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Abstract

Introduction: *Enterococcus spp.* are typically intestinal commensals that may colonize the vagina and are associated with obstetric complications such as preterm birth, low birth weight, and puerperal sepsis. Vancomycin-resistant *Enterococci* (VRE) have emerged as a global health concern. This study aimed to determine the prevalence of vaginal colonization and aerobic vaginitis (AV) caused by *Enterococcus* spp., in third-trimester pregnant women in Taiz city, Yemen, and assess the antimicrobial resistance pattern, including inducible clindamycin resistance.

Materials and Methods: This cross-sectional study involved 200 third-trimester pregnant women who attended one of three antenatal clinics in Taiz, Yemen, from April 2023 to June 2024. Vaginal swabs were inoculated on appropriate culture media for isolation. Antimicrobial susceptibility tests were performed by disk diffusion, and AV was diagnosed using Donder's criteria.

Results: *Enterococcus* species were isolated from 56 (28%) participants. *Enterococcus faecium* was identified in 30 isolates, and *Enterococcus faecalis* in 26 isolates. Among the 56 *Enterococcus*-colonized women, 33 (58.9%) fulfilled Donders' criteria for AV. Mild AV (81.8%, 27/33) was predominantly associated with E. faecium, while moderate AV (18.2%, 6/33) involved both species equally. Antimicrobial resistance was highest for penicillin G (89.3%), erythromycin (87.5%), and vancomycin (58.9%). Amoxicillin-Clavulanate showed the highest sensitivity (91.1%).

Conclusion: The high burden of multidrug-resistant *Enterococcus spp*., including vancomycin-resistant strains, underscores the urgent need for antimicrobial stewardship and routine screening for aerobic vaginitis during prenatal care in low-resource settings.

Keywords: *Enterococcus spp*; Vaginal colonization; Aerobic vaginitis; Pregnancy; Antimicrobial resistance.

Introduction

Enterococci are Gram-positive facultative anaerobic bacteria typically found in pairs or short chains. They are catalase-negative, bile salts tolerant, and capable of hydrolyzing esculin [1]. These organisms are constituents of both gastrointestinal and vaginal microbiota [2, 3]. The genus Enterococcus includes over 40 recognized species, with *E. faecium* and *E. faecalis* ranked as the third and fourth most prevalent human pathogens globally [4].

Enterococcus species have been linked to some hazardous conditions, including spontaneous abortion, premature birth, puerperal sepsis, abscesses, and urinary tract infections (UTIs) [5]. Neonates born to infected mothers are at increased risk of low birth weight and/or puerperal sepsis, contributing to elevated morbidity and mortality. *E. faecalis* was reported as the most common agent of AV, causing 32.26% of cases, followed by *E. coli* (8-25%) [6, 7].

Aerobic vaginitis is a vaginal inflammation characterized by marked reduction in the protective lactobacilli with overgrowth of some opportunistic pathogens, as *E. faecalis*, *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Escherichia coli* [3]. It was reported that *E. faecalis* has been associated with a 6% neonatal mortality rate in early-onset septicemia (EOS), increasing to 15% in late-onset (LOS), and implicated in 7% to 50% of cases of fatal sepsis [8, 9].

The pathogenicity of *Enterococci* is linked to their important virulence factors, such as surface proteins and aggregation substance, which promote adhesion, colonization, and biofilm formation. Multiple enzymes, such as hyaluronidase, cytolysin, and gelatinase, these enzymes facilitate tissue invasion and host damage [10-13]. Biofilm plays a role in the protection of *Enterococci* from phagocytosis and allows the exchange of virulence genes in multispecies [14]. Enterococci are known for acquiring resistance to the most commonly used antimicrobial agents, including ampicillin, aminoglycosides, clindamycin, and cephalosporins. This makes treatment of these infections a significant health challenge [8, 15]. Worldwide increases of vancomycin resistance among Enterococci (VRE) have been reported as a serious public health hazard by the World Health Organization (WHO) [16].

