The Bacterial Causative Agents of Aerobic Vaginitis in Sexually Active Nonpregnant Women and their Antibiotic Sensitivity Pattern

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المسببات البكتيرية لالتهاب المهبل الهوائي (Aerobic Vaginitis) في النساء النشطات جنسيا غير الحوامل ونمط حساسيتها للمضادات الحيوية

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الخلاصة

تم الحصول على نمو معنوي للبكتريا الهوائية واللاهوائية الاختيارية من 43 عينة جمعت من نساء نشطات جنسيا وغير حوامل (بعمر 20-40 سنة) مصابات بالتهاب المهبل الهوائي (Aerobic vaginitis). عزلت البكتريا الموجبة لملون غرام من 24 حالة (55.8%) بينما عزلت العصيات المعوية السالبة لملون غرام من 14 حالة (32.55%). عزلت المكورات العنقودية الذهبية (*Staphylococcus aureus* والمكورات العنقودية السالبة للكواكيوليز(Coagulase-negative staphylococci: CNS) من 12 حالة لكل منهما (27.90%) تلتها بكتريا الأشيرشية القولونية (*Escherichia coli*) 11 (25.58) ثم بكتريا *Enterobacter* spp 2 (4.65%) وبكتريا *Klebsiella pneumoniae* 1 (2.32 %) . كانت الإصابة مختلطة في أربع حالات : *S. aureus* مع .sp *Streptococcus*1 (2.32%) و CNSمع .sp *Streptococcus* 1 (2.32%) وCNS مع *E. coli* 2 (4.65%). تم عزل الخمائر من حالة واحدة (2.32%). أظهر عدد قليل من المضادات التقليدية المتوفرة فعالية جيدة (نسبة الحساسية >80%) ضد أي من البكتريا الشائعة كمسبب لالتهاب المهبل. كان مضاد اميبينيم الأكثر فعالية ضد *S. aureus* و CNS بنسبة 100% و83.3% على التوالي. بينما كان اغلبها مقاومة للبنسلينات والجيل الثالث من السيفالوسبورينات والميثيسلين والمترونايدازول والفانكومايسين. اما عزلات بكتريا *E. coli* فكانت جميعها حساسة للمضاد اميبينيم (100%) و 84.6% للكلورامفينيكول و 76.9% لكل من النورفلوكساسين والجينتامايسين. من ناحية أخرى كانت أغلب عزلات بكتريا *E. coli* مقاومة للبنسلينات والجيل الثالث من السيفالوسبورينات والتتراسايكلينات. نستنتج من هذه الدراسة أن التهاب المهبل الهوائي يمكن إن يكون مصدراً" لإصابات أعمق وأكثر خطورة في هؤلاء المريضات ولهذا يجب أخذ مثل هذه الإصابات بنظر الاعتبار وإجراء زرع روتيني لها للتحري عن المسببات وإجراء اختبار الحساسية للمضادات في مختبراتنا السريرية.

Abstract

Significant growth of aerobic and facultative anaerobic bacteria was obtained from 43 specimens collected from sexually active nonpregnant women (20-40 years old) with aerobic vaginitis. Gram positive bacteria were isolated from 24 cases (55.8%), whereas Gram negative enteric rods were isolated from 14 cases (32.55%). *Staphylococcus. aureus* and coagulase negative staphylococci (CNS) were isolated from 12 cases for each (27.90%) followed by *Escherichia coli* 11 (25.58%), *Enterobacter* spp. 2 (4.65%), and *Klebsiella pneumoniae* 1 (2.32%). Mixed infection was noted in 4 cases: *S. aureus* with *Streptococcus* sp. 1 (2.32%); CNS with *Streptococcus* sp. 1 (2.32%); and CNS with *E. coli* 2 (4.65%). Yeast was isolated from one case (2.32%). There were few antibiotics among the conventionally available antibacterial agents possessing good sensitivity (>80%) against any one of the common aerobic vaginal pathogens. For *S. aureus* and CNS, imipenem was the most effective 100% and 83.3%, respectively. Most of them were resistant to penicillis, third generation cephalosporins, methicillin, metronidazol, and vancomycin. For *E. coli*, 100% of the isolates were sensitive to imipenem, 84.6% to chloramphenico, and 76.9% to norfloxacin and gentamicin. On the other hand most of *E. coli* isolates were resistant to penicillins ,third generation cephalosporins and tetracyclines. In conclusion, aerobic vaginitis can be an origin of more serious deeper infections in these patients, for that such cases must be taken in account and routine cultures and antibiotic sensitivity tests must be done for such cases in our clinical laboratories.

