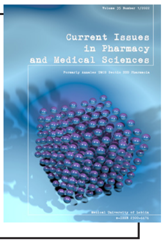
DOI: 10.2478/cipms-2023-0031 Curr. Issues Pharm. Med. Sci., Vol. 36, No. 3, Pages 180-184

Current Issues in Pharmacy and Medical Sciences
Formerly ANNALES UNIVERSITATIS MARIAE CURIE-SKLODOWSKA, SECTIO DDD, PHARMACIA

journal homepage: http://www.curipms.umlub.pl/



Prevalence of aerobically growing opportunistic bacteria in the nasopharyngeal microbiota of pregnant women living in rural and urban areas

Sylwia Andrzejczuk[1]*, Joanna Frend[2], Malgorzata Tomczak[2], Julia Cwiklak[2], Natalia Krakowiak[2], Magdalena Maciocha[2], Dorota Pietras-Ozga[3], Dominik Franciszek Dluski[4]

[1] Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland
[2] Student's Research Group at the Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland
[3] Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin, Poland
[4] Department of Obstetrics and Perinatology, Medical University of Lublin, Poland

ARTICLE INFO

Received 06 June 2023
Accepted 16 July 2023

Keywords:
nasopharyngeal microbiota,
pregnant women,
pregnancy,
place of residence.

ABSTRACT

The upper respiratory tract, including the nasopharynx, is inhabited by many microbial species. Still, the nasopharyngeal microbiota (or microbiome) of pregnant women is not well understood and may be influenced by a number of factors, including place of residence. Many changes occur during pregnancy, and their association with the respiratory microbiota is important for the health of the mother and for the developing fetus. The aim of this study was to compare the colonisation of the nasopharyngeal cavity by culturable aerobic bacteria in the microbiota of women with a physiological pregnancy course according to rural or urban place of residence. Nasopharyngeal swabs were collected from 37 pregnant women (17 from rural and 20 from urban areas) to detect the colonisation of aerobically growing culturable bacteria. Isolates were identified using microbiological culture methods and the MALDI-TOF MS technique. Bacteria were colonisers of the nasopharynx cavity in all cases tested. Accordingly, 97.3% (36/37) of the pregnant women were colonised by Gram-positive bacteria, mainly coagulase-negative (CoNS) and -positive (CoPS) *Staphylococcus* spp. and *Corynebacterium* spp. in both the rural and urban groups. Gram-negative *Klebsiella variicola*, *Proteus mirabilis* and *Pseudomonas congelans* colonised only 17.6% (3/17) of rural women and *Stenotrophomonas maltophilia* only 5.0% (1/20) of the urban women. To summarise, all pregnant women were colonised with bacteria in the nasopharyngeal cavity. The majority from both rural and urban residences were colonised with Gram-positive bacteria only. Gram-negative bacteria were isolated mainly from samples in the rural group.

INTRODUCTION

We share our body space with microorganisms that form the microbiota. It is a fact that microbiota and microbiome composition are associated with various diseases, including infections, and for this reason the research community wants to identify microbial and/or human host biomarkers for diagnostic and clinical purposes. Population-based variation in microbiota composition, especially respiratory microbiota between healthy individuals, is rarely investigated. Variously defined, the microbiome is the combined genetic material of microorganisms in a given environment. The newly proposed definition of microbiota and microbiome is based on both biotic and abiotic factors that modulate interactions between microbial cells and cells of the host organism [1], and also encompasses the broad range of activities of the microbiome, called "the theatre of activity" [2]. The microbiota comprises all the microorganisms in a given ecological niche of the host body, whereas the human microbiome is a characteristic community of microorganisms (including bacteria, archaea, lower and higher eukaryotes and viruses) occupying a fairly well-defined habitat, with clearly specific physicochemical properties [1]. This definition of the microbiome includes all the structural elements of microorganisms, as well as the environmental conditions in which

---

* **Corresponding author**
e-mail: sylwia.andrzejczuk@umlub.pl

a given microbial population resides, and it is now even considered to be our "last organ" [3].

While the composition and health effects of the gut microbiota are well studied, little is known about the microbiota of the upper respiratory tract in healthy individuals. The human respiratory tract is physiologically divided into two parts. One part is the upper respiratory tract (URT), which consists of the oropharynx, nasopharynx and nasal cavity, and the other part is the lower respiratory tract (LRT), which includes the lungs. The upper respiratory tract is inhabited by many microbial species [4,5] that directly influence the maintenance of host body homeostasis and the proper functioning of the immune system [6].

