotic

1 Background

Women perceive cesarean delivery as a labor-free and safest mode of delivery. Cesarean delivery is an abdominal surgery that invites risks like surgical complications and admission to the neonatal intensive care unit. It is also more expensive as compared to vaginal delivery. In global statistical analysis, the cost of unnecessary c-section amounted approximately to US\$2.32 billion and of needed c-section was around US\$432 million [1]. A woman with a primary cesarean has a 10% chance of vaginal birth for subsequent deliveries [2]. In the USA and most Western countries, women have the right to opt for the mode of delivery. In the USA, one in three women opts for c-section. The UK National Health Service has stated that the death rate of women in cesarean delivery was three times more as compared to vaginal delivery. In India, National Family and Health Survey has reported that the c-section rate increased from 10.6

% in 2006 to 17.2% in 2017 [3]. There is an alarming difference between the c-sections conducted in public and private institutions. Private institutions conduct more c-sections than public institutions. There is a greater chance of unnecessary cesarean being conducted by private institutions for their economic motives [4]. This statistical analysis is of concern and has predisposed several risks to the development and health of the child. Some of these include neonatal depression due to general anesthesia, fetal injury during hysterectomy, respiratory distress, and breastfeeding complications. With the increasing trend of c-section, there has been an increase in the occurrences of autoimmune diseases like Crohn's disease, type 1 diabetes, multiple sclerosis, allergic diseases like asthma, rhinitis, and atopic dermatitis. These diseases have now been affluent in Western and industrialized countries [5].

The fetal gastrointestinal tract is considered to be sterile [6–8]. The colonization of microorganisms in the newborn begins during delivery and also within minutes after birth [9]. The establishing microorganisms originate from

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the vaginal and gastrointestinal flora of the mother, from environmental sources and consumption of breast milk along with formula milk. Different types of maternal vaginal bacteria come in contact with the mouth of the infant, and they act as an inoculum for the development of the gut flora. The intestinal microbiota of a newborn is highly dependent on the mode of delivery. The immune system undergoes a major development during infancy. This development is highly related to the colonization of the gut flora of the infant. It has been observed that vaginally delivered infants have a diverse microflora than c-section-delivered infants. C-section was considered as a life-saving procedure during complex deliveries. The c-section delivery often contributes to prolonged mother-infant separation, longer hospital stays, and delayed or shorter breastfeeding periods [10]. This favors the outgrowth of unwanted, harmful, and pathogenic bacteria. These factors lead to delayed intestinal colonization of these infants. It has also been observed that the beneficial bacteria were not found in these infants until 6 months of birth. Moreover, women who undergo premature labor or c-section are exposed to a broad spectrum of antibiotic treatment. This, in turn, causes the reduction of useful intestinal microbiota [11].

Enterococci are commonly found on the first day of life and are among the first microorganisms detected in the stool of infants. *Enterococcus* genus accounts for up to 2% of the total vaginal flora. This family has been targeted for its probiotic properties. *Enterococcus* and *Escherichia* are the first to colonize the infant's gut flora and establish an anaerobic environment which, in turn, establish favorable conditions for the growth of Firmicutes and other useful bacteria [12, 13]. The enterococci are present up to 10^8 cfu/g in stool samples and constitute part of the normal human intestinal flora. These bacteria are also found in the human genital tract and oral cavity [14, 15]. Many strains of *E. faecalis* produce bacteriocins called enterocins, a family of safe [16] and ribosomally synthesized antimicrobial peptides that inhibit pathogenic organisms [17]. There are many factors known to influence the composition of the microbial flora in infants during and after birth [1, 2, 4]. Several researchers have observed that preterm infants and c-section-delivered infants exhibited a delayed intestinal colonization along with a smaller number of species variability and a higher rate of potentially pathogenic microorganisms [6, 8, 9, 18]. Treatment with antibiotics decreases the amount of anaerobic bacteria while increasing the number of *Enterobacteriaceae* in the infant's stool [9]. Pre-term births are likely predisposed to necrotizing enterocolitis (NEC) than term births [19]. However, interestingly, one study revealed that infants who developed NEC carried less *E. faecalis* [20]. A study relating to allergy development in infants during the first

