The Prevalence of Vaginal Candida Colonization among Female Students in Bingham University

Maikenti James Ishaku¹, Adogo Lillian Yami¹*, Koggie Amos Zamfara¹ and Shawulu Godiya Nganjiwa¹

¹Department of Biological Sciences, Bingham University, Nigeria.

Authors’ contributions

This work was carried out in collaboration between all authors. Author MJI designed the study, author ALY performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors KAZ and SGN managed the analyses of the study and literature searches. All authors have read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/22843

Editor(s): (1) Eggehard Holler, Cedars-Sinai Medical Center, Department of Neurosurgery, Los Angeles, USA and University of Regensburg, Germany.

Reviewers: (1) Md. Golam Hafiz, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.
(2) Maen Mahfouz, Arab American University of Jenin, Palestine.
(3) Natthanje Luplertlop, Mahidol University, Bangkok, Thailand.

Complete Peer review History: http://sciencedomain.org/review-history/12730

ABSTRACT

Aim: To determine the prevalence of vaginal Candida colonization among female students in Bingham University.

Place and Duration of Study: This study was carried out in Bingham University, Auta balefi, Karu LGA, Nasarawa State between the months of March and July 2015.

Research Hypothesis: The prevalence of Candida albicans is not significantly high in the high vagina swab of most females; therefore the null hypothesis will be that the prevalence of Candida albicans is highly significant in the high vagina swab of most females.

Materials and Methods: High vagina swab of ninety six female students was collected and screened for the presence of Candida albicans. Questionnaires were issued to obtain demographic information. Inoculation was carried out on Sabourad Dextrose Agar, incubated for 48 hours at 37°C and positive growth was checked for on Corn Meal Agar after 72 hours of incubation at 37°C, positive growth was identified further by the use of the germ tube test using human serum and incubated between 2 to 4 hours.

Received 30th October 2015
Accepted 8th December 2015
Published 18th December 2015
Results: Ninety six females were screened and 25(26.0%) was infected. This reveals that the prevalence of *Candida albicans* was highly significant at $X^2 = 1.758, df = 2$ at .05, $P = .05$. The highest prevalence was recorded among female students within the age group of 16-20 years, (15.6%).

Conclusion: This study reveals that candidiasis caused by *C. albicans* is a major health challenge among female students. Therefore the practice of good personal hygiene is highly recommended in order to prevent the spread of the infection.

Keywords: *Candida albicans*; students; vaginal discharge; prevalence; high vagina swab.

1. INTRODUCTION

*Candida albicans* is an opportunistic fungal pathogen that is responsible for candidiasis in the human hosts [1]. Among microbial diseases, fungal diseases are emerging rapidly, especially in the last twenty five years. Candida although a commensal fungi is the most prevalent fungal pathogen causing both mucosal and systemic infections [2,3]. Candida species are reported as common cause of vaginal discharge worldwide [4,5]. *Candida albicans* occurs as a harmless commensal in the gastrointestinal and genitourinary tracts in about 70% of humans and 75% of women will suffer from *candida* or vaginal yeast infection at least once in their lives. Virtually 40 to 50 percent of women will also experience multiple infection [6]. *Candida* species are part of the normal flora of the lower genital tract in 20-50% of healthy asymptomatic women [7,8] which causes diverse disorders that may include thrush, *Candida enteritis*, urinary tract candidiasis, mucocutaneous candidiasis and invasive candidiasis [9]. Candida may cause urinary tract infections which accounts 10-15% of the total reported urinary tract infections [10]. It may also cause male infertility [11] vulvovaginal candidiasis [12,13], oropharyngeal candidiasis, systemic candidiasis and cutaneous candidiasis [14]. Carrier rates are higher in women treated with broad spectrum antibiotics [15] pregnant women, diabetic women [16,17] and those with HIV/AIDS [18,19].

The commonest sign of infection is vaginal discharge which could be generally profuse, white, yellow or green, thick, and odorless. Several factors can be associated with increased rate of vaginal colonization by *Candida albicans*, these include pregnancy, use of high estrogen content and oral contraceptives [20], uncontrolled diabetes mellitus [21], poor dietary habits, poor personal hygiene and prolonged use of broad spectrum antibiotics [22] which kill the good and beneficial bacteria, allowing yeast overgrowth.

