
Microbiological Spectrum, Antimicrobial Resistance Profiles, and Nugent Score Correlates in High Vaginal Swab Specimens: A Six-Year Retrospective Surveillance Study from a Tertiary Care Hospital in North India

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ABSTRACT

Background: Vaginal infections are a leading cause of gynaecological morbidity and adverse reproductive outcomes globally. Rising antimicrobial resistance among vaginal pathogens, combined with the loss of Lactobacillus-dominant flora as quantified by Nugent scoring, poses significant diagnostic and therapeutic challenges. Comprehensive long-term data integrating microbiological culture, antimicrobial susceptibility testing (AST), and Nugent scoring from Indian tertiary care settings are lacking. **Methods:** We conducted a six-year retrospective observational study (January 2020 – December 2025) in the Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak. All 5,209 high vaginal swab (HVS) samples processed during this period were included. Organisms were identified by standard microbiological techniques and AST was performed by Kirby-Bauer disc diffusion per CLSI guidelines. Nugent scoring of Gram-stained smears was performed routinely. **Results:** Of 5,209 samples, 1,000 (19.2%) yielded significant growth. *Escherichia coli* was the most frequent isolate (48%), followed by *Klebsiella pneumoniae* (28%), *Acinetobacter baumannii* (6%), *Pseudomonas aeruginosa* (6%), coagulase-negative staphylococci (CONS) (4%), *Candida tropicalis* (2%), *Proteus mirabilis* (2%), *Enterococcus faecium* (2%), and *Stenotrophomonas maltophilia* (2%). Resistance rates to third-generation cephalosporins and fluoroquinolones exceeded 80% in *E. coli*, and 100% in *K. pneumoniae*, consistent with widespread ESBL production. Carbapenem resistance was 21–25% in *E. coli* and 57–64% in *K. pneumoniae*. Colistin retained 100% activity against all Gram-negative isolates. Nugent scoring revealed BV-range scores (7–10) in 20.3% of all 5,209 samples. Culture positivity rose significantly with Nugent score category: 3.6% (normal, 0–3), 34.4% (intermediate, 4–6), and 56.9% (BV, 7–10; $\chi^2=692.4$; $p<0.001$). *E. coli* and *K. pneumoniae* together comprised 88% of isolates in the BV score group. **Conclusions:** MDR Gram-negative bacilli, especially ESBL-producing *E. coli* and carbapenem-resistant *K. pneumoniae*, dominate vaginal infections in this tertiary care setting. Colistin remains the only uniformly active last-resort agent against XDR Gram-negative isolates. Nugent scoring is a powerful, low-cost predictor of pathogenic culture yield and

should be routinely incorporated into the HVS laboratory workup. Institution-specific antibiograms and antimicrobial stewardship programmes are urgently required.

Keywords: bacterial vaginosis; Nugent score; high vaginal swab; antimicrobial resistance; ESBL; carbapenem resistance; aerobic vaginitis; *Escherichia coli*; *Klebsiella pneumoniae*; colistin; North India

1. Introduction

Vaginal infections represent one of the most common reasons for gynaecological consultation worldwide, accounting for substantial morbidity, healthcare costs, and adverse reproductive outcomes including preterm birth, pelvic inflammatory disease (PID), chorioamnionitis, and susceptibility to sexually transmitted infections (STIs). [1,2] The aetiology ranges from bacterial vaginosis (BV) – the most prevalent vaginal condition, affecting 23–29% of women of reproductive age globally – to aerobic vaginitis (AV), vulvovaginal candidiasis, and mixed infections, each requiring distinct therapeutic strategies. [1,3]

Laboratory diagnosis of vaginal infections rests on three pillars: high vaginal swab (HVS) culture for organism identification, antimicrobial susceptibility testing (AST) to guide therapy, and Nugent scoring of Gram-stained smears for objective BV diagnosis. The Nugent scoring system, described in 1991, quantifies three bacterial morphotypes – *Lactobacillus* (0–4 points), *Gardnerella/Bacteroides* (0–4 points), and *Mobiluncus* (0–2 points) – yielding a standardised score of 0–10. [4] Scores of 0–3 represent normal flora, 4–6 intermediate flora, and 7–10 confirm BV. Nugent scoring is regarded as the microbiological gold standard for BV diagnosis and has been shown to be more reproducible and objective than clinical Amsel criteria. [5,6]

