Prevalence of antibiotic-resistant bacterial colonization in neonatal intensive care in Europe

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Introduction

Infants colonized with antibiotic-resistant bacteria (ARB) are at **high-risk** of developing **severe bacterial infections**, that can ultimately lead to sepsis, particularly when born at <32 weeks gestation (high-risk). However, overall **prevalence and burden** of specific antibiotic resistance genes (ARGs) in a high-income setting is largely **unknown**.

Methodology

In the pan-European NeoIPC project, **skin swabs** (N=929) and **stool samples** (N=754) were collected during **four point-prevalence surveys** (PPSs) interspaced with 4-, 7-, or 14-day intervals from all infants (<1 year, N=468) present in 14 NICUs across six European countries. DNA was extracted (NucliSENS easyMAG, bioMérieux) followed by **RT-qPCR detection** of **carbapenemases** (CBPs), **extended-spectrum-beta-lactamases** (ESBLs), and **vancomycin-resistant enterococci** (VREs) in stools, as well as **methicillin-resistant *Staphylococcus aureus*** (MRSA) in skin swabs (**Table 1**). Collected anonymized data were analyzed per sample per PPS.

Abbreviations
ARB: antibiotic-resistant bacteria
ARG: antibiotic resistance gene
PPS: point-prevalence survey
CBP: carbapenemase
ESBL: extended-spectrum beta-lactamase

VRE: vancomycin-resistant enterococci
MRSA: methicillin-resistant *Staphylococcus aureus*
MRCoNS: methicillin-resistant coagulase-negative staphylococci

Table 1. Overview of ARBs/ARGs detected, assays used, and sample matrix utilized for analysis.

| Sample matrix	ARB Type	Gene target	Assay
Stool	CBP	bla_{KPC} bla_{NDM} bla_{VIM} bla_{IMP} bla_{OXA-48}	Carbaplex-IVD PCR (Bruker)
	ESBL	bla_{CTX-M} group1 bla_{CTX-M} group9	Ba04646149_s1, Ba04646127_s1 (ThermoFisher)
	VRE	$vanA$ $vanB$	VRE (Geneproof)
Skin swab	MRSA	$mecA/mecC$ $SCCmec/orfX$	Diarella MRSA-SeqC (Gerbion)
	MRCoNS	$mecA/mecC$	

Results

Table 2. Antibiotic-resistant bacterial (ARB) colonization patterns across four point-prevalence surveys (PPSs). ARB/ARG colonization rates were determined per PPS by performing PCRs detecting ESBLs, CBPs, and VREs (stool screening), as well as MRSA and MRCoNS (skin swab screening).

