wjpmr, 2019,5(4), 207-116

SJIF Impact Factor: 4.639

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

BACTERIAL ISOLATES RECOVERED FROM BREAST MILK SAMPLES OF HIV PATIENTS IN AKURE, ONDO STATE, NIGERIA.

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Article Received on 20/02/2019 **Article Revised on 10/03/2019** **Article Accepted on 31/03/2019**

ABSTRACT

Background: Bacterial colonization of human breast milk samples is of public health concern. In sub-Saharan countries where hygiene is poor among most patients, infants often come in contact with the flora of their mothers' breast which often colonize their nostrils. Because of the new-born immature immunological state, contamination of the breast milk may result in opportunistic infection and as such have serious impact on the new-born that can lead to death. **Methods:** The study was carried out among 70 HIV infected pregnant women and their neonates in Akure south and Ifedore local government area (LGA) of Ondo State between November 2015-December 2016. Human immunodeficiency virus (HIV) status of each woman was determined using the determine test kit and confirmed by the Abbott enzyme-linked immunosorbent assay (ELISA) Seventy (70) breast milk samples were collected immediately after birth (1- 14 days of delivery) from HIV seropositive lactating mothers. One millilitre (1 ml) of each breast milk sample and nasal swab of each neonate was inoculated into freshly prepared sterile thioglycollate medium and processed following standard microbiological procedures. Antibiotic sensitivity testing of selected pathogens were tested using the Kirby-Bauer method. Screening for Extended spectrum β- lactamase (ESBL) was carried out by using double disk approximation method / synergy test (DDST). **Results**: Altogether, 518 bacterial isolates were recovered from the anterior nares of neonates of HIV seropositive women and their breast milk samples. *S. aureus* was selectively studied as the single predominant pathogen recovered from both sample sites. The antibiotypes of *S. aureus* isolates tested showed varying degree of resistance to the antibiotics tested. Furthermore, 61.1% of the isolates were methicillin resistant *Staphylococcus aureus* (MRSA) and 38.8% methicillin sensitive *Staphylococcus aureus* (MRSS). Of the breast milk isolates tested, none of the MRSA produced ESBL while one isolate produced ESBL in the MSSA group. Similarly, 6 MRSA of the nares of neonates produced 2 ESBL strains and 1 ESBL from the MSSA. **Conclusion**: Regular bacterial colonization examination in HIV infected lactating women will help reduce vertical transmission of pathogens from mother- to- child. Additionally, the development and spread of ESBL producing organism is worrisome because of rapid spread of resistance to same species and even to other genera. The pressure of unregulated misuse of antibiotics with poor quality of drugs may have resulted in the widesspread of resistant strains generally.

KEYWORDS: Breast milk, neonatal nares, HIV, antibiotics, MRSA & ESBL.

INTRODUCTION

Breastfeeding is an active mechanical process that involves maternal skin contact and her infant pair. If breastfeeding appears to be a plausible route of bacterial transmission, this simply means that bacterial agent(s) may be vertically transmitted from a lactating mother to her infant. This is particularly important especially in developing countries, where infections are frequent and breastfeeding is common. Infants often come in contact with the flora of their mothers' breast which may colonize their nostrils and because of their immature immunological state may result in opportunistic infections.[1] In HIV infected patients, Mother-to-child transmission (MTCT) of HIV infection have been pointed to come from intrauterine transmission during pregnancy, vaginal delivery and post-partum through breast feeding. The high incidence of nipple fissures and sore which occurs as a result of cell inflammation often results in mastitis of the breast in these immunocompromised women.[2] This condition has been reported to be associated with S. aureus and more frequently, MRSA.[3] The aftermath of this colonization repeatedly results in painful lactation and can be problematic because it may lead to the discontinuation of breast-feeding, which provides optimal infant nutrition. Although pathogenic organism colonization in breast milk samples results in diseases, some studies have reported beneficial properties of commensals cultured in Bovine milk samples.[4] Lactic acid bacteria (LAB), propionibacteria and other closely related Gram-positive bacteria for example, have been reported to be associated

