**This is a pre- or post-print of an article published in
Heideking, M., Lander, F., Hufnagel, M., Pfeifer, Y.,
Wicker, E., Krause, G., Berner, R.
Antibiotic susceptibility profiles of neonatal invasive
isolates of Escherichia coli from a 2-year nationwide
surveillance study in Germany, 2009-2010
(2013) European Journal of Clinical Microbiology and
Infectious Diseases, 32 (9), pp. 1221-1223.**

Antibiotic Susceptibility Profiles of Neonatal Invasive Isolates of *Escherichia coli* from a 2-Year Nationwide Surveillance Study in Germany, 2009-2010

Martin Heideking¹, Fabian Lander⁴, Markus Hufnagel¹, Yvonne Pfeifer², Esther Wicker³, Gérard Krause³, Reinhard Berner^{4*}

Center for Pediatrics and Adolescent Medicine, University Medical Center Freiburg, Freiburg¹, Germany, Robert Koch-Institute, Nosocomial Infections, Wernigerode², Germany, Robert Koch Institute, Infectious Disease Epidemiology, Berlin³, Germany, Center for Pediatrics and Adolescent Medicine, University Medical Center Dresden⁴, Dresden, Germany

* Corresponding author. Mailing address: Center for Pediatrics and Adolescent Medicine, University Medical Center Dresden, Fetscherstr. 74, 01307 Dresden, Germany. Phone: +49-351458-2440. Fax +49-351458-4384. E-mail: reinhard.berner@uniklinikum-dresden.de.

Abstract

A nationwide 2-year surveillance study on invasive neonatal *Escherichia coli* infections in Germany was conducted; 158 isolates were tested for antibiotic susceptibility . The empirical

treatment regimen of ampicillin plus gentamicin for neonatal sepsis appears to remain effective, but emerging resistance needs to be closely monitored.

Keywords: *Escherichia coli*, neonatal, sepsis, resistance, national surveillance.

Background

Escherichia coli is the leading gram-negative organism causing neonatal sepsis and meningitis with high rates of morbidity and mortality despite advances in neonatal intensive care [1, 2]. . In *E. coli*, attention concerning antibiotic susceptibility mainly has been focused on the development of ampicillin-resistance in early onset sepsis (EOS) of preterm infants [3, 4], whereas few studies have systematically examined susceptibility to other classes of antibiotics in neonatal sepsis. We present the first nationwide surveillance data on antibiotic susceptibility of neonatal invasive *E. coli* strains isolated in Germany.

Methods

Active surveillance for invasive *E. coli* infections in all neonates up to the age of three months was performed from January 2009 through December 2010. Monthly questionnaires were sent out by the Robert Koch Institute (RKI), Berlin, Germany, to all microbiological laboratories in Germany serving neonatal wards and willing to participate in the study.

Strains were considered definitely invasive when isolated from blood or cerebrospinal fluid (CSF) culture or from other sterile compartments. *E. coli* isolates from non-sterile material in a clinically septic infant were considered to be suspected invasive. The study design targeted laboratories directly and personal data protection did not allow for retrieval of the patients' age at acquisition of the isolate nor any additional clinical information.

Antimicrobial susceptibility to piperacillin/tazobactam, cefuroxime, meropenem, ciprofloxacin, cotrimoxazole, tetracycline and tigecycline was assessed using disk diffusion

tests (BioMérieux, Nuertingen, Germany). The minimal inhibitory concentration (MIC) of ampicillin, piperacillin, cefotaxime and gentamicin was determined using Etest strips (BioMérieux, Nuertingen, Germany). Susceptibility was defined as “susceptible”, “intermediately-susceptible” or “resistant” according to EUCAST guidelines [5]. Isolates resistant to cefotaxime were further evaluated by double disk diffusion test for phenotypical confirmation of extended-spectrum β -lactamase (ESBL) production. Relevant ESBL genes (*bla*_{TEM}, *bla*_{SHV}, *bla*_{CTX-M}) were amplified by multiplex PCR and sequenced [6, 7]. Strain typing was performed for ESBL-positive *E. coli* using pulsed-field gel electrophoresis (PFGE) with *Xba*I-digested whole genomic DNA.

