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Bacterial colonization of the vagina, oropharynx, breast milk and anterior nares of neonates among HIV seropositive pregnant women and seronegative pregnant counterparts

Abstract

Introduction: The human Immunodefiency virus/acquired immune deficiency syndrome (HIV/AIDS) is a scourge to mankind because the disease has devastated and decimated communities worldwide in the last two or more decades, yet there is no cure. Incidence of HIV/AIDS in Nigeria is high, where 10% of the global incidence occurs. The study determined the colonization of the vagina, oropharynx, breast milk and nares of neonates in clinics at Akure South and Ifedore Local Government Area (LGA) in Ondo State, Nigeria from November 2015 to December 2016.

Methodology: Two hundred and forty pregnant women aged 19-43 years were involved in the study. 114 HIV seropositive subjects mean age 31.81 years and 126 HIV seronegative subjects mean age 29.05 years as controls. HIV serostatus of each participant was determined by HIV-1/2 strip and confirmed by the Abbott enzyme-linked immunosorbent assay procedure. High vaginal sawbs, breast milk samples, oropharynx swabs and nares of each neonate were collected using sterile cotton-tipped applicator and introducing each into freshly prepared thioglycollate fluid medium for growth. Bacterial isolates were charcterised by standard microbiological methods and API kits.

Results: 2,148 bacterial isolates were recovered from the four study sites, HVS 1,156(53.8%), oropharynx 466(21.7%), breast milk samples 153(7.1%) and anterior nares of neonates 373(17.4%). Our study showed 60.5% against 39.5%, 68.2% compared to 31.8% and 73.8% against 26.2% of the predominant pathogens encountered among HIV seropositive and seroneagtive subjects respectively.

Conclusion: HVS constituted the major isolates (53.8%), oropharynx (21.7%), breast milk (7.1%) and nares of neonates (17.4%).

Keywords: HIV, bacterial colonization, HVS, oropharynx, breast milk, anterior nares of neonates

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Introduction

The human Immunodefiency virus/acquired immune deficiency syndrome (HIV/AIDS) is a scourge to mankind because the disease has devastated and decimated communities worldwide in the last two or more decades.[1,2] The etiological agent was identified separately by Luc Montanier (Pasteur Institute, Paris), who shared the Nobel Prize for his discovery in 2008, and Robert Gallo National Institutes of health (NIH). Bethesda Maryland, USA.[3] Recent advancements in molecular biology and gene technology have contributed to the understanding of enormous complexity of HIV genome which has invariably led to the rapid development of tools and strategies to reduce HIV transmission, high rates of mortality and increase in life expectancy among sufferers.[2] In the last ten years, great advancements have been made in understanding the genomic structure of the virus and related subtypes like the simian immunodeficiency virus (SIV)[2,4] and understanding of the genome[2] of the HIV and associate subtypes which have given scientist insights to the organization of the virus and has led to the formulation of ARV drugs which reduced the burden of infection in patients. Globally approximately 35.3 million people suffers from HIV/AIDS in addition, 2 million new cases occurred in 2012.[4] Nigeria accounts for 10% of global HIV/AIDS,[5,6] of which 1.72 millions are women within the ages of 35-49 years mostly in their reproductive years.

Therefore, Mother-To-Child-Transmission (MTCT) is a major concern in Nigeria which ranks highest in children acquiring HIV in the world.[7] HIV/AIDS is becoming a disease of poverty in developing countries especially in sub- Saharan Africa which bears a disproportionate number of the population.[5–7] The illicit sex trade practices particularly among young girls and women have made women and young girls vulnerable.[7] Worldwide, Nigeria has the second highest rates of new infections reported each year with an estimated 3.7% of the population living with HIV.[6] In sub -Saharan Africa about 60% of people living with HIV/AIDS are females and 50% of the global population living with HIV/AIDS are females.[4] Each year 55% of AIDS deaths occur among women and girls. There are many risk factors that contribute to the spread of HIV including prostitution, high risk practices among itinerant sex workers, high prevalence of sexually transmitted diseases, high risk heterosexual and homosexual practices in Nigeria.[4,6] The microbiota of the woman vagina is complex consisting of microbial communities that are

useful to her health, some are indigenous/normal micro flora that can become opportunists.[8] HIV/SIV infection has been shown to be associated with microbiome shift and immune activation that may affect the outcome of the disease progression.[8] Similarly, it has been reported that altered microbiome and inflammation are associated with increased risk of HIV acquisition suggesting the role of microbiome in HIV transmission[8] and MTCT.

