Asian Journal of Biological and Life Sciences | Original Research

Bacterial colonies in cervicovaginal mucosa of normal spontaneous intraoperative delivery patients 15 to 41 Years Old

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Submitted : 09.05.2015 Accepted : 10.07.2015 Published : 30.08.2015

Abstract

To identify common organism colonizing the cervico-vaginal mucosa of normal spontaneous intraoperative delivery patient's swabs were collected from 50 subjects prior to child birth. Swabs were used for Gram-stained slide preparation and bacterial cultures. Swabs for culture were placed in Thioglycollate broth, incubated at 35^0 C for 24 hours and streaked on Blood agar plates (BAP). BAPs were incubated at 35^0 C for 24 hours.Colonies yielded 97 bacterial and 1 fungal isolates. Bacteria isolated were *Lactobacilli* spp. 36 (36.73%), *E. coli* 19 (19.31%), CoNS 17 (17.35%), *S. aureus* 13 (13.27%), *K. pneumoniae* 11 (11.22%), and *P. mirabilis* 1 (1.02%). Multiple colonies of organisms were observed; infected subjects yielded higher rates of multiple colonization 8 (40%) with *E. coli* and *S. aureus* found to be predominantly co-infecting subjects with greater than 10 pus cells LPF. Microscopic evaluation of pus cells were insufficient to rule out bacterial colonization highlighting the importance of culture methodologies. Multiple colonization of organism suggests molecular influences on growth and biofilm formation aside from specie to specie interaction. Application of 2% erythromycin bath suggests inhibition of colonization of infants after birth *thereby negating* the formation of inflammatory response. These data can provide information for maternal prophylaxis and post-delivery infant management.

Key words : *Escherichia coli*, Coagulase-negative Staphylococci, Erythromycin, Pus cells, *Streptococcus agalactiae.*

INTRODUCTION

It is approximated that there are 131.4 million births per year with an average of four births each second everyday[1]. In the USA alone 24,000 infants died in 2011 with the most common factors ranging from serious birth defect, preterm birth, Sudden Infant Death Syndrome (SIDS), maternal complications of pregnancy, and injury (suffocation) [2]. In 2014 infant mortality rate in the Philippines is estimated at 17.64 deaths/1,000 live births. These statistics are often used as indicators of the level of health in a country [3] and could be significantly decreased with the advocacy of knowledge in each of the determining factors which predominate in the strata of society concerned.

The leading cause of the death in term-infants is bacterial in origin: pneumonia being responsible for 9%, neonatal tetanus for 14%, sepsis/meningitis for 7% and diarrhea for 2% of the deaths. The incidence of neonatal sepsis ranges from 7.8 to 21.8/1,000 live births, with case fatality rates as high as 38%. Appropriate preventive and therapeutic intervention must be done in order to prevent this high mortality. However, lack of etiological data from rural areas has blocked the progress [4, 5]. In low and middle income countries, maternal morbidity and mortality has remained remarkably high because the important determinants to poor pregnancy outcomes are sparse and poor. This includes information on bacterial and viral maternal infections. Lack of information can also undesirably impact donor interest and international commitment [6]. Lack of knowledge of the common etiological agents compounded by the scarcity of laboratory and culture facilities in most primary and secondary health facilities and delay in and reluctance of the family to seek care which often results in most babies succumbing to serious infections within their homes without coming to seek for any medical intervention; these factors cause neonatal sepsis in different communities and are important aspects to consider in order to devise community-based strategies for managing serious infections [7]. *Escherichia coli* and Group B Streptococcus (GBS) are the usual causative agents in the early onset sepsis which is regarded as maternally-acquired and usually found in the maternal genital tract, whereas late onset sepsis is considered environmental in origin either hospital or community acquired. Coagulase-negative Staphylococci (CoNS), *Staphylococcus aureus*, Gram-negative organisms such as *Klebsiella* spp. and *Pseudomonas* spp. are commonly implicated organism in hospital-acquired infections [7]. *Streptococcus agalactiae* can be recovered from various sites in healthy adults. The urethra, vagina, perineum and anorectal region have all been suggested as the prime site of carriage. GBS colonize the vaginal and rectal areas of 10% to 30% of pregnant women. The most important determining factor is its presence in the mother's vagina. Cases of *S. agalactiae* in the Philippines have been documented and it is said to be frequent in the low income population [8].

One of the most useful intervention strategies among the high-risk women would be the earlier recognition of the invasive pathogen. CDC's guidelines recommend that a pregnant woman be tested, or screened, for GBS in her vagina and rectum when she is 35 to 37 weeks pregnant [9]. Detection of heavy colonization has traditionally relied on culture methods, which may not be feasible in some health care settings and require 1-3 days before results are available [10]. Thus reliable statistics on common colonizing agents will provide adequate information for prophylactic treatment of mothers and post-delivery management of infants.

