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Bacteriological assortment in oral microbiome of postnatal females

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***Abstract*---**In this study, women who had recently given birth were very likely to have dental problems. In girls, 28.1% had dental caries and 27.3% had gingivitis. The number of APOs was also high: LWB was 22.5 percent, PTB was 21.7 percent, and preeclampsia was 11.6%. After giving birth, women with oral problems, poor oral

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hygiene, or APOs were more likely to have high levels of S. mutans (p0.0001) and S. sobrinus colonisation. Even though S. mutans and S. sobrinus are the most common bacteria that cause cavities, S. mutans was found in only a small number of samples (BK1=0.004%, BK2 and BKC2=0.001%, and BKC1=0.004%) when tested without a culture. Instead, Streptococcus, Yersinia, Haemophilus, Prevotella, Neisseria, Fusobacterium, Gemella, Prevotella, Aggregatibacter, Rothia, Veillonella, Granulicatella, and Actinomyces were found to be most common in females with dental caries and gingivitis. This suggests that these isolates may play a role in causing dental caries. Using the culture-based method, the most common types of bacteria found in the poop of both healthy and postpartum women were Staphylococcus, Streptococcus, Enterobacteriaceae, Neisseria, and Lactobacillus. 65.1% of the 267 pregnant women who were tested for Staphylococcus species in culture were found to have it.

***Keywords*---**oral microbiome, postnatal females, types of bacteria.

Introduction

Oral microbiota is very malleable and contains microorganisms that can cause disease when the environment is appropriate (Cobb et al., 2017). It is the second most complex community after the colon. It is made up of more than 1000 different microbial species that live in balance with the immune system of the host. At any given time, it is made up of both allochthonous (moving) and autochthonous (stable) species. Microbes can colonise both the soft (mucosa) and hard (teeth) surfaces of the mouth as biofilms. In these biofilms, they work together and compete with other species to keep the ecosystem stable. Most of the oral flora cannot be cultivated. Oral species identification is not enough to understand how a host and microbes interact when the host is healthy or sick (disease). This is because resident microbes have different genes and the environment has a strong influence on them all the time. Commensal oral bacteria help keep your mouth and body healthy by stopping opportunistic pathogens from taking over (colonization resistance). Microbes play different roles depending on the condition of the mouth and the effects of outside factors. For example, when antimicrobials kill off oral bacteria, the risk of Candida and Staphylococcus aureus infections goes up. Both of these bacteria are often found in the mouth, but they become dangerous when they grow too much. Periodontitis happens when Streptococcus salivary strain K12 is stopped from growing. This is because Streptococcus salivary strain K12 makes bacteriocin, which stops periodontopathogens from growing (Zaura et al., 2009) Periodontal disease and dental caries are two important oral diseases that are often linked to oral microbes (Wade, 2013). Oral health can change into an ecosystem full of pathogens if, for example, a lot of antibiotics are used, the host's defences are lowered, oxygen tension is lowered, which makes biofilms thicker, and there are changes in nutrition, metabolism, and structure. When the immune system reacts to this colonisation with inflammation, the gingival and tooth surfaces are damaged. This can lead to periodontal disease and dental caries in the long run. Microbes in the mouth can spread, according to the "focal infection theory." This

