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Bacterial vaginosis pattern and antibiotic susceptibility testing in female patients using high vaginal swabs

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Abstract. *Al-Kraety IAA, Al-Muhanna SG, Banoon SR, Ghasemian A. 2022. Bacterial vaginosis pattern and antibiotic susceptibility testing in female patients using high vaginal swabs. Biodiversitas 23: 2838-2844.* Bacterial species found in the vaginal environment encompass a wide variety of species. A common cause of vaginal discharge in women is bacterial vaginosis BV (BV). Various Gram-positive and Gram-negative rod shaped bacteria, including *E. coli, Klebsiella* spp., *Enterococcus* spp., *Enterobacter* spp., *Raoultella ornithinolytica*, and *Staphylococcus* spp. contribute significantly to bacterial vaginosis. In this study, vaginal swabs (VS) were obtained from 50 individuals with symptoms of vaginal discharge. The swabs were inoculated on blood, Mannitol, and MacConkey agar culture media. Biochemical tests were performed after an overnight incubation period to determine growth and colonial morphology. In addition to VITEK® 2 compact system and PCR technique by using a 16s RNA gene where all bacteria isolates were positive for this gene. Antibiotic sensitivity was investigated through compact VITEK® 2 and sensitivity cards (AST-P580), (AST-N222), and (AST-GN76). The bacterial isolates including 20 (43.4%) of *E. coli*, 8 (17.3%) of *Klebsiella* spp. and 8 (17.3%) of *Staphylococcus* spp. were investigated in present study. Additionally, 4 (8.6%) isolates of *Enterobacter* spp., 3 (6.5%) of *E. faecalis*, and 3 (6.5%) of *R. ornithinolytica*. The *E. coli*, *Staphylococcus* spp., *Enterobacter* spp., *E. faecalis* and *R. ornithinolytica* isolates were found resistant to several antibiotics and considered multi-resistance (MDR).

Keywords*:* 16s rRNA gene, bacterial contamination, multi-drug resistance, vaginal swap, VITEK-2 system

INTRODUCTION

Bacterial vaginosis is a significant inflammatory condition due to its serious consequences, including preterm birth, immaturity, rupture of uterine membranes, spontaneous abortions, and the risk of sexually transmitted diseases (Joyisa et al. 2019). Bacteria are naturally present in the vagina, and their proliferation results in vaginitis and increased excretions. The normal vaginal flora maintains the vaginal environment by establishing acidic conditions, producing metabolites such as hydrogen peroxide and bacteriocin, and competing with epithelial cells for mannose receptors (Aduloju et al. 2019). *Lactobacillus* is the predominant genus in the normal vaginal micro-flora. Notably, bacterial number fluctuations lead to changes in vaginal fluid, an increase in vaginal secretions, and the induction of odor (Machado et al. 2017). Abed and Kandala (2016) found that bacterial vaginosis included 69.5% of the Gram-negative and 30.5% of the Gram-positive species, where *E. coli*, *Klebsiella* spp., *S. aureus*, and *Streptococcus agalactiae* were the most prevalent bacteria, all of which are antibiotic-resistant.

Staphylococcus epidermidis, *Enterococcus faecalis*, and a few Alpha-hemolytic streptococci inhabit the anterior urethra, where sterile urine and its acidity make it difficult for bacteria to reach and establish (Roine et al. 2014; Kline et al. 2016). Variegated microbial communities often seen in the human vaginal environment include the typical vaginal microbiota and the mycobiota. *Lactobacillus*, which includes *L. crispatus*, *L. gasseri*, *L. iners*, and *L. jensenii*, is the most often isolated bacteria from the healthy human vagina. Using vaginal lactobacilli to control pathogen populations has been promoted as a means of preventing invasion (Chee et al. 2020).