METHODS

Respondents

From a single Department of Health (DOH) accredited tertiary hospital,50 females who qualified under normal spontaneous intraoperative delivery were chosen for the study. All respondents consented to be part of the study as facilitated by the obstetrics department with permission from the hospital administration. Patients ranged from 15 to 41 years of age and presented with no illness during the time of delivery. Patients

were given a corresponding code to denote their sample and to safeguard the anonymity of their identity. Additionally, monitoring of infants was done for 1 month after delivery to check for any reports of post-delivery infection.

Collection of Cervicovaginal swab

Subjects were at their lithotomy position prior to child birth; clinical staff responsible for specimen collection were trained and licensed as obstetrician gynecologists. Removal of excess mucus from the vaginal area using sterile cotton pledget was done prior to specimen collection during the crowning of the neonate. A new swab was then inserted into the vaginal canal and swabbed onto the cervix and walls of the vagina. Two swabs were collected; the first swab was streaked on a dry clean glass slide for Gram staining and the second swab was placed in two ml of Thioglycollate broth. Gram stained slides were examined under low power objective (LPO) for white blood cell counts.

Microscopic examination

Licensed obstetrician gynecologists examined Gram stained slides under LPO to count the number of polymorphonuclear neutrophils (pus cells) to determine whether the patient is exhibiting a non-inflammatory or inflammatory cellular response during the time of collection. Patients with pus cells less than 8-10/LPF were considered negative for infection while counts of greater than 10/LPF were considered positive for infection. Other parameters noted were epithelial cells and Gram reaction and morphology of bacterial/fungal isolates. Plump Alpha-hemolytic Gram-positive bacilli were presumptively identified as *Lactobacilli* spp. and yeast cells were not subjected to any further identification.

Culture

Thioglycollate broths were incubated at 35^0 C for 24 hours and streaked on blood agar plates (BAP).BAPs were incubated at 35^0C for 24 hours. Growth was observed according to quantity, colonial morphology and hemolytic reaction. Microscopic examination was done to verify Gram reaction, morphology and arrangement of bacterial growth as being cocci or bacilli and fungal growth were described by the presence of yeast cells.

Biochemical Identification

Conventional biochemical testing panel of Citrate, Triple sugar iron (TSI), Lysine iron agar (LIA), Methyl-red (MR), Voges-Proskauer (VP), Sulfide, Indole and Motility (SIM), and urease were done to verify the identity of Gram-negative bacilli; Catalase and Coagulase testing was done to verify the identity of Gram-positive cocci. Additionally, CAMP test was done on all Beta-hemolytic colonies to confirm whether isolates are *S. agalactiae.*

RESULTS

Vaginal smears subjected for microscopic examination were able to identify the following parameters respectively: Epithelial cells, Pus cells and Bacterial cells. Epithelial cells ranged from rare to many per LPF. Pus cells ranged from 0 to greater than 25 cells / LPF. Gram positive cocci, Gram positive bacilli, Gram negative bacilli and yeast cells were observed, cultured and biochemically tested except for yeast cells. From 50 respondents a total of 97 bacterial isolates and 1 fungal isolate were observed in BAPs. Isolates observed were *Lactobacilli* spp. 36 (36.73%), *Escherichia coli* 19 (19.31%), Coagulase-negative Staphylococci 17 (17.35%), *Staphylococcus aureus* 13 (13.27%), *Klebsiella pneumoniae* 11 (11.22%), *Proteus mirabilis* 1 (1.02%), and yeast cells 1 (1.09%).12 (12.30%) Beta-hemolytic Gram positive cocci colonies were isolated, all of which are CAMP test negative indicating that there is no *Streptococcus agalactiae* colonization in all the members of the study group. Distribution of hemolytic colonies were as follows: Alpha (α): 36 (37.11%), Beta (β): 31 (31.96%), Gamma (γ): 31 (30.93%). Except for *Lactobacilli* spp. which is a normal flora of the vaginal canal, single and multiple presences of bacteria in combinations were observed: Lactobacilli only 3 (6%), single colonization 31 (62%) and multiple colonizations16 (32%).

