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Molecular determination of the microbial diversity associated with vaginitis and testing their sensitivity to selected antimicrobials

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Abstract. *Al-Zaidi MHH, Al-Tamimi WH, Saleh AAA. 2023. Molecular determination of the microbial diversity associated with vaginitis and testing their sensitivity to selected antimicrobials. Biodiversitas 24: 4253-4261.* Millions of women suffer from bacterial vaginitis is associated with several serious health problems. The present study aimed to investigate the microbial diversity and their sensitivity to different antimicrobial agents. Bacterial isolates were identified by genotyping using the 16S rRNA gene sequencing and BLASTn analysis, while conventional mycological methods identified *Candida* spp. The disc diffusion method was used to study the antimicrobial susceptibility patterns. The molecular identification showed that *Escherichia coli* (20.8%) and Staphylococcus haemolyticus (20.8%) were the most frequent species, followed by *Klebsiella pneumoniae* (16.7%). At the same time, *Macrococcus caseolyticus, Streptococcus agalactiae, Staphylococcus aureus*, and *Bacillus cereus* were the least prevalent bacteria (4.16%)—all *Candida* sp. against the selected antimicrobial agents. Thus, molecular assays are important in monitoring microorganisms associated with vaginitis. Prospective genotyping studies are needed to determine these microbes' resistant genes and understand their mode of action and response to drug therapy.

Keywords: 16S rRNA gene sequencing, bacterial diversity, *Candida albicans*, susceptibility vaginosis

INTRODUCTION

The most common cause of vaginal discharge is bacterial vaginosis (BV), which has prevalence rates of 23 to 29% in different parts of the world (Peebles et al. 2019). It is characterized by a change in the vaginal microbiota from a dominant *Lactobacillus* sp. to a broad array of facultative and strictly anaerobic bacteria that form a multi-species biofilm on vaginal epithelial cells; there is a significant rate of recurrence even after treatment (Muzny et al. 2022). BV has also been linked to several potential side effects, including a higher risk of preterm birth and contracting infections of human immunodeficiency virus, human papillomavirus infection, other sexually transmitted diseases, and pelvic inflammatory disease (Muzny et al. 2019). A change in the vaginal microbiome can result in bacterial vaginosis (BV), often accompanied by inflammation (Redelinghuys et al. 2020).

Vaginitis is the most frequent gynecologic diagnosis in general care, and the majority of women experience it at least once in their lifetimes; therefore, the importance of standardized methods for diagnosing and treating vaginitis is stressed in both primary care and among gynecologists (Eleutério et al. 2023). Many pathogenic processes, each mediated by one or more types of vaginal pathogens, result in mixed vaginitis (Xiao et al. 2022). Aerobic vaginitis (AV) (Donders et al. 2017) differs from BV. It is marked by vaginal epithelial inflammation as well as aberrant vaginal microbiota that includes aerobic and Enterobacteria such as *Acinetobacter* spp., *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp., *Staphylococcus* spp., and group B *Streptococcus* (GBS). Nevertheless, AV and BV are vaginal dysbioses with reduced *lactobacilli* (Son et al. 2018). The vaginal microbiota of non-pregnant AV patients was examined by Wang et al. (2020) through next-generation sequencing of the 16S rRNA gene. They discovered that the relative abundance of some aerobes in the AV group subjects, including *Aerococcus christensenii, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas putida, Streptococcus agalactiae, S. anginosus, S. luteciae*, and *S. mitis* are significantly raised compared to those in healthy women. One of the infections, vulvovaginal candidiasis (VVC), affects roughly 75% of reproductive-aged women at some point in their life, with recurrences in about 5% of these women (Xia et al. 2016; Ceccarani et al. 2019).

Under certain circumstances, *Candida* species transform into a robust opportunistic fungal pathogen instead of a normal component of the vaginal microflora, with *C. albicans* causing 80-92% of VVC cases (Peters et al. 2014). Antimicrobial treatment guidelines for BV symptoms have been established and are readily available, in addition to the significant research for efficient medicines (Mendling et al. 2022). The BV treatment goal is to balance out the vaginal flora to stop the spread of dangerous pathogens. Antibiotics, such as metronidazole or clindamycin, are prescribed. Research on vaginitis is becoming more intense because of largely missing knowledge surrounding BV's origin, the high recurrence rate, the inadequate treatment options, and the clinical

management, which is frequently insensitive and uneven (Abou et al. 2022). Most affected women seek alternative therapies like probiotics (*Lactobacillus*) and boric acid because BV is linked to poor quality of life (Chow et al. 2023).