Currently, there is no standardized treatment protocol for vaginal infection caused by *E. faecalis* Han *et al.* recommended tailoring therapy based on susceptibility pattern in addition to topical antibiotic administration. Some studies support using ampicillin combined with aminoglycosides for effective eradication [6, 22]. A clinical study for treating *E. faecalis* and *E. coli* vaginitis using nitrofurantoin at a dose of 500 mg daily for 6 days showed that symptom resolution was reported in about 72% of cases [17]. During pregnancy, clindamycin is always preferred. Fluoroquinolones, such as ciprofloxacin and ofloxacin, could also be used to treat AV due to their minimal effect on the vaginal microbiota, potentially facilitating rapid recovery from AV [18, 19].

Although increasing reports of multidrug-resistant *Enterococcus* spp., in addition to the association of these bacteria with adverse pregnancy outcomes, little data exists on their vaginal colonization or antimicrobial resistance (AMR) patterns in Yemen. This gap highlights the need for regional studies and investigations, particularly in under-resourced settings.

Materials and Methods

Study design, population, and place

This cross-sectional study included 200 third-trimester pregnant women who attended AL-Thawra Hospital, Republican Hospital-Taiz, and a private gynecology and obstetrics clinic in Taiz, Yemen, from April 2023 to June 2024.

The sample size was determined using Cochran's formula for proportions [20].

$$n = (Z^2 \times p(1-p)) / d^2$$

Where:

- 1.96 (95% confidence level).
- p = expected prevalence of Enterococcus colonization (15%)
- d = desired precision (0.05).

The calculated sample size was approximately 196. For ensuring adequate power and to cover possible attrition, 200 participants were enrolled.

Microbiological examination and analysis were conducted at the National Center for Public Health Laboratories, Taiz Branch. Ethical approval was obtained, and the study adhered to standards established by the institutional committee for research.

Specimens and data collection

Following the acquisition of consent, data were gathered through face-to-face interviews using a pre-designed questionnaire. Inclusion criteria required participants to be in the third trimester of pregnancy, agree to participate, and have not utilized antimicrobials for at least one week before specimen collection. Exclusion criteria included non-pregnant women, pregnant women not yet in the third trimester, recent antimicrobials use, and those who refused to participate.

Three vaginal swabs were collected from each participant by a gynecologist. The first swab was placed immediately in Amies transport media with charcoal (Hi-MEDIA) for culture. The second swab was used to measure vaginal hydrogen ion concentration (pH) (BIOTEST-RIGHTSIGN, CHINA), and the third swab was used for wet mount and Gram staining to identify AV using Donders' criteria, including the presence of pus cells, polymorphonuclear cells (PMNs), and parabasal cells. Gram-stained smears were examined for large Gram-positive bacilli, Gram-positive cocci, Gram-negative bacilli, parabasal cells, and pus cells for the diagnosis of AV.

Isolation and identification of bacteria

Vaginal swabs were inoculated on neomycin blood agar [21], Columbia agar supplemented with 5% human blood, MacConkey agar with bile salt, mannitol bile salt agar (MSA), and chocolate agar [22] [HI-MEDIA, India]. *Enterococcus* spp. are salt-tolerant and capable of growing on MSA [23].

After overnight aerobic incubation at 37 °C, preliminary identification of *Enterococcus* spp. was based on colony morphology, hemolytic pattern, Gram stain, catalase, esculin bile salt, types of mannitol fermenter, growth characteristics on mannitol salt agar. Final confirmation was performed using Lancefield group D antigen testing (Microgen Bioproducts, UK) [24].

Antimicrobial susceptibility pattern

Antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar supplemented with 5% human blood, following Clinical and Laboratory Standards Institute (CLSI) guidelines [30]. Antibacterial agents used in this study were amikacin (AK), amoxiclav (MAC), ceftriaxone (CTR), ciprofloxacin (CIP), clindamycin (CD), erythromycin (E), tetracycline (TE), vancomycin (VA), and penicillin G (P).