The nasopharyngeal microbiota (or microbiome) of pregnant women is not well understood and may be influenced by a number of factors, including place of residence. Pregnancy, as a physiological state of a woman's body, involves many changes, and their association with the respiratory microbiota is important not only for the health of the mother, but also for the developing fetus [7-9]. Some researchers have attempted to study the composition of the microbiota in the respiratory system of the pregnant woman's body, resulting in several research papers that help to expand current knowledge in this area (Table 1).

Table 1. Summary of distribution of individual microorganisms in the respiratory tract of pregnant and non-pregnant women

Respiratory area	Women		Ref.
	Pregnant group	Non-pregnant group	
front nostril	*Corynebacterium*, *Staphylococcus*, *Propionibacterium*, *Moraxella*, *Streptococcus*	*Corynebacterium*, *Propionibacterium*, *Staphylococcus*	[10]
	-	*Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Streptococcus*, *Moraxella*, *Dolosigranulum*	[11]
nasopharynx	*Staphylococcus* (*S. epidermidis*, *S. aureus*)	-	[12]
	-	*Staphylococcus*, *Corynebacterium*, *Propionibacterium*, *Streptococcus*, *Moraxella*, *Dolosigranulum*	[11]
oral part of the throat	-	*Streptococcus*, *Prevotella*, *Veillonella*, *Neisseria*, *Fusobacterium*, *Actinomyces*, *Gemella*, *Haemophilus*, *Granulicatella*, *Leptotrichia*, *Rothia*	[11]
mucous membrane of the posterior pharyngeal wall	*Staphylococcus aureus*	-	[13]

Abbreviations: underlined – species/genus/cluster dominant in the area

The aim of our investigation was to determine whether place of residence influences the composition of aerobic-growing bacteria in the nasopharyngeal microbiota of pregnant women. This has potential implications for pregnant women's respiratory microbiota and wider health, particularly in relation to infectious diseases.

MATERIALS AND METHODS

Thirty-seven pregnant women in the third trimester of pregnancy from rural (45.9%, 17/37 cases) and urban (54.1%, 20/37 cases) areas, from the Lublin region, Poland, were enrolled in our study to determine the differences in the colonisation of the nasopharyngeal cavity by aerobically growing bacteria. Pregnant women were diagnosed at the Department of the Obstetrics and Perinatology, Medical University of Lublin, Poland. Samples were collected using sterile cotton swabs with the Amies medium (Copan Liquid Amies Elution Swab, eSwab, Copan, Italy). The collected samples were immediately transferred to the Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland. Inclusion criteria for the PW group were: single pregnancy, lack of chronic disease or autoimmune disease, and lack of infection. The exclusion criteria for the PW group were as follows: multiple pregnancy, presence of chronic disease (e.g. diabetes, hypertension, hypo- or hyperthyroidism), presence of autoimmune disease (e.g. lupus erythematosus), of hereditary thrombophilia, of adverse pregnancy outcomes in the patient's history (e.g. stillbirth, preterm delivery, miscarriage, fetal growth restriction), and presence of infection of any origin. Inclusion criteria for the NPW group were good health without autoimmune or chronic diseases and the absence of infections. None of the patients enrolled in the study had been treated with anticoagulants and antimicrobials for at least two months. The protocol was approved by the institutional review board (KE-0254/59/2016, Ethics Committee of the Medical University of Lublin, Poland). To protect the confidentiality of the cases, no information that could identify the pregnant women was included in the case record form.

Bacterial components of the nasopharyngeal microbiota were isolated and identified from 37 nasopharyngeal swabs taken from asymptomatic pregnant women aged 21-34 years old (mean age 29.14±3.69 years). All participants were in good health condition, without symptoms of infection or antimicrobial treatment. Collected nasopharyngeal samples were cultured on the various agar media routinely used for selective growth of microorganisms: blood agar plate with 5% sheep blood (MHF, Biomaxima, Poland), Mueller-Hinton agar medium (MHA, Oxoid, Ireland) and incubated for 24-48 h at 35±2°C under aerobic conditions. After incubation, selected isolates were initially identified phenotypically based on their characteristics, e.g.: macro- and microscopic characteristics, biochemical features. Final species identification was based on the protein profile detection by matrix-assisted laser desorption-ionization time of flight mass spectrometry (MALDI-TOF MS) and MALDI Biotyper 3.1 database – Build (Bruker, Germany) at the Department of Epizootiology and Clinic of Infectious Diseases, University of Life Sciences in Lublin, Poland.