The information on the genetic identification of bacteria that cause BV in Iraq is generally lacking; hence, the current study aimed to present a thorough description of the community structure of the vaginal microbiota in women with vaginal discharge and determine the antimicrobial susceptibility profiles for as-needed therapy, and also to identify the bacterial isolates genotypically using polymerase chain reaction (PCR) and comparing the 16S rRNA gene sequences information of each bacterium with data from the NCBI GenBank.

MATERIAL AND METHODS

Samples collection

Samples were collected from 52 women diagnosed with vaginitis by the specialist doctor at Al-Fyehaa and Ibn Ghazwan Hospital in Basrah Government, Iraq, from February to April 2022. The vaginal swabs were collected using sterile cotton and provided with a transport medium. The swabs were inserted 2 cm into the vagina and rotated gently towards the vaginal wall. Samples were immediately transported to the laboratory for bacterial and yeast isolation and characterization.

Bacterial culturing conditions

All collected vaginal swabs were plated onto blood agar, MacConkey agar, and chocolate agar to isolate aerobic bacteria from infected women. The inoculated plates were incubated aerobically at 37°C for 24 h. Single, isolated colonies were streaked on a nutrient agar plate to obtain pure isolates. The isolates were maintained on slants agar. All isolates were identified using well-established microbiological techniques, including assessment of morphological characteristics, Gram-staining, and genetic methods.

Molecular identification of bacteria

DNA extraction

The genomic DNA of all bacterial isolates was extracted using the Promega Wizard Bacteria Kit (Promega, USA), following the manufacturer's protocol. The genomic DNA concentrations were visualized by 0.8% agarose gel electrophoresis.

Amplification of 16S rRNA gene

To amplify the 16S rRNA gene, PCR was performed using a universal 27F forward primer (5´-AGAGTTTGATCCTGGCTCAG-3') and 1492R reverse primer (5´-GGTTACCTTGTTACGACTT-3'). The PCR mixture contained primer solution (1 µL each), template DNA (2µL), nuclease-free water (1 µL), and PCR master mix (20 µL). A thermal cycle was used for PCR, and the amplification involved initial denaturation for 2 min at 94°C, 35 cycles of denaturation at 94°C for 40 sec, annealing at 55°C for 30 sec, extension at 72°C for 1 min, and a final elongation step at 72°C for 10 min (EL-Sheshtawy et al. 2015). PCR products with an estimated size of around 1500 bp were electrophoresed on a 2% (w/v) agarose gel with a 100-base pair DNA ladder tag at a voltage 65 and a current of 120 mA for 40 min. The fragments obtained by the PCR of the gene coding for 16S rRNA were purified at Macrogen Company (South Korea) for gene sequencing detection using the same primer. The 16S rRNA gene sequences were compared to nucleotide sequences in NCBI using the program BLAST (http://www.ncbi.nlm.nih.gov/BLAST) to determine homology scores and characterize bacterial strains. The genetic similarity of the identified isolates to the sequences available in the GenBank was assessed by preparing a phylogenetic tree of the isolated and closely related species using MEGA 11 software (Tamura et al. 2021).

Identification of *Candida* spp. isolates

Gram-staining showed that some isolated colonies had the characteristics of *Candida* sp. These isolates were identified morphologically and by some biochemical tests. The isolates were first activated by inoculation into Sabouraud dextrose agar (SDA) medium and incubated at 37°C for 48 h. Subsequently, they were streaked on chromogenic *Candida* agar and incubated at 37°C for 48 h. The identification of yeast was based on the color of the growing colonies. Next, to evaluate their ability to germ tube formation, the yeast isolates were cultured on an SDA medium for 1-3 days at 37°C. Then, a small portion of the activated colony was added to 0.5 mL of serum tubes and incubated at 37°C for 3 h. After the incubation period, the samples were examined by adding a drop of inoculum on the slide, covered with a coverslip, and examined microscopically (10X and 40X magnification) to detect the formation of the elongated tube without constriction from mother cells, indicating germ tube formation. For chlamydospore production, 24-48 hours old yeast colonies were inoculated on the plates of corn meal agar-Tween 80 medium, covered with a sterile cover slide, and incubated at 25°C for 2-6 days. The growth was stained with lactophenol cotton blue and examined microscopically to detect chlamydospore formation. The identified isolates were confirmed by subculture on tobacco agar medium; this medium was prepared as described earlier (Tendolkar et al. 2003). In brief, 50 g of tobacco from a commercial cigarette containing 6 mg tar and 0.5 mg nicotine was dissolved in 1,000 mL of distilled water, boiled for 30 min, and filtered by clean and dry gauze. Then, 20 g of agar was added to the filtrate, and the volume was maintained at 1 L. Tobacco agar plates were streaked with a small amount of fresh subculture incubated at 30°C and observed daily for up to 4 days for colony characteristics, especially the color and formation of fringes.

