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Original Article

Ampicillin versus penicillin in the empiric therapy of extremely low-birthweight neonates at risk of early onset sepsis

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Abstract

Background: There are no comparative data on the impact of different empiric antibiotic regimens on early bowel colonization as well as on clinical efficacy in extremely low-birthweight (ELBW) neonates at risk of early onset sepsis (EOS).

Methods: A subgroup analysis was carried out of ELBW neonates recruited into a two-center, prospective, cluster randomized study comparing ampicillin and penicillin both combined with gentamicin, within the first 72 h of life. A composite primary end-point (need for change of antibiotics within 72 h and/or 7 day all-cause mortality) and the rate and duration of colonization by opportunistic aerobic microorganisms were assessed using hierarchical models corrected for study center and period.

Results: In the ampicillin ($n = 36$) and penicillin ($n = 39$) groups change of antibiotics, 7 day mortality and the composite end-point occurred at similar rates. Neonatal intensive care unit mortality for infants with gestational age <26 weeks was lower in the ampicillin group. Ampicillin treatment was associated with a higher colonization rate by *Klebsiella pneumoniae*, including ampicillin-resistant strains.

Conclusion: Preliminary data indicate an urgent need for adequately powered studies of early antibiotic therapy in the subpopulation of ELBW neonates at risk of EOS.

Key words antibiotics, extremely low-birthweight neonate, gut colonization.

The empiric antibacterial choices for the treatment of early onset sepsis (EOS) in extremely low-birthweight (ELBW) neonates are based on data originating from studies performed in larger infants, and date from times when ELBW babies were not even there to be studied.[1] We are not aware of any comparative studies on the clinical efficacy or impact on bowel colonization of empiric antibiotics (AB) in this population, although differences in bacterial etiology of EOS,[2–5] as well as in host response, compared to term infants have been described.[6,7]

Ampicillin (AMP) and penicillin G (PEN) in combination with gentamicin (GEN) are the most widely recommended and used regimens in the empiric treatment of neonatal EOS.[1,8] The two treatments differ in their antibacterial coverage, with AMP having greater efficacy against Gram-negative microorganisms such as *Escherichia coli.* This distinction is likely most relevant in the treatment of ELBW neonates, because Gram-negative pathogens, particularly *E. coli*, have been found to be dominant among EOS-causative microorganisms in this population.[2,4,5,9] In contrast, AMP resistance is increasing among *E. coli* strains,[10–12] already reaching 80% in some centers.[9,12] AMP has also been associated with the spread of extended spectrum beta-lactamase-positive *Klebsiella pneumoniae* strains in the neonatal intensive care (NICU) environment, induction of beta-lactam resistance in neonatal strains of *Enterobacter cloacae* and probably with *Candida* colonization – all carrying high risk for ELBW neonates.[13–16] Narrow-spectrum penicillins such as PEN, in contrast, have the least potential to interfere with normal gut colonization.[17,18] It is not clear, however, whether these disadvantages of AMP have outweighed the potential of better Gram-negative coverage, because the two regimens have never been compared in ELBW neonates.

Based on these considerations and the lack of any data in the field, we performed a subgroup analysis of ELBW neonates recruited in a larger two-center study, comparing the clinical efficacy and impact on early gut colonization of AMP versus PEN both combined with GEN in the empirical treatment for risk of EOS.[19]

Methods

Study design

A subgroup analysis included all ELBW neonates (birthweight <1000 g) participating in a prospective, open label, cluster-randomized study conducted in two third-level NICU in Estonia

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from 2 August 2006 to 30 November 2007. The study design, together with inclusion and exclusion criteria, is described in detail elsewhere.[19] Briefly, the study included all neonates admitted to NICU within 72 h of life needing empiric AB for EOS or for risk factors of infection according to CDC criteria (e.g. maternal chorioamnionitis and/or maternal risk factors of infection and/or preterm labor in <35 weeks of gestation)[20] and not transferred within the first 24 h.