Multiple colonization observed in our study with common combinations of organisms were CoNS and *E. coli* 6 (37.5%), *S. aureus* and *E. coli* 5 (31.25%), CoNS and *K. pneumoniae* 3 (18.75%), *S.aureus* and *K. pneumoniae* 2 (12.50%). As observed in our results, multiple colonizations is higher in 20 respondents with greater than 10 pus cells per LPF 8 (40.00%) compared to 30 respondents with less than 8 pus cells per LPF 5 (17%). Multiple colonization observed in our normal respondents were CoNS and *E. coli* 2 (40%), *S. aureus* and *K. pneumoniae* 2 (40%), *S. aureus* and *E.coli* 1 (20%) while multiple colonization observed in 30 infected respondents were *S. aureus* and *E. coli* 4 (50%), CoNS and *K. pneumoniae* 3 (38%), CoNS and *E. coli* 1 (12%).

Normal

From 50 (antibiotic free) respondents, 30 (60%) aged 15 to 41 years old were identified to be without infection (pus cells less than 8-10/LPF) and a total of 54 bacterial isolates were observed in BAP after incubation at 35^0 C for 24 hours. Isolates were identified as follows: *Lactobacilli* spp. 21 (38.89%), *S. aureus* 9 (16.67%), CoNS 9 (16.67%), *E. coli* 9 (16.66%), *L.pneumoniae* 6 (11.11%). 16 colonies which exhibited Beta-hemolytic patterns were isolated and were identified as *S. aureus* 8 (50%) and, *E. coli* 8 (50%). Gram-positive Beta-hemolytic cocci positive for CAMP test and Gram-positive Alpha-hemolytic cocci suspected of being *S. pneumoniae* were absent.

Infected

From 50 (antibiotic free) respondents, 20 (40%) aged 19 to 37 years old were identified to be with infection (pus cells greater than 10/LPF), 9 (45%) of which presented with greater than 25 pus cells per LPF. None of these respondents presented with any signs or symptoms of inflammatory response at the time. A total of 43 bacterial isolates and one fungal growth were observed in BAP after incubation at 35^0 C for 24 hours. Isolates were identified as follows: *Lactobacilli spp*. 15 (34.09%), *Escherichia coli* 11 (25.00%), Coagulase-negative Staphylococci 8 (18.18%), *Staphylococcus aureus* 4 (09.09%), *Klebsiella pneumoniae* 4 (09.09%), *Proteus mirabilis* 1 (02.27%), and yeast cells 1 (02.27%).14 colonies which exhibited beta-hemolytic patterns were isolated and were identified as *S. aureus* 4 (29%) and *E. coli* 10 (71%). Gram-positive Beta-hemolytic cocci positive for CAMP test and Gram-positive Alpha-hemolytic cocci suspected of being *S. pneumoniae* were absent.

DISCUSSION

A total of 50 respondents are considered sufficient to support the evaluation of any intrauterine infections as a major cause of preterm labor[11]. The results of our study showed that the predominant pathogenic organism to colonize the cervico-vagina is *E. coli* with 19 (19.31%) isolates from a total population of 97 bacterial isolates. This result is consistent with data published by Saini *et al.,* 2008 titled Microbiological Surveillance in Antenatal

Table 1. CONSOLIDATED MICROSCOPIC AND CULTURE DATA RESULT FROM 50 NORMAL SPONTANEOUS INTRAOPERATIVE DELIVERY PATIENTS FROM RICARDO P. RODRIGUEZ MEMORIAL HOSPITAL 2013

PUS CELLS LESS THAN 8-10/LPF (NEGATIVE FOR INFECTION)

Control No.	Age	Micro-organism/ Cells	Gram Stain		BLOOD AGAR PLATE					BIOCHEMICAL										CAMP	Identified Organism
			Value	Morphology	Growth	Colony Description	Hemolytic Pattern			Catalase	Coagulase	Citrate	TSI	MR	VP	LIA	SIM				
							α	β	γ								S	I	M		
		G(+) Cocci																			
NSID	32	G (+) Bacilli	Mod		Many	Medium,gray	X			Neg											*Lactobacilli*
13 - 001		G (-) Bacilli																			
		EC	Few																		
		PC	2-4/lpf																		
	18	G(+) Cocci	Mod	Singly	Many	Creamy, Butter looking		X		Pos	Pos									Neg	*S. aureus*
NSID		G (+) Bacilli																			
13 - 002		G (-) Bacilli																			
		EC	Mod																		
		PC	0-1/lpf																		
	27	G(+) Cocci	Few	Singly	Mod	Creamy, Butter looking		X		Pos	Pos									Neg	*S. aureus*
NSID		G (+) Bacilli	Few		Few	Medium, Creamy	X			Neg											*Lactobacilli*
13 - 003		G (-) Bacilli																			
		EC	Few																		
		PC	1-2/lpf																		
	21	G(+) Cocci																			
NSID		G (+) Bacilli	Mod		Many	Medium, gray	X			Neg											*Lactobacilli*
13 - 007		G (-) Bacilli																			
		EC	Rare																		
		PC	2-5/lpf																		
	32	G(+) Cocci	Many	Singly	Many	Creamy, butter looking		X		Pos	Pos									Neg	*S. aureus*
NSID		G (+) Bacilli																			
13 - 008		G (-) Bacilli																			
		EC	Mod																		
		PC	0-1/lpf																		
	26	G(+) Cocci	Few	Singly/ Cocci	Few	White creamy			X	Pos	Neg										*CoagNeg*
NSID		G (+) Bacilli	Mod		Mod	Medium gray	X			Neg											*Lactobacilli*
13 - 010		G (-) Bacilli																			
		EC	Rare																		
		PC	2-4/lpf																		
	34	G(+) Cocci																			
NSID		G (+) Bacilli	Few		Few	Medium gray	X			Neg											*Lactobacilli*
13 - 013		G (-) Bacilli	Few		Mod	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg		*K. pneumoniae*
		EC	Few																		
		PC	0-2/lpf																		
NSID	25	G(+) Cocci																			
13 - 014		G (+) Bacilli	Many		Many	Medium, gray	X			Neg											*Lactobacilli*
		G (-) Bacilli																			
		EC	Few																		
		PC	0-3/lpf																		
	26	G(+) Cocci	Many	Pairs	Few	White, creamy			X	Pos	Neg										*CoagNeg*
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg											*Lactobacilli*
13 - 018		G (-) Bacilli	Mod		Few	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Few																		
		PC	5-8/lpf																		

