Antibacterial susceptibility patterns of *Klebsiella pneumoniae* isolates from vaginitis cases of pregnant women in Baghdad city

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الخلاصة

تعد البكتريا واحدة من أعداد كبيرة من الكائنات الحية الدقيقة، وقد تشكل احيانا الغالبية العظمى منها ، ومن المعروف عنها انها تعمل على الاستعمار وتشكيل المجتمعات المعقدة في مواقع مختلفة داخل وعلى الجسم البشري المصاب بها مسببه عدوى وإمراضيه معقدة في الاجسام . ولعل التهاب المهبل الجرثومي (البكتيري) هو أحد عدوى الجهاز التناسلي الأكثر انتشارا لدى النساء في سن الإنجاب في جميع أنحاء العالم.اجريت الدراسة الحالية لغرض الكشف عن بكتيريا الكلبسيلا الرئوية بوصفها قد تكون احدى عوامل العدوى المسببة لالتهاب المهبل البكتيري عند النساء الحوامل بالإضافة الى دراسة نمط فعالية المضادات البكتيرية المتنوعة على العينات السريرية التي جمعت خلال عام 2012 من مستشفيات متعددة في مدينة بغداد. ولتحقيق غرض الدراسة فقد جمعت 450 مسحات مهبلية من النساء الحوامل (في الأشهر الثلاثة الأولى من الحمل) اللواتي يعانين من التهاب المهبل مع وجود لتاريخ مرضي من الحمى والإفرازات المهبلية والأعراض السريرية الأخرى المرافقة للإصابة.، وقد شخصت الحالات المصابة جميعها من الاطباء والطبيبات الاختصاص بأمراض النسائية والتوليد أو الجهاز التناسلي. تم تشخيص العزلات السريرية لبكتيريا الكلبسيلا الرئوية باستخدام الاوساط الزرعية البكتيرية المختلفة لتحديد وتشخيص العزلات البكتيرية. تمكنت الدراسة من عزل وتشخيص 128 عزلة بكتيرية توزعت بواقع المكورات العنقودية 50 (39.06٪)، الإشريشية القولونية 29 (22.6٪)، الكلبسيلة الرئوية 19 (14.8٪)، المكورات السبحية 14(10.9٪)، العصيات اللبنية 8(6.2٪)، الليمونية الفروينية 6 (4.6٪)، والسرشية 2(1.5٪). أشارت نتائج الدراسة الى ان بكتريا الكلبسيلة الرئوية فقط أظهرت حساسية عالية لمضاد سيبروفلوكساسين وبنسبة مطلقة100 ٪ في المختبر. بينما أظهرت بقية المضادات البكتيرية كلاريثروميسين، أموكسيسيلين، ببراسين، الستربتوميسين، ميثوبريم، سيفوتاكسيم،أزيتريونام ، ايجيمنتين وجنتاميسين استجابات حساسية أو مقاومة متباينة. وتشير هذه النتائج إلى زيادة عوامل المقاومة لهذه المضادة البكتيرية التي تستخدم عادة لالتهاب المهبل والذي يؤدي الى تزايد اعداد وانواع(عتر) وضراوة بكتريا الكلبسيلة الرئوية وبالتالي تصبح مقاومة للمضادات مؤدية بذلك الى كونها ستصبح مصدر قلق للصحة العامة. يلاحظ من نتائج الدراسة الى اهمية اجراء فحوص التشخيص البكتيرية الروتينية في حالات التهاب المهبل البكتيري واختبار حساسيتها للمضادات البكتيرية المختلفة باعتبارها الطريق العلمي السليم في الكشف المبكر عن الاصابة علاوة على مساهمة ذلك في توفير الرعاية الصحية الملائمة للحفاظ على الجهاز التناسلي والتكاثري للنساء سليمة مما يجعل حياتهم أكثر سعادة في مجتمعات صحية صحيحة.

Abstract

There are large numbers of microorganisms, the vast majority of which are bacterial species, are known to colonize and form complex communities, or microbiota, at various sites within and on the human body. Bacterial vaginosis (BV) is the most prevalent lower genital tract infection in women of reproductive age throughout the world.

