

## Vaginal Bacterial Flora and its Antibiogram in Bitches with Vaginitis in Ibadan

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**Abstract:** Vaginal swab cultures are useful in diagnosing and treating certain diseases of the reproductive tracts. We therefore conducted a study to determine the bacterial flora present in some bitches with vaginitis presented for treatment in a clinic in Ibadan. In all, eight of 36 bitches presented during the study period had visible abnormal vaginal discharges ranging from thick to mucoid in consistency and brown to creamy and milky in colour and were selected for the study. Vaginal swabs were aseptically obtained and inoculated onto Blood and McConkey agar and the plates were incubated at 37°C for 24 hours. Gram staining and other routine biochemical tests as well as drug sensitivity tests were further carried out in the identification of the bacterial organisms. Our findings showed the presence of *Escherichia coli* (33.33%), *Klebsiella spp* (33.33%), *Streptococcus pyogenes* (20.00%) and *Staphylococcus aureus* (13.33%). The isolated bacteria were, to a very large extent resistant to most of the commonly used antibiotics like tetracycline, ampicillin, streptomycin and septrin, but highly susceptible to tarivid, ciprotab, augumentin and rocephine. It therefore suffices to say that vaginal bacterial cultures and drug sensitivity tests are sine qua non before initiating any treatment in bitches with vaginitis.

**Key words:** Vaginitis · Microflora · Bitch

### INTRODUCTION

Many bacterial organisms have been isolated from the canine vagina. Though, it can be difficult to distinguish pathogenic bacteria from normal vaginal flora, a heavy growth of a uniform bacterium is more suggestive of a pathogen [1]. The two forms of vaginitis recognized in dogs are juvenile or puppy vaginitis and adult-onset vaginitis [2]. The type of organisms isolated may therefore vary with the age of bitch [3]. A higher percentage of prepuberal bitches have been suggested to have coagulase-positive *Staphylococcus* than do post puberal bitches [4]. The commonly isolated organisms include *E. coli*, *Staphylococcus spp.*, *Streptococcus spp.*, *Pasteurella spp.*, *Proteus spp.*, *Bacillus spp.*, *Corynebacterium spp.*, *Pseudomonas spp.* and *Micrococcus spp.* Others include *Neisseria spp.*, *Klebsiella spp.* and *Moraxella spp.* [3, 5-8]. Some of these organisms have been cultured from healthy as well as infertile bitches [9].

Frequently, vaginal cultures are obtained in an attempt to correlate various diseases (e.g. inflammation of the clitoral fossa, vestibule, vagina, cervix or uterus; infertility, abortion, neonatal deaths) with specific infectious agents [7], but it is difficult to associate disease with a specific bacterial isolate since a variety of organisms are present in the vagina of bitches with or without reproductive diseases [3]. In a study, Gandotra *et al.* [8] isolated *Staphylococcus aureus* in 38.47%, *Streptococcus spp.* in 30.77%, *Corynebacterium pyogenes* in 23.07% and *E. coli* in 7.59% in 24 cases of canine pyometra. In another study, vaginal cultures from young and mature bitches with chronic vaginitis yielded mixed populations of *Staphylococcus spp.*, *Streptococcus spp.*, *E. coli*, *Proteus spp.*, *Pasteurella spp.*, *Citrobacter spp.* and *Enterobacter spp.* These organisms are qualitatively the same as the normal bacterial flora of the canine vagina [10]. It has also been observed that the only consistent difference between the bacterial flora from the vagina of clinically normal bitches and those with

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vaginitis was the relative higher number of bacterial population in the latter [5].

Treatment of mild cases of vaginitis in bitches usually involves oral administration of systemic alkalizers like disodium hydrogen citrate. Johnston *et al.* [11] opined that vaginal douches with antibiotic or antiseptic agents are ineffective in flushing out significant amounts of vaginal discharge and may be irritating to the vaginal mucosa, worsening the vaginitis.

Knowledge on vaginal microflora and their antibiogram is of paramount significance in the astute selection of the antibiotic to tackle vaginitis in bitches. This study was therefore conducted in order to identify the vaginal bacterial flora of bitches with vaginitis and its antibiogram so as to help the veterinarians in effective treatment of vaginitis in these valuable pets.