Variable	Total samples[1]	Total	Survey-1	Survey-2	Survey-3	Survey-4
Stool colonization	754	217 (29%)	47 (26%)	53 (27%)	57 (31%)	60 (32%)
Number of positive gene targets[1]	754					
1		151 (20%)	36 (20%)	40 (21%)	35 (19%)	40 (21%)
2		48 (6.4%)	9 (4.9%)	10 (5.1%)	14 (7.5%)	15 (7.9%)
3		15 (2.0%)	2 (1.1%)	3 (1.5%)	6 (3.2%)	4 (2.1%)
4		3 (0.4%)	0 (0%)	0 (0%)	2 (1.1%)	1 (0.5%)
VRE detected	754	54 (7.2%)	7 (3.8%)	8 (4.1%)	15 (8.1%)	24 (13%)
vanA		54 (7.2%)	7 (3.8%)	8 (4.1%)	15 (8.1%)	24 (13%)
vanB		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
CBP detected	754	79 (10%)	15 (8.2%)	19 (9.7%)	23 (12%)	22 (12%)
bla_KPC		54 (7.2%)	11 (6.0%)	12 (6.2%)	17 (9.1%)	14 (7.4%)
bla_NDM		2 (0.3%)	1 (0.5%)	0 (0%)	0 (0%)	1 (0.5%)
bla_VIM		44 (5.8%)	8 (4.3%)	10 (5.1%)	17 (9.1%)	9 (4.8%)
bla_IMP		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
bla_OXA-48		5 (0.7%)	2 (1.1%)	0 (0%)	0 (0%)	3 (1.6%)
ESBL detected	754	145 (19%)	31 (17%)	39 (20%)	40 (22%)	35 (19%)
bla_CTX-M group1		144 (19%)	31 (17%)	39 (20%)	40 (22%)	34 (18%)
bla_CTX-M group9		1 (0.1%)	0 (0%)	0 (0%)	0 (0%)	1 (0.5%)
MRSA detected[2]	929	25 (2.7%)	9 (3.9%)	6 (2.5%)	7 (3.0%)	3 (1.3%)
MRCoNS detected[3]	929	821 (88%)	209 (91%)	209 (87%)	200 (87%)	203 (88%)
Risk group[1]	754					
High risk		86 (40%)	22 (47%)	23 (43%)	17 (30%)	24 (40%)
Non-high risk		131 (60%)	25 (53%)	30 (57%)	40 (70%)	36 (60%)
Country[1]	754					
Estonia (*N*=20)		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
ES1 (*N*=20)		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Germany		7 (7.1%)	2 (6.9%)	1 (4.2%)	2 (11.1%)	2 (7.1%)
GE1 (*N*=99)		7 (7.1%)	2 (6.9%)	1 (4.2%)	2 (11.1%)	2 (7.1%)
Greece (*N*=295)		140 (48%)	33 (46.5%)	35 (43.8%)	39 (50.6%)	33 (49.3%)
GR1 (*N*=27)		14 (51.9%)	3 (60 %)	2 (25%)	7 (88%)	2 (33%)
GR2 (*N*=69)		54 (78.3%)	11 (65%)	15 (79%)	17 (94%)	11 (73%)
GR3 (*N*=82)		51 (62.2%)	15 (71%)	13 (59%)	10 (48%)	13 (72%)
GR4 (*N*=83)		11 (13.3%)	3 (14%)	3 (14%)	2 (9.5%)	3 (16%)
GR5 (*N*=34)		10 (29.4%)	1 (14%)	2 (22%)	3 (33%)	4 (44%)
Italy (*N*=70)		19 (27.1%)	3 (17.6%)	4 (22.2%)	5 (27.8%)	7 (41.2%)
IT1 (*N*=43)		7 (16.3%)	2 (17%)	2 (22%)	0 (0%)	3 (27%)
IT2 (*N*=27)		12 (44.4%)	1 (20%)	2 (22%)	5 (71%)	4 (67%)
Spain (*N*=216)		49 (22.7%)	9 (18.0%)	11 (20.8%)	11 (21.2%)	18 (29.5%)
SP1 (*N*=54)		16 (29.6%)	4 (31%)	3 (25%)	4 (36%)	5 (28%)
SP2 (*N*=66)		7 (10.6%)	2 (14%)	0 (0%)	0 (0.0%)	5 (29%)
SP3 (*N*=96)		26 (27.1%)	3 (13%)	8 (36%)	7 (28%)	8 (31%)
Switzerland (*N*=54)		2 (3.7%)	0 (0%)	2 (14.3%)	0 (0%)	0 (0%)
SW1 (*N*=44)		2 (4.5%)	0 (0%)	2 (15%)	0 (0%)	0 (0%)
SW2 (*N*=10)		0 (0%)	0 (0%)	0 (0 %)	0 (0%)	0 (0%)

