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REGULAR ARTICLE

Comparison of ampicillin plus gentamicin vs. penicillin plus gentamicin in empiric treatment of neonates at risk of early onset sepsis

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Abstract

Aim: We aimed to compare the clinical efficacy of ampicillin (AMP) vs. penicillin (PEN) both combined with gentamicin in the empirical treatment of neonates at risk of early onset neonatal sepsis (EOS).

Methods: We performed an open label cluster randomized equivalence study in both Estonian neonatal intensive care units, including neonates with suspected EOS, aged less than 72 h. Primary end-point was clinical failure rate, expressed by need for change of antibiotic regimen within 72 h and/or 7-day all cause mortality. Bowel colonization was followed with biweekly perineal swab cultures.

Results: Incidence of proven EOS was 4.9%. Among neonates receiving AMP (n = 142) or PEN (n = 141) change of antibiotic regimen within 72 h (10/142 vs. 10/141; OR 1.02; 95% CI 0.40–2.59), 7-day mortality (11/142 vs. 14/141; OR 0.76; 95% CI 0.33–1.75) and over-all treatment failure (20/142 vs. 20/141; OR 1.01; 95% CI 0.52–1.97) occurred at similar rates. The only differences in gut colonization were lower number of patients colonised with enterococci, *S. aureus* and AMP resistant *Acinetobacter* spp. in AMP and lower number of those with *S. haemolyticus* and *S. hominis* in PEN arm.

Conclusions: AMP and PEN combined with gentamicin have similar effectiveness in the empiric treatment of suspected neonatal EOS.

INTRODUCTION

As signs of early onset neonatal sepsis (EOS) are non-specific and prompt treatment with antibiotics has been shown to reduce mortality (1,2) a large population of neonates receives empiric therapy based on risk factor driven decisions. The scientific evidence of which treatment regimen should be preferred is poor, with only a few underpowered prospective trials addressing the issue (2).

A recent Cochrane review identified only two studies that specifically compared antibiotic regimens for suspected EOS, both performed more than 15 years ago, small in sample size and with antibiotics some of which are no longer in routine use (2). More recently Clark et al. conducted a retrospective review involving 128 914 neonates in Untied States and found that the use of cefotaxime was associated with greater risk of death than ampicillin plus gentamicin (AMP) regimen (3). Still, due to the retrospective design in this study several confounding factors like higher rates of organ dysfunction and other serious complications in the cefotaxime group were not included in the multivariate analysis (4).

Although not evaluated in appropriately powered clinical trials, the combination of aminoglycoside and AMP or PEN has remained the treatment of choice for EOS in many nurseries world-wide (2,3). Recent changes in the bacterial aetiology of EOS, with decreasing rates of group B streptococci (GBS) and increasing *Escherichia coli* raise the issue of potential differences between the two regimens. The predominance of Gram-negative rods in bacterial aetiology of EOS among preterm neonates, reported in Europe (5) and United States (6,7), as well as in Israel (8), suggests higher potential efficacy of AMP at least in this subpopulation.

At the same time, AMP intrapartum prophylaxis of GBS may underlie the 'out-selection' of AMP resistant Gram-negative micro-organisms, especially Escherichia coli (5–7),

Abbreviations

AB, antibiotic; AMP, ampicillin; BPD, bronchopulmonary dysplasia; CoNS, coagulase negative staphylococci; ELBW, extremely low birth weight (birth weight below 1000 g); EOS, early onset neonatal sepsis; ESBL, extended spectrum B-lactamase; GA, gestational age; GBS, group B streptococci; IRDS, infant respiratory distress syndrome; IVH, intraventricular haemorrhage; LOS, late onset neonatal sepsis; MRSA, methicillin resistant *Staphylococcus aureus;* MSSA, methicillin susceptible *Staphylococcus aureus;* NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PEN, penicillin; VLBW, very low birth weight (birth weight below 1500 g).

although this effect has not been uniformly confirmed (9). Increasing resistance will likely abolish the advantage of AMP's broader spectrum in antibacterial activity; treatment failure rate as high as 70% has been reported (10).

Antibiotics have also been shown to interfere with the development of gut microbiota. Studies suggest an association between AMP use and the spread of broad spectrum beta-lactamase producing Gram-negative rods like *Klebsiella pneumoniae* among colonising as well as invasive strains in neonatal intensive care patients (11) and induction of beta-lactam resistance in neonatal strains of *Enterobacter cloacae* (12). Penicillin G, at the same time has been suggested to have the least impact on neonatal bowel colonization with resistant microorganisms (13,14) – an issue to be considered, bearing in mind the large population of neonates receiving antibiotics according to the present best practice guidelines and the high probability of translocation, especially from the immature bowel (15).

We hypothesized that in the empiric therapy of neonates with or at risk of EOS the two most widely used antibiotic regimens AMP and PEN are equivalent in terms of need for antibiotic change within 72 h and/or 7-day all cause mortality, but PEN regimen has less impact on bowel colonization with AMP resistant Gram-negative bacteria.