Brenchley et al.,[9] and Hunt[10] have shown that persistent activation was a key factor of HIV and markers of inflammation which predict clinical outcome than viral load. Brenchley et al.,[11] have reported activation and inflammatory markers declined with antiretroviral therapy (ART) but remained high compared to healthy individuals. An HIV-infected individual suffers from significant gastrointestinal pathology than HIV-uninfected individuals.[12] The gut -associated lymphoid tissue (GALT) is a unique site for early HIV replication and severe CD4 cell depletion. Studies have revealed that the breakdown of the mucosal surface can lead to microbial translocation, a major factor that complicates HIV/AIDS progression.[13] Therefore, persistent viral replication in the GALT has been shown to be responsible for replenishment and maintenance of the viral reservoirs seen in an HIV/AIDS patient. MTCT is increased in neonates because of the permeability of HIV across the thin gastrointestinal epithelium in neonates.[14] Therefore, knowledge of the bacterial agent(s) colonizing the vagina, oropharynx and breast milk, among HIV seropositive women compared to HIV seronegative participants that severed as controls at healthcare clinics in Akure south and Ifedore LGA in Ondo State is desirable, hence our study that isolated, identified, compared communities involved. We are not aware of similar studies done in this environment. We believe our findings will assist clinicians and researchers to understand the changing microbial communities in these sites, their role in pathogenesis and treatment in event of an epidemic among HIV seropositive and seronegative pregnant mothers. We believe our findings will enable clinicians to better manage treatment and provide data base which hitherto does not exist in this environment thus strengthening the research capacity in the region.

Materials and methods

HIV screening among cohort

A 5mL volume of whole blood was collected in a sterile vacuette EDTA tubes K3 and sterile 38x0.8mm needles from each participant. A small aliquot was applied onto the HIV-1/2 strip (Determine Test, Alere, London, England, UK) for the preliminary determination of HIV serostatus. Confirmatory test for HIV infection was performed using the Abbott enzyme-linked immunosorbent assay (ELISA) procedure (Abbott Laboratories, Chicago, IL, USA).

Study centres

The participants, including controls were pregnant women at their third trimester of pregnancy that attended the ante-natal clinics of four selected healthcare centres in Akure south and Ifedore local government areas in Ondo State from November 2015 to December 2016. The study was approved by the Ondo State Hospital's Management and ethical board. Information relating to each participant was obtained through verbal interview, questionnaire responses and case files managed by the attending physicians.

Inclusion criteria

Human immunodeficiency virus (HIV) status of each subject was determined through blood screening at the HIV centre of each clinic and was a requirement for inclusion in the study. The screening was done at the onset of the study during which subjects were at their third trimester of pregnancy. Participants were also encouraged to keep all physician appointments throughout the study after consenting and fully registered.

Exclusion criteria

Those subjects who did not fall into the above categories, i.e subjects (whose HIV status was known) that did not comply with revisit and those who declined involvement in the study were not included.

Collection of High Vaginal Swab

Isolation and identification of bacterial isolates

A sample of high vaginal swab was collected from the posterior fornix from each pregnant women by the attending physician using sterile bivalve speculum (Changzhou Huankang Medical Devices Co. Ltd, Changzhou City 213116, Jiangsu Province, China) and sterile cotton-tipped applicator (Evepon, Industrial Limited, Onitsha, Anambra State, Nigeria) into freshly prepared sterile thioglycollate medium and incubated at 37°C for 24 h for growth. After growth was observed, a loopful of the sample was streaked initially with the aid of heat- flamed standard aluminium wire loop (delivering 0.001 ml on to freshly prepared agar plates - Blood agar, Proteose peptone agar and Mannitol salt agar). Thereafter, the plates were incubated aerobically at 37°C for 24 h and anaerobically in AnaeroPack jar 2.5 Litre, Order No. 50-25, product of Mitsubishi Gas Chemical Company Co., Inc. Japan (all samples were analysed within 24 hours of collection) for growth. Only plates on which colonies appeared were examined. Each distinct colony appearing on agar plates was picked and further studied. Each colony was classified based on cultural and morphological characteristics such as size, elevation, opacity and colour on media plates. Initial Gram's stain was prepared for each colony and further identification of each colony was based on their reaction on conventional enriched, selective and differential media. Colonies in clusters resembling staphylococci were inoculated onto mannitol salt agar (MSA) and those colonies that fermented mannitol on MSA were presumptively identified as *Staphylococcus aureus* (the prominent isolate from HIV infected patients) and confirmed as such by the coagulase slide and tube agglutination tests with pooled human plasma. Further confirmatory test was done on DNase and RNase agar. Coagulase negative staphylococci (CONS) were identified using sugar fermentation test.

