



## Mucosal surveillance cultures in predicting Gram-negative late-onset sepsis in neonatal intensive care units

Ü. Parm, T. Metsvaht, E. Sepp, M.-L. Ilmoja, H. Pisarev, M. Pauskar, I. Lutsar\*

Department of Microbiology, Medical Faculty, University of Tartu, Tartu, Estonia

### ARTICLE INFO

#### Article history:

Received 30 March 2010

Accepted 11 March 2011

by S.J. Dancer

Available online 20 June 2011

#### Keywords:

Bacterial colonization

Neonatal sepsis

Neonate

Surveillance culture

### SUMMARY

This study aimed to examine the spectrum and time course of gut and nasopharyngeal colonization with Gram-negative micro-organisms, and to define the value of surveillance cultures in predicting late-onset sepsis in neonates admitted to neonatal intensive care units. Nasopharyngeal and rectal swabs were collected on admission and twice weekly thereafter in 278 neonates admitted within the first 72 h of life with risk factors of early-onset sepsis. Sterile body fluid cultures were obtained on admission and subsequently as clinically indicated. Approximately half of the rectal (693/1250, 55%) and nasopharyngeal (558/1153, 48%) samples but only 6% (32/555) of the sterile fluid samples in 26 patients were culture positive for Gram-negative organisms. In total, 2108 invasive and mucosal culture pairs were analysed. The overall sensitivity, specificity, and positive and negative predictive values of a mucosal sample to predict late-onset sepsis were 27%, 66%, 4% and 94%, respectively. Patients with pre-existing colonization with *Klebsiella pneumoniae* ( $P = 0.011$ ), *Klebsiella oxytoca* ( $P = 0.002$ ), *Escherichia coli* ( $P = 0.003$ ), *Stenotrophomonas* spp. ( $P = 0.003$ ) and *Pseudomonas* spp. ( $P \leq 0.001$ ) were more likely to develop late-onset sepsis. No such association was found for *Acinetobacter baumannii*, *Serratia* spp. or *Enterobacter cloacae*. In conclusion, routine mucosal cultures are inefficient for the prediction of Gram-negative late-onset sepsis in neonatal intensive care units. However, targeted screening for specific organisms in an outbreak (e.g. *Klebsiella* spp., *E. coli*, *Stenotrophomonas* spp. and *Pseudomonas* spp.) may offer an opportunity to improve infection control measures and enable timely initiation of appropriate antibiotic therapy.

© 2011 The Healthcare Infection Society. Published by Elsevier Ltd. All rights reserved.

### Introduction

Between 2% and 10% of patients admitted to neonatal intensive care units (NICUs) experience at least one episode of Gram-negative sepsis.<sup>1</sup> Determination of the most appropriate therapy in the NICU setting is not easy, as cultures may remain negative, and even positive culture results typically take 48 h to be reported.

Mucosal colonization with potentially pathogenic Gram-negative micro-organisms is common in NICUs. By the end of the first week of life, more than 40% of high-risk neonates are colonized; this increases to nearly 100% by 30 days of life.<sup>2–4</sup> Due to disturbed barrier integrity, colonization may lead to invasive

disease; concordance of colonizing and invasive strains has been demonstrated previously.<sup>5</sup>

For decades, clinicians and researchers have attempted to use surveillance cultures to predict invasive infections, but the issue remains controversial. Surveillance cultures of the gastrointestinal tract, pharynx and/or skin have been studied in an effort to identify infants who will develop infections. Three decades ago, Harris *et al.* and Sprunt *et al.* reported the clinical relevance of surveillance cultures in neonates, but pointed out that collection is labour intensive and expensive.<sup>6,7</sup> Others have suggested that mucosal samples taken once or twice a week enable the identification of patients at risk of nosocomial infection.<sup>8–10</sup>

Two previous studies investigating the sensitivity and specificity of surveillance cultures for the prediction of invasive disease in the NICU setting have found them to be suboptimal.<sup>11,12</sup> However, these studies included all hospitalized neonates, the majority of whom are at low risk for nosocomial infection. In adults admitted to intensive care units (ICUs), sensitivity, specificity, and positive and

\* Corresponding author. Address: Department of Microbiology, Medical Faculty, University of Tartu, Ravila 19, 50411 Tartu, Estonia. Tel.: +372 7 374 170; fax: +372 7 374 172.

E-mail address: [Irja.Lutsar@ut.ee](mailto:Irja.Lutsar@ut.ee) (I. Lutsar).

negative predictive values (PPV and NPV, respectively) of 54%, 90%, 28% and 97%, respectively, have been reported for mucosal samples in the detection of further positive deep site cultures with identical pulsed-field gel electrophoresis (PFGE) patterns.<sup>13</sup>

This study aimed to examine the spectrum and time course of gut and nasopharyngeal colonization with Gram-negative micro-organisms, and to define the value of twice weekly surveillance cultures in predicting late-onset sepsis in high-risk neonates.