PATIENTS AND METHODS

A two-centre prospective open label cluster-randomized study was conducted in two third level neonatal intensive care units (NICU) in Estonia from 2 August 2006 to 30 November 2007. All maternity hospitals referring neonates to the units used risk-factor based intrapartum antibiotic prophylaxis for GBS.

The study was approved by the Ethics Committee of the University of Tartu and registered at ClinicalTrials.gov identifier: NCT00487019.

Patients

During the study period all neonates admitted within 72 h of life, needing early empiric antibiotic treatment for EOS or risk factors of infection according to the CDC criteria (e.g. maternal chorionamnionitis and/or maternal risk factors of infection and/or preterm labour in <35 weeks of gestation) (16) and not transferred within the following 24 h, were eligible. The exclusion criteria were prior administration of a different antibiotic regimen for more than 24 h or presence of suspected or proven meningitis, necrotizing enterocolitis (NEC), peritonitis, severe sepsis (defined as clinical and laboratory symptoms of sepsis and signs of at least one organ dysfunction) or septic shock with isolation of micro-organisms resistant to the study regimen in maternal urinary tract or birth canal or other situations that required different antibacterial treatment.

Treatment and monitoring of patients

The order of the study regimens in the two participating units was assigned randomly by flipping the coin (IL). During the first phase of the study (from 2 August 2006 to 20 March 2007) gentamicin [4–5 mg/kg 24–48 hourly, based on gestational (GA) and postnatal age (PNA)] (17) was combined with AMP (25 mg/kg 8–12 hourly, based on GA and PNA) (17) in unit A, and with penicillin G (25 000 IU/kg 8–12 hourly, based on GA and PNA) (18,19) in unit B. In the second phase (from 21 March 2007 to 30 November 2007), after half of the patients were recruited, beta-lactam antibiotics were switched. All clinical and laboratory findings were recorded according to the clinical routine. Infants were followed up until discharge from NICU or until 60 days of life, whichever was earlier.

Microbiological samples from blood were collected on admission and thereafter from sterile body sites as clinically indicated. Monitoring of mucosal colonization had been implemented by infection control service prior to the study with swabs obtained from the perineum on admission and twice a week thereafter. All microbiological samples were analysed using routine methods and evaluated according to the CSLI criteria (20); any Gram-negative micro-organism that grew on MacConkey agar with 16 μg/mL of AMP was termed ampicillin-resistant.

Diagnostic criteria of neonatal sepsis

Neonatal sepsis (proven or clinical) was diagnosed in the presence of at least two clinical (hyper- or hypothermia, apnoea or bradycardia spells, increased oxygen requirement, feeding intolerance, abdominal distension, lethargy and hypotonia, hypotension, skin and subcutaneous lesions such as petechial rash, abscesses, sclerema) and two laboratory criteria (WBC count <5000 or >20 000 $\times$ 10^9 cells/L; immature to total WBC ratio (I/T) >0.2; platelet count <100 000 $\times$ 10^9/L; CRP >10 mg/L). Proven sepsis included cases where in addition to clinical and laboratory signs a pathogen [except coagulase-negative staphylococci (CoNS) that had to be isolated from at least two different specimens or with only one positive culture adequate antibiotic treatment had to be given for more than 72 h] was isolated from a normally sterile body fluid. All other cases were termed clinical sepsis. Sepsis occurring within the first 72 h was classified as EOS and all other cases as late onset sepsis (LOS).

Statistical analysis

The study was planned as an equivalence trial assuming that the lower and upper boundary of the two-sided 95% CI for the difference in the treatment failure between the study arms will not exceed ±10%. The power calculation was based on a retrospective analysis of medical records in 2003–2004 showing that about 10% of patients with suspected EOS need change of early empiric antibiotic regimen (21). Accepting a two-sided type I error rate of ≤0.05 and a power of 80% both treatment arms were to enrol at least 140 patients (22).

Primary endpoint was treatment failure as expressed by the need for a change in the initial empiric antibiotic regimen within 72 h (widely accepted limit between EOS and LOS) and/or any death within 7 days. The following situations were pre-specified to require change of antibiotic regimen: (i) suspicion of meningitis or abdominal infection/NEC; (ii) isolation of bacteria resistant to the empiric antibiotic

regimen from a neonate with sepsis; (iii) isolation of bacteria resistant to the empiric antibiotic regimen from maternal urinary tract/birth canal of a neonate with sepsis; (iv) no improvement or deterioration of clinical status; (v) suspicion of nosocomial infection (age more than 72 h); and (vi) other situations where the treating physician considered change of antibiotic regimen necessary – detailed reasons were documented.

Secondary end-points were 28-day and NICU mortality, NICU and hospital stay, duration of early empiric antibiotic treatment, duration of respiratory support and vasoactive treatment, rate of LOS and use of additional antibacterial therapy, the presence of NEC stage II–III (23), patent arterial duct (PDA) requiring surgery, threshold retinopathy of prematurity requiring laser-therapy (ROP), severe intraventricular haemorrhage (IVH stage III–IV) (24) and severe bronchopulmonary dysplasia (BPD) (25).