Both units admit patients up to 16 years of age; approximately 60–65% of them are neonates cared for in a separate area. The units are divided into four and five rooms, respectively; the number of neonates in a room varies from three to six. The nursing staff/infant ratio in the units is 1:2, but can be 1:3 occasionally. Gloves, gowns, caps and masks are used routinely in all aseptic procedures. Both units follow similar hospital infection prevention guidelines and strict AB policy, in which narrow-spectrum AB and short courses are preferred. Fluconazole prophylaxis is used in one of the participating units in ELBW neonates during broad-spectrum AB coverage. Patients colonized with alert microorganisms are isolated in separate rooms and cared for by separate nurses. Both units practice early introduction of enteral feeding with preference given to fresh or frozen own mother's milk. Formula, if needed, is prepared centrally; donor milk is not used. In all referring maternity hospitals risk factor-based intrapartum AB prophylaxis of group B streptococci (GBS) is used.

Treatment and monitoring of patients

During the first phase of the study (2 August 2006–20 March 2007) GEN (4 mg/kg every 48 h)[21] was combined with AMP (25 mg/kg every 12 h)[21,22] in unit A, and with PEN G (25 000 IU/kg every 12 h)[23] in unit B. In the second phase (21 March 2007–30 November 2007), after half of the power calculation-based study population was recruited, beta-lactam AB were switched. All clinical and laboratory findings were recorded according to the clinical routine. Feeding regimen was documented on days 1, 3 and 7 with patients categorized into the following groups based on the route of nutrition and the character of enteral feeds: (i) total parenteral nutrition (enteral calories constituted <10% of total daily calories); (ii) breastfeeding (breast milk constituted >10% of enteral feeds); and (iii) formula feeding (formula constituted >89% of enteral feeds). Additional parenteral nutrition supplying up to 89% of daily caloric intake was accepted in the two latter groups.

Microbiological samples from blood were collected on admission and thereafter from sterile body sites as clinically indicated. Gut colonization was monitored with swabs obtained from the perineum on admission and twice a week thereafter.[19] The swabs were stored at −20°C for a maximum of 1 week and processed in batches; the results were not reflected in AB prescription. All microbiological studies followed routine methods according to the CSLI criteria.[24] AMP resistance of colonizing Gram-negative microorganisms was assessed by the growth on MacConkey agar with 16 mg/L of AMP.

Diagnostic criteria of neonatal sepsis

The diagnostic criteria of neonatal sepsis have been published previously.[19] All sepsis cases were categorized as proven when, in addition to at least two clinical and laboratory signs, a pathogen was isolated from a normally sterile body fluid (or in the case of coagulase-negative staphylococci: either two positive cultures, or one positive culture plus adequate AB treatment for >72 h); and as clinical sepsis in all remaining cases. Sepsis occurring within the first 72 h was classified as EOS and all other cases as late-onset sepsis (LOS).[25]

The study was approved by the Ethics Committee of the University of Tartu and registered at ClinicalTrials.gov (identifier: NCT00487019). All parents were informed about the study. Informed consent was not considered necessary by the Ethics Committee, because both AB regimens were equally used in both centers and routine colonization surveillance using rectal swabs had been implemented prior to the study. Therefore the study was associated neither with any changes in routine treatment practice nor with additional sampling. Separate informed consent was required for all participants with birthweight <1200 g for additional colonization studies (stool sampling at day 1–3, 1 month and 2 months of age).

End-points

The primary end-point was AB treatment failure defined as need for change of the initial empiric AB regimen within 72 h, based on pre-specified situations,[19] and/or any death within 7 days.

Secondary end-points were 28 day mortality and NICU mortality and duration of NICU stay, hospital stay, early empiric AB treatment, respiratory support and vasoactive treatment, rate of LOS and use of additional AB therapy, the presence of necrotizing enterocolitis (NEC) stage II–III,[26] patent ductus arteriosus (PDA) requiring surgical treatment, threshold retinopathy of prematurity (ROP) requiring laser therapy, severe intraventricular hemorrhage (IVH; stage III–IV),[27] severe bronchopulmonary dysplasia[28] and colonization with Gram-negative AMP-resistant bacteria and fungi. Colonization pattern was assessed as the rate of colonized patients and colonization duration (CD); the latter describing the ratio of colonizing days per 100 NICU days.

