Isolation and Characterization of *Streptococcus agalactiae* from Woman Patients with Vaginitis in Hilla Province

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Abstract

In this study, 120 vaginal swabs obtained from women suffering from vaginitis, and admitted to Babylon Hospital of Delivery and Maternal in Hilla Province were included. It was found that only three isolates of *Streptococcus agalactiae* were identified. All isolates underwent culture and biochemical tests to confirm diagnosis, and it was reveled that all isolates gave the same cultural and biochemical characters except in their ability to grow in 6.5% NaCl. However, other types of bacteria and yeasts were also isolated. *Streptococcus agalactiae* isolates were isolated mainly from non-pregnant women but there was no isolates obtained from pregnant women.

The ability of the bacterial isolates to produce bacteriocin was also investigated and it was found that only one isolate had the ability to produce bacteriocin that had effect on other *Streptococcus agalactiae* strains isolated in this study.

The effect of lactic acid on the bacterial growth was likewise studied. It was concluded that lactic acid at high concentration >20µg/ml could cause inhibition to *Streptococcus agalactiae* growth.

الخلاصة

في هذه الدراسة, تم الحصول على 120 مسحة مهبلية من النساء المراجعات إلى مستشفى الولادة والأطفال في الحلة واللواتي يعانين من التهاب المهبل. وقد عزلت فقط ثلاث سلالات من بكتيريا *Streptococcus agalactiae* والتي تمتلك نفس المواصفات الزرعية والبايوكيماوية باستثناء قابليتها على النمو بوجود كلوريد الصوديوم بنسبة 6.5%, ولوحظ أيضاً بان جميع العزلات قد تم عزلها من النساء المتزوجات غير الحوامل في حين لم تعزل أي عزلة من النساء الحوامل.

لوحظ بان عزلة واحدة من البكتريا لها القابلية على إنتاج البكتريوسين والتي لها القدرة على التأثير على العزلات الأخرى التابعة لنفس البكتريا. وأظهرت النتائج بأن حامض اللاكتيك عند التراكيز التي تفوق 20 مايكروكرام/مل يمتلك تأثير تثبيطيا على نمو عزلات البكتريا.

Introduction

Streptococcus agalactiae or Group B *Streptococcus* (GBS) is gram-positive coccus which appears in chain or pairs. It is usually beta-hemolytic and reliably identified by its production of Lancefield group B antigen [1]. *Streptococcus agalactiae* has been classified serologically into 9 serotypes (Ia, Ib, and II-VIII) according to difference in capsular polysaccharide [2]. The gastrointestinal tract is the most likely human reservoir for *Streptococcus agalactiae*, whereas the genitourinary tract is the most common site of secondary spread. It is a member of the normal flora of the female genital tract, and in most studies from 10%-30% of pregnant women are colonized with

Streptococcus agalactiae in the vaginal or rectal area [3]. However, this agent is frequently implicated as an important cause of a severe invasive disease primarily in newborns, pregnant women, and adults with underlying disease [4].

Most of the infection with this bacteria can be prevented by using antibiotics such as penicillin or ampicillin [5].

There is no independent study on this bacteria conducted in Iraq; this work therefore, aims to study the isolation and identification of *Streptococcus agalactiae* associated with vaginitis.

Patients and Methods

Patients

One hundred and twenty samples are collected from woman suffering from severe to moderate vaginitis. The period is from October 2003 to March 2004.

Collection of specimens

The specimens were collected from patients with vaginitis. The swabs were inserted into the upper part of the vagina and rotated there before withdrawing it, so that exudates was collected from the upper as well as the lower vaginal wall. An endocervical swab must be collected. A vaginal speculum must be used to provide a clear sight of the cervix and the swab was rubbed in and around the introitus of the cervix and withdrawn without contamination from the vaginal wall.

Swab for culture should be placed in tubes containing normal saline to maintain the swab moist until taken to laboratory. The swab has been inoculated on culture media and incubated aerobically for 24hr. at 37°C.

Isolation and Identification

A colony that is gray has been selected with hemolysis blood agar (growing on blood agar) and showing beta hemolysis on blood agar. It has been identified depending on its morphology (shape, size, color) and then examined under microscope after staining it with gram stain (It appears in pair or in chain and gram positive).

After staining the bacteria, its specific shape, color, aggregation and specific intracellular compound have observed. Biochemical tests have been done to reach final identification according to Bergy's Manual For Determinative Bacteriology [6].

Growth in NaCl

Two to three colonies were inoculated into a tube of nutrient broth containing various concentrations of NaCl (4.5%, 5%, 5.5%, 6%, 6.5%, 7%) and the tube is then incubated at 35°C for 24hr. The growth is judged by the turbidity seen after dispersing any sediment indicated to positive growth, otherwise the growth is negative [7].