*Candida albicans* and other candida species have been isolated from several clinical specimens in different parts of Nigeria [23]. In a study conducted by Ojiyi et al. 2012, a prevalence rate of 14.75% was recorded among sexually active women in Maiduguri, Nigeria [24]. Throughout the world, prevalence rates for Candidiasis and Candidemia are similar [25], and this has led to the general consensus that *Candida albicans* is the most commonly isolated species however; there has been a growing trend of recovery of non-*albicans* species.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out in Bingham university Auta-balefi Karu, Nasarawa State. This community is situated in the middle belt of Nigeria at longitude 8º32’N 8º18’E and Latitude 8.533ºN 8.300ºE and is characterized by a tropical sub-humid climate with two distinct seasons; wet and dry seasons. Monthly temperature ranges from 20ºC to 34ºC and annual rainfall ranging from 1100 mm to about 2000 m [26]. It occupies a land mass of over 200 square meters, found at 23 kilometers away from Abuja [27].

2.2 Sample Collection

Ninety-six high vagina swab collected was obtained from female students between the ages of 16-30 years. The study was conducted in compliance with Bingham University’s ethical committee approval.

Sterile swab sticks were labeled and accompanied with a questionnaire and was randomly given to any interested female students in the hostel. High vaginal swabs were obtained from apparently healthy students who indicated their interest in the research as there was no criterion for inclusion or exclusion in the study. A
brief enlightenment on the purpose, importance of participating and how to collect the right sample from the vagina were properly explained.

2.3 Culture

The high vagina swab collected was used to inoculate on Sabouraud Dextrose Agar (SDA) and incubated aerobically at 37°C for 48 hours and Candida species were checked for on Corn Meal Agar (CMA) plates and incubated at 37°C for 48 hours. First, an inoculum pool was made with the swab specimen then; a sterile wire loop was used to spread the inoculum by streaking in quadrant to obtain discrete colonies. Incubation was at 37°C for 48 hours under aerobic conditions. Colonies which appeared white to cream in colour with a smooth border and a pasty and moist consistency were observed.

2.4 Germ Tube Test

Germ tube experiment was used as a rapid tool for identification of Candida albicans. Using a sterile loop, a small portion of a pure colony of Candida albicans was harvested and inoculated in to sterile test tubes containing 0.5 ml of human serum. The resulting suspension was incubated aerobically at 37°C for 3 hours. A drop of the yeast-serum suspension was placed on a clean microscope slide with 1 drop of cotton blue lactophenol stain and covered with a cover slip and examined microscopically, using the x10 and x40 objective lenses of an olympus microscope. The appearance of small, sprouting tube-like outgrowths or filaments projecting from the cell surface confirmed production of germ tubes [28].

2.5 Statistical Analysis

The statistical sampling method of the results were analyzed using, simple percentage and the relationship between risk factors and microbiological results was compared and using SPSS version 20 on windows 7 packages which was relevant to Chi-square statistics. The significant prevalence of Candida albicans was determined by $X^2$ at $P=.05$. 

Fig. 1. Growth of Candida albicans on Sabouraud Dextrose Agar (SDA)
3. RESULTS

Table 1 shows the overall prevalence of *Candida albicans* among female students in Bingham University. The total number examined was 96(100%), 25(26%) were negative and 71(74.0%) was negative.

**Table 1. Prevalence of *Candida albicans* among female students**

| Overall prevalence of *Candida albicans* and percentage |
| --- | --- | --- |
| Total number examined | 96 | 100.0% |
| Total number positive | 25 | 26.0% |
| Total number negative | 71 | 74.0% |

Table 2 shows the prevalence of *Candida albicans* in relation to age of the subjects. The total prevalence rate based on age was 26%. Students between the age of 16-20 years had the highest prevalence rate of (15.6%) while those between the ages of 21-25 years had a prevalence rate of (10.4%).

Table 3 shows the prevalence of *Candida albicans* in relation to antibiotic chemotherapy, trend of diabetes, vagina discharge/discomfort, the use soap to wash their genital area and the use of the hostel toilet to urinate.

