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PATHOTYPING $bla_{\mathrm{CTX\text{-}M}}$ *ESCHERICHIA COLI* FROM NIGERIA

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Background: Escherichia coli have become the enterobacteriaceae species most affected by extended-spectrum β-lactamases (ESBLs) in view of the emergence of CTX-M-type ESBLs. These CTX-M-positive *E. coli* have been reported in numerous regions worldwide. Virulence determinants of already reported CTX-M-positive *E. coli* were investigated. *Methodology:* To gain insights into the mechanism underlying this phenomenon, we assessed serogroup, susceptibility pattern and diversity of virulence profiles within a collection of nine $bla_{\mathrm{CTX\text{-}M}}$-positive *E. coli* strains and their virulent determinant using miniaturized DNA microarray techniques. The nine ESBL-positive *E. coli* isolates were from eight male and one female patient(s) selected for study based on previous work. Virulence potential was inferred by detection of 63 virulence factor (VF) genes. *Results:* Four (44.4%) of the 9 *E. coli* isolates exhibited the same set of core characteristics: serotype O8:Hnt, while all were positive for OXA-1, ciprofloxacin resistance. Five of the isolates exhibited highly similar (91% to 100%) VF profiles. *Conclusion:* The findings describe a broadly disseminated, $bla_{\mathrm{CTX\text{-}M}}$-positive and virulent *E. coli* serogroup with highly homogeneous virulence genotypes, suggesting recent emergence in this zone. Understanding how this clone has emerged and successfully disseminated within the hospital and community, including across national boundaries, should be a public health priority.

Keywords: $bla_{\mathrm{CTX\text{-}M}}$-positive, virulent *E. coli,* miniaturized DNA microarray, pathotyping, resistance

Introduction

Pathogenic *Escherichia coli* strains constitute a significant public health problem worldwide [1]. In contrast to their nonpathogenic counterparts, these strains have acquired specific virulence attributes that allow them to cause a spectrum of human and animal illnesses [2, 3]. Numerous methods exist for the detection of pathogenic *E. coli*, including geno- and phenotypic marker assays for the detection of virulence genes and their products [4–7]. *Escherichia coli* represents a versatile and diverse enterobacterial species which can be subdivided into (i) nonpathogenic, commensal, (ii) intestinal pathogenic, and (iii) extraintestinal pathogenic strains. This classification is mainly based on the presence or absence of DNA regions which are frequently associated with certain pathotypes. In most cases, this genetic information has been horizontally acquired and belongs to the flexible *E. coli* genome, such as plasmids, bacteriophages, and genomic islands. These genomic regions contribute to the rapid evolution of *E. coli* variants as they are frequently subject to rearrangements, excision and transfer as well as further acquisition of additional DNA thus contributing to the creation of new (pathogenic) variants. Genetic diversity and genome plasticity of *E. coli* has been underestimated. The accumulating amount of sequence information generated in the era of "genomics" helps to increase our understanding of factors and mechanisms that are involved in diversification of this bacterial species as well as in those that may direct host specificity.

Nevertheless, *Escherichia coli* recently has gained wider scope of studies based on their virulence and ability to hydrolyze the oxy-imino cephalosporins. The earlier ESBLs, which were first reported in 1985, consisted

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of plasmid-mediated TEM-1, TEM-2, and SHV-1 derivatives and were primarily a hospital-based problem [8]. However, since 2000, ESBLs increasingly have also appeared in the community [9]. This phenomenon coincided with the emergence of a new group of plasmid-mediated ESBLs, namely, the CTX-M enzymes, which seem to be taking over as the main ESBL type in some locales [10, 11]. Likewise, it has been reported that most bla_{CTX-M} from *E. coli* strains involved in outbreaks in different countries also showed that they additionally carried other antibiotic resistance genes, such as *blaOXA-1, blaTEM-1, tetA, aac(6)-Ib*, and *aac(3)-II* and sometimes a class 1 integron [12–14].

The aim of this work was for pathotyping; to investigate the virulence pattern of already reported bla_{CTX-M} enzymes of *E. coli* isolates from a tertiary health care facility in Nigeria [15]. We also sought to assess these strains' molecularly inferred virulence potential, a possible contributor (in addition to antimicrobial resistance) to their recent emergence and dissemination as successful pathogens in this region.