Key words: Aerobic vaginitis, nonpregnant women, antibiotic susceptibility.

Introduction

Vaginal infections are often accompanied by vaginitis, which is an inflammation of the vagina characterized by discharge, irritation, and/or itching (1). The frequent cause of vaginal discharge is an infection or colonization with different microorganisms (2). Vaginal infections are frequent causes of distress and discomfort in adult women. The most common vaginal infections are bacterial vaginosis, trichomoniasis, and vulvovaginal candidiasis. Other causes include allergic and irritative factors or other sexually transmitted diseases (1). In recent years, another type of vaginitis is described 'aerobic vaginitis' which is caused by aerobic normal vaginal flora previously not considered as pathogens. Aerobic Vaginitis is a term proposed to describe purulent vaginal discharge with predominance of abnormal aerobic flora (3). The intermediate flora is addressed separately, and a new entity-'Aerobic Vaginitis' is- discussed (4). Aerobic vaginitis is associated with aerobic microorganisms and its characteristics are different from those of bacterial vaginosis and elicit an important host response and genital complaints are those of a real vaginitis (red inflammation, yellow discharge, vaginal dyspareunia) (5 , 6).

Vaginitis is the most gynecological problem encountered in clinical practice. It may be caused by protozoa, bacteria, fungus, or viruses, single or in combination (7). Group B streptococci (GBS), *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Trichomonas vaginalis* are frequently cultured from cases of aerobic vaginitis (3, 5, 6). Vaginal microorganisms associated with aerobic vaginitis were found to be mainly GBS, *S. aureus*, and *E. coli* which were three to five times more frequent in aerobic vaginitis than in the normal flora (8).

Clothing, personal habits, sexual activity, oral contraceptives, antibiotics, and pregnancy are the most contemplated predisposing factors to vaginitis (7). Some pathogenic conditions causing vaginitis are well defined, yet 7-72% of women with vaginitis may remain undiagnosed and such forms of abnormal vaginal flora, neither considered as normal, nor can be called bacterial vaginosis, have been termed as 'intermediate flora; and its management probably differ from that of bacterial vaginosis. It is of crucial importance in pregnant females at risk of preterm delivery (9).

The aim of this study was to detect the bacterial causative agents of aerobic vaginitis in sexually active nonpregnant women of age group 20-40 years and to estimate the susceptibility of the isolates to selected antibiotics and chemotherapies.

Materials and Methods

Patients

This study included sexually active non-pregnant women (aged 20 to 40 years) with vaginitis, attending private Obstetrics and Gynecology Clinic in Al-Kut/Wassit Province/Iraq.

Specimen Collection and Processing

Specimens were collected during May 2008 to May 2009 according to Vandepitte *et al*. (10). High vaginal swabs were collected by the Gynecologist and streaked immediately after collection on eosine methylene blue agar (EMB) (Himedia) and on blood agar (Oxford) plates.

EMB plates were incubated at 37 °C for 24 hours at ambient air while blood agar plates were incubated at 37 °C for 48 hours at 5% CO_2 atmosphere.

Identification of the Isolates

All isolates (Gram-positive and Gram-negative aerobic bacteria) were diagnosed biochemically according to Forbes *et al.* (11) and according to methods described by MacFaddin (12).

Antibiotic Susceptibility Test

It was carried out using agar diffusion method (Forbes *et al.*, 2002). For this purpose inocula were prepared by diluting overnight cultures in sterile sodium chloride (0.9%) suspension and then match with the Macfarlane index. Bacterial suspensions were then plated onto Mueller-Hinton agar (HiMedia) and the

commercially available antibiotic discs were placed on lawn of culture and the plates were incubated over night at 37^{o}C. Sensitivity, intermediate sensitivity, and resistances were determined by the zone of complete growth inhibition around each disk according to reference standards.

Results and Discussion

Culture Results

From 43 sexually active nonpregnant women (aged 20-40 years) with vaginitis, significant growth (heavy growth) of aerobic bacteria was obtained. Since most of the causative agents of aerobic vaginitis are part of normal vaginal flora, so that we considered only those samples that gave heavy growth as infection. Cook *et al*. (13) and Lawson (14) reported that vaginitis-associated isolates represented the predominant vaginal flora present concurrent with symptoms. Larsen and Monif, (15) reviewed that for disease to occur, exogenous or endogenous bacteria that possess pathogenic prerequisites must attain replicative dominance. The microbial load for a given organism appears to influence the relative risk of symptomatic infection. For endogenous bacteria of the female genital tract, the microbiological environment may affect the bacterial expression of virulence factors. Theoretically, if a virulent factor is constitutive, the number of organisms present will determine the amount of the virulence factor available to promote infection (15).