After the recruitment of each quarter of the study population an interim safety analysis assessing mortality and the rate of major neonatal complications (see secondary endpoints), involving two paediatricians not associated with the study. No significant differences between the two treatment regimens were observed and the study was completed as planned.

Statistical analysis was performed using statistical software R 2.4.0. Treatment success (reverse treatment failure rate) was assessed by Kaplan–Meier curves. For comparisons between groups hierarchical models appropriate for cluster randomized cross-over design incorporating the effect of study centre and treatment period were used (26).

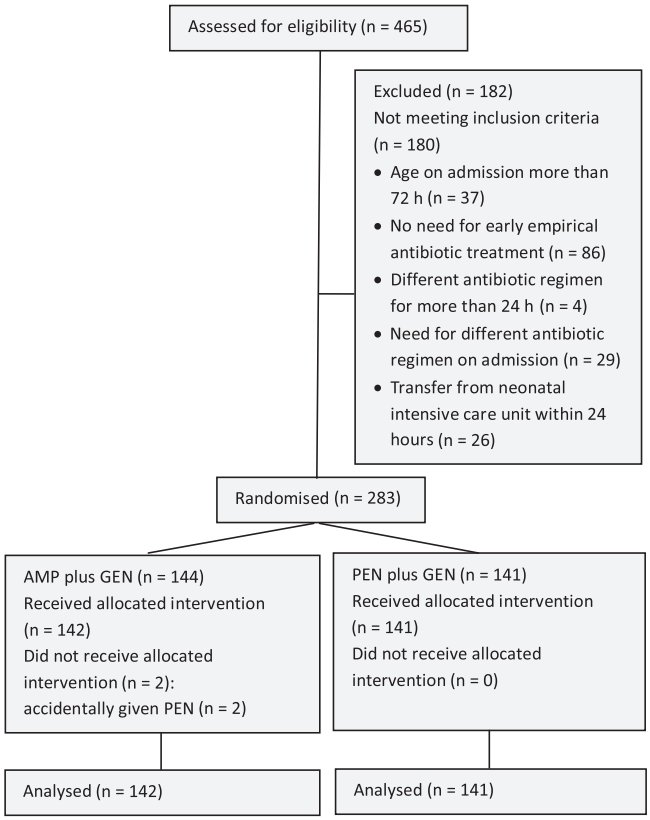

Figure 1 Study outline and reasons for exclusion from the study.

RESULTS

Patients' characteristics

A total of 465 neonates were admitted to units A and B throughout the study period; 29 of them needed other antibiotics than the study regimen – 18 required preoperative antibacterial prophylaxis with cefazolin; four had suspected NEC and/or peritonitis and received metronidazole, ampicillin-sulbactam and/or piperacillin-tazobactam; three received cefotaxime for suspected meningitis and one for severe renal failure; a neonate received fluconazole antifungal treatment for candidiasis. Additional two neonates accidentally received penicillin G during the AMP period.

Similar number of neonates in unit A (43%) and B (34%), were excluded. Absence of need for early empiric antibiotic treatment was more common in unit A than in unit B [odds ratio (OR) 3.78; 95% CI 2.18–6.53], likely reflecting a difference in admitted population. A total of 283 (60.9%) neonates, 142 in AMP and 141 in PEN group comprised the study population (Fig. 1). With regards to demographic characteristics both treatment arms were well balanced except for ventilatory support that was more commonly used in PEN group (OR 0.44; 95% CI 0.24–0.81) (Table 1).

Table 1 Demographic data of the study groups

	AMP (n = 142)	PEN (n = 141)
Gestational age (weeks)		
Median (quartiles)	31 (27–34)	31 (28–35)
>36 weeks – n (%)	29 (20)	28 (20)
<28 weeks – n (%)	41 (29)	34 (24)
<26 weeks – n (%)	24 (17)	21 (15)
Birthweight (g)		
Median (quartiles)	1467 (920–2553)	1500 (960–2343)
<1501 g – n (%)	73 (51.4)	72 (51.1)
<1001 g – n (%)	36 (25.4)	39 (27.7)
<751 g – n (%)	19 (13)	15 (11)
M/F sex – n	78/64	85/56
Mean Apgar score at 5 min	6.6 ± 1.5	6.3 ± 1.6
Ventilated – n (%)	99 (70)	116 (82)
Surfactant – n (%)	81 (56)	88 (62)
Caesarean section – n (%)	77 (54)	80 (57)
Multiple pregnancies – n (%)	34 (24)	23 (17)
Chorionamnionitis – n (%)	21 (15)	30 (21)
PROM >16 h – n (%)	25 (18)	28 (20)
Prenatal glycocorticoids	86 (61)	71 (50)
Maternal antibiotic therapy – n (%)		
During pregnancy	38 (27)	27 (19)
During delivery	51 (36)	46 (33)

PROM, premature rupture of membranes.

