



**MICROBIOLOGICAL SCREENING AND ANTIBIOTIC SENSITIVITY PROFILING OF
HIGH VAGINAL SWAB FROM SYMPTOMATIC WOMEN OF DIFFERENT AGE
GROUP**

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ABSTRACT

Vaginal infections are also known as vaginosis. The incident of vaginosis is very common and occurs several times in whole life of a woman. *Gardnerella vaginalis*, *Prevotella*, *Peptostreptococcus*, *Staphylococcus* spp., *Streptococcus* spp., *Escherichia coli*, *Bacteroides* spp., *Haemophilus influenza* etc. are some most common agents to cause vaginosis. Vaginosis especially occurs during the young age starting for early twenty's to late thirty's. Because of the most sexual activity occurs in this age period and lacking in knowledge of proper personal hygiene, poor socioeconomic condition are responsible for such diseases. In the current study, about hundred bacterial isolates were collected from hundreds individual patients having vaginosis and their drug resistance pattern was observed. Six types of bacteria was found with most predominant *Escherichia coli* (48 isolates), *Staphylococcus aureus* (26 isolates), *Streptococcus pyogenes* (15 isolates). After doing drug resistance pattern it was quite shocking that all of the isolates are already resistant to multiple antibiotic drugs in use at present. Among them about 23 isolates (*Escherichia coli*-8, *Staphylococcus aureus*- 9, *Streptococcus pyogenes*-3, *Haemophilus influenzae*-3) showed most resistance to the antibiotics. To prevent vaginosis it is needed to use protection during intercourse, having only one sexual partner, personal hygiene maintenance, eating probiotic containing food like curd, cheese for supplementation of more *Lactobacillus* spp. which helps keeping low pH in vagina making it difficult for growth of pathogens and seeking for treatment when symptoms of vaginosis appears. Sooner the treatment starts better the physical status.

KEY WORDS: Vaginosis, sexual activity, hygiene, drug resistance.

INTRODUCTION

Vaginal infections are very common for the women seeking medical treatment for gynecological problems. As many women do not often bother their problems in genital areas, the situation becomes complicated and finally they end up in hospitals. The abnormal physiological conditions (unusual vaginal secretion, inflammation itching, painful urination, burning sensation), infections like vaginosis caused by bacteria, trichomoniasis, candidiasis), viral genital infections, sexually transmitted disorders, other complications like cervical ectopy, cancer etc) are signs of need for treatment.^[1-4] Normally the reproductive age group women have acidic vaginal environment (≤ 4.5). Such condition help to protect this area from pathogenic bacterial infections. *Lactobacillus* spp. is a commensals of vagina which can thrive in low acidic environment and produce acid to maintain this condition in vagina. Bacterial infection is not common in ≤ 4.5 pH but *Candida* spp. can cause candidiasis. Protozoal infection

caused by *Trichomonas vaginalis* can elevate the pH (>4.5) of vagina which encourage the secondary infection by bacteria.^[5] *Trichomonas vaginalis* infected women ranging from 57-180 million between the age group of 20 to 40 years in developing countries characterized by foul smelling greenish-yellow discharge with itching and dyspareunia.^[6] As this infection increases the vaginal pH, other aerobic, anaerobic and microaerophilic mixed bacteria start infection replacing *Lactobacillus* spp.^[7] Aerobic bacteria like group B streptococci, *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella* spp and anaerobic bacteria like *Gardnerella vaginalis*, *Prevotella* spp., *Mycoplasma hominis*, *Mobiluncus* spp. *Escherichia coli* can infect the infants causing sepsis, meningitis after invading chorioamnion while delivering through birth canal or by amniotic fluid during intra uterine life.^[8] Group B streptococci can cause preterm labor, low weight infant, post natal infections, life threatening conditions in infants (meningitis, septicemia, osteomyelitis,