Table 4 shows the prevalence of *Candida albicans* in relation to type of underwear and age distribution. 14(56%) make use of cotton under wears; those that make use of tight under wears had a prevalence of 16(64%).

Table 5 shows the prevalence of *Candida albicans* in relation to method of vaginal clean up and age distribution. The use of baby wipe by the female students to clean their genital area had the highest prevalence of 5(20%) while the use of water, tissue and water had a total prevalence of 4(16%).

4. DISCUSSION

The overall prevalence of *Candida albicans* in this research reveals that out of a total of 96(100%) females examined, 25(26.0%) were found to be positive and 71(74.0%) were negative, which shows that the prevalence rate of *Candida albicans* among the subjects was significantly high at $X^2=1.758$, df $=2$ at $P=.05$, ($P=5.991$). Those within the age group of 16-20 years had the highest record of the infection 15(15.6%) while those within the age group 21-25 years had a prevalence rate of 10(10.4%). The Statistical analysis (Chi-square) shows a significant difference within the age group of 21-25 at $P = .05$. These findings revealed that the infection was almost uniformly distributed in the subjected age groups indicating that *Candida albicans* is more frequent within the age range of 16-25 years and those within this age range were found to be sexually active. This can be attributed to several factors such as lack of personal hygiene and wearing tight under wears. This could also be due to the fact that as girls mature, hormonal changes takes place thereby making them more vulnerable to *Candida albicans* infection. This can also be due to the high oestrogen content of the vaginal epithelia [29].

The prevalence rate of 25(26.0%) obtained from this study was lower when compared to the findings of [30,31] who reported a prevalence rate of 28.0% and 52.5% respectively. The prevalence rate of 77.0% reported by [32] among HIV-infected women in Sagamu, Ogun state, Nigeria and the 70.0% reported by [33] among females of reproductive age in Kano, Nigeria was higher than the prevalence rate recorded in this study.

This study also reveals that out of 25(26.0%) who tested positive for *Candida albicans* infections, 8(32%) responded to the use of antibiotic chemotherapy such as; Flucomol-50 mg, Mycoten, Ketoconazole, Folic acid, Palutrine, Tegretol, Atamet, Nemoxil (Amoxicillin Tryhistrate), while 17(68%) with the infection were not on any antibiotic therapy. In a study carried out by Cutler [34], candida infections was common in patients with long history of antibiotic usage or indiscriminate administration of broad spectrum antibiotics which has a side effect of destroying resident friendly flora in the bowel, thus resulting in an imbalance of the micro flora occurring in the bowel.

The prevalence rate of 25(26.0%) obtained from this study was lower when compared to the findings of [30,31] who reported a prevalence rate of 28.0% and 52.5% respectively. The prevalence rate of 77.0% reported by [32] among HIV-infected women in Sagamu, Ogun state, Nigeria and the 70.0% reported by [33] among females of reproductive age in Kano, Nigeria was higher than the prevalence rate recorded in this study.

This study also reveals that out of 25(26.0%) who tested positive for *Candida albicans* infections, 8(32%) responded to the use of antibiotic chemotherapy such as; Flucomol-50 mg, Mycoten, Ketoconazole, Folic acid, Palutrine, Tegretol, Atamet, Nemoxil (Amoxicillin Tryhistrate), while 17(68%) with the infection were not on any antibiotic therapy. In a study carried out by Cutler [34], candida infections was common in patients with long history of antibiotic usage or indiscriminate administration of broad spectrum antibiotics which has a side effect of destroying resident friendly flora in the bowel, thus resulting in an imbalance of the micro flora occurring in the bowel.

**Table 2. Prevalence of *Candida albicans* in relation to age of the subjects**

| Age group | Number of females tested | Number positive | Number negative |
| --- | --- | --- | --- |
| 16-20 | 64(66.7%) | 15(15.6%) | 49(51.0%) |
| 21-25 | 29(30.2%) | 10(10.4%) | 19(19.8%) |
| 26-30 | 3(3.1%) | - | 3(3.1%) |
| Total | 96(100%) | 25(26%) | 71(74%) |
In this study, the prevalence rate of *Candida albicans* in relation to vaginal discharge or discomfort, shows that 6(24%) had vagina discomfort/vaginal discharge and this agrees with the findings of [35]. Vagina discharge or discomfort is one of the most frequent gynecological problems encountered in females especially during their reproductive stage as an alteration in the normal vagina flora can cause the over growth of bacteria that creates vaginal discharge and this is usually common among sexually active women.