Detection of AV by Donders' criteria

Aerobic vaginitis was diagnosed using Donders’ criteria as follows:

a. **Sample collection**: swabs were collected from the vagina using sterile swabs. Wet and gram-stained smears were prepared.

b. **Microscopic examination:** Five parameters were ranked (0, 1, or 2) under the microscope.
   - **Lactobacillary Grade**: 0 (predominant *Lactobacilli*), 1 (reduced *Lactobacilli*), or 2 (no *Lactobacilli*).
   - **Leukocyte Count**: 0 (<10 leukocytes per high-power field [HPF]), 1 or 2 (>10 leukocytes per epithelial cell).
   - **Toxic Leukocytes**: 0 (none/sporadic), 1 (≤50% of leukocytes showing toxicity), or 2 (>50% toxic leukocytes).
   - **Background Flora**: 0 (unremarkable/cytolysis), 1 (small coliform bacilli), or 2 (cocci or chains).
   - **Parabasal Cells**: 0 (none/<1%), 1 (≤10%), or 2 (>10%).

c. **Total Score Calculation**: Scores from all five parameters were summed.

d. **Interpretation**: AV severity was classified as:

e. **No AV** (score <3), **Mild AV** (3–4), **Moderate AV** (5–6), or **Severe AV** (score >6) [25].

Data Analysis

Data were analyzed using IBM SPSS Statistics 26.0. Descriptive statistics included frequencies and cross-tabulation. Chi-square and Fisher's exact tests were used for assessment of statistical significance. The significance level (*P* value) of less than 0.05 was considered a significant result.

Results

This cross-sectional study included 200 third-trimester pregnant women attending AL-Thawra Hospital, Republican Hospital, and one private gynecology and obstetrics clinic in Taiz. Participants ranged from 17 to 45 years, with a mean age of 29.25 years. Vaginal colonization by *Enterococcus* spp. was detected in 56 participants (28%). *E. faecium* was isolated in 30 cases (15%) and *E. faecalis* in 26 cases (13%). The highest isolation rates were observed in the age group 25-35 years (17.6% for *E. faecium*) and >35years (18.8% for *E. faecalis*).

Among 56 women colonized by *Enterococcus* spp., 33 (58.9%) fulfilled Donders' criteria for AV. Of these 33, 27 had mild AV and 6 had moderate AV. No severe AV cases were observed, as shown in Table 1.

Table 1:

Distribution of mild, moderate, and severe AV among Enterococcus-colonized women.

Variables	No. of cases	Total colonization	*E. faecium*		*E. faecalis*		*P value*
			N	%	N	%	
Normal	119 (59.5%)	23 (19.3%)	10	8.4%	13	10.9%	< 0.001
Mild aerobic vaginitis	52 (26%)	27 (51.9%)	17	32.7%	10	19.2%	
Moderate aerobic vaginitis	18 (9%)	6 (33.3%)	3	16.7%	3	16.7%	
Severe aerobic vaginitis	11 (5.5%)	00	0	00	0	00	
Total	200 (100)	56 (28%)	30	15%	26	13%	

The D-test was positive in 5 cases, 4 of them were resistant to vancomycin (one belongs to *E. faecalis*, and three belong to *E. faecium*), and only one was sensitive. (Table 2).

Among *E. faecium* isolates, the highest proportion was found in the age group 25-35 (17.6%, n=15). For *E. faecalis*, the majority was found in the age group >35 (18.8%, n=9).

Table 2:

D-TEST results: Clindamycin and Erythromycin Interaction

Isolated bacteria			Clindamycin and erythromycin interaction		Total
			Positive	Negative	
Enterococcus Spp.	*E. faecium*	N	4	26	30
		%	7.1%	46.4%	53.6%
	E. faecalis	N	1	25	26
		%	1.8%	44.6%	46.4%
Total		N	5	51	56
		%	8.9%	91.1%	100.0%