pneumonia, septic arthritis).^[9] Being the most frequent infection, candidal vaginitis (CV) infects approximately 75 % of adult women during their life time with recurrence for several episodes.^[10] Whatever the reason for vaginitis is responsible, if untreated can result in infertility, pelvic inflammatory disease, cervical dysplasia, reproductive malfunctioning, ectopic pregnancy etc.^[11-14] To detect the infection causing agent is important to give proper medication to the patients. Usually high vaginal swab (HVS) test is performed to culture the responsible organism. This test is suggested during the complicated cases of vaginal discomfort during postnatal, post miscarriage, after contraceptive insertion, vaginitis, abnormal vaginal discharge, pre or post termination of pregnancy etc.^[15-17] In the current study about 100 patients were selected to determine the pathogenic isolates from female patients of different ages groups randomly. We aimed to determine the presence of bacteria in HVS samples. This work was conducted to determine different types of antibiotic resistant bacteria causing vaginal infection.

MATERIALS AND METHOD

Study area and sampling

About 100 female patients of different age group were randomly selected who were seeking treatments for vaginal discomforts. The experiment was conducted between June, 2018 to December, 2018. High vaginal swab (HVS) samples were aseptically collected and transferred to different culture media immediately. Personal protective clothing and equipments were used to avoid any accidental exposure of hazards and transmissions.

Detection of bacteria from high vaginal swab

HVS samples were inoculated aseptically on blood agar, chocolate agar and SDA media plates to detect the bacterial isolates present in the swab samples. After 24 hours of incubation at 37° C all the bacterial colonies were subjected to biochemical identification.

Antibiotic susceptibility of the isolates

To determine the susceptibility of the isolates against the commonly prescribed antibiotics used in Bangladesh, bacterial suspensions of the isolated pathogens from the HVS were prepared in normal saline separately and incubated at 37°C until matched with McFarland solution. After that, lawn of bacterial suspension was made onto the Mueller Hinton agar plates. Finally 22 different antibiotic discs (Amikacin (AK 10 µg), Ampicillin (AM-10 µg), Amoxycylave (AMC 30 µg), Aztreonam (ATM-30 µg), Azithromycin (AZM 15 µg), Cephadrine (CD-30 µg), Cefuroxime (CXM-30 µg), Cefoxitin (FOX-30 µg), Ceftazidime (CAZ-30 µg), Cefotaxim (CTX-30 µg), Cefepime (30 µg), Ciprofloxacin (CIP-5 µg), Co-trimoxazole (SXT-25 µg), Gentamicin (CN-10 µg), Imipenem (IPM-10 µg), Levofloxacin (LEV-5 µg), Linezolid (LZD-30 µg), Nitrofurantoin (F-300 µg), Norfloxacin (NOR-10 µg), Oxacillin (OX-1 µg), Penicillin G (P-10 µg), Piperacillin/Tazobactam (PT-75/10µg), were positioned over the bacterial lawn with enough space between them for better interpretation of results with clear zone of inhibition. After 24 hours incubation at 37°C, the plates were observed for the presence of clear zone of inhibition surrounding the antibiotic discs and measured in millimeter to determine the sensitivity or resistance of the isolates against those antibiotics using CLSI guidelines

RESULTS

About 100 patients were selected for the HVS sample prescribed by the doctors and the swap from every female was collected for the detection of the responsible bacteria causing gynecological problems among the females. At the same time antibiotic susceptibility test was done for determination of the antibiotic sensitivity for suggesting them proper antibiotic treatment to cure the disease.

Table 1: Drug resistance trait of the isolates collected from HVS sample.