Soap which is alkaline can change the normal acidic environment of the vagina, soaps are not only disruptive to the vaginal environment, but can damage mucus membranes. The use of perfumes and dyes can also trigger allergic reaction that lead to vaginal yeast infection. The use of soap in relation to *Candida albicans* was 22(88%).

This study also reveals that females that who use cotton under wears had the highest frequency

---

### Table 3. Prevalence of *Candida albicans* in relation to clinical presentation

| Antibiotic chemotherapy | Prevalence % |
|-------------------------|-------------|
| Yes                     | 8 (32%)     |
| No                      | 17 (68%)    |
| Total                   | 25 (100%)   |

| Trend of diabetics      | Prevalence % |
|-------------------------|-------------|
| Yes                     | 4 (16%)     |
| No                      | 21 (84%)    |
| Total                   | 25 (100%)   |

| Vagina discharge/discomfort | Prevalence % |
|-----------------------------|-------------|
| Yes                         | 6 (24%)     |
| No                          | 19 (76%)    |
| Total                       | 25 (100%)   |

| Use of soap                  | Prevalence % |
|------------------------------|-------------|
| Yes                          | 22 (88%)    |
| No                           | 3 (12%)     |
| Total                        | 25 (100%)   |

| Use of hostel toilet         | Prevalence % |
|------------------------------|-------------|
| Yes                          | 18 (72%)    |
| No                           | 7 (28%)     |
| Total                        | 25 (100%)   |

---

### Table 4. Prevalence of *Candida albicans* in relation to type of underwear and age distribution

| Types of underwear | 16 – 20 (%) | 21 – 25 (%) | Prevalence (%) |
|--------------------|-------------|-------------|----------------|
| Nylon              | 1 (4%)      | -           | 1 (4%)         |
| Silk               | 1 (4%)      | 1 (4%)      | 2 (8%)         |
| Cotton             | 8 (32%)     | 6 (24%)     | 14 (56%)       |
| Nylon and cotton   | 2 (8%)      | 1 (4%)      | 3 (12%)        |
| Silk and cotton    | 2 (8%)      | 2 (8%)      | 4 (16%)        |
| Nylon, silk and cotton | 1 (4%) | -           | 1 (4%)         |
| Total              | 15 (60%)    | 10 (40%)    | 25 (100%)      |

| Underwear used      | Age range 16 – 20 (%) | Age range 21 – 25 (%) | Prevalence (%) |
|---------------------|-----------------------|-----------------------|----------------|
| Tight               | 10 (40%)              | 6 (24%)               | 16 (64%)       |
| Very tight          | 1 (4%)                | -                     | 1 (4%)         |
| Loose               | 2 (8%)                | 1 (4%)                | 3 (12%)        |
| Tight and loose     | 2 (8%)                | 2 (8%)                | 4 (16%)        |
| Very tight and loose| 1 (4%)                | -                     | 1 (4%)         |
| Total               | 16 (64%)              | 9 (36%)               | 25 (100%)      |

---

### Table 5. Prevalence of *Candida albicans* in relation to method of vaginal clean up and age distribution

| Method of vaginal clean up | Age range 16 – 20 (%) | Age range 21 – 25 (%) | Prevalence (%) |
|----------------------------|-----------------------|-----------------------|----------------|
| Baby wipe                  | 5 (20%)               | -                     | 5 (20%)        |
| Tissue                     | 3 (12%)               | -                     | 3 (12%)        |
| Water                      | 3 (12%)               | 1 (4%)                | 4 (16%)        |
| Towel                      | -                     | -                     | -              |
| Baby wipe and tissue       | -                     | 1 (4%)                | 1 (4%)         |
| Baby wipe and water        | 1 (4%)                | 1 (4%)                | 2 (8%)         |
| Tissue and water           | 1 (4%)                | -                     | 1 (4%)         |
| Tissue and towel           | 2 (8%)                | 2 (8%)                | 4 (16%)        |
| Tissue, water and towel    | 1 (4%)                | -                     | 1 (4%)         |
| Baby wipe, tissue and water| 1 (4%)                | 2 (8%)                | 3 (12%)        |
| Total                      | 17 (68%)              | 8 (32%)               | 25 (100%)      |
14(56%) and also a high frequency of 16(64%) was recorded for the use of tight under wears. The use of synthetic under wears and tight under wears reduces airflow which may increase moisture and warmth thereby encouraging yeast infections. Some females have allergies to synthetic material which may cause health changes that encourage yeast infections; tight garments can also reduce healthy blood circulation. This agrees with the findings made by [36].