Primary endpoint

The overall treatment failure rates (14.2% in PEN vs. 14.1% in AMP group) as well as the proportions of the individual components of the primary endpoint were similar in both arms with 95% CI remaining within the pre-specified range

Table 2 Primary endpoint, early onset sepsis and reasons for antibiotic change – an univariate model corrected for participating unit and treatment period

	AMP (n = 142)	PEN (n = 141)	OR (95% CI)	Treatment difference (%; 95% CI)
Treatment failure (composite) – n (%)	20 (14.1)	20 (14.2)	1.01 (0.52–1.97)	0.1 (−8.1; 8.3)
Components of the composite endpoint:				
AB change in 72 h – n/died	10/1	10/4	1.02 (0.40–2.59)	0.05 (−6.3; 6.4)
Death in 7 days – n (%)	11 (7.7)	14 (9.9)	0.76 (0.33–1.75)	2.2 (−4.7; 9.1)
EOS (proven+clinical) – n (%)	34 (23.9)	33 (23.4)	1.03 (0.60–1.78)	
Proven EOS – n (%)/died in 7 days	6 (4.2)/1	8 (5.7)/3	0.73 (0.25–2.17)	
Clinical EOS – n (%)/died in 7 days	28 (19.7)/5	25 (17.7)/5	1.14 (0.63–2.07)	
Reasons for AB change within 72 h – n/died				
No improvement/deteriorating condition	5/1	4/4	NA	
Meningitis or suspicion of meningitis	3/-	4/-	NA	
NEC or other abdominal infection	2/-	2/-	NA	
Resistant isolate from the baby	-/-	-/-	NA	
Resistant isolate from the mother	-/-	-/-	NA	

of ± 10% (Table 2). Kaplan-Meier analysis revealed almost identical treatment success rate for both treatment regimens (p = 0.92). There were no differences in treatment failure rates between centres (12.0% in unit A vs. 15.6% in unit B) and treatment periods (13.5% in first vs. 14.1% in second period).

Early onset sepsis

The incidence of proven EOS in both groups was similar (6/142 in the AMP vs. 8/141 in the PEN arm; Table 2); among extremely low birth weight (ELBW) infants it was significantly more frequent in PEN than in AMP group (5/39 vs. 0/36, respectively).

Reasons for changing early empiric antibiotic regimen within 72 h together with outcome of EOS are shown in Table 2. After 72 h empiric antibiotic regimen was changed in another six neonates in the AMP and 18 in the PEN group (OR 0.30; 95% CI 0.12–0.78). Clinical or proven LOS and/or NEC as a reason for antibiotic change accounted for the majority of between-group difference (four vs. 14 in the AMP and PEN groups, respectively, OR 0.26; 95% CI 0.084–0.82).

There were a total of 14 micro-organisms isolated from patients with EOS. All three cases of *Staphylococcus epidermidis* sepsis were diagnosed in preterm neonates with GA ≤28 weeks and the diagnosis was based on at least two positive blood cultures not more than 72 h apart. The only case of *S. haemolyticus* EOS was diagnosed based on a single positive blood culture in a term neonate with clinical and laboratory signs of sepsis (CRP 151 mg/L, I/T ratio 0.65); unfortunately no further blood cultures were taken from the baby within the next 72 h. In the AMP group all six isolates were susceptible to at least one component of the empiric antibiotic regimen compared to three out of eight in the PEN group (OR 0.080; 95% CI 0.0095–0.67). Difference between the two groups was accounted for by the resistance of all four CoNS strains and *Candida albicans* to both empiric antibiotics in the PEN group (Table 3). Both *Enterobacter cloacae* isolates and the *E. coli* strain in AMP arm were resistant to AMP, but none of the Gram-negative isolates were resistant to gentamicin.

Table 3 Bacterial aetiology of early and late onset sepsis by treatment group

Micro-organisms	EOS – n of episodes (died)		LOS – n of episodes	
	AMP (n = 142)	PEN (n = 141)	AMP (n = 142)	PEN (n = 141)
Gram-positive	4 (0)	4 (0)	17	28
Staphylococcus epidermidis	–	3 (0)	5	14
Staphylococcus haemolyticus	–	1 (0)	7	4
Staphylococcus hominis	–	–	–	1
Other CoNS	–	–	–	1
*Staphylococcus aureu:*MSSA	–	–	1	2
MRSA	–	–	1	3 ¶
Enterococcus spp.	–	–	3	1
Streptococcus agalactiae	4 (0)	–	–	–
Streptococcus salivarius	–	–	–	1
Gram-negative	2 (1)	3 (2)	13	13
Acinetobacter baumannii	–	–	4	3
Escherichia coli	1 (0)	1 (1)	2	1
Enterobacter cloacae	1 (1)	1 (1)	2	2
Klebsiella oxytoca	–	–	–	3
Klebsiella pneumoniae	–	–	5*	1
Pseudomonas spp.	–	–	–	1
Stenotrophomonas spp.	–	–	–	1
Serratia spp.	–	–	–	1
Haemophilus influenzae	–	1 (0)	–	–
Yeasts	–	1 (1)	3	1
Candida albicans	–	1 (1)	3	–
Candida parapsilosis	–	–	–	1
Total episodes	6 (1)	8 (3)	33	42

An outbreak of MRSA was diagnosed in study centre B during the PEN period, which lasted to the beginning of the AMP period; *An outbreak of *K. pneumoniae* (ESBL negative) was diagnosed in study centre A during the AMP period.