with prevention of infection in breast feeding infants. Similarly, bifidobacteria,[5] such as Lactobacillus gasseri and Enterococcus faecium have been used as probiotics.[6,7] In vitro studies have also shown that commensal LAB from human breast milk inhibits HIV-1 virus, suggesting a possible role of these bacteria in mucosal protection against HIV in an exposed breastfed infant.[8,9] Furthermore, in HIV-infected children probiotics may restore the microbiota, increase naïve CD4 T-cell counts, and protect against inflammation and chronic immune activation of the gastrointestinal immune system.[10] While this has been reported, the mechanism of action still remains contentious. It is also important to understand that apart from the nutritive role of breast milk, microbial colonization of breast milk samples may influence the host immune system and possible transmission of pathogens from mother to infant pair. It has been reported that the natural microbiota of the human mammary gland may also be colonized with pathogenic staphylococci and streptococci, which are a threat in the leading cause of a disease.[11]

Therefore, continuous exposure to pathogenic bacteria obtained from infected maternal breast milk can result in resistant strains and a threat to neonatal life. Extented spectrum beta- lactamase (ESBL) producing bacteria has become widespread not only with the gram negative bacilli but with pathogenic *S. aureus.*[12] They have been associated with nosocomial and community acquired infections where they cause a variety of infections and severe implications with corresponding multiple antibiotic resistance. Strains of bacterial isolates expressing these β-lactamases often present a host a therapeutic challenge in treatment regime.[13] This first β-lactamase was first identified in *E. coli* prior to the introduction of the use of penicillins as antibiotics. The emergence of resistance in penicillin was rapid because of a plasmid encoded penicillinase in *S. aureus*.[14]

To date, there have been no studies to the best of our knowledge demonstrating bacterial diversity colonization associated with vertical neonatal transmission of pathogenic bacterial isolates from breast milk samples of HIV positive women in our environment. Additionally, our study evaluated the antibiotic resistant pattern and ESBL production of selected pathogens recovered from maternal breast milk and the anterior nares of their neonates to a wide range of antibiotics. This study we believe will provide database on the diversity of bacterial population colonizing human breast milk samples in our environment and their antibiotic resistant pattern to commonly employed antibiotics. We are also optimistic that knowledge on the acquisition of pathogenic organism through vertical transfer of pathogens from mother-to-child will assist clinicians on how to better manage patients in this environment. We believe that this research also will open research gaps that need to be explored in other to proffer better preventative and management strategy of MTCT of HIV in our region.

METHODS

The study was carried out among HIV infected pregnant women in Akure south and Ifedore local government areas in Ondo State between November 2015-December 2016. Human immunodeficiency virus (HIV) status of each subject was determined through blood screening at the HIV antenatal clinic of each local government area. Each participant was screened for HIV serostatus at the third trimester of pregnancy using the determine test kit (London, England, United Kingdom (UK) and confirmed by the Abbott enzyme-linked immunosorbent assay (ELISA) procedure (Abbott Laboratories, Chicago, IL, USA).

Seventy (70) breast milk samples were collected immediately after birth (1- 14 days of delivery) from HIV seropositive lactating mothers. Criteria for collection was strict as subjects were requested to clean their hands and the nipple of their breasts with sterile warm water and manually express their breast milk into sterile calibrated test tube. One millilitre (1 ml) of each breast milk sample was inoculated into freshly prepared sterile thioglycollate medium and incubated at 37^{o}C for 24 h for growth. Thereafter, a loopful of breast milk was streaked with the aid of heat- flamed standard aluminum wire loop (delivering 0.001 ml on to duplicates of freshly prepared agar plates - Blood agar, cysteine lactose electrolyte deficient agar and Mannitol salt agar, Lactic acid bacterial (LAB) medium) and McConkey agar. Afterwards, the plates were incubated both aerobically and anaerobically at 37^{o}C for 24 h for growth. Colonies that grew on the media were picked and studied.

