

Epidemiology and Healthcare Factors Associated with Neonatal Enterococcal Infections

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ABBREVIATIONS

ANNP	Advanced Neonatal Nurse Practitioners
BAPM	British Association of Perinatal Medicine
BSI	bloodstream infection
CoNS	coagulase-negative <i>Staphylococcus</i> spp.
CRP	C-reactive protein
EOS	early-onset sepsis
LOS	late-onset sepsis
NEC	necrotising enterocolitis
TPN	total parenteral nutrition

ABSTRACT

Objective: To investigate the epidemiology and healthcare factors associated with late-onset neonatal enterococcal infections.

Design: Multi-centre, multi-national retrospective cohort study using prospectively collected infection data from a neonatal infection surveillance network (neonIN) between 2004-2016; this was supplemented with healthcare data from a questionnaire distributed to participating neonatal units.

Setting: 60 neonatal units across Europe (UK, Greece, Estonia) and Australia.

Patients: Infants admitted to participating neonatal units who had a positive culture of blood, cerebrospinal fluid or urine after 48 hours of life.

Results: 414 episodes of invasive *Enterococcus* spp. infection were reported in 388 infants (10.1% of a total 4,083 episodes in 3,602 infants). *Enterococcus* spp. were the second most common cause of late-onset infection after coagulase-negative *Staphylococcus* spp. (CoNS) and were strongly associated with necrotising enterocolitis (NEC) (adjusted OR 1.44, 95% CI 1.02-2.03, p=0.038), total parenteral nutrition (TPN) (adjusted OR 1.34, 95% CI 1.06-1.70, p=0.016), increasing postnatal age (per 1 week increase: adjusted OR 1.04, 95% CI 1.02-1.06, p<0.001) and decreasing birthweight (per 1 kg increase: adjusted OR 0.85, 95% CI 0.74-0.97, p=0.017). There was no evidence that inadequate nurse to patient staffing ratios in high dependency units were associated with a higher risk of enterococcal infections.

Conclusions: *Enterococcus* spp. were the second most frequent cause of late-onset infections. The association between enterococcal infections, NEC and TPN may inform empiric antimicrobial regimens in these contexts and provide insights into reducing these infections.

INTRODUCTION

Although part of the normal gastrointestinal and genitourinary microbiota, members of the *Enterococcus* species are increasingly recognised as a cause of nosocomial infections, especially urinary tract and wound infections.[1, 2] They can cause bacteraemia, endocarditis, meningitis and gastrointestinal infections,[2] and are an increasing concern in neonatal intensive care units, causing both late-onset sepsis (LOS; onset after 48 hours' postnatal age) and occasionally early-onset sepsis (EOS; onset within 48 hours of birth).[3]

Enterococci can endure high salt concentrations, a wide range of temperatures (10-45°C) and survive on inanimate surfaces for prolonged periods,[1] enhancing their ability to be transferred between patients.

Despite increasing interest in neonatal enterococcal infections, there remain significant gaps in our knowledge. The published literature primarily consists of observational studies and case series which describe disease groups rather than specific aetiologies. While patient risk factors for nosocomial infection are well known (e.g. prematurity, invasive procedures),[4] further investigation of healthcare risk factors is required. Increased admissions, infrastructural changes, excessive workload and understaffing are healthcare factors which have been associated with an increased risk of infections.[5-9]

This study aimed to investigate the epidemiology and healthcare factors associated with neonatal enterococcal infections with a view to informing infection management and prevention strategies.

METHODS

The neonatal infection surveillance network (neonIN) (www.neonin.org.uk) is a multi-national network that prospectively collects data on invasive neonatal infections from participating neonatal units. Since its inception in 2004, neonIN has grown and currently receives data from 60 neonatal units worldwide.

Details on infection episodes from every participating unit from 2004-2016 were extracted from the neonIN database. Infection was defined as a positive culture of blood, cerebrospinal fluid or urine

(obtained in a sterile manner, e.g. via suprapubic aspirate). Early- and late-onset infections were defined as infection with onset within or after 48 hours of birth respectively, and uni- and multi-variate analyses were performed for the late-onset cases. All cultures growing the same organism within 7 days (or 10 days for coagulase-negative *Staphylococcus* spp. (CoNS) and fungi) were considered to be part of the same episode. Cultures obtained at greater than 6 months of postnatal age were excluded from the dataset. Organism identification and antimicrobial sensitivity analyses were completed according to routine procedures at each participating institution.

