

VIRULENCE FACTORS OF *STREPTOCOCCUS MUTANS* ISOLATED FROM PREGNANT WOMEN WITH ACUTE VAGINITIS⁺

بعض عوامل الضراوة لجرثومة *Streptococcus mutans* المعزولة من النساء الحوامل

المصابات بالتهاب المهبل الحاد

Mohammed Sh. Jebur *

Abstract:

Streptococcus mutans, known to be an etiologic agent of dental caries, also causes infective endocarditic. In the present study, acute vaginitis samples (vaginal swabs) were investigated from pregnant women patients whom admitted to many hospitals of Delivery and Maternal in Baghdad city. Ages of the women ranged from 20-30 years (only marriage one), while all samples were collected during March to July 2009.

One hundred and fifty vaginal swabs were collected and cultured on Blood agar plate, Nutrient agar plates, Chocolate agar plates and MacConkey agar plates, then incubated aerobically and anaerobically for 24 hrs. Bacterial diagnosis were done according to macroscopic, microscopic and biochemical tests.

The results of recent study were showed that the prevalence of bacterial acute vaginitis in pregnant women, 18(12%) as positive cases, while no growth was seen in the rest vaginal swabs 132 (88%). Results of bacterial isolation and identification were showed that out of 18 isolates only 8 isolates diagnosed as *Streptococcus mutans* , *Staphylococcus epidermidis* 8 isolates, *Staphylococcus aureus* 6 isolates, and 5 isolates for each *Streptococcus agalactiae*, *Streptococcus salivaris* and *Proteus spp.*

Results of blood hemolysis of *Strep. mutans* isolates as virulence factors were showed that only two isolates (25%) having ability to produce hemolysin enzyme, and noticed that four isolates (50%) of *Strep. mutans* were having ability to produce extracellular protease enzyme on Mg media. While it have (100%) ability to produce siderophores.

Results of antibiotics sensitivity test of *Strep. mutans* isolates showed that all isolates were resistant (100%)to Ampicillin and Amoxicillin, whereas seven isolates were resistant in a lesser degree (87.5%) to tetracycline and Gentamycin (75%) to Cefotaxime and Ciprofloxacin and (62.5%) to Erythromycin. But also all isolates showed highly sensitivity (100%) to Amoxiclave, Doxycyclin and Ampiclox antibiotics.

The study concluded that *Strep. mutans* isolates were showed moderate prevalence in the distribution of pathogenic microorganism of acute vaginitis. These isolates were having (25%) ability of blood hemolysis, (50%) produce protease enzyme and (100%) siderophores production. *Strep. mutans* vaginal isolates were appeared high sensitivity(100%) to Amoxiclave, Doxycyclin and Ampiclox antibiotics, but it had 100% resistance to synthetic Penicillin(Ampicillin & Amoxicillin) and there were different susceptibility to rest antibiotics that used in the study.

The study recommended that isolation and identification of the causative agent as well as its virulence factors are very important and also using of sensitivity test having high value to set the scientific and medical antibacterial drugs to patients.

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*Prof . / Institute of Medical Technology.

المستخلص:

تعرف بكتريا *Streptococcus mutans* بكونها المسبب المرضي لتسوس الأسنان والتهاب شغاف القلب. في الدراسة الحالية تم جمع مسحات مهبليّة من نساء حوامل يعانون من التهاب المهبل راجعوا مستشفيات النسائية والتوليد في مدينة بغداد للفترة من آذار إلى تموز 2009. تم فحص وتشخيص جميع الحالات المدروسة من قبل الطبيبة الاختصاص على أنها حالات مرضية (مصابة بالتهاب المهبل). وقد تراوحت أعمار النساء الحوامل المصابات ما بين 20 إلى 30 سنة.

جمعت مائة وخمسون مسحة مهبليّة وزرعت على أوساط نمو الزرع البكتيري أكار الدم والآكار المغذي وآكار الجوكليت وآكار الماكونكي وحضنت بدرجة حرارة 37 °م ولمدة 24 ساعة. تضمنت فحوص العزل والتشخيص الفحص المظهري للمستعمرات النامية والفحص المجهرى بعد صبغها بصبغة كرام والفحوص الكيموحيوية (Biochemical) .

