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Plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal *ampC* genes: prevalence at a Swiss university hospital and occurrence of the different molecular types in Switzerland

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Sir,

Since 1989, plasmid-mediated AmpC β-lactamases have been known to exist in various species lacking inducible chromosomal *ampC* genes such as *Klebsiella* spp., *Escherichia coli*, *Proteus mirabilis* and *Salmonella*. They descend from chromosomal *ampC* genes and fall into six phylogenetic groups. Origins are the *ampC* genes of *Hafnia alvei*, *Morganella morganii*, *Citrobacter freundii*, *Enterobacter cloacae* and two unknown organisms.[1] Organisms producing plasmid-mediated AmpC β-lactamases raise special concerns because of the high rate of clinical failure among infected patients.[2] We report here on the prevalence of plasmid-mediated AmpC β-lactamases in isolates of Enterobacteriaceae lacking an inducible chromosomal *ampC* gene at University Hospital Basel and the occurrence of different molecular types of plasmid-mediated AmpC β-lactamases in Switzerland.

Between 27 January 2006 and 27 January 2007, a total of 3217 consecutive clinical isolates of various species of Enterobacteriaceae lacking inducible chromosomal *ampC* genes (i.e. 2434 *E. coli*, 174 *Klebsiella oxytoca*, 459 *Klebsiella pneumoniae*, 8 *Klebsiella* spp., 134 *P. mirabilis*, 7 *Salmonella enterica* ssp. *enterica* and 1 *Shigella flexneri*) were screened for resistance to cefoxitin with the disc diffusion test according to CLSI guidelines.[3] Isolates with an inhibition zone diameter of <18 mm were considered putative AmpC producers and were stored at −70°C for further investigation. Additionally, 45 clinical isolates suspected to harbour a plasmid-mediated AmpC β-lactamase were collected from 5 laboratories situated in Switzerland. *ampC* genes were identified by a *ampC* multiplex PCR with primers specific for the genes of six different phylogenetic groups.[1] For sequencing, the *ampC* genes were amplified by PCR using primer pairs as described previously for bla_{CMY} and bla_{DHA}, respectively.[4,5] In addition, bla_{CMY-31} was amplified using a second pair of primers.[6] PCR products were purified using Montage PCR Units (Millipore, Zug, Switzerland) and sequencing reactions were carried out using a BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Rotkreuz, Switzerland) as described by the manufacturer. Sequencing products were purified with Dye Ex 2.0 Spin Kit (Qiagen, Hombrechtikon, Switzerland) and electrophoresis was performed with the 3130 Genetic Analyzer (Applied Biosystems). The isoelectric point (pI) of the new β-lactamase was determined by isoelectric focusing, applying the supernatants of crude sonic cell extracts to Phast gels (GE HealthCare, Fairfield, CT, USA) with a pH gradient of 3–9 in a Phast system (GE HealthCare). Extended-spectrum β-lactamases (ESBLs) with known pI values (TEM-1, TEM-12, SHV-12 and CTX-M15) were included as pI markers. A filter paper containing 2.5% flucloxacillin (GlaxoSmithKline, Munchenbuchsee, Switzerland) as an inhibitor of the AmpC β-lactamase was applied for 2 min to one of the gels before staining with nitrocefin (Oxoid, Basel, Switzerland).

Of 3217 consecutive clinical isolates that were obtained at our hospital, 124 (3.8%; 103 *E. coli*, 3 *K. oxytoca* and 18 *K. pneumoniae*) were resistant to cefoxitin and were thus considered putative AmpC producers. Among these, five isolates (all of them *E. coli*) carrying an *ampC* gene known to be plasmid-encoded were found by *ampC* multiplex PCR. Thus, the prevalence of plasmid-mediated AmpC β-lactamases at University Hospital Basel was 0.16% for Enterobacteriaceae lacking an inducible chromosomal *ampC* gene (0.2% for *E. coli*).

Overall, plasmid-mediated AmpC β-lactamases were found in 17 isolates obtained from five Swiss laboratories. Details are given in Table 1. Fourteen of the isolates were *E. coli*, two were *K. pneumoniae* and one was *P. mirabilis*. Fifteen of the plasmid-mediated AmpC β-lactamases were CIT enzymes, a phylogenetic group that has its origin in the chromosomal *ampC* gene of *C. freundii*. Fourteen of them were CMY-2. For one isolate of *K. pneumoniae*, sequencing of the full gene revealed a unique sequence that has been designated CMY-31 (GenBank accession no. EF622224). The derived amino acid sequence differed from CMY-2 by one amino acid (Q235R). Isoelectric focusing revealed a β-lactamase with a pI between 8.8 and 9.0. Enzyme activity of CMY-31 was inhibited by flucloxacillin which is indicative of an AmpC β-lactamase. Enzyme activity of the ESBLs that served as pI markers was retained. This is the first report of the isolation and characterization of CMY-31, a new β-lactamase which is closely related to CMY-2. DHA-1, a plasmid-mediated AmpC β-lactamase that has its origin in the chromosomal *ampC* gene