As many as 75% of women have the potential to have a vaginal infection at some point in their lives, and the vaginal tract can be infected by a variety of common microorganisms including *Enterobacteriaceae* sp., *Enterococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., *Lactobacillus* sp., and *Candida albicans*. A variety of variables, including antibiotic use, can alter the vaginal environment, and it has been found that taking therapy without doing a susceptibility test may be a contributing cause to the rise in resistance patterns (Stokholm et al. 2014). According to Sari and Nugraheni (2013), the fungus Candida albicans can also cause vaginal infections, paronychia and thrush. Toxic shock syndrome patients were found to have *Staphylococcus* sp. in nearly all of their vaginal cultures, while healthy women had Group B *Streptococci* in 5% to 25% of their vaginal cultures. There are several research focusing on the female vaginal microflora of Staphylococcus and Streptococcal bacteria,

as these infections can cause neonatal sepsis in pregnant women (Mohammed and Hamadamin 2021).

Staphylococci are Gram-positive cocci, facultative and aerobic bacteria that can be isolated from animals and various environmental sources such as soil, sand, dust, air, natural water, and clothing (Götz et al. 2006; Banoon et al. 2019; Al-Muhanna et al. 2021). Some *Staphylococci* species are opportunistic, causing various infections such as inflammatory bowel, cystitis, bacteremia, and vaginitis (Muzny et al. 2019). *Staphylococcal* spp. possesses several virulence factors such as accumulation associated protein (Aap), Laminin binding protein (eno), and Extracellular matrix binding protein (Embp). *Enterococcus* spp. is a genus of Gram-positive cocci that occurs in short chains or pairs and is facultatively anaerobic (Mukherjee et al. 2016, Braiek and Smaoui 2019). It is prevalent in the oral cavity, vaginal tract, and hospital environment (Mukherjee et al. 2016), where it causes health care infections, most commonly infections of the urinary tract and soft tissue of the host, as well as infections caused by their adhesion to the surfaces of medical instruments. Moreover, the species possesses various virulence factors, including *Enterococcal* surface protein (ESP) as an adhesion factor, β-hemolysin, gelatinase, S-layer and biofilm formation (Igbinosa and Bashiru 2019), aggregation substance (agg), cytolysin (cyl), extracellular surface protein (esp), and adhesion to collagen (ace) (Semedo-Lemsaddek et al. 2016).

Bacteria isolated from various environments are resistant to commonly used human medicine, limiting treatment options and putting affected individuals' lives at risk (Banoon et al. 2020). Thus, this study aimed to isolate bacteria from vaginal swabs and determine their antibiotic sensitivity profile.

MATERIALS AND METHODS

During the study period from September 2021 to December 2021, twenty-five females with symptoms of vaginal discharge provided 50 vaginal swabs (VS); 46 (92%) samples of them contained bacterial growth, while 4 (8%) samples had no bacterial growth. Bacterial growth was isolated using MacConkey agar (Merk), Mannitol agar, and blood agar incubated aerobically overnight at 37°C and subjected to biochemical examinations. The final coincidence was accomplished by using Gram-positive and Gram-Negative-Identification (GN-ID) cards and integrated VITEK® 2 compact devices.

Antibiotic susceptibility testing

Using the automated VITEK® 2 compact device and sensitivity cards (AST-P580), (AST-N222), and (AST-GN76); this card was composed of the following antibiotics: Amikacin, Tigecycline, Gentamycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Imipenem, Meropenem, Ertapenem, Ampicillin, Cefazolin, Trimethoprim/ Sulfamethoxazole, Linezolid, Oxacillin, Benzylpenicillin, Clindamycin, Erythromycin, Tetracycline, Piperacillin/ Tazobactam, Ceftriaxone, Ceftazidime, Cefepime, Amikacin, Cefoxitin, and Teicoplanin plus Vancomycin.

Molecular identification

Gel electrophoresis was used to detect genomic DNA using a UV transilluminator. As indicated in Table 2, the bacteria responsible for the symptoms were identified using the polymerase chain reaction (PCR) method. This primer was developed by Alpha DNA (USA)(Table 1). Afterward, the gel was stained with ethidium bromide (Promega, USA) and operated at 85 volts for 1.5 hours. A UV light transilluminator (Cleaver, UK) illuminated a single band in the desired location, and bands were photographed using the gel documentation method (Cleaver, UK). Subsequently, a 1000bp ladder was used to determine the molecular weights of amplified products.

