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Original Article

Antimicrobial Sensitivity Pattern of Microorganisms Isolated from Vaginal Infections at a Tertiary Hospital in Bangalore, India

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Abstract

Background: The vagina contains dozens of microbiological species in variable quantities and is, therefore, considered a complex environment. Among the microorganisms, bacteria have important repercussions on women's health. The present study was conducted to elucidate this type of vaginal isolates and their sensitivity towards currently used antibiotics. **Methods:** This was a retrospective study conducted at the Department of Obstetrics and Gynaecology, Sapthagiri Hospital, Bangalore, India from January 2012 to December 2013. All symptomatic women who had a high vaginal swab taken for culture and sensitivity testing were included in this study. Antibiotic susceptibility was tested using disc diffusion method (modified Kirby-Bauer's method). The antibiotic sensitivity patterns of isolated microorganisms were studied. **Results:** Out of 200 patients, 95% had positive vaginal cultures. Fifteen types of microorganisms were isolated. The highest frequency of infection was seen at the age of 20-30 years, followed by 41-50 years and 31-40 years, and a low frequency of infection was observed above 50 years of age. The most prevalent pathogen was *Escherichia coli*, followed by *Streptococcus agalactiae* and diphtheroids with equal incidence. Among the antibiotics tested, isolated pathogens were completely resistant to nalidixic acid and highly sensitive to meropenem and imepenem. **Conclusion:** The high prevalence of gynaecological infections demands that patients with symptoms undergo thorough investigation with cultures and sensitivity essays. Changes in treatment protocols are required to treat vaginal infections effectively.

Keywords: Vaginitis, Microbiota, Anti-Bacterial Agents, Microbial Sensitivity Tests (Source: MeSH-NLM).

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Introduction

The vagina is a complex ecosystem containing a variety of micro-organisms.[1] This unique environment undergoes significant changes throughout life, from birth to puberty and menopause.[2] Females are more prone to urinary and vaginal infections because of the anatomical and functional proximity to the anal canal and due to the short urethra.[3] The sex steroid hormones play a vital role in stabilizing this environment. In normal women, the estrogen accounts for the maturation of vaginal epithelium, resulting in the accumulation of glycogen which helps in the maintenance of vaginal pH.[4]

The causative organisms can be endogenous, iatrogenic or sexually transmitted. The human body harbours hundreds of organisms of gram-positive and gram-negative varieties in the lower one-third of vagina. A key protective role is played by the lactic acid-producing bacteria in keeping the vaginal pH low.[5]

Common organisms are *Neisseria gonorrhoea*, *Trichomonas vaginalis*, *Streptococcus agalactiae* (group B Streptococcus) and *Chlamydia trachomatis*. Presentation includes itching and pain in the external genitalia and vagina, painful sexual intercourse, and the presence of abnormal vaginal discharge.[6]

The balanced vaginal environment varies with practices like douching, dressing, use of contraceptives and sexual activity.[7] Coexistent factors like diabetes and pregnancy also play a role in the vaginal ecosystem imbalance. Surprisingly, not many studies have investigated the prevalence of vaginal infections in relation to age and that of the antibiotic sensitivity pattern.[8] Morbidity associated with these infections also affects the economic productivity and quality of life of many individual women and consequently of communities as a whole.

Many women believe that such infections are normal and part of the female experience and do not seek care due to shame or lack of information.[9] In the pregnant woman, these infections lead to preterm labour, chorioamnionitis, premature rupture of membranes and low birth weight of the neonate, leading to high perinatal mortality.[10]

These infections can be easily detected by simple tests such as a vaginal swab for culture and sensitivity testing, which inform us the causative organism as well the antibiotic to use against it.[11] As per the Centre for Disease Control and Prevention (CDC) guidelines, the management includes therapy based on susceptibility pattern, partner notification, follow-up and health promotion. The aim of our study was to identify the common organisms in vaginal infections and to discuss the sensitivity pattern based on culture sensitivity. By studying sensitivity pattern to antibiotics in these strains, we may revise the recommendations for treatment protocol in such patients.

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Methods

This is a retrospective study conducted at Department of Obstetrics and Gynaecology, Sapthagiri Hospital (a tertiary health care centre), Bangalore from January 2012 to December 2013. Two hundred women aged between 20 to 65 years who had presented with symptoms of vaginal infections were included based on the previous records of culture and sensitivity obtained from the Department of Microbiology. They were labelled with a unique identifier to ensure confidentiality and freedom from bias. All the women had presented with one or more of the following symptoms: vaginal discharge, dyspareunia, malodour, dysuria, itching and fever. Those using antibiotics during the last two weeks and those with a recent history of vaginal instrumentation were excluded from the study.