**MATERIALS AND METHODS**

**Sample Collection:** Out of thirty-six (36) bitches presented for treatment for various health reasons at a Veterinary Clinic in Ibadan, Nigeria; those with abnormal vaginal discharges (n=8) formed the materials for this investigation. These bitches were all adult with varying history and presentations (Table 1). The animals were adequately restrained on lateral recumbency on an examination table. The perivaginal area was scrubbed with a potent antiseptic (TCP®, Pfizer products). Sterile swab sticks (Evepon Sterile Swabs®, Nigeria) were passed into the vagina with the vaginal labia parted. The stick was

directed cranio-dorsally initially avoiding the clitoral fossa and the urethral orifices and then longitudinally through a length of about 12 cm as described by Olson *et al.* [12] and Holst [13]. The swab was then rolled around the vaginal wall gently and withdrawn carefully into its adequately labeled sterile container for transport to the laboratory. The transport medium used was peptone water in order to preserve the samples.

**Innoculation:** The swab samples were each inoculated onto 5% sheep blood and McConkey agar and the plates incubated at 37°C for 24-48 hours after which discrete colony growths were observed and recorded, having cultured each of the samples on two different plates aerobically and anaerobically. Gram staining and other routine biochemical tests were further employed in the identification of bacteria based on standard procedures [14, 15]. The bacterial isolates were then subjected to drug sensitivity test during which fifteen (15) different antibiotics were used.

**RESULTS**

Bacterial growth was appreciated in all of the eight (8) vaginal discharge samples aseptically collected and subjected to culturing, from where fifteen isolates of four different bacteria were obtained. The organisms identified were *E. coli* (33.33%), *Klebsiella spp* (33.33%), *Streptococcus pyogenes* (20.00%) and *Staphylococcus aureus* (13.33%) (Table 2).

Table 1: History of bitches with vaginitis and the nature of their discharges

Dog identification	History	Nature of discharge
I	Abnormal vaginal discharge few days after mating	Brown and mucoid
II	Abnormal vaginal discharge few days after mating	Creamy and mucoid
III	Milky fluid vaginal discharge after whelping	Creamy and thick
IV	Anorexia and creamy vaginal discharge	Brown and mucoid
V	No prior sign noticed by the owner before abnormal vaginal discharge	Brown and mucoid
VI	No prior sign noticed by the owner before abnormal vaginal discharge	Creamy and thick
VII	No prior sign noticed by the owner before abnormal vaginal discharge	Brown and mucoid
VIII	Abortion after few days of mating	Brown and mucoid

Table 2: Distribution of vaginal bacterial isolates among bitches suffering from vaginitis

Dogs	*Types of isolates				Total
	<i>E.coli</i>	<i>Klebsiella</i>	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>	
I	-	+	-	+	2
II	+	+	-	+	3
III	-	+	+	-	2
IV	+	+	-	-	2
V	+	-	-	-	1
VI	-	+	+	-	2
VII	+	-	-	-	1
VIII	+	-	+	-	2
Total	5	5	3	2	15
Percentage	33.33	33.33	20.00	13.33	100.00

+: Bacterial isolate present  
 -: Bacterial isolate absent

Table 3: Details of organisms isolated from vaginal discharge of bitches with vaginitis and their antibiogram patterns

S/N	Organism isolated	<sup>a</sup> Bacterial to which sensitive	Antibiotics to which resistant
1	<i>E. coli</i> (n =5)	Peflotab +++ Tarivid +++ Ciprotab +++ Rocephine +++ Augumentin ++ Gentamycin +	Tetracycline Ampicillin Septrin Streptomycin Amoxyl Nalidixic acid Nitrofurantin
2	<i>Klebsiella spp</i> (n = 5)	Peflotab +++ Tarivid +++ Ciprotab +++ Rocephine +++ Augumentin ++ Zanoxin +++ Gentamycin ++	Tetracycline Ampicillin Septrin Streptomycin Amoxyl
3	<i>Streptococcus pyogenes</i> (n = 3)	Peflotab +++ Tarivid +++ Ciprotab +++ Rocephine +++ Zanoxin +++ Augumentin ++	Septrin Fortin Amoxyl Tetracycline Nitrofurantin
4	<i>Staphylococcus aureus</i> (n = 2)	Peflotab +++ Tarivid +++ Gentamycin ++	Tetracycline Ampicillin Septrin Streptomycin