## Methods

### Study population and design

This study was based on a prospective, cluster-randomized trial that aimed to compare the efficacy of gentamicin combined with either ampicillin or penicillin in neonates at risk of early-onset sepsis, conducted in two third-level NICUs in Estonia from 2 August 2006 to 30 November 2007. The results of the trial have been presented elsewhere.<sup>14</sup>

### Sample collection for microbiological studies

Normally sterile body fluids (typically 0.5 mL blood and/or 0.2 mL cerebrospinal fluid) were cultured on admission and subsequently if the clinical condition worsened and symptoms suggestive of neonatal sepsis developed.<sup>14</sup> Rectal and nasopharyngeal samples, or tracheal aspirates in intubated patients, were collected with transport swabs (Nuova Aptaca, Canelli, Italy) on admission and then twice weekly until discharge or 60 days of admission, whichever came first. Samples were stored at  $-20^{\circ}\text{C}$  for a maximum of two weeks and analysed in batches. In the pilot study conducted to compare the recovery rates of frozen swabs with the freshly cultured samples, the sensitivity and specificity of frozen samples were 100% [95% confidence interval (CI) 79–100] and 81% (95% CI 54–95), respectively, and the recovery rate was 86%.

### Bacteriological methods

Transport swabs were thawed and plated on to blood agar, MacConkey agar, MacConkey agar with 16  $\mu\text{g}/\text{mL}$  ampicillin, and Mueller–Hinton agar with 6  $\mu\text{g}/\text{mL}$  gentamicin, and incubated at  $37^{\circ}\text{C}$  in air for 24–48 h.<sup>15</sup> Each morphologically different colony type was identified to species and genus level using standard laboratory methods, and confirmed with API 20E kits (bioMérieux, Marcy l'Etoile, France). Sterile fluid samples were processed using a BACTEC 9240 system (Bac Tec, LLC, Lincoln, USA) in the microbiology laboratories of the University Clinics of Tartu and the North Estonian Medical Centre. Microbes were identified with the VITEK 2 system (Biomérieux, Durham, France). All isolates were stored in skimmed milk at  $-80^{\circ}\text{C}$  before molecular typing.

Mucosal and invasive strains of *Klebsiella pneumoniae* and *Enterobacter cloacae* were typed by PFGE method according to the manufacturer's instructions, using *SpeI* enzyme restriction (Gene-path, Bio-Rad, Marnes-la-Coquette).

Electrophoresis was performed using a CHEF-DR II system (Bio-Rad). Gel images were processed with Gene Tools software syngene version 1.2 (Syngene, Cambridge, UK). PFGE typing of *Acinetobacter baumannii* was performed using the enzyme *ApaI*.<sup>16</sup>

### Definitions

Late-onset sepsis was diagnosed if Gram-negative organisms were cultured from normally sterile body fluids, and clinical and laboratory signs of sepsis were present at  $\geq 72$  h of life.<sup>14</sup>

If different species were cultured in the same patient more than two weeks apart, they were considered as separate episodes. Pre-existing mucosal colonization was defined as the presence of Gram-negative micro-organisms on mucosal surfaces two to 14 days before the collection of sterile fluid cultures.

Phenotypically similar micro-organisms from mucosa and sterile body fluids were considered to be concordant. PFGE results, if available, took precedence over phenotypic culture data.

### Statistical analysis

Sigma Stat for Windows 2.0 (Jandel Corporation, San Rafael, CA, USA) and R 2.6.2 (The R Foundation for Statistical Computing, Vienna, Austria <http://www.r-project.org>) were used for statistical analysis.

### Sensitivity, specificity, and positive and negative predictive values

For each mucosal and invasive sample pair, the following algorithm was used to calculate the predictive value:

- (1) true-positive (TP), when both samples yielded phenotypically similar organisms;
- (2) true-negative (TN), when both samples were negative;
- (3) false-positive (FP), when a positive mucosal sample was paired with a negative blood culture or with a different organism from a positive blood culture; and
- (4) false-negative (FN), when a negative mucosal sample was paired with a positive blood culture.

Separate analyses were conducted for rectal and nasopharyngeal samples, and for the combined dataset. In the combined analysis, if a sample from one site was TP and a sample from the other site was FN, it was labelled TP. If a sample from one site was FP and a sample from the other site was TN, it was labelled FP.