Three swabs were collected for each woman. The first sample was a high vaginal swab from which culture was performed using blood agar for gram-positive bacteria, MacConkey agar for gram-negative bacteria (especially the Enterobacteriaceae), and Sabouraud's dextrose agar for fungi. They were incubated for 48 hours. The second sample was used for direct microscopic examination of *Trichomonas vaginalis*. The cervical samples were collected in suspected cases only and processed for the detection of *Chlamydia trachomatis*. Once the samples were obtained, they were transported to the Microbiology Laboratory Department at Sapthagiri Hospital, Bangalore.

Identification of microorganisms: isolated bacteria were identified using conventional methods, including colonial morphology, Gram stain, motility, germ tube test and biochemical tests (DNAse, catalase, Indole, coagulase, lactose fermentation, urease, oxidase, sugar fermentation, citrate utilization). Identification of *Streptococci* was based on haemolysis and thereby categorized according to Lancefield's grouping.

Antibiotic susceptibility was tested using disc diffusion method (modified Kirby-Bauer's method). Antimicrobials tested for sensitivity were amikacin, ampicillin, amoxicillin, imepenem, meropenem, ceftazidime, ceftriaxone, tigecycline, ciprofloxacin, cefpodoxime, nalidixic acid and cefixime.

Data obtained were presented as distribution of microorganisms with respect to age, number and percentage of patients from which the microorganisms were isolated, and antimicrobial sensitivity patterns. Analysis was done using Microsoft Excel program. However, the analysis was done with respect to age only, as details of social factors were not available. Based on data obtained, observations were drawn regarding the age-specific infective rates and present status of antimicrobial sensitivity patterns.

The ethical approval for this study was given by the Ethics and Research Committee of Sapthagiri Institute of Medical Sciences and Research Centre, Bangalore.

Results

Out of 200 women enrolled in the study, 95% had positive vaginal cultures. Fifteen microorganisms, including gram-negative bacteria, were isolated. The highest infection rate was observed among women aged between 20 and 30 years (39.5%), followed by those aged 31-40 years (19.0%) and 41-50 years (31.0%), and the lowest frequency of infection was observed among those aged between 51 and 65 years (5.5%), as shown in ***Table 1***.

Table 1. Distribution of Microorganisms with Respect to Age and Infection Rates.

Age (years)	Frequency (%)	Organisms
20-30	79 (39.5%)	*Commensals, other gram-negative bacteria, extended-spectrum beta-lactamase-producing Escherichia coli, coagulase-negative Staphylococcus aureus, diphtheroids, Candida albicans, Acinetobacter.*
31-40	38 (19.0%)	*Commensals, Escherichia coli, Streptococcus agalactiae, Gardnerella vaginalis, Klebsiella pneumoniae, Citrobacter.*
41-50	62 (31.0%)	*Proteus vulgaris, Escherichia coli, Klebsiella pneumoniae, commensals, Micrococci, methicillin-resistant Staphylococcus aureus.*
>50	11 (5.5%)	*Escherichia coli, Enterococci, Klebsiella pneumoniae, commensals, Pseudomonas aeruginosa, Proteus vulgaris, coagulase-negative Staphylococcus aureus.*

Table 2. Types and Proportions of Microorganisms Recovered from Women with Vaginal Infections at Department of Obstetrics and Gynaecology, Sapthagiri Hospital, Bangalore.

Organism	Frequency (%)
Escherichia coli	**23 (11.5%)**
Extended-spectrum beta-lactamase-producing Escherichia coli	13 (6.5%)
Streptococcus agalactiae	12 (6.0%)
Diphtheroids	12 (6.0%)
Pseudomonas aeruginosa	10 (5.0%)
Methicillin-resistant Staphylococcus aureus	10 (5.0%)
Gram-negative bacteria	10 (5.0%)
Gardnerella vaginalis	10 (5.0%)
Citrobacter	10 (5.0%)
Enterococci	8 (4.0%)
Proteus vulgaris	8 (4.0%)
Coagulase-negative Staphylococcus aureus	8 (4.0%)
Micrococci	7 (3.5%)
Klebsiella pneumoniae	7 (3.5%)
Acinetobacter	7 (3.5%)
Candida albicans	5 (2.5%)
Mixed growth	2 (1.0%)

