# Comparison Of Oral Microbiota In Various Modes During Births – Viz., Normal, Induced, Episiotomy, Forceps, Cesarean And Premature Neonates

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#### **Abstract**

Aim/Objective: Observation of bacteria transference from mother's birth canal to infant's oral cavity during various birth delivery modesMaterials and Methods: 150 healthy pregnant women, selected cognitively for the study were divided into six groups, each comprising 25 subjects. The delivery modes studied were Normal (group-I), Induced (group-11), Episiotomy (group-III), Forceps (group-IV), Cesarean (group-V) and Premature delivery (group-VI) of 25 each. Parturient canal sample was obtained prior to the delivery, whereas Infant oral cavity samples were obtained immediately after birth. The samples were transported to the laboratory for the microbiological assays and data was tabulated and statistically analyzed using normality test (Z-test) and Chi-square test. Results: In group-I, In Groups I and II, bacteriodes were not transferred to the infant oral cavity while Group III had statistically significant enterococci etc. Conclusion: In all cases, regardless of birth delivery mode, the mother's vagina was found to be contaminated and the infant's oral cavity was contaminated by microorganism.

## INTRODUCTION

Myriads of bacteria abound in the world, and these and other single cell creatures represent the major diversity of life on our planet. Bacteria also constitute the communal or normal microflora and populate the mucosal surfaces of the oral cavity, gastrointestinal tract, urogenital tract and skin surface<sup>1</sup>.

The microflora of the vagina normally varies during different phases of a woman's life, but they can also be strikingly different in individual women during the fertile age. Two major patterns of vaginal microflora exist among reproductive age group women. The normal or physiologic pattern in the birth canal is dominated by facultative lactobacilli. The other major pattern is synonymous with the clinical entity known as bacterial vaginitis (BV), which is characterized by mixed anaerobic and facultative bacteria. The lactobacilli most frequently present in normal vaginal secretions are Lactobacillus crispatus, Lactobacillus gasseri and Lactobacillus jensenii <sup>2</sup>.

The possibility of the maternal microflora being transferred to the infant during childbirth has been observed in different

birth modes. The present study was, therefore, undertaken with the following aims and objectives:

To compare the vaginal microflora of mothers just before delivery and the oral microflora of the newborns immediately after birth.

To compare the microbiota obtained from the mouth of the neonates at birth, born via normal delivery, episiotomy delivery, induced delivery, forceps delivery, cesarean delivery and premature delivery.

To compare the microbiota obtained from the mother's birth canal during normal delivery, episiotomy delivery, induced delivery, forceps delivery, caesarean delivery and premature delivery.

# **MATERIALS AND METHODS**

This study was conducted in the Obstetrics and Gynecology department of Women and Child Hospital, K.R. Market, Davangere, in collaboration with the Department of Microbiology, J.J.M.M.C. Davangere, after obtaining ethical clearance. This was an observational study in which the newborn's oral cavity, was observed for microorganism

transfer from pregnant women's birth canals during various delivery modes. The present study involved 150 healthy, pregnant women, with no history of antibiotic usage. Deliveries resulting in twins, triplets, quadruplets etc. were excluded from the study. Infants born by Normal, Induced, Episiotomy, Forceps, Cesarean, and Premature deliveries alone were studied. Deliveries resulting in infant death were excluded from the study.

The procedures, the possible discomforts, risks and benefits were explained fully to the human subjects involved and informed consent for the study was obtained from the families of the pregnant women, prior to the investigation.

Parturient canal sample was obtained from each of the 150 pregnant subjects. A sterile cotton swab was used to obtain the vaginal material from the vagina, by rotating and swabbing, just prior to the delivery. Oral cavity samples were obtained immediately following the infant's birth. Sterile swabs were rotated and swabbed from the cheeks, buccal sulci and edentulous ridges; the tongue and hard palate were uniformly sampled with a single swab obtained using a wooden stick. Both the birth canal and oral cavity samples, before transferring to the transportation medium, were used to make a smear on a sterile glass slide, which was then air dried, fixed with alcohol and finally transferred to the sterile leak-proof test tube containing 5% glucose broth. This was stored on ice and transported to the microbiological laboratory for analysis. Z test and Chisquare test were applied for statistical analysis.

#### MICROBIOLOGICAL PROCEDURES

The glass slides with the smear were stained with Gram's stain and microscopically observed for organism morphology. The specimens inoculated in the 5% glucose broth were then placed in the incubator for 18-24 hours and then inoculated into the blood agar and McConkey media, using a sterile inoculating loop. The media were incubated for 18-24 hours. After 24 hours, the culture media were observed for growth. If no growth was evident, it was further incubated. Colonies from the growth were observed, to study colony morphology. The colonies were further processed for sub-culture. A further biochemical reaction was performed to confirm the identity of the organisms.

