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Isolation and Identification of some Bacterial Species From Cervix Infected Women in Salahuddin Governorate

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Abstract :

This study was carried out for isolating some pathogenic types of positive and negative bacteria for Gram stain, which cause the infection of the vaginitis in infected women and its diagnosis by routine laboratory methods and confirm the diagnosis using Vitek2compact system by collecting fifty samples of the swab of the cervix in the period between October 2015 till April 2016 from the in patients and out patients women in Salahuddin Hospital for the age groups between (14-50) years. The results of isolation show the percentage of bacterial growth was 78%, it reached samples in 78%. The results of the diagnosis of routine diagnostic tests, which were confirmed by using VITEK compact2 system that there was a diversity of bacterial species including bacterial species from the source of isolation of vaginal infection, where the *Escherichia coli* bacteria had the highest isolation rate of isolation in 22%, followed by other bacterial species with different isolations. It was observed that there was a variation in the isolation rates and bacterial infections species of the vagina where bacterial isolates were isolated *Proteus mirabilis* isolates in 20.5% and as *Klebsiella pneumonia* showed isolations in 10.3%. For *Salmonicida Aeromonas* and *Staphylococcus hominis* Bacteria, the isolates was in 7.7%, While *Pseudomonas luteola*, *Pantoos spp*, *Pseudomonas oryzihabitans*, *Ochrobactrum anthropic*, *Sphingomonas paucimbilis* bacteria, isolations were isolated in 5.1 %. All isolates were characterized by their high resistance to ten different types of antibiotics, which represent: Gentamicin, Oxacillin, Tetracyclin, Cefotaxme, Amplicillin Novabiocin, Chloramphenicol, Rifampin, Trimethoprim and Azithromycin. The bacterial isolates gave absolute resistance to 100% of the majority of antibiotics used in our current study, while most isolates were sensitive to antagonists Gentamicin

المستخلص

أجريت هذه الدراسة لغرض عزل بعض أنواع البكتيريا الموجبة والسالبة لصبغة جرام المسببة لالتهاب المهبل لدى النساء وتشخيصها بالطرق المختبرية الروتينية وتأكيد التشخيص باستعمال جهاز Vitek2compact system، تم جمع خمسون مسحة من عنق الرحم في الفترة ما بين شهر تشرين الاول 2015 الى نيسان 2016 من النساء الراقداً والمراجعات الى مستشفى صلاح الدين وكانت اعمار النساء تتراوح ما بين (14-50) سنة، أظهرت نتائج العزل، إن العينات التي اعطت نمواً بكتيريا والمأخوذة من عنق الرحم فبلغت تسعة وثلاثون عينة من مجموع 50 عينة ونسبة (78%)، أظهرت نتائج التشخيص بالاختبارات التشخيصية الروتينية والتي تم تأكيدها باستخدام جهاز VITEK compact2 System ان هناك تنوع في الاجناس والانواع البكتيرية المعزولة من عنق المهبل حيث أظهرت النتائج ان بكتيريا *Escherichia coli* احتلت اعلى نسبة عزل بواقع 11 عزله بنسبة (22%) من التهاب عنق المهبل، بينما تلتها الانواع البكتيرية الاخرى حيث لوحظ أن هناك تنوع في نسب العزل و الاجناس البكتيرية المسببة لالتهاب المهبل حيث عزلت بكتيريا *Proteus mirabilis* بواقع (8) عزلات بنسبة 20.5%، أما

Aeromonas بكتريا لكل من بكتريا *Klebsiella pneumoniae* ظهرت بعدد (4) عزلات بنسبة 10.3% ، أما بالنسبة لكل من بكتريا *Staphylococcus hominis* , *salmonicida* فقد كانت بعدد (3) عزلات بنسبة 7.7% ، في حين شكلت كل من بكتريا *Pseudomonas luteola* , *Pantoea spp* , *Pseudomonas oryzihabitans* , *anthropic Ochrobactrum Sphingomonas paucimobilis* ، عزلتين بنسبة 5.1% ، إن العزلات البكتيرية اعطت مقاومة مطلقة 100% لغالبية المضادات الحيوية المستخدمة في دراستنا الحالية، بينما اغلب العزلات كانت حساسة للـ Gentamicin .