A nasal swab from each neonate of the women was obtained from within the first to 14 days of birth after each neonate had been introduced to breast milk feeding. Each swab was initially moistened with a sterile normal saline and introduced into the anterior nares of each neonate by careful rotation by the clinician/nurse. Each sample was thereafter introduced into freshly prepared sterile thioglycollate medium and incubated at 37^{o}C for 24 h for growth. Thereafter, a loopful was streaked with the aid of heat- flamed standard aluminium wire loop (delivering 0.001 ml on to duplicates of freshly prepared agar plates - blood agar, cysteine lactose electrolyte deficient agar (CLED) and mannitol salt agar, LAB medium and MacConkey agar). The plates were incubated both aerobically and anaerobically at 37^{o}C for 24 h for growth. Colonies that grew on the media were picked and studied.

Antibiotics sensitivity testing

Antibiotic sensitivity testing of randomly selected pathogens were tested to 23 antibiotics of varying classes using the Kirby-Bauer method. The antibiotics used were obtained from Oxoid (Basingstoke, UK) and included amoxicillin/clavulanic acid AMC (30 µg), ampicillin AMP (10 µg), penicillin G P (1 IU), oxacillin OX (1 µg), ceftriaxone CRO (30 µg), cefuroxime CXM (30 µg), chloramphenicol C (30 µg),imipenem IPM (10 µg),

tetracycline TE (30 µg), erythromycin E (15 µg), gentamycin CN (10 µg), kanamycin K (30 µg), streptomycin S (10 µg), vancomycin VA (5 µg), bacitracin BA (10 IU), optochin OPT (5 µg), nalidixic acid NA (30 µg), ciprofloxacin CIP (5 µg), ofloxacin OFX (5 µg), nitrofurantoin F (300 µg), fusidic acid FD (5 µg), sulphomethoxazole/ trimethoprim SXT (25µg) and mupirocin MUP (200 µg). *S. aureus* ATCC 25923 and *Enterobacter aerogenes* ATCC 13042 (American Type Culture Collection, Rockville, USA) were used as control organisms.

Detection of ESBL from *S. aureus*

Screening for Extended spectrum β- lactamase was done by using double disk approximation method / synergy test (DDST) according to the Clinical and Laboratory Standards Institute protocol.[15] Test isolates were incubated in nutrient broth at 37^{o}C adjusted to an optical density of 0.5 Mc Farland turbidity standards for 24 h. This suspension was seeded onto a Mueller-Hinton Agar (Oxoid, Basingstoke, Hampshire, England, UK) plate by swabbing with sterile cotton-tipped applicator and placing ceftazidime (30µg) and cefotaxime (30µg) at 20mm apart (centre to centre) from a centre disc containing augmentin 30µg (amoxicillin (20 µg)/clavulanic acid (10µg) and incubated at 37^{o}C for 24 h. Potentiation in the zone of inhibition of any of the antibiotic disc towards the centre disc (augumentin) indicated the presence of ESBL.