Secondary endpoints

With regards to secondary endpoints both treatment arms were equal except for events highlighted below (Fig. 2). In univariate analysis there was a trend towards lower incidence rate of proven LOS caused by Gram-positive micro-organisms per 1000 patient days in the AMP arm [9.0 vs. 15.2; relative risk (RR) 0.60; 95% CI 0.33–1.10] and a significant difference in rate of *S. epidermidis* sepsis (2.7 vs. 7.6 in the AMP and PEN arm, respectively, RR 0.32; 95% CI 0.19–0.55) (Table 3). Of note, in three out of five *S. epidermidis*

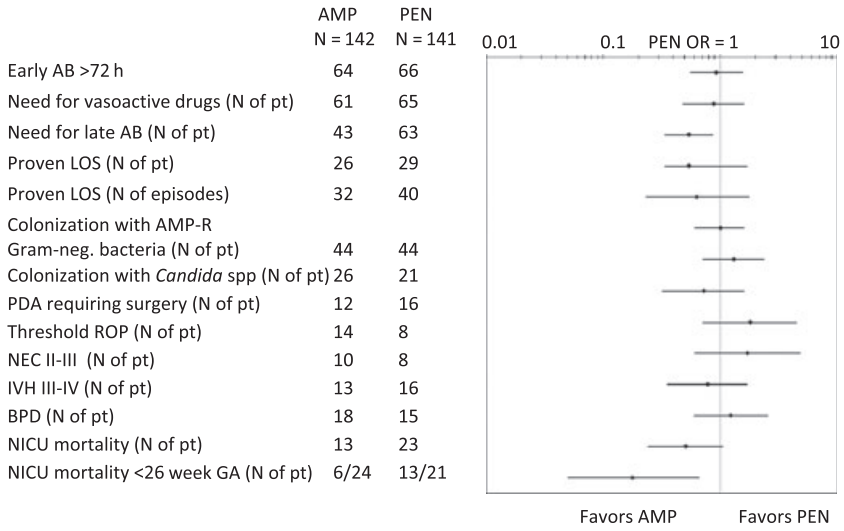

Figure 2 Forest plot of secondary endpoints. The data are presented as number of cases per treatment arm and point estimates of odds ratios (OR) (indicated by diamonds) with the 95% CI (indicated by lines). For the mortality of infants born before 26th week of gestation number of deaths/number of patients per treatment arm is shown. OR of the PEN treatment arm is 1, shift to the left from this line indicates lower risk in the AMP arm and vice versa. NEC II–III – necrotizing enterocolitis stage II–III (23); IVH III–IV – intraventricular haemorrhage stage III–IV (24); †cumulative colonization by 16 days is shown.

episodes in the PEN and 10 out of 14 in the AMP group the diagnosis was based on at least two positive blood cultures. The *Str salivarius* and all but two cases of CoNS LOS were diagnosed in VLBW neonates with GA below 30 weeks; the two larger infants with *S. epidermidis* LOS were born at 36th and 38th week of gestation and required long-term parenteral nutrition for gastroschisis and small bowel atresia, respectively and had repeated positive blood cultures of *S. epidermidis*. However, in the multivariate mixed model adjusted for peri- and neonatal risk factors (GA, route of delivery, chorionamnionitis, maternal antibiotic and prophylactic steroid use, route of feeding, character of enteral feeds, use of central venous and arterial lines and artificial ventilation), AMP early empiric AB did not remain associated with *S. epidermidis* LOS at $p < 0.05$ (z value: −1.742; $p = 0.0815$). AMP regimen was also associated with lower proportion of days on additional antibiotic treatment (31 vs. 42 per 100 patient days; OR 0.63; 95% CI 0.55–0.71).

Both regimens were equally well tolerated with no major differences in adverse events or laboratory abnormalities (including those potentially associated with antibiotic toxicity like creatinine and aminotransferases) registered in either group at any time point studied (data not shown).

