UNIVERSITI PUTRA MALAYSIA

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF GARDNERELLA VAGINALIS ISOLATED FROM WOMEN WITH BACTERIAL VAGINOSIS FROM HOSPITAL KUALA LUMPUR, MALAYSIA

NADA KHAIRI YOUNUS AL-ASSADI

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NADA KHAIRI YOUNUS AL-ASSADI

MASTER OF SCIENCE

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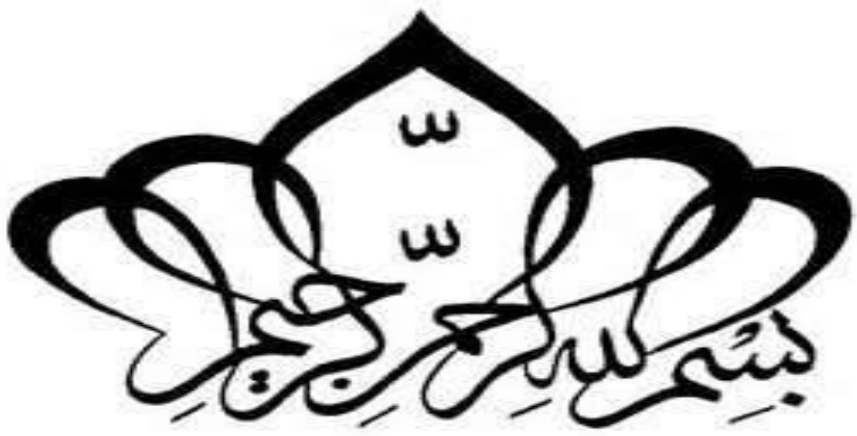
By

NADA KHAIRI YOUNUS AL-ASSADI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirements for the Degree of Master of Science

June 2013



In the Name of God the Compassionate the Merciful

Dedicated to my loving family, my parents and sister Watffa for their unending love, invaluable support and extraordinary courage

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF *GARDNERELLA VAGINALIS* ISOLATED FROM WOMEN WITH BACTERIAL VAGINOSIS FROM HOSPITAL KUALA LUMPUR, MALAYSIA

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NADA KHAIRI YOUNUS AL-ASSADI

June 2013

Chairman: Assoc. Prof. Vasanthakumari Neela, PhD

Faculty: Medicine and Health Sciences

Bacterial vaginosis (BV) is a highly prevalent, but poorly understood polymicrobial disorder of the human vaginal microbiota. Symptoms for BV include vaginal discharge, itching and odor. *Gardnerella vaginalis* which is commonly found in the vaginal mucosa of asymptomatic women is the frequent cause of BV. BV microbiota although dominated by *G. vaginalis,* it also includes a number of anaerobes. The main challenge in the diagnosis of BV is the identification of the correct etiological agents.

Bacterial vaginosis if untreated leads to chronic infectious/inflammatory disease with multiple reproductive tract complications including preterm birth, neonatal infections and suppurative lesions. Treatment options as recommended by Center for Disease Control (CDC) include oral or intravaginal administration of metronidazole or clindamycin. Despite treatment with these antibiotics, treatment failure and recurrent infection rates are still rising.

Therefore, this study is aimed at identifying methods for conclusive diagnosis of BV, and corrects laboratory techniques for isolation of *G. vaginalis* bacterium. Also this study is shed the light on determining the susceptibility of *G. vaginalis* strains against recommended antibiotics and determining the phenotypes and genotypes characteristics of this bacterium with its virulence properties.

Two-hundred and seven vaginal swabs were collected from patients' attending the gynecological clinic at Hospital Kuala Lumpur (HKL), Malaysia from 16th February to 20th July 2012 and transported to the Medical Microbiology Laboratory, Universiti Putra Malaysia. One hundred and sixty (77.2%) out of 207 samples were recorded as symptomatic biased on clinical observation and due to the presence of vaginal discharge, while the remaining 47 (22.7%) were recorded as asymptomatic as they did not show any vaginal discharge.

A patient is diagnosed for BV based on the Amsel-clinical criteria and Nugent-Gram staining. In the present study, comparison of Amsel-clinical criteria and Nugent-Gram stain in the diagnosis of BV showed the later to be more sensitive (91%) than the former (53%). Among the 160 samples, 47 (73.4%) were confirmed as *G. vaginalis* positive by phenotypic (culture, Gram stain and biochemical) and genotypic (16S rRNA) methods.