Coagulase, catalase tests, growth on Bile aesculine agar (Oxoid, Basingstoke, Hampshire, England, UK) and sensitivity to Taxo A disc (0.04 units of bacitracin) and Taxo P disc (5µg) ethylhydrocupreine hydrochloride (optochin; BD Diagnostics, Difco Laboratories, Detriot, MI, USA) were also employed for identification of streptococci and enterococci. Furthermore, isolates resembling *Neisseria* species were cultured on protease peptone agar (Difco Laboratories, Detroit, Michigan, USA) supplemented with serum and determined by sugar fermentation test including; glucose, maltose, sucrose and lactose. Only gram negative diplococci isolates with bean shape colonies and positive for glucose fermentation were confirmed *Neisseria gonorrhoeae.* Colonies that were Gram negative rods were identified as lactose or non-lactose fermenters using eosin methylene blue (EMB) and MacConkey agar. Further speciation of isolates were based on their activities on convectional media such as triple sugar iron agar (TSI), Koser's citrate medium, sulphide, indole and motility agar (SIM) and urea agar (Oxoid, Basingstoke, Hampshire, England, UK) and according to methods described by Barrow and Feltham,

(1993). Gram positive rods that grew on conventional media were also classified as spore formers and non spore formers. The API kits used include API 20E and API Staph (bioMéerieux, France).

Collection and processing of oropharyngeal samples

A sample of oropharyngeal swab was obtained from each subject by the attending physician using a sterile cotton-tipped applicator that was initially dipped in sterile saline and inoculated into duplicate sterile thioglycollate fluid media and separately incubated at 37°C aerobically for 24 h and in anaerobic jars for 48 h (AnaeroPack Jar 2.5 Litre, Order No.50-25, product of Mitsubishi Gas Chemical Company Co., Inc. Japan. For bacterial identification, a loopful of each culture was streaked onto blood agar (BA), chocolate agar (CA), mannitol salt agar (MSA), eosin methylene blue agar (EMB), sulphide indole motility agar (SIM), koser citrate agar and triple sugar iron agar (TSI) (Oxoid LTD, Basingstoke, Hampshire, England). The plates were incubated aerobically and anaerobically at 37° C for 48 h. Colonies that grew on each culture medium were Gram stained and processed for biochemical identification using the Analytical Profile Index (API) 20E and API Staph (Biomerieux, France).

Collection and processing of breast milk samples

Breast milk sample (102 in all) was collected from each lactating mother made up of 99 samples collected from HIV seropositive women and only 3 samples each from HIV seronegative mothers because of restrain by the participants. Breast milk samples were collected from HIV seropositive mothers at three periodic intervals: first, 70 samples were collected immediately after birth (1-14 days of delivery), 18 samples were collected 180 days later and 11 samples 270 days later. However, only 3 breast milk samples were collected from HIV seronegative lactating mothers because of restraint. Criteria for collection was strict as subjects were requested to clean their hands and the breasts with sterile warm water, as each sample was collected into sterile calibrated test tube by manual expression. One millilitre (1ml) of each breast milk sample was inoculated into freshly prepared sterile thioglycollate medium and incubated at 37°C for 24 h for growth. Thereafter, samples were analysed according to method used in processing HVS.

Nasal carriage of bacterial isolates in neonates

In addition, a nasal swab from each neonate was obtained from within the first to 14 days of birth. Each swab was initially moistened with a sterile normal saline and gently introduced into the anterior nares of each neonate by careful rotation by the clinician/nurse. Such a sample was thereafter introduced into freshly prepared sterile thioglycollate medium and incubated at 37°C for 24 h for growth. Thereafter, samples were analysed similar to those isolates recovered from oropharynx.

Results

Table 1 depicts the distribution of bacterial isolates from high vaginal swab of HIV seropositive and HIV seronegative pregnant women from the centres. The data show that 1,156 bacterial isolates were cultured from both HIV seropositive and HIV seronegative pregnant women. 549(47.5%) bacterial isolates were recovered from HIV seropositive women while 607 (52.5%) bacterial isolates were cultured from HIV seronegative women. The data also showed that the mean bacteria per subject for HIV seropositive was 4.81 (549/114) per subject compared to the control 4.81(607/126) per subject. Among the staphylococci, pathogenic *Staphylococcus aureus* isolates were the single predominant isolates 32/327(9.8%) recovered from HVS of HIV seropositive subjects compared to 6/327(7.9%) for HIV seronegative subjects. The results also showed among the coagulase negative staphylococci (CONs) 21.7% against 60.5% of the CONs were cultured from HIV seropositive and HIV seronegative subjects respectively. The data revealed a statistical significance difference between the two cohorts (p=0.002). Furthermore, the results showed that Gram positive rods constituted 458/1156 (39.6%) of which the spore formers constituted 22/1156 (1.9%) while the non spore formers constituted 436/1156 (37.7%). Among the spore formers, *B. subtilis* constituted 2/22(9.1%) for HIV seropositive subjects while 13/229(59.1%) of *B. subtilis* was cultured from HIV seronegative subjects. This was followed by *B. cereus* 2/22(9.1%), *B. mycoides* 4/22(18.2%) and *B. sphaericus* 1/22(4.5%) for the HIV seronegative pregnant subjects. There was statistical significance difference within the two cohorts (p=0.026). Of the gram positive non spore formers, bacterial isolates cultured from high vaginal swab of HIV seropositive pregnant subjects, *Arcanobacterium haemolyticum* was 58/436(13.6%) followed by the corynebacteria isolates which constituted 26.63% of the total bacterial isolates cultured from the seropositives subjects compared to 39.7% for the seronegative subjects. The data also showed a significant difference between the cohorts with p=0.001.