Introduction

The natural Microbial flora of the vagina is currently considered as an important factor to protect the vagina from different types of causing the pathogenic diseases, where there are many types of microorganisms, the most important of which are lactic acid bacilli that contribute to maintaining the acidity of the vagina throughout the production of acid by fermenting to the collagen in the lining cells. These mechanisms seem to help preventing various types of pathogenic bacteria from settling in the vagina and causing infection as well as producing many inhibitors such as hydrogen peroxide H_2O_2 bacterocin (^{1,2}), vaginitis occurs because of the excessive growth of bacteria, Increase in vaginal secretions, most often, it smells odors or fish and the secretions are white or gray and cause heartburn when urinating and itching(^{3,4}). Bacterial vaginosis B.V is known as an imbalance in vaginal microorganisms with a decrease in the number of Lactobacilli, where most cases begin with the formation of Biofilm which helps in the growth and prosperity of opportunistic bacteria (^{4,5,6}). Vaginal infection increases the risk of early birth among pregnant infected patient women. The infected patient women aged between (14-49) years affected where the B.V is more common in infected patient women of reproductive age (⁷). Vaginitis may sometimes affect infected patient women after menopause and iron deficiency may be associated with bacterial vaginosis at an early stage of

pregnancy (⁸). The existence of female partner increases the risk of B.V with rate 60% where the bacteria were isolated associated with the occurrence B.V of the male genitalia and transmitted after intercourse from female to male (⁹).

- 1 - Isolation of some bacterial species that affect women in vagina.
- 2 - Diagnosis of isolated bacterial species and determine their sensitivity to ten types of antibiotics.
- 3 - confirm the diagnosis of bacterial isolates using the Vitek Compant 2 system.

MATERIALS AND METHODS OF WORK

Collection of Samples

There are (50) samples which have taken from the vaginal area of infected patient women for the period from October 2015 into April 2016, and between the ages of (14-50) years of the in patients and out patients in Salahuddin Hospital who suffer from symptoms of vaginitis The samples were taken by a cotton swab from infected patients with vaginitis. Information relating to age, previous infections of the vagina and antibiotics were recorded.

Insulation and Diagnosis

The bacteria were diagnosed using the blood agar, MacConkey agar, Mannitol salt agar, and a group of biochemical tests. Then, the diagnosis was confirmed using the Vietick compact 2 system.

- 1- Methyl red test
2. Voges - proskauer test
3. Urease test
- 4 - Citrate utilization test
5. The production of H₂S gas Test
6. Motility test
7. Test of blue methylene and Eosin
8. Indol test

Antibiotics Sensitivity Test Using Disc Diffusion Method

The antibiotic sensitivity test was performed on Agar Muller Hinton Medium using 10 types of antibiotic disc. The bacterium genus took one colony to 5 ml from the nutrient agar and incubated at 37 ° C for 24 hours.

The growth curve was contrasted with the previously recorded standard McFarland solution, (⁸10 x 1.5) cell/ml, and then spread 0.1 ml of the bacterial cultured above in Agar Muller Hinton Medium by a sterile glass diffuser and then leave to dry at room its temperature for 10-15 minutes, force the (Forceps) into the dishes by 6 tablets for each dish and then incubate dishes at 37 ° C for 24 hours, the diameter measuring Damping areas (including disk diameter) and divided the isolates into 3 categories are sensitive and medium sensitivity and resistance based on global measurements of (^{11,10}).

Table(1): the Sensitivity of Bacterial Isolates to antibiotics.