year of life showed that babies with allergies were less often colonized with *Enterococcus* during the first month of life when compared to healthy infants [21]. This implicates the importance of *Enterococcus* in intestinal immune development in earlier months of life. However, some reports have shown that they act as opportunistic pathogens, but their probiotic properties cannot be ignored [22]. Selection of avirulent enterococci from maternal source with probiotic properties would help in the reconstitution of the gut flora of c-section babies. Various non-culturable techniques have been adopted recently to study the organisms transferred during delivery [23].

In a previous study, we have already characterized the enterococci strains obtained from mother-infant transmission as potential probiotic candidates [24, 25]. The literature has reported very few studies on the vertical transmission of *Enterococcus* from mother to infant during delivery. In the present study, we investigate the transmission, isolation, and identification of enterococci during delivery.

2 Methods

2.1 Sampling

Thirty-five mother-infant pairs were enrolled in this study. Out of these, 23 women had vaginal delivery while 12 women underwent c-section. The women were explained the study and an informed consent was obtained from them. These women were healthy, in their reproductive age (20–35 years) with weight 40–70 kg. Their diet consisted of cereals, legumes, vegetables, dairy products, and/or meat. They were not on antibiotics or any probiotic supplements. Cases including rupture of membranes or chorioamnionitis were excluded. Women with vaginal infections, vaginal hemorrhage, past history of any sexual diseases, HIV positive, or hepatitis B positive were excluded from the study. Also, women with smoking, alcoholism, and other such addictions were excluded from this study. The study was approved by Lokmanya Tilak Municipal Corporation General Hospital, Mumbai, India.

In this study, vaginal swabs of healthy pregnant women in their third trimester were obtained. The samples were collected randomly during 5 days prior to delivery. The vaginal lateral wall was swabbed, inoculated into Amies transport medium, stored in icepacks, and was transported to the lab within 2 h. The samples were serially diluted in PBS (pH 7) and plated on Mann-Rogosa-Sharpe (MRS) agar plates followed by 48 h of anaerobic incubation at 37 °C. The first and fresh meconium samples of the neonates were obtained and immediately transported to the laboratory. One gram of sample was serially diluted up to 10^8 using sterile PBS (pH 7.0) and viable counts were carried out on MRS agar

plates after incubating for 48 h at 37 °C anaerobically. The colonies were characterized by Gram staining and a catalase test. Gram-positive and catalase-negative cocci were selected and inoculated in MRS broth with 30% glycerol as cryo-protectant and stored at – 80 °C. All the protocols were carried out in duplicates [26].

2.2 Biochemical characterization

The strains obtained were screened for their physiological, morphological, and biochemical characteristics as described in Bergey's Manual of Systematic Bacteriology. The colonies were examined by Gram staining and for catalase production. The strains that were Gram-positive cocci and catalase-negative were further studied for their biochemical characteristics. The strains were screened for their sugar fermenting abilities using various sugars discs in peptone water. Sugars like arabinose, cellobiose, sorbitol, mannitol, mannose, lactose, maltose, glucose, melibiose, raffinose, ribose, salicin, rhamnose, sorbitol, xylose, tagatose, and trehalose at a final concentration of 1% were used for the study.

The strains were observed for temperature tolerance by growing in MRS broth at 4 °C, 15 °C, and 45 °C. Tolerance at 6% NaCl and 3% bile concentration was carried out. Growth on potassium tellurite agar and sodium azide was carried out. The strains were also screened for ammonia production from arginine.