Sensitivity, specificity, PPV and NPV were calculated for all Gram-negative species according to the time frame of sample collection (2–6, 7–10 or 11–14 days prior to infection). For the per-patient analysis, odds ratios (OR) with 95% CIs were calculated. Patients with positive mucosal and invasive cultures with concordant organisms were assigned to TP, those with negative mucosal and invasive cultures were assigned to TN, those with positive mucosal cultures and negative invasive cultures were assigned to FN, and all remaining patients were assigned to FP.

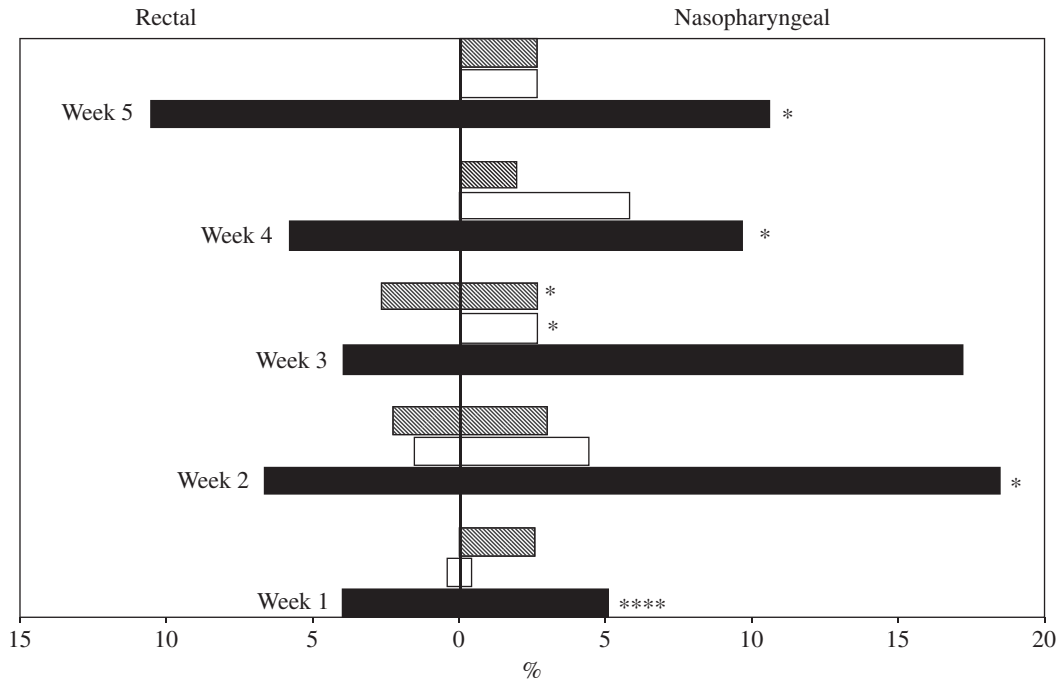
A multi-variate mixed model adjusted for study centre, treatment period and individual subject was used to identify factors improving the accuracy of prediction of invasive disease. The model included the following parameters: time from mucosal sample to invasive sample, number of TP mucosal samples prior to invasive sample, gestational age, collection site and postnatal age.

The study was approved by the Ethics Committee of the University of Tartu.

## Results

### Patients

Of 465 neonates admitted during the study period, 283 met the inclusion criteria. Mucosal cultures were available for 278 of these neonates. The presence of risk factors for late-onset sepsis, as defined by Perlman *et al.*,<sup>17</sup> was as follows: 75 (27%) subjects had birthweight  $< 1000$  g and 144 (52%) had birthweight  $< 1500$  g, 59 (21%) subjects had total parenteral nutrition during the first week of life, 240 (86%) subjects had central venous catheters, and 215 (77%) subjects were mechanically ventilated.