No	Antibiotics	code	(µg/disk content)	Diameter of the standard inhibition area (mm)		
				sensitive	medium	Resistive
1	Rifampin	RA	5 mg	≥ 19	18-12	≤ 11
2	Ampicillin	AM	25 mg	≥ 14	13-12	≤ 11
3	Tetracyclin	TE	10 mg	≥ 18	17-15	≤ 14
4	Cefotaxime	CEF	30 mg	≥ 26	25-23	≤ 22
5	Gentamicin	GN	10 mg	≥ 15	14-13	≤ 12
6	Chloramphenicol	C	10 mg	≥18	13-17	≤12
7	Oxacillin	OX	5 mg	≥ 13	11-12	≤ 10
8	Novabiocin	Nb	30 mg	≥22	18-21	≤ 17
9	Trimethoprim	TM	5 mg	≥16	15-11	≤ 14
10	Azithromycin	AZM	15 mg	≥ 18	17-14	≤ 13

Results and Discussion

Isolation and Identification

The current study included 50 samples of infected patient women with vaginitis from the inpatients and outpatients of Salahuddin Hospital, aged between 14 and 50 years between October 2015 and April 2016. These samples were cultured on the blood

medium in the middle of the Blood agar, MacConkey agar, Mannitol salt agar medium.

The results showed that the number of samples taken from the vagina, which showed a bacterial growth, was 39 samples, i.e. (78%). This percentage was similar to that of the researcher (¹²). Who showed that, the percentage

of bacterial vaginitis was 75%. Bacterial growth may be viral or fungal or anaerobic bacteria that cannot be isolated by conventional transplant methods used in this study, which may require special cultured and development, or because infected patient women use doses of antibiotics or may be due to differences in nature and size samples taken as well as

variations in grading Heat and humidity⁽¹³⁾.

isolates were diagnosed microscopically and macroscopically based on microscopic and visual tests and then further confirmed using Vitek Compact2 System. The results of the diagnosis showed differences in the types, numbers and proportions of isolated bacteria.

Table (2): The results of initial isolation of Vaginal Swabs Samples and Percentages

Results of Transplantation	Number	Percentages
Pathogenic bacteria	39	%78
Not Pathogenic bacteria	11	%22
Total Number	50	%100

Bacterial colonies were identified on the medium of blood agars and in the MacConkey agars and in the medium of the salt Mannitol through microscopic, morphology and biochemical tests, which included tests of the intestinal family (catalases, oxidaes, simons, Red-methyl, Voges -

proskauer, movement, Indol, Urea test). Staph included (Catalase, Coagulase, Manitol salt agar, Urease). The Vitek compacte2 System was used to confirm the results of the biochemical diagnosis. The results of this test were identical to the above biochemical tests.

Table(3): of Bacterial Isolates taken from Infected patient women with vaginitis

Bacterial Isolates	Number of Isolates	percentage For total isolation*	percentage For qualitative isolation**
<i>E.coli</i>	11	%22	28.3
<i>Proteus mirabilis</i>	8	%16	20.5
<i>Staph.hominais</i>	3	%6	7.7
<i>Ochrobactrum anthropi</i>	2	%4	5.1
<i>Aeromonas salmonicida</i>	3	%6	7.7
<i>Pseudomonas oryzihabitans</i>	2	%4	5.1
<i>Pseudomonas luteola</i>	2	%4	5.1
<i>Pantoea spp</i>	2	%4	5.1
<i>Klebsella pnemonia</i>	4	%8	10.3
<i>Sphingomonas paucimobilis</i>	2	%4	5.1
Total NO.	39	%78	% 100

* Percentage of total isolation = number of isolates / total number of samples.

