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Assessing the prevalence and antibiotic susceptibility patterns of S. aureus bacteria isolated from Iraqi women with vaginosis

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Abstract

Bacterial vaginosis (BV) is a common infection that occurs when the number of *lactobacillus spp.* bacteria (vaginal flora) decreases in the vaginal canal. The study aimed to determine the prevalence of *Staphylococcus aureus* within vaginosis in order to emphasize the importance of early detection and treatment. Totally, 90 vaginal swabs were collected using speculum and swabbing. The vaginal swabs were subjected to standard microbiological testing, which included microscopy, cultures (Blood agar and Mannitol salt agar), and antibiotic sensitivity testing. The results showed that out of 90 samples, only 40 *S.aureus* isolates were collected. *S. aureus* isolates showed maximum sensitivity to gentamicin and high resistance to Cefoxitin, Erythromycin, Azithromycin, and moderate resistance to Clindamycin, Rifampicin, Ciprofloxacin, and Doxycycline. Regarding molecular detection of *mecA*, the results revealed that out of 40 *S. aureus* isolates only 22 isolates have this gene. This study concludes that *S. aureus* (MRSA) was the most prevalent within vaginosis with high resistance to most antibiotics.

Keywords: Vaginal infection, MRSA, antibiotics resistance.

تقيم انتشار و نمط الحساسية للمضادات الحيوية لبكتيريا المكورات العنقودية بين العراقيات المصابات بالتهاب المهبل

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الخلاصه

التهاب المهبل الجرثومي (BV) هو عدوى شائعة تحدث عندما ينخفض عدد بكتيريا العصيات اللبنية(الفلورا المهبلية) في القناة المهبلية .هدفت الدراسة إلى تحديد مدى انتشار المكورات العنقودية الذهبية في التهاب المهبل من أجل التأكيد على أهمية الاكتشاف المبكر والعلاج .تم جمع العينات بالكامل(90) عينة باستخدام الموسع ومسحة .تم إخضاع المسحات المهبلية للاختبارات الميكروبيولوجية المعيارية والتي اشتملت على الفحص المجهري والزرع (أجار الدم وأجار مانيتول الملح) واختبار حساسية المضادات الحيوية .أظهرت النتائج أنه من بين 90 مسحة ، تم جمع 40 فقط من المكورات العنقودية الذهبية .أظهرت بكتريا المكورات العنقودية حساسية قصوى للجنتاميسين ومقاومة عالية لسيفوكسيتين وإريثروميسين وأزيثروميسين ومقاومة متوسطة للكليندامايسين وريفامبيسين وسيبروفلوكساسين ودوكسيسيكلين .فيما يتعلق بالكشف الجزيئي لـ*mecA*

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، أظهرت النتائج أن من أصل 40 عزلة من المكورات العنقودية الذهبية فقط 22 عزلة تحمل هذا الجين .
كانت العزلات تحمل هذا الجين ، وخلصت هذه الدراسة إلى أن المكورات العنقودية الذهبية المقاومة للميثيسيلين
(MRSA)كانت الأكثر انتشارًا في التهاب المهبل مع مقاومة عالية لمعظم المضادات الحيوية.

Introduction

Vaginal dysbacteriosis is a disorder that results from bacterial vaginosis (BV) among women of reproductive age**,** causing discharge, odor, and irritation. Bacterial vaginosis is caused by complex bacterial communities colonizing the vaginal cavity [1], many of which are non-cultivable and have interdependent metabolism, with anaerobic populations likely playing a key role in pathogenesis. *Lactobacillus* species account for more than 95 percent of all bacteria in the vaginal normal flora, which includes both aerobic and anaerobic bacteria [2].