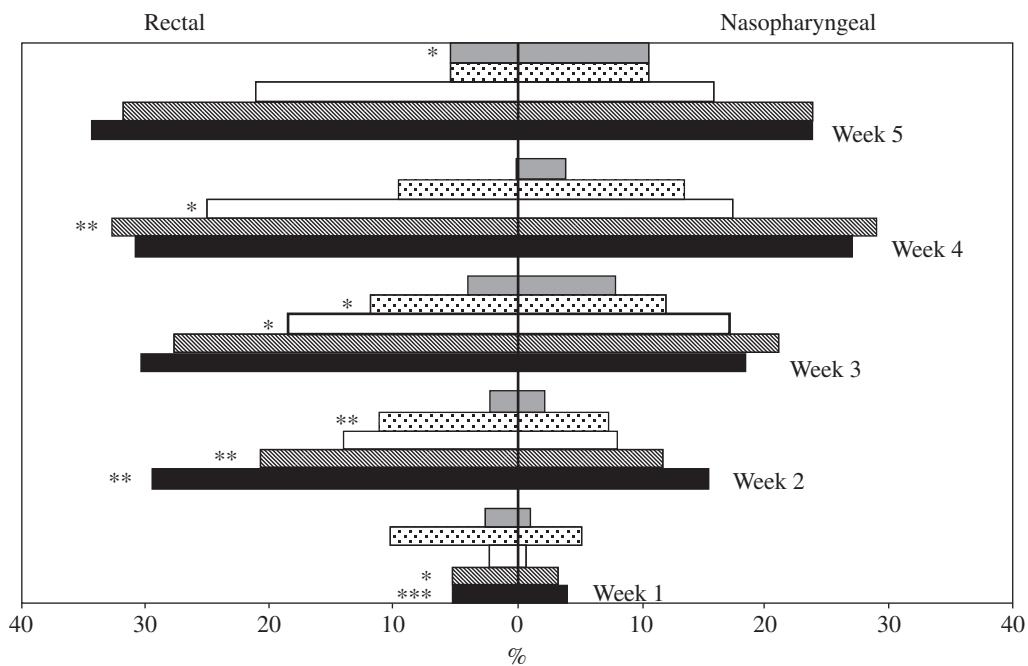


**Figure 1.** Timing of rectal and nasopharyngeal colonization with non-fermentive Gram-negative micro-organisms during the first five weeks of life. x-axis represents percentage of colonized patients. *Pseudomonas* spp., striped bars; *Stenotrophomonas* spp., open bars; *Acinetobacter* spp., solid bars. Asterisks indicate cases of late-onset sepsis. The following differences between nasopharyngeal and rectal colonization were statistically significant: *Acinetobacter* spp. at two weeks ( $P = 0.006$ ), *Acinetobacter* spp. at three weeks ( $P = 0.017$ ) and *Pseudomonas* spp. at one week ( $P = 0.022$ ).

*Sterile body fluid cultures*

In total, 555 sterile body fluid samples (514 blood, 36 cerebrospinal fluid and five others) were collected, with a median of two

samples per patient (range 0–24); of these, 32 (6%) contained Gram-negative organisms. The prevalence of Gram-negative late-onset sepsis was 10% ( $N = 27$ ); this was caused by *Enterobacteriaceae* in 18 cases and by non-fermentative organisms in nine



**Figure 2.** Timing of rectal and nasopharyngeal colonization with Enterobacteriaceae during the first five weeks of life. x-axis represents percentage of colonized patients. *Serratia* spp., grey bars; *Escherichia coli*, stippled bars; *Klebsiella oxytoca*, open bars; *Klebsiella pneumoniae*, striped bars; *Enterobacter cloacae*, solid bars. Asterisks indicate cases of late-onset sepsis. The following differences between nasopharyngeal and rectal colonization were statistically significant: *K. pneumoniae* at one week ( $P = 0.047$ ), *E. cloacae* at two weeks ( $P = 0.009$ ) and *E. coli* at one week ( $P < 0.001$ ).

**Table 1**  
Microbes causing late-onset sepsis (LOS) and their association with mucosal colonization

	Total colonized	No. of patients with LOS	Colonized and with LOS	OR (95% CI)	Colonized prior to LOS	OR (95% CI)
<i>Enterobacter cloacae</i>	76	5	3	4.11 (0.55–35.96)	1	0.66 (0.28–6.15)
<i>Klebsiella pneumoniae</i>	51	6	5	24.57 (2.69–587.93)	4	9.57 (1.45–77.74)
<i>Escherichia coli</i>	49	3	3	34.55 (2.03–20,474.48)	3	34.55 (2.03–20,474.48)
<i>Acinetobacter</i> spp.	47	7	5	13.63 (2.41–105.29)	2	2.01 (0.26–12.20)
<i>Klebsiella oxytoca</i>	46	3	3	37.41 (2.20–22,188.59)	3	37.41 (2.20–22,188.59)
<i>Serratia</i> spp.	31	1	1	24.34 (0.81–16,921.25)	1	24.34 (0.81–16,921.25)
<i>Stenotrophomonas</i> spp.	12	1	1	69.52 (2.19–49,681.68)	1	69.52 (2.18–49,681.68)
<i>Pseudomonas aeruginosa</i>	7	1	1	125.31 (3.69–92,781.90)	1	125.31 (3.69–92,781.90)

OR, odds ratio; CI, confidence interval.

cases. The median time of occurrence of sepsis was 13 days after mucosal sample collection; sepsis occurred earliest for *E. cloacae* and latest for *Klebsiella oxytoca* (median eight and 30 days, respectively).