theory says that bacteria, their toxins, and by-products can enter the systemic circulation from a localised, asymptomatic lesion and contribute to the development of pulmonary disorders, cardiovascular disorders, and bad pregnancy outcomes (APOs) (Baig et al., 2013) Periodontal diseases include gingivitis and periodontitis. Gingivitis is an infection of the gums caused by the growth of bacteria and other germs (Kinane and Bouchard, 2008). At first, gingivitis causes the gums to swell up and turn red. Later, bleeding gums happen mostly when you brush your teeth, but the tooth structure stays the same even though the gums are irritated. Gingivitis needs to be treated as soon as possible because it can lead to periodontitis, which is a more serious disease. In this stage, the inner layers of gums and bones are removed from the teeth, leaving small spaces between the gums and teeth called periodontal pockets. These pockets are the source of disease because microbes get into them (Pihlstrom et al., 2005). Gram-negative anaerobic bacteria that grow in the subgingival area are what cause gum diseases. Porphyromonas gingivalis, Tannerella forsythia, Aggregatibacter actinomycetemcomitans, Campylobacter rectus, Prevotella intermedia, Streptococcus intermedius, and Treponema species are some of these. It is now well known that these organisms can also be found in a healthy mouth. There is also a study that says P. gingivalis was not found in half of the patients with aggressive periodontitis (Curtis et al., 2011; Pozhitkov et al., 2015). When someone is sick, the number of bacteria is up to ten times higher than when they are healthy (Lovegrove, 2003). Along with periodontal pathogenic microorganisms, the response of the host immune system makes the situation worse, causing severe inflammation and damage to the tissues that surround and support the teeth. In addition to anaerobic bacteria, other things that can cause periodontal diseases are smoking, diabetes, a weakened immune system from things like AIDS or chemotherapy, eating more sugary foods, and changes in hormones (Piscoya et al., 2012). The periodontal tissues are affected by pregnancy and the hormonal changes that come with it. For example, higher levels of oestrogen and progesterone during pregnancy increase blood flow to the gingiva and lower the immune response, which makes it easier for periodontal disease to develop. Depending on the study, the number of pregnant women with gingivitis ranges from 35% to 100%. (Dhaliwal et al., 2013).

Hypothesis

During pregnancy, the oral microbiome changes, which is just one of the many physical and hormonal changes that a woman's body goes through. This change in oral flora can be especially harmful near the end of pregnancy. It can lead to a number of oral disorders, which in turn can affect the development and delivery of the baby.

Aim of the Study

The purpose of this research was to describe the oral microbiome of postpartum women in (city name) and figure out which bacteria and fungi could be grown in a lab and which could not.

Objectives of the Study

Objectives of the study were.

1) To study demographic, oral health, and obstetric characteristics of postpartum females
2) To study Mutans streptococci in postpartum females as an indicator of oral health status
3) To study culturable and unculturable oral bacterial diversity and its pathogenic potential in postpartum females
4) To study culturable and unculturable oral fungal diversity and its pathogenic potential in postpartum females
5) To study fungal-bacterial interaction in dual-species biofilm for pathogenesis

Literature Review

Physiologically, biologically, and at the molecular level, a woman's body goes through a lot of changes during pregnancy. Depending on how healthy both the baby and the mother are overall, these differences can lead to either normal or APOs (Muwazi et al., 2014). Most women in industrialised countries are less likely to have problems while pregnant or after giving birth. These women know that their health and the health of their unborn child are linked. In developing countries, on the other hand, pregnancy-related problems like PTB, LWB, and preeclampsia are getting worse, and the number of fetal-maternal deaths is slowly going up. The high cost of treatment, the lack of hospitals, doctors, and paramedical staff, and the fact that patients don't know where to find OPDs, wards, and labs in hospitals, especially in rural areas, are some of the problems these women face. Poor nutrition and a lack of medical health facilities are also problems. Women who are pregnant should take extra care of their teeth and gums because bacteria can enter the body through the mouth. This means that the mouth is a major factor in the overall health of the mother and her unborn child. Pathogenic microbes and the waste products they make during metabolism can get through the mucosal barrier in the mouth. This can cause both local and systemic infections. Periodontal disease, gingival hyperplasia, gum lesions, dental caries, and changes in saliva are all common oral problems during pregnancy. All of these have been linked to systemic illnesses like APOs (Tarsitano and Rollings, 1993)