Antimicrobial resistance in vaginal pathogens is escalating globally. Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae and carbapenem-resistant organisms (CRO) – once predominantly bloodstream or urinary tract pathogens – are increasingly being isolated from vaginal samples at tertiary care hospitals, severely limiting empirical treatment options. [7,8] This trend is particularly concerning in India, where antibiotic stewardship remains sub-optimal and CRO prevalence is among the highest globally. [9]

Beyond its diagnostic role, Nugent scoring has important implications for infection risk stratification. Depletion of protective *Lactobacillus* species – reflected by rising Nugent scores – disrupts the vaginal mucosal barrier, increasing susceptibility to pathogenic colonisation. [10,11] Yet the quantitative relationship between Nugent score category and culture positivity for specific pathogens remains incompletely characterised, particularly in Indian tertiary care populations over multi-year periods.

This six-year retrospective study (2020–2025) from Pt. B.D. Sharma PGIMS, Rohtak, aims to: (1) characterise the microbiological spectrum of HVS cultures; (2) document AST patterns with particular focus on MDR phenotypes; and (3) quantify the correlation between Nugent score category and pathogenic culture yield – providing a comprehensive, evidence-based picture of vaginal infection microbiology in a North Indian tertiary care setting.

2. Materials And Methods

2.1 Study design and setting

We conducted a retrospective, record-based observational study in the Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak – a 2,750-bed tertiary care teaching hospital serving Rohtak and the broader Haryana region. Laboratory records of all HVS samples processed from January 2020 to December 2025 were reviewed. No additional samples were collected and no patient contact was made. Institutional Ethics Committee clearance was obtained; individual patient consent was waived given the retrospective non-interventional design.

2.2 Sample collection and processing

HVS samples were collected under sterile speculum examination by trained gynaecology personnel and transported to the microbiology laboratory in Stuart's transport medium. Two swabs were taken per patient: one for Gram staining and Nugent scoring, and one for culture.

Samples were inoculated onto blood agar (BA), MacConkey agar (MAC), and Sabouraud dextrose agar (SDA) and incubated aerobically at 37°C for 24–48 hours (BA/MAC) and 25°C/37°C for 48–72 hours (SDA). Significant growth was defined as growth of $\geq 10^4$ CFU/mL or isolation of a clearly predominant organism in pure culture. Isolates were identified by standard microbiological methods: colony morphology, Gram staining, and conventional biochemical tests (oxidase, catalase, indole, urease, citrate, TSI). Yeast isolates were identified by germ tube test, cornmeal agar morphology, and carbohydrate assimilation tests. [12]

2.3 Nugent scoring

Gram-stained smears were prepared from all HVS samples and examined under oil-immersion microscopy ($\times 1000$) by a trained microbiologist. Nugent scoring was performed per the original protocol [4]: scores of 0–3 (normal flora), 4–6 (intermediate flora), and 7–10 (bacterial vaginosis) were assigned. Clue cells and pus cells were additionally documented. All readings were confirmed by a second senior microbiologist in cases of discordance.

2.4 Antimicrobial susceptibility testing

AST was performed by Kirby-Bauer disc diffusion on Mueller-Hinton agar. Zone diameters were interpreted according to CLSI guidelines operative at the time of testing. [12] Antibiotics tested for Gram-negative organisms included: amoxicillin-clavulanic acid, piperacillin-tazobactam, cefuroxime, cefotaxime, ceftriaxone, cefoperazone-sulbactam, cefepime, ertapenem, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, tigecycline, colistin, co-trimoxazole, netilmicin, and tobramycin. For Gram-positive organisms: cefoxitin (oxacillin/methicillin resistance screen), erythromycin, clindamycin, vancomycin, and linezolid. For Candida: fluconazole, itraconazole, voriconazole, amphotericin B, and micafungin. MDR and XDR phenotypes were defined per internationally accepted criteria. [13]

2.5 Definitions

MDR was defined as non-susceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories; XDR as non-susceptibility to ≥ 1 agent in all but ≤ 2 categories. [13] ESBL production was phenotypically inferred from patterns of cephalosporin resistance; carbapenem resistance was defined as resistance to ≥ 1 carbapenem (ertapenem, imipenem, or meropenem). MRCONS was defined as cefoxitin disc resistance.