Genetic analysis

To detect genetic relatedness of mucosal and invasive strains of *K. pneumoniae* and *E. cloacae*, pulse field gel electrophoresis (PFGE) was used according to the manufacturer's instructions (Bio-Rad, France). DNA was extracted with commercially available PFGE kits using SpeI enzyme (Genepath; Bio-Rad). Electrophoresis was performed with the CHEF-DR II (Bio-Rad). The images of the gels were processed with the software Gene Tools version 1.2 (Syngene, UK). The PFGE typing of *Acinetobacter baumannii* was performed as previously described using enzyme ApaI.[29] The results were estimated as discriminatory if at least a 10-banding pattern appeared; up to three band differences in PFGE were considered to represent clonally related strains.

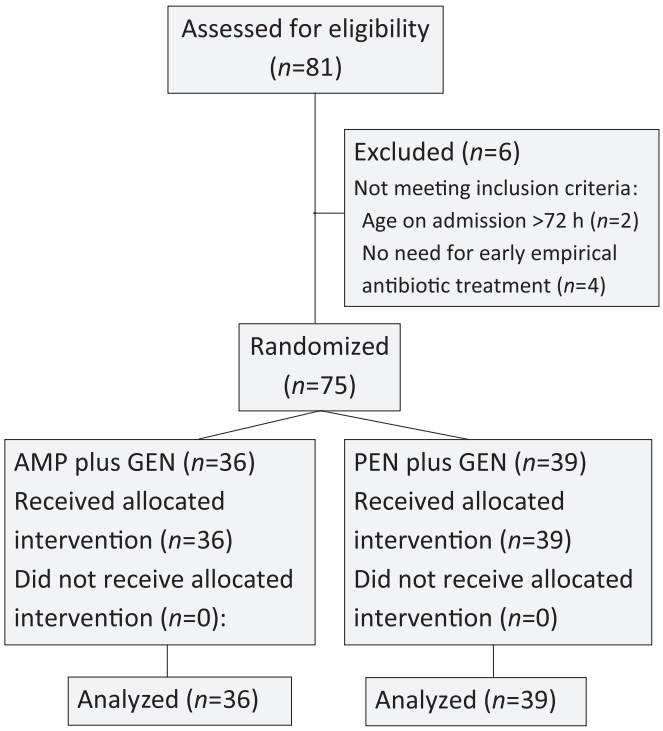

Fig. 1 Flowchart of subject selection. AMP, ampicillin; GEN, gentamicin; PEN, penicillin G.

Statistical analysis

Statistical analysis was performed using R 2.4.0 (Web-based free software). For all comparisons between groups, hierarchical models appropriate for cluster-randomized cross-over design incorporating the effect of study center and treatment period were used. Differences significant at $P < 0.1$ were further evaluated on multivariate mixed model analysis. To assess the impact of empiric AB regimen on mortality the model was adjusted for previously described risk factors of mortality in ELBW neonates, identified from the literature.[30,31] The impact of empiric AB regimen on bowel colonization was further assessed on multivariate mixed models adjusted for gestational age (GA), mode of delivery, maternal chorioamnionitis, rupture of membranes for >18 h, use of antenatal steroids and AB, type of feeding, mechanical ventilation, presence of culture-proven EOS, prior use of carbapenems, third- and fourth-generation cephalosporins, beta-lactamase-resistant penicillins and duration of NICU stay.

After the recruitment of each quarter of the study population, safety analysis evaluating mortality and major neonatal complications was conducted. No significant differences between the two regimens were observed and the study was completed as planned.

Results

Patient flow and reasons for exclusion are presented in Figure 1. A total of 81 ELBW neonates was admitted during the study period and screened for eligibility; 75 fulfilled the inclusion criteria and were recruited. All four neonates not needing empirical AB treatment were small for gestational age and were born near or at term. As shown in Table 1, with regards to demographical data the study groups were similar in all parameters.

Primary outcome and early onset sepsis

There was no difference in the composite primary end-point or either of its components between the two treatment groups (Table 2). Apart from EOS, six early neonatal deaths were caused by complications of respiratory distress syndrome (RDS; hypoxic respiratory failure in two infants and IVH III–IV in one infant in both treatment arms).