أوضحت نتائج الدراسة أن نسبة الإصابة بالتهاب المهبل الحاد المتسبب من البكتيريا كان 12% حيث كانت المسحات الموجبة للفحوص البكتيرية 18 مسحة ، في حين كانت المسحات السالبة 88% بواقع 132 مسحة والتي قد تكون لأسباب أخرى غير بكتيرية . أما نتائج العزل والتشخيص فقد سجلت أعلى نسبة عزل لبكتريا *Streptococcus mutans* بواقع 8 عزلات من مجموع 18 عزلة بكتيرية ، في حين عزلت *Staphylococcus epidermidis* و *Staphylococcus aureus* و *Streptococcus agalactiae* و *Streptococcus salivaris* و *Proteus spp* بواقع 8، 6 و 5 (لأنواع الثلاثة الأخيرة) على التوالي .

أظهرت نتائج فحوص الضراوة أن عزلتين (25%) من *Streptococcus mutans* كانت لها القابلية على تحلل الدم بينما كانت لأربع عزلات (50%) القدرة على إفراز إنزيم تحلل البروتين على وسط Mg أزرعي . وأظهرت جميع العزلات (100%) قابلية على إنتاج السايروفور (Siderophores) .

أوضحت نتائج فحص الحساسية أن جميع عزلات جرثومة *Streptococcus mutans* كانت مقاومة لل Gentamycin و Ampicillin و Amoxcillin وبنسبة 100% . وكانت أيضا مقاومة لل Tetracycline و Gentamycin بدرجة أقل وبنسبة 87.5% . بينما كانت المقاومة بنسبة 75% لكل من Ciprofloxacin و Cefotaxime وبنسبة 62.5% لل Erythromycin . وأظهرت جميع العزلات حساسية عالية جدا (100%) لل Amoxiclave و Doxycyclin و Ampiclox .

نستنتج من نتائج الدراسة أن عزلات جرثومة *Strep. mutans* توزعت بنسب قليلة نسبيا (12%) عند النساء المصابات بالتهاب المهبل الحاد. ولوحظ أن (25%) من هذه العزلات لها القابلية على تحلل الدم في الوسط الزرعي، (50%) إنتاج إنزيم تحلل البروتين و(100%) إنتاج السايروفور. بينما تفاوتت حساسية ومقاومة الجرثومة للمضادات الحياتية المستخدمة .

توصي الدراسة بأهمية العزل والتشخيص المختبري لجرثومة *Strep. mutans* ومعرفة عوامل الضراوة ونمط الحساسية والمقاومة للمضادات الحياتية والتي تقود الى توصيف المضاد الحياتي المناسب لعلاج الحالات المصابة بشكل علمي دقيق.

Introduction:

Vaginitis is usually characterized by a vaginal discharge or vulvar itching and irritation; a vaginal odor may be present. The three common diseases associated with vaginal infection include trichomoniasis (15-20%), bacterial vaginosis (40-45%), and vulvovaginal candidiasis (20-25%) or, not infrequently, a combination. Other causes of vaginal discharge or irritation include mucopurulent cervicitis caused by *Chlamydia trachomatis* or *Neisseria gonorrhoeae*, or herpes simplex virus, atrophic vaginitis, allergic or irritant reactions (spermicides, deodorants, minipad adhesive), vulvar vestibulitis, lichen simplex chronicus and lichen sclerosis (especially pruritis) and foreign bodies [1,2].

The vagina is a dynamic ecosystem that normally contains approximately 10^9 bacterial colony forming units per gram of vaginal fluid. The normal bacterial flora is dominated by lactobacilli, but a variety of other organisms, including some potential pathogens, are also present at lower levels. Lactic acids and other organic acids are metabolized from glycogen by the lactobacilli, maintaining the vaginal pH between 3.8 and 4.2. The acidic environment inhibits the overgrowth of bacteria and other organisms with pathogenic potential. The normal vaginal discharge is clear to white, odorless, and of high viscosity [3, 4].

One of the most common agents bacteriologically to vaginitis is *Streptococcus mutans*, which is gram positive bacteria, spherical that form pairs or chains during its growth; some are member of normal flora of oral cavity, intestine and vagina [4]. The major route for early acquisition of *Streptococcus mutans* is vertical transmission from mother to child [4].

Virulence factors of *Strep. mutans* help to protect the bacterium against possible defenses and maintain its ecological niche in the sites of growth. Probable virulence factors include adhesions, acid tolerance, protease production and production of glycosyltransferase and intracellular polysaccharides[5].

Pathogenic *Streptococcus mutans* produce hemolysin enzyme as a virulence factor, which plays a role in blood invasion and in supplying the bacteria with their requirements of iron[6]. Antibiotic resistances of the bacteria consider in some cases as virulence factors because of the ability of *Strep. mutans* to avoid the action of these antibiotics and have another way to grow and multiply in the sites of infections [7].

The recent study aimed to isolate, identify *Strep. mutans* isolates which are associated with vaginitis infection in women patients. Also studying some virulence factors of these isolates such as production of hemolysin enzyme, protease enzyme and profile of their antibiotics resistance.