Materials and methods

Bacterial strains

Nine *E. coli* which harbored bla_{CTX}-genes were already reported [15]. They were selected from a total of 116 *E. coli* isolates that were recovered from 906 clinical samples between December 2006 and January 2007 within Ladoke Akintola University Teaching Hospital (LAUTECH), Osogbo, Nigeria and subjected to further testing for the existence of ESBLs by agar diffusion test using guideline of CLSI [16]. Of these nine ESBLs producers, eight were from urinary tract infection and one from a patient with sepsis. The presence of virulence genes factors using microarray techniques was further investigated on these *E. coli* isolates already reported as carrying bla_{CTX}-genes.

Antibiotic susceptibility

Susceptibility to the following non-β-lactam molecules was determined by disc diffusion: Ciprofloxacin, Gentamicin, Amikacin, Tetracyline, Chloramphenicol, and Sulfamethoxazole/Trimethoprim. Isolates were defined as resistant or susceptible according to the standards of the CLSI 2002 [16].

Screening for ESBLs

According to the CLSI guidelines, isolates inhibition zone of ≤22 mm with Ceftazidime (30 µg), ≤25 mm with Ceftriaxone (30 µg), and ≤27 mm with Cefotaxime (30 µg) were identified as potential producers and shortlisted for confirmation of ESBL production.

Phenotypic confirmation test with combination disk for ESBLs

This test requires the use of a third-generation cephalosporin antibiotic disk alone and in combination with clavulanic acid. In this study, a disk of Ceftazidime (30 µg) alone and a disk of Ceftazidine + Clavulanic acid (30 µg/10 µg) were used. Both the disks were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37 °C. Difference in zone diameters with and without clavulanic acid was measured. When there is an increase of ≥5 mm in inhibition zone diameter around combination disk of Ceftazidine + Clavulanic acid versus the inhibition zone diameter around Ceftazidine disk alone, it confirms the ESBLs production.

Serotyping

The determination of O and H antigens was carried out by using the method previously described by Guine et al. [17], in which all available O (O1–O185) and H (H1–H56) antisera were tested. All antisera were obtained and absorbed with the corresponding cross-reacting antigens to remove the non-specific agglutinins.

PCR amplification and blaOXA-1 *gene sequencing*

A 10-µl aliquot of an overnight culture of the test isolate was diluted 1:10 with water and boiled, from a suspension of colonies from an overnight culture on Nutrient Agar (Biolab, Germany).

Bacterial DNA was prepared by heating this suspension at 95 °C for 5 min, PCR amplification was then performed with 10 µl of this dilution as the DNA template. PCR amplifications were then performed in a Gene Amp PCR System (Applied Biosystems, California, USA). The primers were supplied by Operon (Germany) and sequences of the sets are described in *Table 1*. The amplification of *blaOXA* genes was carried out as described previously by Bert et al. [18]. PCR products were resolved on 1% agarose gels, stained with ethidium bromide, and photography with UV illumination.

Microarray

A miniaturized *E. coli* oligonucleotide virulence array that had previously been designed was used for this work [19]. Eighteen genes were specific to a particular *E. coli* pathotype, 13 were common between two more pathotypes, and seven were unassigned. Genomic DNA was extracted from cells grown aerobically overnight at 37 °C in LB broth, using a DNA assay tissue kit (catalog no. 69504; QIAGEN). One microgram of genomic DNA