	34	G(+) Cocci	Few		Mod	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli	Rare		Few	Medium, gray	X			Neg												*Lactobacilli*
13- 019		G (-) Bacilli																				
		EC	Few																			
		PC	0-1/lpf																			
	25	G(+) Cocci																				
NSID		G (+) Bacilli	Many		Many	Medium, gray	X			Neg												*Lactobacilli*
13 - 021		G (-) Bacilli																				
		EC	Few																			
		PC	3-5/lpf																			
	30	G(+) Cocci																				
NSID		G (+) Bacilli	Few		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 022		G (-) Bacilli	Mod		Mod	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coi*
		EC	Few																			
		PC	4-8/lpf																			
	28	G(+) Cocci	Mod	Short chains	Few	Creamy, butter-looking		X		Pos	Pos									Neg	*S. aureus*	
NSID																						
13 - 023		G (+) Bacilli	Many		Few	Medium, gray	X			Neg												*Lactobacilli*
		G (-) Bacilli	Mod		Few	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg			*K. pneumoniae*
		EC	Few																			
		PC	2-5/lpf																			
	41	G(+) Cocci																				
NSID		G (+) Bacilli	Mod		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 024		G (-) Bacilli	Few		Mod	Large, moist,gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Mod																			
		PC	2-5/lpf																			
	24	G(+) Cocci																				
NSID		G (+) Bacilli																				
	15	G(+) Cocci	Few		Few	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli	Rare		Mod	Medium, gray	X			Neg												*Lactobacilli*
13 - 027		G (-) Bacilli																				
		EC	Mod																			
		PC	0-3/lpf																			
	20	G(+) Cocci	Rare	Singly	Few	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli	Rare		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 029		G (-) Bacilli	Few		Few	Large, moist, gray		X		Pos		Neg	A/A w/ gas	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Many																			
		PC	2-4/lpf																			
	24	G(+) Cocci	Few		Few	Creamy, butter		X		Pos	Pos									Neg	*S. aureus*	
NSID		G (+) Bacilli																				
13 - 030		G (-) Bacilli	Mod		Mod	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC																				
		PC																				
	23	G(+) Cocci	Mod	Singly	Mod	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 031		G (-) Bacilli																				
		EC	Rare																			
		PC	8-10/lpf																			
	24	G(+) Cocci																				
NSID		G (+) Bacilli																				
13 - 032		G (-) Bacilli	Mod		Many	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg			*K. pneumoniae*
		EC	Mod																			
		PC	3-5/lpf																			
	26	G(+) Cocci																				
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 033		G (-) Bacilli	Many		Mod	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg			*K. pneumniae*
		EC	Mod																			
		PC	2-4/lpf																			