Table 1. Occurrence of various molecular types of AmpC

Molecular type of AmpC	Number of isolates	Species
CMY-2	12	*E. coli*
CMY-2	1	*K. pneumoniae*
CMY-2	1	*P. mirabilis*
DHA-1	2	*E. coli*
CMY-31	1	*K. pneumoniae*

of *M. morganii*, was detected in two isolates of *E. coli*. DHA-1 is an inducible plasmid-mediated AmpC β-lactamase whose emergence raises concerns because the mortality of patients infected with organisms that produce DHA-1 has been shown to be higher than that of patients infected with organisms that produce CMY-1.[2]

In conclusion, we have demonstrated that plasmid-mediated AmpC β-lactamases have emerged in Switzerland. The prevalence of 0.16% is still low. However, the occurrence of DHA-1, an inducible type of enzyme, raises clinical concerns. Additionally, a novel plasmid-mediated AmpC β-lactamase, which was designated CMY-31, was found.

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Transparency declarations

None to declare.

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Prevalence and characterization of macrolide-lincomycin-streptogramin B-resistant *Staphylococcus aureus* in Korean hospitals

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Sir,

Resistance to macrolide, lincosamide and streptogramin B (MLS_B) antibiotics in *Staphylococcus* spp. is mediated by a methylase encoded by erythromycin ribosome methylation (*erm*) genes or ATP transporter efflux pumps encoded by the *msr* or *mef* genes. The methylation of the 50S ribosomal subunit by *erm* gene products causes constitutive or inducible resistance to MLS_B antibiotics. Constitutively resistant MLS_B (MLS_Bc) strains and inducibly resistant MLS_B (MLS_Bi) strains express *erm* genes, *erm*(A) or/and *erm*(C). In this study, we investigated and characterized the constitutive and inducible resistance, and the susceptibility to clindamycin of *Staphylococcus aureus* isolated from non-tertiary hospitals.

We collected 508 *S. aureus* strains from 157 hospitals nationwide from January to June 2005 in Korea. Antimicrobial susceptibility to oxacillin, erythromycin, clindamycin and quinupristin/dalfopristin and the MICs were evaluated according to the guidelines of the CLSI. Of the 508 *S. aureus* isolates, 46.1% (234 isolates) were resistant to oxacillin, 55.1% (280 isolates) were resistant to erythromycin, which comprised 98.3% (230/234) methicillin-resistant *S. aureus* (MRSA) strains and 18.2% (50/274) methicillin-susceptible *S. aureus* (MSSA) strains, and 37.4% (190 isolates) were resistant to clindamycin. No strains were susceptible to erythromycin and resistant to clindamycin. One hundred and eighty-six MRSA (80.9%) and four MSSA (8.0%) were MLS_Bc (resistant to erythromycin and clindamycin; Table 1). D-test according to Steward's method was used for isolates with erythromycin resistance and clindamycin susceptibility.[1] Forty-three MRSA (18.7%) and 40 MSSA (80.0%) were MLS_Bi (showing the D form on the D-test), and 1 MRSA (0.4%) and 6 MSSA (12.0%) were MLS_Bs (susceptible to clindamycin).

The MLS_B resistance genes, *erm*(A), *erm*(B), *erm*(C), *msr*(A), *msr*(B) and *mef*, were detected using a PCR method.[2] *erm*(A) was detected in 250 strains, *erm*(C) in 14 strains, *erm*(B) in 1 strain, *msr*(A) in 3 strains, *erm*(A) and *erm*(C) in 2 strains, *erm*(A) and *msr*(A) in 3 strains and no genes in 7 strains.

Most MLS_Bc and MLS_Bi strains carried the *erm*(A) or *erm*(C) gene (Table 1). The *erm*(A) gene (in 250 isolates) was detected far more frequently than *erm*(C) (in 14 strains). However, the *erm*(C) gene was distributed more frequently in MLS_Bi strains (12 isolates) than in MLS_Bc strains (2 isolates). Using OLIGO primers designed by Arthur *et al*.,[3] the PCR products were detected in three MLS_Bc or MLS_Bi strains lacking *erm*(A), *erm*(B) or *erm*(C). It was possible that the three strains carry an *erm* gene besides *erm*(A), *erm*(B) or *erm*(C). *msr*(A)