Microorganism with the highest frequency of infection was *Escherichia coli* (11.5%), followed by extended-spectrum beta-lactamase-producing *Escherichia coli* (6.5%), *diphtheroids* (6.0%), *Streptococcus agalactiae* (6.0%), *Pseudomonas aeruginosa* (5.0%), *Gardnerella vaginalis* (5.0%), methicillin-resistant *Staphylococcus aureus* (MRSA) (5.0%), *Citrobacter* (5.0%), and other gram-negative bacteria (5.0%). *Candida albicans* was isolated in 2.5% of the cases. Other organisms, such as *Proteus vulgaris*, coagulase-negative *Staphylococcus aureus*, *Acinetobacter*, *Klebsiella pneumoniae*, Micrococci and Enterococci, were also isolated (***Table 2***).

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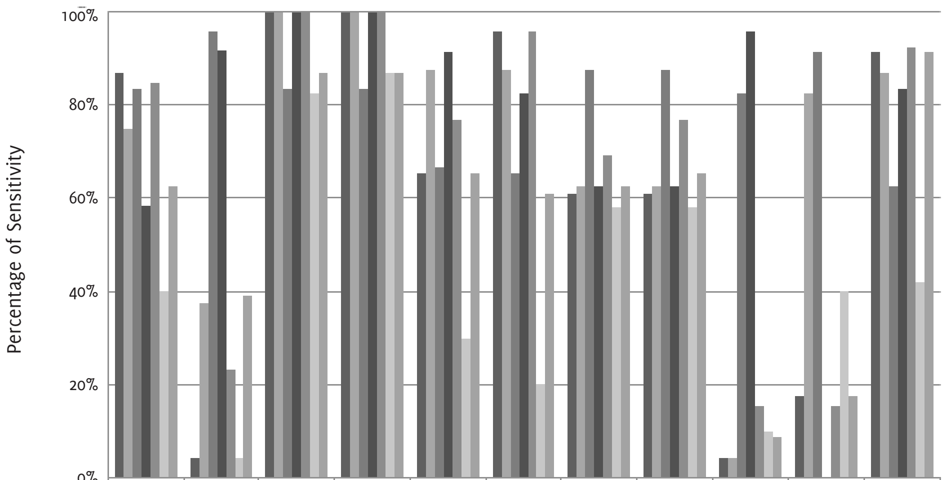
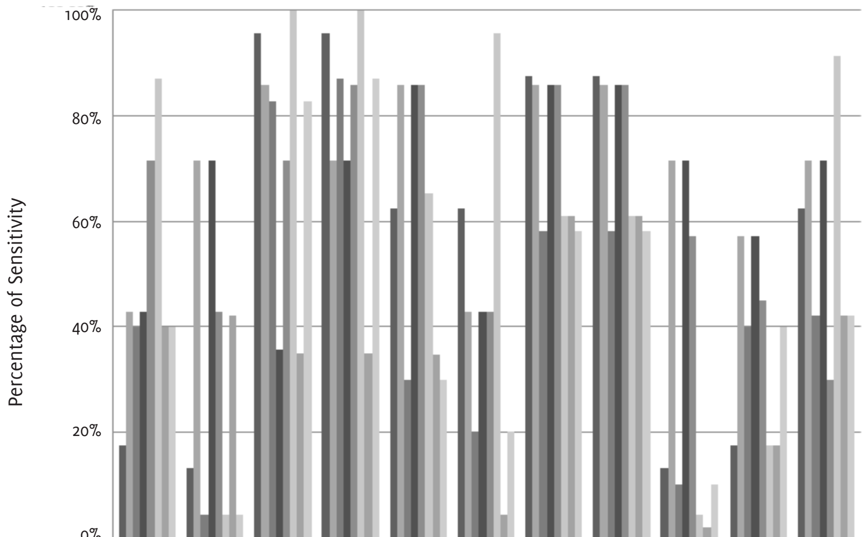
Figure 1. Antibiotic Sensitivity Pattern of the Isolated E. Coli, Proteus, Diphtheroids, Streptococci, Micrococci, Pseudomonas and Enterococci to 11 Antimicrobial Agents.