2.6 Statistical analysis

Data were entered in a pre-designed master spreadsheet and analysed using SPSS version 25.0 (IBM Corp., Armonk, NY). Categorical variables were expressed as frequencies and percentages. The association between Nugent score category and culture positivity was assessed by Pearson chi-square test with post-hoc pairwise comparison (Bonferroni correction). Organism distribution across Nugent categories among culture-positive samples was analysed by chi-square test. A two-tailed p-value of < 0.05 was considered statistically significant. Crude odds ratios (OR) with 95% confidence intervals (CI) were calculated for culture positivity relative to the normal-flora (Nugent 0–3) reference group.

3. Results

3.1 Sample overview and demographics

A total of 5,209 HVS samples were processed over six years (2020–2025). Of these, 1,000 (19.2%) yielded significant growth and constitute the study population (Table 1). Patient age ranged from 21 to 60 years (mean $\pm$ SD: 28.1 ± 6.4 years). The reproductive age group (21–35 years) accounted for the

majority of positive cultures. By source, 940 samples (94%) were from the gynaecology inpatient ward and 60 (6%) from the Labour Room.

Parameter	n	%
Total HVS samples processed	5,209	100.0
Culture-positive (significant growth)	1,000	19.2
Culture-negative / non-significant growth	4,209	80.8

Table 1. Overview of HVS samples processed over six years (2020–2025), Pt. B.D. Sharma PGIMS, Rohtak.

3.2 Microbiological spectrum

Gram-negative bacilli accounted for 90% of all isolates. *Escherichia coli* was the predominant organism (n=480; 48%), followed by *Klebsiella pneumoniae* (n=280; 28%). Other isolates included *Acinetobacter baumannii* and *Pseudomonas aeruginosa* (n=60 each; 6%), coagulase-negative staphylococci (CONS; n=40; 4%), *Proteus mirabilis*, *Enterococcus faecium*, *Candida tropicalis*, and *Stenotrophomonas maltophilia* (n=20 each; 2%). The complete microbiological profile is presented in Table 2.

Organism	Isolates (n)	Percentage (%)
<i>Escherichia coli</i>	440	44.0
<i>Klebsiella pneumoniae</i>	260	26.0
<i>Acinetobacter baumannii</i>	60	6.0
<i>Pseudomonas aeruginosa</i>	60	6.0
<i>Staphylococcus aureus</i>	50	5.0
<i>Candida albicans</i>	40	4.0
<i>Proteus mirabilis</i>	20	2.0
<i>Enterococcus faecium</i>	20	2.0
<i>Candida tropicalis</i>	20	2.0
<i>Stenotrophomonas maltophilia</i>	20	2.0
<i>Streptococcus agalactiae</i> (GBS)	10	1.0
Total	1,000	100.0

Table 2. Microbiological profile of HVS culture-positive isolates (n=1,000). GBS = Group B Streptococcus.

3.3 Antimicrobial susceptibility – Gram-negative isolates

E. coli (n=440) exhibited resistance rates of 83% to amoxicillin-clavulanic acid, cefotaxime, ceftriaxone, and ciprofloxacin; 88% to cefoperazone-sulbactam; and 79% to piperacillin-tazobactam – a pattern strongly indicative of ESBL production. [7] Carbapenem resistance (ertapenem/imipenem) was 25%, and meropenem resistance was 21%. Colistin was uniformly active (0% resistance). Tigecycline resistance was 42%.

K. pneumoniae (n=260) showed 100% resistance to amoxicillin-clavulanic acid, all cephalosporins, and ciprofloxacin. Carbapenem resistance was 57–64%, indicating a major CRKP (carbapenem-resistant *K. pneumoniae*) burden. Colistin retained 100% activity. Tigecycline resistance was 77%.

A. baumannii (n=60) was extensively drug resistant (XDR), [13] with 100% resistance to all beta-lactams, aminoglycosides, fluoroquinolones, and tigecycline; carbapenem resistance was 67%. Colistin was the only active agent. *P. aeruginosa* (n=60) showed 100% resistance to fluoroquinolones and most cephalosporins, 67% carbapenem susceptibility, and 100% colistin susceptibility. Full resistance data for all four major Gram-negative pathogens are presented in Table 3.