2.3 Molecular identification of the strains

After biochemical characterization, 15 representative strains from each group were selected for further molecular identification by 16S rRNA sequencing. Genomic DNA was isolated from the culture by using Sigma's Gene Elute Bacterial Genomic DNA Kit. Around 600 bp of V₃–V₅ hypervariable region was amplified using the following combination of primers – FDD2 – RPP2 (universal primers for 1.5 kB fragment amplification for eubacteria) Template DNA – Genomic DNA. Sequences of the primer pair used for amplification were

RPP2 – CCAAGCTTCTAGACGGITACCTTGTTAC GACTT, FDD2-CCGGATCCGTTCGACAGAGTTTGAT CITGGCTCAG

The PCR products obtained from above reactions were then processed for cycle sequencing reaction using an Eppendorf Gradient Mastercycler system with a cycle of 94 °C for 5 min, 30 cycles of 94 °C, and 60 °C and 72 °C for 10 min. The mixture was then held at 4 °C. The PCR product was precipitated using polyethylene glycol (PEG 6000, 8.5%), washed thrice using 70% ethanol, and then was dissolved in Tris-HCl (10 mM, pH 8.0). Following the above reaction, the samples were cleaned up and loaded on the sequencer Avant 3100 Gene Analyzer. The sequence output was compared with type strain *Enterococcus faecium* ATCC 19454 in the EzTaxon

database of the National Center for Biotechnology Information GenBank entries by using BLAST algorithm [27].

The phylogenetic tree was created with Mega 7 software using the neighbor-joining method. The sample sequences and affiliated known sequences from public databases were aligned, checked, and corrected using the Clustal W program in BioEdit. The evolutionary tree was inferred by bootstrapping 1000 replicates. The evolutionary distances were compared using the Maximum Composite Likelihood method and are in the units of the number of transversal substitutions per site. The differences in the composition bias among sequences were considered in evolutionary comparisons. The analysis consisted of 15 test nucleotide sequences. For comparison, 22 nucleotide sequences were procured from the GenBank database. All ambiguous positions were removed from each pair. There were total 1533 positions in the final dataset. Bootstrap values above 50% were displayed as percentages.

3 Results

3.1 Sampling

The viable counts of mother-infant pairs were calculated. The vaginally delivered babies showed good bacterial counts. The c-section-delivered babies showed no bacterial counts in their feces. Table 1 shows the number of species passed in the mother-infant pairs. Out of 23 vaginally delivered infants, 21 neonates shared at least one species from the mother. *Enterococcus canintestini*, *Enterococcus dispar*, and *Enterococcus rivorum* showed higher transmission among the pairs.

3.2 Biochemical characterization

Around 78 strains were selected from mother-infant pairs. The Gram-positive cocci were co-cultured and purified for biochemical studies. The strains were checked for their distinguishing fermentation abilities. Table 2 shows the biochemical characterization according to Bergey's Manual Of Systematic Bacteriology.

3.3 Molecular characterization

After the preliminary biochemical characterization, 15 strains were selected as representatives of each group and characterized by 16S rRNA amplification and sequencing. The strains showed similarity to several species of the genus *Enterococcus*. The strains were compared with type strain *Enterococcus faecium* ATCC 19434. All the strains showed 98–100% similarity with the type strain. The species determination was carried out considering both phenetic characteristics and the sequence similarity with affiliated strains in the database. Figure 1 shows the phylogenetic tree constructed by neighbor-joining method. Strain SB2 shows 99.86% similarity with *E. villorum*; strains AB3 and S26B show

Table 1 Comparison of bacterial counts in mother-infant pair and species shared during delivery