	AMK	AMP	MRP	IMP	CFX	TGC	CFZ	CFP	AMX	CFT	CIPRO
ECOLI	86,95%	4,35%	100%	100%	65,21%	95,65%	60,86%	60,86%	4,35%	17,40%	91,30%
PROTEUS	75%	37,50%	100%	100%	87,50%	87,50%	62,50%	62,50%	4,35%	82,60%	86,95%
DIPTHEROIDS	83,30%	95,65%	83,30%	83,30%	66,66%	65,21%	87,50%	87,50%	82,60%	91,30%	62,50%
STREPTOCOCCI	58,33%	91,66%	100%	100%	91,30%	82,60%	62,50%	62,50%	95,65%	0%	83,30%
MICROCOCCI	84,61%	23,07%	100%	100%	76,92%	95,65%	69,23%	76,92%	15,38%	15,39%	92,30%
PSEUDOMONAS	40%	4,35%	82,60%	86,95%	30%	20%	58%	58%	10%	40%	42%
ENTEROCOCCI	62,50%	39,14%	86,95%	86,95%	65,21%	60,86%	62,50%	65,21%	8,70%	17,40%	91,30%

Legend: ECOLI-Escherichia coli. Antibiotics: AMK-Amikacin, AMP-Ampicillin, MRP-Meropenem, IMP-Imipenem, CFX-Cefixime, TGC-Tigecycline, CFZ-Ceftazidime, CFP-Cefpodoxime, AMX-Amoxicillin, NA-Nalidixic acid, CFT-Ceftriaxone, CIPRO-Ciprofloxacin.

Figure 2. Antibiotic Sensitivity Pattern of the Isolated Coagulase-Negative Staphylococcus Aureus, Acinetobacter, Citrobacter, Klebsiella, ESBL-producing E. Coli, Gardnerella Vaginalis, MRSA and other GNB to 11 Antimicrobial Agents.



	AMK	AMP	MRP	IMP	CFX	TGC	CFZ	CFP	AMX	CFT	CIPRO
CONS	17,40%	13,05%	95,65%	95,65%	62,50%	62,50%	87,50%	87,50%	13,05%	17,40%	62,50%
ACINETOBACTER	42,85%	71,42%	85,71%	71,42%	85,71%	42,85%	85,71%	85,71%	71,42%	57,14%	71,42%
CITROBACTER	40%	4,35%	82,60%	86,95%	30%	20%	58%	58%	10%	40%	42%
KLEBSIELLA	42,85%	71,42%	35,71%	71,42%	85,71%	42,85%	85,71%	85,71%	71,42%	57,14%	71,42%
ESBL E.COLI	71,42%	42,85%	71,42%	85,71%	85,71%	42,86%	85,71%	85,71%	57,14%	45%	30%
GARDNERELLA VAGINALIS	86,95%	4,35%	100%	100%	65,21%	95,65%	60,86%	60,86%	4,35%	17,40%	91,30%
MRSA	40%	42%	35%	35%	34,79%	4,35%	60,86%	60,86%	2%	17,40%	42%
other GNB	40%	4,35%	82,60%	86,95%	30%	20%	58%	58%	10%	40%	42%

Legend: CONS-Coagulase-negative *Staphylococcus aureus*, ESBL E.COLI-Extended-spectrum beta-lactamases-producing *Escherichia coli*, MRSA-Methicillin-resistant *Staphylococcus aureus*, GNB-Gram-negative bacteria. Antibiotics: AMK-Amikacin, AMP-Ampicillin, MRP-Meropenem, IMP-Imipenem, CFX-Cefixime, TGC-Tigecycline, CFZ-Ceftazidime, CFP-Cefpodoxime, AMX-Amoxicillin, NA-Nalidixic acid, CFT-Ceftriaxone, CIPRO-Ciprofloxacin.

Table 3. Antimicrobial Sensitivity Pattern of Isolated Microorganims.

Antiobiotic	Microorganisms						
	Escherichia coli	*Proteus vulgaris*	*Diphtheroids*	*Streptococcus agalactiae*	Micrococci	*Pseudomonas*	Enterococci
AMK	+++++	++++	+++++	+++	++++	+++	++++
AMP	+	++	+++++	+++++	++	+	++
MRP	+++++	+++++	+++++	+++++	++++	+++++	+++++
IMP	+++++	+++++	+++++	+++++	++++	+++++	+++++
CFX	++++	+++++	++++	+++++	++++	++	++++
TGC	+++++	+++++	++++	+++++	+++++	+	++++
CFZ	++++	++++	+++++	++++	++++	+++	++++
CFP	++++	++++	+++++	++++	++++	+++	++++
AMX	+	+	+++++	+++++	+	+	+
NA	+	+	+	+	+	+	+
CFT	+	+++++	+++++	+	+	++	+
CIPRO	+++++	+++++	+++++	+++++	+++++	+++	+++++

Legend: Legend: Sensitivity rates: 80%-100%: +++++, 60%-80%: ++++, 40%-60%: +++, 20%-40%: ++, 0%-20%: +. Antibiotics: AMK-Amikacin, AMP-Ampicillin, MRP-Meropenem, IMP-Imipenem, CFX-Cefixime, TGC-Tigecycline, CFZ-Ceftazidime, CFP-Cefpodoxime, AMX-Amoxicillin, NA-Nalidixic acid, CFT-Ceftriaxone, CIPRO-Ciprofloxacin.