RESULTS

Our results showed a total of 145 bacterial isolates were cultured from the 70 breast milk samples of HIV lactating seropositive women, averaging 2.07 bacteria per subject. The results showed, 5.5% of the total isolates, consisted of pathogenic *Staphylococcus aureus* and 21.4% coagulase negative staphylococci. However, among the coagulase negative staphylococci isolated, *Staphylococcus epidermidis* 38.8%, *Staphylococcus saprophyticus* 29%, *Staphylococcus haemolyticus* 16.1%, *Staphylococcus cohnii* 9.6% while *Staphylococcus lentus* and *Staphylococcus warneri* 3.2% each of the staphylococci. Similarly, the micrococci comprised 6.2%, streptococci and enterococci 0.7% each of the total isolates recovered with *Micrococcus luteus* accounting for 55.6%, *Micrococcus agilis* and *Micrococcus lylae* 11.1% each while *Micrococcus roseus* was 22.2%. *Streptococcus salivarius* was the only species isolated among the streptococci and *Enterococus faceium.* Furthermore, while the gram positive rods consisted of spore formers 7.5% and non- spore formers 57.9% of the total bacterial isolates cultured, *Bacillus sphaericus* 45.4% each and *Bacillus mycodies* 9.0%. Among the non-spore formers *Arcanobacterium haemolyticum* was 23.8%, *Corynebacterium ulcerans* 4.7%, *Corynebacterium xerosis* 9.5%, *Corynebacterium jeikieum* 22.6%, *Corynebacterium diphtheriae* 5.9%, *Corynebacterium pseudotuberculosis* 3.5% and *Corynebacterium amycolatum* 5.9%. However, *Lactobacillus reuteri* provided 3.5%, *Lactobacillus brevis* 5.9%, *Lactobacillus acidophilus* 3.5% *Lactobacillus salivarius* 1.1%, and *Lactobacillus* casei 3.5% non-spore formers. *Listeria monocytogenes* 5.9% however was also recovered from the breast milk sample of the lactating mothers. In addition, no gram negative bacterial isolates was recovered from the breast milk sample.

Similarly, 373 bacterial isolates were cultured from the anterior nares of 70 neonates of HIV seropositive mothers from the centres. This reveals that the mean bacterial isolates recovered from each neonate was 5.3 bacteria per subject. Of these isolates, Staphylococci constituted 24.4% of the total bacterial isolates recovered, which reflects that pathogenic *S. aureus* isolates was 21.11% and among the coagulase negative staphylococci, *Staphylococcus xylosus* was the predominant bacterial isolates 24.17%, *S. epidermidis* 19.8%, *S. saprophyticus* 7.69%. *S. capitis* and *S. haemolyticus* constituted 6.6% each, *Staphylococcus warneri* constituted 5.49%, *S. schleiferi* 3.3%, *S. simulans* and *S. sciuri* constituted 2.2% each while S. *hominis* 1.09% (p=0.002).

Micrococci also constituted 16.1% of the total bacterial isolates, *Micrococcus luteus* was the predominant micrococci seen at 71.7% followed by *M. lylae* 25% followed by *M. agilis* and *M. roseus* 1.66% each. Enterococci also constituted 0.54% made up of *Enterococcus faecium* and *E. faecalis* 50% each. Furthermore, Gram positive rods constituted 41.01% of which the spore formers constituted 5.88% while the non-spore formers constituted 94.1%. Among the spore formers, *B. substilis* constituted 55.6% *B. sphaericus* 33.3% and *B. cereus* 11.1%. Of the Gram positive non spore formers bacterial isolates cultured from anterior nares of neonates of HIV seropositive mothers, *C. xerosis* constituted 35.4%, followed by *A. haemolyticum* 22.9%, *C. ulcerans* 13.9%, *C. amycolatum* 6.9%, *C. pseudotuberculosis* 2.1%, *C. pseudodiphtheriticum* 6.25%, *C. jeikieum* 9.02%, *C. diphtheriae* 0.69%. *Lactobacillus reuteri* 1.38%, *L. brevis* 0.69% and *Actinomyces israelii* 0.69%.

Furthermore, Gram negative rods constituted 17.96% of the total bacterial isolates, of which the lactose constituted 6.7% and non lactose fermenters 11.3%. Among the lactose fermenters, *E. coli* constituted 20%, *Klebsiella pneumonia*e 12%, *Enterobacter aerogenes* 40%, *Enterobacter cloacae* 8%. *Citrobacter freundii* 16% and *Citrobacter diversus* 4%. Among the non lactose fermenters, *Providencia rettgeri* constituted 38.1%, *Pseudomonas aeruginosa* 26.2%, *Pseudomonas fluorescens* 4.7%, *Proteus mirabilis* 14.2%, *Proteus vulgaris* 7.1% and *Salmonella typhimurium* 9.5%. Table 1.