It has been shown that Lactobacilli has a role in preventing infections, by its ability to maintain vaginal acidic conditions (PH) and ensure the presence of hydrogen peroxide in the vaginal environment. Furthermore, a recent study on BV pathogenesis reported that Lactobacilli can form a biofilm on the epithelium cells of the vagina that serves as a "scaffold" to which many other bacterial species can adhere in a symbiotic manner and then colonize the vaginal cavity [3]. On the other hand, polymicrobial infection is caused by a decrease in lactobacilli and an increase in pathogenic bacteria, primarily anaerobic or microaerophilic species including *Gardnerella spp*, *Mobiluncus spp, Bacteroide spp, Prevotella species, and Mycoplasma spp.* are among these organisms [4]. Changes in the vaginal flora seen in BV have been linked to preterm birth, spontaneous abortion, postpartum endometritis, HIV, and other sexually transmitted infections [5,6]. Methicillin-resistant *S. aureus* (MRSA) infections have been considered primarily bacteria in nosocomial infection [7].

The infections could be severe, leading to long-period hospitalization and a high rate of mortality. Carriage sites of patients and personnel are the main sources of MRSA.*S. aureus*(MRSA) infections are most reported in patients with burns, and abdominal or vascular surgery [8]. The present case describes a vaginal infection with MRSA. The purpose of this study was to find out how common *S. aureus* bacteria is in vaginal vaginitis and their antibiotic resistance.

Methods

Samples collection

All specimens (90) were collected using a sterile speculum during a vaginal examination. A sampling swab was taken by inserting the swab into the vaginal canal and rotating them for at least 8 sec. Standard microbiological analysis was performed on the vaginal swabs, including microscopy, culturing on (blood and mannitol salt agar), and antibiotic susceptibility testing. 33% (30) of women involved in this study were aged younger than 22, 45% (40) were aged younger than 35, and 22% (20) were younger than 40.

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Antibiotic susceptibility test

The antibiotic susceptibility for *S. aureus was* determined using the Kirby Bauer method. This method was used to determine the susceptibility of bacteria to Gentamycin, Clindamycin, Doxycycline, Ciprofloxacin, Azithromycin, Rifampicin, Erythromycin, Cefoxitin, and Vancomycin and the result was interpreted according to the guideline of CLSI [9].

Molecular detection of *mecA* gene

The bacterial DNA was extracted from bacterial isolates using WizPrep gDNA Mini Kit (Korea). The prevalence of *mecA* gene was detected using Conventional PCR technique. The primer used for detection of these genes was illustrated in Table 1.1 [10]. as follows: final concentration volume 25µl which was prepared by mixing 12.5µl of master mix with 2µl of primers, 3µl of DNA and the volume was completed to 25µl by the addition 7.5µ of nuclease-free water. The mixture was placed in the thermo-cycler (Bioneer Korea), and the steps of amplification were as follows: one cycle at 95°C for 5 min, then 35 cycles in the 30s at 95 °C, annealing step at 53 °C for mecA 30 S and extension at 72 °C for 7 min, then final extension at 72°C for 10 min.

Table 1: Primer sequences of *mecA* gene that used in this study

Primer	Sequence	Tm	Size
mecA-F	5`- GTAGAAATGACTGAACGTCCGATAA-3`	53	310 bp
mecA-R	5`- CCAATTCCACATTGTTTCGGTCTAA-3`		

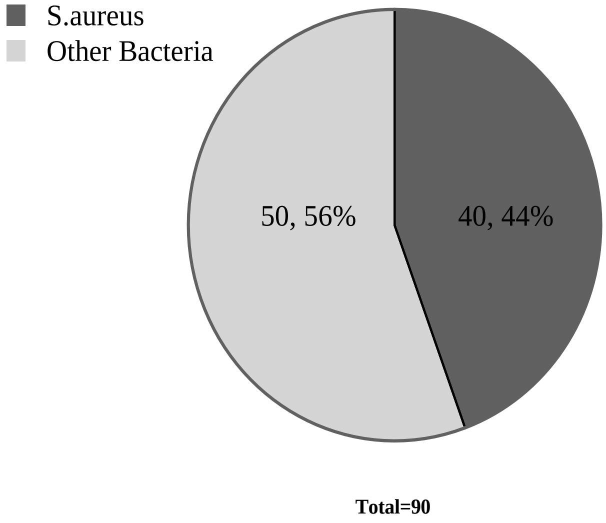

Figure 1: Prevalence of *S. aureus* among participants

Results and discussion

S. aureus prevalence among participants

Among the 90 vaginal specimens that were collected, only 44% (40 isolates) were confirmed as *S. aureus* based on the result obtained from microscopy and biochemical tests Figure 1.

