RESEARCH ARTICLE

CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE OF VAGINAL MICROBIOTA IN MARES FROM LAGOS AND OYO STATES, NIGERIA

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ABSTRACT

Despite the importance of microbiota in mammalian reproductive health, research on equine vaginal microbiota remains limited, particularly in tropical regions. The composition of mares' vaginal microbiota in Lagos and Oyo States, Nigeria is undocumented. Microbiota profiles are essential for developing effective reproductive health management strategies. We aim to characterize vaginal microbiota composition and determine antimicrobial resistance profiles of isolated bacteria in mares from Lagos and Oyo States, Nigeria. Vaginal swabs from 81 apparently healthy mares, comprising Nigerian indigenous breeds, Sudanese breeds, and Argentine Polo Ponies, were collected using sterile guarded culture swabs. Samples underwent bacterial isolation on selective and differential media including Trypticase soy, Mannitol salt, Eosine methylene blue, Muller-Hinton, Salmonella-Shigella, and MacConkey agar. Bacterial identification involved morphological and biochemical tests, including oxidase, catalase, motility, Gram staining, and sugar fermentation. Antimicrobial susceptibility was tested against 18 antimicrobials using the Kirby-Bauer disc diffusion method following CLSI standards. Analysis revealed distinct bacterial distribution patterns between locations. Of 81 samples, *Staphylococcus aureus* dominated (66.7%), while *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* each represented 1.2%, 30.9% showed no growth. Biochemical characterization demonstrated typical profiles for each species. *S. aureus* showed complete sensitivity to imipenem and streptomycin, but resistance to cefuroxime, penicillin-streptomycin, enrofloxacin, ciprofloxacin, and metronidazole. *P. aeruginosa* was sensitive to clindamycin and imipenem, while resistant to most other antibiotics. Given the high presence of drug-resistant *S. aureus*, veterinarians should prioritize aminoglycosides and carbapenems for treating mare vaginal infections, using other antibiotics only with susceptibility testing guidance.

Keywords: Antimicrobial resistance, equine reproduction, Nigerian mares, *Staphylococcus aureus*, vaginal microbiota

INTRODUCTION

The reproductive well-being of mammals is closely connected to various microbial communities that inhabit their reproductive systems. These microorganisms, referred to as the microbiota, are essential for sustaining reproductive health and fitness (Malaluang et al., 2024).

The lower female genital area contains a dynamic microbial ecosystem, featuring numerous microorganisms that can inhabit both the vagina and uterus in healthy animals (Adekunle et al., 2024). Various factors affect the composition and stability of equine vaginal microbiota. These consist of physiological elements, like the phase of the oestrous cycle, age, and reproductive condition; environmental elements such as geographical area, climate, and seasonal changes; and management aspects like nutrition, veterinary care, and previous use of antimicrobial drugs (Gil-Miranda et al., 2024; Malaluang et al., 2024). The dynamic characteristics of the bacterial community are also highlighted by its reactions to interventions like antibiotic therapy or breeding practices, along with pathological states such as uterine infections. This complexity highlights the significance of comprehending these interactions, since dysbiosis—an imbalance in bacterial populations—can result in pathogen growth and ensuing reproductive health problems (Gil-Miranda et al., 2024).

Earlier research has recognized various prevalent bacterial species within mares' vaginal microbiota, such as *Escherichia coli, Staphylococcus capitis, Streptococcus equisimilis*, *Streptococcus thoraltensis*, and *Streptococcus zooepidemicus* (Malaluang et al., 2024). Nonetheless, these results mainly originate from research conducted in a different area, and the bacterial composition of mares in Lagos and Oyo States, Nigeria, is still not documented.

This research aims to characterize the vaginal microbiota composition and determine the antimicrobial resistance profile of isolated bacteria in mares from Lagos and Oyo States, Nigeria. This information is especially important for veterinary professionals and researchers aiming to tackle infertility and subfertility challenges in mares in tropical settings. Moreover, comprehending the patterns of dysbiosis and microbial interactions will aid in creating more efficient treatment approaches for reproductive tract infections.

This research hypothesizes that the vaginal microbiota of mares in the studied regions possesses unique characteristics, shaped by the tropical climate, nutritional resources, and management practices. These factors are expected to influence bacterial diversity and the prevalence and distribution of bacterial species from mare vagina.