Serial No.	Age	Bacteria	Akikacin	Ampicillin	Amoxycylave	Aztreonam	Azithromycin	Cephadrine	Cefuroxime	Cefoxitin	Ceftazidime	Cefotaxim	Cefepime	Ciprofloxacin	Co-trimazole	Gentamicin	Imipenem	Levofloxacin	Linezolid	Nitrofurantoin	Norfloxacin	Oxacillin	Penicillin G	Piperacillin/Tazobactam
1	42	<i>Escherichia coli</i>	R	R	S	S	R	S	R	S	S	S	S	S	S	R	S	S		S				
2	35	<i>Escherichia coli</i>	R	R	R	S	R	S	S	S	S	S	S	S	R	R	S	S		S				
3	30	<i>Haemophilus influenzae</i>	R	S	S	R	R	R	R	R	R	R	R	R	S	R	S	R		S				
4	28	<i>Haemophilus influenzae</i>	R	S	S	R	R	R	R	R	R	R	R	R	R	R	S	R		S				
5	26	<i>Escherichia coli</i>	R	R	S	R	R	S	S	R	R	S	S	S	R	R	S	S		R				
6	19	<i>Staphylococcus aureus</i>						R	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R	

7	30	<i>Haemophilus influenzae</i>	R	S	R	R	R	R	R	R	R	R	R	R	R	R	S	R		R	R	R		S
8	37	<i>Escherichia coli</i>	S	R	R	R	R	R	R	S	S	S	R	R	S	S	S		S	R				S
9	21	<i>Escherichia coli</i>	R	R	R	R	R	S	R	R	S	S	S	R	R	R	S	R		R	R			S
10	45	<i>Escherichia coli</i>	S	S	R	R	R	S	R	R	S	S	R	R	S	R	S	R		S	R	S		R
11	36	<i>Haemophilus influenzae</i>	R	R	R	R	R	S	S	S	R	S	S	R	R	S	S	R		S	R			S
12	40	<i>Haemophilus influenzae</i>	R	S	R	S	R	S	S	S	S	S	R	R	R	S	R		R	R				S
13	27	<i>Escherichia coli</i>	R	R	S	S	R	R	R	S	R	R	R	R	R	R	S	R		R	S			S
14	22	<i>Escherichia coli</i>	S	R	R	S	R	R	S	S	S	R	S	R	R	S	S	R		R	R			R
15	32	<i>Escherichia coli</i>	S	S	S	R	R	R	R	S	R	S	S	R	S	S	S	R		S	R			R
16	36	<i>Escherichia coli</i>	S	R	S	R	R	R	S	R	R	S	S	S	S	S	S	R		S	S			S
17	43	<i>Escherichia coli</i>	R	R	R	S	R	R	R	S	S	S	R	R	R	R	R	R		S	R			S
18	38	<i>Escherichia coli</i>	R	S	R	R	R	R	S	S	R	S	R	R	R	R	S	R		R	R			S
19	32	<i>Escherichia coli</i>	R	R	S	R	R	R	R	R	R	R	R	R	R	R	S	R		S	R			S
20	18	<i>Escherichia coli</i>	R	R	S	S	R	S	R	S	R	S	S	R	R	R	R	R		R	S			R
21	40	<i>Staphylococcus aureus</i>						S	S	S	R	S	R	R	R	R	S	R	S	R	R	R	R	
22	30	<i>Escherichia coli</i>	S	S	S	R	R	S	S	S	R	S	S	R	R	R	S	S		R	R			S
23	17	<i>Streptococcus pyogenes</i>		R			S	R	S	S	S	S	R	R	S	S	R		R		S			
24	24	<i>Staphylococcus aureus</i>					R	S	S	S	S	S	R	S	R	R	S	R	R	R	R	S	R	
25	22	<i>Escherichia coli</i>	R	R	R	R	R	R	R	S	R	S	R	R	S	S	S		S	S				R
26	31	<i>Staphylococcus aureus</i>					R	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S		
27	40	<i>Escherichia coli</i>	R	R	S	S	R	S	S	S	S	R	S	S	R	R	S	S	S		S	S		S
28	35	<i>Staphylococcus aureus</i>					R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R		
29	25	<i>Escherichia coli</i> S	S	S	S	R	R	S	S	S	S	S	R	R	R	R	S	R		S	R	R		S
30	18	<i>Escherichia coli</i>	S	R	S	R	R	S	S	S	R	S	S	R	R	S	R		R	R	R	R		S
31	22	<i>Streptococcus pyogenes</i>		S			R	S	S	S	R	R	S	S	S	S	R	R	R	S	S	S	S	
32	22	<i>Streptococcus pyogenes</i>		S			R	R	R	R	R	R	R	R	R	R	S	R	S		R	R		S
33	30	<i>Escherichia coli</i>	R	S	S	R	R	S	S	R	S	S	R	R	R	R	S	R				R	R	S
34	37	<i>Escherichia coli</i>	S	R	S	R	R	R	S	S	R	S	R	R	R	R	S	R		S	R	R		
35	21	<i>Streptococcus pyogenes</i>		S			R	S	S	S	S	S	S	S	S	S	R	S	S	R	S		R	
36	30	<i>Streptococcus</i>		R				R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	R	