Periodontal diseases

Periodontal diseases cause inflammation of the tissues that hold and surround the teeth. The gingiva, the alveolar bone, and the periodontal tissue are all a part of the periodontium. Gram-negative bacteria can cause illness directly or indirectly by making toxins. In gingivitis, the types of bacteria changed. Common Streptococcus species were replaced by Actinomycetes, Eikenella, Prevotella, Capnocytophaga, Fusobacterium, and Campylobacter. These species can damage the alveolar bone, gums, gingival ligament, and cementum, which can lead to tooth loss (Pihlstrom et al., 2005). Gingivitis affects most people, but between 10% and 15% of the population also has periodontitis (Preshaw et al., 2012). A sudden change in the community of beneficial microbes can turn a healthy state into a

diseased one. Actinomyces and Streptococcus are the most common genera in a healthy mouth. In a dysbiotic mouth, however, anaerobic genera from the groups Proteobacteria, Bacteroidetes, Firmicutes, Spirochetes, and Synergizes are the most common. Periodontitis-causing bacteria tend to hide in the spaces between teeth and gums. Several things can cause periodontitis to get worse, such as dysbiosis, the host's genes, stress, diet, and behaviour. Several cytokines and their receptors may affect how and when a disease starts or gets worse. These include interleukin-1 beta, interleukin-6, tumour necrosis factor-alpha, Fcgamma receptor IIa, complement component 5 (C5), Wnt5a, and complement component 14. (Hajishengallis, 2015).

Pregnancy as a risk factor for periodontal diseases

Sixty to seventy percent of women who are pregnant could get periodontitis (Dommisch et al., 2015). Changes in the amounts of two hormones, oestrogen and progesterone, can be linked to inflammation of the gums. When a woman is pregnant, her body makes more of these hormones, which are thought to affect the healing process, speed up the progression of periodontal disease, make the gums more bloody and weaken the immune system. When these chemicals were released, the species of Prevotella that cause gum disease grew faster. Gingivitis usually starts to show up in a woman's second month of pregnancy, and the symptoms get worse as the pregnancy goes on (Gajendra & Kumar, 2004). If you don't treat gingivitis, it can lead to periodontitis, which can destroy the bone and gums that hold your teeth in place. About one-third of pregnant women have periodontitis. Tooth movement, which is another name for tooth loss, happens when the minerals in the tissues that support the teeth change. Changes in the gums caused by higher levels of progesterone and other hormones can also cause this loss. Pregnancy granulomas are sores on the gums that happen in 0% to 9.6% of pregnant women. They are caused by a mix of hormone changes, bacteria, and local irritants. There is nothing rough about these red sores. First, these sores show up on the gums. Then, they spread to the soft palate, the mucosa of the cheeks, and the tongue. During the first trimester, the chance of getting a tumour is the highest. Another periodontal problem that can be made worse by vomiting often is the erosion of teeth and gums (Giglio et al., 2009).

Prevalence of periodontal diseases

Different countries and even parts of the same country had different rates of periodontitis. Adults in developed countries are less likely to have periodontal disease now than they were in the past, but people in emerging countries still have it because they don't have access to dental care or know how important it is. As suggested by the World Health Organization, the Decayed Missing Filled Teeth (DMFT) measure is used to figure out how healthy your teeth are. In 2008, the Pakistan National Health Survey found that the DMFT measure was much higher in women between the ages of 24 and 30 than in men of the same age. When the indicator values were high, it meant that the oral health was bad. Shahzad et al. (2015) found that the risk of getting periodontitis is about 18.2% higher in women than in men. After gingivitis and dental caries, which are also types of periodontal disease, periodontitis is the third most common disease overall. The Islamic International Dental Hospital in Pakistan found that 69% of the people in the

study had gingivitis and 31% had periodontitis. Gingivitis was more common in women than in men (Ali et al., 2012).