Mother-infant pair	Viable counts log ₁₀ mother	Viable counts log ₁₀ cfu/ml infants		Bacterial species shared by the pair								N		
		VD	CD	<i>E. riv</i>	<i>E. dis</i>	<i>E. can</i>	<i>E. phe</i>	<i>E. vil</i>	<i>E. ita</i>	<i>E. rat</i>	<i>E. viik</i>		<i>E. tha</i>	
1	5.67 ± 0.1		ND		+/-	+/-								0
2	5.95 ± 0.07	2.34 ± 0.07					+/+	+/+	-/+					2
3	5.85 ± 0.12	2.28 ± 0.13			-/+		+/+				+/+			2
4	4.68 ± 0.03		ND				+/-							0
5	6.078 ± 0.31	3.56 ± 0.15											+/+	1
6	6.15 ± 0.41	2.68 ± 0.06			+/-									0
7	6.20 ± 0.27	2.43 ± 0.14					-/+							0
8	5.78 ± 0.07	3.21 ± 0.27											+/-	0
9	5.85 ± 0.07		ND				+/+							1
10	5.43 ± 0.15	3.06 ± 0.38									+/+			1
11	4.48 ± 0.06	3.24 ± 0.21								+/+				1
12	5.30 ± 0.02		ND			+/-								0
13	4.78 ± 0.12		ND				+/-							0
14	5.77 ± 0.04		ND		+/-		+/-							0
15	5.65 ± 0.04	3.36 ± 0.19								+/+			-/+	1
16	5.67 ± 0.45	3.45 ± 0.21			+/+	+/+								2
17	5.32 ± 0.33	3.76 ± 0.16								+/+		+/+		2
18	4.98 ± 0.52	3.56 ± 0.27					+/+							1
19	6.31 ± 0.41	3.77 ± 0.19			+/+									1
20	5.54 ± 0.26		ND			+/-		+/+					+/-	0
21	6.89 ± 0.23	2.94 ± 0.09							+/+					1
22	5.95 ± 0.34	3.25 ± 0.14			+/+									1
23	4.80 ± 0.08	3.66 ± 0.23					+/+			-/+				1
24	5.76 ± 0.02		ND		+/-									0
25	5.38 ± 0.24	2.98 ± 0.33					+/+							1
26	5.81 ± 0.01		ND			+/-	+/-							0
27	6.02 ± 0.03	3.44 ± 0.21					+/+	+/+						2
28	6.45 ± 0.09	2.95 ± 0.05			-/+		+/+							1
29	5.20 ± 0.01		ND		+/+									1
30	6.91 ± 0.37	2.48 ± 0.35			+/+		-/+					+/+		2
31	6.11 ± 0.46	3.16 ± 0.11											+/+	1
32	5.97 ± 0.48		ND							+/+				0
33	5.54 ± 0.43	2.79 ± 0.26					+/+							1
34	6.70 ± 0.36	3.24 ± 0.13			+/+			+/+						2
35	4.78 ± 0.05	3.67 ± 0.17					+/-							0

Legend: Values are mean log₁₀ cfu/mL (vaginal fluid) or log₁₀ cfu/g (feces) ± standard deviation

E. riv, *Enterococcus rivorum*; *E. dis*, *Enterococcus dispar*; *E. can*, *Enterococcus canintestini*; *E. phe*, *Enterococcus pheoniculicola*; *E. vil*, *E villorum*; *E. ita*, *Enterococcus italicus*; *E. rat*, *Enterococcus ratti*; *E. viik*, *Enterococcus viikiensis*; *E. thai*, *Enterococcus thailandicus*; VD, vaginal delivery; CD, cesarean delivery; N, number of bacterial species shared by the pair; +/+, bacterial species was present in both the both mother and infant sample; +/-, bacterial species was present in the mother but absent in the infant; -/+, bacterial species absent in mother but present in infant; ND, not detected