** Percentage of qualitative isolation = number of isolates / total number of isolates.

E.coli was characterized by the formation of small dry pink colonies on the MacConkey medium, while in the development of the EMB medium, it was shown as a green metallic sheen. In addition to the IMVC test, it was negative for the Voges–Proskauer test and negative for the citrate test, and positive for Andol test and Methyl red test. It is a fermented bacteria of sugars and produced acid and gas, but there was no black deposit of H₂S on the medium of iron trioxide TSI and gave a negative result of the urease test that was consistent with what is mentioned (¹⁴). The results showed that

these bacteria were one of the most common causes of vaginitis. The isolates were isolated by 28.3%. This result was agreed with (¹⁵), which found 28.8% of *E. coli* infection in the vagina. The presence of special receptors of the type of glycosphingolipid on the surface of cells epithelial urinary tracts strongly associated with *E. coli* strongly and also possess many of (the factors of ferocity) the most important cilia, which helps to adhere to cells lining epithelium of the vagina, *Proteus mirabilis* bacteria showed pale colonies on the MacConkey medium, a For the

bacteria Swarming phenomenon on the medium of blood sugar, on the medium of MacConkey medium did not show this phenomenon because the salts of bile limit the spread and is positive for the test of urease and non-fermented lactose sugar. The results in our current study showed these bacteria with 8 isolates and 20.5% in patient infected patient women with vaginitis. This result was associated with (16), 16.66%, while *Klebsiella pneumonia* bacteria was characterized by the formation of rosy mucous colonies because they contained a portfolio which is negative for the oxidase test, positive for the catalase test, positive for urease, and negative for the red-methyl test, and positive for the Voges-Proskauer test and fermented glucose sugar, Mannitol, Ramenor, Arbinos and Meli Baioz (17), where the number of isolates was 4 by 10.3% in infected patient women with vaginitis (12), which recorded the percentage of vaginal infection with *Klebsiella* by 14.8%, and *Aeromonas Salmonicida* bacteria, which is non-motile bacteria, and glucose-fermented bacteria with gas and negative test for the Indol test, Voges-Proskauer test, Citrate test, coagulase test, Gelatin and Methyl red test and when developed on the medium of blood agars are fully analyzed for red blood cells of any type β -haemolysis and colonies that appear on the MacConkey are pale color because it is not fermented for lactose sugar (18). Then, three isolates appeared with (7.7%) in infected patient women with vaginitis, and *Pseudomonas oryzihabitans*. These bacteria are under the microscope in a rod-negative form of oxidase and blood-thinner on the blood agar medium and have the ability to form biofilms (19). We have found these bacteria with two isolates in infected patient infected patient women with vaginitis 5.1%, while *Pseudomonas*

luteola is a negative chromosomal polycyclic bacteria through multiple polysaccharides ranging from 0.8 to 2.5 microns, and are positive for the test of oxidase and urease and negative for H₂S and grow and the optimal temperature for its growth is 30 m. When growing on the MacConkey, its colonies produce a yellow-orange color (20). The bacteria were found to be isolated by 3.2% in infected patient women with vaginitis, while *Ochrobactrum anthropic* are shown under a moving rod microscope by oceanic lows. The colonies appear on circular nutritious and non-pigmented soft cores, which are positive for the test of oxides and catalase and are reduced to nitrates (21). Where the two isolates in the vagina by 5.1%, while the bacteria *Sphingomonas paucimobilis* characteristically under the microscope and has a polar whip one of their colonies produce yellow or creamy pigments on the medium of blood agars and do not produce these pigments on the medium of the MacConkey and is positive for the test of oxidase and catalase. The number of isolated isolates was two with 5.1% isolates from infected patient women with vaginitis. while the *Pantoea spp* was characterized by coccobacilli under the microscope and was moving by positive oceanic lactation to test the catalase and fermented glucose with acid and fermented production of mannitol, mannose, lactose, three isolates in the treatment of infected patient women with urinary tract infection (3.2%) and isolates of the vagina by (2). 5.1%. It has isolated from infected patient women with vaginitis. The *Pantoea spp* bacteria were characterized by a coccobacilli under the microscope. They were moved by positive ocean lactates to test the catalase and fermented glucose sugar with acid and fermented production of mannitol, mannose and

lactose. It was negative for the Oxidase test, the decay of arginine and the urea decay and produced a yellow pigment when its colonies grew on nutritious drainage and colonies were mucous. These bacteria emerged

during the current study results of (5) isolates, (3) isolates in the administration of infected patient women with UTIs by 3.2% and isolation of the vagina by 5.1%.