Dental caries

Caries, also called cavities, are a common problem with teeth that can happen to anyone at any time. There is a chance of getting cavities, but there is also a chance of getting periapical and pulp infections. Streptococcus mutans has gotten the most attention as a possible cause of dental caries in young children. However, a number of other bacterial genera, such as Streptococcus, Actinomyces, Veillonella, Granulicatella, Thiomonas, Leptotrichia, Prevotella, and Bifidobacterium, have also been linked to this disease. Plaque from adults with caries usually has bacteria from the families Streptococcus, Actinomycetes, Lactobacillus, Veillonella, and Propionibacterium. The bacteria of the genera Atopobium, Pseudoramibacter, Olsenella, Selenomonas, and Propionibacterium cause root caries in older people. In another study, ratios of Rothia aeria, Veillonella parvula, and Rothia dentocariosa were found to be signs of poor oral hygiene and caries risk in the population. Al-hebshi et al. (2018) found that the bacteria Prevotella, Veillonella, an as-yet-unnamed Actinomyces species, and Atopobium were most likely to be linked to caries in children. S. mutans, which is often found in mature dental biofilm communities, can make people sick if the conditions are right. Tooth caries are caused by acid made by cariogenic microbes when they break down carbohydrates in the diet and by the formation of branched extracellular polysaccharides (glucans) that help trap acidic metabolites in the matrix. When acid is made in the mouth, the pH drops, which starts the process of dental caries, which breaks down enamel and dentine and leads to cavities. The pH of 5.5 is close to the perfect level for removing minerals. Caries start when certain bacteria are in the mouth, the teeth are weak, there isn't enough saliva, or the bacteria don't have enough time to ferment the carbohydrates in the food (Pannu et al., 2013).

Systemic disorders associated with oral local infections

Oral bacteria have been linked to ischemic stroke, heart disease, cancer, bacterial endocarditis, paediatric Crohn's disease, pneumonia, type 2 diabetes, and obesity, among other diseases. Poor dental care and inflammation caused by the normal flora in the mouth have been linked to several types of cancer, such as colorectal and pancreatic cancer. In the same way, people with periodontitis are more likely to get heart disease due to the constant inflammation they experience (CVD). Oral pathogens contribute to CVD by throwing off the immune system, making inflammation worse, and messing up the surface of endothelial cells. Poor dental hygiene has been linked to infectious endocarditis, atherosclerosis, bacteremia, stroke, and heart attack. Several anaerobic mouth species, like Actinomycetemcomitans and Streptococcus constellatus, have been linked to the risk of getting pneumonia. Most people now agree that inflammation caused by gum disease could play a role in the development of Alzheimer's disease (Shoemark and Allen, 2015).

Oral microbiome

Scientists thought that humans have about 100,000 chromosomes before the Human Genome Project (HGP) was finished. The results of HGP, on the other hand, were surprising. They showed that the human body has only about 20,000 genes that code for proteins. Recent research on microbiomes, on the other hand, has shown that every human host also has a number of microbes, both good and bad, that is equal to the number of human cells. The group of microscopic organisms that live on and in people is called the "human microbiome." In the microbiota of a person, there are genomes from bacteria, fungi, archaea, protists, and viruses. The genomes of these microorganisms give the human body abilities that humans did not have before they met these microorganisms. They help humans absorb nutrients, keep their immune systems in check, and fight off pathogenic microbes. The human body is a "super-organism" or "holobiont" because it has both human cells and their microbial partners, genes from both the human genome and the microbiome, and metabolic qualities that are shaped by both human and microbial traits. A study from 2016 (Blaser & Dominguez-bello), The epidermis, the mouth, the digestive tract, the urogenital tract, and the respiratory system all offer different places for bacteria to live. The microbiome of the stomach has been studied the most. The microbiomes of the vaginal, oral, and skin areas have been studied much less. Each niche has a unique mix of microorganisms that thrive there. Even though the microbiome can change quickly, the characteristics of a community tend to stay the same over many years. To keep the beneficial symbiotic relationship between the host and the commensal microbes, microbial sensing is tightly controlled to stop the host's immune system from attacking the commensal microbes. When the natural immune system isn't able to recognise commensal microbes, the mutually beneficial relationship between the host and the microbes breaks down. This can lead to dysbiosis and other health problems. Because of this, it is important for a healthy homeostatic balance that innate immunity and microorganisms talk to each other. Because of these organisms, dysbiosis can lead to diseases like Crohn's disease, necrotizing enterocolitis, type 1 and type 2 diabetes, different types of cancer, and asthma (Ahn et al., 2012). The oral microbiome is the second most diverse group of microorganisms because it has both cultureable and unculturable microbiota. The most common types of mouth bacteria are Streptococci, Lactobacilli, Staphylococcus, and Corynebacterium. Most of these bacteria live in biofilms, which you can find on your teeth, tongue, gingival sulcus, and buccal mucosa. When a local or systemic infection is caused by taking too many medications, strange microorganisms start to take over, and some microorganisms that are usually harmless, like Staphylococcus, become dangerous (Dewhirst et al., 2010).