# **RESULTS**

In (Group-1) Normal Delivery and (Group-2) Induced Delivery: Escherichia coli and Candida species were significant (P<0.05). Enterococci were very significant in the

birth canal. Bacteroides species were highly significant in the vagina (P<0.001) (Table-1 and 2).

Figure 1

TABLE NO.1: COMPARISON OF MICROFLORA BETWEEN BIRTH CANAL AND ORAL CAVITY IN NORMAL DELIVERY (GROUP-1)

Microorganism	Birth Canal (N=25)		Oral Cavity (N=25)		Difference in transfer of Microorganism		Significance
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	16	64	15	60	1	4	NS
Staphylococcus aureus	19	76	13	52	6	24	NS
Staphylococcus epidermidis	16	64	10	40	6	24	NS
Klebsiella	15	60	12	48	3	12	NS
Escherichia coli	9	36	6	24	3	12	P<0.05
Candida Species	7	28	4	16	3	12	P<0.05
Enterococci	10	40	0	0	0	0	P<0.1
Bacteriodes species	17	68	0	0	0	0	P<0.001
Pseudomonas	13	52	28	28	6	24	NS

Figure 2

TABLE NO.2: COMPARISON OF MICROFLORA BETWEEN BIRTH CANAL AND ORAL CAVITY IN INDUCED DELIVERY (GROUP-2)

Microorganism	Birth Canal (N-25)		Oral Cavity (N-25)		Difference in transfer of Microorganism		Significance
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	16	64	10	40	6	24	NS
Staphylococcus aureus	16	64	10	40	6	24	NS
Staphylococcus epidermidis	14	56	9	36	5	20	NS
Klebsiella	16	52	8	32	8	20	NS
Escherichia coli	12	48	5	20	7	28	P<0.05
Candida Species	9	36	3	12	6	24	P<0.05
Enterococci	8	32	0	0	0	0	P<0.1
Bacteriodes species	16	64	0	0	0	0	P<0.001
Pseudomonas	16	44	9	36	7	8	NS

In (Group-3) Episiotomy Delivery: Enterococci and Bacteroides species were transferred to the oral cavity, which was highly significant (P<0.01). Pseudomonas was transferred to the mouth which was statistically significant (P<0.05) (Table-3).

Figure 3

TABLE NO.3: COMPARISON OF MICROFLORA
BETWEEN BIRTH CANAL AND ORAL CAVITY IN
EPISIOTOMY DELIVERY (GROUP-3)

Microorganism	Birth Cana (N-25)	1	Oral Cavity (N-25)  Difference in transfer of Microorganism		Significance		
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	15	60	10	40	5	20	NS
Staphylococcus aureus	16	64	12	48	4	16	NS
Staphylococcus epidermidis	12	48	9	36	3	8	NS
Klebsiella	10	40	7	28	3	12	NS
α-heamolylic streptococci	5	20	3	12	2	28	NS
Escherichia coli	12	48	7	28	5	20	NS
Diphtheroids	8	32	5	20	3	12	NS
Neisseria species	8	32	4	16	4	16	NS
Candida species	9	36	4	16	5	20	NS
Enterococci	16	64	5	20	11	44	P<0.01
Bacteriodes species	15	60	4	16	11	44	P<0.01
Pseudomonas	9	36	3	12	6	24	P<0.05
Staphylococcus citrius	6	24	4	16	2	8	NS
Acinetobacter	4	16	7	28	3*	12	NS

In Forceps Delivery (Group-4): Bacteroides species were significant (P<0.05). Acinetobacter was not present in the birth canal; however, it was observed in the oral cavity, which was statistically significant (P<0.05) (Table-4).

Figure 4

TABLE NO.4: COMPARISON OF MICROFLORA
BETWEEN BIRTH CANAL AND ORAL CAVITY IN
FORCEPS DELIVERY (GROUP-4)

Microorganism	Birth Canal (N-25)		Oral Cavity (N-25)		Difference in transfer of Microorganism		Significance
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	11	44	8	32	3	12	NS
Staphylococcus aureus	15	60	10	40	5	20	NS
Staphylococcus epidermidis	11	44	10	40	1	4	NS
Klebsiella	14	56	10	40	4	16	NS
α-heamolylic streptococci	7	28	4	16	3	12	NS
Escherichia coli	8	32	5	20	3	12	NS
Diphtheroids	9	36	4	16	5	20	NS
Neisseria species	9	36	7	28	2	8	NS
Candida species	8	32	5	20	3	12	NS
Enterococci	11	44	7	28	4	16	NS
Bacteriodes species	14	56	6	24	8	32	P<0.05
Pseudomonas	11	44	7	28	4	16	NS
Staphylococcus citrius	4	16	2	8	2	8	NS
Acinetobacter	0	0	6	24	-	-	P<0.05

