Gram-negative opportunistic microorganisms colonising the skin and gut of neonates and breast milk of their mothers

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Background

Breast milk (BM) does not normally contain Gram-negative (GN) microorganisms but if it does it could be a source of infection in premature neonates.

Aim

To describe the prevalence of GNs, their antibiotic resistance (AR) and genetic relatedness (GR) between strains colonising BM of mothers and skin and gut of healthy term and hospitalised preterm neonates.

Material and Methods

Study conducting	January 2014 and December 2015
Included neonates and mothers	Preterm neonates (mean GA 28.3 w; n=49) hospitalised in neonatal intensive care unit if BM-feeding was initiated within 7 days of life; term neonates (mean GA 39.6 w; n=20); and their mothers
Sampling	Gut and skin samples from neonates and BM from mothers once a week
Isolation of GN	Cultured onto MacConkey agar
Identification to the species level	MALDI-TOF MS
Microbes used in this study	All GN-s isolated from skin and BM and the same species isolated from gut samples
AR and GR of gut, skin and BM isolates	E-tests; PFGE (when the same species was in different locations)

Results

GRAM-NEGATIVES ON SKIN AND GUT OF NEONATES AND IN BM OF MOTHERS

Colonisation by GN-s occurred at similar frequency in term and preterm neonates in studied locations, and we did not find non-fermentative microbes in gut (Table 1).

Altogether 93 strains (75 *Enterobacteriacae* and 18 non-fermentatives) were isolated. The most frequently isolated organism was *E. cloacae* (35.5%) and *E. coli* (9.7%).

ANTIBIOTIC-RESISTANCE

While all microbes were susceptible to meropenem and ciprofloxacin the AR enterobacterial species were isolated from hospitalised preterm and from two healthy term neonates with no differences in prevalence between isolation sites.

Table 1. Frequency of colonisation and resistance to relevant antibiotics of colonising strains in term- and preterm neonates

	Preterm neonates N = 49			Term neonates N = 20		
	BM	Skin	Gut	BM	Skin	Gut
	Number (%) colonised patients					
Enterobactriaceae	9 (18)	13 (27)	13 (27)	1 (5)	3 (15)	2 (10)
Non-fermentative organisms	8 (16)	1 (2)	0	2 (10)	3 (15)	0
	Number (%) of isolates					
Enterobactriaceae	10 (20)	12 (25)	13 (27)	1 (5)	3 (15)	2 (10)
Non-fermentative organisms	7 (14)	1 (2)	0	2 (10)	3 (15)	0
CTX-AR *Enterobacteriaceae*	2 (7)	2 (10)	4 (13)	0	0	0
GEN-AR *Enterobacteriaceae*	3 (11)	2 (10)	3 (10)	0	0	0
CXM-AR *Enterobacteriaceae*	1 (4)	4 (20)	4 (10)	0	0	0.0
AMC-AR *Enterobaceriaceae*	10 (37)	10 (50)	13 (42)	1 (33)	0	2 (67)
GEN-AR nonfermentative	1 (4)	0.0	0.0	0	0	0

CTX – cefotaxime; GEN – gentamicin; CXM – cefuroxime; AMC – amoxicillin/clavulanic acid

GENOTYPICAL SIMILARITY IN NEONATE-MOTHER MICROORGANISM PAIRS

In 16 neonate-mother pairs the phenotypically same GN microbe in different sites (BM, skin and gut) were observed. In 2 childs`s in skin and 3 mother`s in BM swabs two different GNs were detected. In two child-BM pairs the phenotypically same microbe were isolated in all three sites.
Genotypically similar (results of PFGE) Enterobacteriaceae were detected in 5/9 preterm neonates in BM-gut; in 9/11 in skin-gut, and in 1/1 skin/BM (A21) swabs pairs but no between-site similarity was observed among non-fermentative organisms (Table 2).
There was one case of neonatal sepsis in which genetically similar *K. oxytoca* (B06) was found in BM and blood.

Table 2. Genotypical similarity of GN-s in neonate-mother gut-BM (A) and neonate gut-skin (B) swab pairs.

BM - breast milk; S – skin; G – gut

The intensity of different colours present different genotypes in one neonate-mother pair;
* - the same genotype in different neonate-mother pair;
ID "D" presents term neonates

A

ID	Gut			BM	
A21	Ecoli	Ecoli		Ecoli	Ecoli
B03	Ecl			Ecl	Ecl
B06	Kpn	Kpn		Kpn	Kpn
B14	Ent	Kox		Ent	Kox
B22	Kox	Kox	Kox	Kox*	
B23	Kox	Kox *		Kox*	
C10	Ecl			Ecl	
D23	Kox			Kox	

B

ID	Gut				Skin		
A02	Kox	Kox			Kox	Kox	
A19	Ecoli	Ecoli	Ecoli		Ecoli		
A21	Ecoli	Ecoli			Ecoli		
A23	Ecl				Ecl		
B08	Ecl	Ecl	Ecl		Ecl	Ecl	
B16	Ent	Ent	Ent	Ecl	Ent	Ecl	Ecl
B19	Ecl	Ecl	Ecl	Ecl	Ecl		
C10	Ecl				Ecl		
D14	Ecl	Ecl			Ecl		
D23	Kox				Kox		

Ecl: *Enterobacter cloacae;* Ent: *Entetrobacter asburiae;* Ecoli: *Escherichia coli;* Kox - *Klebsiella oxytoca;* Kpn - *Klebsiella pneumoniae*

Conclusions

In neonates BM is not a source of colonisation with nonfermentative microbes. However, Enterobacteriacae colonising BM may sometimes end up in neonatal gut and eventually cause infection in neonates. Colonisation of BM and gut/skin with AR strains is exclusively observed in hospitalised preterm neonates and is not site specific.