Bacterial vaginal infection is the most common disorder in the lower genital tract, and it is a common cause of sexually transmitted infections STIs like HIV, and bad pregnancies. (11). It has also been linked to preterm labor with birth and late miscarriage in pregnant women, as well as making other gynecologic infections spread more easily [12]. Bacterial vaginosis has a known cause, but it is thought to be sexually assisted and transmitted.

According to the result of this study, of the 90 vaginal swabs the prevalence of *S. aureus* was 44.4% (40 isolates) and detected at ages ranging from 16-60, our result is consistent with Stanley *et al* [13] who had reported that the percentage of *S. aureus* was 57.3%. Our finding is in an agreement with studies mentioned that *S. aureus* isolates are the most prevalent vaginal pathogens.

Antibiotics susceptibility test

The fast development of *S. aureus* to become broadly multidrug-resistant (as a result of the incorrect use of antibiotics) has become a major critical problem. This important threat makes these bacteria the focus of this study. The result of Kirby-Bauer method demonstrated that *S. aureus* was resistant to Azithromycin, Erythromycin, Cifoxitin, Rifampicin, Doxycycline, Clindamycin and Ciprofloxacin with a percentage (92.5%, 87.5%, 97.5%,77.5%,72.5%,72.5%, and 48%) respectively. while 77.5% were sensitive to Gentamycin as shown in Figure 2.

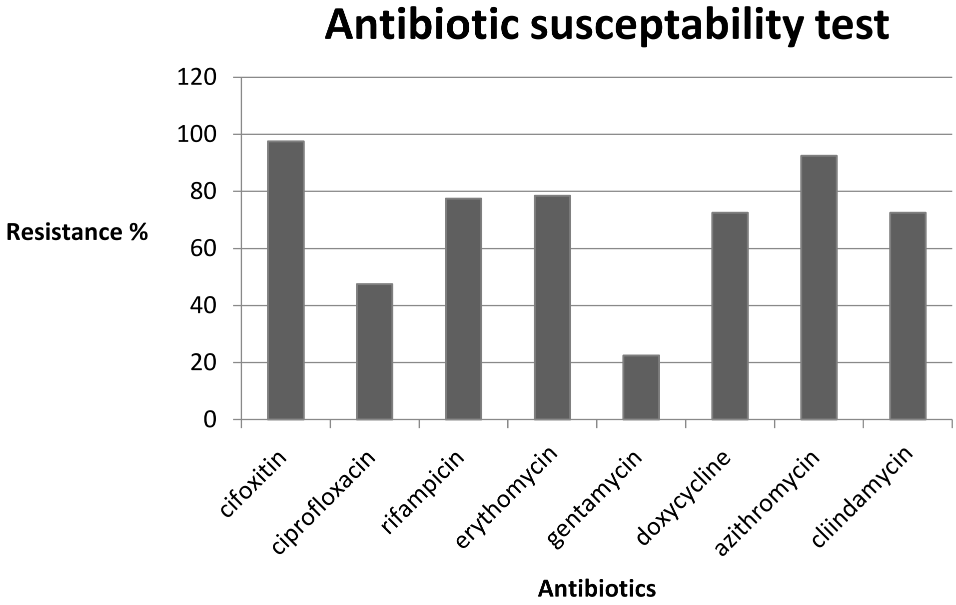

Figure 2: The antibiotic resistance test of *S. aureus*. Gentamycin, Clindamycin, Doxycycline, Ciprofloxacin, Azithromycin, Rifampicin, Erythromycin, Cefoxitin, and Vancomycin

The bacterial isolates (MRSA) showed high resistance to all β-lactam groups due to the presence of *mecA* gene which codes for a penicillin-binding protein with low affinity to β-lactam antibiotics [14]. additionally, different antibiotic discs were used to determine the susceptibility of isolated bacteria. All isolates showed high resistance to β-lactam group and high sensitivity to aminoglycosides group. Our findings are inconsistent with other studies. A stud found that 100% of *S. aureus* were sensitive to β- lactam (14). Another study reported that the isolates were resistant to gentamicin, ciprofloxacin, and cefoxitin [15], while Abdul Jabbar and Al-warid [16] reported that most of the isolates showed sensitivity against penicillin and Gentamicin.