		<i>pyogenes</i>																						
98	26	<i>Staphylococcus aureus</i>					R	R	R	R	R	R	R	R	R	S	R	S	S	R	R	R		
99	23	<i>Staphylococcus aureus</i>					R	S	S	S	R	S	R	S	S	R	R	S	R	R	S	R	S	S
100	23	<i>Escherichia coli</i>	R	S	S		R	S		S	R	S	S	S	S	R	S	S	S	S			R	S

From the result table it is visible that few bacteria were responsible for the gynecological problem among the patients of various ages. Patients were ranged from below twenty to sixty years of age. The causative agent of their disease includes *Escherichia coli*, *Staphylococcus aureus*, *streptococcus pyogenes*, *Haemophilus influenza*, *Klebsiella spp.* and *Enterococcus spp.* Every isolates from all of the hundred patient were subjected to antibiotic sensitivity test and all of these bacteria showed multidrug resistance. From the graph we can see that most of the patients were infected with *Escherichia coli* (48 patients), and then gradually the number of patients decreased simultaneously for

Staphylococcus aureus (26 patients), *Streptococcus pyogenes* (15 patients), *Haemophilus influenza* (7 patients), *Klebsiella spp.* (3 patients) and *Enterococcus spp.* (1 patient). According to the graph, 21-30 years age group is mostly infected mostly by *Escherichia coli* (maximum cases of infection), *Staphylococcus aureus*, *Streptococcus pyogenes* and *Hemophilus influenza* (gradually decreased rates) respectively. *Klebsiella spp.* was found only in one patient and *Enterococcus spp.* was absent for all age groups except 31-40 years. 21-30 years and 31-40 years age group was seen to be most vulnerable for infections. >40 years age group women were the lowest in getting infected.

Table 2: Summary of antibiotic resistance traits of the isolates.

Antibiotics	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>		<i>Streptococcus pyogenes</i>		<i>Haemophilus influenzae</i>		<i>Klebsiella spp.</i>		<i>Enterococcus spp.</i>	
	R	S	R	S	R	S	R	S	R	S	R	S
Amikacin	71%	29%	-	-	-	-	100%	0	67%	33%	-	-
Ampicillin	62%	38%	-	-	53%	47%	43%	57%	100%	0	100%	0%
Amoxycylave	35%	65%	-	-	-	-	57%	43%	67%	33%	0%	100%
Aztreonam	73%	27%	-	-	-	-	86%	14%	100%	0	-	-
Azithromycin	77%	23%	-	-	-	-	100%	0	67%	33%	-	-
Cephadrine	52%	48%	61%	39%	60%	40%	43%	57%	33%	67%	100%	0%
Cefuroxime	25%	75%	50%	46%	33%	47%	57%	43%	67%	33%	0%	100%
Cefoxitin	41%	59%	50%	50%	47%	47%	57%	43%	0%	100%	0%	100%
Ceftazidime	50%	50%	77%	15%	60%	27%	89%	14%	0%	100%	-	-
Cefotaxin	31%	69%	38%	38%	27%	60%	43%	57%	0%	100%	-	-
Cefepime	31%	69%	65%	8%	60%	33%	57%	43%	67%	33%	-	-
Ciprofloxacin	64%	36%	77%	11%	47%	33%	100%	0%	67%	33%	-	-
Co-trimazole	69%	31%	92%	8%	53%	27%	71%	50%	67%	33%	10%	0%
Gentamicin	52%	48%	92%	8%	40%	40%	71%	50%	0	100%	-	-
Imipenem	16%	84%	23%	73%	66%	40%	0%	89%	33%	67%	0%	100%
Levofloxacin	14%	86%	54%	38%	67%	93%	100%	0%	33%	0%	10%	0%
Linezolid	14%	86%	54%	35%	40%	47%	-	-	67%	0%	0%	100%
Nitrofurantoin	37%	63%	65%	23%	33%	33%	50%	57%	33%	33%	10%	0%
Norfloxacin	48%	52%	65%	27%	20%	53%	57%	0%	33%	33%	0%	100%
Oxacillin	33%	67%	61%	31%	47%	27%	14%	0%	0%	67%	0%	100%
Penicillin G	19%	81%	42%	46%	47%	27%	0%	50%	-	-	100%	0%
Piperacillin/Tazobactam	27%	52%	8%	4%	0%	20%	0%	71%	-	-	-	-