RESULTS AND DISCUSSION

Isolation of pathogenic bacteria

Between September to December 2021, a total of 50 vaginal swabs (VS) were collected from patients with symptoms of vaginal discharge; 46 (92%) of the samples contained bacterial growth, while 4 (8%) of the samples did not exhibit bacterial growth. As shown in Table 3, using biochemical tests, 20 (43.4%) *E. coli* isolates were found to be involved in infection (Winn et al. 2006), followed by 8 (17.3%) *Klebsiella* sp. (Mody et al. 2015), 8 (17.3%) *Staphylococcus* sp. (Chelikani et al. 2004), 4 (8.6%) *Enterobacter* isolates, 3 (6.5%) isolates of *E. faecalis*, and 3 (6.5%) isolates of *R. ornithinolytica*.

Table 1. The study's primers

Gene name	Gene	Primer sequence (5'-3')	Amplicon size (bp)	Reference
Universal	*16s rRNA*	F:5-AGAGTTTGATCCTGGCTCAG-3 R:5-GGTTACCTTGTTACGACTT-3	1470	Lu et al.2015

Table 2. AmpC primer PCR program applied in a thermocycler

Gene	Temperature (˚C) / time					Number of cycles
	Initial denaturation	Cycling conditions			Final extension	
		Denaturation	Annealing	Extension		
16s rRNA	94/5 min	94/30 sec	54/30 sec	72/105 sec	72/5 min	35

Table 3. The rate of bacterial species isolated from vaginal discharge

Type	Number (total 46)
E. coli	20
Klebsiella spp.	8
Staphylococcus	8
Enterobacter spp.	4
E. faecalis	3
R. ornithinolytica	3

Identification of bacterial species

We identified 80 isolates, including Gram-negative and Gram-positive bacteria, using gram staining, an automated VITEK® 2 compact system (64 biochemical tests), and PCR (Almayali et al. 2018; Almayali and Al -Kraety 2019; Chelikani et al. 2004). For example, bacitracin, coagulase, and mannitol fermentation were used to identify *Staphylococci* (Tiwari et al. 2008). Our findings corroborated previous findings that numerous bacterial species are involved in bacterial vaginosis (Anukam and Reid 2007; Coleman and Gaydos 2018; Ranjit et al. 2018).

Additionally, three *Enterococcus faecalis* isolates (6.5%) were identified. This bacterium is a nosocomial pathogen that causes endocarditis, surgical wound infection, urinary tract infection, and respiratory tract infection. The enterococcal surface protein (Esp) confers virulence on *E. faecalis* by facilitating aggregation and biofilm formation (Van Wamel et al. 2007).

We identified 38 Gram-negative bacteria from all isolated isolates as *E. coli*, *Klebsiella* spp. and *Enterobacter* spp. on MacConkey agar (Winn et al. 2006). These bacteria appear negative for Gram stain, and the distinction between them is dependable on the IMIVC test.

We detected 18 (12.5%) isolates identified as *K. pneumonia* as a mucoid lactose fermentation on MacConkey agar, consistent with Al-Kraety et al.(2020). When grown on MacConkey agar, *K. pneumoniae* can ferment lactose sugar and produce indole negative and citrate positive compounds (Citrate agar is used to test an organism's ability to use citrate as an energy source) (Winn et al. 2006). In this survey, *Klebsiella* species were found to be uncommon. Members of the *Enterobacteriaceae* were identified on the surface or in the core of tonsils (Brook and Kiran 2001; Kurien et al. 2000). Additionally, 3 (6.5%) *R. ornithinolytica* with oxidase-negative, aerobic, non-motility, and capsule formation were identified (Drancourt et al. 2001).

Molecular detection of bacteria via 16s rRNA

The molecular identification of all bacterial isolates using 16s rRNA gene was carried out by PCR technique, the result of PCR amplification revalued all bacteria isolates gave positive bands with product size of 1470 bp. As illustrated in Figure 1.

Antimicrobial susceptibility

A total of 50 swab samples were collected from patients. Overall, 46 (92%) isolates were positive, and 4 (8%) were negative. Sensitivity was determined using the VITEK® 2 system card (AST-P580), (AST-N222), and (AST-GN76). As shown in Table 4, *E.coli* is highly sensitive to Amikacin, Tigecycline, Gentamycin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Imipenem, Meropenem, and Ertapenem, but has a higher resistance to Ampicillin, Cefazolin, and Trimethoprim/ Sulfamethoxazole while, other antibiotics exhibited variable results (Michie et al. 2003). *E. coli* had demonstrated a relatively high resistance rate.