NSID 13 - 034	34	G(+) Cocci	Mod	Clusters, Singly	Mod	Large, white			X	Pos	Pos									Neg	*S. aureus*
		G (+) Bacilli																			
		G (-) Bacilli																			
		EC	Few																		
		PC	0-1/lpf																		
NSID 13 - 036	25	G(+) Cocci	Rare		Few	Creamy, butter-looking		X		Pos	Pos									Neg	*S. aureus*
		G (+) Bacilli	Rare		Few	Medium, gray	X			Neg											*Lactobacilli*
		G (-) Bacilli	Mod		Few	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg		*K. pneumoniae*
		EC	Rare																		
		PC	0-2/lpf																		
NSID 13 - 038	24	G(+) Cocci	Mod	Pairs, singly	Many	Creamy, butter-looking		X		Pos	Pos									Neg	*S. aureus*
		G (+) Bacilli																			
		G (-) Bacilli																			
		EC	Few																		
		PC	2-4/lpf																		
NSID 13 - 039	25	G(+) Cocci	Mod	Singly	Mod	Creamy, butter-looking		X		Pos	Pos									Neg	*S. aureus*
		G (+) Bacilli	Many		Few	Medium, gray	X			Neg											*Lactobacilli*
		G (-) Bacilli																			
		EC	Few																		
		PC	2-4/lpf																		
NSID 13 - 041	27	G(+) Cocci	Mod	Clusters, singly	Many	White, creamy			X	Pos	Neg										*CoagNeg*
		G (+) Bacilli																			
		G (-) Bacilli																			
		EC	Mod																		
		PC	0-2/lpf																		
NSID 13 - 045	25	G(+) Cocci	Rare	Singly	Few	White, creamy			X	Pos	Neg										*CoagNeg*
		G (+) Bacilli																			
		G (-) Bacilli	Few		Mod	Large, moist, gray		X		Pos		Neg	A/A w/G	Neg	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Many																		
		PC	2-5/lpf																		
NSID 13 - 046	28	G(+) Cocci	Few	Singly	Few	White, creamy			X	Pos	Neg										*CoagNeg*
		G (+) Bacilli	Few		Few	Medium, gray	X			Neg											*Lactobacilli*
		G (-) Bacilli	Few		Few	Large, mucoid, gray			X	Pos		Pos	K/A	Neg	Pos	K/A	Neg	Pos	Pos		*E. coli*
		EC	Many																		
		PC	3-6/lpf																		
NSID 13 - 047	28	G(+) Cocci																			
		G (+) Bacilli	Rare		Few	Medium, gray	X			Neg											*Lactobacilli*
		G (-) Bacilli	Rare		Mod	Large, moist, gray		X		Pos		Neg	A/A	Pos	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Mod																		
		PC	6-10/lpf																		
NSID 13 - 049	22	G(+) Cocci																			
		G (+) Bacilli	Rare		Mod	Medium, gray	X			Neg											*Lactobacilli*
		G (-) Bacilli	Rare		Few	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg		*K. pneumoniae*
		EC	Mod																		
		PC	2-4/lpf																		

α = Alpha-hemolytic, β = Beta-hemolytic, γ = Gamma-hemolytic (non-hemolytic), SIM = Sulfide, Indole, Motility, EC = Epithelia cells, PC = Pus cells, H_2S = Hydrogen sulfide, G = gas, Red boxes = 0-2/LPF *K. pneumoniae*, Orange boxes = β *S.aureus*, Blue Boxes = β *E. coli*

Care to Prevent Preterm Labor where *E. coli* was the predominant aerobic bacterial isolate (15 out of 50)[11]. Further, Ekanem *et al.*,2012 demonstrated 17%-59% isolation of *E. coli*, CoNS, and Staphylococcus from 225 subjects with *Lactobacilli* spp. being 75.6% of the total isolates [12] which also corroborates the data of our study with *Lactobacilli* spp. being the predominant nonpathogenic bacterial isolate at 36.73%. Additionally, it was also noted in their data that *Proteus* spp. along with *Clostridium* spp. are the less frequently isolated organisms which were also consistent with our study wherein *Proteus mirabilis* was only 1.02% of the total bacterial isolates.

In our 30 non-infected respondents, *Lactobacilli* spp. predominated as the nonpathogenic bacterial isolate at 38.89% while *E. coli, S. aureus* and CoNS all had the same isolation rates of 16.67% with *K. pneumoniae* as the least isolated organism at 11.11% from a total of 54 bacterial isolates. Bayo *et al.,* 2009

Table 2. CONSOLIDATED MICROSCOPIC AND CULTURE DATA RESULT FROM 50 NORMAL SPONTANEOUS INTRAOPERATIVE DELIVERY PATIENTS FROM RICARDO P. RODRIGUEZ MEMORIAL HOSPITAL 2013

PUS CELLS GREATER THAN 8-10/LPF (POSITIVE FOR INFECTION)