<sup>a</sup> + : degree of sensitivity

The details of the antibiogram pattern obtained for the organisms isolated are summarized in Table 3. Of these bacteria tested for antibiotic sensitivity, 100% each were found to be sensitive to peflotab and tarivid; 75% to ciprotab, rocephine, augumentin, gentamycin, and 50% to zanoxin while 100% were resistant to tetracycline and septrin; 75% each to ampicillin, streptomycin, and amoxyl whereas 50% were resistant to nitrofurantin and 25% to nalidixic acid and fortin.

### DISCUSSION

Our findings reveal the isolation of *E. coli*, *Klebsiella spp*, *Streptococcus pyogenes* and *Staphylococcus aureus* as some of the bacterial flora incriminated in bitches suffering from vaginitis which were resistant to most of the commonly used antibiotics. Similar findings have also been reported by John *et al.* [16] and Bjurström [17] who reported isolation of *E. coli*, *Klebsiella* and others from bitches with infertility and vaginitis, respectively. This is further buttressed by the reports of Gandotra *et al.* [8] who isolated *Staphylococcus aureus* and *E. coli* from cases of pyometra; and Johnson [10], in a study of chronic vaginitis where isolated bacterial agents included pure cultures of *Streptococcus spp.*, *Staphylococcus spp.*, among others.

The report from the work of Mshelia *et al.* [18] showed the isolation of *Klebsiella* and *Staphylococcus* from clinically healthy bitches in a study. This, therefore, showed that the mere isolation of bacterial agents in the presence of an on-going genital disease may not necessarily be incriminative of those organisms and this is in agreement with the report of Olson *et al.* [7]. This notwithstanding, bacterial isolation in diseased or healthy bitches is important because opportunistic bacteria can become pathogenic when an enabling condition is created. This therefore implies that in the presence of any form of trauma, these bacteria may invade the genitalia giving rise to genital inflammatory conditions and septicemia. Their isolation, therefore, can be of great significance in the clinical diagnosis and management of reproductive diseases in the bitch.

Considering the antibiogram pattern of the drug sensitivity, 100% each were found to be sensitive to peflotab and Tarivid; 75% to ciprotab, rocephine, augumentin, gentamycin, and 50% to zanoxin while 100% were resistant to tetracycline and Septrin; 75% each to ampicillin, streptomycin, and amoxyl whereas 50% were resistant to nitrofurantin and 25% to nalidixic acid and fortin. However, extreme care must be exercised in the judicious selection of the antibiotic of choice when antibiotics are to be used to treat genital tract infections

as some of them can cause serious detrimental effects on sperm, fertilized oocytes or early developing embryos. For instance, as gentamycin cannot be administered by oral route on many occasions; it may not be the preferred antibiotic for the treatment of vaginitis in bitches. As seen in this study, most of the organisms isolated were resistant to most commonly used antibiotics such as tetracycline and septrin, but were only sensitive to more potent ones like peflotab and ciprotab. Therefore, in situations which warrant the use of potent antibiotics, much attention should be paid to ensure proper withdrawal time as there is possibility of persistence of these antibiotics in the animal's body at the time of breeding.

Our findings notwithstanding, this study had some limitations. First, the number of dogs sampled is relatively few as this would have probably reduced the possibility of isolating some other bacterial organisms. Second, identification of conditions known to be primary factors in dogs with vaginitis, such as vaginal anatomical anomalies, concurrent systemic disease, and, potentially, aotopsy, which would better permit practitioners to understand and treat vaginitis were not carried out. Nonetheless, our results showed that out of the eight bitches studied, 100% were found to be positive for bacterial growth and that these organisms were resistant to most of the commonly used antibiotics.

In conclusion, bacterial isolation and drug sensitivity profiles can be of great significance in the clinical diagnosis and management of vaginitis in the bitch.

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