Furthermore, the table showed (Table 1) gram negative rods were also 178/1156 (15.4%) of the total bacterial isolates cultured from high vaginal swabs of HIV seropositive and seronegative pregnant subjects, contained lactose and non lactose fermenters 89/1156 (7.7%) each (Table 1).

Table 1 Comparative analysis of the distribution of bacterial isolates cultured from high vaginal swabs (HVS) of HIV seropositive and HIV seronegative pregnant women

	Bacterial Isolates	Total No. cultured from HVS	No. cultured from HIV seropositive	No. cultured from HIV seronegative	P value
Gram Positive Bacterial					
Gram positive cocci					
Pathogenic Staphylococci (coagulase positive)	*Staphylococcus aureus*	58	32	26	
Coagulase negative staphylococci	*Staphylococcus xylosus*	41	26	15	
	Staphylococcus epidermidis	17	8	9	

(Table 1 continue...)

	Staphylococcus capitis	18	8	10	
	Staphylococcus warneri	28	7	21	
	Staphylococcus simulans	19	5	14	P=0.002
	Staphylococcus lentus	11	5	6	
	Staphylococcus saprophyticus	19	2	17	
	Staphylococcus hominis	23	2	21	
	Staphylococcus kloosii	9	2	7	
	Staphylococcus caseolyticus	17	2	15	
	Staphylococcus haemolyticus	20	1	19	
	Staphylococcus sciuri	6	1	5	
	Staphylococcus caprae	11	1	10	
	Staphylococcus hyicus	5	1	4	
	Staphylococcus cohnii	6	0	6	
	Staphylococcus saccharolyticus	4	0	4	
	Staphlococcus carnosus	1	0	1	
	Staphylococcus intermedius	10	0	10	
	Staphylococcus schleiferi	4	0	4	
Micrococci					
	Micrococcus luteus	95	70	25	
	Micrococcus lylae	27	16	11	P=0.018
	Micrococcus agilis	4	2	2	
	Micrococcus roseus	3	1	2	
Enterococci					
	Enterococcus faecium	7	5	2	P=1.0
	Enterococcus faecalis	13	5	8	
Streptococci					
	Streptococcus pneumonia	8	4	4	
	Group B Streptococci (viridians)	21	2	19	P=0.232
	Streptococcus pyogenes	8	0	8	
Gram Negative cocci					
	Neisseria gonorrhoeae	7	5	2	
Gram positive Rods (spore formers)					
	Bacillus subtilis	15	2	13	
	Bacillus cereus	2	0	2	P=0.026
	Bacillus mycoides	4	0	4	
	Bacillus sphaericus	1	0	1	
Gram positive rods (Non spore formers)					

(Table 1 continue...)

	Arcanobacterium haemolyticum	108	58	50	
	Corynebacterium xerosis	150	72	78	
	Corynebacterium ulcerans	37	21	16	
	Corynebacterium pseudotuberculosis	41	18	23	
	Corynebacterium amycolatum	6	3	3	P=0.001
	Corynebacterium pseudodiphtheriticum	7	2	5	
	Corynebacterium jeikieum	1	1	0	
	Corynebacterium diphtheria	1	0	1	
	Gardnerella vaginalis	10	5	5	
	Listeria monocytogenes	46	27	19	
	Lactobacillus acidophilus	29	15	14	
Gram Negative Rods					
Lactose fermenters					
	Escherichia coli	33	14	19	
	Klebsiella pneumoniae	15	10	5	P=0.05
	Klebsiella oxytoca	5	4	1	
	Enterobacter aerogenes	22	14	8	
	Enterobacter cloacae	3	0	3	
	Citrobacter freundii	8	6	2	
	Citrobacter diversus	3	1	2	
Non lactose fermenters					
	Providencia rettgeri	21	18	3	
	Pseudomonas aeruginosa	22	17	5	
	Pseudomonas fluorescens	5	4	1	
	Proteus mirabilis	12	7	5	P=0.055
	Proteus vulgaris	10	6	4	
	Salmonella typhimurium	9	4	5	
	Plesiomonas shigelloides	7	4	3	
	Chryseomonas luteola	3	3	0	
Total		1,156	549	607	