MATERIALS AND METHODS

Ethical Approval

The study was conducted in strict accordance with ethical guidelines for animal research and was approved by the Animal Care, Use and Research Ethics Committee of the University of Ibadan, Nigeria (Approval No.: NHREC/UIACUREC/07/11/2025B). Informed consent was also obtained from all animal owners.

Animal and Sample Collection

A total of 81 adult mares comprising Nigerian indigenous breed, Sudanese, and Argentine polo ponies were used for this study. All mares were apparently healthy at the time of sampling. Vaginal swabs were collected from these mares in Lagos and Oyo States, Nigeria. Prior to sample collection, each mare was properly restrained with minimal stress to ensure safety and proper sample collection. The tail was wrapped and secured away from the perineal area using a clean tail bandage. The perineal area was thoroughly cleaned with mild soap and water, rinsed with clean water, and dried using sterile gauze to prevent contamination.

A sterile, long-handled guarded culture swab was used to collect samples from the vaginal wall, approximately 10-15 cm cranial to the vestibulo-vaginal junction. The swab was rotated against

the vaginal mucosa with gentle pressure to ensure adequate sample collection. Care was taken to avoid contamination from the vestibule during withdrawal of the swab.

Immediately after collection, each sample was properly labelled with a unique identification number, the date and time of collection, and the mare's details. The samples were maintained at 4°C in a cold chain and transported to the laboratory within 2 hours of collection for microbiological analysis.

Aseptic techniques were maintained throughout the sampling process to prevent cross-contamination between samples and from the environment. All personnel involved in sample collection wore clean disposable gloves, which were changed between animals.

Bacterial isolation and characterisation

The sterile swabs were placed in 10 ml of buffered peptone water for pre-enrichment, then streaked on selective and differential media, including Trypticase soy agar (HiMEDIA®, USA), Mannitol salt (MSA), Eosine methylene blue (EMB), Muller-Hinton, Salmonella-Shigella, and MacConkey (MAC) agar. The samples were then incubated at 37°C for 24 hours. The main colonies on selective and differential agar were continuously transferred to Trypticase soy agar (TSA) to analyse their colonial morphology and purity (Anifowose et al., 2023).

Bacterial isolates were identified based on morphological and biochemical tests, including oxidase, catalase, motility, Gram staining, simmon citrate, indole, glucose (gas), hydrogen sulfide production, lysine, ornithine and urease, methyl red, vogues proskauer, triple sugar Iron agar slant culture as well as sugar fermentation tests (Anifowose et al., 2023)

Antimicrobial Susceptibility Testing

Each isolate was analyzed for antibiotic susceptibility. By using the agar disc diffusion method, which was carried out in accordance with CLSI standards Mo2, pure isolates of the identified bacteria were evaluated for their sensitivity and resistance to antibiotics (CLSI, 2020). The susceptibility was evaluated against 18 antimicrobials (micrograms) from the following ten classes: penicillin (amoxicillin– clavulanic acid 30 µg (AC); ampicillin 10 µg (AP, amplicox 25 µg (AM)), penicillin (penstrep 25 µg), cephalosporin (third-generation) ceftriaxone 30 µg (CEF), cefuroxime 30 µg (CEX), tetracycline (doxycycline 30 µg (D), tetracycline 30 µg (T), chloramphenicol 30 µg (CH), aminoglycoside: gentamicin 10 µg (G)) streptomycin 10 µg (S), metronidazole 5 µg (M), lincomycin (clindamycin 2 µg (CLI), carbapenem (imipenem 10 µg (IPM), fluoroquinolone (ciprofloxacin 5 µg (CIP), (ofloxacin 5 µg (OF)), enrofloxacin 5 µg (ENR)), and glycopeptide: vancomycin 30 µg (V) based on the Kirby-Bauer disc diffusion method (CLSI, 2020; Gbanamou et al., 2024).

Preparation of Antibiotic Disc

The disc was constructed in accordance with the CLSI Mo2 disc diffusion guide; six 6-mm sterilized Whatman filter paper discs were placed on a 100-mm plate, and the discs were impregnated with the known antimicrobial concentrations stated above. Each isolate on a sterile Mueller-Hinton agar plate was made and appropriately labelled with an indelible marker (CLSI, 2020).