Table 1. Virulence factors of the nine *E. coli* strains

Virulence genes	Genes present in CTX-M strains									
	IMT 12574	IMT 12588	IMT 12595	IMT 12600	IMT 12603	IMT 12667	IMT 13264	IMT 13267	IMT 13268	CS
ast A	–	–	–	–	–	–	–	–	–	–
bfpA	–	–	–	–	–	–	–	–	–	–
cdtB-$_{40}$	–	–	–	–	–	–	–	–	–	–
cdtB-$_{50}$	–	–	–	–	–	–	–	–	–	–
Celb	–	–	–	–	–	–	–	–	–	–
cfaC	–	–	–	–	–	X	–	–	–	–
Cma	–	–	–	–	X	–	–	–	–	–
Enf	–	–	–	–	–	–	–	–	–	–
eae-$_{10}$	–	–	–	–	–	–	–	–	–	–
eae-$_{20}$	–	–	–	–	–	–	–	–	–	–
eae-$_{30}$	–	–	–	–	–	–	–	–	–	–
eae-$_{40}$	–	–	–	–	–	–	–	–	–	–
f17A-$_{40}$	–	–	–	–	–	–	–	–	–	–
f17A-$_{50}$	–	–	–	–	–	–	–	–	–	–
f17A-$_{60}$	–	–	–	–	–	–	–	–	–	–
f17G	–	–	–	–	–	–	–	–	–	–
fan A	–	–	–	–	–	–	–	–	–	–
fasA	–	–	–	–	–	–	–	–	–	–
fim41a	–	–	–	–	–	–	–	–	–	–
gad-$_{10}$	x	x	x	x	x	x	x	X	X	–
ehx(hlyA)	–	–	–	–	–	–	–	–	–	–
hlyE	–	–	–	–	–	–	–	–	–	–
ipaH9.8	–	–	–	–	–	–	–	–	–	–
ireA	–	–	–	–	–	–	–	–	–	–
iron	–	–	–	–	X	–	–	–	–	–
Iss	X	–	–	–	X	–	x	x	X	
K88ab	–	–	–	–	–	–	–	–	–	
lngA	–	–	–	–	–	–	–	–	–	
lthA	–	–	–	–	–	–	–	–	–	
mchB	–	–	–	–	–	–	–	–	–	
mchC	–	–	–	–	–	–	–	–	–	
mchF	–	–	–	–	X	–	–	–	–	
mcmA	–	–	–	–	–	–	–	–	–	
perA-$_{10}$	–	–	–	–	–	–	–	–	–	
perA-$_{20}$	–	–	–	–	–	–	–	–	–	
Pet	–	–	–	–	–	–	–	–	–	
prfB/papB	–	–	–	–	X	–	–	–	–	
senB	–	–	–	–	–	–	–	–	–	
sfaS	–	–	–	–	–	–	–	–	–	
sta1A	–	–	–	–	–	–	–	–	–	
sta1B	–	–	–	–	–	–	–	–	–	

Table 1. *(cont.)*

Virulence genes	Genes present in CTX-M strains									
	IMT 12574	IMT 12588	IMT 12595	IMT 12600	IMT 12603	IMT 12667	IMT 13264	IMT 13267	IMT 13268	CS
Stb	–	–	–	–	–	–	–	–	–	
stx1A	–	–	–	–	–	–	–	–	–	
stx2A	–	–	–	–	–	–	–	–	–	–
virF	–	–	–	–	–	–	–	–	–	–
rrl_0101_0177_10	x	x	x	x	x	x	x	x	X	
rrl_0101_0177_20	x	x	*–	–	x	x	x	x	X	
rrl_0260_0330_10	x	x	–	x	x	–	x	x	X	
rrl_0260_0330_20	x	x	–	x	x	–	x	x	X	
rrl_0260_0330_30	x	x	–	x	x	–	x	x	X	
rrl_0520_0580_10	x	–	–	–	–	–	x	x	X	
rrl_0520_0580_20	-x	–	–	–	–	–	–x	–x	–x	
rrl_1480_1560_coli_10	x	X	–	x	x	–	x	x	X	
rrl_1480_1560_coli_20	x	x	–	x	x	–	x	x	X	
rrl_1480_1560_coli_30	x	x	x	x	x	x	x	x	X	
rrl_1480_1560_shig_40	x	–	–	*–	*–	–	x	x	X	
rrl_1690_1770_coli_10	x	x	–	–	–	–	x	x	X	
rrl_1690_1770_freu_30	x	x	–	–	–	–	x	x	X	
rrl_1690_1770_shig_20	x	–*	–	–	–	–	x	x	X	

from each strain was used as a template in a multiplex linear amplification and labeling reaction with the set of 60 primers. The amplified products were added to Array Tubes for hybridization, and the data were analyzed as previously described [19].