Antibiotic susceptibility of bacteria and *Candida* sp. isolates

The Kirby-Bauer disc diffusion method was used for antimicrobial susceptibility testing. All bacterial isolates were inoculated in nutrient broth and maintained for 24 h,

while yeast was cultivated in SDA for 24 h. Then, 0.1 mL of each bacteria inoculum containing 10^8 CFU/mL (0.5 McFarland turbidity stander) was swabbed on Mueller Hinton agar. The antibiotics (Oxoid, England) ceftriaxone (CTR) 30 µg azithromycin (AT) 15 µg, doxycycline (DO) 30 µg, ciprofloxacin (CIP) 5 µg, gentamicin (GEN) 10 µg, and amoxicillin (AMC) 30 µg were used for bacterial strains. The antifungals used for *Candida* spp. were nystatin (NS) 15 µg, colistin sulfate (CS) 10 µg, fluconazole (FLU) 25µg, and posaconazole (POS) 5 µg (Italy). The diameter of the inhibition zone was measured in millimeters according to Clinical and Laboratory Standards Institute (2021) guidelines. Resistance, sensitivity, and intermediate data were interpreted.

RESULTS AND DISCUSSION

Microbial prevalence in vaginal samples

This study investigated the prevalence, composition, and diversity of potential aerobic vaginal pathogens in symptomatic women with vaginal infections. Samples were collected - from 52 women with vaginal infections; finally, 54 isolates were obtained. Some of the samples we collected were duplicates, which is why we ended up with two isolates more than the number of unique samples. Among these, only 45 gave positive culture; microscope investigation revealed six isolates (13.3%) with characteristics of *Candida* sp., and 39 (86.7%) isolates were identified as bacteria. The incidence of Gram-positive bacteria was observed in 19 cases (42.2%), whereas Gram-negative bacteria were recorded in 20 cases (44.5%) (Figure 1). These findings were consistent with the results previously reported by Serretiello et al. (2021). They contradicted those stated by Ghiasi et al. (2014), who mentioned that Gram-positive bacteria were significantly higher in number than the Gram-negative bacteria in the examined vaginal samples. The cause of vaginal dysbiosis is unknown, but the health effects are severe, including obstetrical problems and an increased risk of sexually transmitted infections and urogenital infections (Abou et al. 2022).

PCR amplification, sequencing, and phylogenetic analysis

The electrophoresis technique showed the pure quality of the isolated genomic DNA from all isolates (Figure 2); this preparation was used for 16S rRNA gene sequencing using a set of universal primers, 27F, and 1492R. The PCR products were visualized by agarose gel electrophoresis, with bands corresponding with 1500 bp compared to the bands of the 100 bp DNA ladder (Figure 3). This finding was consistent with the results reported by Al-Tamimi and Mehdi (2015), EL-sheshtawy et al. (2015), and Hafsari and Purnawan (2020).