Material and Methods

Study Design, Settings and Data Collection

The retrospective case-control research done here was approved by the ethical review boards at Capital Hospital in Islamabad, Holy Family Hospital-Rawalpindi Medical University in Rawalpindi, and Quaid-i-Azam University in Islamabad (QAU-BRC). All of the women gave their written permission (Appendix A). In the

Gynecology Departments of Capital Hospital in Islamabad and Holy Family Hospital in Rawalpindi, between January 2016 and March 2018, we enrolled women who had just given birth and healthy women who were not pregnant. Using a standard questionnaire, women who were not pregnant and women who had just given birth within the past week were asked their opinions (Appendix B). From the patient's medical history, we also learned about their general health. The exams were done by trained dentists. Everyone who was pregnant and had dental problems was taken into account. A dentist said that gingivitis was present in females with a mean periodontal pocket depth of 3–4 millimetres, bleeding gums when flossing and brushing, red, swollen gums, no tooth mobility, and bone loss. The state of females' dental caries was written down, along with information about decayed, missing, filled, and filled surfaces of teeth (DMFT and DMFS).

Results and Data Analysis

Table 1: Demographic characteristics of postpartum females (n=267) and healthy control group (n=54)

Factors		Postpartum females *n (%)*	Healthy control females *n (%)*
Age (years)	16-20	12 (4.50)	03 (5.5)
	21-25	88 (33.0)	21 (39.0)
	26-30	88 (33.0)	23 (42.6)
	31-35	64 (24.0)	04 (7.40)
	36-40	15 (5.60)	03 (5.5)
Residential area	Rural	118 (44.2)	05 (9.3)
	Urban	149 (55.8)	49 (90.7)
Education level	No	66 (24.7)	03 (5.5)
	Primary	39 (14.6)	02 (3.7)
	Middle	32 (12.0)	00 (0.00)
	Metric	27 (10.1)	02 (3.7)
	Intermediate	65 (24.3)	01 (1.9)
	Graduation	34 (12.7)	09 (16.7)
	Higher level	04 (1.50)	37 (68.5)
Socio-economic status	Low	64 (24.0)	07 (12.9)
	Middle	203 (76.0)	42 (77.8)
	High	00 (0.00)	05 (9.3)
Body mass index (BMI)	Normal	140 (52.4)	36 (66.7)
	Overweight	126 (47.2)	05 (9.3)
	Underweight	01 (0.40)	13 (24.0)
Total		267 (100)	54 (100)

A total of 549 women took part in this study. This included 267 women who had just given birth (mean age: 27.844.876 years) and 54 women who were thought to be in good health (mean age: 25.904.27 years). Most of these women were between 21 and 30 years old, came from middle-class backgrounds, and lived in cities. Most of the women who had just given birth did not have a high school diploma (24.7%) and were overweight (47.2%). (Table 3.1).