#### Mucosal colonization

One hundred and seventy-one (62%) patients had mucosal colonization with Gram-negative micro-organisms; the number of different species ranged from one to eight (median 3). Approximately half of the rectal (693/1250, 55%) and nasopharyngeal (558/1153, 48%) samples were culture positive. In addition, 60 tracheal samples (median 0.22, range 0–7) contained 32 organisms.

As shown in Figures 1 and 2, carriage of Enterobacteriaceae increased with the duration of NICU stay. In the first week of life, *K. pneumoniae* and *Escherichia coli* colonized the gut more often than the nasopharynx (OR 2.55, 95% CI 1.08–6.03 and OR 2.19, 95% CI 1.11–4.31, respectively), but no relevant differences were observed between the two sites in the second week of life, except for *E. cloacae* (OR 2.28, 95% CI 1.26–4.13). No association was found between non-fermentative micro-organisms and duration of NICU stay, but *Acinetobacter* spp. tended to colonize the nasopharynx more often than the gut (OR 3.18, 95% CI 1.42–7.10 and OR 5.02, 95% CI 1.37–18.42, respectively) in the second and third weeks of life.

#### Associations between infection and colonization

In 22 of 27 cases of late-onset sepsis, mucosal and invasive strains were phenotypically identical, and preceded invasive disease in 16 of 22 colonizations. The median duration of prior colonization with a concordant strain was 10.5 days (range 2–36) for Enterobacteriaceae and seven days (range 2–29) for non-fermentative micro-organisms. Nine of 16 patients were colonized with several Gram-negative strains (range 2–6). All invasive infections were mono-infections.

**Table 2**  
Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of mucosal and invasive sample pairs overall and by site and time of colonization

	Sensitivity		Specificity		PPV		NPV	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
All samples (N = 2108)	26.8	19.1–36.1	65.7	63.6–67.8	4.2	2.9–6.0	94.1	92.7–95.3
Enterobacteriaceae	37.3	26.2–49.8	65.8	65.5–66.3	3.5	2.5–4.7	96.9	96.3–97.5
Non-fermentative micro-organisms	11.1	4.2–24.7	66.5	66.3–66.8	0.8	0.3–1.7	97.0	96.8–97.5
Site of colonization								
Nasopharyngeal (N = 1046)	27.6	17.0–41.1	65.8	62.7–68.7	4.5	2.7–7.4	93.9	91.8–95.5
Rectal (N = 1062)	25.9	15.4–39.9	65.7	62.6–68.6	3.9	2.2–6.6	94.3	92.3–95.8
Any mucosa (N = 1053)	29.3	26.8–53.2	49.2	46.3–52.1	3.6	2.3–5.5	94.4	92.1–96.0
Sampling time (days prior to infection)								
Days 2–6 (N = 975)	36.2	23.1–51.5	61.9	58.6–64.9	4.6	2.8–7.4	95.0	92.9–96.6
Days 7–10 (N = 635)	18.9	8.6–35.7	68.1	64.1–71.8	3.5	1.6–7.4	93.1	90.2–95.2
Days 11–14 (N = 498)	21.4	9.0–41.5	70.4	66.0–74.5	4.1	1.7–9.2	93.8	90.6–96.0

'Any mucosa', positive sample in at least one mucosal site (e.g. nasopharyngeal and/or rectal); CI, confidence interval.

Prior mucosal colonization with Gram-negative organisms other than *E. cloacae* and *Serratia* spp. was associated with invasive infection (Table 1). The genetic relatedness of colonizing and infecting strains of *K. pneumoniae*, *E. cloacae* and *A. baumannii* was confirmed by PFGE analysis in all cases.

#### Predictive value of mucosal site cultures

In total, 2108 invasive and mucosal culture pairs were assessed. Overall sensitivity, specificity, PPV and NPV were 27%, 66%, 4% and 94%, respectively (Table 2). Overall sensitivity (37% vs 11%) and PPV (4% vs 1%) were greater for Enterobacteriaceae compared with non-fermentative organisms, and at 2–6 days prior to infection compared with 7–10 and 11–14 days prior to infection (sensitivity: 2–6 days 50% vs 16%, 7–10 days 26% vs 7%, 11–14 days 31% vs 8%; and PPV: 2–6 days 4% vs 1%, 7–10 days 3% vs 0.5%, and 11–14 days 3.5% vs 1%, respectively). All other parameters were similar for both sites and all time periods. In the multi-variate mixed model analysis, only the number of TP surveillance cultures prior to invasive disease (OR 4.66, 95% CI 2.28–9.55;  $P < 0.0001$ ) and younger postnatal age (per additional day, OR 0.94, 95% CI 0.88–1.00;  $P = 0.047$ ) improved the accuracy of prediction.