For the statistical analysis of some differences in bacterial colonisation, Fisher's exact test was applied with a significance level of $p<0.05$ (GraphPad InStat ver. 3.0, GraphPad Software, USA).

RESULTS

To estimate the differences in bacterial colonization, thirty-seven women in the third trimester of pregnancy, aged 21-35 years, were included in this study. Of the tested pregnant women, 45.9% (17/37) were recruited from the rural areas, and 54.0% (20/37) from the urban area. The mean age of pregnant women from the rural and urban areas was 29.47±3.81 (range: 21-34) years old and 28.85±3.66 (range: 21-35) years old, respectively.

All pregnant women were colonised with bacteria in the nasopharyngeal cavity. The majority of pregnant women from both rural and urban residences were colonised exclusively with Gram-positive bacteria (Figure 1).

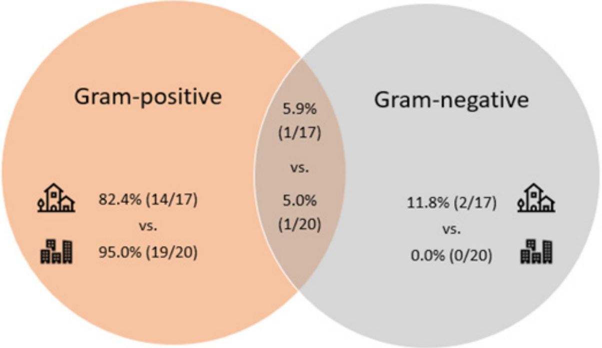


Figure 1. Prevalence of aerobically growing bacteria colonisation of the nasopharyngeal cavity in rural versus urban pregnant women in the third trimester of pregnancy

As evident in Table 2, among the pregnant women living in the rural condition, only 11.8% (2/17) were not colonised by *Staphylococcus* spp. Moreover, different staphylococci species colonised the nasopharyngeal cavity of 52.9% (9/17) persons without detection of other aerobic bacteria. Accordingly, the nasopharynxes of 35.3% (6/17) were colonised by *Staphylococcus* spp. (both CoNS and CoPS) and other Gram-positive bacteria (*Corynebacterium* spp., *Bacillus cereus* and *Micrococcus luteus*). Furthermore, coagulase-negative staphylococci (CoNS), mainly *S. epidermidis*, were the predominating colonisers in this group of pregnant women, selected from 76.5% (13/17) cases with or without other bacteria, while the coagulase-positive opportunistic pathogen *S. aureus* (CoPSA) was isolated from 41.2% (7/17) of the nasopharyngeal samples. Three species of *Corynebacterium* were detected in 17.6% (3/17) women. Additionally, one women was colonized simultaneously by two species of this genus. In the group of pregnant women living in the rural area, 17.6% (3/17) were colonised by Gram-negative bacteria: *Klebsiella variicola* (one case), *Proteus mirabilis* (one case) and *Pseudomonas congelans* (one case), as shown in Table 2.

Certain differences were observed in the group of pregnant women with urban residence (Table 2). In the urban group of pregnant women, 95.0% (19/20) were colonised by different staphylococcal species, including 30.0% (6/20) colonised by *Staphylococcus* spp. and 60.0% (12/20) by *Staphylococcus* spp. and other Gram-positive species (*Corynebacterium* spp., *Micrococcus luteus* and *Bacillus cereus*). Only 5.0% (1/20) of all women were colonised by Gram-negative *Stenotrophomonas maltophilia*, together with two species of staphylococci, *S. hominis* and *S. warneri*. What is more, the coagulase-positive *S. aureus* was detected in 20.0% (4/20) of the nasopharyngeal samples in the urban group.