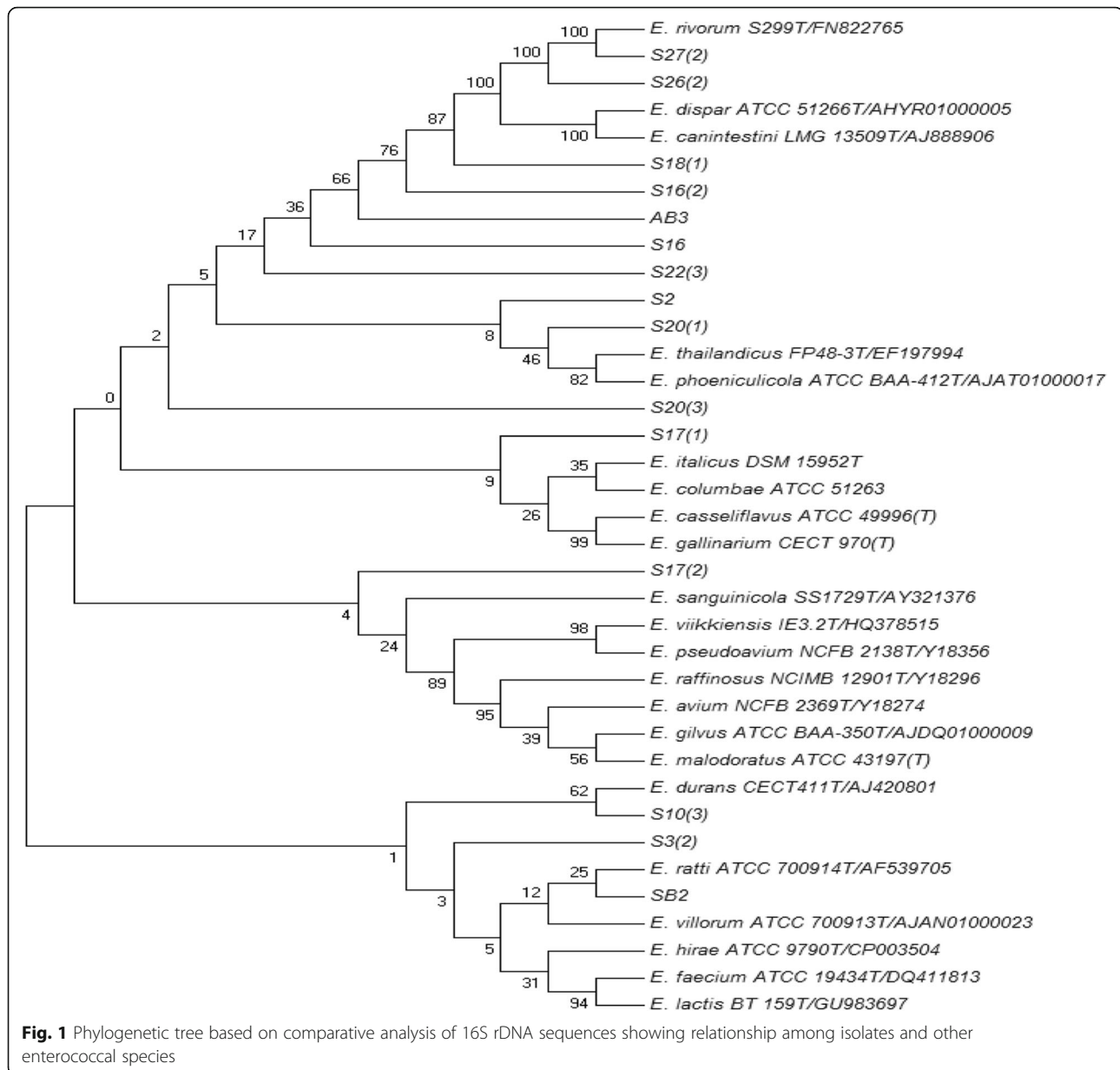
98.56% and 99.65% with *E. canintestini*; and strains S20A and S2 show 99.84 % and 98.51% similarity with *E. pheoniculicola*, respectively. Strains S16 and S22C show 99.37% and 99.53% similarity with *E. rivorum*, respectively. On the other hand, strains S10C, S16B, S27B,