The profile of bacterial isolates cultured from breast milk of lactating HIV seropositive women in selected centres is shown in Table 2. The results showed 145 isolates were cultured from 70 breast milk samples of lactating HIV seropositive women averaging 2.07 bacteria per subject at the first collection, while no bacterial isolate was cultured from the 18 samples collected at 180 days after delivery and from 11 samples collected after 270 days after delivery. The initial breast milk sample collected consisted of gram positive cocci in clusters made up of pathogenic *Staphylococcus aureus* 8/145(5.5%) and coagulase negative staphylococci 31/145(21.4%), micrococci 9/145(6.2%), streptococci and enterococci 1/145(0.7%) each while the gram positive rods consisted of spore formers 11/145(7.5%) and non spore formers 84/145(57.9%).

Table 2 Profile of bacterial isolates cultured from breast milk of lactating HIV seropositive women in selected centres

	Bacterial Isolates	Total Number cultured from breast milk	No. cultured from HIV seropositive	No. cultured from HIV seronegative	P value
Gram Positive Bacteria	Gram Positive cocci				
Staphylococci					
Pathogenic Staphylococci	*Staphylococcus aureus*	8	8	0	
	Staphylococcus epidermidis	14	12	2	
	Staphylococcus saprophyticus	9	9	0	
	Staphylococcus haemolyticus	5	5	0	0.013
	Staphylococcus cohnii	3	3	0	
	Staphylococcus lentus	1	1	0	
	Staphylococcus warneri	1	1	0	
	Staphylococcus capitis	1	0	1	
Micrococcus					
	Micrococcus luteus	5	5	0	0.09
	Micrococcus agilis	1	1	0	
	Micrococcus roseus	2	2	0	
	Micrococcus lylae	1	1	0	
Streptococcus					
	Streptococcus salivarius	1	1	0	
Enterococcus					
	Enterococcus feacium	1	1	0	
Gram positive Rods					
Spore formers					
	Bacillus subtilis	7	5	2	
	Bacillus sphaericus	5	5	0	0.11
	Bacillus mycoides	1	1	0	
Non spore formers					
	Arcanobacterium haemolyticum	21	20	1	
	Corynebacterium ulcerans	6	4	2	
	Corynebacterium xerosis	8	8	0	
	Corynebacterium jeikieum	19	19	0	
	Corynebacterium diphtheriae	5	5	0	0.002
	Corynebacterium pseudotuberculosis	3	3	0	
	Corynebacterium amycolatum	5	5	0	
	Lactobacillus reuteri	3	3	0	
	Lactobacillus brevis	5	5	0	
	Lactobacillus acidophilus	3	3	0	
	Lactobacillus salivarius	1	1	0	
	Lactobacillus casei	3	3	0	
	Listeria monocytogenes	5	5	0	
Total		153	145	8	

Citation: Ako-Nai KA, Omo-Omorodion B. Bacterial colonization of the vagina, oropharynx, breast milk and anterior nares of neonates among HIV seropositive pregnant women and seronegative pregnant counterparts. *J Microbiol Exp.* 2019;7(2):116–124. DOI: 10.15406/jmen.2019.07.00250

Table 3 reveals the distribution of bacterial isolates from oropharynx of HIV seropositive and HIV seronegative pregnant women from the centres. The data show that 466 bacterial isolates were cultured from both HIV seropositive and HIV seronegative pregnant women. 217(46.6%) bacterial isolates were recovered from HIV seropositive women while 249(53.4%) bacterial isolates were cultured from HIV seronegative women. The data also show that the mean bacteria per subject for HIV seropositive was 1.90 (217/114) per subject compared to the control 1.97 (249/126) per subject. The profile of distribution of the isolates is shown in Table 3.

Table 3 Comparative analysis of the distribution of bacterial isolates cultured from oropharynx of HIV seropositive and HIV seronegative pregnant women

	Bacterial Isolates	**Total No. cultured from nares of neonates**	**No. cultured from nares of neonates**
Gram Positive Bacterial			
Gram positive cocci			
Pathogenic Staphylococci (coagulase positive)	*Staphylococcus aureus*	19	19
Coagulase negative staphylococci	*Staphylococcus xylosus*	22	22
	Staphylococcus epidermidis	18	18
	Staphylococcus capitis	6	6
	Staphylococcus warneri	5	5
	Staphylococcus simulans	2	2
	Staphylococcus lentus	0	0
	Staphylococcus saprophyticus	7	7
	Staphylococcus hominis	1	1
	Staphylococcus kloosii	0	0
	Staphylococcus caseolyticus	0	0
	Staphylococcus haemolyticus	6	6
	Staphylococcus sciuri	2	2
	Staphylococcus caprae	0	0
	Staphylococcus hyicus	0	0
	Staphylococcus cohnii	0	0
	Staphylococcus saccharolyticus	0	0
	Staphlococcus carnosus	0	0
	Staphylococcus intermedius	0	0
	Staphylococcus schleiferi	3	3
Micrococci			
	Micrococcus luteus	43	43
	Micrococcus lylae	15	15
	Micrococcus agilis	1	1
	Micrococcus roseus	1	1
Enterococci			
	Enterococcus faecium	1	1
	Enterococcus faecalis	1	1
Streptococci			
	Streptococcus pneumoniae	0	0
	Group B Streptococci (viridians)	0	0
	Streptococcus pyogenes	0	0
Gram Negative cocci	*Streptococcus salivarus*	0	0
Gram positive Rods (spore formers)	*Neisseria gonorrhoeae*	0	0