However, Antibiotics' unrestricted over-the-counter availability, as well as their increased use in domestic and commercial animal farming, contribute significantly to the antimicrobial resistance problem, especially in resource-constrained settings [17].

Detection of methicillin resistant S. *aureus* in vaginosis

DNA of *S. aureus* isolates were scanned for the presence of *mecA* gene by using gene-specific PCR primers. Results are considered positive if a distinct band at 310 bp is observed

on 1.5 % agarose gel. out of 40 *S. aureus* isolates, only 55% (22 isolates) have *mecA* within their chromosome Figures 3 and 4.
Figure 4,the percentage of MRSA among Patients

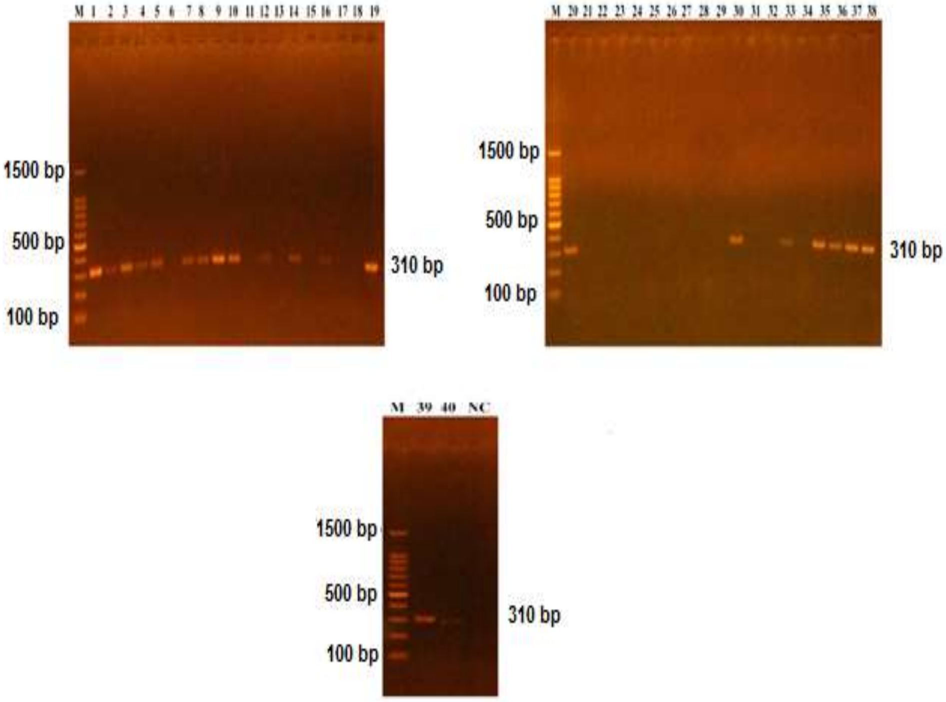

Figure 4: The amplification results of *mecA* gene on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-40 resemble 310bp PCR products

The Opportunistic pathogen *S aureus* infection occurs when the number of *Lactobacillus*, main vaginal microbial flora, changes within women vagina [18]. In the present study, we observed that women with vaginosis were more likely to be colonized by MDR or MRSA. The result revealed that the percentage of MRSA isolates was 55% (22 isolates) of *S. aureus*, compared with other studies that concluded that the percentage of MRSA isolates was very low [19]. while another study reported 14.3% of the vagina colonized bacteria [20].

Conclusion;

This study concludes that *S. aureus* (MRSA) was the most prevalent within vaginosis which has *mecA* gene and showed high resistance to most antibiotics.

Ethical clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq.

Conflict of interest:

The authors declare that they have no conflict of interest.

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