Results

Bacterial strains

Nine previously reported *E. coli* isolates, from patients in Nigeria, carrying bla_{CTX-M} genes were analyzed.

Antibiogram, screening for ESBLs and phenotypic confirmation, blaOXA-1 *gene sequencing and serotyping*

The antibiotic susceptibility pattern of these strains to commonly used antibiotics has been reported [15]. The nine CTX-M ESBL-producing strains showed similar phenotypic resistance patterns. All were resistant to the tested cephalosporins (cefotaxime, ceftazidime, cefpodoxime, ceftriaxone, and cefalexin) but susceptible to the combinations of cefotaxime or ceftazidime with clavulanic acid. They were also resistant to other β-lactams such as ampicillin and aztreonam as well as the non-β-lactam antibiotics ciprofloxacin, gentamicin, tetracycline, and tobramycin. None of the isolates showed imipenem resistance. The only differences in resistance were found against chloramphenicol (7/9) and sulfamethoxazole/trimethoprim (8/9). PCR amplification further revealed *blaTEM* and *blaOXA* group III genes in all nine isolates, while genes coding for SHV enzymes were not present in these strains.

Two isolates showed special peculiarity in their susceptibility to commonly used antibiotics, while Isolate IMT 12588 was susceptible to chloramphenicol and sulfamethoxazole/trimethoprime, IMT 12595 also showed susceptibility to chloramphenicol, both from case of urinary tracts infection from male patients.

In the present study, all the isolates were OXA-1-derived ESBLs positive and appear to be important for ceftazidime hydrolysis as earlier reported [15].

Four different serogroup were identified during the study, respectively. (O8:Hnt, O102:H6, O86Hnt, O25:H4). Six out of nine strain harboring *CTX-M-* genes (6/9) having serogroup O8:Hnt, while (2/9) of the total strain harboring *CTX-M-* genes IMT 12588 and IMT 12600 both belong to serogroup O102:H6 while strain (1/9) IMT 12603 belong to O25:H6.

Microarray

Table 1 shows panels of the nine previously reported bla_{CTM}-*E. coli* clinical isolates which were pathotyped using the virulence miniaturized microarray. Three of the nine isolates hybridized to one or more virulence determinants and were readily designated with a recognized pathotyped. Strains IMT 12574, IMT 13264, IMT 13267, and IMT 13268 they all harbored the *iss* gene, which is very wide spread. IMT 12603 harbored *cma*, *iroN*, *iss*, *mchF*, *prfB/papB* genes, while IMT 12667 harbored *cfaC* gene too. Although the rest of the strains not carrying any virulence, or bacteriocin genes, were seen to hybridize with the control (rrl and gad), these isolates may harbor virulence genes not present on our array used. The strain IMT 12603 expressed more virulence factors often encountered in ExPEC strains.