Materials and methods:

1- Collection of specimens :

One hundred Fifty vaginal swabs were collected from pregnant women who attended the gynecology clinic, with symptoms of acute vaginitis .These patients were admitted to many hospitals of Delivery and Maternal in Baghdad city. The women aged from 20-30 years (only married one). All samples were collected during March to July 2009.

All patients had not taken any antibiotics within the previous (5-10) days before attending the hospital. The swabs were taken by a gynecologist from vaginal fonices with the use of a sterile speculum. Swabs for culture were placed in sterile tubes containing 5 ml of normal saline to maintain and have bacterial suspension, until being examined in the laboratory [8].

Fifty micro liter(μL) of bacterial suspension were immediately inoculated on Blood agar plates, Nutrient agar plates, Chocolate agar plates and MacConkey agar plates, and then incubated aerobically and anaerobically for 24 hrs. [9]. All media used for isolation and diagnosis taken from (Difeco company) and prepared according to the recommendation of the company.

2- *Strep. mutans* isolation:

Nutrient agar supplemented by sodium azid with concentration 1:16000 to inhibit gram negative bacteria and crystal violate with concentration 1:500000 to inhibit *Staphylococci* that became enrichment and selective media for Streptococcal growth [10].

3- **Bacterial diagnosis** : All procedures were done according [10, 11, 12] through :

A: Macroscopic examination of single colony which was taken from pure bacterial culture that depended on its morphology (size, shape).

B: Microscopic examination of selected colony which investigated by gram stain to observe specific features of the isolated bacteria (gram positive bacteria).

C: Biochemical tests: Specific biochemical tests (Oxidase, Catalase, Coagulase, Vancomycin susceptibility and Motility test) were done to have the final identification.

4-**Virulence factors of *Strep. mutants*** isolates mentioned by Blood Hemolysin test (using sheep blood) Protease production test and Antibiotics Profile Resistance. Antibiotics resistance was done according to disc diffusion method [13] by using pure culture of four previously identified isolates. The inoculums which be used were prepared through 1ml of bacterial suspension (1.5×10^8 CFU/ml), inoculated by streaking on Mueller-Hinton agar, antibiotics discs (listed in table-) were placed on the surface of cultures then incubated overnight with 37°C . Antibiotic zone of inhibitions were measured and compared to standard zones (as the instruction of the company) to determine the sensitivity or resistance of these isolates to each antibiotic.

5- **Statistical analysis was carried out with excel tools.**

Table (1): Antibiotic discs with its potency (from Oxiod Company).

No	Antibiotics	Symbol	Disk-potency $\mu\text{g/ml}$
1	Gentamycin	GEN	10
2	Cefotaxime	CTX	30
3	Tetracycline	TET	30
4	Ampicillin	AMP	10
5	Erythromycin	ERY	30
6	Amoxillin	AMX	10
7	Doxicyclin	DOX	30
8	Ciprofloxacin	CIP	30
9	Amoxiclave	AMC	15
10	Ampiclox	APX	15

Results and Discussion:

The results were showed that the prevalence of bacterial acute vaginitis of pregnant women (in different months of pregnancy), 18(12%) as positive cases. These results had indicated the susceptibility of women for infection, and this might be due to alteration in female hormones during pregnancy and menstruation periods [14] and explain that in these two physiological conditions the level of progesterone increased causing vasodilatations which leading to the inflammations of tissues [15].

All swabs were subjected for culturing on available media showed out of total 150 samples, only 18(12%) gave positive bacterial culture , while no *Strep. mutans* growth was seen in the rest vaginal swabs 132 (88%). These results also indicated the presence of other pathogenic non bacterial agents. (Table-2).

Results of culturing showed that most vaginal swabs gave mixed cultures 15(83%) this might be attributed to the fact that vaginal media provide favorable environments for colonization of more species, aerobic, facultative anaerobic bacteria and other pathogenic non bacterial agents [16].

The results of bacterial isolation and identification according to macroscopic, microscopic finding and biochemical tests, showed that the mixed cultures were 15(83%) and out of 18 isolates there were only 8 isolates diagnosed as *Streptococcus mutans*, while other isolates distributed to *Staphylococcus epidermidis* 8 isolates, *Staphylococcus aureus* 6 isolates, 5 isolates for each *Streptococcus agalactiae*, *Streptococcus salivaris* and *Proteus spp.* The results reported also the presence of two isolates of facultative aerobic bacteria *Streptococcus pyogenes* and anaerobic bacteria *Neisseria spp.* (Table-3). These results were obtained from pregnant woman suffering of acute vaginitis might consider normal flora of vagina [17] but those patients had a symptoms of pelvic pains [18], with disappearance of *Lactobacillus spp.* bacteria which explained the opportunistic nature of these isolates [19] and present in coaggregation each other that plays critical role in the ecology of bacterial growth to vaginal site.

