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ARTICLE

Antibiotic susceptibility profiles of neonatal invasive isolates of *Escherichia coli* from a 2-year nationwide surveillance study in Germany, 2009–2010

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Abstract A nationwide 2-year surveillance study on invasive neonatal *Escherichia coli* infections in Germany was conducted. A total of 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.

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 has mainly 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 the 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 3 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 to be 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 minimum 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 the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines

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[5]. Isolates resistant to cefotaxime were further evaluated by the 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 polymerase chain reaction (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 1,230 laboratories were identified as target laboratories. Of these, 128 (10.4 %) laboratories responded to serving neonatal wards and performing antibiotic susceptibility testing on neonatal *E. coli* isolates. During the 2-year study period, 93 laboratories continuously provided data and 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 to be 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 intra-abdominal abscess. No significant differences in antibiotic susceptibility were found between invasive and suspected invasive isolates.

The resistance rates are shown in Table 1. The MIC of ampicillin ranged from 0.5 to >256 μg/ml, with an MIC_{50} of 3 μg/ml and an MIC_{90} of >256 μg/ml (Table 2). The MIC of piperacillin ranged from 0.038 to >256 μg/ml, with an MIC_{50} of 1.5 μg/ml and an MIC_{90} of 64 μg/ml. Six isolates (3.8 %) were resistant to cefotaxime, with MIC ranging from 0.016 to 128 μg/ml, an MIC_{50} of 0.047 μg/ml and an MIC_{90} of 0.094 μg/ml. Among these six isolates, two harbored the 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 (Fig. 1).

Gentamicin MICs ranged from 0.19 to 96 μg/ml, with an MIC_{50} of 0.5 μg/ml and an MIC_{90} of 1.0 μg/ml. All 158 isolates were uniformly susceptible to meropenem, piperacillin/tazobactam, and tigecycline.

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

Antibiotic	Susceptible (%)	Intermediately susceptible (%)	Resistant (%)
Ampicillin	87 (55.1)	–	71 (44.9)
Piperacillin	89 (56.3)	25 (15.8)	44 (27.9)
Tazobactam	158 (100)	–	–
Cefuroxime	151 (95.6)	–	7 (4.4)
Cefotaxime	152 (96.2)	–	6 (3.8)
Meropenem	158 (100)	–	–
Gentamicin	151 (95.6)	–	7 (4.4)
Cotrimoxazole	127 (80.4)	1 (0.6)	30 (19)
Ciprofloxacin	151 (95.6)	–	7 (4.4)
Tetracycline	104 (65.8)	6 (3.8)	48 (30.4)
Tigecycline	158 (100)	–	–

Table 2 Minimal inhibitory concentrations (MICs) of selected antibiotics for 158 neonatal invasive *E. coli* strains

Antibiotic	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	Range (μg/ml)
Ampicillin	3	>256	0.5 to >256
Piperacillin	1.5	64	0.38 to >256
Cefotaxime	0.047	0.094	0.016–128
Gentamicin	0.5	1	0.19–96

Discussion

We present the first national surveillance study on antibiotic susceptibility in neonatal invasive *E. coli* strains 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 the published data of resistance rates of 40–65 % 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, likewise, 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 in 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 %

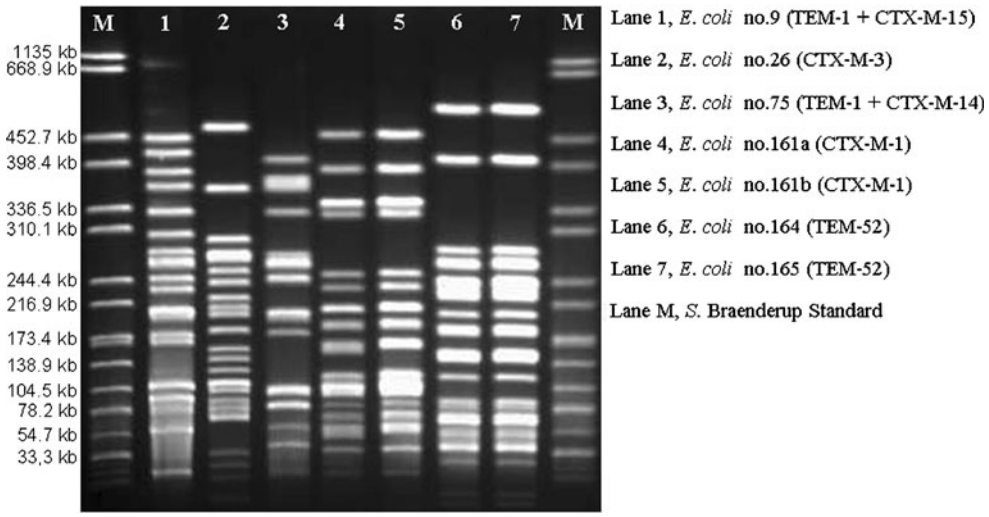

Fig. 1 Macrorestriction patterns [*Xba*I pulsed-field gel electrophoresis (PFGE)] of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolated from six neonates. *E. coli* strains 4 and 5 were isolated from the same patient. Isolates 6 and 7 with identical patterns were isolated from separate patients in different hospitals in the same geographic region

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 to 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.

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Conflict of interest The authors declare that they have no conflict of interest.

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