Discussion

Several *E. coli* virulence arrays for genotyping have been described previously including ones similar to what we employed in our study [20–27]. According to our study, several virulence factors differed in prevalence among the organisms that produced CTX-M enzymes *(Table 1)*. Studies have shown that CTX-M producers are more commonly positive for *afa/dra* (Dr-binding adhesions), *iha* (putative adhesion-siderophore receptor,) *sat* (secreted autotransporter toxin), and *kps*MII. In contrast, non-CTX-M producers significantly more often exhibited *ireA* (iron-regulated element) and cvaC (colicin [microcin] V). On balance, aggregate virulence factor scores were similar among CTX-M producers. We found that bla_{CTX-M} strains in addition to having resistance genes can also carry virulence genes. This study demonstrates clearly that virulence genes are present in our isolates studied with the miniature microarray technique used and such genes can coexist on the same strain as seen in strains 12603 and 12667. Although our sample size is too small for statistical conclusion, we can safely infer that an appreciable proportion of these isolate carry virulence genes on them. We also compared isolates originating from septicemia and found that this isolate did not appear to be different from others in terms of serogroups, resistance profile, and virulence factor. In the present study, all strains were OXA-I-derived ESBLs which appears to be important for ceftazidime hydrolysis as reported earlier [19]. Their presence seems to influence the level of resistance to penicillins conferred to isolates. The most common acquired B-lactamase in *E. coli* isolates revealed the distribution of various OXA-I group enzymes in our hospital. A recent study of hospital-acquired *E. coli* isolates producing various types of ESBLs from different parts of France showed that whereas the preponderance of the SHV-and TEM-producing strains (approximately 60% and 37%, respectively) were from group B2, the greater proportion of CTX-M producers (40%) was from group D [28] In that study, isolates from group B2 exhibited numerous virulence factors but were usually susceptible to fluoroquinolones, while the group D strain lacked virulence factors but were resistant to fluoroquinolones [28]. Our results support some of these findings, as all of CTX-M-enzyme producers were resistant to ciprofloxacin as earlier reported [15]. The fact that the majority of our CTX-M producers may be clonally related, although subject to further studies, could in part explain the similarity in their virulence pattern. The presence of fluoroquinolence resistance in addition to ESBL production among these isolates with multiple virulence factors is a worrisome development that deserves close monitoring. This might also in part explain the worldwide appearance and propagation of bla_{CTX-M}-producing organisms. Notable limitation of this study includes small numbers and limited clinical and epidemiological data. Accordingly, we could not explore relationships among patients, such as family contracts to assess the possibility of a common source or transmission between individuals within the community. To address these deficits, we have initiated a large prospective study at our center to more extensively explore the role of several risk factors and to increase the sample size within the different subgroups so that novel virulence combinations can be detected and addressed before strains carrying such spread become a health problem.

Conclusion

Comparison of the microarray method with the traditional serotyping method revealed several advantages of the microarray method. Labeling with specific primers located in proximity to the target oligonucleotides improved the signal-to-background ratio compared to that obtained with random primers (data not shown). Furthermore, it resulted in a higher specificity and shorter labeling times, together with the short template preparation protocol, it is possible to obtain clear array results within a few hours starting from a single colony. Hence, the chip allows fast and parallel detection of serotypes in a single experiment. Moreover, the microarray is able to detect unexpressed potential H antigens even in the case of a phenotypically non-motile strain. Therefore, the microarray technique provides a powerful tool for further characterization and epidemiological tracing of motile and non-motile strains, a feature that renders it clearly superior to phenotypic serotyping.

References

1. Mead PS, Slutsker L, Dietz V et al.: Food-related illness and death in the United States. Emerg Infect Dis 5, 607–625 (1999)
2. Kaper JB, Nataro JP, Mobley HL: Pathogenic *Escherichia coli*. Nat Rev Microbiol 2, 123–140 (2004)
3. Nataro JP, Kaper JB: Diarrheagenic *Escherichia coli*. Clin Microbiol Rev 11, 142–201 (1998)