Results

A total of 1230 laboratories were identified as target laboratories . Of these, 128 (10.4%) laboratories responded to serve neonatal wards and to perform antibiotic susceptibility testing on neonatal *E. coli* isolates. During the 2-year period 93 laboratories continuously provided data, 18 provided data only for a limited period ranging from 8 to 20 months. The capture areas of the laboratories include all 16 federal states of Germany. A total of 180 *E. coli* strains were collected of which 115 (63.9%) were identified as definitely invasive and a further 43 (23.9%) strains were considered suspected invasive pathogens as defined above. The remaining 22 (12.2%) strains were either identified as non-invasive or could not be evaluated and were thus excluded from the analysis. Of the 115 confirmed invasive strains 95 (82.6%) were isolated from blood, 16 (13.9%) from CSF, 2 (1.7%) from pleural effusion and one from an intraabdominal abscess. No significant differences in antibiotic susceptibility were found between invasive and suspected invasive isolates.

Resistance rates are shown in table 1a. The MIC of ampicillin ranged from 0.5 to >256 μ g/ml, with a MIC₅₀ of 3 μ g/ml and a MIC₉₀ of >256 μ g/ml (table 1b). MICs of Piperacillin ranged from 0.038 to >256 μ g/ml with a MIC₅₀ of 1.5 μ g/ml and a MIC₉₀ of 64 μ g/ml. Six isolates

(3.8%) were resistant to cefotaxime, the MIC ranging from 0.016 to 128 µg/ml with a MIC₅₀ of 0.047 µg/ml and a MIC₉₀ of 0.094 µg/ml. Among these six isolates, two harbored ESBL gene *bla*_{TEM-52} and the remaining four harbored *bla*_{CTX-M-1}, *bla*_{CTX-M-3}, *bla*_{CTX-M-14}, and *bla*_{CTX-M-15}, respectively. Macrorestriction patterns (PFGE analysis) of the ESBL strains showed no genetic relationship except for the two TEM-52 producing *E. coli* that were isolated from two different patients in two different hospitals in the same geographic area (Figure 1).

Gentamicin MICs ranged from 0.19 to 96 µg/ml with a MIC₅₀ of 0.5 µg/ml and a MIC₉₀ of 1.0 µg/ml. All 158 isolates were uniformly susceptible to meropenem, piperacillin/tazobactam and tigecycline.

Discussion

We present the first national surveillance study on antibiotic susceptibility in neonatal invasive *E. coli* conducted in Germany to date. Empirical treatment regimens for neonatal sepsis combine ampicillin with either an aminoglycoside or a cephalosporin of the cefotaxime-group. The proportion of ampicillin-resistance in this study was comparable to published data of resistance rates of 40% to 65% in *E. coli* EOS [3, 4], whereas susceptibility to gentamicin and cefotaxime was shown to remain high throughout Germany. However, 3.8% of the strains proved to be resistant to the combination of ampicillin and gentamicin, and another 3.8% were resistant to the combination of ampicillin and cefotaxime. The emergence of ESBL-producing strains needs to be closely monitored in the future, considering its potential impact on the choice of empirical therapy. Outbreaks of ESBL-producing *E. coli* strains on neonatal intensive care units did not occur during the study period, but have recently been reported from elsewhere in Europe [8, 9].

Few studies presenting nationwide susceptibility profiles of neonatal invasive *E. coli* in Europe have been published. A current publication from England showed a slightly higher rate of cephalosporin-resistance of 5% in late onset sepsis (LOS) *E. coli* strains [10]. The

same study showed that 93% of LOS *E. coli* strains remained susceptible to a combination of amoxicillin and gentamicin. In contrast, an English multicenter surveillance study found resistance rates to amoxicillin plus cefotaxime of approximately 16% among *E. coli* LOS strains [11].