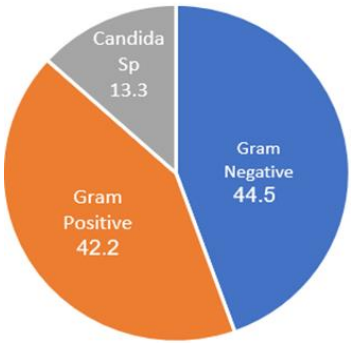

Figure 1. Percentage of bacteria and *Candida* sp. isolated from vaginal samples

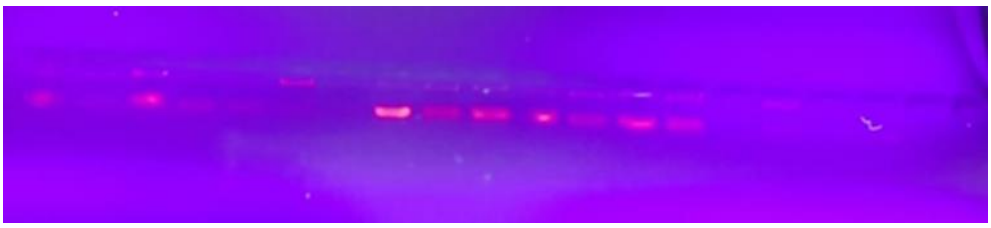

Figure 2. Electrophoresis of genomic DNA

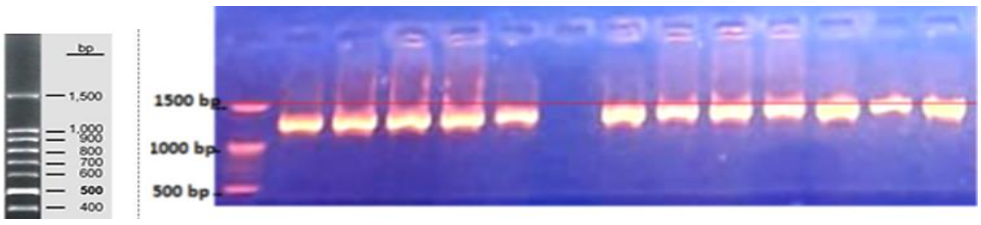

Figure 3. Polymerase chain reaction-amplified products of the 16S rRNA gene for bacterial isolates

Out of the 39 bacteria isolates tested, only 24 could be successfully identified through PCR amplification and 16S rRNA gene sequencing. The 16S rRNA gene sequencing results of isolates were analyzed and compared with their reference strains from the NCBI GenBank database using the BLASTn tool. The analysis showed that bacterial isolates belonged to seven genera and at ten levels of species (Table 1). The genera identified were as follows: *Staphylococcus* spp., *Escherichia* spp., *Klebsiella* spp., *Bacillus* spp., *Enterococcus* spp., *Streptococcus* spp., and *Macrococcus* spp. NCBI BLAST analysis of 16S rRNA gene sequencing of the obtained bacterial isolates showed that the sequence identity with the reference strains was 99 % to 100%. The length in bp ranged from 545 for *E. coli* to 1148 for *E. faecalis.* These results were simulated with those reported previously by Anderson et al. (2014). Molecular technologies are objective, can detect fastidious bacteria, enable quantitation, and are ideal for the self-collection of vaginal swabs (Coleman and Gaydos 2018).

Eight new bacterial strains were identified based on the 16S rRNA gene sequences and the available information. Alignment of the sequenced 16S rRNA gene with those of other 16S rRNA gene sequences from GenBank displayed significant levels of similarity with the reference strains (99%). The 16S rRNA gene sequences were deposited at National Center for Biotechnological Information (NCBI) under the GenBank accession number (Table 2).

The evolutionary relationship between the 24 bacterial isolates and their reference strains was demonstrated by a phylogenetic tree (Figure 4). The dendrogram clustered the genotypes into two main clusters. Cluster I was observed at 64% similarities, comprising Gram-positive micro-organisms, while cluster II was observed at 11.3% similarities and included Gram-negative bacteria. Similar results were reported by Alsanie et al. (2018).

Frequency of bacterial isolates

In the present study, the most frequent species were five strains (20.8%) of each *Staphylococcus haemolyticus* and *Escherichia coli*, followed by four strains (16.7%) of *Klebsiella pneumoniae* (Table 3). Earlier studies noted similar results (Al-Kraety et al. 2022; Yalew et al. 2022).

Table 2. The list of new bacterial strains identified

New strain in GenBank	Accession no.
Staphylococcus haemolyticus BASMWA1	OP646606
Staphylococcus haemolyticus BASMWA2	OP646607
Staphylococcus hominis BASMWA3	OP646608
Enterococcus faecalis BASMWA4	OP646609
Escherichia coli BASMWA5	OP646610
Escherichia coli BASMWA6	OP646611
Klebsiella pneumoniae strain BASMWA7	OP646612
Streptococcus agalactiae BASMWA8	OP646613

Table 1. Genetic identification of pathogenic bacteria isolated from vaginal samples