Table 4. Antimicrobial Sensitivity Pattern of Isolated Microorganims.

Antiobiotic†	Microorganisms‡							
	CoNS	*Acinetobacter*	*Citrobacter*	*Klebsiella*	*ESBL-producing E. coli*	*Gardnerella vaginalis*	MRSA	GNB
AMK	+	+++	+++	+++	++++	+++++	+++	+++
AMP	+	++++	+	++++	+++	+	+++	+
MRP	+++++	+++++	+++++	++	++++	+++++	++	+++++
IMP	+++++	++++	+++++	++++	+++++	+++++	++	+++++
CFX	++++	+++++	++	+++++	+++++	++++	++	++
TGC	++++	+++	+	+++	+++	+++++	+	+
CFZ	+++++	+++++	+++	+++++	+++++	++++	++++	+++
CFP	+++++	+++++	+++	+++++	+++++	++++	++++	+++
AMX	+	++++	+	++++	+++	+	+	+
NA	+	+	+	+	+	+	+	+
CFT	+	+++	+++	+++	+++	+	+	+++
CIPRO	++++	++++	+++	++++	++	+++++	+++	+++

Legend: Legend: Sensitivity rates: 80%-100%: +++++, 60%-80%: ++++, 40%-60%: +++, 20%-40%: ++, 0%-20%: +. † AMK-Amikacin, AMP-Ampicillin, MRP-Meropenem, IMP-Imipenem, CFX-Cefixime, TGC-Tigecycline, CFZ-Ceftazidime, CFP-Cefpodoxime, AMX-Amoxicillin, NA-Nalidixic acid, CFT-Ceftriaxone, CIPRO-Ciprofloxacin. ‡ CoNS-Coagulase-negative Staphylococci, ESBL-Extended-spectrum beta-lactamase, MRSA-Methicillin-resistant *Staphylococcus aureus*, GNB-Gram-negative bacilli.

Escherichia coli (***Figure 1*** and ***Table 3***) was most sensitive to meropenem (100%) and imipenem (100%) and most resistant to amoxicillin (4.4%). Proteus vulgaris showed sensitivity to meropenem (100%), imipenem (100%), cefixime (87.5%), tigecycline (87.5%) and ciprofloxacin (87.0%). Diphtheroids showed sensitivity to ampicillin (95.7%), ceftriaxone (91.3%), cefpodoxime (87.5%), ceftazidime (87.5%), and others. *Streptococcus agalactiae* showed sensitivity to meropenem (100%), imipenem (100%), amoxicillin (95.7%), ampicillin (91.7%) and cefixime (91.3%). Micrococci showed 100% sensitivity to meropenem and imipenem. *Pseudomonas aeruginosa* and Enterococci were sensitive to meropenem and imipenem, as shown in ***Figure 1*** and ***Table 3***. Complete resistance to nalidixic acid was noted with all organisms.

As shown in ***Figure 2*** and ***Table 4***, coagulase-negative *Staphylococcus aureus* showed sensitivity to meropenem (95.7%), imipenem (95.7%), cefpodoxime (87.5%), ceftazidime (87.5%), cefixime (62.5%) and tigecycline (62.5%). Acinetobacter showed 85.7% sensitivity to meropenem, cefixime and cefpodoxime; Citrobacter and other gram-negative bacteria showed sensitivity to imipenem (87.0%) and meropenem (82.6%). *Klebsiella pneumoniae* showed sensitivity to ceftazidime and cefpodoxime. Extended-spectrum beta-lactamase-producing *Escherichia coli* showed sensitivity to imipenem and cefixime, whereas *Gardnerella vaginalis* showed sensitivity to meropenem (100%) and imipenem (100%). MRSA strains were sensitive to ceftazidime (60.9%) and cefpodoxime (60.9%).