Healthcare factors data were obtained through a questionnaire distributed to all neonIN units. Questions covered basic unit characteristics and healthcare factors from 2011-2016, and broadly fell into three categories:

1. General information – live births, unit level, levels of care, provision of neonatal surgery, number of neonatal intensive care cots, central line days (2015), neonatal admissions (2015), care level days by unit
2. Staffing – nursing staff (numbers and staff to patient ratios), and full-time equivalent neonatal consultants, trainees and Advanced Neonatal Nurse Practitioners (ANNPs). Standards were based on those specified by the British Association of Perinatal Medicine (BAPM).[10]
3. Policies & practice – infection/infection-control protocols, regular surveillance screening, antimicrobial stewardship policies, routine intrapartum antibiotic prophylaxis, empiric sepsis regimens, time to nappy (diaper) disposal

Questionnaire results were collated using REDCap,[11] then synthesised with case data and analysed using Stata[®] 14. Categorical variables were analysed using the Chi-squared or Fisher's exact test, while continuous variables were analysed using the Mann Whitney U-test. Variables statistically significant on univariate analysis and clinically significant variables were included in multivariate models. Multivariate analyses were completed via logistic regression, with Hosmer-Lemeshow's goodness-of-fit test finding that both the patient and healthcare factor models fitted the data well. Missing data were handled by Stata's inbuilt functions. A p-value <0.05 was considered statistically significant.

NeonIN has ethics approval from the Health Research Authority (05/Q0806/34+5).

RESULTS

414 episodes of *Enterococcus* spp. infection were reported in 388 infants (10.1% of a total 4,083 infection episodes in 3,602 infants). 31 of these 414 episodes (7.5%) were early-onset, while the remaining 383 episodes (92.5%) were late-onset. Enterococci were the fourth most common cause of all infections (416/4,303 isolates) and the second most common cause of late-onset infections (385/3,481 isolates). Enterococcal infections most commonly affected infants of very or extremely low birthweight (<1.5 kg or <1 kg respectively) (see *Figure 1A*).

The majority of enterococcal isolates were *E. faecalis* (72%), followed by *E. faecium* (7%), *E. gallinarum* (0.2%) and enterococci of unspecified species (21%).

Twenty-four units completed the healthcare factors questionnaire (response rate 40%), four of which were from two hospitals (i.e. two hospitals had two respondent units each). From these twenty-four units, 172 episodes of enterococcal infection were reported in 160 infants.

Patient and culture characteristics

Characteristics of late-onset enterococcal versus non-enterococcal infections are compared in *Figure 1* and *Table 1*. Infants with *Enterococcus* spp. infections had a lower median gestational age (26 weeks, IQR 24-31 weeks, $p<0.01$) and birthweight (840g, IQR 684-1422g, $p<0.01$) and their infections occurred at a later postnatal age (median 22 days, IQR 11-52 days, $p<0.01$). These infants were more likely to have had associated necrotising enterocolitis (NEC), defined as modified Bell II A & B (11.7% vs 8.4%, $p=0.03$), received total parenteral nutrition (TPN) within 24 hours prior to culture (66.6% vs 59.5%, $p=0.01$) and to have had an arterial and/or venous central line *in situ* at the time of culture (54.6% vs 46.5%, $p=0.01$).

Insert Figure 1 and Table 1 here

A multivariate analysis found four patient-related factors to be associated with an increased risk of enterococcal versus other infection (see *Table 2*): NEC (adjusted OR 1.44, 95% CI 1.02-2.03, p=0.038), administration of TPN (adjusted OR 1.34, 95% CI 1.06-1.70, p=0.016), decreasing birthweight (per 1kg decrease: adjusted OR 1.18, 95% CI 1.03-1.35, p=0.017) and increasing postnatal age, with a 4% increase per week of life (adjusted OR 1.04, 95% CI 1.02-1.06, p<0.001).

Insert Table 2 here

Unit characteristics and healthcare factors

Twenty of the twenty-four units were designated as Level 3 (these units provide intensive care, although Level 2 units can also provide short-term intensive care).[12] Thirteen units were from the UK, seven from Greece, three from Estonia and one was from Australia. From 2011 to 2015, the mean annual number of live births among respondent unit hospitals ranged from 4125 to 4331. Additional unit characteristics are shown in *Supplementary Table 1*.

EOS antimicrobial regimens were relatively consistent between respondents, with units using penicillin or ampicillin, plus gentamicin. There was greater variation in choice of LOS empirical regimens, with a combination of flucloxacillin and gentamicin being the most common choice.