Table 2. Colonisation of the nasopharyngeal cavity of women in the third trimester of pregnancy according to the rural or urban area of residence

Number (%) of pregnant women colonised in nasopharyngeal cavity (n=37)		
Bacterial genus/species colonisation	Rural group (n=17)	Urban group (n=20)
Staphylococccus spp.	9 (52.9)	6 (30.0)
Bacillus cereus	0 (0)	1 (5.0)
Staphylococccus spp. + *Corynebacterium* spp.	1 (5.9)	7 (35.0)
Staphylococccus spp. + *Micrococcus luteus*	1 (5.9)	2 (10.0)
Staphylococccus spp. + *Bacillus cereus*	1 (5.9)	3 (15.0)
Staphylococccus spp. + *Streptococcus pneumoniae* + *Bacillus cereus*	1 (5.9)	0 (0)
Staphylococccus spp. + *Corynebacterium* spp. + *Pseudomonas congilans*	1 (5.9)	0 (0)
Staphylococccus spp. + *Micrococcus luteus* + *Bacillus cereus* + *Corynebacterium* spp.	1 (5.9)	0 (0)
Staphylococccus spp. + *Stenotrophomonas maltophilia*	0 (0)	1 (5.0)
Klebsiella variicola	1 (5.9)	0 (0)
Proteus mirabilis	1 (5.9)	0 (0)

All culturable and aerobically growing bacterial isolates from the nasopharyngeal cavity of pregnant women were defined as colonisers and they were not etiological agents of any infection. Among the nasopharyngeal microbiota, staphylococci dominated in both groups of pregnant women (Figure 2).

In the rural group, 47.0% (8/17) of all women were colonised by one *Staphylococcus* species, 23.5% (4/17) by two species, 11.8% (2/17) by three species, and 5.9% (1/17) by five species. *S. epidermidis* and *S. aureus* were the most common bacterial species colonising nasopharyngeal cavity in pregnant women living both in rural (76.5%, 13/17 *vs.* 41.2%, 7/17 of those colonised) and urban (85.0%, 17/20 *vs.* 20.0%, 4/20 of those colonised) areas. *Corynebacterium* spp. was observed as the second major group of bacteria colonising the nasopharynx of most women in the third trimester of pregnancy. The presence of these Gram-positive bacteria were confirmed in 17.6% (3/17) and 35.0% (7/20) cases in the rural and urban groups, respectively.

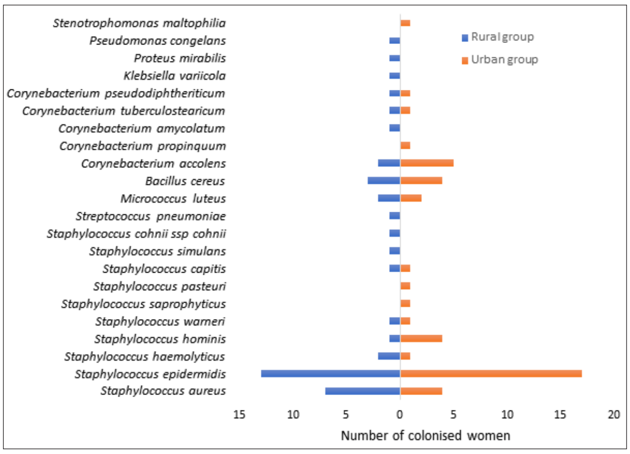


Figure 2. Number of pregnant women colonised by selected species of aerobic bacteria in the nasopharyngeal cavity

We did not find statistically significant differences both in *S. aureus* (p=0.279) and *S. epidermidis* (p=0.680) or Gram-negative bacteria (p=0.315) colonisation based on Fisher's exact test results.

DISCUSSION

One of the fundamental issues in the microbiota/microbiome research is the characterisation of the healthy human microbiota and microbial diversity as a function of various factors. Residence and place of living have a major impact on the respiratory microbiota, including the nasopharyngeal cavity as a mucosal surface in constant and direct contact with the surrounding environment. This has been clearly demonstrated by the current study of Callewaert *et al*. [14], where skin microbiota/microbiomes and microbiota composition was proven to change due to the external conditions such as weather and climate and, therefore, depended on the residence of people. Other authors also wrote about the microbiota depending on the place of human residence [15].

Recent studies have elucidated the influence of the local environmental (rural/urban) on the differences in the composition of the nasopharyngeal microbiota of healthy pregnant women in the third trimester of the physiological course of pregnancy. This study investigated the aerobically growing, including selected bacterial species that can be potent opportunistic pathogens. Our study showed that the nasopharyngeal cavity of all pregnant women living in both rural and urban conditions was colonised by culturable and aerobically growing bacteria, mainly *Staphylococcus* spp. and *Corynebacterium* spp. They were not an etiological agent of any infection, but were derived from respiratory microbiota as colonisers.