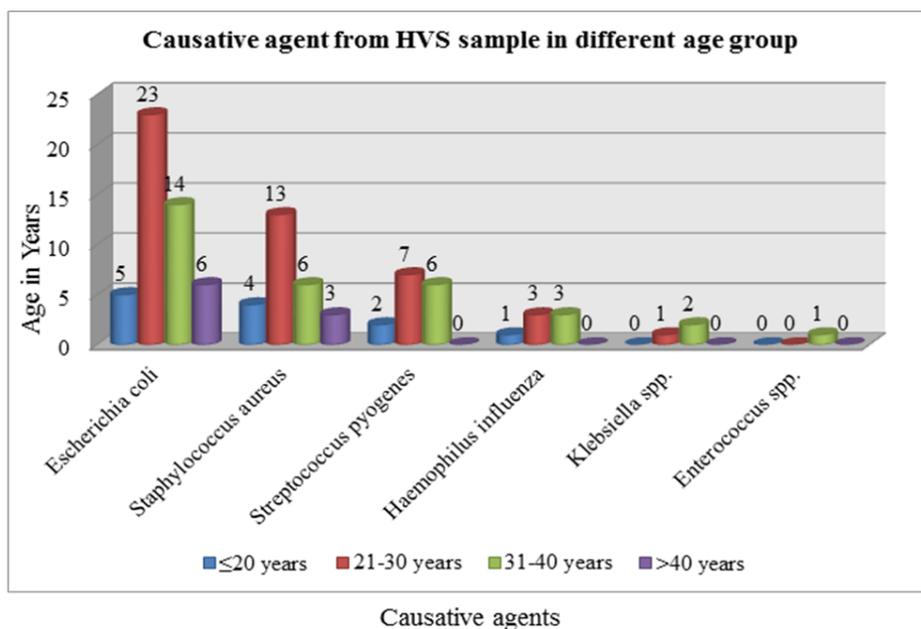


Table 3: Total number of isolates among the patients.

Isolates	Total number of patients
<i>Escherichia coli</i>	48
<i>Staphylococcus aureus</i>	26
<i>Streptococcus pyogenes</i>	15
<i>Haemophilus influenza</i>	7
<i>Klebsiella spp.</i>	3
<i>Enterococcus spp.</i>	1
Total	100