We recognize limitations to our study. Although we initiated a comprehensive recruitment of laboratories, we cannot claim to have a full coverage of all laboratories in question and we cannot assure full representativeness of the participating sample. Yet we have no indication that our data are subject of a systematic recruiting bias. Since the design of this study targeted laboratories directly, clinical information could not be retrieved, making it impossible to differentiate susceptibility profiles between EOS and LOS. In summary, susceptibility to aminoglycosides and cefotaxime among *E. coli* isolates causing neonatal sepsis in Germany remains high. Empirical therapy combining ampicillin with either an aminoglycoside or a cephalosporin of the cefotaxime-group can thus still be considered effective. The emergence of ESBL-producing *E. coli* strains among the neonatal population should be closely monitored through future surveillance studies.

Funding

This work was supported by the Walter-Marget-Foundation.

Acknowledgments

We extend special thanks to Sybille Müller-Bertling and Christine Günther for performing genotypical analyses of the ESBL-producing *E. coli* isolates.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Gaschignard J, Levy C, Romain O, et al (2011) Neonatal Bacterial Meningitis: 444 Cases in 7 Years. *Pediatr Infect Dis J* 30:212-7
2. Stoll BJ, Hansen NI, Sanchez PJ, et al (2011) Early onset neonatal sepsis: the burden of group B Streptococcal and *E. coli* disease continues. *Pediatrics* 127:817-26.
3. Alarcon A, Pena P, Salas S, Sancha M, Omenaca F (2004) Neonatal early onset *Escherichia coli* sepsis: trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. *Pediatr Infect Dis J* 23:295-9.
4. Bizzarro MJ, Dembry LM, Baltimore RS, Gallagher PG (2008) Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 121:689-96.

5. European Committee on Antimicrobial Susceptibility Testing of the European Society of Clinical Microbiology and Infectious Diseases (2012) Breakpoint tables for interpretation of MICs and zone diameters.
http://www.eucast.org/clinical_breakpoints.
6. Grobner S, Linke D, Schutz W, et al (2009) Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. J Med Microbiol 58:912-22.
7. Pfeifer Y, Matten J, Rabsch W (2009) *Salmonella enterica* serovar Typhi with CTX-M beta-lactamase, Germany. Emerg Infect Dis 15:1533-5.
8. Moissenet D, Salauze B, Clermont O, et al. (2010) Meningitis caused by *Escherichia coli* producing TEM-52 extended-spectrum beta-lactamase within an extensive outbreak in a neonatal ward: epidemiological investigation and characterization of the strain. J Clin Microbiol 48:2459-63.
9. Tschudin-Sutter S, Frei R, Battegay M, Hoesli I, Widmer AF (2010) Extended spectrum beta-lactamase-producing *Escherichia coli* in neonatal care unit. Emerg Infect Dis 16:1758-60.
10. Muller-Pebody B, Johnson AP, Heath PT, Gilbert RE, Henderson KL, Sharland M (2011) Empirical treatment of neonatal sepsis: are the current guidelines adequate? Arch Dis Child Fetal Neonatal Ed 96:F4-F8.
11. Vergnano S, Menson E, Kennea N, et al (2011) Neonatal infections in England: the NeonIN surveillance network. Arch Dis Child Fetal Neonatal Ed 96:F9-F14.

Figure 1. Macrorestriction patterns (*Xba*I-PFGE) of ESBL-producing *E. coli* isolated from six neonates. *E. coli* strains 4 and 5 were isolated from the same patient. Isolates 6 and 7 with identical patterns were isolated from separate patients in different hospitals in the same geographic region.

Table 1a. Antibiotic susceptibility profiles of 158 neonatal invasive *E. coli* strains isolated in a national surveillance study in Germany between January 2009 and December 2010.

Table 1b. Minimal inhibitory concentrations of selected antibiotics for 158 neonatal invasive *E. coli* strains.