Bowel colonization

There was no difference between the treatment arms in the number of patients colonized with ampicillin resistant Gram-negative *Enterobacteriaceae*, CoNS or *Candida* spp. at any time-point (Fig. 2). The number of patients colonised with *E. cloacae* (31/142 vs. 38/141; OR 0.58; 95% CI 0.31–1.08) and *K. pneumoniae* (30/142 vs. 17/141; OR 1.9; 95% CI 0.85–4.25) overall or with resistant strains (21/142 vs. 19/141; OR 1.09; 95% CI 0.49–2.42 and 21/142 vs. 12/141; OR 1.46; 95%CI 0.62–3.44, respectively) was similar in the AMP and PEN groups. There was no difference in the proportion of resistant strains of *K. pneumoniae* (70% vs. 71%) between the two treatment arms. The proportion of ampicillin resistant strains among all *E. cloacae* strains tended to be higher, although not statistically significantly, in the AMP compared to the PEN group (68 vs. 50%; OR 2.1; 95% CI 0.78–5.63). However, among Gram-positive microorganisms differences on species level were seen. Compared with PEN, AMP treatment was associated with greater risk of colonization by *S. haemolyticus* (43/142 vs. 25/141; OR 2.22; 95% CI 1.20–4.12) and *S. hominis* (22/142 vs. 4/141; OR 6.46; 95% CI 2.12–19.67) but lower risk of colonization by enterococci (36/142 vs. 55/141; OR 0.5; 95% CI 0.3–0.83), *S. aureus* (6/142 vs. 16/141; OR 0.34; 95% CI 0.13–0.91) and ampicillin resistant *Acinetobacter* spp. (0/142 vs. 8/141; $p = 0.008$). The colonization data obtained in this study will be discussed in detail elsewhere (27).

NICU mortality

The mortality rate of EOS was similar in both treatment arms (Table 2). However, as shown in Fig. 2 there was a trend towards higher all cause mortality in the PEN group, which was significant for neonates born before 26th week of gestation. Detailed analyses of reasons for death revealed that among neonates with respiratory distress syndrome mortality was lower in the AMP than in PEN group (3/72 vs. 12/63; OR 0.2; 95% CI 0.05–0.70).

DISCUSSION

In this prospective cluster randomised two centre efficacy study, by comparing AMP with PEN, both combined with gentamicin, in patients at risk for EOS, we demonstrate that in terms of a composite endpoint – need for change of

antibiotic regimen within 72 h and/or first 7-day all cause mortality – both treatments are equal. In initial bowel colonisation minor differences predominantly within Gram-positive micro-organisms occur, but neither regimen was associated with higher rates of colonization by ampicillin resistant *Enterobacteraceae* or *Candida* spp.

Although neonatal sepsis is a common disease and antibiotics are widely used in NICUs, this study is one of the very few prospective comparative studies in the field. Previous studies by contrast have been either small in sample size, have not distinguished between patients with EOS and LOS, have compared antibiotics that are currently not in use (e.g. ticarcillin) (2) or have had retrospective or observational design (3).

The strength of this study is the complex approach with simultaneous evaluation of the clinical efficacy and influence of antibiotic regimens on development of gut microflora. As opportunistic bacteria colonizing the gastrointestinal tract of neonates may persist for several months (28) and serve as a predominant source of subsequent bloodstream infections (15), systematic investigations in the field may have a role in understanding the changing spectrum of bacteria involved in LOS and possibly other long-term outcomes such as allergic diseases (29). Hence, given the nature of the changing field, this is the area that certainly would need more studies.

We selected a prospective cluster randomized design as this allowed us to include the possible effects of the study regimens on the entire NICU environment and to look at associated bowel colonization patterns, which would have been impossible in individual randomization with the two regimens running in both wards at the same time. It also enabled to handle better the open label design, chosen due to logistic reasons; all antibiotic doses are prepared in the ward. We believe that the final results were not influenced by the design as throughout the study the number of patients, considered to require antibiotics other than the study regimen, was similar, suggesting that physicians did not have any preferences in selecting empiric therapy. In addition, prior to the study both empiric regimens were equally used in both participating units.

In order to avoid misinterpretation of the clustering effect, we have applied hierarchical models, stratifying the results for random effects of treatment period and study centre (26). Inclusion of all neonates, assigned to early empiric antibiotic treatment with AMP or PEN, to our mind, allows drawing meaningful conclusions for the entire population, recruited according to the present clinical approach. We would still like to point out, that the results of the present study, undertaken based on these routines, as well as the many reports on the different bacterial aetiology of EOS in different GA groups, lead to the question, whether 'one size really fits all' and indicate the need for separate studies in different patient populations.

Although we have shown, that in respect to primary endpoint these regimens are similar, there are secondary endpoints in which they differed, like the numerically lower incidence of *S. epidermidis*-caused LOS and lower need for late antibiotic therapy found in the AMP compared to PEN treatment arm. Still, the cause-result relationship is more difficult to establish, because the development of LOS in a neonate is affected by a large variety of additional factors (5,30). In this respect it is noteworthy to emphasize that AMP containing early empiric AB regimen did not remain a significant risk factor of *S. epidermidis* LOS ($p = 0.08$), when data were compared in multivariate models corrected for peri- and neonatal risk-factors of LOS. We cannot exclude the clonal spread of more virulent *S. epidermidis* strain(s) during the PEN treatment period, like described in other settings (30). These findings are further supported by the fact that the difference between the two regimens originated predominantly from one of the participating units, which also had significantly higher overall CoNS colonization rates.