4. Clark CG, Johnson ST, Easy RH, Campbell JL Rodgers FG: PCR for detection of cdt-III and the relative frequencies of cytolethal distending toxin variant-producing Escherichia coli isolates from humans and cattle. J Clin Microbiol 40, 2671–2674 (2002)
5. Osek J: Detection of the enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 (EAST1) gene and its relationship with fimbrial and enterotoxin markers in *E. coli* isolates from pigs with diarrhoea. Vet Microbiol 91, 65–72 (2003)
6. Sharma VK: Detection and quantitation of enterohemorrhagic *Escherichia coli* O157, O111, and O26 in beef and bovine feces by real-time polymerase chain reaction. J Food Prot 65, 1371–1380 (2002)
7. Wang G, Clark CG, Rodgers FG: Detection in *Escherichia coli* of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type 2 Shiga toxin family by multiplex PCR. J Clin Microbiol 40, 3613–3619 (2002)
8. Paterson D, Bonomo R: Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 18, 657–686 (2005)
9. Pitout JD, Nordmann P, Laupland KB et al.: Emergence of Enterobacteriaceae producing extended-spectrum b-lactamases (ESBLs) in the community. J Antimicrob Chemother 56, 52–59 (2005)
10. Canton R, Coque TM: The CTX-M b-lactamase pandemic. Curr Opin Microbiol 9, 466–475 (2006)
11. Bonnet R: Growing group of extended-spectrum b-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 48, 1–14 (2004)
12. Boyd DA, Tyler S, Christianson S et al.: Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum β-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrob Agents Chemother 48, 3758–3764 (2004)
13. Karisik E, Ellington MJ, Pike R, Warren RE, Livermore DM Woodford N: Molecular characterization of plasmids encoding CTX-M-15 ß-lactamase from *Escherichia coli* strains in the United Kingdom. J Antimicrob Chemother 58, 665–668 (2006)
14. Lavollay M, Mamlouk K, Frank T et al.: Clonal dissemination of a CTX-M-15 β-lactamase-producing *Escherichia coli* strain in the Paris area, Tunis, and Bangui. Antimicrob Agents Chemother 50, 2433–2438 (2006)
15. Olowe OA, Grobbel M, Büchter B, Lübke-Becker A, Fruth A, Wieler LH: Detection of $bla_{CTX-M-15}$ extended-spectrum β-lactamase genes in *Escherichia coli* from hospital patients in Nigeria. Int J Antimicrob Agents 3, 206–207 (2010)
16. National Committee for Clinical Laboratory Standards Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Approved standard M31-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, PA (2002)
17. Guinée PAM, Jansen WH, Wadström T et al. (1981): *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. In: Laboratory Diagnosis in Neonatal Calf and Pig Diarrhoea: Current Topics in Veterinary and Animal Science, eds Leeww PW, Guinée PAM, Martinus-Nijhoff, The Hague, The Netherlands, pp. 126–62.
18. Bert F, Branger C, Lambert-Zechovsky N: Identification of PSE and OXA beta-lactamase genes in *Pseudomonas aeruginosa* using PCR-restriction fragment length polymorphism. J Antimicrob Chemother 50, 11–18 (2002)
19. Anjum MF, Mafura, M Slickers P et al.: Pathotyping *Escherichia coli* by using miniaturized DNA microarrays. Appl Environ Microbiol 73, 5692–5697 (2007)
20. Ballmer K, Korczak BM, Kuhnert P, Slickers P, Ehricht R, Hachler H: Fast DNA serotyping of *Escherichia coli* by use of an oligonucleotide microarray. J Clin Microbiol 45, 370–379 (2007)
21. Monecke S, Ehricht R: Rapid genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolates using miniaturised oligonucleotide arrays. Clin Microbiol Infect 11, 825–833 (2005)
22. Bruant, G, Maynard C, Bekal S, Gaucher I, Masson L, Brousseau R, Harel J: Development and validation of an oligonucleotide microarray for detection of multiple virulence and antimicrobial resistance genes in *Escherichia coli*. Appl Environ Microbiol 72, 3780–3784 (2006)
23. Call, DR, Brockman FJ, Chandler DP: Detecting and genotyping *Escherichia coli* O157:H7 using multiplexed PCR and nucleic acid microarrays. Int J Food Microbiol 67, 71–80 (2001)
24. Chen S, Zhao S, McDermott PF, Schroeder CM, White DG, Meng J A: DNA microarray for identification of virulence and antimicrobial resistance genes in *Salmonella serovars* and *Escherichia coli*. Mol Cell Probes 19, 195–201 (2005)
25. Chizhikov V, Rasooly A, Chumakov K, Levy DD: Microarray analysis of microbial virulence factors. Appl Environ Microbiol 67, 3258–3263 (2001).
26. Jenkins C, van Ijperen C, Dudley E G, Chart H, Willshaw GA, Cheasty T., Smith HR, Nataro JP: Use of a microarray to assess the distribution of plasmid and chromosomal virulence genes in strains of enteroaggregative *Escherichia coli*. FEMS Microbiol Lett 253, 119–124 (2005)
27. Korczak B, Frey J, Schrenzel J et al.: Use of diagnostic microarrays for determination of virulence gene patterns of *Escherichia coli* K1, a major cause of neonatal meningitis. J Clin Microbiol 43, 1024–1031 (2005)
28. Branger CO, Zamfir S, Geoffroy G et al.: Genetic background of *Escherichia coli* and extended-spectrum β-lactamase type. Emerg Infect Dis 11, 54–61 (2005)