The purpose of this study was to detect *Klebsiella pneumoniae* bacterium as a causative agent of bacterial vaginosis of pregnant women and its antibacterial pattern in Baghdad city.

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A cross sectional study to assess the incidence and the antibacterial susceptibility patterns of *Klebsiella pneumoniae* isolates in pregnant women who were presented at many hospitals of Obstetrics and Gynecology in Baghdad city during 2012.

A total of 450 vaginal swabs from pregnant women (in first three months of pregnancy) with history of fever, vaginal discharge and other clinical symptoms were investigated , by using different bacterial cultures to identified and diagnose the bacterial isolates.

The frequency of 128 bacterial isolates were diagnosed *Staphylococcus epidermidis* 50 (39.06%), *Escherichia coli* 29 (22.6%), *Klebsiella pneumoniae19* (14.8%), *Streptococcus spp.*14 (10.9%), *Lactobacillus spp.*8 (6.2%), *Citrobacter freundii* 6 (4.6%), and *Serratia spp*.2 (1.5%). The antibacterial susceptibility patterns of *Klebsiella pneumoniae* only Ciprofloxacin showed 100% sensitivity *in-vitro*. While Clarithromycin, Amoxicillin, Pipracin, Streptomycin, Trimethoprim, Cefotaxime, Azitreonam, Augmentin and Gentamicin showed varied sensitivity / resistance responses. These findings suggest an increasing of resistance to the antibacterial agents that commonly used for vaginitis and the rate at which bacteria become resistant to antibacterial agents are of public health concern. This calls for routine bacteriological culture and sensitivity test in the management of bacterial vaginitis. Further, the provisions of adequate health care that keeping the women vagina healthy and maintain human public health.

Keyword: Vaginitis, Antibacterial susceptibility profile, *Klebsiella pneumonia*.

Introduction

Vaginitis is a common gynecology problem found in women of all ages that can be caused by several different microorganisms. (1) Unequilibrium of vaginal normal microbiota increased of one or more pathogenic microorganisms and decreased or absence *Lactobacillus spp*. from the vagina often caused bacterial vaginitis. (2) Therefore .bacterial vaginitis (BV) is worldwide the most important perturbation of the normal vaginal condition in women (3). BV affects pregnant women or women in reproductive age leading to high public health costs and associated complications, such as pelvic inflammatory disease, preterm birth, postoperative infections and an increased risk of acquisition and transmission of sexually transmitted diseases. (3, 4) *Klebsiella pneumoniae* consider one of causative agents of bacterial vaginitis, that having multiresistance to antibacterial especially beta – lactam antibiotics. (5) Bacteriologists were confirmed that *K. pneumonia* is opportunistic pathogenic bacteria, which infect all ages and consider one of nosocomial

pathogenic agents that can be easily grow and multiplication in different environments such as soil, hospitals and become the main cause of urinary infections, wounds and burns (6). *Klebsiella* infections caused high morbidity and mortality which indicate the virulence factors of it and also to its highly resistance to antibacterial substance that coded on many plasmids. (7) Bacteria use several strategies to avoid the effects of antimicrobial agents, and have evolved highly efficient means for clonal spread and for the dissemination of resistance traits. (8) The resistant organisms are now a worldwide problem (9, 10) and their incidence is being continuously increasing with limited treatment alternatives. It becomes necessary to know the prevalence of these organisms and to formulate treatment policy. (9)

The current investigation was undertaken to determine the bacterial *K. pneumoniae* incidence in bacterial vaginitis and its antibacterial susceptibility patterns at many hospitals of Baghdad city, so these needs for prompt investigations and management of vaginitis in them and possibly any changes in the recommendations for treatment protocol in such patients.

Material and methods

Patients and bacterial isolates

This study included 450 vaginal swabs which were collected from pregnant women (aged 17–45 years) clinically diagnosed as having symptomatic genital tract infection especially acute vaginitis as it was made clear by the Gynecologists. The symptoms were characterized by purulent vaginal discharge and all patients had not taken any drugs within the previous (5 – 7) days before attending the hospital.