Citation: Ako-Nai KA, Omo-Omorodion B. Bacterial colonization of the vagina, oropharynx, breast milk and anterior nares of neonates among HIV seropositive pregnant women and seronegative pregnant counterparts. *J Microbiol Exp.* 2019;7(2):116–124. DOI: 10.15406/jmen.2019.07.00250

(Table 3 continue...)

	Bacillus subtilis	5	5
	Bacillus cereus	1	1
	Bacillus mycoides	0	0
Gram positive rods (Non spore formers)	*Bacillus sphaericus*	3	3
	Arcanobacterium haemolyticum	33	33
	Corynebacterium xerosis	51	51
	Corynebacterium ulcerans	20	20
	Corynebacterium pseudotuberculosis	3	3
	Corynebacterium amycolatum	10	10
	Corynebacterium pseudodiphtheriticum	9	9
	Corynebacterium jeikieum	13	13
	Corynebacterium diphtheriae	1	1
	Gardnerella vaginalis	0	0
	Listeria monocytogenes	0	0
Gram Negative Rods	*Lactobacillus acidophilus*	0	0
Lactose fermenters	*Lactobacillus reuteri*	2	2
	Lactobacillus brevis	1	1
	Lactobacillus salivarius	0	0
	Lactobacillus casei	0	0
	Lactobacillus spp	0	0
	Actinomyces israelii	1	1
Non lactose fermenters	*Escherichia coli*	5	5
	Klebsiella pneumoniae	3	3
	Klebsiella oxytoca	0	0
	Enterobacter aerogenes	10	10
	Enterobacter cloacae	2	2
	Citrobacter freundii	4	4
	Citrobacter diversus	1	1
	Providencia rettgeri	16	16
	Pseudomonas aeruginosa	11	11
	Pseudomonas fluorescens	2	2
	Proteus mirabilis	6	6
	Proteus vulgaris	3	3
	Proteus penneri		
	Serratia marcescens	0	0
	Salmonella typhimurium	4	4
	Plesiomonas shigelloides	0	0
	Chryseomonas luteola	0	0
	Shigella dysenteriae	0	0
	Streptobacillus moniliformis	0	0
	Salmonella enteritidis	0	0
	Sarcina spp		
Total		373	373

Table 4 depicts the distribution of bacterial isolates cultured from anterior nares of 70 neonates of HIV seropositive mothers from the centres. The data show 373 bacterial isolates were cultured from anterior nares of the neonates. The data also showed that the mean bacteria per subject was (373/70)=5.3. The profile of distribution of the isolates encountered is shown in Table 4.

Table 4 Profile distribution of bacterial isolates cultured from anterior nares of neonates of HIV seropositive women

	Bacterial Isolates	Total No. cultured from nares of neonates	No. cultured from nares of neonates
Gram Positive Bacterial			
Gram positive cocci			
Pathogenic Staphylococci (coagulase positive)	*Staphylococcus aureus*	19	19
Coagulase negative staphylococci	*Staphylococcus xylosus*	22	22
	Staphylococcus epidermidis	18	18
	Staphylococcus capitis	6	6
	Staphylococcus warneri	5	5
	Staphylococcus simulans	2	2
	Staphylococcus lentus	0	0
	Staphylococcus saprophyticus	7	7
	Staphylococcus hominis	1	1
	Staphylococcus kloosii	0	0
	Staphylococcus caseolyticus	0	0
	Staphylococcus haemolyticus	6	6
	Staphylococcus sciuri	2	2
	Staphylococcus caprae	0	0
	Staphylococcus hyicus	0	0
	Staphylococcus cohnii	0	0
	Staphylococcus saccharolyticus	0	0
	Staphlococcus carnosus	0	0
	Staphylococcus intermedius	0	0
	Staphylococcus schleiferi	3	3
Micrococci			
	Micrococcus luteus	43	43
	Micrococcus lylae	15	15
	Micrococcus agilis	1	1
	Micrococcus roseus	1	1
Enterococci			
	Enterococcus faecium	1	1
	Enterococcus faecalis	1	1
Streptococci			
	Streptococcus pneumoniae	0	0
	Group B Streptococci (viridians)	0	0
	Streptococcus pyogenes	0	0
Gram Negative cocci	*Streptococcus salivarus*	0	0
Gram positive Rods (spore formers)	*Neisseria gonorrhoeae*	0	0
	Bacillus subtilis	5	5

(Table 4 continue...)