S17B, and S3B show similarity with *E. ratti* (99.86%), *E. dispar* (98%, 98.78%), *E viikkiensis* (99.56%) and *E. ratti* (99.26%), respectively. These strains were biochemically similar to the reported biochemical characteristics of the same species [28]. Seventy-eight strains were classified

Table 2 Phenotypic characterization and molecular identification of the isolates

Strain	Clones	6% NaCl	Na ₂ S ₂ O ₃	Trehalose	Arabinose	Raffinose	Rhamnose	Ribose	Tagatose	Xylose	Glycerol	Inulin	Mannitol	Sorbitol	Melibiose	Fucose	Cellobiose	Arginine	16S rRNA similarity, %	Species identification	Accession number
S2	5	+	+	+	+	-	+	+	+	+	+	-	-	-	+	-	+	+	99.86	<i>E. villorum</i>	KX 830968
AB3	6	-	+	+	-	-	-	-	+	-	-	+	-	-	-	-	w	+	98.56	<i>E. canintestini</i>	KX 830969
S10C	2	+	+	+	-	+	+	+	+	-	-	-	+	+	+	-	+	+	99.86	<i>E. ratti</i>	KX 830970
S22C	8	+	+	+	-	-	w	+	-	+	+	-	+	-	+	w	+	+	99.53	<i>E. rivorum</i>	KX 830971
S2	4	+	+	+	+	-	-	-	+	-	+	-	+	+	w	-	-	+	99.84	<i>E. phoeniculicola</i>	KX 830972
S16	5	+	+	+	-	-	-	+	+	-	+	-	+	+	w	w	+	+	99.37	<i>E. rivorum</i>	KX 830973
S27B	5	+	+	+	-	-	-	+	+	-	-	-	-	-	+	-	+	+	98.78	<i>E. dispar</i>	KX 830974
S20A	3	+	+	+	+	+	-	+	-	-	-	-	+	-	+	-	+	+	99.84	<i>E. phoeniculicola</i>	KX 830975
S26B	8	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	+	+	99.65	<i>E. canintestini</i>	KX 830976
S20C	7	+	+	+	-	-	-	+	-	+	+	-	+	-	+	-	+	+	99.40	<i>E. thailandicus</i>	KX 830977
S17A	4	+	+	+	-	+	+	+	-	-	-	-	-	-	+	-	+	+	98.84	<i>E. italicus</i>	KX 830978
S18A	6	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	+	+	99.84	<i>E. canintestini</i>	KX 830979
S16B	11	-	+	+	-	-	-	+	-	-	+	-	-	-	+	-	-	+	98.00	<i>E. dispar</i>	KX 830980
S17B	2	+	+	+	-	-	-	-	-	-	+	-	-	-	w	-	-	+	99.56	<i>E. viikkiensis</i>	KX 830981
S3B	2	+	+	+	-	+	-	+	+	-	-	-	+	+	+	-	+	+	99.26	<i>E. ratti</i>	KX 830982

Legend: +, positive reaction; -, negative reaction; w, weak reaction



as 26% *E. canintestini*, 20% *E. dispar*, 17% *E. rivorum*, 9% *E. phoeniculicola*, 9% *E. thailandicus*, 6% *E. villorum*, 5% *E. ratti*, 5% *E. italicus*, and 3% *E. viikkiensis*. *Enterococcus canintestini* was the predominant strain. Similar results were observed by Belma et al. while profiling the presence of *Lactobacillus* species in the vagina [29]. Figure 2 shows the percentage of transmission of *Enterococcus* species in the mother-infant pairs. *E. canintestini*, *E. rivorum*, and *E. dispar* were transferred frequently within the pairs. All the strain sequences were submitted to the NCBI GenBank Database under accession numbers from KX830968–KX830982.