Metronidazole (MTZ), which is a broad spectrum antibiotic against candidiasis and giardiasis is given as the first choice of drug for the treatment of BV. Among the studied 47 isolates, three showed resistance to MTZ and carried the *nim* gene which mediates MTZ resistance.

A simple and reproducible scheme for identifying biotypes of *G. vaginalis* identified eight biotypes based on reaction for hippurate hydrolase, ONPG test and lipase test. Among the isolates studied, biotype 1 was the most prevalent (18/47 or 38%), followed by biotype 7 (17/47 or 36%), biotype 5 (8/47 or 17%), and finally biotype 8 (4/47 or 8.5%). Biotypes 2, 3, 4 and 6 were not observed. The biotypes did not show any significant association with the clinical reasons (abortion, minor surgery and vaginal fluid) for suspecting BV.

Genotyping of the microbial pathogens is important in the management of infections as it gives useful information about the spread of pathogens. In addition, monitoring of endemic pathogens determines the adequacy of the general hygienic measures

performed. All 47 *G. vaginalis* isolates when genotyped by amplified ribosomal DNA restriction analysis (ARDRA) produced three different genotypes (I, II and III). Similar to the biotypes, the genotypes also did not show any significant association with clinical data. Twelve (25%) isolates belonged to genotype I, 22 (46%) isolates to genotype II and 13 (27.6%) to genotype III. Isolates that were biotype 7 matched well with genotype II. *G. vaginalis* isolates when further screened for virulence factors, such as vaginolysin (*vly,* gene that mediate pore formation and cytotoxicity) and sialidase gene (mediate biofilm production), it was found that 25 (53%) and 26 (55%) carried sialidase and *vly* gene respectively. All *G. vaginalis* isolates of genotype I harbor the sialidase gene 12 (25.5%), and contain also *vly* gene 11 (23.4%). This result suggest of recording these strains as a virulent strains not as a commensally strains.

In conclusion, characterization of *G. vaginalis* isolated from patients with BV revealed that for the accurate identification of *G. vaginalis* associated BV, combination of phenotypic and genotypic methods should be implemented in the routine diagnostic laboratory. As the *G. vaginalis* strains are highly diverse, susceptibilities towards recommended antibiotics are decreasing and frequently carry virulent and resistant genes, routine monitoring of the *G. vaginalis* important for the efficient management of bacterial vaginosis infection.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

FENOTIP DAN PENCIRIAN GENOTIP VAGINALIS GARDNERELLA TERPINGGIR DARI WANITA DENGAN VAGINOSIS BAKTERIA DARI HOSPITAL KUALA LUMPUR, MALAYSIA

Oleh

NADA KHAIRI YOUNUS AL-ASSADI

Jun 2013

Pengerusi: Profesor Madya Vasanthakumari Neela, PhD

Fakulti: Perubatan dan Sains Kesihatan

Vaginosis bakteria (BV) adalah gangguan yang sangat berleluasa, tetapi kurang difahami polymicrobial daripada microbiota faraj manusia. Gejala untuk BV termasuk keputihan, gatal-gatal dan bau. *Gardnerella vaginalis* yang biasanya ditemui di mukosa faraj wanita asimptomatik adalah punca kerap BV. Vaginosis bakteria microbiota walaupun dikuasai oleh *G. vaginalis*, ia juga termasuk beberapa anaerobes. Cabaran utama dalam diagnosis BV adalah pengenalan ejen etiological betul.

Vaginosis bakteria jika tidak dirawat membawa kepada penyakit kronik berjangkit/radang dengan saluran pembiakan pelbagai komplikasi termasuk kelahiran pramatang, jangkitan neonatal dan luka-luka bernanah. Pilihan rawatan seperti yang

disyorkan oleh Pusat Kawalan Penyakit (CDC) termasuk pentadbiran lisan atau intravaginal metronidazole atau clindamycin. Walaupun rawatan dengan antibiotik, kegagalan rawatan dan kadar jangkitan berulang masih meningkat.

Oleh itu, kajian ini bertujuan untuk mengenal pasti kaedah untuk diagnosis muktamad *G. vaginalis* menyebabkan BV, faktor risiko, kecenderungan *G. vaginalis* terhadap antibiotik yang disyorkan, menentukan fenotip dan genotip dan harta kebisaan.