Isolates code	Closet strain	Identity	Length (bp)	Accession no. of closest species
M1	*Staphylococcus haemolyticus* yasmun69	99%	1050	OK632095
M3	*Staphylococcus haemolyticus* Hakim 1980	99%	623	MT622589
M5	*Bacillus cereus* Gvt-Sh-12	100%	905	OP456397
M6	*Staphylococcus hominis* R14	99%	820	KM017979
M11	*Staphylococcus haemolyticus* EE103-B1	100%	1061	MN581166
M12	*Staphylococcus haemolyticus* yasmun69	100%	1061	OK632095
M13	*Enterococcus faecalis* UFVCC1180	99 %	1148	KY630662
M15	*Escherichia coli* 06P2R2D2E3	100%	869	ON054407
M16	*Staphylococcus hominis* subsp. *novobiosepticus*	100%	1086	LN774616
M17	*Staphylococcus epidermidis* Y19	100%	1105	MW672196
M18	*Staphylococcus epidermidis* BP11	100%	1109	MT482624
M22	*Escherichia coli* 01P2R2D2E5	100%	1120	ON054352
M23	*Klebsiella pneumoniae* strain TZT-18-63	100%	853	MH930397
M24	*Staphylococcus aureus* S21	100%	935	MT154227
M26	*Klebsiella pneumoniae* M1	100%	1131	KP178218
M27	*Escherichia coli* IAUK 8735	99 %	945	MK560846
M28	*Enterococcus faecalis* ABC3	100%	1148	ON631062
M31	*Macrococcus caseolyticus* ZY02	100%	1040	MG996517
M33	*Escherichia coli* strain 152-a blue	100%	545	MN208228
M34	*Escherichia coli* EC87E	99%	1048	MT453882
M35	*Klebsiella pneumoniae* KP1713	99%	857	MK386786
M36	*Klebsiella pneumoniae* NK 2.bp-1	100%	934	EU352755
M37	*Staphylococcus haemolyticus* OB058	100%	1027	KY623006
M39	*Streptococcus agalactiae* 149	99 %	901	MZ670748

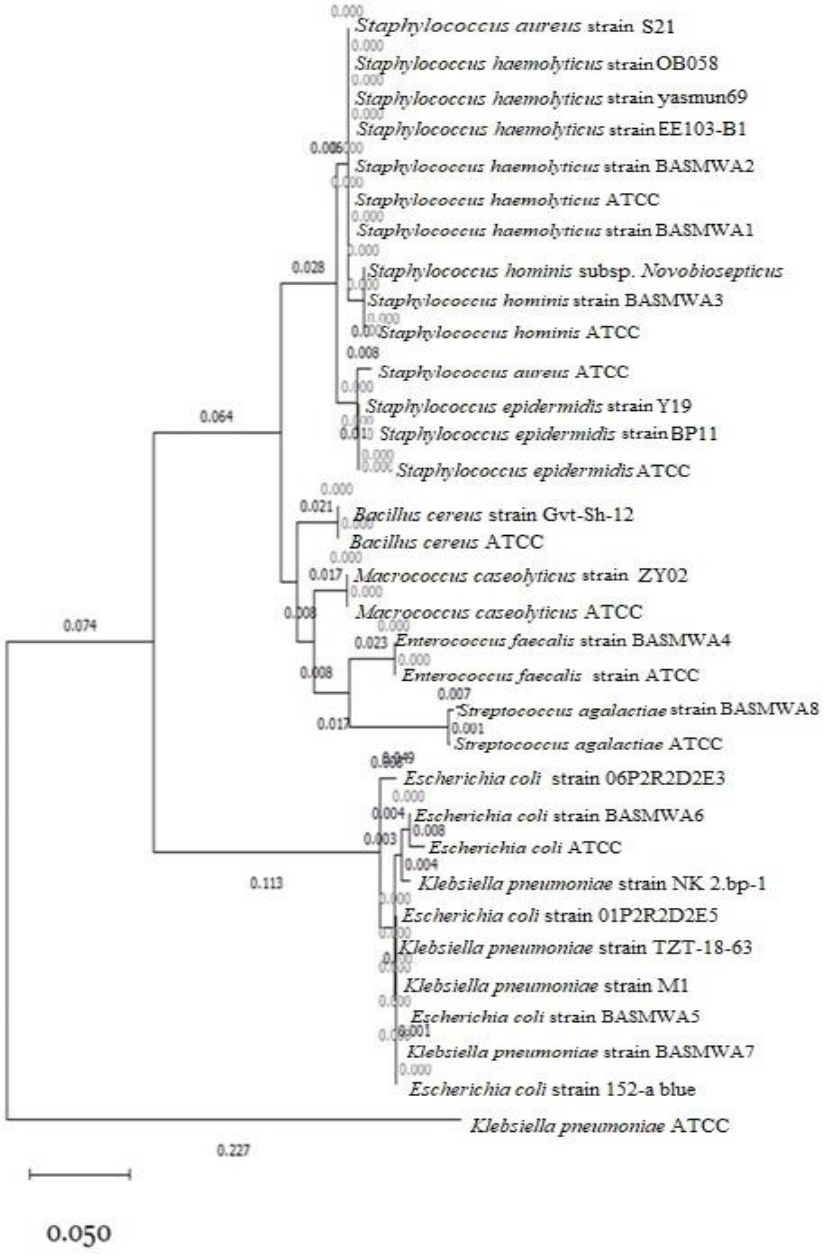

Figure 4. Neighbor-joining phylogenetic reconstruction tree showing evolutionary relationships between bacterial pathogenic isolated from the vagina and sequences of type strains available in the NCBI GenBank database