On multivariate analysis (see *Table 3*), meeting BAPM standards for nurse:patient ratios in the high dependency unit (i.e. $\geq 1:2$) was associated with an increased risk of enterococcal infection (adjusted OR 2.26, 95% CI 1.08-4.70, p=0.030).

Insert Table 3 here

Antimicrobial susceptibility data

Antimicrobial susceptibility data were reported for 288/416 (69%) of all enterococcal isolates. There was high susceptibility to vancomycin (97%), while susceptibility to ampicillin and gentamicin was lower (83% and 62% respectively).

DISCUSSION

We report the results of a large multi-national study of enterococcal infections from 2004-2016 using an infection surveillance network database. There have been limited previous investigations into epidemiology and healthcare factors related specifically to enterococcal infections, thus our study provides unique insights. The patient and healthcare risk factors identified for enterococcal infection are likely generalisable to neonatal units across the developed world as neonIN encompasses multiple units from Europe and Australia.

Patient factors

Infants with enterococcal infections were of a lower birthweight and greater postnatal age compared to infants with other infections. It seems likely that this reflects longer lengths-of-stay and perhaps a greater number of comorbidities. These infants may also have had greater exposure to antimicrobials.

Enterococcal infection was strongly associated with NEC. While a retrospective analysis cannot assess causation, it is biologically plausible that enterococci may translocate from the gut.[1] There are several studies that have assessed the relationship between NEC and bloodstream infection (BSI), but these have yielded variable results, suggesting that BSI may be a risk factor for NEC, or occur concurrently with or following NEC.[13-15] Bizzaro *et al.* found that 158 of 410 infants with NEC had at least one episode of BSI. Of 126 BSI episodes, enterococci were responsible for 2/57 (3.5%) NEC-associated episodes and 13/69 (18.8%) post-NEC episodes. Further mapping of the chronological relationship between NEC episodes and enterococcal BSIs would be of value, for example in refining empiric antibiotic policies for NEC. Enterococcal infection concurrent with NEC may be covered by empiric regimens containing ampicillin or gentamicin, although there was moderate resistance to these agents in this study. Conversely, other empiric LOS regimens commonly used, such as cefotaxime or flucloxacillin, will not be effective against enterococci.

Enterococcal infection was also associated with concurrent receipt of TPN. TPN has previously been identified as a risk factor for BSI: among neonates with a central catheter, Perlman *et al.* reported a 4.69

relative risk of BSI in those who did versus those who did not receive TPN.[16] Further, Mahieu *et al.* found increasing duration of TPN with catheter use to be significantly associated with risk of catheter-associated BSI.[17]

It has been suggested that TPN may compromise the neonatal gastrointestinal mucosal barrier, leading to “gut-derived” sepsis.[18] This could explain an increased risk of enterococcal BSI. Others have suggested that TPN adversely affects neutrophil phagocytosis, thereby predisposing infants to infection,[19] and TPN is delivered centrally, hence central lines themselves may be implicated. It is unknown in most cases where there is a central line in situ, whether the likely source of the BSI is the gut or the central line.

Conventionally, efforts to decrease infection rates have primarily focused on improving infection control and related strategies, e.g. central line care. These are important, but given the persistently high rate of nosocomial infections, it may also be reasonable to identify aetiology-targeted infection prevention strategies. Enterococci are an apt starting point, being the second most common cause of late-onset infection in our study.

The possible routes of invasive disease caused by enterococci include the gastrointestinal tract, urinary tract infections, central line infections and ventilator-associated pneumonia. Our study suggests that for critically-ill neonates, improving NEC prevention may have a vital role in reducing rates of *Enterococcus* spp. infections, particularly BSIs, and further research is necessary to clarify the association between TPN and enterococcal BSI.

Research into NEC prevention includes studies of feeding rate, and lactoferrin and probiotic supplementation to ‘re-shape’ gut flora.[20, 21] The PiPS trial showed that use of the single-strain *Bifidobacterium breve* BBG-001 was ineffective in preventing NEC in preterm infants up to 32 weeks’ gestation.[22] However, meta-analyses strongly support probiotic use to prevent NEC, with most evidence of benefit coming from dual-strain probiotic combinations.[23-25] Well-established strategies for NEC prevention should continue to be reinforced, such as giving human breast milk rather than

formula, and use of standardised feeding regimens.[20, 26] Furthermore, there is evidence that use of probiotics can prevent LOS in high-risk preterm infants.[24, 27, 28]

Healthcare factors

Decreased staffing has been associated with an increased infection risk and mortality;[8, 9, 29] it increases the workload among available staff, which in turn may lead to decreased adherence to infection prevention processes.[9, 30] Counterintuitively, our study found that achieving BAPM nurse:patient ratio guidelines in the high dependency unit was associated with an *increased* risk of enterococcal infection (adjusted OR 2.26, 95% CI 1.08-4.70, p=0.030). While this may be a chance finding, it may also indicate that those units which are more likely to have babies with increased susceptibility to enterococcal infections are also those that are better staffed.