Similarly to our studies, a research on the nasopharyngeal microbiota conducted by Kosikowska *et al*. [12] showed a high prevalence of *Staphylococcus* spp. representatives in pregnant women, together with 42% of women colonized simultaneously by more than one *Staphylococcus* species. A total of 71 species of the genus *Staphylococcus* were isolated, with the vast majority being coagulase-negative staphylococci (*S. epidermidis*, 79%) and coagulase-positive staphylococci (*S. aureus*, 30%) [12]. The microbiota of the posterior pharyngeal mucosa of pregnant women with and without symptoms of acute respiratory tract infection has been shown to be dominated by Gram-positive bacteria. Meshcheriakova *et al*. [13] isolated 111 groups of microorganisms, of which Gram-positive bacteria were the most numerous, accounting for 79% of all cultured microorganisms, followed by Gram-negative bacteria (18%) and *Candida* spp. (3%). Moreover, pregnant women without any symptoms of infection exhibit a higher *S. aureus* colonization rate (31%) compared to sick women (2%).

Changes occurring in the respiratory microbiota of pregnant women were observed earlier on the basis of the quantitative composition of the oral microbiota [16,17]. In the subgingival plaque of women who are in the first and second trimesters of pregnancy, there is a significant increase in the abundance of the pathogenic bacterial species *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* compared to non-pregnant women. In addition, *Candida* is more common in women in the middle and late stages of pregnancy [16]. Attempts have been made to elucidate the mechanisms by which pregnancy leads to changes in the composition of the oral microbiota, but these remain unclear. It has been suggested that progesterone and estrogen affect the microbiota of this area of the female body, but this has not been sufficiently proven. It is hypothesised that the general immune status of a pregnant woman's body is responsible for the increase in the number of bacterial species in the oral cavity. Balan *et al*. [18] observed that the oral cavity is dominated by pathogenic bacterial species during pregnancy, whereas the normal microbiota is restored in the postpartum period. These fluctuations may be the result of complex interactions between the host and the microbiota that are influenced by many elements, including sex hormone activity and/or immune factors.

Unfavourable changes in the respiratory microbiota of pregnant women may play a role in the occurrence of complications during pregnancy and may even predispose to their occurrence in the future [16,19]. The most serious consequence of the adverse effects of bacteria from the mother's oral cavity on the fetus is miscarriage. Han *et al*. [9] were the first to demonstrate that *F. nucleatum* from maternal subgingival plaque caused acute inflammation and subsequent fetal death after crossing the placenta into the fetus. The bacteria were able to cross freely into the placenta probably because the pregnant woman's immune system was weakened by a mild upper respiratory tract infection [9].

The physiological changes that occur in a woman's body, especially during the first months of pregnancy, promote the proliferation of microorganisms in the oral cavity, and facilitate the colonisation of this area of the human body by pathogenic microorganisms that cause periodontal disease, such as *P. gingivalis* and *A. actinomycetemcomitans* [20]. Sometimes these microbial changes can have serious consequences for both for the mother's body and the fetus, including the onset of gingivitis and periodontitis in women, premature birth and even pregnancy loss. Therefore, it is very important to investigate the changes in the qualitative and quantitative composition of the respiratory microbiota of pregnant women and their importance for maternal health and fetal development.

CONCLUSIONS

There are many scientific publications in which the authors present the results of ongoing studies to define the quantitative and qualitative composition of the gut microbiota and the reproductive tract of pregnant women. In contrast, the respiratory tract of pregnant women is rarely the subject of such research work. In our study, Gram-positive bacteria, mainly CoNS and CoPS staphylococci and *Corynebacterium* spp. were found to be predominant in both rural and urban groups. All pregnant women were colonised with bacteria in the nasopharyngeal cavity. The majority of both rural and urban women were only colonised with gram-positive bacteria, while. Gram-negative bacteria were mainly isolated from samples from the rural group. As a result, there is still a lack of scientific data that provides information on how pregnancy affects the biodiversity of the respiratory microbiota.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID iDs

Sylwia Andrzejczuk https://orcid.org/0000-0001-6301-6059
Dorota Pietras-Ożga https://orcid.org/0000-0002-1580-9254
Dominik Franciszek Dłuski https://orcid.org/0000-0001-5886-2664