The antibiotic sensitivity test was performed and multi drug resistant bacteria was found in almost all cases. Highest infecting bacteria was *Escherichia coli* (48 out of 100). Maximum number of isolates of this type of bacteria was found for Amikacin (71%), Aztreonam (73%), Azithromycin (77%), Ciprofloxacin (64%), Co-trimazole (69%). Second predominant bacteria was *Staphylococcus aureus* (26 out of 100). The isolates were highly resistant to Ceftazidime (77%), Ciprofloxacin (77%), Co-trimazole (92%) and Gentamicin (92%). *Streptococcus pyogenes* (15 out of 100) were mostly resistant to Cephadrine (60%), Ceftazidime (60%), Imipenem (66%), Levofloxacin (67%). *Haemophilus influenza* (7 out of 100) was 100% resistant to Ampicillin, Azithromycin, Ciprofloxacin, Levofloxacin and 71% resistant to Piperacillin/Tazobactam. *Klebsiella spp.* (3 out of 100) was 100% resistant for Ampicillin, Aztreonam and finally *Enterococcus spp.* (1 out of 100) was resistant to Ampicillin, Cephadrine, Co-trimazole, Levofloxacin, Nitrofurantoin respectively.

DISCUSSION

Bacterial vaginosis is very common among sexually active females which occurs during unprotected sex, sexual transmission of STDs, lack of proper cleanliness and alteration of vaginal normal flora by other bacteria.^[18] In this context we took 100 patients seeking

treatment for vaginosis and who did HVS test and got positive results for bacterial infection. Among these patients most predominating bacteria was *Escherichia coli* (48). It is a fecal indicator coliform bacteria which can easily get its entrance from anus to the vagina due to the physiological position (being so close with vagina). If proper hygiene is not maintained *E. coli* can get into vagina easily and cause vaginosis if other conditions favor its colonization like scaring, sexual contact, pH change due to hormonal imbalance, decolonization of normal flora like *Lactobacillus spp.* *Staphylococcus aureus* (found in 26 samples) is another most common agent of vaginosis.^[19] It is a part of normal flora and can get access inside vagina at anytime, especially during intercourse and failure to maintain wearing healthy cotton underwear, using tampon or other sanitary napkins, long use of those napkins, childbirth etc.^[20] *Klebsiella spp.* (3 isolates) can also infect from contaminated water and intercourse as well. As *Haemophilus influenza* (7 isolates) is a pathogen of respiratory tract, cases have been found it to cause vaginosis and considered as both maternal and neonatal pathogen.^[21] *Streptococcus pyogenes* (15 isolates), and *Enterococcus spp.* (1 isolates) were also found in HVS samples and it has been considered that *Streptococcus pyogenes* and *Enterococcus spp.* are part of female genital normal flora but can cause disease (vaginosis) acquiring some virulence factor or getting chance to

overpopulate themselves and under different environmental conditions.^[21] A male partner having pathogenic genital bacteria can interchange with the female partner and using condom/protection this incident can be managed. Upon infection it is needed to receive proper treatment to cure the disease. But as we found in this study, lots of antibiotics have become resistant to these bacteria causing vaginosis. Yet there are several drugs left to be resistant, currently patients have some options to recover from vaginosis. But there exists some bacterial isolates which are going to be resistant soon for all the current antibiotics available. Such isolates include *Escherichia coli* from sample 4,17,19,25,49,60,61,92; *Staphylococcus aureus* from sample 6,26,28,63,74,75,81,84,98; *Streptococcus pyogenes* from sample 32,36,86; and *Haemophilus influenza* from sample 3,4 and 7. These isolates are resistant to most of the antibiotics in use today and are a threat to transfer their resistance gene to other isolates which are still sensitive by the help of plasmids and virus or mutations in the genetic material.

CONCLUSION

Vaginosis depends on some factors to occur like black ethnicity, smoking, changing sexual partner, using IUD contraception etc. Using lactate gel to intra vaginally after each period for three consecutive days maintain the low pH in case of abnormally high pH condition of vagina can reduce the chance of vaginosis and enhance the growth of vaginal *Lactobacillus* spp. Maintaining good personal hygiene, wearing clean cotton underwear, having fixed sexual partner and using protection during intercourses, completing the antibiotic dose in case of vaginosis, avoiding the use of tampon etc. can reduce the cases of vaginosis.

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