Preparation of Inoculums

By adjusting the suspension of each isolate colony in sterile normal saline to the density of a McFarland 0.5 turbidity standard, the direct colony suspension method was employed. Each isolate was grown overnight on Nutrient agar, and pure cultures were harvested using a sterile loop. The colonies were suspended in sterile normal saline that had been blended to a consistent turbidity. Saline or additional bacteria were added in order to get the density of each isolate suspension to McFarland 0.5. After that, Muller-Hinton agar was streaked with the suspended each isolate colony. Quality control was performed using the previously identified *Escherichia coli* ATCC (CLSI, 2020).

Inoculation of Agar Plates

The sterile pointed forceps were used to pick one antibiotic disc (for each bacterial isolate) and

applied to the surface of Mueller-Hinton agar plates. This allowed the discs to effectively touch the medium. Afterward, the plates were incubated for 18 hours at 37^0C (CLSI, 2020). For the purpose of identifying which antibiotics the isolate was sensitive to or resistant to, the diameter of the visible zone of growth inhibition surrounding the several discs was measured in millimetres. Each isolate's resistance pattern was created using the antibiogram (Anifowose et al., 2024).

Data Analysis

The data was analyzed using IBM SPSS Statistics software version 26. Descriptive statistics were employed to analyze and present the data. Frequency distributions and percentages were calculated for distribution of bacterial isolates by location (Oyo State and Lagos State), prevalence of different bacterial species (*S. aureus*, *P. aeruginosa*, and *S. epidermidis*) and antimicrobial susceptibility patterns.

RESULTS

Distribution of Bacterial Isolates by Location

Based on Table 1, a total of 81 vaginal swab samples were collected from apparently healthy mares across two locations in Southwestern Nigeria, with 56 bacterial isolates successfully obtained overall. Lagos State contributed the majority of samples (n=60) and yielded 48 bacterial isolates, while Oyo State provided 21 samples with 8 bacterial isolates recovered. The chi-square analysis ($\chi^2 = 12.85$, df = 1, $p < 0.001$) reveals a statistically significant association between sampling location and bacterial isolation patterns from the vaginal microflora of these mares.

Prevalence of Isolated Bacterial Species

The prevalence data showed that *S. aureus* was the dominant organism, representing 66.7% of all samples from the vaginal swabs, while *P. aeruginosa* and *S. epidermidis* each represented 1.2% of the isolates. Notably, 25 samples (30.9%) showed no bacterial growth (Table 2).

Biochemical Characteristics of Bacterial Isolates

The biochemical characterization revealed distinct profiles for each bacterial species isolated from the mare vaginal swabs. Both *Staphylococcus* species demonstrated typical Gram-positive cocci characteristics, showing positive results for catalase and negative for oxidase. *S. aureus* and *S. epidermidis* could be differentiated by their behaviour on Mannitol salt agar, with *S. aureus* producing yellowish colonies and *S. epidermidis* forming pinkish colonies. Both species showed similar sugar fermentation patterns, being positive for glucose, maltose, lactose, mannitol, xylose, sucrose, and arabinose. *P. aeruginosa* displayed characteristic Gram-negative rod morphology with unique biochemical features including positive oxidase and motility tests. It showed limited sugar fermentation capabilities compared to the *Staphylococcus* species, being positive only for glucose and mannitol, while producing a distinctive greenish-blue pigment on Mueller-Hinton agar (Table 3).

Antimicrobial Susceptibility Patterns

The antimicrobial susceptibility testing revealed varying patterns of resistance and sensitivity among the isolated bacteria. For *S. aureus* isolates (Table 4), complete sensitivity was observed to imipenem and streptomycin, while gentamicin showed moderate effectiveness with over half of the isolates being sensitive. However, concerning resistance patterns were noted against multiple antibiotics including cefuroxime, penicillin-streptomycin, enrofloxacin, ciprofloxacin, and metronidazole. The single *S. epidermidis* isolate (Table 5) demonstrated complete sensitivity to gentamicin and streptomycin but showed resistance to several antibiotics, including cefuroxime, ampicillin, and various fluoroquinolones. *P. aeruginosa* isolate (Table 6) showed a distinct susceptibility pattern, being completely sensitive to clindamycin and imipenem, while demonstrating resistance to most other tested antibiotics. This comprehensive resistance profile suggests the need for careful antibiotic selection in treating vaginal infections in

mares, with aminoglycosides and carbapenems appearing to be the most reliable treatment options across all isolated species.