The prevalence of EOS (clinical and proven), in 15 (42%) and 16 (41%) infants in the AMP and PEN groups, respectively, was similar. All six cases of proven EOS occurred in the PEN group (odds ratio [OR], 0.1; 95% confidence interval [CI]: 0.01–0.6; Table 2) and were caused by the following microorganisms: *Staphylococcus epidermidis*, $n = 2$; *E. coli*, $n = 1$; *E. cloacae*, $n = 2$; *Candida albicans*, $n = 1$. Four of the six neonates with proven EOS died. Both *S. epidermidis* strains were resistant to PEN and GEN (not tested for AMP) and *E. cloacae* to AMP. The only *E. coli* isolate was susceptible to AMP and GEN.

Secondary outcome

There was a trend towards increased NICU mortality in the PEN group, which was significant for neonates born before the 26th week of gestation (Fig. 2). In the PEN arm six neonates died after 7 days of life; among them, only one neonate with the primary diagnosis of EOS died of IVH III–IV complications. The other five had the primary diagnosis of RDS; three died of LOS

Table 1 Subject demographic data

	AMP ($n = 36$) *n* (%)	PEN ($n = 39$) *n* (%)
Gestational age (weeks), median (IQR)	25 (24–26)	25 (25–27)
<28	33 (92)	29 (74)
<26	24 (67)	21 (54)
Birthweight (g), median (IQR)	770 (694–876)	810 (693–866)
<751	19 (53)	15 (38)
M/F sex	19 (53)	22 (56)
Mean Apgar score at 5 min (mean ± SD)	6.2 ± 1.7	5.9 ± 1.4
Ventilated	36 (100)	39 (100)
Surfactant	36 (100)	38 (97)
Cesarean section	13 (36)	20 (51)
Multiple pregnancies	5 (14)	7 (18)
Chorioamnionitis	12 (33)	19 (49)
PROM >18 h	9 (25)	13 (33)
Prenatal glycocorticoids	30 (83)	32 (82)
Maternal antibiotic therapy		
During pregnancy	7 (19)	10 (26)
During delivery	19 (53)	20 (56)
TPN/BM/Formula	18/7/11	18/10/11
Broad spectrum antibiotics	26 (72)	26 (67)
Fluconazole prophylaxis	12 (33)	7 (18)

AMP, ampicillin group; BM, breast milk; IQR, interquartile range; PEN, penicillin G group; PROM, premature rupture of membranes; TPN, total parenteral nutrition.

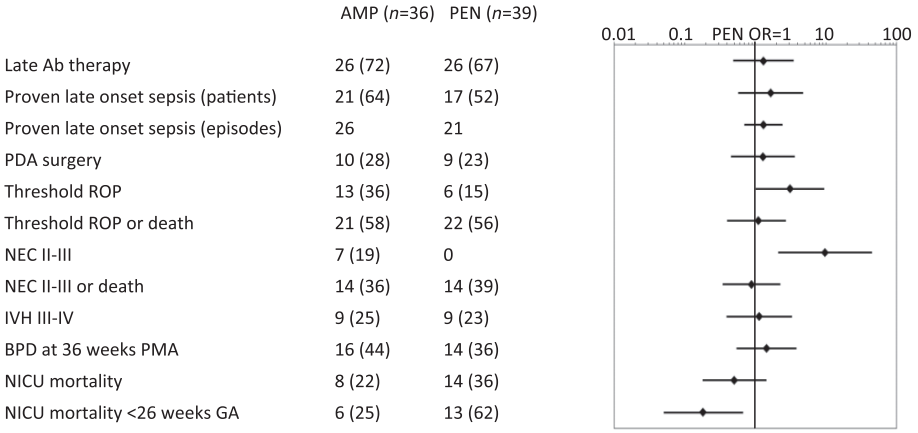

Fig. 2 Secondary outcome: odds ratios (OR) from hierarchical model analysis adjusted for study center and treatment period. Data given as *n* or as *n* (%). AB, antibiotics; AMP, ampicillin; BPD, bronchopulmonary dysplasia; GA, gestational age; IVH, intraventricular hemorrhage; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PDA, patent ductus ateriosus; PEN, penicillin G; ROP, retinopathy of prematurity.

