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PATTERN OF MICROBIAL COLONIZATION OF THE VAGINA OF DIABETICS IN IBADAN, NIGERIA

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ABSTRACT

The pattern of microbial flora of the vagina of diabetics was studied, to advise on empirical regimen for the treatment of sepsis in diabetics with the lower genital tract as source. In 2003, microscopy, culture and sensitivity of high vaginal swabs from 60 consecutive non-insulin-dependent diabetics and 20 non-diabetics attending Oluyoro Catholic Hospital, Ibadan were done. The fasting plasma glucose was estimated.

The prevalence of micro-organisms decreased with duration of diabetes. The isolates were *Gardnerella vaginalis, Candida* species, *Staphylococcus epidermidis*, *Klebsiella* species, *Enterococcus faecalis*, and viridans Streptococcus. *Klebsiella* species and *Escherichia coli* were incriminated in Gram negative bacilli (GNB) bacteraemia in diabetics in this environment.

In addition to metronidazole, ceftazidime or ceftriazone should be used as first line drugs, while the quinolones should be reserved for the treatment of sepsis in diabetics where lower genital tract is the likely source of infection.

KEYWORDS: Microbes, vagina, diabetics.

INTRODUCTION

Immediately post-delivery, circulating maternal oestrogen may result in microbial flora in babies, similar to that in adults. This changes in approximately two weeks as oestrogens are metabolised, so that throughout childhood the vaginal epithelium lacks glycogen, and so carries a scanty background flora of skin organisms and upper respiratory tract commensals [1]. The post-pubertal vaginal epithelium has a high glycogen concentration due to the influence of circulating oestrogens. This is metabolised by lactobacilli to lactic acid, producing a low pH. The vaginal flora may superficially resemble faecal flora due to the transient peri-anal contamination, but many organisms have a very close association with the lower genital tract e.g. *Bacteroides bivius (*now *Prevotella bivia), Gardnerella vaginalis* and *Mycoplasma hominis*[1]. In vaginal secretions, anaerobes outnumber aerobes by 10^9 to 10^8per ml. The commonest anaerobes found are lactobacilli, *Bacteroides* spp. and anaerobic Gram-positive cocci. Diphtheroids and coagulase-negative Staphylococci are the commonest aerobic bacteria[1]. *Staphylococcus aureus* is recovered from the vagina of only about 5% of healthy women[4]. Yeasts are carried by about 15 –

20% of healthy women[4]. With the onset of sexual activity, statistically significant increases are observed in the prevalence of *Gardnerella vaginalis*, lactobacilli, Mycoplasma and Ureaplasma, but the prevalence of group B Streptococci, *Stapylococcus aureus* and yeasts are not significantly altered[6].

Although our descriptive knowledge of vaginal microbiology has increased, our understanding of the factors controlling the flora remains primitive[7]. Specific and non-specific vaginal host defenses have been catalogued but again, their precise significance is unclear[8].

Diabetes mellitus is a constellation of abnormalities caused by a relative or absolute lack of insulin[9]. It is a state of chronic hyperglycaemia[10]. A random venous plasma glucose of 11.1 mmol/l or more on two occasions or a fasting value of 6.7 mmol/l or more on two occasions is diagnostic[10,11,12]. In the temperate regions, there are two common types of Diabetes mellitus. Type 1 (insulin dependent) and type 2 (non-insulin dependent) accounting for about 15% and 85% of cases respectively. These also occur in the tropics. A third type of the disease seen in the tropical countries has been termed variously as J-type, Z-type, type III or tropical pancreatic diabetes. Such cases are often associated with malnutrition. Some term this as malnutrition–related diabetes mellitus. Type II diabetes is by far the more common of the two main types of the disease[13].

Diabetics are more susceptible to infection than non-diabetics because of the immune response resulting from combined factors such as increased glucose content of blood and tissue, impairment of chemotaxis, serum opsonisation and phagocytosis and lower capacity of tissue reaction to antigenic stimuli[14]. Numerous localized infections are often accompanied by transient phase of bacteraemia[15], some common infections in diabetics include urinary tract infections, gram-negative pneumonia, malignant otitis externa, acute pyelonephritis, diabetic foot, tuberculosis and acute cholecystitis[16]. The lower genital flora may act as the endogenous source of infection that occurs in diabetics.