The study was carried out at 2012 among pregnant women who were presented at many hospital of Delivery and Maternal in Baghdad city.

Swabs for culture should be placed in tubes containing normal saline to maintain the swabs moist until checked in laboratory. Then the swabs had been inoculated on culture media of MacConkey, Nutrient and Blood agar (from Himedia – India), which incubated aerobically at 37°C for 24 hours. (11) The cultures with detectable bacterial growth were subculture on Blood agar and MacConkey agar plates that incubated aerobically at 23°C for 24 - 48 hours. Suspected *Klebsiella spp.* bacteria were identified morphologically and biochemically according to standard bacteriological methods (12).

Susceptibility testing

Disk-diffusion tests were carried out with antibiotic containing disks (Bioanalyse) on Mueller-Hinton agar plate (Himedia). The results were expressed as susceptible or resistant according to the criteria recommended by the Clinical Laboratory Standards Institute (CLSI) (13). Bacterial inoculum to be used in this test is prepared by adding growth from 5 isolated colonies grown on Blood agar plate to 5ml of broth. This culture was then incubated for

12 hrs. to produce bacterial suspension of moderate turbidity. A Sterile swab was obtained an inoculum from standardized culture. This inoculum was then streaked on a Mueller – Hinton plate (from Himedia – India). Antibacterial discs were placed on the surface of the medium at evenly spaced intervals with flamed disc applicator (14). Incubation of cultured plates was overnight with optimal time being 14hrs. at 37°C, zones of inhibition were measured by using caliper. Zone size was compared to standard zones to determine the sensitivity or resistance of *Klebsiella pneumoniae* isolates to each antibacterial which listed in table-1.

The data were analyzed to determine the percentage of incidence of the bacterial isolates and antibacterial susceptibility of *Klebsiella pneumoniae* respectively.

Table-1: Antibacterial discs (from Bioanalyse – Turkey) which used in sensitivity test.

Antibacterials	Synonym	Disc potency µg/disc	Zone of inhibition*		
			Sensitivity	Moderate	resistant
Clarithromycin	CLR	5	18	14 -17	13
Amoxicillin	AX	25	14	12 -13	11
Pipracillin	PRL	100	18	15 -17	14
Streptomycin	ST	10	15	12 -14	11
Trimethoprim	TMP	5	16	11 - 15	10
Cefotaxime	CTX	30	23	15 -22	14
Azitreonam	ATM	30	22	16 - 21	15
Augmontin	AUG	20 / 10	18	-	-
Ciprofloxacin	CIP	5	21	16 - 20	15
Gentamicin	GN	10	15	13 -15	12

***Calculated with millimeter (mm).**

Results

In this, 450 vaginal swabs obtaining from pregnant women were subjected for culturing on available media found that only 72 samples showed positive bacterial culture (16%), while there were 378 samples showed negative results (84%). Results of isolation and identification of 128 bacterial isolates (mixed infections) were distributed as, *Staphylococcus epidermidis* 50 isolates (39.06%), *Escherichia coli* 29 isolates (22.6%), *Klebsiella pneumoniae* 19 isolates (14.8%), *Streptococcus spp.* 14 isolates (10.9%), *Lactobacillus spp*. 8 isolates (6.2%), *Citrobacter freundii* 6 isolates (4.6%) and *Serratia spp*. 2 isolates (1.5%). (Table-2)

Table-2: Frequency of bacterial isolates from total 128 positive cultures.

Organisms	No. of isolates	Percentage (%)
Staph. aureus	50	39.06
E. coli	29	22.6
K. pneumoniae	19	14.8
Strep. spp.	14	10.9
Lacto. Spp.	8	6.2
Citrobacter freundii	6	4.6
Serratia spp.	2	1.5
Total	128	100%

Results of *in – vitro* antibacterial susceptibility patterns of selected *K. pneumoniae* isolates showed that there were 100% of isolates sensitive to only Ciprofloxacin antibiotic. While other demonstrated antibiotics (Clarithromycin, Amoxicillin, pipracillin, Streptomycin, Trimethoprim, Cefotaxime, Azitreonam, Augmentin and Gentamicin showed varied sensitivity or resistance as shown in table-3.