Table 2 Primary outcome, early onset sepsis and early neonatal mortality

	AMP (*n* = 36)	PEN (*n* = 39)	OR (95%CI)
Treatment failure (composite), *n* (%)	10 (31)	10 (41)	1.1 (0.4–3.1)
Components of the composite end-point:			
Antibiotic change in 72 h (*n*/died)	4/1	3/1	1.5 (0.3–9.3)
Death in 7 days, *n* (%)	7 (22)	8 (21)	0.9 (0.3–2.9)
EOS (proven + clinical), *n* (%)	15 (42)	16 (41)	1.1 (0.5–2.9)
Died, *n* (% EOS early neonatal mortality)	4 (27)	5 (31)	NA
Proven EOS (*n*/died)	0	6/4	0.1 (0.01–0.6)
Clinical EOS (*n*/died)	15/4	10/1	NA

AMP, ampicillin group; CI, confidence interval; EOS, early onset sepsis; NA, not applicable; OR, odds ratio; PEN, penicillin G group.

(*Stenotrophomonas*; *S. aureus* and *K. oxytoca*), one of IVH III-IV complications and another of PDA complications. In the AMP arm the only case of late neonatal death was caused by LOS due to *K. pneumoniae* in a neonate with the primary diagnosis of EOS. The overall NICU mortality of infants with EOS of 5/15 (33%) and 6/16 (38%) in the AMP and PEN arm was similar. Multivariate mixed model found greater GA, full-course steroid prophylaxis and singleton pregnancy to be associated with improved survival, while early AB treatment with AMP plus GEN regimen remained of borderline significance (Table 3).

The duration of the empiric AB regimen (median, interquartile range: 67 h, 58–126 h and 60 h, 48–86 h in the AMP and PEN groups, respectively; $P = 0.871$), vasoactive and respiratory support and duration of intensive care (data not shown) as well as the number of neonates requiring initial empiric AB regimen for >72 h (36% in both groups; OR, 1.27; 95%CI: 0.46–3.51) or additional AB treatment were similar in both groups (Fig. 2). Threshold ROP and NEC stage II–III were diagnosed more frequently in the AMP group, but the difference disappeared when combined with death (Fig. 2).

Table 3 Independent risk factors of NICU mortality

	OR (95%CI)	*P*
Glycocorticoid prophylaxis		
Full course	0.08 (0.01–0.5)	0.005
Partial course	0.4 (0.06–2.2)	0.276
Gestation (per week increase)	0.6 (0.4–0.9)	0.025
Singleton pregnancy	0.1 (0.02–0.9)	0.036
Treated with AMP	0.3 (0.07–1.0)	0.055
Sex (M vs F)	1.5 (0.4–5.7)	0.547

Adjusted OR from multivariate mixed model analysis

AMP, ampicillin; CI, confidence interval; NICU, neonatal intensive care unit; OR, odds ratio.

Bacterial etiology of LOS and gut colonization

The incidence of LOS was similar in both treatment arms: 21 neonates in the AMP and 16 in the PEN group had a total of 26 and 20 LOS episodes, respectively. On multiple mixed model analysis adjusted for gender, GA, presence of EOS and age on NICU admission, neither the treatment arm (OR for penicillin regimen, 1.99; 95%CI: 0.59–6.70; $P = 0.27$) nor the duration of early empiric AB treatment (OR per each additional h 1.01; 95%CI: 1.00–1.02, $P = 0.07$) was associated with the development of proven LOS (all pathogens included). The bacterial etiology of LOS is presented in Table 4. There were four cases of *K. pneumoniae* infection, caused by a single strain during an outbreak in the AMP group versus none in the PEN group. All three cases of *Candida* infection also occurred in the AMP group.