We studied the pattern of microbial flora of the vagina of diabetics without symptoms and signs of genital tract infection, and their antibiotics sensitivities to advise on empirical regimen for sepsis in diabetics with the lower genital tract as the likely source.

PATIENTS, MATERIALS AND METHODS.
From January to April 2003, consenting 60 consecutive known non – insulin-dependent diabetic and 20 non-diabetic women attending Oluyoro Catholic Hospital, Ibadan were studied. Intake of either an antibiotic or anti-fungal agents within the previous two months and the presence of symptoms and signs of genital tract infections were exclusion criteria.

The age, duration of diabetes, type of anti-diabetic drug, history of vaginal discharge and itching were recorded. Clinical examination for features of lower genito-urinary tract infection was done. High vaginal swab (HVS) was collected with sterile cotton-tipped applicator for microscopy, culture and sensitivity. The pH of the vaginal secretion was done by using a narrow range pH paper, the Potassium hydroxide (KOH), whiff test, was done. Two mls of venous blood was collected from each participant for fasting plasma glucose (FPG) estimation.

Laboratory Procedures
Wet smear microscopy of HVS was prepared for detecting inflammatory cells, motile trichomonads, yeast cell and characteristic "clue" cells. Inoculation was made on Blood, MacConkey and Chocolate agars. The Blood and MacConkey plates were incubated aerobically,while Chocolate plates were in candle extension jar, at 37^{o}C overnight. All yeast and bacterial isolates were identified by conventional laboratory methods[10]. The anti-biotic sensitivities were determined by using Stoke's disc diffusion technique[17]. FPG estimation was done according to standard method[18].

RESULTS

Most diabetics (26.7%) were within age range 21 – 30 and 41 – 50(Table 1, Panel 1). The patient's FPG ranged from a mean of 6.8 – 11.1 to 22.3-27.8mmol/L, with the control group having 6.7mmol/L (Table I, panel 2). At the mean FPG of 8.4mmol/L, the isolates gave a prevalence of 45%, while at 13.9mmol/L and 25.1mmol/L the percentages were 90% and 100% respectively, a statistically significant finding ($p<0.00001$). Greatest isolates were from the elderly diabetics (28.4% of 51-60 years), then from those in the age range of 31-40 years (24.3%).

Forty-five percent of diabetics (27 out of 60) were not sure of the duration of their ailment, 41.9% of the isolates were from this group. With increasing duration of diabetes, there was a decrease in the yield of isolates (24.3%, 18.9%, 10.9% and 4.1%) with a statistically significant finding ($p<0.00001$) (Table I, panel 3). Twenty-two (36.6%) of the patients had 'clue cells' on wet preparation, while none of the controls had, a statistically significant finding ($p= 0.0008$). A total of 74 microbial isolates were from the 60 patients, while 12 were from the 20 controls. The isolates are as shown in table II. Out of the 74 microbial isolates, 54 were bacteria and 20 were fungi; 18 *Candida albicans* and 2 *Candida tropica`lis* The ratio of Gram negative to Gram positive was 1.0:1.5.

The Gram-negative bacteria were *Escherichia coli* (21.6%) and *Klesiella* species (8.1%), while the Gram positive cocci were *Staphylococcus auerus* (24.3%), *Staphylococcus epidermidis* (9.5%), *Enterococcus faecalis* (5.4%) and viridans Streptococci (4.1%). The fungi were *Candida albicans* (24.3%) and *Candida tropicalis* (2.7%). Ten percent of the participants had multiple agents. Augumentin, gentimicin and cefuroxime were active against an average of half of the bacterial isolates, over two-third of the isolates were sensitive to ceftazidime and ceftriazone while the quinolones (ofloxacin and ciprofloxacin) were active against 78 – 100% of the bacterial isolates.

DISCUSSION

In this study, the yield in microbial isolates from the vagina of diabetic decreased with increasing duration of the ailment. This indicates that the diabetics with longer duration of ailment on oral hypoglycaemic agents would have achieved and maintained euglycaemic control, a situation required for control of infection in diabetics. This is explained further by the statement that secondary abnormalities in leukocyte function (chemotaxis, phagocytosis, and intracellular killing) related to glycemic control may contribute to poor host responses to fungal infections[19]; and other infections. Hence emphasis should be placed on timely and adequate control of diabetes mellitus to prevent infection of the lower genital tract and complications such as bacteraemia and sepsis syndromes. The antibiotic sensitivity pattern of the bacterial isolates is shown in table III. Co-trimoxazole, tetracycline and amoxycillin were active against about a third of the bacterial isolates.