The analysis of different species indicated that sensitivity was above 60% for *K. oxytoca*, *E. coli* and *Pseudomonas aeruginosa*, but was lower for all other species (Table 3). This was most likely due to the small sample size, and there was wide variation in the 95% CIs. In contrast, specificity exceeded 92% for all organisms. In addition, high NPVs (>98% for all species) were observed, enabling the identification of patients at low risk of late-onset sepsis.

#### Discussion

This study of neonates at high risk of healthcare-associated infections demonstrated that prior mucosal colonization with Gram-negative organisms other than *E. cloacae*, *Serratia* spp. and

**Table III**  
Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) of mucosal and invasive sample pairs by bacterial species

Microbes	Sensitivity		Specificity		PPV		NPV	
	%	95% CI	%	95% CI	%	95% CI	%	95% CI
<i>Klebsiella oxytoca</i>	75.0	43.3–93.3	92.8	92.5–93.0	10.3	6.0–12.9	99.7	99.3–99.9
<i>Pseudomonas aeruginosa</i>	66.7	12.7–98.2	99.0	98.8–99.1	15.4	2.9–22.7	99.9	99.8–100
<i>Escherichia coli</i>	62.5	26.2–89.7	94.8	94.5–95.0	8.1	3.4–11.6	99.7	99.4–99.9
<i>Klebsiella pneumoniae</i>	35.7	14.1–63.8	94.0	93.8–94.4	7.1	2.8–12.8	99.1	98.9–99.5
<i>Serratia</i> spp.	33.3	1.8–87.3	98.6	98.5–98.8	6.3	0.3–16.4	99.8	99.7–100
<i>Stenotrophomonas</i> spp.	33.3	1.8–87.3	97.9	97.8–98.0	4.2	0.2–10.9	99.8	99.7–100
<i>Enterobacter cloacae</i>	7.7	0.4–37.2	93.2	93.1–93.5	1.3	0.1–6.5	98.8	98.7–99.2
<i>Acinetobacter</i> spp.	4.8	0.2–25.1	93.8	93.7–94.2	1.5	0.1–7.7	98.1	98.0–98.5

Both mucosal sampling sites (nasopharyngeal and rectal) and all time points (from two to 14 days prior to infection) are included.

*Acinetobacter* spp. may lead to invasive disease. However, as found in previous studies, the sensitivity, specificity and PPVs of mucosal surveillance samples in predicting invasive disease were moderate for Enterobacteriaceae (37%, 66% and 4%, respectively) and suboptimal for non-fermentative micro-organisms (11%, 67% and 1%, respectively).<sup>11,12</sup>

In order to initiate prompt appropriate antibacterial therapy, physicians and scientists have studied the value of regular surveillance cultures over several decades.<sup>6,7,9–11,13,18,19</sup> Two types of study have been published: those looking at the predictive value of individual mucosal samples, and those comparing colonized and uncolonized patients.<sup>6,7,9–11,13,18</sup> Both methods were used in this study.

Research based on the correlation of mucosal and invasive sample pairs is more precise and scientifically sound, as it allows estimation of the predictive value of each individual sample. However, this approach results in suboptimal sensitivity, specificity and PPVs of mucosal samples.<sup>11,12</sup> In addition, this method is far from the real-life situation; if surveillance samples are implemented, they are collected regularly and decisions are rarely based on a single sample. Therefore, a per-patient analysis was also used in this study. The results confirmed previous findings in adult ICUs, indicating that if colonized patients and specific organisms are considered, instead of a single sample and specific organisms, the sensitivity and specificity of mucosal cultures are acceptable; this may aid in guiding empirical antimicrobial treatment and in preventing the spread of organisms that are difficult to manage, such as *P. aeruginosa*.<sup>20,21</sup> However, it is important to emphasize that in the NICU setting (at least in this study), most patients have several micro-organisms on the mucosal surfaces, and this method does not allow discrimination between colonizing and invasive strains.