The effective mechanisms of *E. coli* include the transfer of genes encoding plasmid extended-spectrum β-lactamases (conferring resistance to broad-spectrum cephalosporins), carbapenems (conferring carbapenem resistance), 16S rRNA methylases (conferring pan-resistance to aminoglycosides), and plasmid-mediated quinolone resistance (PMQR) genes (conferring quinolone resistance) (Karkman et al. 2018).

As shown in Table 5, *Klebsiella* is highly sensitive to Amikacin, Tigecycline, Gentamycin, Cefazolin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, Imipenem, Meropenem, and Ertapenem, but also has exhibited a high resistance to Ampicillin and Trimethoprim/ Sulfamethoxazole (Michie et al. 2003). In these cases, Gram-negative bacilli such as *Escherichia coli. Salmonella* spp., *Mycobacteria* spp., and fungi such as *Candida* spp. and *Cryptococcus* spp. have been identified. Treatment options have been limited due to the widespread resistance of strains such as K. pneumonia in hospital- and community-acquired infections (Prestinaci et al. 2015).

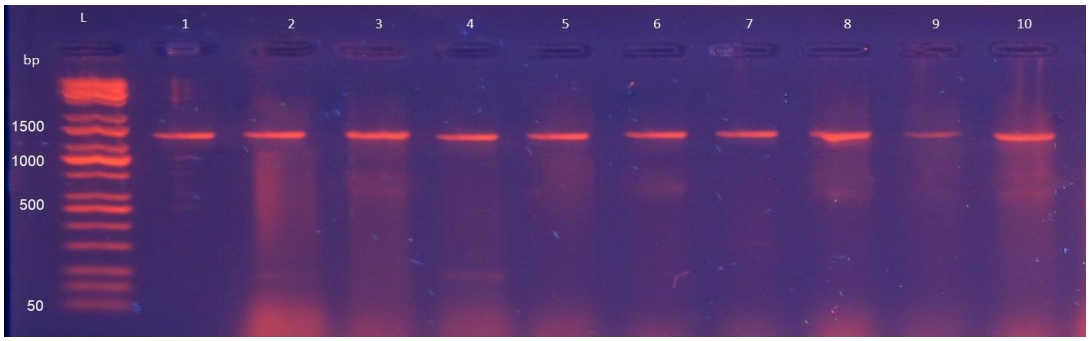

Figure 1. PCR amplification products of bacteria isolates in this study amplified with 16s r RNA gene primers with product 1470 bp. Lane (L), DNA molecular size marker (100-bp ladder), all specimen shows positive results

According to Table 6, *Staphylococcus* is highly sensitive to Tigecycline and Linezolid and exhibited high resistance to Oxacillin, Benzylpenicillin, Levofloxacin Nitrofurantoin, Trimethoprim/Sulfamethoxazole, Clindamycin, Erythromycin, Tigecycline, and Tetracycline. Various resistance mechanisms have been employed by *S. aureus*. In particular, the localization of resistance genes on transferable genetic elements such as plasmids and transposons facilitate horizontal resistance transfer between bacteria (van Hoek et al. 2011; Woodford and Sundsfjord 2005). However, we did not decipher the molecular mechanisms of vaginosis isolates.

According to Table 7, *Enterobacter cloacae* is highly sensitive to Tigecycline, Gentamycin, Trimethoprim/ Sulfamethoxazole, Piperacillin/Tazobactam, Levofloxacin, Ciprofloxacin, Ertapenem, Ceftriaxone, Imipenem, Ceftazidime, Cefepime, and Amikacin, but also exhibits high resistance to Cefazolin. *E. cloacae*, as the family's leading agent, also represents resistance via the production of ESBLs such as AmpC-type, CTX-M, TEM, and SHV (Ito et al. 2018; Szabó et al. 2005), as well as quinolone resistance (*qnr* genes) (Corkill et al. 2005; de Jong et al. 2018).