[1]Calculated based on stool ARG colonization
[2]MRSA-positive skin swab samples tested positive for both *mecA/mecC* and *SCCmec/orfX*
[3]MRCoNS-positive skin swab samples tested positive only for *mecA/mecC*
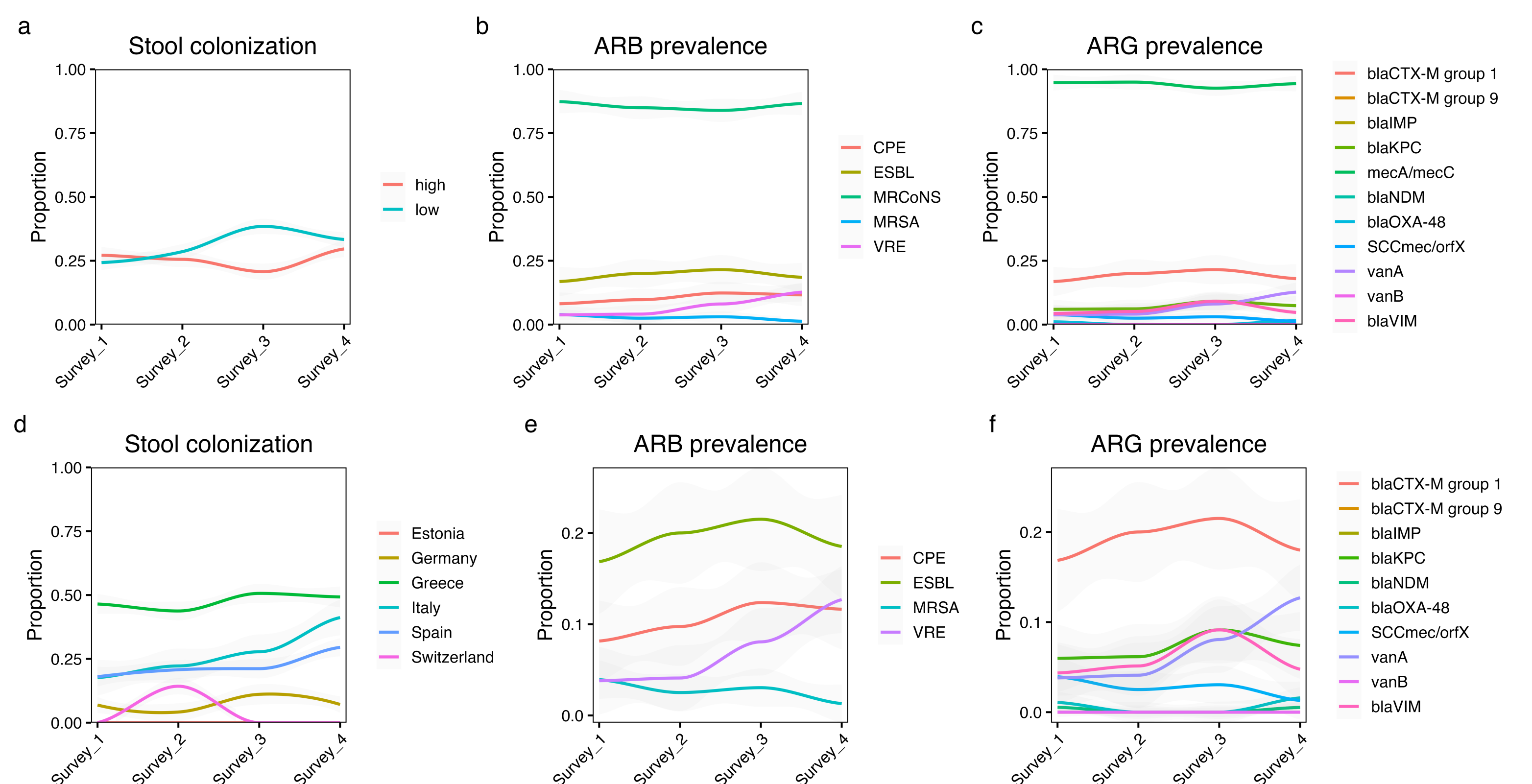
Figure 1. ARB colonization patterns over time in infants admitted to 14 European NICUs. Panels (a) and (d) display proportion of ARG-positive (CPE, ESBL, or VRE) stool samples defined by infant risk status and country of collection, respectively. Panels (b) and (e) display proportion of ARG/ARB-positive samples with or without MRCoNS, respectively. Finally, panels (c) and (f) display proportion of samples where individual ARGs were detected with and without *mecA/mecC*, respectively.

Overall, **26%** (217/754) stool samples were **ARG-positive** (ESBL, CBP, or VRE) across four PPSs, and in 8.8% (66/754) ≥2 ARGs were detected (**Table 2**). **ESBLs** (19%, bla_{CTX-M} group1) were **most prevalent**, followed by **CBPs** (10%; mainly bla_{KPC} and bla_{VIM}), and VREs (7.2%, *vanA*). In contrast, **MRSA** skin colonization was **rare** (2.7%). While not specifically screened for, methicillin-resistant coagulase-negative staphylococci (**MRCoNS**) were detected by the MRSA assay in **majority** of the skin swabs (88%, 821/929).

40% of high-risk infants were colonized, compared to 60% of non-high-risk infants (Pearson's χ^2, p=0.3), and **high-risk infants** were more frequently **ESBL-colonized** (21% vs. 16%, p=0.086). Gut ARG colonization **varied by country and site** (p<0.001), ranging from 0.0%-94% for individual sites, and was most common in Greece (**Figure 1**).

Colonization rate **stability** over time was found to be **ARG/ARB-dependent**; only **VRE** colonization **increased** from 3.8%-13% during the study period (p=0.002), while the **CBP, MRSA, ESBL**, and **MRCoNS** positivity remained **stable** over time (p>0.05).

Conclusion

- We observed remarkable variation in ARG prevalence across countries and NICU sites.
- Stability of colonization rate/PPS was found to vary for different ARGs.
- Although non-significant, gut colonization with ESBLs, specifically bla_{CTX-M} group 1, was more frequent in high-risk infants.

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