Another secondary endpoint in which these treatment regimens differed was the numerically higher NICU mortality in PEN as compared with AMP group that was statistically significant in premature babies born before 26th week of gestation and in infants with the primary diagnosis of IRDS. Still, caution should be taken when interpreting these data; our study does not have a power to draw conclusions in subgroup analysis. These results may just reflect the unequal distribution of proven EOS among ELBW infants as well as the somewhat higher use of prenatal glycocorticoids and lower need for invasive respiratory support in the AMP as compared to PEN group. The problem warrants further clarification, as similarly higher case fatality rate for PEN plus tobramycin regimen compared to amoxicillin plus cefotaxime was observed in a previous study on empiric antibacterial treatment of EOS in two Dutch NICUs (14). Further and adequately powered studies should confirm the validity of these results in ELBW babies.

In contrast to de Man et al. (14) we did not detect major differences in colonization with ampicilllin resistant Gram-negative bacilli between the two regimens However, like in the study by Burman et al. (12) a trend, though statistically insignificant, towards higher proportion of ampicillin-resistant *E.cloaceae* among all colonizing strains in AMP compared with PEN arm was observed. This may suggests that despite AMP potentially being associated with the induction of resistance among *E. cloacae* (12) it is not associated with increased spread of resistant strains in NICU environment. With this our findings are similar to those by Jauréguy et al. who failed to find association between intrapartum amoxicillin prophylaxis and outselection of beta-lactam resistant enterobacteria in neonates (31). At present time most gut colonizing *Enterobacteriaceae* but *E. coli* are resistant to PEN as well to AMP. Thus, it is not surprising that these two antibiotic regimes appeared to have similar effect on Gram-negative colonization. Our ongoing studies should demonstrate whether there are differences between AMP and PEN containing regimes in molecular-genetic level or whether either of these regimens is involved in the induction of antibiotic resistance as hinted by Burman et al. (12). In addition, the clinical relevance of differences in early Gram-positive colonization,

seen in our study remains to be answered in further studies.

In addition to the open label design the limitation of the study was the selection of a surrogate primary endpoint, which in our opinion did not prevent drawing appropriate conclusions. The use of a surrogate marker has been suggested before and was chosen because the occurrence of culture proven EOS (4.9% in this study) or EOS related mortality are extremely rare (2). We do believe, that both components of the selected primary endpoint adequately described the efficacy of the antibiotic regimen, since clinical status of septic neonates deteriorates rapidly with inadequate treatment and the all cause mortality within the first 7 days avoids skipping any unrecognized cases. Another limitation of this, but also of most previous studies conducted in the field, is the high number of culture negative cases (about 95% in both arms) among those included based on the risk factors. This may have introduced a significant bias as patients without bacterial infection would respond similarly to all antibiotics provided that these agents are equally well tolerated. On the other hand, however, the population selected by us well resembles the one treated with empiric antibiotics in clinical practice and reflects adequately the overall treatment efficacy of neonates at risk of EOS. We would like to emphasize that results of this study are applicable to the population involved and should not be transferred to other settings, like treatment of proven EOS. Furthermore the problem of poor recognition of sepsis is equally important in studies and clinical practice and will not be solved until reliable and rapid diagnostic methods are available. Until then several patients will be unnecessarily exposed to antibiotics that they may not need, which in turn prioritizes the safety and narrow antibacterial spectrum of possible choices.

In conclusion, we have shown that in patients admitted to NICU AMP and penicillin, both combined with gentamicin, could be equally used in the empiric treatment of suspected EOS, as these two agents do not differ with regards to clinical efficacy and have similar influence on the development of gut microflora. Which of these two regimens is to be preferred should depend on the local distribution of EOS causing micro-organisms together with their antibacterial susceptibility. The suitability of these regimens in extremely prematurely born neonates and in areas with high level of beta-lactam resistance needs further confirmation in clinical trials adequately powered to address these issues.