Resistance varied by antimicrobials, with ciprofloxacin resistance observed in 58.9% of isolates (*E. faecium*: 63.3%, *E. faecalis*: 53.9%), while ceftriaxone resistance was 48.2% overall (*E. faecalis*: 34.6%, *E. faecium*: 60%). Tetracycline resistance was noticed in 51.8% of isolates (*E. faecium*: 56.67%, *E. faecalis*: 46.15%). Clindamycin resistance differed markedly between species, with *E. faecalis* showing 23.08% resistance compared to 36.67% in *E. faecium*. Inducible clindamycin resistance (D-test positivity) was identified in 8.9% of isolates (4 *E. faecium*, 1 *E. faecalis*). Amoxicillin-clavulanate (AMC) demonstrated the highest sensitivity (91.1%), with *E. faecalis* showing slightly greater susceptibility (92.31%) than *E. faecium* (90%). Amikacin sensitivity was 69.6%, with *E. faecalis* exhibiting higher susceptibility (84.62%) than *E. faecium* (56.67%). Multidrug resistance (MDR), defined as resistance to ≥3 antimicrobial classes, was observed in more than 85.7% of isolates (Table 3), with *E. faecium* displaying resistance to a broader range of agents compared to *E. faecalis*.

Table 3:
Antimicrobial resistance pattern of E. faecium and E. faecalis

Antimicrobial	Sensitivity among Species		Resistance among species		Total sensitivity among genus	Total resistance among genus	*P value*
	E. faecium	*E. faecalis*	*E. faecium*	*E. faecalis*			
AK	**17 (56.67)**	**22 (84.62)**	**13 (43.33%)**	**4 (15.38%)**	**39 (69.6%)**	**17 (30.4%)**	**0.023**
AMC	**27 (90.0%)**	**24 (92.31)**	**3 (10.0%)**	**2 (7.69%)**	**51 (91.1%)**	**5 (8.9%)**	**0.569**
CTR	**12 (40.0%)**	**17 (65.38)**	**18 (60.0%)**	**9 (34.62%)**	**29 (51.8%)**	**27 (48.2%)**	**0.051**
CIP	**11 (36.67%)**	**12 (46.15)**	**19 (63.33%)**	**14 (53.85%)**	**23 (41.1%)**	**33 (58.9%)**	**0.327**
TE	**13 (43.33)**	**14 (53.85)**	**17 (56.67%)**	**12 (46.15%)**	**27 (48.2%)**	**29 (51.8%)**	**0.303**
VA	**13 (43.33%)**	**10 (38.46%)**	**17 (56.67%)**	**16 (61.54%)**	**23 (41.1%)**	**33 (58.9%)**	**0.462**
P	**6 (20.0%)**	**0 (00.0)**	**24 (80.0%)**	**26 (100.0 %)**	**6 (10.7%)**	**50 (89.3%)**	**0.018**
***CD**	**19 (63.33%)**	**20 (76.92%)**	**11 (36.67%)**	**6 (23.08%)**	**39 (69.6%)**	**17 (30.4%)**	**0.209**
***E**	**4 (13.33%)**	**3 (11.54%)**	**26 (86.67%)**	**23 (88.46%)**	**7 (12.5%)**	**49 (87.5%)**	**0.582**

Discussion

The present study found a 28% (56 of 200) prevalence of vaginal colonization by *Enterococcus* spp. among third-trimester pregnant women in Taiz, Yemen, with slightly more common *E. faecium* (15% of isolates) than *E. faecalis* (13%). The highest *E. faecium* colonization occurred in women aged 25-35 years, while *E. faecalis* was more frequent in women over

35 years. A lower colonization rate of *Enterococcus spp.* was reported by a similar study made in Kerman City, Iran; 8.14%, the predominant species were *E. faecalis* 89.8%, *E. faecium* 6.1%, and other *Enterococcus* spp. 4.1% [26], and also exceed the prevalence reported from Nigeria [8].

The comparatively high prevalence of *Enterococcus spp*. and the predominance of *E. faecium* in this study, unlike lower rates and predominance of *E. faecalis* in some other parts of the world, could reflect widespread antibiotic misuse, which diminishes its efficacy and also favors resilient species, and may indicate stricter antibiotic regulations and differing diagnostic protocols.