Our results also showed that the antibiotypes of these bacterial isolates tested to antibiotics from breast milk samples showed resistance to beta lactam antibiotics

such as ampicillin, penicillin G, oxacillin and cefuroxime. Other antibiotics to which resistance was demonstrated were: nalidixic acid, erythromycin, fusidic acid, chloramphenicol, vancomycin, tetracycline and sulphamethoxazole/trimethoprim. Similarly, bacterial isolates cultured from the anterior nares of the neonats showed 100% resistance to penicillin, nalidixic acid, vancomycin, optochin and varying resistances to other antibiotics employed. Table 2.

Furthermore, our results showed that of the 8 S aureus isolates recovered from the breast milk samples, 5 of the isolates were methicillin resistant *Staphylococcus aureus* (MRSA) and 3 methicillin sensitive *Staphylococcus aureus* (MRSS) While 6 MRSA and 4 MSSA isolates were recovered from the anterior nares of the neonates. These isolates were further grouped into ESBL producing and non-producing strains, our results showed that from the breast milk isolates tested, none of the MRSA produced ESBL while one isolate was ESBL producing strain of the MSSA group. Similarly, 2 ESBL producing S aureus was recovered from the 6 MRSA of the nares of neonates and 1 ESBL from the MSSA. Table 3.

Table 1: Profile of Bacterial Isolates cultured from Breast milk samples of HIV seropositive and the anterior nares of their neonates.

Gram Positive Bacterial	Gram positive cocci	Type of Isolates	Grand total No. cultured	Total No. cultured from Breast milk	Total No. cultured from neonatal nares
	Pathogenic Staphylococci (coagulase positive)	*Staphylococcus aureus*	27	8	19
	Coagulase negative staphylococci	*Staphylococcus xylosus*	22	0	22
		Staphylococcus epidermidis	30	12	18
		Staphylococcus capitis	6	0	6
		Staphylococcus warneri	6	1	5
		Staphylococcus simulans	2	-	2
		Staphylococcus lentus	1	1	0
		Staphylococcus saprophyticus	16	9	7
		Staphylococcus hominis	1	-	1
		Staphylococcus haemolyticus	11	5	6
		Staphylococcus sciuri	2	-	2
		Staphylococcus cohnii	3	3	0
		Staphylococcus schleiferi	3	-	3
	Micrococci			-	
		Micrococcus luteus	48	5	43
		Micrococcus lylae	16	1	15
		Micrococcus agilis	2	1	1
		Micrococcus roseus	3	2	1
	Enterococci				
		Enterococcus faecium	2	1	1
		Enterococcus faecalis	1	-	1
	Streptococci				
		Streptococcus salivarus	1	1	0
	Gram positive Rods (spore formers)				
		Bacillus subtilis	10	5	5
		Bacillus cereus	1	0	1
		Bacillus mycoides	1	1	0
		Bacillus sphaericus	8	5	3
	Gram positive rods (Non spore formers)				
		Arcanobacterium haemolyticum	53	**20**	**33**
		Corynebacterium xerosis	61	10	51
		Corynebacterium ulcerans	22	2	20
		Corynebacterium pseudotuberculosis	6	3	3
		Corynebacterium amycolatum	15	5	10
		Corynebacterium pseudodiphtheriticum	9	-	9
		Corynebacterium jeikieum	32	19	13