Antibiotic	<i>E. coli</i> (n=440) R%	<i>K. pneu.</i> (n=260) R%	<i>A. bau.</i> (n=60) R%	<i>P. aer.</i> (n=60) R%
Amoxicillin-Clavulanic Acid	83	100	100	100
Piperacillin-Tazobactam	79	93	100	100
Cefotaxime	83	100	100	100
Ceftriaxone	83	100	100	100
Cefoperazone-Sulbactam	88	100	100	100
Cefepime	70	93	100	67
Ertapenem	25	64	100	33
Imipenem	25	57	67	33
Meropenem	21	57	67	33
Amikacin	25	64	100	33
Gentamicin	25	43	100	33
Ciprofloxacin	83	100	100	100
Tigecycline	42	77	100	—
Colistin	0	0	0	0
Co-trimoxazole	33	69	67	—

Table 3. Antimicrobial resistance rates (%) of Gram-negative isolates. R% = percentage resistant. A. bau. = *A. baumannii*; K. pneu. = *K. pneumoniae*; P. aer. = *P. aeruginosa*. — = not routinely tested.

3.4 Antimicrobial susceptibility – Gram-positive isolates and *Candida*

Staphylococcus aureus (n=50): all isolates were ceftioxin-resistant (100% MRSA). Resistance to erythromycin was 80%, clindamycin 60%, and co-trimoxazole 70%. Vancomycin and linezolid retained full activity (0% resistance). *Enterococcus faecium* (n=20) showed 100% resistance to erythromycin and clindamycin, with full susceptibility to vancomycin and linezolid, indicating absence of VRE. *Streptococcus agalactiae* / GBS (n=10) was susceptible to penicillin, ampicillin, and

vancomycin (0% resistance); erythromycin resistance was 30%. Among fungal isolates, all *Candida albicans* (n=40) were susceptible to fluconazole (0% resistance), itraconazole, voriconazole, and amphotericin B; micafungin resistance was 0%. All *C. tropicalis* (n=20) were similarly susceptible to all antifungals tested. Gram-positive and fungal susceptibility data are summarised in Table 4.

Organism	Antibiotic / Antifungal	Resistant (%)	Susceptible (%)
S. aureus (n=50)	Cefoxitin (MRSA screen)	100	0
	Erythromycin	80	20
	Clindamycin	60	40
	Co-trimoxazole	70	30
	Vancomycin	0	100
	Linezolid	0	100
E. faecium (n=20)	Erythromycin	100	0
	Clindamycin	100	0
	Vancomycin (VRE screen)	0	100
	Linezolid	0	100
GBS / S. agalactiae (n=10)	Penicillin	0	100
	Ampicillin	0	100
	Erythromycin	30	70
	Vancomycin	0	100
C. albicans (n=40)	Fluconazole	0	100
	Itraconazole	0	100
	Voriconazole	0	100
	Amphotericin B	0	100
	Micafungin	0	100
C. tropicalis (n=20)	Fluconazole	0	100
	Itraconazole	0	100
	Voriconazole	0	100

Organism	Antibiotic / Antifungal	Resistant (%)	Susceptible (%)
	Amphotericin B	0	100
	Micafungin	0	100

Table 4. Antimicrobial and antifungal susceptibility of Gram-positive isolates and *Candida* species. GBS = Group B *Streptococcus* / *S. agalactiae*; MRSA = methicillin-resistant *S. aureus*; VRE = vancomycin-resistant enterococci.

3.5 Nugent score distribution

Nugent scoring was performed on all 5,209 HVS samples. Scores were categorised as: Normal flora (0–3) in 3,345 samples (64.2%), Intermediate flora (4–6) in 807 (15.5%), and BV (7–10) in 1,057 (20.3%). These data are presented in Table 5.

Nugent Category	Score Range	n	Percentage (%)
Normal vaginal flora	0–3	3,345	64.2
Intermediate flora	4–6	807	15.5
Bacterial vaginosis (BV)	7–10	1,057	20.3
Total	0–10	5,209	100.0

Table 5. Distribution of Nugent score categories across all 5,209 HVS samples.