With the exception of *E. cloacae*, mucosal colonization with a phenotypically identical strain preceded all invasive infections due to *Enterobacteriaceae* and the only case of *Stenotrophomonas* LOS. In the only case of *E. cloacae* LOS, colonization with a phenotypically identical strain was detected only 2 weeks after invasive disease. With *A. baumannii*, colonization occurred prior to invasive disease in one case. The genetic relatedness of colo-

Table 4 Bacterial etiology of late onset sepsis

	AMP (n = 36)	PEN (n = 39)
Total no. patients/no. isolates	21/27	17/22
Gram-positive	15	16
CoNS	10	9
Staphylococcus aureus (MSSA)	1	2
MRSA	1	3
Enterococcus spp.	3	1
Streptococcus salivarius	0	1
Gram-negative	9	6
Acinetobacter baumannii	2	2
Escherichia coli	2	0
Enterobacter cloacae	1	0
Klebsiella oxytoca	0	2
Klebsiella pneumoniae	4	0
Stenotrophomonas spp.	0	1
Serratia spp.	0	1
Yeasts	3	0
Candida albicans	3	0

Two neonates in the PEN group had two isolates in a single sepsis episode.

AMP, ampicillin group; CoNS, coagulase-negative staphylococci; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; PEN, penicillin G group.

nizing and infecting strains of *K. pneumoniae*, *E. cloacae* and *A. baumannii* was further confirmed on PFGE analysis in all cases.

The gut colonization pattern in both treatment arms including AMP-resistant Gram-negative microorganisms was largely similar (Table 5), except that neonates receiving the AMP-containing regimen were more likely to have colonization and had greater CD with *K. pneumoniae*, including AMP-resistant strains, and with *S. haemolyticus* and *S. hominis*, compared to those in the PEN group. There was a trend towards higher colonization rate with any *Candida* spp. ($P = 0.077$), but no difference in colonization with *C. albicans* ($P = 0.332$) was seen. On multivariate mixed model analysis AMP treatment remained an independent risk factor for colonization with *K. pneumonia* overall (OR, 5.18; 95%CI: 1.45–18.54) and with AMP-resistant strains (OR, 5.83; 95%CI: 1.63–20.84) and with *S. hominis* (OR, 12.57; 95%CI: 1.92–82.34). Neither the duration of initial empiric AB regimen (OR per additional hour, 1; 95%CI: 0.99–1.01, $P = 0.59$) nor the daily dosage (OR per additional mg/kg per day, 0.91; 95%CI: 0.77 1.08; $P = 0.28$) remained independently associated with the risk of *Klebsiella* colonization.

Discussion

This analysis, to the best of our knowledge, is the first to compare AMP plus GEN versus PEN plus GEN regimens in terms of clinical efficacy and impact on early gut colonization in the risk factor-based empiric treatment of suspected EOS in ELBW neonates. We found no difference in early neonatal mortality and/or change of AB regimen within 72 h, but found lower incidence of proven EOS, a trend towards lower NICU mortality, and a higher colonization rate with *K. pneumoniae* including AMP-resistant strains, associated with the use of the AMP-containing regimen.

The choice of relatively narrow-spectrum empiric AB for suspected EOS is likely prompted by the fact that today only approximately 2–4% of treated neonates develop culture-proven

Table 5 Mixed model of colonization rate and duration, corrected for study center and period