		Corynebacterium diphtheriae	6	5	1
		Listeria monocytogenes	5	5	0
		Lactobacillus acidophilus	3	3	0
		Lactobacillus reuteri	5	3	2
		Lactobacillus brevis	6	5	1
		Lactobacillus salivarius	1	1	0
		Lactobacillus casei	3	3	0
		Actinomyces israelii	1	0	1
Gram Negative Rods					
	Lactose fermenters				
		Escherichia coli	5	-	5
		Klebsiella pneumoniae	3	-	3
		Enterobacter aerogenes	10	-	10
		Enterobacter cloacae	2	-	2
		Citrobacter freundii	4	-	4
		Citrobacter diversus	1	-	1
	Non lactose fermenters				
		Providencia rettgeri	16	-	16
		Pseudomonas aeruginosa	11	-	11
		Pseudomonas fluorescens	2	-	2
		Proteus mirabilis	6	-	6
		Proteus vulgaris	3	-	3
		Salmonella typhimurium	4	-	4
Total		**Total**	**518**	**145**	**373**

Table 2: Antibiotyping of Bacterial isolates cultured from Breast milk samples of HIV seropositive and the anterior nares of their neonates.

		From breast milk samples		From anterior nares of neonates	
Antibiotypes	Bacterial Isolates	Antibiogram	No. (%) of isolates recovered	Antibiogram	No. (%)
A	*Staphylococcus aureus*	AMP, P, OX, CXM, NAL, ERY, FD, CHL, VAN, TET, SXT	1(20)	P,AMP, OX,CMX,CRO,S,VAN,TET,C,E,NA,OFX,CIP,OPT, BA,SXT,FD	1(10)
B	*Staphylococcus aureus*	AMP, P, OX CXM, OFL, NAL, FD, VAN	1(20)	P, OX, CMX, S, VAN, TET, C, E, NA, OFX, CIP, OPT, FD	1(10)
C	*Staphylococcus aureus*	AMP, P, OX, CXM, NAL, FD, VAN	2(40)	P, OX, VAN, TET,NA, OFX, CIP, OPT, FD	1(10)
D	*Staphylococcus aureus*	AMP, P, CXM, OX, OFL, NAL, FD, VAN, TET	1(20)	P, OX, VAN, TET, NA, CIP, OPT, FD	3(30)
E	*Staphylococcus aureus*	AMP, P, CXM, NAL, S, FD, BA, CHL, VAN, MUP	1(33.3)	P, VAN, NA, CIP, OPT, FD	1(10)
F	*Staphylococcus aureus*	CXM, NAL, S, CHL, MUP, SXT	1(33.3)	P, VAN, NA, OPT	2(20)
G	*Staphylococcus aureus*	AMP, CXM NAL, S, CHL, VAN, TET, K, SXT	1(33.3)	P, NA, OPT	1(10)

Table 3: Comparative distribution of ESBL and MRSA *S. aureus* isolates recovered from Breast milk samples of HIV seropositive and the anterior nares of their neonates

	Maternal Breast milk				From anterior nares of neonates			
Antibiotypes	Antibiogram	No. (%) of isolates recovered	Presence of MRSA	Presence of ESBL	Antibiogram	Presence of MRSA	Presence of ESBL	No. (%)
A	AMP, P, OX, CXM, NAL, ERY, FD, CHL, VAN, TET, SXT	1(20)	MRSA	-	P,AMP, OX,CMX,CRO,S,VAN,TET,C,E,NA,OFX,CIP,OPT, BA,SXT,FD	MRSA	-	1(10)
B	AMP, P, OX CXM, OFL, NAL, FD, VAN	1(20)	MRSA	-	P, OX, CMX, S, VAN, TET, C, E, NA, OFX, CIP, OPT, FD	MRSA	-	1(10)
C	AMP, P, OX, CXM, NAL, FD, VAN	2(40)	MRSA	-	P, OX, VAN, TET,NA, OFX, CIP, OPT, FD	MRSA	ESBL	1(10)
D	AMP, P, CXM, OX, OFL, NAL, FD, VAN, TET	1(20)	MRSA	-	P, OX, VAN, TET, NA, CIP, OPT, FD	MRSA	ESBL	3(30)
E	AMP, P, CXM, NAL, S, FD, BA, CHL, VAN, MUP	1(33.3)	MSSA	-	P, VAN, NA, CIP, OPT, FD	MSSA	-	1(10)
F	CXM, NAL, S, CHL, MUP, SXT	1(33.3)	MSSA	ESBL	P, VAN, NA, OPT	MSSA	ESBL	2(20)
G	AMP, CXM NAL, S, CHL, VAN, TET, K, SXT	1(33.3)	MSSA	ESBL	P, NA, OPT	MSSA		1(10)