An important finding in this study is the rise of antimicrobial resistance. The resistance for penicillin G, erythromycin, and vancomycin was 89.3%, 87.5%, and 58.9%, respectively. D-test positivity (the inducible clindamycin resistance) was detected in 8.9% of isolates. Amoxicillin-Clavulanate appeared to be the most effective antimicrobial agent. Enterococci showed minimal resistance to this combination at 8.9%.

The observed high resistance patterns demonstrate a critical community health challenge. Vancomycin resistance (58.9%) highly exceeds rates reported in Iran (14%%) [27], and Italy that reported that the frequency of Vancomycin-resistant Enterococci was 1.3% in 2017, 1.8% in 2018, and 1.9% in 2019 for *E. faecalis* and 3.7% in 2017, 1.6% in 2018, and 6.1% in 2019 [28]. Vancomycin failure threatens neonatal sepsis management in resource-limited settings. Penicillin and erythromycin resistance (89.3% and 87.5%) further reflect indiscriminate prescribing practices without susceptibility testing. It is also noted that *E. faecium* showed broader resistance than *E. faecalis;* these results are consistent with global trends [26, 28, 29] and may be attributed to its enhanced *biofilm formation* and the ability to acquire resistance genes in hospital environments vs *E. faecalis.*

The high MDR rate underscores the urgent need for antimicrobial stewardship programs to limit inappropriate antibiotic use. The D-test positivity (8.9%) indicates electable clindamycin resistance and necessitates caution in empirical clindamycin use to avoid management failure. Biofilm formation and intrinsic resistance mechanisms, such as altered penicillin-binding proteins, may further complicate treatment [10, 13].

According to Donders' criteria 76.8% *Enterococcus*-colonized women met the diagnostic threshold for true AV**,** which indicates pathological inflammation, not only asymptomatic colonization. Among these AV cases, mild AV predominated (81.8%, n=27/33), with *E. faecium* implicated in 17/27 cases (62.96%) and *E. faecalis* in 10/27 (37.0%). Moderate AV in 18.2% (n=6/33), with both species equally represented (3 cases each). Notably, no severe AV cases were observed, contrasting with studies in Iran and India, where severe AV correlated with polymicrobial infections or broader-spectrum resistance [26, 29]. This discrepancy may reflect the unique pathogenic profile of *Enterococcus* spp. The Yemeni population showed high antimicrobial resistance may suppress competing flora, limiting inflammation severity [19].

While this study provides valuable data from Yemen, some limitations include a single-center design and a lack of molecular characterization of resistance genes. It is better to perform future multicenter studies to explore genetic determinants of resistance and to assess interventions like routine susceptibility testing and clinician education. Public health authorities should plan policies that prioritize regulating antibiotic sales, promoting hygiene, and integrating AV screening into prenatal care.

Limitations

This study, while giving a valuable understanding, has many limitations. It is designed as a cross-sectional study establishing causality between Enterococcus colonization, aerobic vaginitis, and adverse pregnancy outcomes, yet longitudinal studies are needed. The findings are from one region (Taiz), which limits generalization to other parts of the country, especially those with different epidemiological or health care practices. The genetic mechanisms of antimicrobial resistance were not assessed; assessment of these mechanisms would provide deeper insight into resistance epidemiology. Lastly, the absence of clinical follow-up makes this study unable to link directly between colonization or vaginitis and specific maternal or neonatal outcomes, highlighting the need for further studies to determine this aspect.

Conclusion

Our study emphasized the high prevalence of multidrug-resistant *Enterococcus* spp. in the third trimester of pregnancy among women in Taiz, Yemen, with *E. faecium* presenting a significant threat due to high resistance. These findings clarify the need for immediate action to design a plan for antimicrobial administration stewardship, improve diagnostic capacity, and design effective local treatment guidelines. Determining and managing these challenges is critically important to reduce the spread of antimicrobial-resistant strains and minimize maternal-neonatal morbidity in vulnerable populations.

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Ethical Approval:

The study protocol was approved by the Research Ethics Committee, Faculty of Medicine and Health Sciences, Taiz University.
Approval No: HREC-04-2023, dated 15 March 2023.

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