Control No.	Age	Micro-organism/ Cells	Gram Stain		BLOOD AGAR PLATE					BIOCHEMICAL										CAMP	Identified Organism
			Value	Morphology	Growth	Colony Description	Hemolytic Pattern			Catalase	Coagulase	Citrate	TSI	MR	VP	LIA	SIM				
							α	β	γ								S	I	M		
	19	G(+) Cocci																			
NSID		G (+) Bacilli																			
13 - 004		G (-) Badlli	Many		Many	Large, gray, moist		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Few																		
		PC	10-12/lpf																		
	21	G(+) Cocci																			
NSID		G (+) Bacilli	Many		Few	Medium, Gray	X			Neg											*Lactobacilli*
13 - 005		G (-) Badlli	Many		Mod	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos		*E.coli*
		EC	Many																		
		PC	>25/lpf																		
	25	G(+) Cocci	Mod	Pairs	Few	White, creamy			X	Pos	Neg										*CoagNeg*
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg											*Lactobacilli*
13 - 006		G (-) Badlli	Many		Few	Large, moist, gray		X		Pos		Neg	A/Aw/G	Pos	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Mod																		
		PC	>25/lpf																		
	21	G(+) Cocci	Mod	Short Chains	Mod	White creamy			X	Pos	Neg										*CoagNeg*
NSID		G (+) Bacilli	Few		Few	Medium gray	X														*Lactobacilli*
13 - 009		G (-) Badlli																			
		EC	Mod																		
		PC	15-20/lpf																		
	26	G(+) Cocci	Few	Cluster/Singly	Few	White Creamy			X	Pos	Neg										*CoagNeg*
NSID		G (+) Bacilli	Many		Mod	Medium gray	X			Neg											*Lactobacilli*
13 - 011		G (-) Badlli																			
		EC	Few																		
		PC	22-25/lpf																		
	37	G(+) Cocci																			
NSID		G (+) Bacilli	Mod		Few	Medium gray	X			Neg											*Lactobacilli*
13 - 012		G (-) Badlli	Many		Mod	Large, moist gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Mod																		
		PC	>25/lpf																		
	23	G(+) Cocci																			
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg											*Lactobacilli*
13 - 015		G (-) Badlli	Many		Mod	Large, gray, swarming			X	Pos		Pos	A/A w/H_2S	Pos	Neg	K/K	Pos	Neg	Pos		*P. mirabilis*
		EC	Mod																		
		PC	10-12/lpf																		
	24	G(+) Cocci	Mod	Singly	Few	Creamy, Butter-looking		X		Pos	Pos									Neg	*S. aureus*
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg											*Latobacilli*
13 - 016		G (-) Badlli	Many		Few	Large, moist, gray		X		Pos		Neg	A/A w/ G	Pos	Neg	K/K	Neg	Pos	Pos		*E.coli*
		EC	Mod																		
		PC	10-12/lpf																		
	23	G(+) Cocci	Rare		Few	White, creamy			X	Pos	Neg										*CoagNeg*
NSID		G (+) Bacilli	Rare		Few	Medium, gray	X														*Lactobacilli*
13 - 017		G (-) Badlli	Few		Few	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg		*K. pneumoniae*
		EC	Few																		
		PC	10-12/lpf																		
	20	G(+) Cocci	Mod	Pairs, Short chains	Few	Creamy, butter-looking		X		Pos	Pos									Neg	*S. aureus*
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg											*Lactobacilli*
13 - 020		G (-) Badlli	Many		Few	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos		*E. coli*
		EC	Mod																		
		PC	>25/lpf																		

	26	G(+) Cocci																				
NSID		G (+) Bacilli	Mod		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 025		G (-) Bacilli	Many		Mod	Large, moist,gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Few																			
		PC	12-15/lpf																			
	25	G(+) Cocci																				
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 028		G (-) Bacilli	Many		Mod	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg			*K. pneumoniae*
		EC	Many																			
		PC	12-15/lpf																			
	20	G(+) Cocci	Few	Singly	Mod	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli																				
13 - 035		G (-) Bacilli	Few		Few	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg			*K. pneumoniae*
		EC	Mod																			
		PC	>25/lpf																			
	41	G(+) Cocci	Many	Pairs, singly	Many	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli																				
13 - 037		G (-) Bacilli																				
		EC	Few																			
		PC	12-15/lpf																			
	35	G(+) Cocci	Many		Mod	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli																				
13 - 040		G (-) Bacilli	Mod		few	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Mod																			
		PC	12-15/lpf																			
	30	G(+) Cocci																				
NSID		G (+) Bacilli	Mod		Many	Medium, gray	X			Neg												*Lactobacilli*
13 - 042		G (-) Bacilli																				
		EC	Mod																			
		PC	12-15/lpf																			
	24	G(+) Cocci	Few	Singly, chains	Few	White, creamy			X	Pos	Neg											*CoagNeg*
NSID		G (+) Bacilli	Few		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 043		G (-) Bacilli	Many		Few	Large, mucoid, gray			X	Pos		Pos	A/A w/G	Neg	Pos	K/K	Neg	Neg	Neg			*K. pneumoniae*
		EC	Many																			
		PC	>25/lpf																			
	21	G(+) Cocci	Rare	Singly	Few	Creamy, butter-looking		X		Pos	Pos									Neg		*S. aureus*
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 044		G (-) Bacilli	Many		Few	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Many																			
		PC	>25/lpf																			
NSID	26	G(+) Cocci	Few	Pairs, singly	Mod	Cream, butter		X		Pos	Pos									Neg		*S. aureus*
13 - 048		G (+) Bacilli																				
		G (-) Bacilli	Few		Few				X	Pos		Neg	A/A	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Many																			
		PC	>25/lpf																			*Yeast cells*
		yeast cells	Rare																			
	37	G(+) Cocci																				
NSID		G (+) Bacilli	Many		Few	Medium, gray	X			Neg												*Lactobacilli*
13 - 050		G (-) Bacilli	Many		Mod	Large, moist, gray		X		Pos		Neg	A/A w/G	Pos	Neg	K/K	Neg	Pos	Pos			*E. coli*
		EC	Mod																			
		PC	>25/lpf																			