DISCUSSION

The study examined the biodiversity of bacteria that colonize maternal breast milk and anterior nares of neonates for vertical mother-to-child transmission of pathogens. In addition, the study also determined the antibiogram of pathogenic *S. aureus* recovered from mother and neonate samples to a varying classes of antibiotics and tested for extended beta lactamase in these samples. The result of the study revealed that numerous and diverse isolates were recovered from the anterior nares of neonates (72.0%) and breast milk samples (28%). Similarly, bacterial isolates recovered from both breast milk and anterior nares samples consisted of primarily commensals *Arcanobacterium haemolyticum* and species of corynebacteria and coagulase negative staphylococci.

Of these commensals, corynebacterium which are often reported as part of the skin flora It was previously seen as contaminants in the laboratories. Much interest in these organism is on the increase because it accounts for a major proportion of isolates recovered from breast milk samples, tissue culture, pus and deep wound. Furthermore, Corynebacteria have been reported to be isolated among HIV infected and uninfected patients from rectal swabs, UTI, oropharynx and HVS in our environment.[16,17] Our results showed that *C. jeikeium* was obviously high in the number recovered compared to other Corynebacterium isolated from the breast milk and anterior nares. *C. jeikeium* are well recognised pathogens and have been reported among HIV patients in previous studies done in this region.[16] Our study revealed 10.3% of the bacterial isolates in breast milk of HIV-seropositive mothers were lactobacillus species comprising of 5 *L. brevis*, 3 each of *L. reuteri, L. casei and L. acidophilus*, 1 *L. salivarius* and 20% *Lactobacillus reuteri* isolate, which is interesting as they are major probiotics while 2 *L. reuteri* and 1 *L. brevis* isolate was recovered from the anterior nares of the neonates.

Some studies have shown that human milk lactobacilli play several roles in the infant gut, such as the increase in the production of functional metabolites such as butyrate, which is the main energy source for colonocytes and a relevant compound in the modulation of intestinal function.[18] As a result, they improve the intestinal habitat, with an increase in fecal moisture, and in stool frequency and volume.[19] They also contribute to the reduction of the incidence and severity of infections in the breast-fed infant employing different mechanisms.[20] According to authors by Sinkiewicz and Ljunggren, (2008),[21] 15% of their study population were carriers of *Lactobacillus reuteri* in their breast milk samples which corroborates our study where 20% of the lactobacillus species cultured was *Lactobacillus reuteri*.

This high occurrence of commensals like lactobacilli in breast milk of these women may be associated with microbial competition of nutrients that ward off pathogens such as *S aureus* through production of hydrogen peroxide and bacterocin,[22] a mechanism recognised as bacteriotheraphy in the microbial food system.[23]