In Cesarean Delivery (Group-5): Lactobacilli and Bacteroides species were present only in the birth canal which was highly significant (P<0.001). Diphtheroids also observed in the birth canal were statistically significant (P<0.01). Staphylococcus epidermidis and Pseudomonas were acquired by the infant, which was also statistically significant (P<0.05) (Table-5).

Figure 5

TABLE NO.5: COMPARISON OF MICROFLORA BETWEEN BIRTH CANAL AND ORAL CAVITY IN CESAREAN DELIVERY (GROUP-5)

Microorganism	Birth Canal (N-25)		Oral Cavity (N-25)	Difference in transfer of Microorganism		Significance	
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	15	60	0	0	0	0	P<0.001
Staphylococcus aureus	10	40	9	36	1	4	NS
Staphylococcus epidermidis	17	68	7	28	10	20	P<0.05
Klebsiella	7	28	4	16	3	12	NS
Escherichia coli	6	24	3	12	3	12	NS
Diphtheroids	7	28	0	0	0	0	P<0.01
Candida species	4	16	2	8	2	8	NS
Enterococci	6	24	2	8	4	16	NS
Bacteriodes species	20	80	0	0	0	0	P<0.001
Pseudomonas	13	52	5	20	8	32	P<0.05

In Premature Delivery (Group-6): Staphylococcus aureus was noted, which was statistically significant (P<0.01). Enterococci and Bacteroides species were significant (P<0.05) (Table-6).

# Figure 6

TABLE NO.6: COMPARISON OF MICROFLORA BETWEEN BIRTH CANAL AND ORAL CAVITY IN PREMATURE DELIVERY (GROUP-6)

Microorganism	Birth Canal		Oral Cavity	trans	rence in sfer of organism	Significance	
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	15	60	11	44	4	16	NS
Staphylococcus aureus	20	80	11	44	9	36	P<0.01
Staphylococcus epidermidis	12	48	6	24	6	24	NS
Klebsiella	7	28	3	12	4	16	NS
Escherichia coli	8	32	4	16	4	16	NS
Diphtheroids	7	28	4	16	3	12	NS
Candida species	4	16	4	16	0	0	NS
Enterococci	6	24	0	0	0	0	P<0.05
Bacteriodes species	8	32	0	0	0	0	P<0.05
Pseudomonas	0	0	5	20	0	0	NS

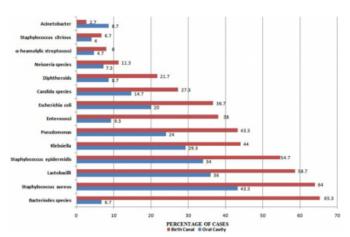
The overall transfer of microorganisms from the birth canal to the oral cavity in all the birth modes were identified as Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Enterococci, Bacteroides species and Pseudomonas, highly significant (P<0.001). Escherichia coli, diphtheroids and Candida species were very significant. Klebsiella and Acinetobacter were significant (Table-7) (Figure-1).

Figure 7

TABLE NO.7: NUMBER AND PERENTAGE OF CASES (MOTHERS AND NEWBORN) HARBOURING VARIOUS MICROORGANISUMS

Microorganism	Birth Cana (N-150)	4	Oral Cavity (N-150)	y	Difference in transfer of Microorganism		Significance
	Microorganism present	96	Microorganism present	96	No.	96	
Lactobacilli	88	58.7	54	26.0	34	22.7	P<0.001
Staphylococcus aureus	96	64.0	65	43.3	31	20.7	P<0.001
Staphylococcus epidermidis	82	54.7	51	34.0	20	14.7	P<0.001
Klebsiella	66	44.0	44	29.3	20	14.7	P<0.05
α-heamolylic streptococci	12	8.0	7	4.7	5	3.3	NS
Escherichia coli	55	36.7	30	20.0	25	16.7	P<0.01
Diphtheroids	31	21.7	13	8.7	18	13.0	P<0.01
Neisseria species	17	11.3	11	7.3	6	4.0	NS
Candida species	41	27.3	22	14.7	19	12.6	P<0.01
Enterococci	57	38.0	14	9.3	43	28.7	P<0.001
Bacteriodes species	98	65.3	10	6.7	88	58.6	P<0.001
Pseudomonas	65	43.3	36	24.0	29	19.3	P<0.001
Staphylococcus citrius	10	6.7	6	4.0	4	2.7	NS
Acinetobacter	4	2.7	13	8.7	9	6	P<0.05