Legend:
α = Alpha-hemolytic, β = Beta-hemolytic, γ = Gamma-hemolytic (non-hemolytic), SIM = Sulfide, Indole, Motility, EC = Epithelia cells, PC = Pus cells, H_2S = Hydrogen sulfide, G = gas, Yellow boxes = PC >25/LPF, Orange boxes = β *S.aureus*, Blue Boxes = β *E. coli*

provided published data from 623 healthy pregnant women with *E. coli* as the second most isolated organism followed by *Proteus* spp., diptheroids, CoNS, *S. aureus*, *S. agalactiae*, and *K. pneumoniae*. *Lactobacilli* spp. was the predominant isolate as it plays a crucial role in maintaining the acidic environment in the vagina which inhibits colonization of pathogenic organisms [13], further *Lactobacilli* spp. are known to form biofilms which effectively hinders the attachment of invading organisms [14].

A study by English and Newton 2007, Young Infant Sepsis: Aetiology, Antibiotic Susceptibility and Clinical Signs identified that hospital acquired infections are mostly caused by Gram-negative bacteria specifically *E. coli*, *K. Pneumoniae* and *Pseudomonas* spp. while community-acquired infections were attributed to Gram-positive organisms like *S. aureus,* GBS, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* with *E.coli, K. pneumoniae*, *S. aureus*, and GBS as the most common causative agents of infection across all settings [15]. This publication supports the result from our 20 infected respondents where the predominant pathogenic isolate was *E. coli* (25.58%) with *S. aureus* and *K. pneumoniae* both at 9.30%. It is also important to note our isolation of CoNS (18.61%) from respondents with less than 8 Pus cells per LPF (16.67%) as it has been well documented to cause morbidity and mortality in very low birth weight (VLBW) infants with sepsis some of whom have been diagnosed with infective endocarditis or aortic thrombi [16]. Khamees, 2010 conducted a study on 360 women respondents with complaints from genital tract infection; bacterial and candida infection were at 75.5% and 13.12% respectively [17]; in comparison isolation of bacteria and fungi in our study was at 99% and 1% respectively. The predominance of *E. coli*, a lactose fermenting member of the family enterobacteriacea as the pathogenic isolate from cervico-vaginal samples particularly from patients with urinary tract infections (UTI) could be attributed to increased levels of amino acids and lactose during pregnancy which encourages bacterial growth; it could also be by fecal contamination due to poor hygiene[18] and contaminated water sources. Similarly, Bayo, *et al*, 2002 isolated *K. pneumoniae* and *Proteus* spp. among 623 pregnant women and suggested it was from contamination by rectal microorganism that may predispose for UTI[13]. Predisposing factors for the colonization of our respondents with the noted bacterial isolates can be attributed to the above-mentioned as majority of our study population belongs to the low-income stratum of society.

Although *Streptococcus agalactiae* is well documented to colonize pregnant women, low incidence of colonization has been consistent in several studies. Crisostomo *et al*., 2007 with 136 parturients showed 10 (7.4%) culture positivity for GBS[19]. Chaudhry *et al*., 2010 with 200 pregnant respondents demonstrated GBS carriage rate of 8.5%. The rate of GBS colonization in pregnant females varies from 5% to 30% with different geographical distribution[20]. Even a molecular study by Lysakowska *et al*., 2011 with 105 pregnant women demonstrated only 9 (11.6%) vaginal colonization [21]. Similarly, our study with 50 respondents yielded low incidence rate with 0% CAMP positivity from 12 (13.40%) isolates of Beta-hemolytic Gram-positive cocci. Monitoring of the infants was done for four weeks after delivery and there were no reported cases of post-delivery infections among the infants even in those patients with pus cells greater than 10/ LPF. This could be attributed to prophylaxis with 2% erythromycin bath immediately after delivery which is part of the hospitals infant care to avert serious infections post-delivery. As part of maternal care mothers should adhere to CDC guidelines call for recto-vaginal cultures from women at 35 to 37 weeks of gestation to screen for group B Streptococcus [22] to ensure safety of the fetus from bacterial colonization and subsequent complications thereof.