Bacteriocin production

The method of Abbot and Shannon [8], developed by Abbot and Graham [9] have been used.The bacteriocin production was scored as growth inhibition at the medial streak line.

Effect of Lactic acid on bacterial growth

1- Nutrient broth was prepared and distributed in tubes and lactic acid was added to each tube at various volumes to gain the final concentrations (10, 20, 40, 60, 80, 100µg/ml)

2- Positive control was prepared by using nutrient broth free from lactic acid.

3- The tubes in item 1 and 2 were inoculated with 0.5 ml of bacterial suspension and then incubation 24hr. at 37°C.

4- After incubation, the absorbance was read at wave length 520nm by using spectrophotometer to show the effect of lactic acid on the growth of bacteria strain.

Results and Discussion

Isolation and Characterization

All swabs were subjected for culturing on available media and it was found out of the total of 120 samples, only 96 samples showed positive cultures, 66 bacterial isolates and 30

yeast isolates. No growth was seen in the other samples (24 samples) which could indicate the presence of microorganisms that might be cultured with diffeclty such as viruses, Chlamydia, and other agents.

The results of bacterial isolation (table 1) showed that only three isolates of *Streptococcus agalactiae* have been isolated from non-pregnant women suffering from vaginitis

Table 1 Isolation of Bacteria from Non-pregnant Women with Vaginitis

ISOLATES	***NON-PREGNANT %***		***CLINCAL SIGNS***
Staphylococcus epidermidis	17	34%	Most of infected women has vaginal discharge and itching
Pseudomonas aeruginosa	15	30%	
Lactobacillus	6	12%	
Klebsiella pnumoniae	4	8%	
Streptococcus agalactiae	3	6%	
Moraxella catarrahlis	3	6%	
Acinetobacter	2	4%	
Total	***50***	***100%***	

In the table (1), the most common types of bacterial isolates from non-pregnant women were *Staphylococcus epidermidis* (17) followed by *Pseudomonas aeruginosa* (15), *Lactobacillus*(6), *Klebsiella pnumoniae*(4), *Streptococcus agalactiae*(3), *Moraxella catarrahlis*(3), and *Acinetobacter* (2). Whereas the most common types of bacterial isolates in pregnant women as shown in table (2) were *Staphylococcus epidermidis* (7) followed by *Lactobacillus* (5), *Klebsiella pnumoniae* (2), and *Pseudomonas aeruginosa* (2).

Table 2 Isolation of Bacteria from Pregnant Women with Vaginitis

ISOLATES	***PREGNANT %***		***CLINCAL SIGNS***
Staphylococcus epidermidis	7	43.75%	Vaginal discharge and itching
Lactobacillus	5	31.25%	
Klebsiella pnumoniae	2	12.5%	
Pseudomonas aeruginosa	2	12.5%	
Total	***16***	***100%***	

The result was correlated with the results obtained by [10], [11] and [12], who have pointed out that the bacterial flora in non-pregnant women are the most common types in vagina. Whereas the results of bacterial types among pregnant women were similar to those obtained by [13] and [14].

This study is concerned with *Streptococcus agalactiae* because there is no sufficient studies carried out on this bacteria in Iraq, although it has been isolated in Najaf and Baghdad [15] and [16].

This bacteria has been isolated in previous studies and most of these studies have stated that this bacteria is mostly prevalent among pregnant women and less frequently in non pregnant women [17].

The results documented in this work were identical with the results obtained by [18] and [19] who have indicated that the prevalence of *Streptococcus agalactiae* among non-pregnant women is higher than in pregnant women.

Whereas other studies have pointed out that the prevalence of this bacteria among pregnant women is higher than that of in non-pregnant women.

However, [20] have proved that the rate of isolation of *Streptococcus agalactiae* from vaginal swabs ranges from 5-40% due to difference in the sample sites and culture method employed.

2- Effect of NaCl on the growth of *Streptococcus agalactiae*

It has been found that all *Streptococcus agalactiae* isolates can grow until 6.5% except one isolate which has failed to grow in 6% or above. Furthermore, all the isolates have failed to grow in 7% of NaCl or above (Table 3).

Table 3 Effect of Different Concentrations of NaCl on the Growth of *Streptococcus agalactiae*

	Concentration of NaCl					
Isolation	***4.5%***	***5%***	***5.5%***	***6%***	***6.5%***	***7%***
1	+	+	+	+	+	-
2	+	+	+	+	+	-
3	+	+	+	-	-	-

(+) GROWTH (-) NO GROWTH

Numerous reference manuals indicate that *Streptococcus agalactiae* is either unable to grow in the media containing 6.5% NaCl [21] or has a variable capacity to do that. [22] have stated that *Streptococcus agalactiae* can grow in a concentration at NaCl up to6.5%. However, this test is not performed routinely by the laboratories in the standard procedure for the identification of beta hemolytic streptococci. Therefore, to establish the proportion of *Streptococcus agalactiae* isolates that were able to grow in 6.5% NaCl, all isolates selected, regardless of the type of hemolysis produced were submitted to this study [23].