Table 2: Oral health status and practices of postpartum females (n=267) and healthy control group (n=54)

Factors		Postpartum females *n (%)*	Healthy control females *n (%)*
Oral health problems	Present	126 (47.2)	00 (0.00)
Gingivitis	Present	73 (27.3)	00 (0.00)
Dental caries	Present	75 (28.1)	00 (0.00)
Dental caries + Gingivitis	Present	22 (8.20)	00 (0.00)
Brushing frequency/day	1 time 2 times 3 times No brushing	162 (60.7) 98 (36.7) 03 (1.10) 04 (1.50)	42 (77.8) 11 (20.3) 01 (1.9) 00 (0.00)
Intake of drinks and juices	Frequently Not frequently No intake	118 (44.2) 88 (22.8) 61 (33.0)	27 (50.0) 20 (37.0) 07 (13.0)
Tea and coffee intake	1-2 times daily 3-4 times daily > 4 times daily No intake	198 (74.2) 40 (15.0) 02 (0.70) 27 (10.1)	24 (44.5) 23 (42.6) 04 (7.40) 03 (5.5)
Smoking, Snuff, Hookah, and Betelnut intake	Yes No	01 (0.40) 266 (99.6)	00 (0.00) 54 (100)
Total		276 (100)	54 (100)

Women in the control group did not have any cases of bad oral hygiene. 47.2% of women who had just given birth to a child had some kind of dental health problem. None of the women who had just given birth were found to have periodontitis, but 27.3% had gingivitis and 28.1% had oral caries. 8.2% of women who had just given birth had both gum disease and tooth decay. Females 16–20 years old had periodontal and dental diseases at a rate of 41.66% (5/12), females 21–25 years old at a rate of 37.5% (33/88), females 26–30 years old at a rate of 46.6% (41/88), females 31–35 years old at a rate of 56.25% (36/64), and females 36–40 years old at a rate of 73.4% (11/15). Sixty-seven percent of these women only brushed their teeth once a day, and none of them used mouthwash or did anything else to keep their teeth clean. Women who had just given birth drank a lot of juices and drinks (44.2%), tea, and coffee. About 74% of women who have gone through menopause said they drink tea or coffee every day. Only one woman who had just given birth was addicted to betelnut. This shows that smoking, snuff, hookahs, and betelnuts are not very common (Table 3.2).

Table 3: Frequency distribution of postpartum females according to obstetric factors (n=267)

Factors		Frequency *n* (%)
Parity	Nulliparous Multiparous	78 (29.2) 189 (70.8)
Mode of delivery	Vaginal Caesarean	92 (34.5) 175 (65.5)
Gestational age	PTB FTB	58 (21.72) 209 (78.28)
Baby weight (Kg)	< 2.5 2.5-4.0 > 4.0	60 (22.5) 203 (76.0) 04 (1.50)
Preeclampsia	Present	31 (11.6)
BP at the time of delivery	High Low Normal	48 (18.0) 28 (10.5) 191 (71.5)
Pregnancy depression	Present	10 (3.70)
Hypertension	Present	06 (2.20)
Orthodontic treatment during pregnancy	Yes No	45 (16.9) 222 (83.1)
Total		267 (100)

Table 4*:* Association of LWB and PTB with other obstetric factors

Factors		Baby weight (kg)			*P*-value (x^2 value)	Gestational Age		*P*-value (x^2 value)
		< 2.5	2.5-4	> 4		FTB	PTB	
Preeclampsia	No Yes	48 12	186 17	02 02	0.003 (11.931)	185 24	51 07	0.902 (0.015)
Gestational hypertension	No Yes	57 03	202 01	02 02	<0.001 (46.438)	205 04	56 02	0.485 (0.487)
Pregnancy Depression	No Yes	59 01	194 09	04 00	0.565 (1.141)	200 09	57 01	0.360 (0.840)

Discussion

Biofilm infections are hard to find and treat because they are usually made up of many different kinds of microorganisms, including both eukaryotic and prokaryotic members of normal flora and nosocomial pathogens. These microbial communities are hard to treat because doctors don't know much about them (Harriott &Noverr, 2011). Biofilm-forming bacteria like to live in the oral cavity and spread to other mucosal and osseous tissues. Biofilms in the mouth can be managed well by making it easier for epithelial cells to leave soft tissues on a regular basis. Biofilm protects microbes from the body's natural immune response, so they can grow and spread even when good oral hygiene is used. If these microbial populations are not routinely gotten rid of, they can cause oral diseases (Jakubovics, 2015). Most of what we know about how biofilms form on