Table 3. Frequency of pathogenic bacteria isolated from vaginal samples

Isolation code	Closet species	Repetition	Percentage (%)
M1, M3, M11, M37, M12	*Staphylococcus haemolyticus*	5	20
M5	*Bacillus cereus*	1	4
M6, M16	*Staphylococcus hominis*	2	8
M15, M27, M33, M34, M22	*Escherichia coli*	5	20
M17, M18	*Staphylococcus epidermidis*	2	8
M24	*Staphylococcus aureus*	1	4
M26, M35, M36, M23	*Klebsiella pneumoniae*	4	16
M13, M28	*Enterococcus faecalis*	2	8
M31	*Macrococcus caseolyticus*	1	4
M39	*Streptococcus agalactiae*	1	4

Escherichia coli was abundantly detected in vaginal samples; although it is an intestinal inhabitant, there is a high likelihood of opportunistic infections due to poor personal hygiene (Xia et al. 2016). In this study, *Staphylococcus haemolyticus* was the most frequent than *Staphylococcus aureus* 1 (4.2%). This pathogen can cause many illnesses, from mild skin infections to sepsis and fatal pneumonia. Antibiotic resistance poses a challenge in treating this pathogen, and there is currently no effective vaccine. The extraordinary amount of toxins and other virulence factors produced by *S. aureus* and their effect on the disease have been the subject of ongoing and growing interest (Cheung et al. 2021). *Bacillus cereus* was detected in 1 case (4.2%), and *Enterococcus faecalis* and *Staphylococcus epidermidis* were observed in 2 cases each (8.3%). *Enterococci* are naturally found in the human intestine and can be transmitted to the vagina (Karimi et al. 2016). They may colonize the female genital system, and vaginal colonization rises in AV patients or after the host microbiome changes. There is growing evidence that connects enterococci to BV and AV. These bacteria were isolated from different environments, including produced water in oil fields (Alyousif et al. 2020; Hamzah et al. 2020; Aboud et al. 2021; Alshami et al. 2022; Alyousif et al. 2022). One of the bacteria detected at a frequency of 1(4.2%) was *Streptococcus agalactiae* strain 149, a significant pathogen in AV and other human diseases. This contrasted with previous studies reporting GBS strains at frequencies of 16.8% and 9.6%, respectively (Sangeetha et al. 2015; Tang et al. 2020). Studies have focused primarily on the *Staphylococcus* and *Streptococcal* bacteria found in the female vagina because they can infect pregnant women and result in neonatal sepsis (Al-Kraety et al. 2022).

Identification of *Candida* spp. isolates

All yeast isolates were purified and identified according to their phenotypic characteristics. The results on chromogenic agar medium showed the light green color of *C. albicans*, while on tobacco agar medium, all isolates developed white to creamy colonies without fringes. After 2 h of incubation in human serum, the isolates were able to form germ tubes, which were observed as along tube-like projections extending from the yeast cells (Figure 5). The germ tube formation in human serum is a rapid method for identifying *Candida* sp. (Deorukhkar et al. 2012). When *C. albicans* is exposed to human serum, it transforms from yeast form to filamentous growth or mycelial form as a result of the production of germ tubes (Biswas et al. 2007). These morphological changes often represent a response of the fungus to changing environmental conditions and may allow the fungus to adapt to different biological niches (Kim et al. 2002). In the case of spore formation on corn meal agar medium, all isolates were positive for Chlamydospores; this test is used to distinguish yeast species of the *Candida* genus. Chlamydospores were observed even after being incubated for more than ten days (Figure 5).