S. aureus isolates are considered as a skin colonizers and inhabit the mucous membranes of humans and animals. Infection with *S aureus* occurs after an aberration in skin or mucosal barriers and are high in occurrence in the hospital environment as hospital acquired- methicillin resistance *S aureus* (HA-MRSA). This HA-MRSA when transferred to the community, spread rapidly among the community and are transferred from person- to- person as community acquired methicillin resistant *S aureus* (CA-MRSA). In recent times, acquisition of MRSA have been traced to strains from livestock and are referred to as Livestock Acquired –Methicillin *S. aureus* (LA-MRSA).[24] These MRSA (HA-MRSA, CA-MRSA, LA-MRSA) in molecular characteristics, harbours some difference especially in SCC*mec* elements, resistant to multiple antibiotics and exotoxin gene and varying mobile genetic element profile.[25] While varying clones of *S. aureus* exit, infected breastfeeding has been reported to be associated with severe neonatal disease, including infantile pneumonia, neonatal sepsis and food poisoning.[26,27] Our study also showed that 62.5 % of the *S. aureus* cultured from breast milk were MRSA. while 60% of their infant nares was also MRSA. MRSA are strains of *S. aureus* that are resistant to methicillin, and related beta-lactam antibiotics (e.g. penicillin and cephalosporin).[28] MRSA have evolved resistance not only to beta-lactam antibiotics, but to several classes of antibiotics.[29] Our result showed that of the 8 *S. aureus* recovered from breast milk samples, 5 of the isolates were MRSA while 3 isolates were MSSA. Similarly, of the 10 *S. aureus* cultured from the anterior nares of the neonates, 6 of the isolates were MRSA while 4 were MSSA. It has been reported that more people now die from MRSA infection than from AIDS.[30] Though, most invasive MRSA infections could be traced to a hospital stay or some other health care exposure, about 15% of invasive infections occurred in people with no known health care risk. Studies by Sibhghatulla *et al.*, have suggested that the high level of multi-resistant isolates are most likely to be extended spectrum beta lactamase producers.[31] In addition to hydrolysing the beta lactam ring, ESBL producing bacterial display resistance to other classes of antibiotics thus posing a therapeutic concern.[32] However, this mechanisms of antibiotics resistance acqusition have been linked to elaboration of enzymes, the presence of resistance and virulence genes that modify antibiotics, bacteria and host cells.[33] Furthermore, the mechanisms of action of ESBLamase confers resistance to the penicillin, third generation cephalosporins and aztreonam, but are inhibited by the clauvunic acid.[34] This implies that ESBL producing bacterial in breast milk sample of HIV patients may be associated with antibiotic treatment failure, especially excessive exposure to the aminoglycosides, quinolones, fluoroquinolones, tetracyclines, chloramphenicol, and

sulfamethoxazole-trimethoprim. Other studies have suggested that the presence of plasmid.[35] on bacterial cells that carry ESBL genes which are often located on transposable elements or intergron results in resistance gene transfer function. This suggest that ESBL organism gene borne/carried on a plasmid can spread multiple antibiotic resistance from between species and to other genera.

Paradoxically, no gram negative isolate was cultured from any of the breast milk samples, whereas 26.19% was recovered from nares of their neonates. *Pseudomonas aeruginosa* isolates may have been acquired from other sources other than breast milk and has emerged as one of the most common causes of Gram negative bacteraemia and pneumonia in HIV-infected hospitalized.[36] which implies that acquisition of *Pseudomonas* species from neonatal nares may be from other sources other than breast milk during breastfeeding.

In conclusion, Breast milk account for complete nutritional diets recommended for infants, pathogenic bacterial colonization and multiple antibiotics resistance in breast milk samples is of great public health concern. This calls for routine bacterial colonization checks in lactating women especially in HIV infected women to reduce vertical transmission of pathogens from mother-to- child.. In addition, the development and spread of ESBL producing organism is worrisome because of rapid spread of resistance to same species and even to other genera. The pressure of unregulated misuse of antibiotics with poor drug quality like in the consumption of fake or expired drugs may have encouraged unprecedented wide spread of resistant strain. This calls that drug policies/law be enacted in our environment to reduce the burden of wide spread of multiple resistant pathogenic *S. aureus*.

ACKNOWLEDGEMENT

The authors acknowledge the Doctors, Dr. P.O. Osho, Dr. V. Koledoye and Dr. Akintan Adesina, Dr. A K Aderoba, Miss Love Awoniyi, nursing staff and laboratory technologists at the four healthcare centres.

Conflicts of Interest: The authors declare no conflicts of interest.

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