nonliving surfaces comes from studies with Candida that were done in a lab. Since the ability of C. albicans to grow on biotic surfaces and, more and more, on indwelling catheters (an abiotic surface), which can lead to fatal systemic infections, it is important to find out what they do on oral surfaces. Due to the different kinds of species in polymicrobial biofilm, it is hard to figure out what part each species plays in making someone sick. Modern molecular techniques, on the other hand, have made it possible to learn about how these communities work. More and more research is being done on how different types of bacteria affect the formation of Candida biofilms. This is shedding light on the cooperative and competitive relationships between these communities (Harriott &Noverr, 2011). Candida albicans isn't the only species that can be grown in the mouth. Other species, like Candida glabrata, Candida Krusei, and Candida parapsilosis, also form biofilms on both soft and hard tissues. There are many different kinds of microorganisms in the mouth, not just fungi. This oral flora can either help other resident flora grow by working together with them in a synergistic way or stop other resident flora from growing by working against them in the biofilm community (Ovchinnikova et al., 2013).

Here, researchers used an in vitro biofilm model made up of CV biofilm biomass and XTT reduction assay to test the ability of multidrug-resistant, weak biofilm-forming former Candida species and other commensal aerobic bacteria isolated from saliva samples of postpartum females to grow and stay alive in a dual-species biofilm. This study used ATCC isolates to look at a biofilm that had formed on a polystyrene surface. The biofilm was made up of two different species: Candida species and oral bacteria. The fact that C. albicans is so good at making biofilms is a big problem, especially for groups that work together (Gulati and Nobile, 2016). Most of the time, researchers look at how bacterial and Candidal species interact in polymicrobial biofilms. However, not much is known about the presence of more than two Candida species in a biofilm. The focus of this study was on how biofilms formed between two different kinds of Candida. Both C. albicans and C. glabrata had less biomass and protein in their biofilms, but their metabolic activity went up in the biofilm with both species. Aspartyl proteinase activity, which is another metabolic marker, was lower in the biofilm of both C. albicans and C. glabrata together than in the biofilm of either species alone. C. krusei made the most biofilm biomass of any Candida species, but this biomass dropped by a lot when both Candida species were used in a biofilm assay. After 48 hours of growth, the two-species biofilm also had less metabolic activity. In established polymicrobial biofilms, different species of Candida could be at odds with each other.

Conclusion

In this study, women who had recently given birth were very likely to have dental problems. In girls, 28.1% had dental caries and 27.3% had gingivitis. The number of APOs was also high: LWB was 22.5 percent, PTB was 21.7 percent, and preeclampsia was 11.6%. After giving birth, women with oral problems, poor oral hygiene, or APOs were more likely to have high levels of S. mutans (p0.0001) and S. sobrinus colonisation. Even though S. mutans and S. sobrinus are the most common bacteria that cause cavities, S. mutans was found in only a small number of samples (BK1=0.004%, BK2 and BKC2=0.001%, and BKC1=0.004%)