Candida albicans is a commensal organism of the human gastrointestinal tract and a prevalent opportunistic pathogen. It exhibits different morphogenic forms to facilitate its survival in different host niches with distinct environmental conditions (Prasad and Tippana 2023). In this study, the frequency of *Candida* sp. was 6 (13%), and all six isolates were of *Candida albicans.* Our findings about the predominant species were in line and comparable with those reported in earlier studies (Bitew and Abebaw 2018; Xiao et al. 2022). Studies have shown that *C. albicans* is responsible for 80-92% of yeast infections; however, our findings are in contrast to the known prevalence of *Candida* sp. infection, accounting for around 67.7% and 56.14% prevalence, respectively (Ghajari et al. 2018; Shekhany 2021). It was previously believed that *Candida* only passively aids in developing an opportunistic fungal infection in the presence of an organic weakness or an immunocompromised host. These yeasts use virulence factors, become more aggressive, and contribute to the etiology of disease (Tamura et al. 2007). Adhesion development of biofilms and production of extracellular hydrolytic enzymes are the most notable virulence factors of *Candida* sp. (Rukadikar et al. 2022). One of the limitations of this study was that the sequencing of the isolates ws performed in South Korea at our own expense, with no external financial assistance. Unfortunately, due to budget constraints, we were unable to perform molecular identification of *Candida*.

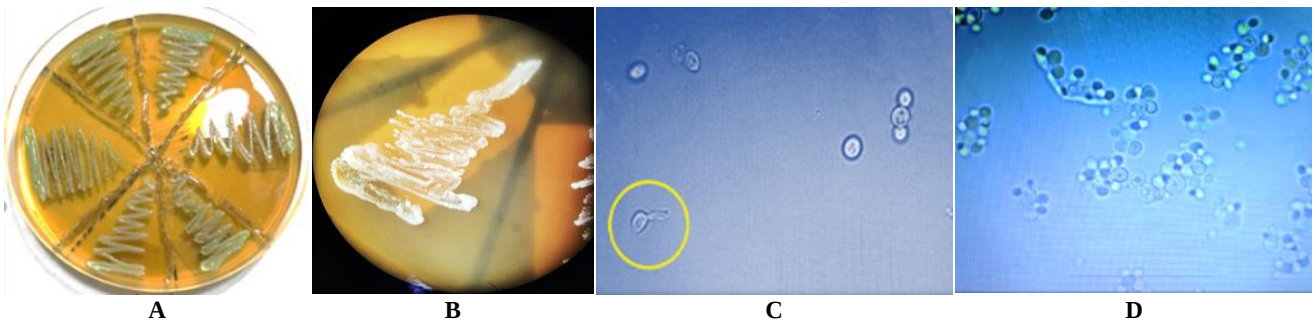

Figure 5. A. Growth of *Candida albicans* on chromogenic *Candida* agar, B. White to cream-colored colonies of *C. albicans* on tobacco agar, C. Germ tube of *C. albicans* grown on human serum (40X magnification), D. Single chlamydospores of *C. albicans* (100X magnification)

Antibiotic resistance pattern of bacterial isolates

Antibiotics are administered as needed to address BV. In the present investigation, sensitivity and resistance testing of identified bacteria were performed on various antibiotic discs. The disk diffusion method was employed to test six types of antibiotics against pathogenic bacteria isolated from vaginal samples, and the results revealed varying levels of resistance between different isolates (Table 4, Figure 7) *Staphylococcus* spp. showed a high resistance (80%) to azithromycin, and *Escherichia coli* exhibited 100% resistance toward ceftriaxone, doxycycline, and amoxicillin/ clavulanic acid. Our result was consistent with a study conducted by Yalew et al. (2022), which reported 100% resistance against amoxicillin/clavulanate acid. *Klebsiella* spp. showed 15% resistance to doxycycline, ciprofloxacin, and amoxicillin/clavulanic acid. *Enterococcus* spp. was 100% resistant to doxycycline and ciprofloxacin, whereas *Bacillus*, *Macrococcus*, and *Streptococcus* showed no resistance to the antibiotics used. Since the middle of the 20th century, numerous scientific theories have been proposed to describe bacterial antibiotic resistance. It is currently thought that bacteria can develop antibiotic resistance by several mechanisms, including actively removing the antibiotic from the cell, modifying the antibiotic through enzymatic processes, altering the antibiotic's target components in the cell, overexpressing an enzyme that the antibiotic renders inactive, changing the permeability of the bacterial cell membranes, producing an alternative metabolic pathway, or increasing the concentration of an antagonistic metabolite (Van Hoek et al. 2011).