Table 1 Distribution of bacterial isolates from vaginal swabs of apparently healthy mares in different locations of Southwestern Nigeria

Location	No. of Samples	No. of Bacterial Isolates	Chi-square (χ^2)	df	P-value
Oyo State	21	8	12.85	1	< 0.001
Lagos State	60	48			
Total	81	56			

Table 2 Prevalence of bacterial species isolated from mare vaginal swabs

Bacterial isolates	Number	Prevalence (%)
Staphylococcus aureus	54	66.7
Pseudomonas aeruginosa	1	1.2
Staphylococcus epidermidis	1	1.2
No growth	25	30.9
Total	81	100

Table 3 Biochemical characteristics of *Staphylococcus aureus* isolates

Biochemical Characteristics	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*
Gram Staining	Gram-positive cocci	Gram-positive cocci	Gram-negative rods
Catalase	Positive	Positive	Positive
Oxidase	Negative	Negative	Positive
Motility	Negative	Negative	Positive
Methyl-red	Positive	Positive	Negative
Hydrogen sulphide production	Negative	Negative	Negative
Indole production	Negative	Negative	Negative
Citrate	Positive	Positive	Positive
Voges-proskauer	Negative	Negative	Negative
Urea Hydrolysis Test	Negative	Negative	Negative
Mannitol Salt agar	Yellowish colonies	Pinkish colonies	-
Haemolysis	Positive	Positive	-
OF (Oxidative-Fermentative)	-	-	Facultative anaerobes
TSI Agar	-	-	Alkaline slant, alkali butt
Mueller-Hinton agar			Greenish-blue pigment
Sugar Fermentation Test			
Glucose	Positive	Positive	Positive

Biochemical Characteristics	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*
Maltose	Positive	Positive	Negative
Lactose	Positive	Positive	Negative
Mannitol	Positive	Positive	Positive
Xylose	Positive	Positive	Negative
Sucrose	Positive	Positive	Negative
Arabinose	Positive	Positive	Negative

Table 4 Frequency of antimicrobial susceptibility for *Staphylococcus aureus*

Number of isolates n = 12

Antimicrobial Class	Antimicrobial Agent	Disk Potency	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Aminoglycoside	Gentamicin	10 µg	7 (58.33)	5 (41.67)	0 (0.00)
Cephalosporin (3rd generation)	Ceftriaxone	30 µg	0 (0.00)	8 (66.67)	4 (33.33)
Cephalosporin (3rd generation)	Cefuroxime	30 µg	0 (0.00)	0 (0.00)	12 (100.00)
Lincomycin	Clindamycin	2 µg	1 (8.33)	11 (91.67)	0 (0.00)
Penicillins	Ampicillin	10 µg	0 (0.00)	12 (0.00)	0 (0.00)
Penicillins	Amoxicillin	25 µg	0 (0.00)	12 (100.00)	0 (0.00)
Penicillins	Amoxicillin Cloxacillin	30 µg	3 (25.00)	9 (75.00)	0 (0.00)
Penicillins	Penstrep	25 µg	0 (0.00)	0 (0.00)	12 (100.00)
Carbapenem	Imipenem	10 µg	12 (100.00)	0 (0.00)	0 (0.00)
Fluoroquinolones	Enrofloxacin	5 µg	0 (0.00)	0 (0.00)	12 (100.00)
Fluoroquinolones	Ciprofloxacin	5 µg	0 (0.00)	0 (0.00)	12 (100.00)
Fluoroquinolones	Ofloxacin	5 µg	0 (0.00)	12 (100.00)	0 (0.00)
Glycopeptide	Vancomycin	30 µg	2 (16.66)	10 (83.33)	0 (0.00)
Tetracyclines	Oxytetracycline	30 µg	0 (0.00)	11 (91.67)	1 (8.33)
Tetracyclines	Doxycycline	30 µg	0 (0.00)	10 (83.33)	2 (16.66)
Phenicols	Chloramphenicol	30 µg	0 (0.00)	12 (100.00)	0 (0.00)
Metronidazole	Metronidazole	5 µg	0 (0.00)	0 (0.00)	12 (100.00)
Aminoglycoside	Streptomycin	10 µg	12 (100.00)	0 (0.00)	0 (0.00)