Another benefit of surveillance cultures is the monitoring of alert micro-organisms and reinforcing infection control measures, including isolation of colonized patients, to interrupt horizontal spread and avoid outbreaks.<sup>13,22</sup> However, collection and processing of surveillance cultures is labour intensive and costly; without a clear target, surveillance cultures will have little use in clinical practice.<sup>11,12</sup> Furthermore, a study in adult ICU patients has reported a negative effect of regular collection of screening samples if therapy guided by these samples results in overuse of antibiotics with consequent increases in costs, antibiotic resistance and potential superinfections.<sup>23</sup>

In agreement with previous research, this study found a low rate of positive blood cultures (6%) despite the fact that it was conducted in a high-risk setting and blood samples were only taken when clinical signs suggested invasive infection.<sup>24,25</sup> However, this is not surprising as the relatively small amounts of blood available for culturing and low bacterial loads have been associated with negative cultures in neonates.<sup>26,27</sup> Some such neonates with late-onset sepsis will therefore be categorized as FP, leading to low sensitivity and PPVs, as seen in this analysis. The value of surveillance cultures should be re-assessed when new and more sensitive

diagnostic tests to identify infecting organisms become available.<sup>28,29</sup>

This study demonstrated that the predictive value of mucosal cultures was particularly poor in infections caused by *A. baumannii*. The ratio of infection to colonization was 1:7, but mucosal colonization led to invasive disease in most cases. *A. baumannii* infection, which usually occurs in patients with prior risk factors, is known to be hospital acquired and outbreaks are not uncommon.<sup>30</sup> The study data suggest that mucosal cultures are not useful for selecting appropriate antibacterial agents for *A. baumannii*, but may have value in avoiding cross-colonization.

Universal screening of mucosal surfaces in NICUs is impractical as it is too expensive and time consuming. Furthermore, selection of antibiotics based on a single positive mucosal culture may even be misleading.<sup>23</sup> However, the collection of rectal or nasopharyngeal swabs may have some value in cohorting chronically colonized patients, or selecting appropriate antibacterial therapy during outbreaks of hospital-acquired infections.<sup>31</sup> It is recommended that surveillance cultures should be taken twice weekly in such circumstances. This study has demonstrated that the prediction of invasive disease depends on the duration of mucosal colonization. Also, the higher the number of positive cultures for a specific organism, the greater the likelihood of this organism being a cause of invasive disease.

In conclusion, mucosal surfaces may be an important source of infection, but routine and non-targeted surveillance cultures are not efficient for the prediction of late-onset sepsis in NICUs. However, targeted screening for specific organisms (e.g. *Klebsiella* spp., *E. coli*, *Stenotrophomonas* spp. and *Pseudomonas* spp.) in an outbreak situation may offer an opportunity to improve infection control measures and enable timely initiation of appropriate antibiotic therapy.

#### Acknowledgements

The authors wish to thank their colleagues from both participating NICUs for their support and help with documenting all clinical decisions, head nurse Tiina Kilg and all nurses for their help with sample collection.

#### Conflict of interest statement

None declared.

#### Funding sources

Estonian Science Foundation Grant No. 6984, Estonian Target Financing No 2726 and ESPID Small Grant Award.

#### References

- Phillips P, Cortina-Borja M, Millar M, Gilbert R. Risk-adjusted surveillance of hospital-acquired infections in neonatal intensive care units: a systematic review. *J Hosp Infect* 2008;**70**:203–211.