TABLE I: AGE, FASTING PLASMA GLUCOSE, DURATION OF DIABETES AND MICROBIAL ISOLATES FROM RESPONDENTS

PANEL 1: AGE OF RESPONDENTS AND MICROBIAL ISOLATES

AGE RANGE (YEARS)		AGE DISTRIBUTION		DISTRIBUTION OF ISOLATES	
		Number	% Distribution	Number	% Distribution
11 – 20		2	3.3	4	5.4
21 – 30		16	26.7	15	20.3
31 – 40		12	20.0	18	24.3
41 – 50		16	26.7	16	21.6
51 – 60		14	23.3	21	28.4
TOTAL		60	100.0	74	100.0

PANEL 2: FASTING PLASMA GLUCOSE (FPG) OF RESPONDENTS AND MICROBIAL ISOLATES

FPG (mmol/L)		FPG DISTRIBUTION		DISTRIBUTION OF ISOLATES	
		Number	% Distribution	Number	% Distribution
6.8 – 11.1		22	36.7	23	31.1
11.2 – 16.6		20	33.3	26	35.1
16.7 – 22.2		16	26.7	20	27.0
22.3 – 27.8		2	3.3	5	6.8
TOTAL		60	100	74	100
(CONTROL)		20		12	

PANEL 3: DURATION OF DAIBETES AND MICROBIAL ISOLATES

DURATION (MONTHS)		DURATION DISTRIBUTION		DISTRIBUTION OF ISOLATES	
		Number	% Distribution	Number	% Distribution
0 – 12		18	30.0	18	24.3
13 – 24		8	13.3	14	18.9
25 – 36		5	8.3	8	10.8
37 – 48		2	3.3	3	4.1
Unknown		27	45.0	31	41.9
TOTAL		60	100.0	74	100.0

From puberty to menopause, the commensals of the vagina include lactobacilli, anaerobic or microaerophilic Streptococci, *Clostridium* species, Bacteroides, *Acinetobacter* species, Fusobacteria. *Gardnerella vaginalis*, Mycoplasma and small numbers of Diphtheroids and Yeasts. After menopause, Diphtheroids, Micrococci, *Staphylococcus epidermidis*, viridans Streptococci, Enterobacteria, *Candida albicans* and other Yeasts[1,10] inhabit the vagina. However, in this study, the resident organisms in the vagina of diabetics, age range 15.5 to 55.5 years, were *Escherichia coli*, *Klebsiella* species, *Staphylococcus auerus, Staphylococcus epidermidis, Enterococcus* faecalis, viridans Streptococci and *Gardnerella vaginalis*, a finding resembling the commensals in post–menopausal women. Whereas it is expected that the flora should consist of microbes found in childbearing adults.

TABLE II: MICROBIAL ISOLATES FROM RESPONDENTS

PANEL 1 : SINGLE ISOLATES

ISOLATES	**FREQUENCY**	**%**
Candida albicans	**18**	**24.3**
Staphylococcus aureus	**18**	**24.3**
Escherichia coli	**16**	**21.6**
Staphylococcus epidermidis	**7**	**9.5**
***Klebsiella* species**	**6**	**8.1**
Enterococcus faecalis	**4**	**5.4**
Viridans Streptococcus	**3**	**4.1**
Candida tropicalis	**2**	**2.7**
Total	**74**	**100.0**

PANEL 2 : MULTIPLE ISOLATES

ISOLATES	**FREQUENCY**	
Klebsiella spp. & E. coli	**2**	
Staphylococcus aureus & E. coli	**2**	
Staphylococcus aureus, Enterococcus faecalis & Candida albicans	**2**	
	6 out of 60 participants	**10**

It is probable that the high glucose content of the tissues favours the growth of this type of flora, and therefore may be one of the factors controlling the flora of the vagina. Further study is required to compare the microbial flora in post-menopausal diabetics in this environment. Candida species was the most prevalent organism of the isolates from the vagina, being 27% (*Candida albicans* 24.3% and Candida *tropicalis* 2.7%). In diabetics, there is a measurable increased rate of bacterial infections in soft tissues secondary to diabetic vascular

disease/neuropathy. Exposure to antibiotics and poor vascular supply can play an important part in the incidence of fungal infections of the soft tissues[19]. Although most of the cases of vaginal candidiasis in diabetics are caused by *Candida albicans*, this group of patients may have a slightly higher incidence of *Candida glabrata* as an aetiological agent[19]. However in our study, *Candida glabrata* was not found.