Table 5 Frequency of antimicrobial susceptibility for *Staphylococcus epidermidis*

Number of isolates n = 1

Antimicrobial Class	Antimicrobial agent	Disc Potency	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Aminoglycoside	Gentamicin	10 µg	1 (100.00)	0 (0.0)	0 (0.00)
Cephalosporin (3rd generation)	Ceftriaxone	30 µg	0 (0.00)	1 (100.00)	0 (0.0)
Cephalosporin (3rd generation)	Cefuroxime	30 µg	0 (0.00)	0 (0.00)	12 (100.00)
Lincomycin	Clindamycin	2 µg	0 (0.00)	1 (100.0)	0 (0.00)
Penicillins	Ampicillin	10 µg	0 (0.00)	0 (0.00)	1 (100.00)
Penicillins	Amoxicillin	25 µg	0 (0.00)	1 (100.00)	0 (0.00)
Penicillins	Amoxicillin Cloxacillin	30 µg	0 (0.00)	1 (100.00)	0 (0.00)
Penicillins	Penstrep	25 µg	0 (0.00)	0 (0.00)	1 (100.00)
Carbapenem	Imipenem	10 µg	0 (100.00)	1 (100.00)	0 (0.00)
Fluoroquinolones	Enrofloxacin	5 µg	0 (0.00)	0 (0.00)	1 (100.00)
Fluoroquinolones	Ciprofloxacin	5 µg	0 (0.00)	0 (0.00)	1 (100.00)
Fluoroquinolones	Ofloxacin	5 µg	0 (0.00)	1 (100.00)	0 (0.00)
Glycopeptide	Vancomycin	30 µg	0 (0.0)	1 (100.0)	0 (0.00)
Tetracyclines	Oxytetracycline	30 µg	0 (0.00)	1 (100.0)	0 (0.0)
Tetracyclines	Doxycycline	30 µg	0 (0.00)	1 (100.0)	0 (0.0)
Phenicols	Chloramphenicol	30 µg	0 (0.00)	1 (100.00)	0 (0.00)
Metronidazole	Metronidazole	5 µg	0 (0.00)	0 (0.00)	1 (100.00)
Aminoglycoside	Streptomycin	10 µg	1 (100.00)	0 (0.00)	0 (0.00)

Table 6 Frequency of antimicrobial susceptibility for *Pseudomonas aeruginosa*

Number of isolates n = 1

Antimicrobial Class	Antimicrobial Agent	Disc Potency	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Aminoglycoside	Gentamicin	10 µg	0 (0.00)	1 (100.0)	0 (0.00)
Cephalosporin (3rd generation)	Ceftriaxone	30 µg	0 (0.00)	0 (0.00)	1 (100.00)
Cephalosporin (3rd generation)	Cefuroxime	30 µg	0 (0.00)	0 (0.00)	1 (100.00)
Lincomycin	Clindamycin	2 µg	1 (100.00)	0 (0.00)	0 (0.00)