Table-3: Antibacterial susceptibility pattern of K. pneumoniae isolates.

Antibacterial	Sensitive isolates		Resistant isolates	
	No.	(%)	No.	(%)
Clarithromycin	2	10.52	17	84.47
Amoxicillin	4	21.05	15	78.94
Pipracillin	5	26.31	14	73.68
Streptomycin	7	36.84	12	63.15
Trimethoprim	9	47.36	10	52.63
Cefotaxime	11	57.89	8	42.10
Azitreonam	6	31.57	13	68.42
Augmentin	4	21.05	15	78.94
Ciprofloxacin	19	100	-	-
Gentamicin	8	42.10	11	57.89

Discussion

The results of the current study indicate that the incidence of bacterial vaginitis was found with 72 (16%) cases of pregnant women from total 450 investigated cases. Dybas *et al*. (15) have reported that bacterial vaginitis carried out the major problem in vaginitis of pregnant women. Also the results explain clearly the distribution of incidence of bacterial species, which were similar to those obtained by (16, 17). The presence of microorganism in the female lower genital tract (LGT) in the concentrations that modify the established normal equilibrium produces different symptoms that make necessary to consult the gynecologist. It is currently accepted that infections of

LGT are due to microorganisms that are normally integrating the internal flora, except for the erroneously called sexually transmitted disease that are caused by external microorganism. This means that all those agents usually present without causing any disease (18).
Eight isolates (6.2%) of *Lactobacillus spp*. have been isolated in the study from total cases of vaginitis in pregnant women. This bacterium is normally present in healthy vagina and their presence is very essential for maintaining vaginal p^H through its acid production, and hence the growth of unwilling or pathogenic bacteria which will be prevented.(19) This fact on contrast with the results of recent study also explains that these microorganisms are potentially opportunistic within the infected vagina, or may become increasingly prevalent upon minor alterations of the vaginal microenvironments. (20)
Nineteen isolates (14.8%) of *K. pneumoniae* have been isolated in vaginitis of pregnant women. This bacterium is rarely present in healthy vagina; however these finding were confirmed the presence of such bacteria in the presence of other pathogenic or nonpathogenic bacteria like *Lactobacilli,* which pointed its ability to produce various types of virulence factors in the infected vagina to obtain their requirements through its pathogeneses of infection. (21, 22)
The study was evaluated *in – vitro* the susceptibility of 10 antibacterial activities against growth of one pathogenic agent of vaginitis *K. pneumoniae*, which showed different results of sensitivity or / and resistance. These results were in agreement of many results obtained by (23, 24), with some variation that we believe it's due to laboratory proceedings, medium, preparations, antibacterial discs manufacturing and local isolates of the bacteria.
Many studies were reported that *K. pneumoniae* had ability to produce wide spread beta – lactam enzymes that resist different antibacterial effects through also different mechanisms of resistance. (25, 26) We think that these variations of susceptibility of bacteria to the investigated antibacterial were explained the random and wide administration of these antibacterial which lead development of bacterial resistance, and virulence factors of bacteria that have high ability to avoid the antibacterial effects.(27) The present study provides important information regarding the vaginal ecology of pregnant women, through that *K. pneumoniae* and other pathogenic bacteria like *E. coli*, *Staph. aureus, Strep. spp, Lacto. Spp.* and *Citrobacter freundii* were found to be the main causes of bacterial vaginitis. So that, the practice of initiating empirical antibacterial treatment for any vaginal infection without taking high vaginal swabs should be reviewed and appropriate therapy after proper diagnosis of the vaginal infection is recommended, and for this categories must be using the standard microbiological methods with determination of complete antimicrobial susceptibility pattern is recommended.

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