4 Discussion

The 16S rRNA sequencing showed that strains belong to groups 1, 2, 3, and 5 of *Enterococcus* genus. The identification of species of this genus is quite tedious. Generally for such strains having matches with distance scores > 0.5% next to the closest species, biochemical and other distinguishing properties hold importance in species identification. Hence, in this study, the identification was done by considering both biochemical characteristics and sequencing results [30, 31]. The comparison of sequences shows 98–100 % similarity with the type strain *Enterococcus faecium* ATCC 19454. The enterococci

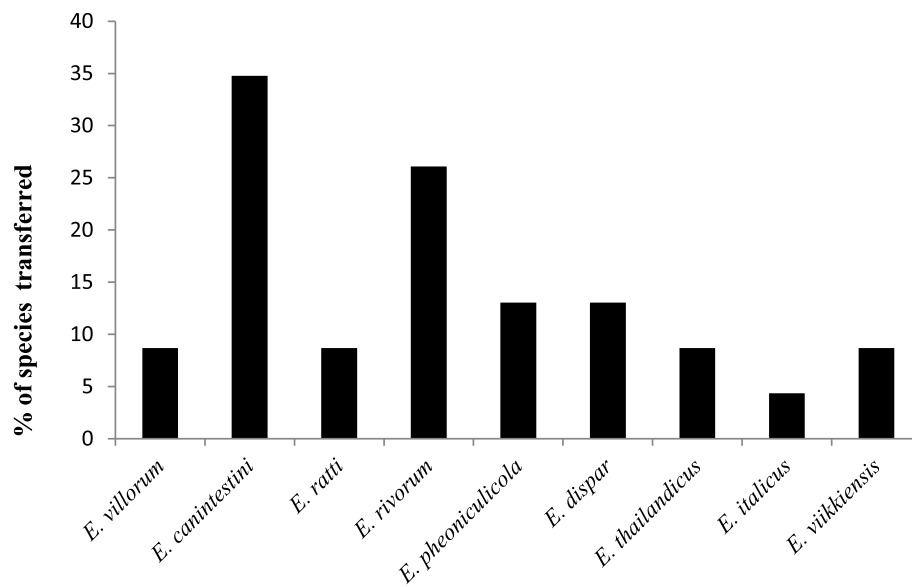


Fig. 2 Percentage distribution of *Enterococcus* species transferred to the infant during vaginal delivery

showed distinguishing fermentation abilities of mannitol, sorbitol, rhamnose, raffinose, arabinose, tagatose, etc. Table 2 shows the enterococci classification based on their sugar fermentation abilities and 16S rRNA sequencing results.

Many of the strains in this study were recently discovered like *E. rivorum*, *E. canintestini*, *E. viikkiensis*, and *E. thailandicus* from various sources [28, 32, 33]. *E. rivorum* was first reported in 2012 from the water source Brooks in Finland which was free from human inhabitation and hence ruled out human feces contamination [34]. *E. canintestini* was first reported in dog feces [35]. This study shows that these species are now widely spread in the human community too. *Enterococcus thailandicus* was first isolated from a fermented sausage in Thailand. It is closely related to *E. hirae*, *E. durans*, and *E. faecium* [36]. *E. pheoniculicola* was first isolated in 2003 from the uropygial gland of the Red-billed Woodhoopoe, *Pheoniculus purpureus* [32].

Figure 2 shows the comparison of the transfer of strains in mother-infant pair with monophyletic transmission. Monophyletic strains are species that are closely related to each other. In most of the vaginally delivered infants, it was observed that similar species were shared by the mother-infant pair. In contrast, c-section delivered infants did not show any transmission. Our findings confirm with earlier understanding that vaginal delivery aids in the transmission of mother's microbiota to the infant. Bacteria can induce a phenomenon called oral tolerance. Oral tolerance is created by the immune system against bacteria transferred orally or ingested. This tolerance helps in reducing over-reactive responses involved in allergies and auto-immune diseases. This study