Antimicrobial Class	Antimicrobial Agent	Disc Potency	Sensitive n (%)	Intermediate n (%)	Resistance n (%)
Penicillins	Ampicillin	10 µg	0 (0.00)	1 (100.00)	0 (0.00)
Penicillins	Amoxicillin	25 µg	0 (0.00)	0 (0.00)	1 (100.00)
Penicillins	Amoxicillin Cloxacillin	30 µg	0 (0.00)	1 (100.00)	0 (0.00)
Penicillins	Penstrep	25 µg	0 (0.00)	0 (0.00)	1 (100.00)
Carbapenem	Imipenem	10 µg	1 (100.00)	0 (0.00)	0 (0.00)
Fluoroquinolones	Enrofloxacin	5 µg	0 (0.00)	0 (0.00)	1 (100.00)
Fluoroquinolones	Ciprofloxacin	5 µg	0 (0.00)	0 (0.00)	1 (100.00)
Fluoroquinolones	Ofloxacin	5 µg	0 (0.00)	0 (0.00)	1 (100.00)
Glycopeptide	Vancomycin	30 µg	0 (0.00)	0 (0.00)	1 (100.00)
Tetracyclines	Oxytetracycline	30 µg	0 (0.00)	0 (0.00)	1 (100.00)
Tetracyclines	Doxycycline	30 µg	0 (0.00)	0 (0.0)	1 (100.00)
Phenicols	Chloramphenicol	30 µg	0 (0.00)	1 (100.00)	0 (0.00)
Metronidazole	Metronidazole	5 µg	0 (0.0)	1 (100.00)	0 (0.00)
Aminoglycoside	Streptomycin	10 µg	0 (0.00)	1 (100.00)	0 (0.00)

DISCUSSION AND CONCLUSION

The investigation of vaginal microbiota in mares and other animals serves as a guide in understanding different conditions that may affect the normal flora and possible intervention to resolve it (Malaluang et al., 2024). In this study, only 3 bacteria organisms were isolated from the vagina of apparently healthy breeding mares as follows: *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* was the most prevalent bacteria organism isolated from the vagina of breeding mares from the two sampled location. The reason for the high prevalence of *Staphylococcus aureus* in the vagina of sampled mares is unknown but may be connected to its commensal nature (Nwobi et al., 2023). Earlier report by Hinrichs et al. (1988) observed the presence of *Staphylococcus* (n = 4), *Streptococcus* (n = 4) and *Arcanobacterium* (n = 10) in investigated vagina samples of clinically normal mares. The report by Hinrichs et al. (1988), is slightly similar to our observation of *Staphylococcus* (n=55) but differs in the area of its abundance without species specification. The present finding is in contrast with an early report on vaginal microbiome of mares from slaughterhouse, in which coliforms and β-haemolytic streptococci were the most abundant (Scott et al., 1971), while a more recent study on vagina microbiota of maiden mares and mares with no history of breeding for over 10 years observed the presence of the following: *Escherichia coli* (40% prevalence in the vaginal samples investigated), *Streptococcus zooepidemicus, Streptococcus equisimilis, Streptococcus thoraltensis* and *Staphylococcus capitis* (Malaluang et al., 2022). Also, Malaluang et al. (2024) reported *Escherichia coli* and *Streptococcus zooepidemicus* as predominate microbes during Days 3, 7 and 14 post-ovulations of the oestrous cycle of healthy mares. These findings by Malaluang et al. (2022) and Malaluang et al. (2024) are in contrast with the vaginal microbes isolated from

the breeding mares in this study. Again, Fraga et al. (2007) observed *Enterococcus faecalis*, *E. faecium*, *Lactobacillus equi*, *L. mucosae* and *L. pantheris* in the vagina, with more *Lactobacillus* identification in 18 out of the 26 sampled healthy mares. Barba et al. (2020) observed the presence of the following genera in the vagina of Arabian mares during oestrous cycle: *Kiritimatiaellae spp*, *Porphyromonas spp, Corynebacterium spp, Streptococcus spp, Campylobacter spp, Arcanobacterium spp, Fusobacterium spp, Lactobacillus spp* and *Akkermansia spp*.

A very low percentage of *Pseudomonas aeruginosa* was identified as part of vagina microbiome of breeding mare in this study. Although, there is a dearth of information on its presence in the mares' vagina in previous reports, Hinrichs et al. (1988) observed *Pseudomonas aeruginosa* in clitoral fossa swab samples of mares. Also, Gil-Miranda et al. (2024) reported it as one of the organisms previously identified in uterine microbiome in mares.

This study also reports a very low composition (1.2%) of *Staphylococcus epidermidis* in the vagina of breeding mare. Similar report by Malaluang et al. (2024) observed a low percentage (0.7%) of *Staphylococcus epidermidis* from the vagina of sampled mares during the oestrous cycle. Though *Staphylococcus epidermidis* belongs to the genus *Staphylococcus,* its role in equine reproduction is not fully known. It had previously been reported that *Staphylococcus epidermidis* plays a symbiotic role in mice embryo implantation (Ono et al., 2015). Iwase et al. (2010) also reported that *Staphylococcus epidermidis* was of a beneficial role in displacing the pathogenic *Staphylococcus aureus* from nasal cavities in humans.