2. Almuneef MA, Baltimore RS, Farrel PA, Reagan-Cirincione P, Dembry LM. Molecular typing demonstrating transmission of Gram-negative rods in a neonatal intensive care unit in the absence of a recognized epidemic. *Clin Infect Dis* 2001;**32**:220–227.
3. Goldmann DA, Leclair J, Maccone A. Bacterial colonization of neonates admitted to an intensive care environment. *J Pediatr* 1978;**93**:288–293.
4. Parm U, Metsvaht T, Sepp E, et al. Impact of empiric antibiotic regimen on bowel colonization in neonates with suspected early onset sepsis. *Eur J Clin Microbiol Infect Dis* 2010;**29**:807–816.
5. Graham 3rd PL, Della-Latta P, Wu F, Zhou J, Saiman L. The gastrointestinal tract serves as the reservoir for Gram-negative pathogens in very low birth weight infants. *Pediatr Infect Dis J* 2007;**26**:1153–1156.
6. Harris H, Wirtschafter D, Cassidy G. Endotracheal intubation and its relationship to bacterial colonization and systemic infection of newborn infants. *Pediatrics* 1976;**58**:816–823.
7. Sprunt K, Leidy G, Redman W. Abnormal colonization of neonates in an intensive care unit: means of identifying neonates at risk of infection. *Pediatr Res* 1978;**12**:998–1002.
8. Silvestri L, Monti Bragadin C, Milanese M, et al. Are most ICU infections really nosocomial? A prospective observational cohort study in mechanically ventilated patients. *J Hosp Infect* 1999;**42**:125–133.
9. Shankar KR, Brown D, Hughes J, et al. Classification and risk-factor analysis of infections in a surgical neonatal unit. *J Pediatr Surg* 2001;**36**:276–281.
10. Pierro A, van Saene HK, Jones MO, Brown D, Nunn AJ, Lloyd DA. Clinical impact of abnormal gut flora in infants receiving parenteral nutrition. *Ann Surg* 1998;**227**:547–552.
11. Evans MS, Federspiel C, Cotton RB, McKee KT, Stratton CW. Sensitivity, specificity, and predictive value of body surface cultures in a neonatal intensive care unit. *JAMA* 1988;**259**:248–252.
12. Choi Y, Saha SK, Ahmed AS, et al. Routine skin cultures in predicting sepsis pathogens among hospitalized preterm neonates in Bangladesh. *Neonatology* 2008;**94**:123–131.
13. Bertrand X, Thouverez M, Talon D, et al. Endemicity, molecular diversity and colonisation routes of *Pseudomonas aeruginosa* in intensive care units. *Intensive Care Med* 2001;**27**:1263–1268.
14. Metsvaht T, Ilmoja ML, Parm U, Maipuu L, Merila M, Lutsar I. Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis. *Acta Paediatr* 2010;**99**:665–672.
15. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; fifteenth informational supplement*. Wayne, PA, USA: Clinical and Laboratory Standards Institute. 2005. pp. 24–25.
16. Antibiotic Resistance Prevention and Control. *PFGE typing protocol recommended by ARPAC for Acinetobacter baumannii*. Schiller Park, IL, USA: Antibiotic Resistance Prevention and Control. 2009.
17. Perlman SE, Saiman L, Larson EL. Risk factors for late-onset health care-associated bloodstream infections in patients in neonatal intensive care units. *Am J Infect Control* 2007;**35**:177–182.
18. Miranda LN, van der Heijden IM, Costa SF, et al. Candida colonisation as a source for candidaemia. *J Hosp Infect* 2009;**72**:9–16.
19. Srivastava S, Shetty N. Healthcare-associated infections in neonatal units: lessons from contrasting worlds. *J Hosp Infect* 2007;**65**:292–306.
20. Blot S, Depuydt P, Vogelaers D, et al. Colonization status and appropriate antibiotic therapy for nosocomial bacteremia caused by antibiotic-resistant Gram-negative bacteria in an intensive care unit. *Infect Control Hosp Epidemiol* 2005;**26**:575–579.
21. Bertrand X, Thouverez M, Patry C, Balvay P, Talon D. *Pseudomonas aeruginosa*: antibiotic susceptibility and genotypic characterization of strains isolated in the intensive care unit. *Clin Microbiol Infect* 2001;**7**:706–708.
22. Mammina C, Di Carlo P, Cipolla D, et al. Surveillance of multidrug-resistant Gram-negative bacilli in a neonatal intensive care unit: prominent role of cross transmission. *Am J Infect Control* 2007;**35**:222–230.
23. Warren MM, Gibb AP, Walsh TS. Antibiotic prescription practice in an intensive care unit using twice-weekly collection of screening specimens: a prospective audit in a large UK teaching hospital. *J Hosp Infect* 2005;**59**:90–95.
24. Tagare A, Kadam S, Vaidya U, Pandit A. Routine antibiotic use in preterm neonates: a randomised controlled trial. *J Hosp Infect* 2010;**74**:332–336.
25. Ronnestad A, Abrahamsen TG, Medbo S, et al. Late-onset septicemia in a Norwegian national cohort of extremely premature infants receiving very early full human milk feeding. *Pediatrics* 2005;**115**:e269–76.
26. Jordan JA, Durso MB. Comparison of 16S rRNA gene PCR and BACTEC 9240 for detection of neonatal bacteremia. *J Clin Microbiol* 2000;**38**:2574–2578.
27. Glynn C, Azadian B. Empiric antimicrobial therapy for severe sepsis in the intensive care unit: in early, hit hard, out early. *Curr Anaesth Crit Care* 2005;**16**:221–230.
28. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999;**69**:1035S–1045S.
29. de La Cochetiere M, Rouge C, Darmaun D, Roze J, Potel G, Leguen C. Intestinal microbiota in neonates and preterm infants: a review. *Curr Pediatr Rev* 2007;**3**:21–34.
30. Zarrilli R, Casillo R, Di Popolo A, et al. Molecular epidemiology of a clonal outbreak of multidrug-resistant *Acinetobacter baumannii* in a university hospital in Italy. *Clin Microbiol Infect* 2007;**13**:481–489.
31. Friedman ND, Kotsanas D, Brett J, Billah B, Korman TM. Investigation of an outbreak of *Serratia marcescens* in a neonatal unit via a case–control study and molecular typing. *Am J Infect Control* 2008;**36**:22–28.