Microorganism	No. patients colonized (%)		OR (95%CI)	CD days per 100 NICU days (mean ± SD)		
	AMP (n = 36)	PEN (n = 39)		AMP (n = 36)	PEN (n = 39)	P
K. pneumoniae	16 (44.4)	8 (20.5)	3.10 (1.12–8.58)	29.9 ± 38.2	9.7 ± 22.0	0.0123
K. pneumoniae AMP R	16 (44.4)	7 (17.9)	3.66 (1.28–10.44)	18.3 ± 26.1	8.4 ± 21.4	0.0212
Klebsiella oxytoca	8 (22.2)	10 (25.6)	0.83 (0.29–2.40)	6.8 ± 14.3	14.9 ± 30.6	0.547
K. oxytoca AMP R	8 (22.2)	10 (25.6)	0.83 (0.29–2.40)	6.4 ± 14.0	14.1 ± 28.7	0.538
Enterobacter cloacae	16 (44.4)	14 (35.9)	1.43 (0.57–3.61)	21.2 ± 31.1	13.3 ± 22.7	0.317
E. cloacae AMP R	11 (30.6)	7 (17.9)	2.01 (0.68–5.94)	14.3 ± 27.5	5.0 ± 13.2	0.139
E. coli	6 (16.7)	7 (17.9)	0.91 (0.28–3.03)	12.1 ± 28.5	7.4 ± 22.2	0.994
E. coli AMP R	2 (5.6)	1 (2.6)	2.24 (0.19–25.77)	4.6 ± 19.5	0.7 ± 4,4	0.502
Serratia	2 (5.6)	5 (12.8)	0.40 (0.07–2.21)	1.8 ± 9.3	4.4 ± 14.4	0.279
Serratia AMP R	0	3 (7.7)	NA	0.0 ± 0	0.9 ± 3.1	0.0947
Acinetobacter	5 (13.9)	6 (15.4)	0.89 (0.25–3.20)	2.1 ± 6.7	6.3 ± 16.8	0.717
Acinetobacter AMP R	0	4 (10.3)	NA	0.0 ± 0	5.1 ± 16.3	0.0515
Stenotrophomonas maltophila	1 (2.8)	2 (5.1)	0.53 (0.05–6.09)	0.9 ± 5.3	1.7 ± 8.6	0.617
Pseudomonas	0	1 (2.6)	NA	0.0 ± 0	0.3 ± 2.1	0.350
S. aureus	3 (8.3)	8 (20.5)	0.35 (0.09–1.45)	4.0 ± 17.0	5.5 ± 13.6	0.158
Enterococcus spp.	20 (55.6)	19 (48.7)	1.32 (0.53–3.27)	32.9 ± 33.7	39.6 ± 35.9	0.430
Streptococcus spp.	1 (2.8)	5 (12.8)	0.19 (0.02–1.75)	1.3 ± 7.9	3.3 ± 10.5	0.128
S. haemolyticus	19 (52.8)	13 (33.3)	2.24 (0.88–5.69)	31.3 ± 34.6	15.4 ± 25.5	0.0253
S. epidermidis	21 (58.3)	17 (43.6)	1.81 (0.72–4.53)	25.1 ± 33.5	22.5 ± 33.9	0.480
S. hominis	12 (33.3)	2 (5.1)	9.25 (1.90–45.03)	8.0 ± 17.0	0.6 ± 2.8	0.0008
Candida albicans	9 (25.0)	7 (17.9)	1.52 (0.50–4.64)	15.6 ± 30.3	6.4 ± 18.7	0.332
Candida spp.	14 (38.9)	8 (20.5)	2.47 (0.88–6.88)	21.4 ± 32.9	9.9 ± 24.6	0.0773

AMP, ampicillin group; AMP R, ampicillin resistant; CD, colonization duration; CI, confidence interval; NICU, neonatal intensive care unit; PEN, penicillin G group; OR, odds ratio

EOS.[2,9] This is not the case, however, among ELBW neonates. As shown previously and also in the present study, up to 40% of at-risk neonates eventually develop clinical or proven EOS. Other likely reasons for the choice of narrow-spectrum AB include fear of alteration to endogenous microflora, with probably irrevocable impact on subsequent immune development and infections with more resistant microorganisms, associated with broader antimicrobial coverage.[32]

In the present study we found minimal differences between AMP- and PEN-containing regimens in initial gut colonization. The only microorganisms in which these two regimens differed were *K. pneumoniae* (both overall and AMP-resistant strains), *S. hominis* and *S. haemolyticus*, all being more common colonizers of the gastrointestinal tract in the AMP compared with the PEN arm. All these organisms had extremely high AMP resistance, ranging from 87.5% for *S. haemolyticus* to 96% for *K. pneumoniae*, but the proportions of resistant strains were similar in both study arms. Although an association between AMP use and the spread of broad-spectrum beta-lactamase-producing Gram-negative rods such as *K. pneumoniae* has been suggested,[13,14] the present results support out-selection of the bacterial species rather than a selective pressure on increasing resistance.[33] The lack of effect of the duration of early empiric AB regimen in the present study should still be interpreted with caution because only approximately one-third of the study patients received early empiric AB therapy for >72 h.[34] AMP has also been shown to facilitate emergence of beta-lactam resistance in neonatal strains of *E. cloacae*.[16] We found a slightly higher proportion of resistance in *E. cloacae* strains (69% vs 50%) in the AMP arm, but no difference either in colonization rate or CD with resistant *E. cloacae* was seen. The present study, however, was probably too short to identify possible time-related changes in circulating microflora in the NICU.