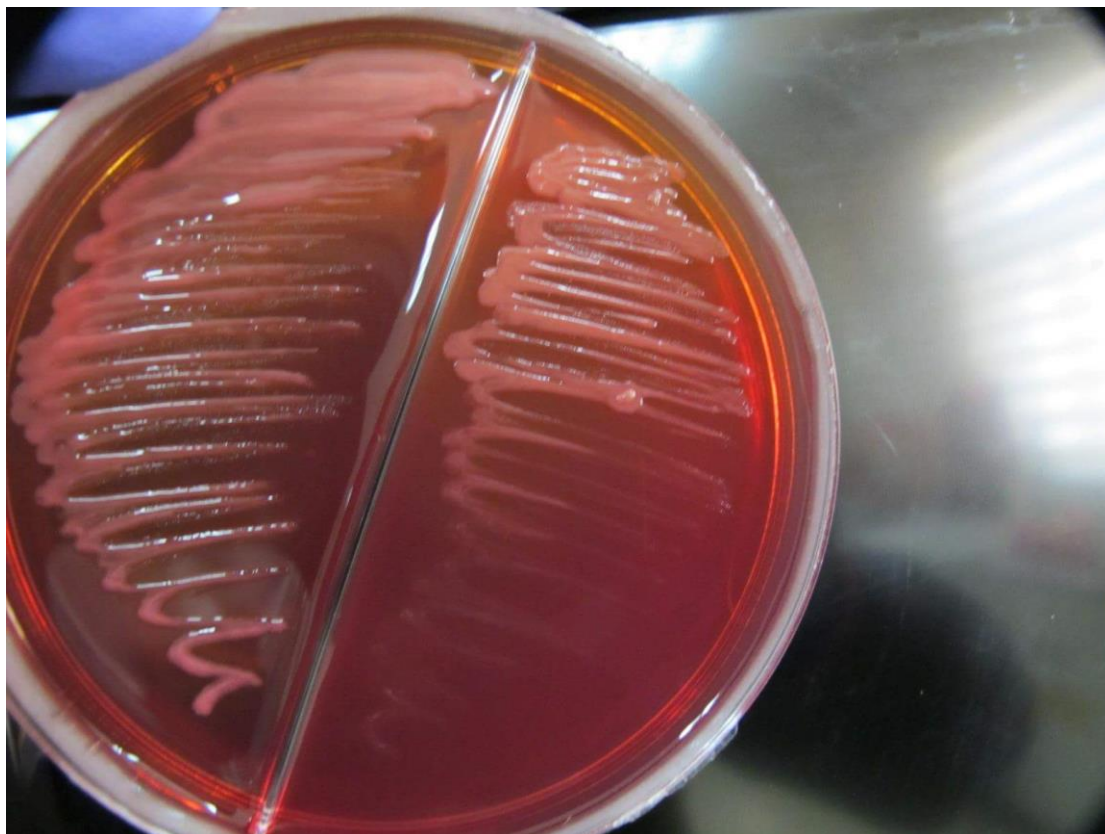


Fig. (1):The Growth of *Ochrobactrum anthropi* Bacteria on the MacConkey agar medium