Table 4. *Escherichia coli* isolates sensitivity from vaginal discharge (AST-N222)

Type of antibiotic (cell wall)	Sensitivity
Ampicillin	R
Piperacillin/Tazobactam	Variable
Imipenem	S
Meropenem	S
Ertapenem	S
Ceftriaxone	Variable
Cefazolin	R
Cefoxitin	Variable
Cefepime	Variable
Ceftrazidime	Variable
Type of antibiotic (DNA synthesis)	Sensitivity
Ciprofloxacin	S
Levofloxacin	S
Nitrofurantion	S
Type of antibiotic (Folic acid metabolism)	Sensitivity
Trimethoprim/Sulfamethoxazole	R
Type of antibiotic (Protein synthesis)	Sensitivity
Amikacin	S
Gentamycin	S
Tigecycline	S

R: Resistant, S: Sensitive

Table 6. *Staphylococcus aureus* isolates sensitivity from vaginal discharge (AST-P580)

Type of antibiotic (cell wall)	Sensitivity
Oxacillin	R
Benzylpenicillin	R
Teicoplanin	Variable
Vancomycin	Variable
Type of antibiotic (DNA synthesis)	Sensitivity
Moxifloxacin	Variable
Levofloxacin	R
Nitrofurantion	R
Type of antibiotic (RNA Synthesis)	
Rifampicin	Variable
Type of antibiotic (Folic acid metabolism)	Sensitivity
Trimethoprim/ Sulfamethoxazole	R
Type of antibiotic (Protein synthesis)	Sensitivity
Clindamycin	R
Erythromycin	R
Tigecycline	S
Tetracycline	R
Linezolid	S

Note: R: Resistant, S: Sensitive

Table 5. *Klebsiella pneumoniae* isolates sensitivity from vaginal discharge (AST-GN76)

Type of antibiotic (cell wall)	Sensitivity
Ampicillin	R
Piperacillin/Tazobactam	S
Imipenem	S
Meropenem	S
Ertapenem	S
Ceftriaxone	S
Cefazolin	S
Cefoxitin	S
Ceftrazidime	S
Cefepime	S
Type of antibiotic (DNA synthesis)	Sensitivity
Ciprofloxacin	S
Levofloxacin	S
Nitrofurantion	S
Type of antibiotic (Folic acid metabolism)	Sensitivity
Trimethoprim/ Sulfamethoxazole	R
Type of antibiotic (Protein synthesis)	Sensitivity
Amikacin	S
Gentamycin	S
Tigecycline	S

Note: R: Resistant, S: Sensitive

Table 7. *Enterobacter cloacae* isolates sensitivity from vaginal discharge (AST-GN76)

Type of antibiotic (cell wall)	Sensitivity
Piperacillin/Tazobactam	S
Imipenem	S
Ertapenem	S
Ceftriaxone	S
Cefazolin	R
Cefoxitin	R
Ceftrazidime	S
Cefepime	S
Type of antibiotic (DNA synthesis)	Sensitivity
Ciprofloxacin	S
Levofloxacin	S
Nitrofurantion	I
Type of antibiotic (Folic acid metabolism)	Sensitivity
Trimethoprim/ Sulfamethoxazole	S
Type of antibiotic (Protein synthesis)	Sensitivity
Amikacin	S
Gentamycin	S
Tigecycline	S

Note: R: Resistant, S: Sensitive

Table 8. *Raoultella ornithinolytica* isolates sensitivity from vaginal discharge (AST-GN76)

Type of antibiotic (cell wall)	Sensitivity
Ampicillin	R
Piperacillin/Tazobactam	I
Imipenem	S
Ertapenem	S
Ceftriaxone	R
Cefazolin	R
Cefoxitin	S
Ceftrazidime	I
Cefepime	S
Type of antibiotic (DNA synthesis)	Sensitivity
Ciprofloxacin	R
Levofloxacin	R
Nitrofurantion	R
Type of antibiotic (Folic acid metabolism)	Sensitivity
Trimethoprim/ Sulfamethoxazole	R
Type of antibiotic (Protein synthesis)	Sensitivity
Amikacin	S
Gentamycin	R
Tigecycline	S