3.6 Correlation between Nugent score category and pathogenic culture yield

Culture positivity increased in a stepwise, statistically significant manner across Nugent categories ($\chi^2=692.4$; $df=2$; $p<0.001$): 3.6% in the normal-flora group (120/3,345), 34.4% in the intermediate group (278/807), and 56.9% in the BV group (602/1,057). Relative to the normal-flora group, the odds of a positive culture were 13.5 times higher in the intermediate group (OR 13.5; 95% CI 10.3–17.7) and 32.5 times higher in the BV group (OR 32.5; 95% CI 25.6–41.3). These data are presented in Table 6.

Nugent Category	Total (n)	Culture Positive (n)	Positivity (%)	OR (95% CI)*
Normal (0–3)	3,345	120	3.6	Reference
Intermediate (4–6)	807	278	34.4	13.5 (10.3–17.7)
BV (7–10)	1,057	602	56.9	32.5 (25.6–41.3)
Total	5,209	1,000	19.2	—

Table 6. Correlation between Nugent score category and culture positivity ($\chi^2=692.4$; $p<0.001$).

*Odds ratio for culture positivity vs. normal-flora reference group (Nugent 0–3). CI = confidence interval.

Among culture-positive BV samples ($n=602$), *E. coli* (58%) and *K. pneumoniae* (30%) were the dominant isolates, together accounting for 88% of BV-group positive cultures. By contrast, in the normal-flora group, Gram-positive organisms and *Candida* accounted for a proportionately larger

share (33%), and Gram-negative environmental organisms (*A. baumannii*, *P. aeruginosa*) were more frequently represented in relative terms.

4. Discussion

This six-year retrospective surveillance study provides a comprehensive characterisation of the microbiological profile, AMR patterns, and Nugent score correlates of vaginal infections at a North Indian tertiary care centre. The principal findings are: (1) Gram-negative enteric bacilli dominate HVS cultures with *E. coli* (44%) and *K. pneumoniae* (26%) as the leading pathogens; (2) resistance rates among these organisms are extremely high, including ESBL-consistent phenotypes and significant carbapenem resistance; (3) colistin retains 100% activity against all Gram-negative isolates; and (4) Nugent score category is a highly significant predictor of pathogenic culture yield, with odds of a positive culture being 32.5 times higher in BV-range samples than in normal-flora samples ($p < 0.001$).

4.1 Microbiological profile

The predominance of *E. coli* (44%) and *K. pneumoniae* (26%) in our series is consistent with the growing literature on aerobic vaginitis (AV) as a distinct clinical entity characterised by enteric Gram-negative colonisation of the vagina in the setting of *Lactobacillus* depletion. [3,14] Marcone et al. (2021), in a six-year Italian retrospective study of 2,069 vaginal swabs, similarly identified *E. coli* (32.5%) as the most frequent Gram-negative isolate, followed by *K. pneumoniae* (7.8%); however, Gram-positive organisms were more prevalent in their cohort (49.6%) than in ours. [8] Indian studies from Puducherry and other centres have consistently reported *E. coli* as the leading aerobic vaginal pathogen, [15,16] reinforcing the predominance of enteric flora in Indian tertiary care settings, possibly linked to proximity of the anal-vaginal corridor, frequent catheterisation, and hospitalisation.

The relatively high proportions of *A. baumannii* (6%) and *P. aeruginosa* (6%) in our study are notable and likely reflect the tertiary care hospital environment, where nosocomial pathogens are endemic. *S. maltophilia* (2%) – an inherently MDR Gram-negative bacillus – was also detected, underscoring the complexity of vaginal infection microbiology in hospitalised women. *S. aureus* (5%) was the leading Gram-positive bacterial isolate; its presence in vaginal cultures from hospitalised women is well-recognised, with Indian studies reporting *S. aureus* as a significant vaginal pathogen particularly in healthcare-associated settings. GBS / *S. agalactiae* (1%) was isolated predominantly from Labour Room samples, consistent with its role as a coloniser in late pregnancy and a potential cause of neonatal early-onset sepsis.

4.2 Antimicrobial resistance

The resistance profile of our *E. coli* isolates – >80% resistance to third-generation cephalosporins and fluoroquinolones – is strongly indicative of widespread ESBL production, a pattern well-documented across Indian hospitals. [7,9] The 21–25% carbapenem resistance in *E. coli* and 57–64% carbapenem resistance in *K. pneumoniae* point to a substantial CRE burden. Carbapenem resistance at this magnitude has profound clinical implications: it leaves colistin and tigecycline as the principal therapeutic options, both of which carry significant toxicity burdens. The 42–77% tigecycline resistance rates observed in our Gram-negative isolates further constrain treatment choices.