when tested without a culture. Instead, Streptococcus, Yersinia, Haemophilus, Prevotella, Neisseria, Fusobacterium, Gemella, Prevotella, Aggregatibacter, Rothia, Veillonella, Granulicatella, and Actinomyces were found to be most common in females with dental caries and gingivitis. This suggests that these isolates may play a role in causing dental caries. Using the culture-based method, the most common types of bacteria found in the poop of both healthy and postpartum women were Staphylococcus, Streptococcus, Enterobacteriaceae, Neisseria, and Lactobacillus. 65.1% of the 267 pregnant women who were tested for Staphylococcus species in culture were found to have it. In the postpartum group, the rate of S. epidermidis growth was faster (P=0.005). Most of the 210 Staphylococcus isolates from postpartum women that showed signs of biofilm also had genes for biofilm formation and were very resistant to most of the antibiotics tested. Streptococcus species were much more common in women who had just given birth than in women who had never been pregnant. Also, 12.73 percent of the sample had Lactobacilli, 10.48 percent had Neisseria meningitidis, 6.36 percent had Klebsiella pneumoniae, 5.61 percent had Enterobacter species, and 2.62 percent had Escherichia coli. Using the MTP method, we were able to confirm that 80% of the Enterobacter species isolates were biofilm-forming, as were 100% of the E. coli isolates and 58.18% of the K. pneumoniae isolates. Klebsiella pneumoniae and Escherichia coli are bacteria that have become very resistant to many of the most common medicines. Using a method based on cultures, the only type of fungus that was found in the females that were looked at was Candida. Females who have just given birth are much more likely to have Candida (55.05%). When compared to a group of people who had never been pregnant, the levels of esterase and phospholipase activity in C. albicans were much higher in postpartum women's saliva samples. The isolates from the postpartum group were more likely to be able to make biofilms (66.87%) and were better at stopping fungal growth than the isolates from the control group. There was a high chance of oral Candidal carriage, which became more dangerous in women who had just given birth. The most common types of fungi in all samples were Candida, Saccharomyces, Phialosimplex, Termitomyces, Penicillium, Aspergillus, Olpidium, Cochliobolus, and Malassezia. Stachybotrys, Geotrichum, Talaromyces, Leucosporidium, Acremonium, Wallemia, Eupenicillium, Septoria, Zymoseptoria, Coniosporium, Phialophora, and Mycosphaerella were only found in the postpartum group. Postpartum women with gingivitis and dental caries had more of the genus Saccharomyces, Phialosimplex, Candida, Olpidium, Cochliobolus, Malaseezia, Hyphodontia, Debaryomyces, Mrakia, and Nakaseomycesas than those with good oral health. After giving birth, women with PLWB and bad oral health had more Saccharomyces, Candida, Hyphodontia, and Malassezia yeasts and less of the good bacteria Propionibacterium and Fusarium sp.

Some isolates that were found using a culture-based method (like Staphylococcus aureus, Staphylococcus saprophyticus, Klebsiella pneumoniae, Escherichia coli, Neisseria meningitidis, and C. krusei) could not be found using a culture-independent method. This difference between culture-dependent and culture-independent methods of identifying diversity raises a number of questions and challenges, such as the need to increase the sensitivity of DNA extraction from samples, to create better conditions for culturing that mimic the oral cavity, and to develop a reliable sequencing method to find these isolates.

Recommendations

The current study was done to find out if there is a link between oral disease in new mothers and how their baby's microbiome develops. To stop pregnancy-related problems, though, there needs to be a thorough look at the oral health of Pakistani pregnant women, both when they are healthy and when they are sick, with a focus on culpable and uncultivable microbial species and how they might affect the outcome of the pregnancy. Since the results of culture-based methods and metagenomic-based methods are very different, it is important to find a more reliable way to extract and sequence DNA so that microbes can be identified down to the strain level. In addition to exploring their diversity, more research needs to be done on how well they work so that we can figure out how they affect groups of microbes. Since saliva was the only part of the mouth that was looked at in this study, future research into oral ecology should look at other mouth parts. It is important to look at the effects of MS on babies as well as how often it happens in mothers. Researchers need to look at the virulence factors made by MS and how they interact with the immune system to learn more about the link between S. mutans colonisation and oral diseases and APOs. Women should be followed up on to find out if S. sobrinus and S. mutans are likely to be passed down from mothers to their children. The results of this study show how important it is to keep an eye on both the mother's and the baby's oral health, since dental caries are often overlooked during pregnancy. To learn more about how oral problems and APOs are related, we need to do more expression and molecular-based studies to find out what makes bacteria and Candida so dangerous. It is important to study how antibacterial and antifungal drugs don't work on these species so that we can stop them from becoming resistant to more than one drug. Biofilms of C. albicans and other mixed-species oral bacteria need to be studied at the molecular and animal model levels to find out how they work together. Interventional studies are also needed to find out how different species help or stop biofilm development for therapeutic purposes.

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