	Bacillus cereus	1	1
	Bacillus mycoides	0	0
Gram positive rods (Non spore formers)	*Bacillus sphaericus*	3	3
	Arcanobacterium haemolyticum	33	33
	Corynebacterium xerosis	51	51
	Corynebacterium ulcerans	20	20
	Corynebacterium pseudotuberculosis	3	3
	Corynebacterium amycolatum	10	10
	Corynebacterium pseudodiphtheriticum	9	9
	Corynebacterium jeikieum	13	13
	Corynebacterium diphtheriae	1	1
	Gardnerella vaginalis	0	0
	Listeria monocytogenes	0	0
Gram Negative Rods	*Lactobacillus acidophilus*	0	0
Lactose fermenters	*Lactobacillus reuteri*	2	2
	Lactobacillus brevis	1	1
	Lactobacillus salivarius	0	0
	Lactobacillus casei	0	0
	Lactobacillus spp	0	0
	Actinomyces israelii	1	1
Non lactose fermenters	*Escherichia coli*	5	5
	Klebsiella pneumoniae	3	3
	Klebsiella oxytoca	0	0
	Enterobacter aerogenes	10	10
	Enterobacter cloacae	2	2
	Citrobacter freundii	4	4
	Citrobacter diversus	1	1
	Providencia rettgeri	16	16
	Pseudomonas aeruginosa	11	11
	Pseudomonas fluorescens	2	2
	Proteus mirabilis	6	6
	Proteus vulgaris	3	3
	Proteus penneri		
	Serratia marcescens	0	0
	Salmonella typhimurium	4	4
	Plesiomonas shigelloides	0	0
	Chryseomonas luteola	0	0
	Shigella dysenteriae	0	0
	Streptobacillus moniliformis	0	0
	Salmonella enteritidis	0	0
	Sarcina spp		
Total		373	373

Discussion

The study compared the bacterial isolates cultured from the vagina, oropharynx and breast milk of HIV seropositive and seronegative mothers and the anterior nares of infants that attended the antenatal and post natal clinics in Akure south and Ifedore LGA clinics in Ondo State South-western Nigeria between Nov 2015-Dec 2016. Two hundred and forty pregnant women aged 19-43 years were recruited for the study. 114 HIV seropositive subjects mean age 31.81 years and 126 HIV seronegative subjects mean age 29.05 years as controls. The results showed altogether 2,148 bacterial isolates were recovered from both cohorts (911 from HIV seropositive, 864 HIV seronegative and 373 from nares of neonates). The number of bacterial isolates recovered from HVS from both HIV seropositive and seronegative mothers 1156/2148=(53.8%), comprising of HVS 549/1156=47.49%, 607/1156=52.51% from HIV seropositive mothers and seronegative mothers respectively. In contrast, oropharynx 466/2148=(21.7%) comprising of 217/466=46.6% for HIV seropositive subjects while 249/466=53.4% for HIV seronegative subjects. Similarly, breast milk samples constituted 153/2148=(7.1%) comprising of 145/153=94.7% for HIV seropositive and 8/153=5.2% for HIV seronegative subjects and the anterior nares of neonates of HIV seropositive mothers 373/2148=(17.36%). The predominant isolates were gram positive rods made up of primarily commensals *Arcanobacterium haemolyticum* and species of corynebacteria in which four were outstanding: *Corynebacterium xerosis*, *Corynebacterium pseudotuberculosis*, *Corynebacterium ulcerans* and *Corynebacterium pseudodiphtheriticum*. Interestingly some of the Corynebacterium often found as commensals have been reported in immunocompromised individuals.[15] *Corynebacterium ulcerans* for example are known to carry beta phages that produce small amount of diphtheria toxins and cause infections with mild toxic manifestations.[16] Similarly, *Corynebacterium diphtheriae* a major pathogen was also isolated from the four sources. 1/31=(3.2%) each from HVS and nares of neonates, 5/31=(16.1%) from breast milk samples and surprisingly 24/31=(77.4%) from oropharynx. The occurrence in strikingly high numbers of *C. diphtheriae* in the oropharynx is of concern because the organism is known to possess potent exotoxins that can cause diphtheria.[17]