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References

1. Dawodu AH, Effiong CE. Neonatal mortality: effects of selective pediatric interventions. *Pediatrics* 1985; 75: 51–7.
2. Mtitimila EI, Cooke RW. Antibiotic regimens for suspected early neonatal sepsis. *Cochrane Database Syst Rev* 2004: CD004495.
3. Clark RH, Bloom BT, Spitzer AR, Gerstmann DR. Empiric use of ampicillin and cefotaxime, compared with ampicillin and gentamicin, for neonates at risk for sepsis is associated with an increased risk of neonatal death. *Pediatrics* 2006; 117: 67–74.
4. Lee PI. Ampicillin and cefotaxime as a risk factor of neonatal death. *Pediatrics* 2006; 117: 1857; author reply 1857–8.
5. Ronnestad A, Abrahamsen TG, Medbo S, et al. Septicemia in the first week of life in a Norwegian national cohort of extremely premature infants. *Pediatrics* 2005; 115: e262–8.
6. Stoll BJ, Hansen NI, Higgins RD, et al. Very low birth weight preterm infants with early onset neonatal sepsis: the predominance of gram-negative infections continues in the National Institute of Child Health and Human Development Neonatal Research Network, 2002-2003. *Pediatr Infect Dis J* 2005; 24: 635–9.
7. Bizzarro MJ, Dembry LM, Baltimore RS, Gallagher PG. Changing patterns in neonatal Escherichia coli sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008; 121: 689–96.
8. Klinger G, Levy I, Sirota L, Boyko V, Reichman B, Lerner-Geva L. Epidemiology and risk factors for early onset sepsis among very-low-birthweight infants. *Am J Obstet Gynecol* 2009; 201: e31–6.
9. Schrag SJ, Hadler JL, Arnold KE, Martell-Cleary P, Reingold A, Schuchat A. Risk factors for invasive, early-onset Escherichia coli infections in the era of widespread intrapartum antibiotic use. *Pediatrics* 2006; 118: 570–6.
10. Reyna J, Ortiz F. Therapeutic failure of the ampicillin plus aminoglycoside scheme in the treatment of early neonatal sepsis. *Arch Med Res* 2008; 39: 546–7.
11. Crivaro V, Bagattini M, Salza MF, et al. Risk factors for extended-spectrum beta-lactamase-producing *Serratia marcescens* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. *J Hosp Infect* 2007; 67: 135–41.
12. Burman LG, Berglund B, Huovinen P, Tullus K. Effect of ampicillin versus cefuroxime on the emergence of beta-lactam resistance in faecal Enterobacter cloacae isolates from neonates. *J Antimicrob Chemother* 1993; 31: 111–16.
13. Bennet R, Eriksson M, Nord CE. The fecal microflora of 1-3-month-old infants during treatment with eight oral antibiotics. *Infection* 2002; 30: 158–60.
14. de Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. *Lancet* 2000; 355: 973–8.
15. Donskey CJ. The role of the intestinal tract as a reservoir and source for transmission of nosocomial pathogens. *Clin Infect Dis* 2004; 39: 219–26.
16. Schrag S, Gorwitz R, Fultz-Butts K, Schuchat A. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC. *MMWR Recomm Rep* 2002; 51: 1–22.
17. Young TE, Mangum B, Neofax A. Manual of drugs used in neonatal care, 17th ed. Raleigh: Acorn Publishing, 2004.

18. Metsvaht T, Oselin K, Ilmoja ML, Anier K, Lutsar I. Pharmacokinetics of penicillin g in very-low-birth-weight neonates. *Antimicrob Agents Chemother* 2007; 51: 1995–2000.
19. Hey EB, C Neonatal Formulary. Drug use in pregnancy and the first year of life. Oxford: Blackwell BMJ Books, 2006.
20. Wilson ML, Mitchell M, Morris AJ. Principles and procedures for blood cultures; approved guideline. *CLSI* 2007; 27: 1–67.
21. Metsvaht T, Padari H, Lang K, Lutsar I. Penicillin + gentamicin versus ampicillin + gentamicin in the empiric treatment of early neonatal sepsis. 16th European Congress of Clinical Microbiology and Infectious Diseases, abstract number: r2260. *Clin Microbiol Infect* 2006; 12 (Suppl 4): 129. Available from: URL: http://www.blackwell publishing.com/eccmid16/PDFs/clm_1432.pdf (accessed January 6, 2010)
22. Hayes RJ, Bennett S. Simple sample size calculation for cluster-randomized trials. *Int J Epidemiol* 1999; 28: 319–26.
23. Bell MJ, Ternberg JL, Feigin RD, et al. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. *Ann Surg* 1978; 187: 1–7.
24. Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular hemorrhage: a study of infants with birth weights less than 1,500 gm. *J Pediatr* 1978; 92: 529–34.
25. Ehrenkranz RA, Walsh MC, Vohr BR, et al. Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. *Pediatrics* 2005; 116: 1353–60.
26. Murray DM, Varnell SP, Blitstein JL. Design and analysis of group-randomized trials: a review of recent methodological developments. *Am J Public Health* 2004; 94: 423–32.
27. Parm Ü, Sepp E, Metsvaht T, et al.. Ampicillin or penicillin for empiric treatment of early-onset neonatal sepsis: influence on bowel colonization by aerobic and facultative anaerobic bacteria. Helsinki: ECCMID, 2009.
28. Millar M, Philpott A, Wilks M, et al. Colonization and persistence of antibiotic-resistant Enterobacteriaceae strains in infants nursed in two neonatal intensive care units in East London, UK. *J Clin Microbiol* 2008; 46: 560–7.
29. Vael C, Nelen V, Verhulst SL, Goossens H, Desager KN. Early intestinal *Bacteroides fragilis* colonisation and development of asthma. *BMC Pulm Med* 2008; 8: 19.
30. Klingenberg C, Ronnestad A, Anderson AS, et al. Persistent strains of coagulase-negative staphylococci in a neonatal intensive care unit: virulence factors and invasiveness. *Clin Microbiol Infect* 2007; 13: 1100–11.
31. Jaureguy F, Carton M, Panel P, Foucaud P, Butel MJ, Doucet-Populaire F. Effects of intrapartum penicillin prophylaxis on intestinal bacterial colonization in infants. *J Clin Microbiol* 2004; 42: 5184–8.