The variations in the organisms detected may be due to several factors that influence the vagina microbiome, such as diet, health status, hormonal status and geographical location. Also, the method of sampling and sample processing may have contributed to the differences observed with our present findings and other studies (Gil-Miranda et al., 2024).

Staphylococcus aureus and *Pseudomonas aeruginosa* had been implicated as part of the causative agents of equine endometritis (LeBlanc, 2010; Canisso et al., 2015). Therefore, their presence in the vagina of breeding mares should be of great concern because of their pathogenic property under unfavourable conditions. A recent study identified multiple drug- and heavy metal-resistant as well as methillicin-resistant *Staphylococcus aureus* isolated from nasal and groin skin swabs of horses slaughtered for meat in Obollo-Afor Southeastern Nigeria (Nwobi et al., 2023). This study by Nwobi et al. (2023) is similar to our present finding of *Staphylococcus aureus* in breeding mare and supports our concern for more vigilance on the reproductive health of breeding mares. Also, *Pseudomonas aeruginosa* has been associated with infertility in inseminated mares (Malaluang et al., 2021). Although these bacteria isolates are located in the vagina, they can invade the uterus during gynaecological evaluation, obstetrical intervention, mating or artificial insemination, despite strict aseptic protocol leading to varying reproductive problems under unfavourable conditions (Malaluang et al., 2021).

Development of antimicrobial resistance by bacteria occurs from either therapeutic or low dose exposure (Malaluang et al., 2021). The three bacteria organisms in this study were all highly resistant to cefuroxime, penicillin-streptomycin, enrofloxacin and ciprofloxacin. Additionally, *Staphylococcus aureus* was also highly resistant to metronidazole and showed very low resistance to ceftriaxone, oxytetracycline and doxycycline, while *Staphylococcus epidermidis* was also highly resistant to ampicillin and metronidazole. Malaluang et al. (2022) observed resistance of isolated *Staphylococcus spp to* erythromycin, oxacillin, penicillin and gentamicin in inseminated mares in Sweden in contrast with present findings. Same report stated that *Staphylococcus epidermidis* was highly resistant to erythromycin, fusidic acid and penicillin.

Pseudomonas aeruginosa showed high level of resistance to most of the antimicrobials (10 out

of 18) tested when compared with other bacteria isolates. It was also highly resistant to ceftriaxone, ofloxacin, amoxicillin, oxytetracycline, vancomycin and doxycycline. The multiple drug resistance exhibited by *Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa* should be of great concern because it limits the spectra of antimicrobials available for treating infection-dependent reproductive problems in mare. Differences in age, previous treatment, location, grouping and personnel may have contributed to the varying resistance to antimicrobials tested in this study. The development of antimicrobial resistance may be acquired or natural through exposure to resistant genes (Malaluang et al., 2022).

Antimicrobial sensitivity for *Staphylococcus aureus* isolates revealed 100% susceptibility to imipenem and streptomycin, below 60% susceptibility to gentamicin and less than 25% to amoxicillin cloxacillin, clindamycin and vancomycin, while *Staphylococcus epidermidis* was highly susceptible to streptomycin and gentamicin, and *Pseudomonas aeruginosa* was highly susceptible to clindamycin and imipenem with intermediate susceptibility to gentamicin, ampicillin, amoxicillin cloxacillin, chloramphenicol, metronidazole and streptomycin. The observation of a few antimicrobial susceptibilities suggests that previous treatment, location, grouping and personnel may have influenced the bacteria presence in the vagina (Malaluang et al., 2022).

A significant limitation of this study is the reliance solely on conventional morphological and biochemical identification methods for bacterial characterization. Molecular techniques such as 16S rRNA gene sequencing, PCR-based identification, or metagenomics approaches are absent in the present study. Future studies should incorporate molecular identification techniques alongside traditional culture methods to provide a more comprehensive understanding of the mare vaginal microbiome and its clinical implications. This study is also limited by unequal sampling between locations (60 mares from Lagos vs. 21 from Oyo) and the absence of critical demographic data, including estrous cycle phase, mare age, and previous antimicrobial exposure history. These factors are known to significantly influence vaginal microbiota composition and antimicrobial resistance patterns, potentially affecting result interpretation. Future studies should employ stratified sampling with equal geographical representation and comprehensive metadata collection to enable more robust analyses.