Figure 8
FIGURE 1: PERCENTAGE OF CASES SHOWING
DIFFERENT MICRO-ORGANISMS IN BIRTH CANAL
AND ORAL CAVITY



When normal delivery was interred compared with Induced Delivery, Episiotomy Delivery, Forceps Delivery, Cesarean Delivery and Premature Delivery, it was not significant. When induced delivery was interred compared with the episiotomy delivery and forceps delivery, it was considered statistically significant (P<0.05) and with cesarean delivery and premature delivery it was not significant. When the episiotomy delivery was interred compared with forceps delivery and premature delivery it was not significant, whereas for the forceps delivery it was statistically significant (P<0.01). When the forceps delivery was interred compared with the cesarean delivery and premature delivery it was statistically significant (P<0.001) and (P<0.05), respectively. When the cesarean delivery was interred compared with premature delivery it was not significant (Table-8).

#### DISCUSSION

The series of movements that occurs on the baby's head, in the process of adaptation during its passage through the pelvis, is termed normal delivery. This delivery is by the vaginal route<sup>3</sup>. Episiotomy is an incision of the perineum. It is performed because the perineum bulges increasingly and the vulvo-vaginal opening becomes progressively more dilated by the fetal head<sup>4</sup>. Induction of labor is an obstetric procedure designed to pre-empt the natural process of labor by initiating its onset artificially before it occurs spontaneously. Forceps delivery is the delivery using mechanical aids. It is indicated to expedite vaginal delivery, which may be slowed due to poor progress or because of some maternal or fetal emergency. Cesarean section is the delivery by the abdominal route. A preterm infant is defined as one born at less than 259 days of pregnancy<sup>5</sup>.

The normal microflora of the vagina include Staphylococcus aureus, Staphylococcus epidermidis, group B Streptococcus, Enterococcus fecalis, Gamerella vaginalis, peptostreptococcus species, peptococcus species, bacteroids species, Fusobacterium species, Prevotella bivia, Prevotella disiens and the Bacteroides fragitis group<sup>6</sup>. In this study, the microorganisms isolated from a pregnant woman's vagina before birth were Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, I-hemolytic Streptococci, Escherichia coli, diphtheroids, Neisseria species, candida, species, enterococci, bacteroids species, pseudomonas, Staphylococcus citrius and Acinetobacter. The microorganisms reported in the oral cavity of the newborn include Streptococci salivanius, 7-9 Pneumococci, 7.8 H. influenzae, 7,8 Lactobacilli, 8,9 Staphylococci, 10 Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella species, P. aeruginosa, Veillonella, E. coli, diphtheroids, Candida albicans, Acinetobacter, S. salivarius

In this study, the oral cavity at birth, in all the delivery types showed, besides the above listed microorganisms, I-hemolytic streptococci, Escherichia coli, diphtheroids, Neisseria species, candida, species, Enterococci, bacteroides species, Pseudomonas, Staphylococcus citrius and Acinetobacter. This may be because of a variation in the birth canal flora among the various socio-economic statuses strata. This study also showed that one or the other microorganisms were present in the birth canal at the time of birth. A similar finding was also reported by Panesar (1997)<sup>11</sup>.

In Group — I (Normal Delivery): In the present study, the infants' mouths were observed to be sterile in 20% of the newborns. The aseptic precautions undertaken in this study, and the vaginal flora could be considered as the possible factors for the higher number of sterile oral cavities at birth, in this type of delivery. Witkowski (1935)<sup>8</sup> reported that the oral cavity was sterile in only 2% infants, a few hours after birth. Gundel and Schwarz (L932)<sup>7</sup> stated that the mouth was sterile in 37.5% within six hours of birth. Panesar J. (1997)<sup>12</sup> noted that the oral cavity was sterile in only 12% of the newborns at birth. Hegde S. (1998)<sup>9</sup> observed that the oral cavity was sterile in 6% of the new born infants, at birth.

In this study, the microorganisms found are Lactobacilli, Staphylococcus aureus, Klebsiella, Pseudomonas, Escherichia coli, candida species, and Staphylococcus epidermidis in relatively low numbers in the infant's mouth as compared with the birth canal of the pregnant woman. This suggests that the mother's vagina could have been the source for these microorganisms for the child. A similar finding is reported by Hegde (1998), Panesar (1997), and Tones-Alipi (1990). In this study, the enterococci and bacteroides species were not transferred to the infant's oral cavity. This could have been because the organism cannot survive in the oral cavity. The degree of contamination of the newborn significantly correlated with the microorganisms found in the mother's vagina. Mandar R. (1996). And the study.