Antifungal susceptibility test of *Candida albicans* isolates

The overall medication resistance pattern of four antifungal drugs tested against the six isolates of *C. albicans* is shown in (Table 5; Figure 8). The highest resistance rate was noticed against colistin (100%); isolates 2 and 5 were resistant to fluconazole and posaconazole. The development of drug tolerance in the infecting *Candida* sp. may lead to therapeutic failures. Mechanisms of drug resistance include mutations that acquire function in the transcription factors controlling membrane transporters, altered cell wall and ergosterol biosynthesis, overexpression of membrane transporters, and ergosterol biosynthesis (Bhattacharya et al. 2020). Similar findings have been reported by Bitew and Abebaw (2018). Our study found that all isolates were 100% susceptible to nystatin, consistent with the finding of Shrestha et al. (2020). Our results suggest that the vaginal ecosystem is diverse, particularly BV-associated bacteria. The composition of the vaginal bacterial community varies widely at the species or genus level, and several bacterial groups, including GBS, *E. coli*, *S. aureus*, and *K. pneumoniae*, are closely associated with BV. Additionally, bacteria showed varying levels of resistance and sensitivity to antibiotics. Although fluconazole seemed effective against some isolates, drug resistance may necessitate searching for different antifungal medications when managing *C. albicans*-related candidiasis.

Figure 7. Antibiotics sensitivity test of pathogenic bacteria. A. *Staphylococcus hemolytic*, B. *Staphylococcus hominis*, and C. *Escherichia coli*

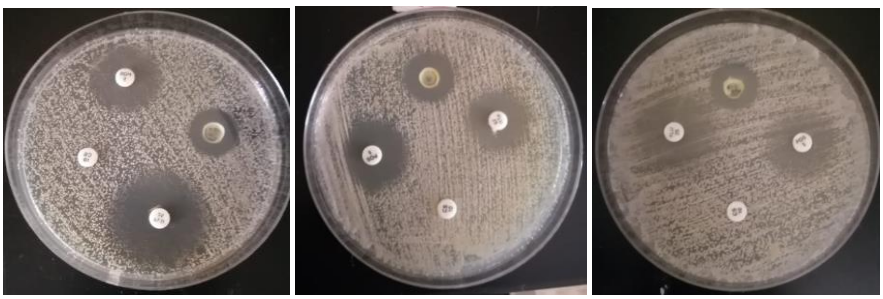

Figure 8. Activities of some antifungals against *Candida albicans* isolate

Table 4. Antimicrobial resistance profiles of bacteria isolated from vaginal infection

Antibiotics	*Staphylococcus* spp.		*Escherichia* spp.		*Klebsiella* spp.		*Enterococcus* spp.		*Macrococcus* spp.		*Streptococcus* spp.		*Bacillus* spp.	
	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %	# T	R %
Ceftriaxone	10	ND	5	100	4	50	2	ND	1	ND	1	ND	1	ND
Azithromycin-	10	80	5	ND	4	ND	2	ND	1	ND	1	0	1	0
Doxycycline	10	10	5	100	4	75	2	100	1	ND	1	ND	1	0
Ciprofloxacin	10	50	5	80	4	75	2	100	1	ND	1	ND	1	0
Gentamicin	10	20	5	40	4	50	2	ND	1	ND	1	ND	1	0
Amoxicillin/clavulanic acid	10	ND	5	100	4	75	2	ND	1	ND	1	ND	1	ND

Note: # T: number of isolates tested against each antibiotic. R %: percent of isolates resistant to antibiotics. ND: not done

Table 5. Susceptibility test of six *Candida albicans* isolates against four antifungal agents

Candida strains	Antifungal / Inhibition zone (mm)			
	Nystatin (15 µg)	Colistin sulfate (10 µg)	Fluconazole (25 µg)	Posaconazole (5 µg)
C. albicans	16	0	20	22
C. albicans	14	0	0	0
C. albicans	14	0	19	14
C. albicans	14	0	22	20
C. albicans	13	0	0	0
C. albicans	15	0	29	21

In conclusion, the bacterial isolates identified through PCR analysis and gene sequencing showed that they were ten species belonging to seven genera. The genera identified were *Staphylococcus* spp., *Escherichia* spp., *Klebsiella* spp., *Bacillus* spp., *Enterococcus* spp., *Streptococcus* spp., and *Macrococcus* spp. The dendrogram of 24 bacterial strains formed two main clusters. Cluster I had 64% similarity and Gram-positive bacteria, while Cluster II had 11.3% similarity and Gram-negative bacteria. Eight bacterial isolates were identified for the first time and were called new strains; their 16S rRNA sequences have been deposited at NCBI GenBank. All *Candida* isolates were *C. albicans*. *Escherichia coli* strains were 100% resistant to ceftriaxone, doxycycline, and amoxicillin/clavulanic acid. In addition, *Enterococcus* spp. displayed 100% resistance for doxycycline and ciprofloxacin, while *C. albicans* isolates exhibited 100% resistance to colistin. Prospective genotyping studies are needed to determine these microbes' resistant genes and understand their action mode and response to drug therapy.

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