A. baumannii XDR phenotype – 100% resistance to all agents except colistin, with 67% carbapenem resistance – mirrors data from ICU-based surveillance across India and globally. [17] The presence of such organisms in vaginal cultures suggests that hospitalised women, particularly in gynaecology wards, are at risk of colonisation with nosocomial XDR pathogens, which may ascend to cause upper genital tract infections with limited treatment options.

The 100% vancomycin and linezolid susceptibility in *E. faecium* and full antifungal susceptibility in *C. tropicalis* are reassuring, suggesting that these organisms have not yet acquired resistance mechanisms prevalent globally. All *S. aureus* isolates were ceftioxin-resistant (100% MRSA), with resistance to erythromycin (80%), clindamycin (60%), and co-trimoxazole (70%). Vancomycin and linezolid retained 100% activity, which is reassuring given global reports of vancomycin-intermediate

and -resistant *S. aureus*. GBS (n=10) showed full susceptibility to penicillin, ampicillin, and vancomycin; 30% erythromycin resistance was noted, consistent with published Indian data on GBS from vaginal specimens. Both *C. albicans* (n=40) and *C. tropicalis* (n=20) were fully susceptible to all antifungal agents tested, suggesting that azole resistance in vaginal *Candida* has not yet emerged at our institution.

4.3 Nugent scoring: clinical utility and correlation with pathogenic growth

The Nugent scoring system has been the gold standard for BV diagnosis since its standardisation in 1991, [4] but its utility as a predictor of pathogenic culture yield has been less systematically explored. Our data demonstrate a striking and statistically significant stepwise association between Nugent category and culture positivity – from 3.6% in normal-flora samples to 34.4% in intermediate samples and 56.9% in BV-range samples – with adjusted ORs of 13.5 and 32.5 respectively. This is one of the largest datasets from an Indian tertiary care centre to quantify this relationship.

The mechanistic basis for this relationship is well-characterised. *Lactobacillus* species (principally *L. crispatus* and *L. iners*) maintain vaginal pH below 4.5 through lactic acid and H₂O₂ production, creating an environment hostile to Gram-negative enteric pathogens. [10] Depletion of *Lactobacillus* – as reflected by rising Nugent score – removes this protective barrier, enabling colonisation by aerobic pathogens. A PCR-based study confirmed positive correlations between the vaginal pathogenic community (*Gardnerella*, *Prevotella*, *Megasphaera*) and both Nugent score and vaginal pH. [11] The enrichment for *E. coli* and *K. pneumoniae* specifically in BV-range samples (88% of BV-group isolates) in our study confirms that Nugent score category is not merely a surrogate for overall microbial density but preferentially predicts enteric Gram-negative pathogenic colonisation.

These findings have direct clinical implications. Nugent scoring, performed on the same Gram-stained smear used for routine microscopy, is low-cost, equipment-free beyond a standard microscope, and delivers a same-day result – unlike culture, which typically requires 48–72 hours. A Nugent score ≥ 7 in our data was associated with a >32-fold increase in the odds of significant pathogenic growth, suggesting it should function as an immediate clinical risk-stratification tool. When a BV-range Nugent score is reported, empirical antimicrobial coverage for MDR Gram-negative organisms should be considered pending culture and AST results. The intermediate category (Nugent 4–6) also carries a 13.5-fold elevated risk and should not be dismissed. This is consistent with data from Sethi et al. (2024) at PGIMER Chandigarh, who demonstrated in 3,531 women that both BV and intermediate Nugent scores were significantly associated with co-existing STIs and vulvovaginal candidiasis, [18] confirming the clinical relevance of the full Nugent score spectrum.

Compared with Amsel's clinical criteria – which have sensitivity of 75–85% and specificity of 92–95% against Nugent scoring as reference standard in published Indian studies [5,6] – Nugent scoring provides a more objective and granular assessment. Its interrater reliability, when performed by trained microscopists, is excellent (intraclass correlation 0.93–0.96). [19] We therefore recommend integrating Nugent scoring into standard HVS laboratory protocols with routine documentation of scores, and using BV or intermediate scores as triggers for prioritising culture and comprehensive AST.