In Group – II (Induced Delivery): The oral cavity was sterile in 32% of the newborns. Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, Escherichia coli, candida species and pseudomonas were found in relatively low amounts in the oral cavity at birth, when compared with the birth canal of pregnant mothers, whereas enterococci and bacteroides species were found only in the mothers' birth canals. Probably, this microorganism cannot survive in the oral cavity because of the unavailability of nutrition there, as well as the pH of the oral cavity environment makes it unsuitable for its survival. The source of these microorganisms could have been the mother's birth canal.

In Group – III (Episiotomy Delivery): The oral cavity was sterile in 8% of the infants. Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, I-hemolytic streptococci, Escherichia coli, diphtheroids, Neisseria species, candida species, enterococci, bacteroides species, pseudomonas and Staphylococcus citrius occurred in

relatively low concentrations in the oral cavity at birth as compared with the birth canals of pregnant women. This clearly shows that all the microorganisms were transferred from the birth canal to the oral cavity of the newborns. Acinetobacter was isolated from the birth canal of the mothers in the most number of cases when compared with the oral cavity of infants. This could be because of the use of contaminated surgical scissors or possible nosocomial infections should also be considered.

Group – IV (Forceps Delivery): The oral cavity was sterile in 4% of the newborns. Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, I-hemolytic streptococci, Escherichia coli, diphtheroids, Neisseria species, candida species, enterococci, bacteroides species, pseudomonas and Staphylococcus citrius were found in relatively low quantities in the oral cavity at birth as compared with the birth canal of the mothers. This implies that some of the microorganisms were transferred to the mouth of the infants. Acinetobacter was found only in the birth canal, which possibly suggests that contamination from the forceps might have taken place. Nosocomial infection should be considered.

In Group – V (Cesarean Delivery): In this study, the oral cavity was sterile in 44% of the newborn infants, whereas Panesar (1997)<sup>11</sup> reported 28%. This difference could be because of the maintenance of a sterile operation theater. Sterilization methods employed in the operation theater, surgical instruments and equipment could also be considered. Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, Escherichia coli, candida species, enterococci and pseudomonas were found in relatively high concentrations in the birth canal when compared with the oral cavity of the neonates. Lactobacilli, diphtheroids, and bacteroides species were found in greater numbers in the mother's birth canal when compared with the infant's mouth. This could be because the microorganisms reside in the birth canal and their transfer to the oral cavity was eliminated. A possible reason is that delivery by cesarean section could have eliminated the acquisition of the microbiota through the birth canal, as the birth is via the abdominal route. Lactobacilli were not present in the oral cavity of the newborns at birth, born by this type of delivery. Similar findings were also reported by Panesar J. (1997)<sup>11</sup>.

In Group — VI (Premature Delivery): The oral cavity was sterile in 24% of the neonates. Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, Escherichia

coli, diphtheroids, and candida, species, were found in relatively high percentages in the mothers' birth canals when compared with their neonate's oral cavity. However, not all the types of microorganisms were transferred to the oral cavity. Gale B. Hill (1998)<sup>2</sup> also observed more numbers of Escherichia coli and diphtheroids in the birth canal in this type of delivery.

#### CONCLUSION

The following conclusions were drawn from this study:

- The oral cavity of the neonates delivered by
   Forceps was found to be the most sterile of all the
   types of deliveries. Neonates delivered by
   Cesarean section were found to have the least
   sterile oral cavities.
- 2. A greater number of mother-child pairs showed the presence of Lactobacilli, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, E. coli, Candida species, enterococci, bacteroides species and pseudomonas. However, □¬hemolytic streptococci, diphtheroids, Neisseria species, Staphylococcus citrius and Acinetobacter were only occasionally isolated from mother-child pairs.
- 3. In all the cases, regardless of the delivery type, the mothers' vagina was contaminated. However, the oral cavity of the newborn was contaminated with the microorganisms from any of three sources by contamination from the birth canal or hospital infection or contaminated instruments.
- 4. In all the birth modes, the oral cavity was contaminated by one or the other microorganism.
- 5. Total or 100% sterile oral cavity was not observed in any of the neonates, in any type of delivery. The degree of contamination in the oral cavity of the

neonates varied from 4-44% in Forceps type and Cesarean type of delivery, respectively.

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