Few studies address the role of nappies in the aetiology of neonatal infection. Disposable compared with cloth nappies reduce the risk of sepsis,[31] and gloves for nappy changes may decrease the risk of spread of infections when used in conjunction with other infection control strategies such as handwashing certification.[32] While gloves may also reduce spread of vancomycin-resistant *Enterococcus* spp. onto healthcare worker hands, they are not fool-proof, rendering handwashing after glove removal essential.[33]

In our study, time to nappy disposal was not significant. This may suggest that faecal transmission (distinguished from NEC) does not have a differential effect on enterococcal infections as opposed to other invasive infections. Alternatively, responses to this question may have been more subjective, despite the remainder of the healthcare questionnaire being objective. On-site collection of time to disposal data could capture exact times and would be a valuable inclusion in future studies.

Antimicrobial susceptibility

There was high susceptibility to vancomycin among enterococcal isolates, as expected with neonatal infection.[34] However, significant proportions of isolates were resistant to common empirical LOS antibiotics, including gentamicin. Enterococci are inherently resistant to flucloxacillin and cefotaxime,

which are also commonly used empirical LOS antibiotics.[35-37] Thus whenever NEC is suspected, the empiric antibiotic regimen should include an agent which is likely to be effective against enterococci.

Limitations

Our study is limited by its retrospective nature. The exact temporal relationship between enterococcal infection and other episodes, e.g. NEC, was also not captured. Further, questionnaire data relating to healthcare factors was limited by response rate, and completion of certain variables was often incomplete and unable to be included (e.g. nursing staff numbers). These excluded variables may be specifically targeted in future studies.

CONCLUSION

Our study provides important and novel insights into the epidemiology and healthcare factors related to neonatal enterococcal infections. In particular, the association with current TPN therapy and NEC may help guide empiric antimicrobial therapy in LOS and warrants further investigation. It also serves to highlight the importance of research into NEC prevention strategies. Improved understanding of neonatal enterococcal infections offers an opportunity to improve both therapeutic and preventive interventions in high-risk newborns.

What is already known on this topic:

- Enterococci are a leading cause of morbidity in neonates and have a significant antibiotic resistance profile.
- There is limited knowledge of the healthcare risk factors associated with enterococcal infections.

What this study adds:

- There were unique and strong associations between enterococcal infection and current or previous necrotising enterocolitis, and with receipt of TPN.
- Enterococci were the second most common cause of late-onset sepsis, but may not be covered by commonly used empirical antimicrobial agents.

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Insert Figure 1 (please refer to attached TIFF file)

Figure 1 Infant demographics. (A) Birthweights, and (B) Gestational ages, of i) Infants who had enterococcal infections, and ii) Infants who had non-enterococcal infections only. Corresponding numerical data for the above histograms can be found at *insert link to corresponding numerical data file here*.

Table 1 Characteristics of late-onset enterococcal versus non-enterococcal infection episodes

	Late-onset cultures positive for <i>Enterococcus</i> spp. (N=383)	Late-onset cultures positive for non- <i>Enterococcus</i> spp. (N=2 899)	p
Median age at infection (days)	22 (11 – 52)	18 (9 – 39)	<0.01
Culture source			
Blood culture	324 (84.6%)	2 513 (86.7%)	0.63
Blood culture & CSF	3 (0.8%)	40 (1.4%)	
Blood culture & urine	5 (1.3%)	41 (1.4%)	
Blood culture, CSF & urine	0 (0.0%)	3 (0.1%)	
CSF	9 (2.4%)	51 (1.8%)	
Urine	31 (8.1%)	177 (6.1%)	
Other/not specified	11 (2.9%)	74 (2.6%)	
Presence of associated conditions			
Abdominal surgery	29 (7.6%)	156 (5.4%)	0.08
Pneumonia	6 (1.6%)	72 (2.5%)	0.27
NEC	45 (11.7%)	244 (8.4%)	0.03
Median maximum CRP within 48h of culture	32 (9 – 88)	55 (20.2 – 108.5)	<0.01
Central line <i>in situ</i> at time of culture			
Yes	209 (54.6%)	1 348 (46.5%)	0.01
No	120 (31.3%)	1 057 (36.5%)	
Unknown	54 (14.1%)	494 (17.0%)	
Feeds			
Had TPN within 24h before culture			
Yes	255 (66.6%)	1 724 (59.5%)	0.01
No	119 (31.1%)	1 083 (37.4%)	
Unknown	9 (2.3%)	92 (3.2%)	
Enteral feeds at time of culture			
No (nil by mouth)	73 (19.1%)	655 (22.6%)	0.10
Yes	298 (77.8%)	2 138 (73.7%)	
Yes (unspecified amount)	5 (1.3%)	18 (0.6%)	
Yes, <50%	129 (33.7%)	907 (31.3%)	
Yes, ≥50%	70 (18.3%)	423 (14.6%)	
Yes, 100% (full)	94 (24.5%)	790 (27.3%)	
Unknown	12 (3.1%)	106 (3.7%)	