Our study revealed numerous isolates in the high vaginal swabs mainly due to the microbiota of the vagina and pregnancy.[18] Altogether, most of these isolates encountered are considered normal flora that protect the host from invaders.[18] Our study showed that 114(5.3%) *Staphylococcus aureus* were recovered from the two sources. *Staphylococcus aureus* is a major pathogen both in adults and in children.[19] *Staphylococcus aureus* manifest its pathogenesis through elaboration of enzymes, toxins and resistant genes that modulate resistance [20]. Similarly, HVS constituted 50.8%, oropharynx 25.4%, anterior nares 16.7% and breast milk 7.0% of *Staphylococcus aureus* recovered. It is interesting to note the high value of 25.4% of *Staphylococcus aureus* in the oropharynx and underscores the probable transmission to neonates. Studies have shown in apparently healthy individuals, colonization with *Staphylococcus aureus* varies from one region to another. Ako-Nai et al.,[21] and Paul et al.,[22] have reported the endemicity of *Staphylococcus aureus* in this region. It is however interesting that this organism constituted 7% in breast milk samples analysed in our study. Transmission of *Staphylococcus aureus* can be horizontal or vertical. It is not unusual to have transmission of *S. aureus* probably at birth or by handlers.[23]

Other pathogens encountered in this study were *Pseudomonas aeruginosa* 42/2148=1.9%, 22/42=(52.38%) of which was recovered from HVS, 9/42=(21.4%) from the oropharynx, 11/42=(26.19%) from nares of neonates and none of *P. aeruginosa* isolate was seen in breast milk samples emphasizing the dominance of pathogens among isolates recovered from HVS. In addition, *Escherichia coli* also constituted 42/2148=1.9% comprising of 33/42=78.6% from the HVS, 11.9% from the nares of neonates, 9.5% from the oropharynx and interestingly no isolate was recovered from the breast milk indicating the sanitary methods used in collecting these samples. Furthermore, *Klebsiella pneumoniae* was also recovered from the two sources 22/2148=1.02% comprising of 15/22=68.2% in HVS, 4/22=18.2% in the oropharynx and 13.6% in the nares of neonates. Interestingly no *K. pneumoniae* was seen in breast milk samples analysed. The high percentage of *E. coli* and *Klebsiella pneumoniae* recovered from HVS is not unusual because of the continual contamination of the vagina (Table 1).[24]. Our study also compared each pathogen recovered from the two sources between cohorts. Among the HIV seropositive individuals, 28.1% of *Staphylococcus aureus* was recovered compared to 22.8% among the seronegative controls. Similarly, 8.7% compared to 16.7% from oropharynx of HIV seropositive and seronegative controls respectively while 7% of *Staphylococcus aureus* isolates was recovered from breast milk samples of seropositive subjects.

However, 16.7% was cultured from the nares of HIV seropositive neonates. Furthermore, among the *E coli* isolates cultured, 33.3% against 45.2% was recovered from HVS, 4.8% each from oropharynx of both HIV seropositive and seronegative subjects respectively while 11.9% from the neonates of HIV seropositive subjects. In the same vein, among the *K. pneumoniae* isolates recovered from HVS samples, 45.5% for seropositive compared to 22.7% for seronegative subjects while 9.1% each from the oropharynx of both cohorts. Similarly, 13.6% of *K. pneumoniae* was recovered from the nares of neonates. The occurrence of lactose fermenters among neonates may have occurred through self contamination or contamination with mothers and handler's flora.[25] Our study also revealed *P. aeruginosa* recovered from both cohorts was as follows: 40.5% against 11.9% for HVS, 7.1% compared to 14.3% for oropharynx for HIV seropositive and seronegative subjects respectively while nares of neonates constituted 26.2%. In summary these comparative values showed higher rates of colonization with *Staphylococcus aureus*(60.5% against 39.5%), *Klebsiella pneumoniae* 68.2% against 31.8% and *Pseudomonas aeruginosa* 73.8% compared to 26.2% recovered from HIV seropositive and seronegative subjects respectively. It is also interesting to note that *E. coli* isolates recovered was 50% each among both cohorts. The dominance of bacterial isolates in HVS have been associated with the epithelia mucosa which is rich in microbiota compared to the other sites.[26]

In conclusion, 2,148 bacterial isolates colonized the four sites (HVS, oropharynx, breast milk samples and nares of neonates) in which HVS constituted the highest due probably to microbiota of the vagina, pregnancy and other related hormones. The incidence of pathogens was high among the HIV seropositive 28.1% of *Staphylococcus aureus* compared to 22.8% among seronegative controls, 7% *Staphylococcus aureus* from breast milk samples of seropositive subjects compared to none from controls. *K. pneumoniae* isolates recovered from HVS samples, 45.5% for seropositive against 22.7% for seronegative subjects. *P. aeruginosa* recovered from both cohorts was as follows: 40.5% against 11.9% for HVS.

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Conflicts of interest

The authors declare no conflicts of interest.

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