Although the clinical relevance of higher colonization rates with *S. haemolyticus* and *S. hominis* is uncertain, selection of *K. pneumoniae* is likely to be important. High-density colonization has been found to facilitate transmission and translocation with subsequent development of invasive infection.[15,35] In the present study these ideas are supported by all four cases of LOS due to *K. pneumoniae* occurring in the AMP treatment group.

The efficacy findings should be treated with caution because one of the major limitations of this subgroup analysis was the low number of participants. Therefore the higher incidence of proven EOS and a trend towards increased NICU mortality in the PEN arm may well be explained by the coincidental unequal distribution of cases between the treatment arms in an underpowered subgroup analysis. Early AB regimen, however, remained a borderline significant predictor of mortality in multivariate analysis adjusted for the random clustering effect (treatment period and study center), as well as for other known risk factors of mortality in ELBW neonates,[31] suggesting a true difference. The potential of empiric AB therapy to prevent culture positivity in preterm neonates without altering the incidence of clinical sepsis has been demonstrated in a recent randomized controlled trial by Tagare *et al*.[36] The effect may be explained by the different antibacterial spectrum of the studied AB regimens. PEN is an AB with a narrow spectrum of activity, limited mostly to Gram-positive organisms; in the context of EOS mainly to GBS.[2] Several recent studies including the present one have shown the predominance of *Enterobacteriaceae* and not GBS in the etiology of EOS in very low-birthweight/ELBW (VLBW/ELBW) neonates.[4,9,25] The issue of which AB regimen should be preferred, however, is further complicated by the high and increasing prevalence of AMP resistance among *Enterobacteriaceae* in the NICU setting,[10] leading to the situation that in many NICU both AB may be equally ineffective against the majority of infecting organisms in EOS. For example, in the present study two of the three Gram-negative isolates were resistant to AMP, this finding being consistent with a recent study in Estonian ICU in which 45% of *E. coli,* 82% of other *Enterobacteriaceae* and 93% of *K. pneumoniae* were AMP resistant.[37]

In light of the high prevalence of EOS among at-risk ELBW infants, the increasing resistance rates and the persistently high mortality of EOS in VLBW/ELBW infants, ranging between 33% and 40% in previous studies[2,25] and 35% in the present study (in culture-proven cases even 4/6), the adequacy of both these presently most widely applied empiric AB choices should be questioned. Although the issue of spreading beta-lactam resistance has been addressed, in part, with concomitant use of GEN, which has preserved high activity against most EOS-causative organisms,[38,39] the widespread empiric use of aminoglycosides in neonates is not problem free either. Among the carriers of a mitochondrial DNA mutation 1555A–G, the prevalence of which in populations of European descent is approximately 0.2%, permanent profound hearing loss develops after aminoglycoside exposure, even when drug levels are within therapeutic range and the duration of treatment is short.[40–42] Because elective genetic screening before aminoglycoside use is not feasible in NICU and maternal screening may not detect low levels of heteroplasmy,[41] a safer choice of AB might be an alternative.

The higher incidence of NEC stage II–III and threshold ROP in the AMP group as compared with the PEN group probably warrants further attention. An association between antenatal exposure to co-amoxiclav and neonatal NEC has been reported,[43,44] but not proven in later case–control studies.[45] Similarly, in the present study there was no difference in the combined outcomes of death and NEC II–III or death and ROP between the two study regimens, suggesting an impact of the improved survival in the AMP group in these findings.

Conclusion

Although in this subgroup analysis AMP plus GEN and PEN plus GEN treatments were similar in terms of early neonatal mortality and/or change of AB regimen within 72 h as well as in general impact on initial gut colonization in ELBW neonates, some trends and differences such as a lower rate of proven EOS and favorable NICU survival among infants born before 26 weeks, but higher colonization rate with *K. pneumoniae*, were associated with AMP treatment. Adequately powered multicenter studies are urgently needed to demonstrate whether these findings indicate the true superiority of the AMP regimen in the empiric treatment of EOS in ELBW neonates, and whether the selection for

K. pneumoniae is associated with changes in LOS etiology, rising from a shift in the circulating microflora in an NICU, which may have been missed in the present study of relatively short duration.

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