In conclusion, the study found a high presence of *Staphylococcus aureus* in mares' vaginal microbiota, with significant multi-drug resistance. However, aminoglycosides and carbapenems showed consistent effectiveness against the isolated bacteria. It is strongly recommended that veterinarians in these regions prioritize the use of aminoglycosides and carbapenems as first-line treatments for vaginal infections in mares, while avoiding the use of antibiotics showing high resistance patterns, unless guided by susceptibility testing.

CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

CONTRIBUTIONS

Concept – DS; Design – DS, ORA; Supervision – OOA[2]; Resources – OGB, TRL; Materials – OGB, LCO; Data Collection and Processing – OOA[1], TRL, DS; Analysis and Interpretation – OOA[1], ORA; Literature Search – ORA, TAO, DS; Writing Manuscript – LCO, MJO, ORA; Critical Review – MJO, DS, OOA[1],

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KARAKTERIZACIJA I ANTIMIKROBNA REZISTENCIJA VAGINALNOG MIKROBIOMA KOD KOBILA IZ DRŽAVA LAGOS I OYO U NIGERIJI

SAŽETAK

Uprkos značaju mikrobioma u reproduktivnom zdravlju sisara, istraživanja o vaginalnom mikrobiomu kod konja ostaju ograničena, posebno u tropskim područjima. Sastav vaginalnog mikrobioma kobila u državama Lagos i Oyo u Nigeriji još nije zabilježen. Profili mikrobioma su neophodni za razvoj učinkovitih strategija upravljanja reproduktivnim zdravljem. Naš cilj je karakterizacija sastava vaginalnog mikrobioma i određivanje profila antimikrobne rezistencije izoliranih bakterija kod kobila u državama Lagos i Oyo u Nigeriji. Korištenjem sterilnih briseva za kulture su prikupljeni vaginalni brisevi od 81 naizgled zdrave kobile, uključujući nigerijske domaće vrste, sudanske pasmine i argentinske Polo ponije. Iz uzoraka su izdvojeni bakterijski izolati na selektivne i diferencijalne podloge, uključujući triptozu sojin agar, manitol slani agar, eozin metilen plavo, Muller-Hinton, salmonelu-šigelu i MacConkey agar. Bakterijska identifikacija je uključivala morfološke i biohemijske testove, uključujući oksidaze, katalaze, motilitet, bojenje po Gramu i fermentaciju šećera. Osjetljivost na antimikrobna sredstva je testirana za 18 antimikrobika korištenjem Kirby-Bauer disk difuzijske metode, prema CLSI standardima. Analiza je pokazala različite obrasce bakterijske distribucije za različite lokacije. U uzorku od 81 kobile je dominirao *Staphylococcus aureus* (66.7%), dok su *Pseudomonas aeruginosa* i *Staphylococcus epidermidis* pojedinačno bili zastupljeni s 1.2%, dok 30.9% uzoraka nije pokazalo nikakav rast. Biohemijska karakterizacija je pokazala tipične profile za svaku od vrsta. *S. aureus* je pokazao potpunu osjetljivost na imipenem i streptomicin, a rezistenciju na cefuroksim, penicilin-streptomicin, enrofloksacin, ciprofloksacin i metronidazol. *P. aeruginosa* je bila osjetljiva na klindamicin i imipenem, dok je na većinu drugih antibiotika bila rezistentna. Imajući u vidu veliku zastupljenost *S. aureusa* rezistentnog na antibiotike, veterinari bi trebali davati prednost aminoglikozidima i karbapenemima kod liječenja vaginalnih infekcija kobila, a koristiti druge antibiotike samo kada se testiranjem dokaže osjetljivost.

Ključne riječi: Antimikrobna rezistencija, nigerijske kobile, reprodukcija konja, *Staphylococcus aureus*, vaginalni mikrobiom