was based on the culture cultivation technique and was focused on specific genera. Hence, these results confirm that vaginal delivery facilitates the mother-infant transmission of enterococci [37]. As monophyletic strains were not observed in infants delivered by c-section, it confirms that monophyletic strains must have been transmitted through the birth canal during vaginal delivery [38]. Similar results were observed while profiling *Bifidobacteria* bacteria during delivery. In Finland, Gronlund et al. studied the transfer of *Bifidobacteria* in 61 mother-infant pairs. These women were supplemented with *Bifidobacterium lactis* and *Lactobacillus GG* until delivery [11]. Chen et al. conducted a similar study with 20 infants vaginally delivered and 20 by c-section. He observed that the mean levels of bifidobacteria were significantly higher in vaginally delivered as compared to c-section infants [39]. Pender et al. also showed that cesarean delivery decreased the counts of bifidobacteria in the gut [40].

The human gut harbor a diverse microflora of numerous genera, species, and bacteria. This early colonization of the beneficial gut flora has an impact on the health of the child. It is highly related to the type of delivery, gestational period, and feeding habitat of the mother [41]. Several studies have confirmed the passage of the gut flora in the mother-infant pairs. These studies have proved that lactic acid bacteria induce a mucosal and humoral immune response. Wild-type lactoflora derived from human origin can be used as probiotics for treating diarrhea and allergies in children. *Enterococcus faecium* strains like SF68, EE3, and M-74 have been tested in animal feeds and they have proved their probiotic efficacy [22, 42]. One study has reported the role of *E. faecalis*,

isolated from newborns, in infant gut protection. *E. faecalis* suppressed pathogen-mediated inflammatory responses in human IECs as well as DSS-induced inflammation in mice model. *E. faecalis* attenuated pro-inflammatory cytokine secretions, especially IL-8, via distinct pathways [13]. This confirms the role of colonizing bacteria in the postembryonic development of the gastrointestinal tract. The postembryonic maturation process is regulated by the commensal bacteria by cross-talking with host cells by recognizing receptors thereby inducing signaling pathways to activate transcription factors like nuclear receptors [43]. Another study has revealed that *E. faecalis* from newborn babies regulate endogenous PPAR γ activity and IL-10 levels in colonic epithelial cells [43]. Franz et al. showed that *E. faecalis* and *E. faecium* are involved in the reduction or prevention of gastrointestinal tract infections [44]. Studies involving *Enterococcus* strains isolated from the meconium of newborn infants have proved their potential for probiotic use [24, 45].

5 Conclusion

Gut microbiota of the neonate plays a crucial role in developing the infant's immunity. The present study confirmed that the mode of delivery plays an important role in the transmission of the gut microbiota at the time of birth. The comparison of bacterial counts of the mother-infant pair confirms that vaginal delivery is responsible for the natural inoculation of the gut of the infant. The study was solely dependent on culturing method and further research may include molecular techniques to investigate the footprint of the exact microbiome. However, the study built a strong foundation for developing a probiotic of vaginal strains from pregnant women for neonates born by cesarean delivery. The development of a probiotic formula might help reconstitute the gut microbiota of c-section-delivered infants. Hence, further research will include a selection of potential probiotic organisms to develop a formula containing probiotic organisms.

Abbreviations

BLAST: Basic local alignment search tool; DNA: Deoxyribose nucleic acid; MRS: Mann-Rogosa-Sharpe; NaCl: Sodium hydrochloride; PBS: Phosphate-buffered saline; PCR: Polymerase chain reaction; PEG: Polyethylene glycol; RNA: Ribose nucleic acid; Tris-HCl: Tris (hydroxymethyl) aminomethane (THAM) hydrochloride

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Authors' contributions

AB performed the experiments and wrote the manuscript. USA supervised the work and edited the manuscript. To this end, both authors have read and approved the final manuscript for publication.

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Availability of data and materials

Not applicable

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Lokmanya Tilak General Municipal Corporation Hospital, Mumbai, India, with reference no. IEC/04/13. A written and informed consent was obtained from all the subjects.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interest.

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