4.4 Implications for antimicrobial stewardship

Our data reveal that the vaginal flora in hospitalised women harbours the same highly resistant organisms encountered in ICU blood or urinary isolates. The detection of CRKP (57–64% carbapenem resistance), XDR *A. baumannii* (67% carbapenem resistance), and MRSA (100%) in vaginal cultures highlights that gynaecology wards are not immune to the AMR crisis. Empirical antibiotic regimens for vaginal infections that rely on fluoroquinolones, cephalosporins, or beta-lactam/inhibitor combinations are likely to be inadequate in >80% of *E. coli* and virtually all *K. pneumoniae* infections at our institution. [9]

Colistin was the only agent with 100% activity against all Gram-negative isolates. However, colistin monotherapy is suboptimal due to nephrotoxicity and its propensity for resistance development; combination regimens (colistin + carbapenem, colistin + rifampicin) as guided by synergy testing and individual AST remain the standard of care for XDR Gram-negative infections. [20] These findings reinforce the urgent need for institution-specific antibiograms, robust antibiotic stewardship programmes with restricted empirical broad-spectrum antibiotic use, and Infection Control interventions targeting genital tract colonisation with nosocomial MDR organisms.

4.5 Strengths and limitations

Strengths of this study include: six-year duration providing robust temporal data; large sample size (5,209 samples; 1,000 positive); comprehensive AST panels including colistin, tigecycline, and antifungals; and integration of Nugent scoring with culture data – a combination rarely reported from Indian settings. Limitations include: (1) retrospective design with potential for incomplete records; (2) absence of year-wise trend analysis, which would enable detection of resistance emergence over time; (3) lack of molecular confirmation of ESBL and carbapenem resistance mechanisms (e.g., bla-KPC, bla-NDM, bla-OXA); (4) absence of clinical outcome data; and (5) single-centre nature limiting generalisability. Future prospective studies incorporating molecular resistance characterisation and clinical correlation are warranted.

5. Conclusion

This six-year surveillance study from a North Indian tertiary care hospital demonstrates that MDR Gram-negative bacilli – predominantly ESBL-producing *E. coli* and carbapenem-resistant *K. pneumoniae* – are the dominant vaginal pathogens, with colistin as the sole uniformly active last-resort agent. XDR *A. baumannii* and high-level MDR *K. pneumoniae* in vaginal cultures highlight the infiltration of nosocomial resistance into gynaecological infections. Critically, Nugent score category is a powerful, independent predictor of pathogenic culture positivity (OR 32.5 for BV vs. normal flora; $p < 0.001$), demonstrating that Nugent scoring should be routinely performed and reported as an integral component of the HVS laboratory workup – not merely as a BV diagnostic tool, but as a real-time clinical risk stratification instrument. Adoption of institution-specific antibiograms, rational antibiotic prescribing guided by local AST data, and systematic Nugent-score-triggered culture prioritisation are urgently needed to improve outcomes in vaginal infections in the era of escalating antimicrobial resistance.

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References

- [1] Workowski KA, Bachmann LH, Chan PA, Johnston C, Muzny CA, Park I, et al. Sexually transmitted infections treatment guidelines, 2021. *MMWR Recomm Rep* 2021;70(4):1–187.
- [2] Koumans EH, Sternberg M, Bruce C, McQuillan G, Kendrick J, Sutton M, et al. The prevalence of bacterial vaginosis in the United States, 2001–2004; associations with symptoms, sexual behaviors, and reproductive health. *Sex Transm Dis* 2007;34(11):864–9.
- [3] Donders GGG, Bellen G, Grinceviciene S, Ruban K, Vieira-Baptista P. Aerobic vaginitis: no longer a stranger. *Res Microbiol* 2017;168(9–10):845–58.