Data are presented as *median (interquartile range)* or *n (%)*. CRP = C-reactive protein.

Table 2 Patient-related risk factors for late-onset culture of *Enterococcus* spp. versus non-*Enterococcus* spp.

Risk factors	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p	Adjusted OR (95% CI)	p
Birthweight (per 1kg increase)	-	<0.001	0.85* (0.74 – 0.97)	0.017
Postnatal age at infection (per 1 week increase)	-	0.002	1.04** (1.02 – 1.06)	<0.001
Gender (male/female)	1.06 (0.85 – 1.32)	0.597	-	-
Associated NEC	1.45 (1.03 – 2.03)	0.031	1.44* (1.02 – 2.03)	0.038
Total parenteral nutrition (within 24 hours prior to culture)	1.36 (1.08 – 1.72)	0.008	1.34* (1.06 – 1.70)	0.016

*Derived from a multivariate model including birthweight, associated NEC and TPN. Birthweight was included over gestational age given its model was a significantly better fit for the data, as assessed by Hosmer-Lemeshow's goodness-of-fit test.

**Derived from a multivariate model including postnatal age at infection, associated NEC and TPN.

Table 3 Healthcare risk factors for late-onset culture of *Enterococcus* spp. versus non-*Enterococcus* spp.

Healthcare factors	Univariate analysis		Multivariate analysis*	
	OR (95% CI)	p	Adjusted OR (95% CI)	p
General				
Number of neonatal admissions (2015) – per 100 increase	-	0.020	1.00 (0.86 – 1.15)	0.973
Unit level (Level-2/Level-3)	0.79 (0.39 – 1.61)	0.519	-	-
Country (UK or Australia/Greece or Estonia)	1.39 (0.92 – 2.11)	0.117	-	-
Provision of neonatal surgery	1.77 (1.04 – 3.02)	0.033	1.90 (0.46 – 7.79)	0.374
Staffing				
Number of staff associated with the unit – per 1 increase				
Consultants	-	0.355	-	-
Trainee medical staff	-	0.009	1.04 (0.98 – 1.11)	0.183
ANNPs	-	0.507	-	-
Nurse staff to patient ratios (ratios at or greater than BAPM recommendations[10]/ratios lower than BAPM recommendations)				
Intensive care unit (1:1/<1:1)	1.92 (1.18 – 3.14)	0.008	1.64 (0.86 – 3.15)	0.135
High dependency unit (≥1:2/<1:2)	1.81 (1.23 – 2.68)	0.003	2.26 (1.08 – 4.70)	0.030
Special care unit (≥1:4/<1:4)	2.10 (1.24 – 3.56)	0.005	0.90 (0.24 – 3.33)	0.870
Policies & clinical practice				
Regular surveillance screening	0.69 (0.48 – 1.01)	0.052	1.88 (0.87 – 4.03)	0.107
Presence of antimicrobial stewardship policies	1.80 (0.71 – 4.54)	0.208	-	-

Longer time to nappy disposal				
“immediately or ≤20minutes” or longer/“immediately”	1.42 (0.85 – 2.36)	0.180	-	-
“≤20minutes” or longer/“immediately” or “immediately or ≤20minutes”	1.24 (0.83 – 1.84)	0.291	-	-
“>20minutes”/“≤20minutes”**	5.60 (1.79 – 17.51)	0.001	-	-

*The multivariate model included all variables with an adjusted OR provided

**This result should be interpreted with caution due to only one center reporting previous disposal of nappies at >20 minutes