Aerobic Bacterial Isolates

Gram positive bacteria were isolated from 24 cases (55.8%) whereas Gram negative enteric rods were isolated from 14 cases (32.55%). Mixed infection was noted in 4 cases (9.30%). These results were consistent with others. Mumtaz *et al*. (9) found that in nonpregnant women 63.8% of cases were caused by gram-positive cocci, and 31.5% were caused by gram-negative enteric rods. Abdul-Rahman *et al.* (16) ,when they studied the causative agents of pelvic inflammatory disease in women, found that 71.43% of the isolates were gram-positive cocci. Khan and Khan (17) showed that gram-positive organisms were more common (71%) in vaginal infections than gram-negative organisms (29%).

S. aureus and CNS were isolated from 12 cases for each (27.90%) (Table 1), followed by *E. coli* 11 (25.58%), *Enterobacter* spp. 2 (4.65%), *Klebsiella pneumoniae* 1 (2.32%), yeast 1 (2.32%). Mixed infection was noted in 4 cases: *Staphylococcus* spp. with *Streptococcus* spp. 2 (4.65%) and *Staphylococcus* spp. with *E. coli* 2 (4.65%).

Table 1: Aerobic and facultative anaerobic organisms prevalent in vaginal isolates from sexually active nonpregnant women with aerobic vaginitis.

Organism	Number of isolates	%
S. aureus CNS	12 12	27.90 27.90
E. coli	11	25.58
Enterobacter spp.	2	4.65
Klebsiella pneumoniae	1	2.32
Mixed infection: - *Staphylococcus* spp. + *E. coli* - *Staphylococcus aureus* + *Streptococcus* sp. - *Staphylococcus* sp. + *Streptococcus* sp.	 2 1 1	 4.65 2.32 2.32
Yeast	1	2.32
Total	**43**	**100**

Macsween and Ridgway (18) found that common commensals which can act as pathogens include *Candida* spp., staphylococci, and β-hemolytic streptococci. Mumtaz *et al*. (9) found that *Staphylococcus aureus* (*S. aureus*) was the most prevalent organism (46%), followed by *E. coli* (13.7%), *Klebsiella pneumonia*e (10.5 %), *Enterobacter* (9.0%), β-hemolytic streptococci (8.8%), *Pseudomonas aeruginosa* (7.3%), and *Candida* spp. (1.0%). Khan and Khan (17) demonstrated that *Enterococcus* infection was the highest (31%) followed by infection with *Streptococcus pyogenes* (22%). *E. coli* was found with the prevalence of 21%. French *et al*. (3) and Donders *et al.* (6) demonstrated that the usual predominant microorganisms are group B streptococci, *E. coli*, and *S. aureus*.

Staphylococci (*S. aureus* and coagulase negative staphylococci) represent part of normal vaginal flora (19, 20). Reid and Bruce (21) ; Demba *et al.* (22); and Schlivert *et al.*(23) demonstrated that the vaginal mucosa of 20.5-23% of the females is coloniozed by *S. aureus*, in whom it predisposes them to toxic shock syndrome. In vaginal cultures most reaserchers considered CNS as contaminants (22, 24). In this work, there is a suspicion regarding the pathogenic potential of these agents as they were isolated as pure heavy growth. da Cunha *et al*. (25) concluded that these microorganisms should not be ignored or classified as mere contaminants. In their study of noramal vaginal flora of women with bacterial vaginosis, Demba *et al.* (22) found that the vaginal flora cultures for aerobic bacteria were *Staphylococcus* spp., and coliforms which were mostly present in a scanty numbers. Rosenstein *et al*. (19) found that the vaginal flora is not static but can convert from a normal state to a grossly abnormal state and back again. Zunin

et al. (26) showed that comparisons among groups (healthy and patients) shows little differences in the microbial population between healthy women and patients with genital tract infections. Otto (27) and Longauerova (28) reviewed that CNS may participate, as commensal flora, in the development of infections only when external barriers (e.g. skin) were damaged due to wounds, inoculation, or implantation of foreign bodies. Most importantly the results that were obtained by Rosenstein *et al.* (29) who reported that the initial disturbance in the vagina causes certain bacteria, such as CNS, to appear first, in large numbers, followed by *Bifidobacterium* spp. However, it is then unclear whether rapid multiplication of these organisms provides an environment conductive to the multiplication of other bacterial spp., such as anaerobic Gram negative rods and Gram positive cocci, then finally *Gardnerella vaginalis* and *Mycoplasma hominis*, or whether it is the alteration of the environment *per se* which causes this sequence of events to occur. Examination of physiological factors, such as hormone and secreted immunoglobulin concentrations in the vagina, might help to distinguish between the two possibilities although, of course, they may not mutually exclusive.