Results of blood hemolysis of *Strep. mutans* isolates as virulence factors were showed that only two isolates (25%) having ability to produce hemolysin enzyme (Table-4), Which explain the flexibility of such bacteria to have number of mechanisms for acquisition of iron from its environments[20]. There is another mechanism for iron acquisition by producing siderophores which chelate iron with a very high affinity and complete effectively with transferring and lactoferrin to mobilize iron for bacterial use [21]. Isolates (100%) of *Strep. mutans* have ability to produce siderophores (Table-4), this ability was tested by siderophore synthesis grown on Mg media containing dipyrindyl [22]. Also the results were showed that all isolates (100%) of *Strep. mutans* having ability to produce siderophores (Table-4).

Furthermore, it is known that bacteria have the ability to produce hemolysin have no ability to produce siderophores, as in our results (high siderophores production with low hemolysin production), such bacteria need only one mechanism for obtaining iron [23].

As shown in table-4, the results noticed that four isolates (50%) of *Strep. mutans* have the ability to produce extracellular protease enzyme on Mg media. When this enzyme reach vaginal mucosa it might often encounter the vaginal fluid IgA, which could inhibit their adherence and attachment to the vaginal tissues, that causes vaginitis and play central role for local and humeral immune response [24]. Protease production from causative agent could contribute directly to pathogenesis of vaginitis by degrading the host defense protein such as immunoglobulin and complement and or cleaving other streptococcal surface protein[25].

Results of antibiotics profile resistance of *Strep. mutans* isolates showed that all isolates(100%) were resistant to Ampicillin and Amoxicillin , whereas seven isolates had shown resistance in a lesser degree (87.5%) to tetracycline and Gentamicin and (75%) to Cefotaxime and Ciprofloxacin and (62.5%) to Erythromycin (Table-5). But all isolates were showed high sensitivity (100%) to Amoxiclave, Ampiclox and Doxycycline antibiotics .These results of wide range susceptibility of *Strep. mutans* to different antibiotics which used in this study (from 100% resistant to 100% sensitivity) may be obtained due to recurrent or random usage of antibiotics in treatment of vaginitis [26],also the sensitivity of some isolates to other antibiotics not widely used in treatment of vaginitis [27].The increasing of resistance due to abuse of antibacterial leading to transferring the resistance through genetic factors such as

plasmid and transposons or due to changing in wall permeability [28] , and this indicate some of virulence effects of *Streptococcus mutans* .

Table-2: Distribution of acute vaginitis cases and cultures.

Culture	Samples	% cultures	Mixed cultures	Single cultures
Positive	150	18(12%)	15(83%)	3(16%)
Negative	150	132(88%)		

Table-3: Bacterial isolates from vaginal swabs of pregnant women with acute vaginitis.

Isolates	Number of isolates	Percentage (%)
<i>Strep. mutans</i>	8	44.4
<i>Staph. epidermidis</i>	8	44.4
<i>Staph. aureus</i>	6	33.3
<i>Strep. agalactiae</i>	5	27.7
<i>Strep. salivaris</i>	5	27.7
<i>Proteus spp.</i>	5	27.7
<i>Strep. pyogens</i>	2	11.1
<i>Neisseria spp.</i>	2	11.1
Total	41	% 100 for each isolate

Table-4: Virulence factors of *Strep. mutans* isolates.

No. of isolates	Hemolysin enzyme	Extracellular Protease	Siderophores production
1	+	+	+
2	-	-	+
3	-	-	+
4	-	+	+
5	-	+	+
6	+	-	+
7	-	+	+
8	-	-	+

Table-5: Antibiotics susceptibility of *Strep. mutans* isolates.

No. of isolates	Antibiotics									
	GEN	CTX	TET	AMP	ERY	AMX	DOX	CIP	AMC	APX
1	S	R	R	R	R	R	S	R	S	S
2	R	R	R	R	R	R	S	R	S	S
3	R	R	R	R	R	R	S	R	S	S
4	R	S	S	R	S	R	S	S	S	S
5	R	S	S	R	S	R	S	S	S	S
6	R	S	R	R	R	R	S	S	S	S
7	R	R	R	R	R	R	S	R	S	S
8	R	R	R	R	S	R	S	R	S	S

R: Resistant isolates. S: Sensitive isolates.

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