Note: R: Resistant, S: Sensitive

Table 9. *Enterococcus* isolates sensitivity from vaginal discharge (AST-P580)

Type of antibiotic (cell wall)	Sensitivity
Teicoplanin	S
Vancomycin	S
Type of antibiotic (DNA synthesis)	Sensitivity
Levofloxacin	S
Nitrofurantion	S
Type of antibiotic (Protein synthesis)	Sensitivity
Erythromycin	R
Tigecycline	S
Tetracycline	R
Linezolid	S

Note: R: Resistant, S: Sensitive

As shown in Table 8, *Raoultella ornithinolytica* is highly sensitive to Amikacin, Cefoxitin, Tigecycline, Cefepime, Imipenem, Meropenem, and Ertapenem, but also exhibits high resistance to Ampicillin, Ceftriaxone, Cefazolin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole.

Similar to some *Klebsiella* sp., *Raoultella* spp. exhibited intrinsic resistance to Ampicillin and ticarcillin conferred by chromosomally encoded beta-lactamases (Sękowska 2017). As previously stated, for the *Enterobacteriaceae* family, various mechanisms have been employed and established (Piccirilli et al. 2019).

As shown in Table 9, *E. faecium* is highly sensitive to Tigecycline, Levofloxacin, Nitrofurantoin, Linezolid, Teicoplanin, and Vancomycin, but is also highly resistant to Erythromycin and Tetracycline.

As clinical evidence demonstrates, *Enterococcus* species are intrinsically resistant to cephalosporins and aminoglycosides (Hollenbeck and Rice 2012). *E. faecium* has a higher resistance rate than *E. faecalis* ($p < 0.05$) observed against Quinupristin/Dalfopristin, Chloramphenicol, Tetracycline, and Minocycline ($p < 0.05$), whereas Linezolid, Vancomycin, and Teicoplanin had a low prevalence of resistance.

Multi-drug resistance

We identified MDR isolates resistant to at least three classes of bacteria, including *E. coli*, *S. aureus*, and *R. ornithinolytica*. We observed that 4.8% of isolates had an MDR index of 1. These findings demonstrated the ineffectiveness of the majority of antibiotics. Indeed, bacterial isolates employ a variety of resistance mechanisms. Additionally, treating vaginosis with resistant bacterial agents will be difficult and ultimately unsuccessful. Javed et al. (2019) and Bitew et al. (2021) demonstrated that shifts in the microbial flora from *Lactobacilli* to opportunistic pathogens and resistance to various antibiotics do not effectively treat bacterial vaginosis. Our study was limited by the absence of molecular identification and quantification of *Lactobacilli* (Lannon et al. 2019).

The significant findings of the current study are summarized as: *E. coli* is the most prevalent bacteria in the vaginal tract. *E. coli*, and *R.ornithinolytica* isolates exhibited high multi-drug resistance. *E. coli* is highly resistant to Ampicillin, Cefazolin, and Trimethoprim/ Sulfamethoxazole. *Klebsiella* spp. exhibited high resistance to Ampicillin and Trimethoprim/Sulfamethoxazole. *Staphylococcus* has a high level of resistance to Oxacillin, Benzylpenicillin, Levofloxacin, Nitrofurantoin, Trimethoprim/ Sulfamethoxazole, Clindamycin, Erythromycin, Tigecycline, and Tetracycline. *Enterobacter* spp. exhibited high resistance to Cefazolin and Cefoxitin. *R. ornithinolytica* demonstrates high resistance to Ampicillin, Ceftriaxone, Cefazolin, Ciprofloxacin, Levofloxacin, Nitrofurantoin, and Trimethoprim/Sulfamethoxazole. *Enterococcus* spp. exhibits high sensitivity to Erythromycin and Tetracycline. Numerous gram-positive and gram-negative rods, including *E.coli*, *Klebsiella* spp., *Enterococcus* spp., *Enterobacter* spp., *R. ornithinolytica*, and *Staphylococcus* spp. contribute significantly to bacterial vaginosis. Bacteria confirmed by 16S rRNA gene sequences comprised 16S rRNA gene sequences that enable bacterial identification.

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