Our results suggest that a considerable proportion of *Streptococcus agalactiae* strains have the ability to grow in 6.5% NaCl. Further research should be conducted to determine if other streptococci isolated from vagina can grow at frequently in NaCl.

3- Effect of Lactic acid on the Growth of *Streptococcus agalactiae*

The effect of lactic acid at different concentrations (10-100µg/ml) on *Str. agalactiae* growth has been investigated

(Figure1). Colorimetric method has been used for this purpose [24].

It has been observed that the growth absorbance of the bacteria without addition of lactic acid is 0.609; the growth rate decreased when lactic acid was added at a concentration 10µg where the absorbance is 0.482. When the lactic acid concentration increases until 100µg/ml, the absorbance decreases to 0.075.

This may be attributed to the presence of lactic acid bacteria and other lactic acid producing microorganism such as *Streptococcus* spp. and yeast.

[25] have pointed out that lactic acid bacteria can prevent the growth of *Streptococcus agalactiae*.

The same results were shown by [26], who have pointed that lactobacilli can control vaginal bacterial microflora through the production of the lactic acid. However, in this study, lactobacilli has been isolated uniquely, where it may prevents the growth of other bacteria and protect the vagina from invasive microorganisms.

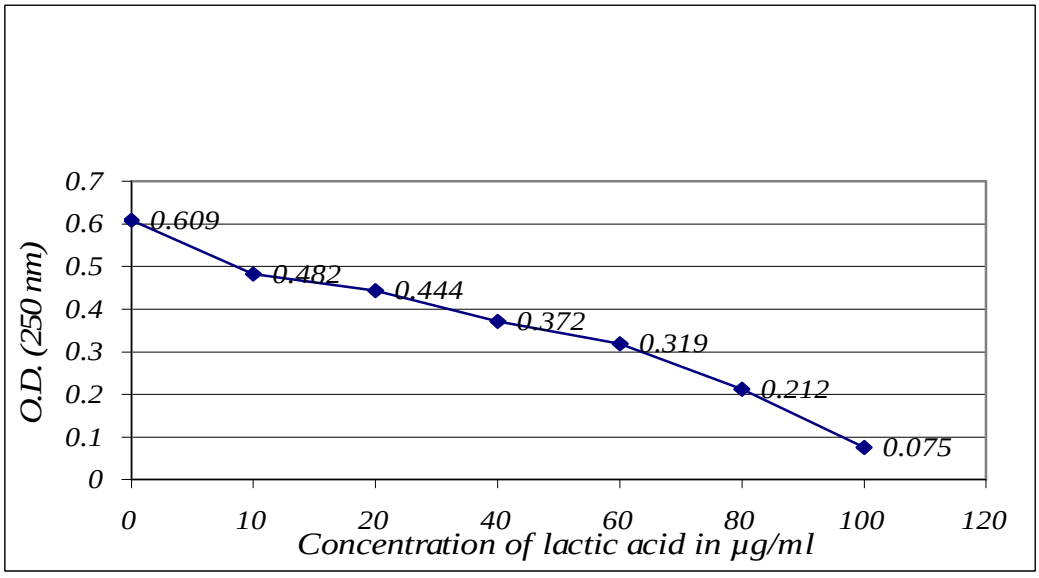

Figure 1 The Effect of Lactic acid on the Growth of *Streptococcus agalactiae*

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- Bacteriocin Production

Bacteriocin is antimicrobial protein produced by bacteria that kill or inhibit the growth of other bacteria related to the same group or species [27].

Bacteriocin production was investigated by using GBS isolates and it was found that only one isolate (no.1) is able to produce bacteriocin by using cross streaking technique, that only one isolate (no.2) is sensitive to it (Figure 2).

This result is identical with the result obtained by [28] who have pointed that GBS is able to produce bacteriocin and it is considered a virulence factor for GBS.

Bacteriocin plays a role in spreading the bacteria inside the host body. This bacteriocin is also produced and secreted without using inducible agents such as mitomycin C widely used in bacteriocin induction. Bacteriocin produced by indigenous bacteria may be critical for the maintenance of normal microflora and host health by preventing invasion by exogenous pathogens (Brook, 1999).

Figure 2 Bacteriocin production by *Streptococcus agalactiae*
* The producer is isolate No. 1
*The sensitive bacteria is isolate No. 2
*The resistant bacteria is isolate No. 3

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