In this study *E. coli* represented 25.58% of cases. This is in agreement with others. *E. coli* is one of the common organisms in the microflora of pregnant as well as nonpregnant women (30). Inter 1(1) demonstrated that vaginitis may also be produced by bowel bacteria such as *E. coli* migrating into the vagina. Vaginal *E. coli* may also cause symptomatic infections (13) such as vaginitis or tubo-ovarian abscess and is associated with life threatening neonatal sepsis and meningitis (31, 32).

Yeast was isolated from one case (2.32 %). This is not consistant with others. Larsen and Monif (15) reviewed that the typical rate of yeast carriage varies among populations and increase both after puberty and during pregnancy, which suggests an important role for host physiology in cases of vaginal candidiasis. Monif and Carson (20) found that *C. albicans* was isolated from 20% of cases. Levett (33) and Puri *et al*. (34) isolated *C. albicans* from 39% and 26.43% of non-pregnant women respectively. The low percentage of yeast isolation in this study, in comparison with studies mentioned above, can be explained by the high rate of bacterial isolation especially staphylococci. Larsen and Monif (15) reviewed that both studies of animal models and observation of humans suggest that there is an inverse relationship between bacterial and yeast floras with respect to prevalence and numerical abundance.

The major difference between this study and previous studies was the low rate of isolation of β-hemolytic streptococci (only 1 case: 2.32%). This difference may be attributed to several reasons including the low percentage of yeast isolation in this study, since Monif and Carson (20) showed that the GBS had a greater probability of coisolation when *C. albicans* was present (30.8% versus 12%). Also in this work, half of staphylococci were coagulase negative which may inhibit the GBS as Carson *et al.* (24) and Chaisilwattana and Monif (35) found that the absence of GBS in the vaginal flora may be the result of mediation by

coagulase-negative staphylococci and selected strains of enterococci. Schuchat *et al.* (36) demonstrated that the various rates of GBS colonization seen in different studies may reflect differences in the underlying population or in ethnicity. In their study, Mumtaz *et al*. (9) accounted the difference between their study and other studies to the prevalent conditions like health education, sanitation, and medical coverage available in each country.

Antibiotic Sensitivity Pattern of the Isolates

The detailed results of the percentage sensitivity of the common isolates against the various antibiotics are shown in Table 2.

Table 2: Percentage of sensitivity of aerobic vaginal isolates to various antibiotics.

Antibiotics	*S. aureus*	CNS	*E. coli*
Penicillins:			
Ampicillin	15.38	16.6	15.38
Amoxicillin	38.46	25	15.38
Amoxicilli-clavulanic acid	41.6	16.6	7.69
Cephalosporins:			
Cefotaxime	7.69	0	15.38
Ceftazidime	0	0	30.76
Carbapenems:			
Imipenem	100	83.3	100
Quinolones:		66.6	
Norfloxacin	53.8	50	76.9
Ciprofloxacin	58.3		
Aminoglycosides:			
Gentamicin	53.84	58.3	76.9
Miscellaneous:			
Co-trimoxazole	58.3	50	
Doxycycline	53.84	75	0
Tetracycline	50	66.6	30.76
Methicilin	0	0	-
Metronidazole	0	0	-
Vancomycin	38.46	25	-
Chloramphenicol	-	-	84.6

The most effective chemotherapeutic against *S. aureus* and CNS in this study was imipenem: 100% and 83.3%, respectively, whereas most of them were resistant to penicillins, third generation cephalosporins (cefotaxime and

ceftazidime), methicillin, metronidazol, and vancomycin . Abdul-Rahman *et al*. (16) showed that most of the gram-positive cocci were sensitive to gentamicin, ciprofloxacin, and tetracycline whereas most of them were resistant to penicillins, third generation cephalosporins, and metronidazole. Mumtaz *et al*. (9) found that the most effective antibiotics against *S. aureus* were imipenem (98.64%) and vancomycin (93.6%), while lesser activity has been noted against penicillins, tetracycline (49.3%), sulphonamides (23.6%), first generation cephalosporins (36.8%) and monobactams (19.13%)