It is very important to identify multiple colonizations with respect and account for common combinations of organisms isolated in a biological system. Dr. Susana Fiorito, M.D. found a prevalence of 1% of gonorrhea among pregnant women, and it is noteworthy that she also found 7% of Chlamydia trachomatis in the same group[23]. Relatively few literatures would present findings of organisms in combinations but this is an important aspect to consider with regard to the influence of each colonizing organism not only to the host but also to other resident or colonizing organisms as well. A study by Bandara *et al.*, 2009 indicated that *E. coli* and *C. albicans* in co-culture mutually modulate biofilm development, both qualitatively and quantitatively, and that *E. coli* lipopolys accharide (LPS) appears to be a key component in mediating these outcomes [24]. Our study showed common combinations of organisms colonizing the cervico-vagina with *S. aureus* and *E. coli* combination to be the most common 4 (50%) of 8 combinations of organisms isolated from 20 infected respondents. These combinations may strongly suggest molecular factors influencing growth and biofilm formation aside from specie to specie interaction and that the growth environment also plays a pivotal role in the up-regulation or down-regulation of signaling molecules responsible for this phenomenon [25].

Finally, microscopic evaluation of pus cell counts less than 8 to 10/LPF as in the results of NSID-13-013 and NSID-13-036 (Table 1.) both of which have pus cell readings of 0-2/LPF shows colonization with moderate to few colonies of *K. pneumoniae* respectively demonstrates that it is not sufficient to rule out bacterial colonization emphasizing the importance of culture methodologies. Additionally, pus cell counts of greater than 25/LPF as in the results of NSID-13-005, 006, 012, 020, 035, 043, 044, 048, and 050 (Table 2.) were not sufficient for inflammatory responses to be evident physiologically, highlighting once again the value of culture methodologies in effective diagnosis of etiologic agents of diseases.

CONCLUSION

To conclude, our data showed absence of GBS colonization in all respondents as well as its transmission to neonates. The study group of 50 pregnant women presented 97 bacterial isolates and 1 yeast cell isolate on BAP. Bacterial isolates were *Lactobacilli* spp. 36 (36.73%), *Escherichia coli* 19 (19.31%), CoNS 17 (17.35%), *S. aureus* 13 (13.27%), *K.pneumoniae* 11 (11.22%), *P. mirabilis* 1 (1.02%), Yeast cells 1 (1.09%). Even though the study group were not able to isolate GBS, the publication of a well-documented absence allows for negative results to be discussed, confirmed or pondered upon by other researchers. Microscopic evaluation of pus cell counts less than 8 to 10/ LPF were not sufficient to rule out bacterial colonization highlighting once again the importance of culture methodologies nor were pus cell counts of greater than 25/LPF sufficient for inflammatory responses to be evident physically. Multiple colonization and combination of organisms may strongly suggest molecular factors influencing growth and biofilm formation aside from specie to specie interaction. Additionally, growth environment plays a pivotal role in the up-regulation or down-regulation of signaling molecules responsible for this phenomenon. Finally, absence of post-delivery complications in infants which were monitored for 4 weeks can be

attributed to proper application and efficacy of 2% erythromycin bath which may have inhibited further colonization of infants after birth thereby negating the formation of an inflammatory response.

Conflict of interest statement

I declare that there is no conflict of interest

Ethics Statement

Written informed consents were obtained from all respondents. The Institutional Review Board approved the collection and use of these samples for research purposes (Ethics Committee of Ricardo P. Rodriguez Memorial Hospital 2013)

Acknowledgements

The author would like to acknowledge his students Ms. Maria Ana Patricia P. Legaspi, Ms. Katherine Joy M. Ong, Ms. Kimberly D. Pagulayan, Ms. Scorch Dominique N. Roldan, and Ms. Tracy Joy D. Sanchez. Also, the author would like to extend his deepest gratitude to Dr. Remedios Ong, head of Pathology Department at Sacred Heart and AUFMC laboratory, Dr. Antonio Ong, Director of Ricardo P. Rodriguez Memorial hospital, and to the staff of Sacred Heart and AUFMC laboratory for selflessly extending their much needed assistance.

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