Discussion

Vaginal infections have wide implications for women's health, being the most common gynaecological problem.[12] It is believed that the lactobacilli play an important role in maintaining normal vaginal ecosystem and preventing the growth of opportunistic bacteria.[13] Our study demonstrates the prevalence of potential vaginal pathogens in symptomatic women.

The results of our study are comparable to the study by Lakshmi K et al., which compared the prevalence of vaginal infections between premenopausal and postmenopausal women.[14]

Increased infections in post-menopausal women are due to the vagina being colonised by pathogenic organisms more than the protective organisms.[15] The highest frequency of infection was noted at an age of 20 to 30 years with a fall in the frequency of infection as age advanced. Similar results with respect to age were seen in a Kenyan study, although it was limited to the study of only one organism.[16]

Several microorganisms were isolated in our study, and those with the highest frequencies were *Escherichia coli*, diphtheroids, *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, MRSA, Micrococci, Enterococci, Acinetobacter, Citrobacter and *Candida albicans*. Commensal growths were found in 14% of cases that need not be treated, but necessary measures such as identification of risk factors (douching, sprays, diabetes) and their prevention have to be carried out.

We also found *Escherichia coli* to be the most common pathogenic bacteria (11.5%) isolated from culture. In fact, *Escherichia coli* was found to be the most prevalent pathogen isolated not only from high vaginal swabs but also urine, pus, blood and wounds, as seen in a study conducted by Dutta S et al., in Dhaka.[17]

Candida albicans is tolerant to the acidic environment and is hence found in the vagina, but the concentrations are too low to cause symptoms. In conditions of decreased local immunity, the hyphae would multiply and transform into infective patterns that result in symptomatic vaginitis.[18] Colonization of Candida species also happens during pregnancy, resulting in symptomatic vaginitis.[19]

Infections with MRSA became a global health issue in 1960s, when the strains were first identified. They may be acquired nosocomially or from the community. What make them difficult to treat are their multiple antibiotic resistant profiles and wide varying prevalence.[20] One should keep in mind that higher antibiotics may be required to treat these infections.

In our study, 6% of the women carried *Streptococcus agalactiae* (Group B Streptococci). However, information regarding their pregnancy status is not available. Maternal group B Streptococci (GBS) colonisation is a major risk factor for GBS disease in neonates.[21] In pregnant women, GBS causes cystitis, amnionitis, endometritis and stillbirth; occasionally, it leads to endocarditis or meningitis.

Coagulase-negative Staphylococci (CoNS), which are considered to be skin commensals, were found in 4% of the cases. No cases of trichomoniasis, Chlamydia infection, and *Neisseria gonorrhoeae* infection were noted.

The presence of co-morbidities like hospitalization, immunosuppression and co-existent reproductive tract infections have to be evaluated accordingly. It is known that vaginal infections, due to a disruption of normal vaginal flora, increase the risk of sexually transmitted infection, especially human immunodeficiency virus (HIV).[22] However, our study did not identify any association with HIV, and diabetic status records were not available. No patients had taken hormone supplements or any other medications that could interfere with the results.

Our study has several limitations. The practice of swab culture is done mostly in clinical microbiological laboratories, and clinical diagnosis may be suboptimal. Only regularly used antibiotics were included in the sensitivity testing, and socio-demographic factors have not been considered. These limitations have to be overcome by future studies, and proper practices have to be implemented in order to preserve these lifesaving drugs for the future.

Diagnosis of these infections based on culture sensitivity is a definite step in treatment of these infections. In regular practice, fixed protocols are followed. Inadequate treatment with antimicrobials due to non-compliance or under the prescription of drugs results in high incidence of recurrence. Extensive resistance rates have emerged among commonly used antibiotics due to indiscriminate use. Newer antibiotics like imipenem and meropenem are highly effective but expensive.[23] Short term effects of antimicrobial regimens have been tested and proved through clinical trials to be effective in achieving clinical and microbiological cure. Newer therapeutic approaches include the development of new drugs, phage therapy (bacterial viruses can be robust anti-bacterial agents in vitro), photodynamic inactivation of micro-organisms and immunomodulators.[24] A significant proportion of pathogens causing vaginal infections are resistant to the conventionally used antibiotics. This study is a step in familiarizing sensitivity and resistance patterns to used antibiotics, preventing resistance and thus preventing the chronic sequelae. Thus, we raise a question of changing the syndromic protocol to treatment protocol based on culture sensitivity. Substantial health gains with a reduction of the disease burden among women should be the long term goal of treatment which should be intended with knowledge of culture sensitivity.

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