It is clear that staphylococci isolated in this study were resistant to most traditionally available antibiotics and this makes treatment of such infections very difficult especially if we note that these organisms represented the most prevalent causative agents. Schmidt and Hensel (32) reviewed that treatment of *Staphylococcus* infections becomes increasingly difficult, since resistance to a growing number of antibiotics has been observed in clinical isolates. Forbes *et al.* (11) stated that although a broad spectrum of agents may be used for therapy, most staphylococci are capable of acquiring and using one or more of the resistance mechanisms. Increasing resistance against antibiotics in staphylococci is an enormous problem for the public health system and one of primary reasons for the in-depth investigation of staphylococcal pathogenicity and resistance factors (27). For a long time, penicillins have been a main stay for the management of a variety of staphylococcal infections but the organism has gradually acquired resistance towards them. In most cases of staphylococci, resistance to penicillin is attributable to β-lactamase production (32, 37). Also these β-lactamase producers are resistant to all β-lactamas, penicillins, cephalosporins,carbapenemes, and penemes (37). Methicillin is an antibiotic of first choice against staphylococci. In nosocomial infections, the most important type of infection linked to *S. epidermidis* and other CNS, about 80% of strains are resistant to methicillin and many strains are resistant to other antibiotics as well. CNS most likely can easily acquire resistance from *S. aureus* and transfer to other species of the genus. Intermediate resistance against glycopeptide antibiotics (vancomycin and teicoplanin) occurs frequently. It is due to alterations of the target of the antibiotic. The very recent alarming finding that high level resistance against vancomycin has been transferred from enterococci to *S. aureus* , very likely means that we will face highly vancomycin-resistant CNS in the very near future. Multiple resistance is also not uncommon (27).

The most effective chemotherapeutic agents against gram-negative rods (*E. coli*) in this study were imipenem(100%), chloraphenicol (84.6%), and norfloxacin and gentamicin (76.9% for each) while most of them were highly resistant to penicillins and third generation cephalosporins. Mumtaz *et al*. (9) found that the most effective chemotherapeutic agents against gram-negative rods (*E. coli*) were imipenem (96.0%) and piperacillin/tazobactam (92.1%) whereas the least active antimicrobials were those beloniging to the groups of penicillins, tetracycline, and sulfonamides.

The sensitivity of *E. coli* to imipenem and their high resistance to penicillins and third generation cephalosporins can be explained by the discovery of what is called extended spectrum β-lactamases (ESBL) which make gram-negative rods resistant to penicillins and third generation cephalosporins but still sensitive to carpabenems (imipenem). The ESBL enzymes are plasmid-mediated enzymes capable of hydrolyzing and inactivating a wide variety of β-lactams, including third generation cephalosporins, penicillins, and aztreonam but have no detectable activity against cephamycins and imipenem. All of these β-lactamase enzymes are commonly found in the Enterobacteriaceae family, most commonly in *Klebsiella* spp., followed by *E. coli* (38, 39). Also in this study *E. coli* showed resistance to multiple drugs, this can also be explained by the possession of ESBL enzymes. Chaudhary and Aggarwal (38) and Sharma *et al*. (39) reviewed that ESBL producing organisms exhibit coresistance to many other classes of antibiotics resulting in limitation of therapeutic options.

Because the causative agents of aerobic vaginitis are part of the host normal flora (opportunistic pathogens), this means that these organisms are continuously exposed to antibiotics used for treating different infections and acquiring resistance to these antibiotics especially if their use is random and sometimes without physician description. Mumtaz *et al*. (9) demonstrated that there were very few antibiotics among the conventially available aminoglycosides, third generation cephalosporins, penicillins, quinolones, sulfonamides, and tetracyclines, possessing good sensitivity (> 80%) against any one of the common aerobic vaginal pathogens. Neu (37) and Karami et al. (40) showed that unnecessary use of antibiotics in humans may be more hazardous than misuse of antibiotics in animal husbandry, because the former targets strains with capacities to persist in the human microbiota and also to cause clinical disease in humans. Also Sharma *et al*. (39) observed a high rate of ESBL production by *E. coli* which may be due to the selective pressure imposed by extensive use of antimicrobials.

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