The Resistance of Bacterial Isolates of Antibiotics

The sensitivity test was performed using ten antibiotic discs to treat bacterial vaginitis for (39) isolated bacterial isolates from Vaginitis for infected patient women over the age of 14. The reason for the selection of modern antibiotics to determine the effect of these different antibiotics on all bacterial isolates in our current study regardless of the age to choose the most effective antagonist to obtain the most effective antagonist, antibiotic isolates which were identified as indicated in (22). The isolates of *E.coli*

showed an absolute resistance to antimicrobial agents such as Trimethoprim, Ampicillin, Cefotaxime, Tetracyclin, Oxacillin, Gentamicin, Rifampin, Novobiocin, Chloramphenicol which have percentage 100% and 66.6% anti-azithromycin. The results of our study with 25 for the antibody Ampicillin, whose isolates had absolute resistance to this antimicrobial and anti-trimethoprim (86.8%), differed as compared to the chloramphenicol, Cefotaxime (14.3%). For Oxacillin, our results agreed with ²³ isolates with absolute resistance to this antibody,

while they differed with Tetracyclin, Novobiocin, which had a resistance in the rate of 60% and 0%, respectively. For the Gentamicin antibody differed, the results of the current study to what referred to ⁽¹⁶⁾ where the isolates are very sensitive to this antidote, as for *Proteus mirabilis* bacteria where shown resistance to antibiotics Cefotaxime, Oxacillin, Chloramphenicol, Trimethoprim, Rifampin 100%, while allergy to azithromycin, Novobiocin, Ampicillin, Gentamicin, Tetracyclin. The results of the current study were similar to those of ⁽²⁴⁾ for Gentamicin, where the sensitivity rate was 80%. It differed with a study ⁽²⁵⁾ at Tikrit Teaching Hospital, which found that these bacteria were very resistant to anti-Ampicillin. Then, ⁽²⁶⁾ for the chloramphenicol and Cefotaxime inhibitors (20% and 40% respectively), while the anti-trimethoprim (90%) and the *oryzihabitans* (*Pseudomonas*) showed resistance to Cefotaxime, Oxacillin, Chloramphenicol, Trimethoprim, Rifampin, Azithromycin, Tetracyclin, Ampicillin, 100%, Gentamicin, Novobiocin, and *Pseudomonas luteola* are resistant to all antibiotics Cefotaxime, Oxacillin, Chloramphenicol, Trimethoprim, Rifampin, Ampicillin, Tetracyclin, Gentamicin, Novobiocin 100% except Azithromycin was 100% sensitive to *Bacteria Klebsiella pneumonia*, which showed a high resistance to antibiotics (Cefotaxime, Oxacillin, Chloramphenicol, Rifampin, Novobiocin, Azithromycin, Gentamicin, 100%) and Ampicillin, Trimethoprim and Tetracyclin. Antibacterials: Ampicillin, Trimethoprim, Gentamicin, Chloramphenicol, Cefotaxime where resistance rates were 100%, 40%, 30%, 20% and 16.3% respectively, while *Sphingomonas paucimobilis* showed high resistance to antibiotics Cefotaxime, Oxacillin, Chloramphenicol, Trimethoprim,

Rifampin , Ampicillin, Tetracyclin, Novobiocin, with 100% resistance while sensitive to Azithromycin, Gentamicin, and *Bactria Antibacterium ocrobactram* showed a high resistance to all antibiotics Ampicillin, Trimethoprim, Oxacillin, Antibacterium, Antibacterium, Amoxicillin, Amoxicillin, Cephotaxime, , Cephotaxime Novobiocin, Azithromycin, Chloramphenicol, 100% resistant, Rifampin 50%, but sensitive to Gentamicin, Tetracyclin, and *Aeromonas salmonicida*. High resistance to antibiotics: Ampicillin, Trimethoprim, Rifampin, Oxacillin, Cefotaxime Novobiocin, Chloramphenicol, Tetracyclin, with 100% resistance while it was sensitive to Azithromycin resistors, gentamicin, while *Staphylococcus hominis* were resistant to all antibiotics Chloramphenicol, Ampicillin, Trimethoprim, Tetracyclin, Oxacillin, Cefotaxime, Rifampin, Azithromycin, Novobiocin. Except Gentamicin, the sensitivity was 100%.

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