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- [4] Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of Gram stain interpretation. *J Clin Microbiol* 1991;29(2):297–301.
- [5] Verma M, Singh R, Kaur G, Sharma S, Rana A. Comparison of Amsel's criteria and Nugent's criteria for diagnosis of bacterial vaginosis in a tertiary care centre. *Int J Reprod Contracept Obstet Gynecol* 2019;8(2):639–43.
- [6] Mittal P, Singh S, Sharma S, Arora R, Malhotra M, Chabra S. Comparison of Amsel's criteria with low and high Nugent's scores for the diagnosis of bacterial vaginosis. *Indian J Sex Transm Dis AIDS* 2022;43(1):49–53.
- [7] Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005;18(4):657–86.
- [8] Marcone V, Calzolari E, Bertini M, Soave D, Ferri R. Prevalence and antibiotic resistance profile of bacterial pathogens in aerobic vaginitis: a retrospective study in Italy. *Antibiotics (Basel)* 2021;10(9):1133.
- [9] Laxminarayan R, Chaudhury RR. Antibiotic resistance in India: drivers and opportunities for action. *PLoS Med* 2016;13(3):e1001974.
- [10] Muzny CA, Schwabke JR. Pathogenesis of bacterial vaginosis: discussion of current hypotheses. *J Infect Dis* 2016;214(Suppl 1):S1–5.
- [11] Ling Z, Liu X, Luo Y, Yuan L, Tong X, Wang Y, et al. Associations between vaginal pathogenic community and bacterial vaginosis in Chinese reproductive-age women. *PLoS One* 2013;8(10):e76589.
- [12] Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, M100. 33rd ed. Wayne, PA: CLSI; 2023.
- [13] Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;18(3):268–81.
- [14] Donders GG, Van Calsteren K, Bellen G, Reybrouck R, Van den Bosch T, Riphagen I, et al. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *BJOG* 2009;116(10):1315–24.
- [15] Ravishankar N, Srinivasan N. Antibigram of bacterial isolates from high vaginal swabs of pregnant women from a tertiary care hospital in Puducherry, India. *Int J Curr Microbiol App Sci* 2017;6(1):882–90.
- [16] Muruges K, Yaji C, Bhatta S, Pramod Kulkarni H, Vijayakumar B. Aerobic bacterial pathogens causing vaginitis in patients attending a tertiary care hospital and their antibiotic susceptibility pattern. *J Pure Appl Microbiol* 2020;14(1):573–80.
- [17] Ayobami O, Willrich N, Suvilampi A, Kimani SM, Osei I, Kreisel K, et al. The epidemiology of carbapenem-resistant *Acinetobacter baumannii* complex across Africa: a systematic review and meta-analysis. *Antimicrob Resist Infect Control* 2022;11(1):60.
- [18] Sethi S, Yadav R, Sharma N, Dadwal R, Chaudary H, Kaur K, et al. Association of intermediate Nugent Score and bacterial vaginosis with sexually transmitted infections and vulvovaginal candidiasis. *Indian J Dermatol Venereol Leprol* 2024;90:296–301.
- [19] Lai NM, Ahmad Kamar A, Choo CM, Kong JY, Ngim CF, Lim KP, et al. A comparison of colorimetric assessment of vaginal pH with Nugent score for the detection of bacterial vaginosis. *J Matern Fetal Neonatal Med* 2017;30(4):420–4.

-
- [20] 20. Paul M, Carmeli Y, Durante-Mangoni E, Mouton JW, Spanu T, Theuretzbacher U, et al. Combination therapy for carbapenem-resistant Gram-negative bacteria. *J Antimicrob Chemother* 2014;69(9):2305–9.
- [21] 21. Muzny CA, Taylor CM, Swords WE, Tamhane A, Chattopadhyay D, Cerca N, et al. An updated conceptual model on the pathogenesis of bacterial vaginosis. *J Infect Dis* 2019;220(9):1399–405.
- [22] 22. Chakraborty A, Pal NK, Sarkar S, Bhattacharya S. Antibiotic resistance pattern of bacteria isolated from cases of vaginal discharge: a study in a teaching hospital of Eastern India. *J Obstet Gynaecol India* 2016;66(Suppl 1):469–74.
- [23] 23. World Health Organization. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: 2022. Geneva: WHO; 2022.
- [24] 24. Gupta N, Mittal N, Srivastava A, Chaudhary K, Raghunath G. High vaginal swab culture and sensitivity pattern in symptomatic patients – a study from a tertiary care centre in Northern India. *J Clin Diagn Res* 2014;8(12):DC04–6.
- [25] 25. Bharadwaj P, Tyagi S, Grover N, Tyagi S, Gupta N. Six-year retrospective study of microbial isolates and antimicrobial susceptibility pattern in high vaginal swab samples from a tertiary care hospital in North India. *J Lab Physicians* 2021;13(2):138–44.