REFERENCES

1. Berg G, Rybakova D, Fischer D, Cernava T, Vergès M-CC, Charles T, et al. *Microbiome definition re-visited: old concepts and new challenges*. Microbiome. England; 2020:103.
2. Whipps J, Lewis K, Cooke R. Mycoparasitism and plant disease control. In: Burge M, editor. *Fungi in biological control systems*. Manchester: Manchester University Press; 1988:161-87.
3. Baquero F, Nombela C. The microbiome as a human organ. *Clin Microbiol Infect.* 2012;18 Suppl 4:2-4.
4. Dickson RP, Erb-Downward JR, Martinez FJ, Huffnagle GB. The microbiome and the respiratory tract. *Annu Rev Physiol.* 2016;78(1):481-504.
5. Kumpitsch C, Koskinen K, Schöpf V, Moissl-Eichinger C. The microbiome of the upper respiratory tract in health and disease. *BMC Biol.* 2019;17(1):87.
6. Hilty M, Burke C, Pedro H, Cardenas P, Bush A, Bossley C, et al. Disordered microbial communities in asthmatic airways. *PLoS One.* 2010;5(1):e8578.
7. Mesa MD, Loureiro B, Iglesia I, Fernandez Gonzalez S, Llurba Olivé E, García Algar O, et al. The evolving microbiome from pregnancy to early infancy: A comprehensive review. *Nutrients.* 2020 Jan;12(1).
8. DiGiulio DB, Callahan BJ, McMurdie PJ, Costello EK, Lyell DJ, Robaczewska A, et al. Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci U S A.* 2015;112(35):11060-5.
9. Han YW, Fardini Y, Chen C, Iacampo KG, Peraino VA, Shamonki JM, et al. Term stillbirth caused by oral Fusobacterium nucleatum. *Obstet Gynecol.* 2010;115(2 Pt 2):442-5.
10. Chu DM, Ma J, Prince AL, Antony KM, Seferovic MD, Aagaard KM. Maturation of the infant microbiome community structure and function across multiple body sites and in relation to mode of delivery. *Nat Med.* 2017;23(3):314-26.
11. Hang J, Zavaljevski N, Yang Y, Desai V, Ruck RC, Macareo LR, et al. Composition and variation of respiratory microbiota in healthy military personnel. *PLoS One.* 2017;12(12):e0188461.
12. Kosikowska U, Chwiejczak E, Dłuski D, Stępień-Pyśniak D, Leszczyńska-Gorzelak B, Malm A. Różnorodność gatunkowa Staphylococcus spp. w mikrobiocie nosogardzieli kobiet ciężarnych. In: III *Ogólnopolska konferencja "Drobnoustroje w świecie człowieka – Drobnoustroje oportunistyczne"*. Bydgoszcz; 2018.
13. Meshcheriakova AK, Kostinov MP, Magarshak OO, Zaĭtseva EV. Species and quantitative characteristics of pharyngeal mucosa microflora in pregnant women. *Zhurnal Mikrobiol Epidemiol Immunobiol.* 2014;(2):93-7.
14. Callewaert C, Ravard Helffer K, Lebaron P. Skin microbiome and its interplay with the environment. *Am J Clin Dermatol.* 2020;21(Suppl 1):4-11.
15. Nuriel-Ohayon M, Neuman H, Koren O, Fujiwara N, Tsuruda K, Iwamoto Y, et al. Geography, ethnicity or subsistence-specific variations in human microbiome composition and diversity. *Front Microbiol.* 2017;8(1):215-29.
16. Nuriel-Ohayon M, Neuman H, Koren O. Microbial changes during pregnancy, birth, and infancy. *Front Microbiol.* 2016;7:1031.
17. Balan P, Brandt BW, Chong YS, Crielaard W, Wong ML, Lopez V, et al. Subgingival microbiota during Healthy Pregnancy and Pregnancy Gingivitis. *JDR Clin Transl Res.* 2021;6(3):343-51.
18. Balan P, Chong YS, Umashankar S, Swarup S, Loke WM, Lopez V, et al. Keystone species in pregnancy gingivitis: A snapshot of oral microbiome during pregnancy and postpartum period. *Front Microbiol.* 2018;9:2360.
19. Amir M, Brown JA, Rager SL, Sanidad KZ, Ananthanarayanan A, Zeng MY. Maternal microbiome and infections in pregnancy. *Microorganisms.* 2020;8(12).
20. Fujiwara N, Tsuruda K, Iwamoto Y, Kato F, Odaki T, Yamane N, et al. Significant increase of oral bacteria in the early pregnancy period in Japanese women. *J Investig Clin Dent.* 2017;8(1).