Dua-ratus dan tujuh swab faraj dikumpulkan daripada 'pesakit menghadiri klinik sakit puan di Hospital Kuala Lumpur (HKL), Malaysia dari 16 Februari hingga 20 Julai 2012 dan diangkut ke Makmal Mikrobiologi Perubatan, Universiti Putra Malaysia. Seratus enam puluh (77.2%) daripada 207 sampel telah direkodkan sebagai gejala akibat kehadiran keputihan, manakala 47 selebihnya (22.7%) telah direkodkan sebagai asimptomatik kerana mereka tidak menunjukkan sebarang keputihan.

Seorang pesakit didiagnosis untuk BV berdasarkan kriteria Amsel klinikal dan pewarnaan Gram Nugent. Dalam kajian ini, perbandingan kriteria klinikal Amsel dan Nugent-Gram noda dalam diagnosis BV menunjukkan kemudian untuk menjadi lebih sensitif (91%) daripada bekas (53%). Antara 160 sampel, 47 (73.4%) telah disahkan sebagai *G. vaginalis* positif dengan kaedah fenotipik (kultur, pewarnaan Gram dan biokimia) dan genotip (16S rRNA).

Metronidazole, yang adalah antibiotik spektrum luas terhadap kandidiasis dan Giardiasis, diberikan sebagai pilihan pertama dadah untuk rawatan BV. Antara 47 pencilan dikaji, tiga menunjukkan kerintangan metronidazole dan dijalankan gen *nim* yang pengantara rintangan metronidazole.

Satu skim yang mudah dan diulang untuk mengenal pasti miang daripada *G. vaginalis* mengenal pasti lapan miang berdasarkan reaksi untuk hippurate hydrolase, ONPG ujian dan ujian lipase. Antara pencilan yang dikaji, biotype 1 adalah 8 yang paling lazim (18/47 atau 38%), diikuti oleh biotype 7 (17/47 atau 36%), biotype 5 (8/47 atau 17%), dan akhirnya biotype (4 / 47 atau 8.5%). Miang 2,3,4 dan 6 tidak dipatuhi. Miang tidak menunjukkan apa-apa hubungan yang signifikan dengan sebab klinikal (pengguguran, pembedahan kecil dan cecair faraj) untuk mengesyaki BV.

Mengenal pasti genotip daripada mikrob patogen adalah penting dalam pengurusan jangkitan kerana ia memberikan maklumat yang berguna tentang penyebaran patogen. Di samping itu, pemantauan patogen endemik menentukan kecukupan langkah-langkah kebersihan umum dilakukan. Semua 47 pencilan untuk *G. vaginalis* apabila digenotaipkan oleh dikuatkan DNA ribosomal sekatan analisis (ARDRA) menghasilkan tiga genotip berbeza (I, II dan III). Seperti kepada miang, genotip juga tidak menunjukkan apa-apa hubungan yang signifikan dengan data klinikal. Dua belas (25%) mengasingkan dipunyai oleh genotip saya, 22 (46%) diasingkan untuk genotip II dan 13 (27.6%) untuk genotip III. Pencilan yang biotype 7 dipadankan dengan II genotip. *G. vaginalis* diasingkan apabila terus disaring untuk faktor kebisaan, seperti vaginolysin

(*vly*, gen bahawa pembentukan liang Mediate dan cytotoxicity) dan sialidase gen (Mediate biofilm pengeluaran) , ia telah mendapati bahawa 25 (53%) dan 26 (55%) dibawa sialidase dan gen *vly*. Tiada pencilan daripada II genotip terkandung gen sialidase. Begitu juga, majoriti pencilan daripada II genotip adalah negatif gen vaginolysin.

Kesimpulannya, pencirian *G. vaginalis* diasingkan daripada pesakit dengan BV mendedahkan bahawa untuk mengenal pasti yang tepat *G. vaginalis* bersekutu BV, gabungan kaedah fenotip dan genotip perlu dilaksanakan di dalam makmal diagnostik rutin.

Sebagai strain *G. vaginalis* adalah sangat pelbagai, mudah dipengaruhi ke arah antibiotik yang disyorkan semakin berkurangan dan sering membawa getir dan tahan gen, pemantauan rutin *G. vaginalis* penting bagi pengurusan yang cekap jangkitan bakteria vaginosis.

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I certify that a Thesis Examination Committee has met on 11.6.2013 to conduct the final examination of **Nada Khairi Younus** on her thesis entitled "**Phenotypic and Genotypic Characterization of *Gardnerella vaginalis* Isolated from Women with Bacterial Vaginosis from Hospital Kuala Lumpur"** in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfillment of requirement for degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for other degree at Universiti Putra Malaysia or other institutions.

NADA KHAIRI YOUNUS AL-ASSADI

Date: 11th June 2013

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