5. LIMITATION OF THE STUDY

The study was limited to students of the University and the laboratory tests carried out. However, due to financial constraint, the laboratory tests were not extended to molecular techniques. The refusal of some female students to participate in the study due to personal reasons posed a challenge to this work.

6. CONCLUSION

This study indicates that the prevalence of vaginal candida colonization among female students is highly significant. Predisposing factors such as the use of tight under wears, indiscriminate administration of antibiotics should be avoided. The practice of good personal hygiene will go a long way to prevent and reduce the spread of the infection.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sudberg P, Gown BJ. The district morphogenetic states of Candida albicans, trends in microbiology. Int. J. STD. AIDS. 2004;13(8):522-539.
2. Aher CS. Species distribution, virulence factors and antifungal susceptibility profile of Candida isolated from oropharyngeal lesions of HIV infected patients. Int. J. Curr. Microbiol. App. Sci. 2014;3(1):453-446.
3. Karima Sdoudi MEB, Rhimou EI, Dorothee D, Naima E, d’Enfert C, Aziza R. Phylogeny and diversity of candida albicans vaginal isolates from three continents. Int. J. Curr. Microbiol. App. Sci. 2014;3(7):471-480.
4. Pirotta M, Fethe KA, Bradshaw CS. Bacterial vaginosis more questions than answers. Aust Fam Physician. 2009;38:6.
5. Abudu OO, Anorlu RI. Textbook of obstetrics and gynecology for medical students. 2nd ed. Heinamann Educational Books Plc Ibadan, Nigeria; 2006.
6. Jack D, Sobel JD. Vulvovaginal candidiasis; 200. 7. McClelland RS, Richardson BA, Hassan WM, Graham SM, Kiarie J, Baeten JM, et al. Prospective study of vaginal bacterial flora and other risk factors for vulvovaginal candidiasis. J. Infect. Dis. 2009;199(12):1883-1890.
8. Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu state, Nigeria. Journal of Medicine and Medical Sciences. 2010;1(10):447-452.
9. Hedayati T, Shafiei G. Candidiasis. eMedicine Specialties; 2010.
10. Sanaa MA, ZMK, Soheir S. Maklad, Manar R, Ameen, Sanaa SZ. Relationship between virulence factors of Candida species with candiduria and myeloperoxidase concentrations. Int. J. Curr. Microbiol. App. Sci. 2015;4(1):108-123.
11. Sasikumar S, Franklin DDA, Rajkumar S. An in-vitro study of effectiveness of Uropathogenic yeast on Male infertility. Int. J. Curr. Microbiol. App. Sci. 2013;2(5):233-246.
12. Saini, SCD. Vulvovaginal candidiasis due to non Albicans Candida: Its species distribution and antifungal susceptibility profile. Int. J. Curr. Microbiol. App. Sci. 2013;2(12):323-328.
13. Solanki MSA. Prevalence of candida infection in pregnant women with and without diabetes. Int. J. Curr. Microbiol. App. Sci. 2014;3(4):605-610.
14. Rajeev SPC. Prevalence of candida from Sputum in HIV infected Patients of Gujrat, India. Int. J. Curr. Microbiol. App. Sci. 2014;3(8):345-357.
15. Singh SI. Treatment of vulvovaginal candidiasis. Clin Rev. 2003;136(9):26-30.
16. Donders GG. Lower genital tract infections in diebetic women. Curr Infect Dis Rep. 2002;6:536-539.
17. De leon EM, Jacober SJ, Sobel JD, Foxman B. Prevalence and risk factors for vaginal candida colonization in women with type1 and type 2 diabetes. BMC Infect Dis. 2002;2(1).
18. Reed BD, Zazove P, Pierson CL, Gorenflo DW, Horrocks J. Candida transmission
and sexual behavior as risks for a repeat episode of Candida vulvovaginitis. J. Women health. 2003;12(10):979-89.

19. Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. Journal of Medicine and Medical Sciences. 2010;1(10):447-452.

20. Alli JAO, Okonko IO, Odu NN, Kolade AF, Nwanze JC. Detection and prevalence of isolates among patients in Ibadan, Southwestern Nigeria. Journal of Microbiology and Biotechnology Research. 2011;1(3):176-184.

21. Centre for Disease Control & Prevention. Sexually transmitted disease guidelines. Morbidity & Mortality Weekly Recommendation Report. 2002;51(RR-6):1-78.

22. Mardh PA, Rodrigues AG, Genc M. Facts and myths on recurrent Vulvovaginal candidosis: A review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. Int. J. STD. AIDS. 2002;13(8):522-539.

23. Donbraye-Emmanuel OOB, Donbraye E, Okonko IO, Alli JA, Ojezele MO, Nwanze JC. Detection and prevalence of candida among pregnant women in Ibadan, Nigeria. World Applied Science Journal. 2010;10(9):986-991.

24. Ojiyi E, Dike E, Okendo C, Anolue F, Audu B, Ngadda H. The prevalence and predictors of genital tract infections in cervical cytology specimens at university teaching hospital, Maiduguri, Nigeria. The intern. J. Gynecol. obstet. 2012;16(1). DOI: 10.5580/2971

25. Hidalgo JA, Vazquez JA, Bronze MS. Candidiasis: Frequency; 2014.

26. National Bureau of Statistics. Population Census. Federal Republic of Nigeria; 2006.

27. Obiekeze SQ, Odun NN, Ogwu D. Aerobe microbiological quality of nono sold in Keffi metropolis. Int. J. Chem. Sci. 2012;5(2):157-162.

28. Elmer WK, Stephen DA, William MJ. Laboratory approach to the diagnosis of fungal infections. 14th ed. Lipincott Co. Philadelphia; 1992.

29. Sobel JD. Vaginitis. N. Engl. J. Med. 1997;337(18):234-236.

30. Khan AS, Amir F, Altaf S, Tanveer R. Evaluation of common organisms causing vaginal discharge. J. Ayub. Med. Coll. Abbottabad. 2009;21(2):90-93.

31. Muvunyi CM, Hernandez CT. Prevalence of bacterial vaginosis in women with vaginal symptoms in south province, Rwanda. Afr. J. Clin. Exper. Microbiol. 2009;10(3):156-153.

32. Oyewole IO, Anyasor GN, Michael-Chikezie EC. Prevalence of STI pathogens in HIV- infected and non-infected women: Implications for acquisition and transmission of HIV in Nigeria. Asian Journal of Medical Sciences. 2010;2(3):163-166.

33. Nwankwo EOK, Kandakai-Olukemi YT, Shuaibu SA. Aetiologic agents of abnormal vaginal discharge among females of reproductive age in kano, Nigeria. Journal of Medicine and Biomedical Sciences. 2010:12-16. ISSN: 2078-0273.

34. Cutler JE. Putative virulence factors of Candida albicans. Annual Review of Microbiology. 1991;45:187-218.

35. Fernández-Limia O, Villar C, Fariñas AT, Betancourt A, De Armas E, Faure R. Prevalence of trichomoniasis, bacterial vaginosis and candidiasis in women attending a sexual transmitted infections and gynaecologic clinic using an immunologic latex agglutination test. The Internet Journal of Gynecology and Obstetrics. 2007;6(2).

36. Nwankwo EOK, Kandakai-Olukemi YT, Shuaibu SA. Aetiologic agents of abnormal vaginal discharge among females of reproductive age in kano, Nigeria. Journal of Medicine and Biomedical Sciences. 2010:12-16. ISSN: 2078-0273.

© 2016 Maikenti et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.