Of the 54 bacterial isolates, the Gram-negative bacilli to Gram-positive cocci ratio were 1:1.5. The Gram-positive cocci were *Staphylococcus auerus* 24.3%, *Enterococcus faecalis* 5.4% and viridans Streptococci 4.0%. It is pertinent to note that the main Gram-negative were *E. coli* and *Klebsiella* species. These findings are important in view of the fact that *E. coli* has been reported as the leading agents of Gram-negative bacilli bacteraemia [20], and in this environment, it was found to be the second leading cause of bacteraemia in diabetics[20]. The lower genital tract should be considered as an important source of Gram-negative bacilli in diabetics with bacteraemia or sepsis.

Ten of the patients had multiple microorganisms. Two (3.3%) each had *Klebsiella* species and *E. coli; Staphylococcus auerus* and *E. coli*; and one had three, *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida albicans*. We did not culture for anaerobes because of technical reasons, however 22 (36.6%) of the patients had 'Clue cells' on wet preparation while none of the controls had. This finding is statistically significant. This indicates that these women were habouring anaerobes, and that the hyperglycaemic state facilitates the growth of *Gardnerella vaginalis* and anaerobes in the vagina.

There was a picture of multidrug resistance by the bacteria to most of the commonly used antibiotics. Co-trimoxazole had <10% to each of the organism, streptomycin invariably recorded 0% while tetracycline and amoxycillin had similar picture. This is a similar pattern to the reported antibiotic sensitivity pattern of isolates from blood of diabetics in the same environment [20]. Augmentin, gentimicin and cefuroxime were active against about half of the bacteria, ceftazidime and ceftriazone against two-third, while most bacteria had good sensitivity to the quinolones (ofloxacin and ciprofloxacin) (78.3-100%). The lower activity of cefuroxime may be as a result of fact that it has been introduced into this environment long before the third generation cephalosporins.

Early antibiotic treatment reduces mortality. It has been documented that delay in introduction of appropriate antibiotics was associated with significant increase in hospital stay and with development of acute organ failure[21]. Therefore, we suggest a timely onset of empirical antibiotics in diabetics with sepsis, whose lower genital tract is the likely source.

The cephalosporins, (ceftazidime or ceftriazone) should be used as first line drug, while the quinolone (ofloxacin or ciprofloxacin) should be used as reserved drugs, to prevent resistance. In view of the fact that 36.6% of these women harbour *Gardnerella vaginalis* and possibly anaerobes, metronidazole should be combined with the antibiotics. This regimen should continue until the substantive antibiotic sensitivity results are available and/or a change of regimen is desirable. In addition, it is important in treatment to achieve and maintain euglycaemic control, because response to therapy can be correlated with glycaemic control[19].

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TABLE III: ANTIBIOTIC SENSITIVITY PATTERN OF THE ISOLATES

Isolates	No	A N T I B I O T I C S											
		Ceftazidime	Ceftriazone	Cefuroxime	Ofloxacin	Ciprofloxacin	Augumentin.	Gentamycin	Tetracycline	Amoxycillin.	Co-trimoxazole.	Sreptomycin.	Erythromycin.
E. coli	16	87	74	60	88	85	50	63	25	38	6	0	NT
Klebsiella spp	6	85	70	58	83	82	47	56	20	38	7	0	NT
Staph. aureus	18	86	78	44	83	82	48	56	23	40	7	0	65
Staph. epidermidis	7	82	71	43	78	80	48	57	18	40	6	0	58
Enterococcus faecalis	4	99	83	59	89	86	38	60	30	38	5	0	55
Viridans Streptococci	3	100	89	61	90	100	68	60	32	42	10	5	70

NT: Not tested